# LABORATORY MANUAL FOR AUSTRALIAN SUGAR MILLS

# VOLUME 2

These methods have been developed over time to suit the conditions and circumstances of the Australian Sugar Industry. Where applicable, the methods have been developed along existing ICUMSA guidelines and include lists of relevant source material.

The Laboratory Manual for Australian Sugar Mills is being made available to the Australian Sugar Industry via Sugar Research Australia

The Laboratory Manual for Australian Sugar Mills will be reviewed from time to time or as required by the Australian Sugar Industry Alliance, seeking appropriate technical input from Australian sugar mills and other qualified experts

# METHOD 1
# CALIBRATION OF POLARIMETER BY QUARTZ PLATE STANDARD

## 1.0 SCOPE AND FIELD OF APPLICATION

This method describes a procedure for calibrating automatic polarimeters using a quartz plate as the standard. The procedure determines the "scale correction" for the polarimeter, which corrects for the calibration error of the polarimeter at 20°C. This correction is added to the polarimeter reading when calculating a polarisation result, using Method 30 "Polarisation - Determination in Raw Sugar using Automatic Polarimeters".

*Scale Corrections outside the range 95°Z to 100°Z*

This method determines the scale correction near 100°Z (95°Z to 105°Z). For readings outside this range a proportional scale correction can be used. For example, if the scale correction at 100°Z (SC) is +0.10°Z, then the scale correction at 30°Z ($SC_{30}$) would be +0.03°Z.

## 2.0 PRINCIPLE OF METHOD

*Initial Scale Correction*

An initial scale correction is determined if:

- the polarimeter scale correction has not been measured for an extended period (more than 15 days).
- maintenance has been carried out on the instrument which is likely to have affected its calibration (for example: removal of the scale or adjustment of the interference filter).

*Running Average Scale Correction*

The "running average scale correction" is the average of the last ten scale correction determinations, and is the scale correction applied during the normal operation of a laboratory. This procedure is intended to "smooth out" the normal variances of individual scale correction determinations. As the calibration of a polarimeter does not normally change suddenly, the average of ten readings will accurately represent the calibration of the polarimeter if all the readings have been taken within the last 15 days.

## 3.0 CAUSES OF CALIBRATION CHANGE

While the calibration stability of automatic polarimeters is good, they may be prone to drift. The most common cause of drift is the ageing of the interference filter used in most automatic circle polarimeters. This is normally a slow process (less than .01°Z per month) but can become as high as .01°Z per day at the end of the filter's life. There are a number of other electronic and optical problems which can cause calibration change.

NOTE: Changing the "REL number" on the "Polartronic" polarimeter will change its calibration and therefore the scale correction. [This change should only be made in accordance with legal metrology requirements.]

## 4.0 DEFINITIONS

- $Q_{20}$ - The certified value of the quartz plate at 20°C.
- $Q_{tq}$ - The polarisation reading (°Z) of the quartz plate at temperature $t_q$.
- $t_q$ - The temperature of the quartz plate (°C) at the time of reading.
- $t_p$ - The ambient temperature of the polarimeter (°C) (assumed to be equal to $t_q$ in this method).
- $C_{tq}$ - The correction to be added to the quartz plate polarisation reading to correct for the temperature of the quartz plate.
- $C_{tp}$ - The correction to be added to the polarisation reading to correct for the temperature of the polarimeter.
- SC - The scale correction of the polarimeter at 100°Z to correct for the scale calibration error at an ambient temperature of 20°C.
- $SC_p$ - The scale correction to be used when the polarisation reading (P) is outside the range 95°Z to 105°Z.

## 5.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

5.1 **Thermometer,** length 20 cm, 10-35°C, graduated in 0.1°C.

5.2 **Certified quartz control plate** in the range 95°Z to 105°Z.
Quartz control plates shall be standard plates which have been certified for use according to legal metrology requirements.

## 6.0 INSTRUMENTS

The polarimeter under test.

6.1 **Care of Polarimeter**. Keep splash glasses clean. If the glasses have to be removed for cleaning, care should be taken when reassembling that they are not overtightened - thus being strained and becoming optically active. Variable readings when a sample is rotated in the trough can indicate stress in the optical components of the sample or the instrument.
Clean the air filters on automatic polarimeters regularly (at least once per month) and keep air outlets clear to allow a free flow of air.

6.2 **Location of Polarimeter**. Locate the polarimeter where it will not be heated by direct radiation (eg sunlight) or be subjected to excessive vibration.

6.3 **Thermometer Location**. For the determination of the temperature of the quartz plate, do not place the thermometer in the trough of automatic polarimeters as this temperature will be above ambient and will not indicate the correct reading. A position near an air *inlet* of the polarimeter is most suitable.

6.4 **Quartz Plate**. Store the quartz plate in a holder near the polarimeter such that it is not heated by direct radiation or by warm air currents from the instrument.
With automatic polarimeters, a position near an air inlet is most suitable. Store the quartz plate in this position for at least 30 minutes before measurements are carried out in order that it reaches thermal equilibrium with the thermometer and the polarimeter.

## 7.0 PROCEDURE

A. SCALE CORRECTION DETERMINATION WITH AUTOMATIC POLARIMETERS

7.1 Record the *temperature of the quartz plate*, $t_q$ to 0.1°C (it is not necessary to record the temperature with quartz wedge instruments — see 8.6). Allow the reading of the empty polarimeter to stabilise and *reset the zero*.

7.2 Place the quartz plate in the polarimeter trough with a minimum of handling and close the lid gently.

7.3 Allow the polarimeter reading to stabilise and record the reading. Obtain four readings rotating the quartz plate through 45° between each reading. Ensure the lid is closed before reading. Average the four readings and record to the nearest 0.01°Z. This is the *quartz plate reading*, $Q_{tq}$ at temperature $t_q$.

7.4 Remove the quartz plate, close the lid and check that the polarimeter returns to zero ± 0.02°Z. If not, repeat 7.1 to 7.4.
NOTE: IF AFTER A SECOND SET OF READINGS THE ZERO STILL DOES NOT RETURN TO ±0.02°Z, A FAULT IN THE INSTRUMENT IS INDICATED. THE INSTRUMENT SHOULD BE CHECKED IN ACCORDANCE WITH LEGAL METROLOGY REQUIREMENTS.

7.5 See Section 8 for the calculation of scale correction.

B. DETERMINATION OF AN INITIAL SCALE CORRECTION

7.6 To determine an "Initial Scale Correction" at least 20 in-tolerance scale corrections are required. To be in tolerance, all results must be within ± .04°Z of the average of the results.

7.7 The scale correction determinations should be carried out over a period of 2 to 15 days. To minimise systematic errors, each of the determinations should be separated by a period of at least 30 minutes.

7.8 It is desirable that all of the sugar analysts who will be using the polarimeter contribute equally to the total of 20 determinations required.

## 8.0 CALCULATIONS

A. CALCULATION OF A SINGLE SCALE CORRECTION VALUE

8.1 The following readings have been recorded:
$Q_{tq}$ — the quartz plate reading at temperature $t_q$
$t_q$ — the temperature of the quartz plate and the polarimeter at the time of reading (required for circle polarimeters only)

$Q_{20}$ — the certified value of the quartz plate at 20°C.

8.2 Calculate the average quartz plate reading, $Q_{tq}$.

8.3 Determine the correction to the quartz plate polarisation for the temperature of the quartz plate, $C_{tq}$ from Table XXVI (required for circle polarimeters only).

8.4 Determine the correction to the polarisation reading for the polarimeter temperature, $C_{tp}$ from Table XXIX (only required for circle polarimeters with an interference filter). Note that $t_p = t_q$.

8.5 Calculate the scale correction at 100°Z (SC), from:

$SC = Q_{20} - (Q_{tq} + C_{tq} + C_{tp})$

NOTES:

1. This equation is an approximation which has an error of less than 0.01°Z when:
   - The scale correction is between -0.2°Z and +0.2°Z.
   - The certified value of the quartz plate is between 95°Z and 105°Z.

   If these conditions are not satisfied then the following proportional (exact) equation should be used:

$$SC = (Q_{20} - (Q_{tq} + C_{tq} + C_{tp}) \times \frac{100}{Q_{20}}$$

2. With quartz wedge compensation polarimeters the terms $C_{tq}$ and $C_{tp}$ will cancel each other in the equation of 8.6 (provided that the quartz plate temperature is the same as the polarimeter ambient temperature). Hence, it is not necessary to measure the temperature with quartz wedge instruments.
3. If a scale correction outside the range 95°Z to 105°Z is required, it can be calculated from the equation:

$$SC_p = \frac{P}{100} \times SC$$

where: SC is the scale correction at 100°Z.
P is the polarisation in °Z at which the instrument is being used,
$SC_p$ is the scale correction for a polarisation reading of P°Z.

B. CALCULATION OF AN INITIAL SCALE CORRECTION

8.6 At least 20 individual scale correction results must be determined (7.14-7.16 and 8.1-8.6). Calculate the average scale correction from all results.

8.7 Determine the result which is furthest from the calculated average scale correction. If this result is more than ± .04°Z from the average scale correction, then eliminate it from the list and recalculate the average scale correction.

8.8 Repeat 8.8 until all results are in tolerance. If there are fewer than 20 results remaining, then further determinations should be made.

8.9 The average of at least 20 in-tolerance scale correction determinations is the initial scale correction for the instrument.

C. CALCULATION OF THE RUNNING AVERAGE SCALE CORRECTION

8.10 The "Running Average Scale Correction" is deter-mined by calculating the average of the last ten scale correction determinations.

8.11 None of the ten values which are used to calculate the running average scale correction should be more than 15 days old. It is desirable to make at least one determination per day. However, all ten values should not be taken on the one day.

8.12 If a scale correction determination is more than ± 0.04°Z from the current running average scale correction then the determination should be repeated. If the repeat determination is also out of tolerance then the polarimeter should be checked, and an initial scale correction should then be established.

**SCALE CORRECTION DETERMINATION**
**WORK SHEET (Method 1)**

Quartz Plate No ..... Analyst ..... Date .....

Polarimeter .....

| READINGS | | | CALCULATIONS | |
|---|---|---|---|---|
| Q.P. | (1) | °Z | Q.P. Certified Value, $Q_{20}$ | °Z |
| | (2) | °Z | | |
| | (3) | °Z | | |
| | (4) | °Z | | |

| | | | |
|---|---|---|---|
| Ave Q.P. reading, $Q_{tq}$ | °Z | Q.P. Reading, $Q_{tq}$ | °Z |
| Q.P. Temp, $t_q$ | °C | Correction $C_{tq}$ (Table XXVI) | °Z |
| Polarimeter Temp. $t_p = t_q$ | | Correction $C_{tp}$ (Table XXIX) | °Z |

CALCULATE THE SCALE CORRECTION AT 100°Z FROM EQUATION:
$SC = Q_{20} - (Q_{tq} + C_{tq} + C_{tp})$
SC =
SC =

**WORKED EXAMPLE**
**SCALE CORRECTION DETERMINATION**
**WORK SHEET (Method 1)**

*Quartz Plate No*. 1487 *Analyst* U.R. Smart *Date* 29.4.12

*Polarimeter:* Polartronic I.D. 401

| READINGS | | | CALCULATIONS | |
|---|---|---|---|---|
| Q.P. | (1) | 98.95 °Z | Q.P. Certified Value, $Q_{20}$ | 98.90 °Z |
| | (2) | 98.90 °Z | | |
| | (3) | 99.05 °Z | | |
| | (4) | 99.00 °Z | | |
| Ave Q.P. reading, $Q_{tq}$ | | 98.98 °Z | Q.P. Reading, $Q_{tq}$ | 98.98 °Z |
| Q.P. Temp, $t_q$ | | 24.8 °C | Correction $C_{tq}$ (Table XXVI) | -0.07 °Z |
| Polarimeter Temp. $t_p = t_q$ | | | Correction $C_{tp}$ (Table XXIX) | - °Z |

CALCULATE THE SCALE CORRECTION AT 100°Z FROM EQUATION:
$SC = Q20 - (Q_{tq} + C_{tq} + C_{tp})$
SC = 98.90 - (98.98 - 0.07 + 0)
SC = -0.01°Z

## 9.0 REFERENCES


9.1 Bunagel R, Rimkus D and Spiegelhalter F (1972): Zucker, 25, 554-559.
9.2 Bettin H and Spieweck F (1990): PTB-Mitteilungen, 100, 195.
9.3 ICUMSA, Proceedings of 20th Session (1990).
9.4 ICUMSA Methods Book (1994): SPS-1.
9.5 Zuckerind, 113, 512-514 (1998).

# METHOD 2
# POL - DETERMINATION IN JUICE
# (& DERIVATION OF POL % JUICE)

**1.0 SCOPE AND FIELD OF APPLICATION**

This Level 3 method has been adopted by the Australian sugar industry for the analysis of first expressed juice. The method is applicable also to the determination of pol in all other cane and mill juices.

**2.0 DEFINITIONS**

Pol: The pol (polarisation) of a solution is the concentration (on a w/v basis) of a solution of pure sucrose in water having the same optical rotation as the sample at the same temperature. The quantity of 26.000g of pure sucrose, weighed in air under standard conditions, dissolved in water to produce 100.00ml of solution, will have a pol of $100^0$Z. For solutions containing only pure sucrose in water, pol is a measure of the concentration of sucrose present; for solutions containing sucrose and other optically active substances, pol is the algebraic sum of the rotations of the constituents present.

Pol % Juice: The Pol % Juice of a solution is the concentration (in g solute per 100g solution) of a solution of pure sucrose in water having the same optical rotation as the sample at the same temperature. For solutions containing pure sucrose in water, pol % juice is the same as sucrose % juice.

**3.0 PRINCIPLE**

A sample of the juice is clarified by adding basic lead acetate powder (which coagulates colloidal impurities and removes some colorant) followed by filtration. The pol of the clarified solution is read in a polarimeter tube of standard length in a sugar polarimeter (self indicating polarimetric saccharimeter).

The pol of a sugar solution (a w/v measure) can be converted to pol % juice (a w/w measure) using the brix value of the solution prior to temperature correction by using Schmitz's Table (Table II).

If the juice from deteriorated cane cannot be clarified by basic lead acetate powder, a solution of lead nitrate/sodium hydroxide (Herles' Reagent) may be used as the clarifying agent.

**4.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

4.1 **Sugar Polarimeter**. Automatic sugar polarimeters should satisfy standards laid down by ICUMSA (1998) SPS-1, Section 7.2.
All sugar polarimeters for first expressed juice analysis for cane payment purposes must meet legal metrology requirements.

4.2 **Quartz Control Plates**. Quartz control plates for first expressed juice analysis for cane payment purposes must meet legal metrology requirements.

4.3 **Polarimeter tube**, 200 mm long, and cover glasses, conforming with ICUMSA (1998) SPS-1, Sections 7.3-7.4, Subject 2, specifications. A standard or a continuous pol tube can be used.
Polarimeter tubes for first expressed juice analysis for cane payment purposes must meet legal metrology requirements.

4.4 **Juice can**, plastic or metal, approximately 1.5 L capacity.

4.5 **Stemless filter funnel**, glass or plastic, 7.5 cm diameter. 100 mL capacity.

4.6 **Filter glass** or conical beakers, 300 mL.

4.7 **Boiling flask** (Florence flask), 250 mL, or suitable container with lid.

4.8 **Filter paper**, Whatman No. 91 or equivalent (15cm or larger).

4.9 **Cold water bath** (typically not needed for 1st expressed juice).

**5.0 REAGENTS**

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity.**
**Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

5.1 Basic lead acetate powder (Horne's Dry Lead), having the following specifications:

| | |
|---|---|
| Basic Lead (as PbO) | : not less than 33% |
| Total Lead (as PbO) | : not less than 75% |
| Basicity | : 41.3 - 50.7% |
| Loss on Drying (2 hr at 105°C) | : not exceeding 1.5% |

Insoluble Matter
(1 g in 50 mL water) : not exceeding 2.0%
Insoluble Matter
(5 g in 100 mL of 10%
acetic acid) : not exceeding 0.05%
Fineness — through 425
micron : 100%
— through 125
micron : not less than 70%

5.2 **Herles' Reagent**

Solution A — Dissolve 50.0 ± 0.1 g sodium hydroxide in water and dilute to 1 litre in a volumetric flask.
Solution B — Dissolve 500.0 ± 0.1 g lead nitrate in water and dilute to 1 litre in a volumetric flask.
To check the reagent, mix equal volumes of solutions A and B and measure the pH of the mixed solution. If the pH is above 7.0, solution A is diluted until the resultant mixed solution gives a pH below 7.0.
*Safety*
Lead is a highly toxic, cumulative poison. Persons who use or are exposed to dry lead over long periods should have an annual blood test through local hospitals or occupational health authorities.
Label containers : Label all containers, in which any lead solution or compounds is stored or used, with the name and also "Poison".
Avoid ingesting lead : Always wash hands thoroughly before eating. Always wear a mask andavoid breathing dust when handling powder. Do not drink from laboratory glass-ware or taps. protective clothing where possible. Keep lead reagent and leaded solutions away from cuts and abrasions.
Display precautions :On laboratory notice board. 131126. Ipecac Syrup APF should be kept on hand.
Disposal of lead waste: Place all wastes containing any lead reagents into a plastic bucket which is half filled with dry soda-ash (sodium carbonate) powder. The bucket should have a tightly fitting lid and be clearly marked "Poison-Lead Waste". Accumulated lead waste should be recycled using arrangements specified for the sugar industry or disposed of to an authorised trade waste authority.

## 6.0 CALIBRATION OF THE SUGAR POLARIMETER

6.1 The sugar polarimeter is calibrated against a certified quartz plate. Refer to Method 1 "Calibration of Polarimeter by Quartz Plate Standard". The calibration of the polarimeter should be adjusted such that the scale correction is insignificant. (Less than ± 0.10°Z).

6.2 The scale correction should be checked regularly preferably at the beginning of each shift. A running record of the scale correction should be kept.

6.3 Before reading a batch of samples, and at least once each shift, the instrument should be set to zero with nothing in the light path (zero on air). The continuous pol tube (4.3) should then be rinsed several times with distilled water, filled with distilled water and a reading taken. A reading outside the range ± 0.02°Z should be investigated and remedied. Dirty or strained cover glasses are the most likely cause.

## 7.0 PROCEDURE

A. JUICE CLARIFICATION

7.1 If necessary, cool the juice to room temperature in a cold water bath as soon as the sample is taken.
(Note: The conversion of pol readings to pol % juice using Schmitz's Table assumes that pol and brix analyses are done at the same temperature. Juice samples other than first expressed juice may be at a temperature higher than ambient and will require cooling prior to analysis.)

7.2 Transfer 150-200 mL to a dry 250 mL boiling flask or suitable container with lid (4.7).

7.3 As soon as practicable, but within 30 minutes of subsampling, add the minimum quantity of dry lead needed to clarify the juice. For normal juices about 1.0 g/200 mL dry lead is sufficient. For juices that are difficult to clarify up to 2.0 g/200 mL may be added.
NOTE: Avoid adding excess dry lead as its use has been shown to inflate the pol reading.

7.4 Shake to mix thoroughly and allow to stand for 2 minutes.

7.5 Filter the solution through a Whatman No. 91 or equivalent filter paper in a clean, dry stemless funnel into a dry filter glass (4.6). Place a cover slip over the filter funnel to minimise evaporation. Discard the first 10-15 mL and collect about 100 mL of clear filtrate.
NOTE: Filter aid e.g.Supercel (about 1 teaspoon or approx. 5 g) may be added to aid poorly filtering juices.

B. READING THE POLARIMETER

7.6 Pour at least 40mL of the clear filtrate (7.5) through the continuous pol tube.
NOTE: If the continuous pol tube was previously filled with water, then rinse the tube with at least 30-50 mL of the clear filtrate prior to reading.

7.7 Allow the pol reading to stabilise. Read and record the pol reading, P, of the clarified juice to 2 decimal places if possible.

C. DETERIORATED JUICE

7.8 Juice from badly deteriorated cane may not give a clear filtrate and in such cases an alternative method of clarification using Herles' reagent (5.2) may be used.

7.9 To 50.0 ± 0.5 mL of the deteriorated juice in a 100 mL flask add 5 ± 0.1 mL of solution B followed by 5 ± 0.1 mL of solution A (5.2). Add water to the neck of the flask and mix by swirling. Make to the mark and mix well.

7.10 Filter and read the pol of the clear juice as in steps 7.5 to 7.9. Multiply the reading by 2 for the alternative clarification method.

**8.0 DETERMINATION OF RESULTS**

8.1 Pol: Pol in juice is taken as the pol reading, P, from 7.7. In the case of deteriorated juice where the alternative clarification method is used, it is necessary to multiply the pol reading P by 2 as per 7.10.

8.2 Pol % Juice: Calculate the % pol of juice from Table II (Schmitz's Table), using the pol reading, P, and the brix of the juice, at the temperature of polarisation. Record to 0.1pol % juice.
(Note:Table II derives the normality of the juice sample from the brix of the juice prior to temperature correctionand hence the % pol of the juice.)

**9.0 RANGE OF RESULTS & PRECISION**

9.1 The expected range of results for pol percent juice is 0-20 units.

9.2 The Australian sugar industry has adopted a within-laboratory repeatability of 0.10°Z for pol readings.

**10.0 REFERENCES**

10.1 ICUMSA, Specification and Standard SPS-1 1998.

# METHOD 3
# BRIX - DETERMINATION IN JUICE BY HYDROMETER

**1.0 SCOPE AND FIELD OF APPLICATION**

This Level 3 method has been adopted by the Australian sugar industry.
For the purpose of cane payment refer to Note 3 – Conventions at the front of this manual.

**2.0 DEFINITION**

Brix: The brix of a solution is the concentration (in grams solute per 100 g solution) of a solution of pure sucrose in water, having the same density and temperature as the solution being analysed.

**3.0 PRINCIPLE OF METHOD**

The sample of juice is cooled if necessary and then allowed to stand so that suspended solids can settle out and air bubbles can escape. A brix hydrometer is floated in the juice and the brix read from the scale. The temperature of the juice is read and, a temperature correction is applied for temperatures other than 20°C.
Unsettled suspended solids e.g. high mud levels can affect the hydrometer reading.

**4.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

4.1 **Brix hydrometer** (spindle), for the standard temperature, 20°C, graduated in 0.1° Bx. Brix hydrometers should be constructed to comply with the ICUMSA (1970) specifications.
All brix spindles used in first expressed juice analysis for cane payment must meet legal metrology requirements.

4.2 **Thermometers**, either mercury in glass, solid stem or a digital temperature indicator system. The scale of the mercury in glass thermometers should not exceed 50°C and must include zero. The maximum graduation should be 0.5°C. Digital units must have a discrimination no greater than 0.1°C.
All thermometers used in first expressed juice analysis for cane payment must meet legal metrology requirements.

4.3 **Juice Can**, metal or plastic, approximately 1.5 L capacity.

4.4 **Brix Cylinder**, metal or plastic. The brix cylinder shall have dimensions of not less than 350 mm internal length and 48 mm internal diameter.

4.5 **Cold Water Bath**.

**5.0 PROCEDURE**

5.1 The juice should not be stirred or otherwise aerated after the sample is taken.

5.2 Fill a clean, well drained brix cylinder with juice from the sample can. Pour the juice through a strainer if there is an appreciable amount of suspended matter present.

5.3 After allowing the juice to stand undisturbed for at least 20 minutes, remove any froth from the top of the cylinder using a spoon or spatula. Alternatively, if the juice is badly aerated, the contents may be placed under a negative pressure of not less than minus sixty five kilopascals (-65 kPa) for a period of five minutes. In the latter case, the juice shall be allowed to remain undisturbed until at least twenty minutes have elapsed since the juice was last disturbed.

5.4 Select a brix hydrometer with the appropriate range for the sample. The lowest range hydrometer suitable for the sample being tested should be used so that depth of immersion is a minimum.

As a ***guide*** only, the ranges of hydrometers to be used are as follows:-

| | |
|---|---|
| For pol reading <70: | 10-20 $^0$hydrometer |
| For pol reading 70-92: | 15-25 $^0$hydrometer |
| For pol reading >92: | 20-30 $^0$hydrometer |

5.5 Hold the hydrometer above the graduated scale between thumb and index finger, and lower it carefully into the juice until the flotation level is reached. Do not wet the stem any further than is necessary to form a natural meniscus.
Withdraw the hydrometer and gently refloat it to the same level as before. The stem should not be wet more than two graduations above the juice level. (This step can be carried out by carefully raising the hydrometer a few centimetres from rest to facilitate the wiping of the stem scale before gently lowering the hydrometer to the former resting point.)

5.6 When the hydrometer has settled, read the brix of the juice. Take the reading (with the eye level with the level of the juice surface), by estimating the point at which the surface would cut the scale.

Alternatively, this step can be carried out by taking the reading at the top of the meniscus and adding 0.2 to the reading of the lowest exposed graduation. Record the brix to 0.1°.

5.7 Record the temperature of the juice in the brix cylinder to 1°C.

**6.0 DETERMINATION OF RESULTS**

6.1 Apply the temperature correction from Table 1 corresponding to the temperature in 5.7 to the brix reading obtained in 5.6 to calculate brix at 20°C. Record to 0.1° brix.

**7.0 RANGE OF RESULTS & PRECISION**

The expected range of results is 12-30$^0$brix.

The Australian sugar industry had adopted a within-laboratory repeatability of 0.2° brix for this method.

**8.0 REFERENCES**

8.1 ICUMSA. Proceedings of 15th Session (1970), General Subject 2, Appendix 1, Sections 4.1 - 4.10.

# METHOD 3A

## BRIX - DETERMINATION IN JUICE BY VIBRATING TUBE DENSITY MEASUREMENT

### 1.0 SCOPE AND FIELD OF APPLICATION

This Level 3 method has been adopted by the Australian sugar industry as a method applicable to the determination of brix in first expressed juice and other mill juices. It has been developed from laboratory and factory research, and has not yet been subjected to rigorous inter-laboratory testing.

### 2.0 DEFINITION

*Brix*: The brix of a solution is the concentration (in grams solute per 100 g solution) of a solution of pure sucrose in water, having the same density as the solution at the same temperature.

### 3.0 PRINCIPLE OF THE METHOD

The method uses a vibrating tube density meter to measure liquid density. The density of a solution is a function of the solution temperature. It is imperative that the temperature used in the conversion of density to brix is a true indication of the juice temperature in the sensor tubes. These instruments are susceptible to fluctuations in temperature. Relatively small temperature differences between the incoming juice and the existing sensor temperature can cause significant errors in brix determination. It is important to ensure the juice and sensor temperatures are within 1°C of each other when measuring the density. It is equally important that the primary calibration, the initial nitric acid calibration and the routine nitric acid checks are conducted under similar conditions. The sample of juice is allowed to stand to allow suspended solids to settle and air bubbles to escape. A predetermined aliquot of the juice sample is then drawn through the mass flow meter by means of a positive displacement pump. This aliquot should be within 1°C of the sensor temperature at the finish of pumping. The pump is stopped, and the juice density and sensor temperature are determined. Brix is calculated using ICUMSA equations in Appendix 2.

### 4.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

4.1 **Vibrating tube density meter** featuring one or more tubes of diameter 5 ± 0.5 mm. Instruments of other dimensions can be applied providing appropriate pumping rate and ancillary equipment are also used. For details see Appendix 3. The accuracy of the instrument should be at least ±0.0005 g/mL.

Materials of construction, including fittings, should be capable of resisting the corrosive effects of cane juice and 2.5 M nitric acid used as a calibration liquid.

**NOTE:** Only nitric acid at the specified strength may be used in this application. Other substances may damage the meter.

Supplementary information for the use of the vibrating tube instrument is provided in Appendix 4.

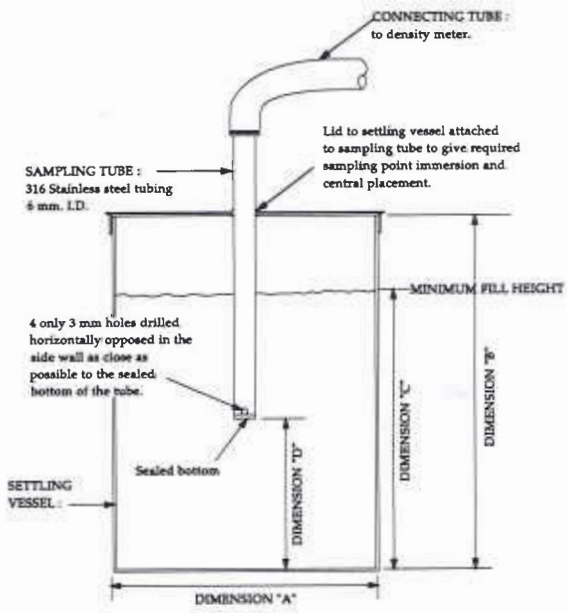
4.2 **A peristaltic pumping system** with a rated capacity of 8 litres/minute and reliably able to deliver 400 ± 10 mL in 50 seconds.

4.3 **Sampling tube.** This should be constructed from 316 stainless steel tubing, approximately 350 mm in length or to suit the settling vessel and 6 mm I.D. sealed at the bottom. Four horizontally opposed 3 mm diameter holes are drilled in the side wall as close as possible to the bottom.

The sampling point should be at approximately the centre of the sampling vessel. It is acceptable to attach a rod to the bottom of the sampling tube to locate the sampling holes at the correct immersion point.

The sampling tube is connected to the meter in the configuration shown in Appendix 1. The lengths of the sampling tube and connecting tubing should be kept to a minimum. For a 400 mL sample volume, the combined volume of sampling tube and connecting tubing should not exceed 40 mL. If this volume is exceeded, the sample volume should be redetermined as per Appendix 3.

TYPICAL SAMPLING TUBE ARRANGEMENT




REFER TO TABLE 1 FOR DIMENSIONS "A", "B", "C" & "D".

N.T.S.

4.4 **Juice Settling Vessel**. Automatic juice sampler cans used in the Australian sugar industry may be suitable for this purpose. Settling vessel dimensions are determined mathematically using equations listed in Appendix 3; and must conform to the requirements of Appendix 3, Part B.

4.5 **Thermometer** with a resolution of 0.1°C and accurate to within ± 1°C (Refer to Appendix 4) to measure the temperature of the juice in the settling vessel. This thermometer is independent of the measuring instrument and is used in determining Apparent Density at solution temperature to enable pol % juice to be calculated.

4.6 **Thermostatically regulated juice temperature control system**, for example a water bath or heat exchanger, able to ensure all samples are within 1°C of the sensor temperature when entering the sensor tubes.

## 5.0 CALIBRATION

This section details three calibration procedures:

- pre-season set up and calibration
- calibration of a new nitric acid working standard
- routine calibration checks as required.

### 5.1 Preparation of 16°Bx, 20°Bx and 24°Bx Standard Solutions

These solutions are to be prepared immediately prior to use and discarded after the calibration is complete.

5.1.1 Spread sufficient refined sugar for a calibration run (approx. 1000g) on a tray in a layer of about 10 mm thick and dry in an oven at 96 - 100°C for 3 hours ± 10 minutes. Transfer to an oven dried beaker and cool to room temperature in a desiccator.

5.1.2 Weigh 171 ± 0.5 g of dried sugar into a dry stoppered conical flask. Record the weight of sugar to 0.01 g ($x_{16}$).

5.1.3 Add 900 ± 0.5 g of distilled water from a measuring cylinder. Record weight of water to 0.01g ($y_{16}$).

5.1.4 Stopper flask and dissolve sugar.
$Bx_{16} = x_{16} \times 100/(x_{16}+y_{16})$

5.1.5 Weigh 284 ± 0.5 g of dried sugar into a dry stoppered conical flask.
Record weight to 0.01 g ($x_{24}$)

5.1.6 Add 900 ± 0.5 g of distilled water from a measuring cylinder.
Record weight to 0.01 g ($y_{24}$)

5.1.7 Stopper flask and dissolve sugar.
$Brix_{24} = x_{24} \times 100/(x_{24} + y_{24})$

5.1.8 Weigh 225 ± 0.5 g of dried sugar into a dry stoppered conical flask.
Record weight to 0.01 g ($x_{20}$)

5.1.9 Add 900 ± 0.5 g of distilled water from a measuring cylinder.
Record weight to 0.01 g ($y_{20}$)

5.1.10 Stopper flask and dissolve sugar.
$Brix_{20} = x_{20} \times 100/(x_{20} + y_{20})$

### 5.2 Preparation of Nitric Acid Working Standard

*Safety*

Nitric acid reacts violently with powerful reducing agents and many organic compounds. It reacts with most metals, in some cases violently. It may ignite combustible material and evolve toxic fumes in fire. It causes severe burns to eyes and skin. If ingested it causes severe internal irritation and damage. It emits an irritating harmful vapour.

Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.

**First Aid**

**Eyes** Irrigate thoroughly with water for at least 10 minutes. Obtain medical attention.

**Lungs** Remove from exposure, rest and keep warm.

**Skin** Drench the skin thoroughly with water. Remove contaminated clothing and wash before re-use. Unless contact has been slight, obtain medical attention.

**Mouth** Wash mouth thoroughly with water and give plenty of water to drink. Obtain medical attention.

Prepare a 2.5M nitric acid working standard of a density which approximates that of a 20° Bx sucrose solution by adding 3200 mL of technical grade 70 % w/w nitric acid, stirring constantly, to 16800 mL potable water in a suitable plastic or glass container. Seal the container, mix well, and allow to cool.

### 5.3 Set Up and Calibration

5.3.1 Flush the meter with potable water to exclude all air.

5.3.2 Calibrate and validate the instrument calibration according to the manufacturer's manual using the standard 16°Bx, 20°Bx and 24°Bx solutions prepared. It is not acceptable to use air and water or a single solution to calibrate the instrument. Solution temperatures should be as close as possible to the sensor temperature at the finish of pumping and must not be more than 1°C from the sensor temperature (3.0).

The following points should be observed:

- Use a flow rate of 8 ± 0.2 mL per second and a 400 ±10 mL sample volume. For the initial 16° brix solution use two 400mL aliquots, the first being designated a 'wash'. This is to avoid any possible dilution from the preceding water in the meter.
- Stop the pump after delivering this volume.
- Allow readings to stabilise.
- Note the sensor temperature.
- Calculate the density of the solution from its known actual brix for the sensor temperature using Equations 1, 2 and 3, Appendix 2.
- Insert this density when requested in the manufacturer's calibration protocol. (eg as D1 or in the 'offset' sequence for a Micromotion instrument).
- Repeat for the second calibration solution as requested (eg as D2 or in the 'slope' sequence).
- Validate the calibration by checking with the 20°Bx solution. This should be within 0.1°Bx of the theoretical value and failing this the calibration should be repeated.

5.4 **Calibration of Nitric Acid Working Standard**

Calibrate the working standard immediately on completion of the instrument calibration. Use two 600 mL aliquots of the nitric acid standard, one designated 'wash' and the other 'test'. Observing the temperature restrictions of Section 3.0, pump 400 mL of the 'wash' followed by a further 400 mL of the 'test' through the meter at the prescribed rate of 8 mL per second. Stop the pump, allow reading to stabilise and note the density reading and sensor temperature. Label the working standard with this information, including the date. The meter should be thoroughly flushed with potable water immediately after calibration.

Carry out a full standardisation with sucrose solutions before use of a newly prepared bulk working standard.

5.5 **Routine calibration checks**

The meter should be checked with the working standard routinely or 'at appropriate intervals' using the same methodology described in Section 5.4. The nitric acid may be pumped after the final juice in a batch if convenient. There is no necessity to flush the meter with water beforehand. If the density after application of a temperature correction varies from that obtained in Section 5.4 by more than 0.0005g/mL, a full recalibration should be undertaken. (Temperature correction = +0.00045 g/mL per degree celsius rise in temperature from the initial check)

**Discard any excess nitric acid. On no account should any acid be returned to the bulk working standard.**

5.6 **Instrument cleaning**

There should be a regular cleaning program in place to prevent the build up of scale in the sensor measuring tubes. Refer Appendix 4.

**6.0 PROCEDURE**

6.1 Pour the juice sample into a clean dry settling vessel. The juice may be poured through a strainer if necessary. Transfer as soon as possible to the water bath if this system of juice temperature control is used(4.6).

6.2 Allow the juice to stand undisturbed for a minimum of 20 minutes.

6.3 Insert the sampling tube to the sampling point and draw 400 mL of juice through the mass flow meter at a flow rate of 8 mL per second. Care must be taken to avoid entrainment of air into the system. Table 1 gives the sampling point and minimum filled height for various juice sampling vessels for a sample size of 400 mL. A 10 mm safety margin has been incorporated into the sampling points to ensure the holes remain submerged after completion of sampling. A 'wash' juice is pumped through the meter at the start of each batch to avoid any possible water dilution effects.

6.4 Stop the pump.

6.5 Allow the sensor temperature and density readings to stabilise. Read and record the temperature to 0.1°C and the density to 0.0001 g/mL. Calculate brix using Equation 4, Appendix 2. An interfaced computer may be used for conversion of density to brix. Random manual calculations (perhaps one or two per week) should be done to ensure the computerised conversion of density to brix is being done correctly.

6.6 Always flush the meter with water at the completion of a batch, even if only a single measurement.

**TABLE 1**
**Minimum Filled Height and Sampling Point**

| DIMENSION A<br>Settling Vessel Diameter (mm)<br>D | DIMENSION B<br>Settling Vessel Height (mm)<br>H | DIMENSION C<br>Minimum Filled Height (mm)<br>h | DIMENSION D<br>Sampling Point (mm) |
|---|---|---|---|
| 95 | 195 | 150 | 80 |
| 100 | 250 | 185 | 120 |
| | 205 | 151 | 87 |
| | 200 | 148 | 84 |
| | 180 | 135 | 71 |
| 125 | 140 | 96 | 50 |
| | 135 | 94 | 49 |
| | 130 | 92 | 47 |
| | 125 | 91 | 46 |
| | 120 | 90 | 45 |
| 130 | 190 | 120 | 77 |
| | 150 | 97 | 55 |
| | 135 | 91 | 49 |
| | 130 | 90 | 47 |
| | 125 | 88 | 46 |
| 135 | 175 | 108 | 68 |
| 140 | 155 | 95 | 57 |
| 150 | 160 | 94 | 59 |
| | 140 | 85 | 51 |

## 7.0 PRECISION

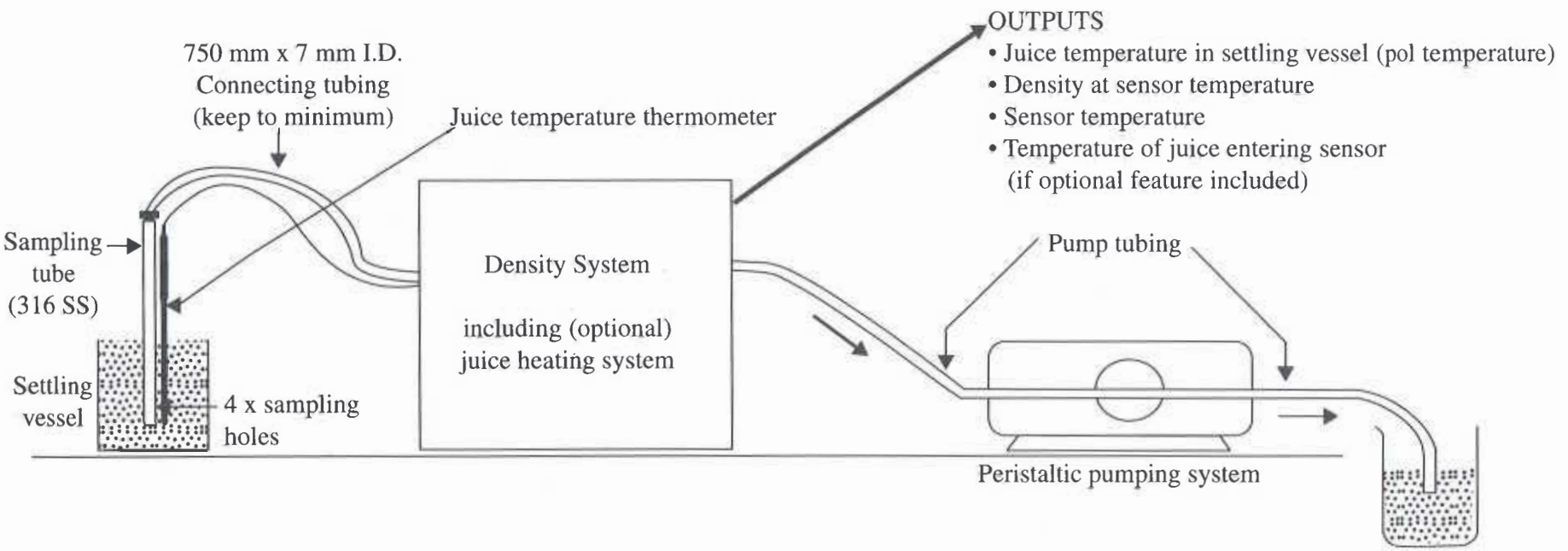
The expected range of results is 12-30°Bx.

| Sample | Concentration Range | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| First Expressed Juice | 12 - 30°Bx | 0.08[2] | Not determined |

## 8.0 REFERENCES

1. Perry's Chemical Engineers' Handbook, Sixth Edition, (1984). Densities of Aqueous Inorganic Solutions, Table 3 - 66, Nitric Acid ($HNO_3$).
2. SRDC Project Report BS133S (October 1997) 'Juice Density Meter Evaluation and System Development'.

# Typical Layout of a Density Determination System



## APPENDIX 2

### MASS FRACTION and DENSITY RELATIONSHIPS

$$\rho = \rho_{H_2O} + 385.1761w + 135.3705w^2 + 40.9299w^3 - 3.9643w^4 + 13.4853w^5 - 17.2890w^6 + (-46.2720w - 7.1720w^2 + 1.1597w^3 + 5.1126w^4 + 17.5254w^5)\tau + (59.7712w + 7.2491w^2 + 12.3630w^3 - 35.4791w^4)\tau^2 + (-47.2207w - 21.6977w^2 + 27.6301w^3)\tau^3 + (18.3184w + 12.3081w^2)\tau^4 \quad (1)^*$$

where $\tau = (t°C - 20)/100$ (2)*

and $\rho_{H_2O}$ is the density of water calculated according to the updated Kell's formula

$$\rho_{H_2O} = (999.83952 + 16.952577t - 7.9905127 \times 10^{-3}t^2 - 46.241757 \times 10^{-6}t^3 + 105.84601 \times 10^{-9}t^4 - 281.03006 \times 10^{-12}t^5)/(1 + 16.887236 \times 10^{-3}t) \quad (3)^{**}$$

w = mass fraction of an aqueous sucrose solution. 'w' must be expressed as a decimal fraction in the formulae.
$\rho$ = Density in $kg/m^3$
t = Temperature °C

$$w = 2.596054r - 2.368154r^2 + 2.529236r^3 - 2.194249r^4 + 1.523668r^5 - 0.479275r^6 + (0.307874r - 0.651523r^2 + 0.689324r^3 - 0.527656r^4 + 0.051049r^5)\tau + (-0.355762r + 0.661862r^2 - 0.594131r^3 + 0.331665r^4)\tau^2 + (0.245401r - 0.366598r^2 + 0.232380r^3)\tau^3 + (-0.075075r - 0.012667r^2)\tau^4 \quad (4)^*$$

where $r = (\rho - \rho_{H_2O})/1000$ (5)*

$$\rho_{App} = \frac{\rho - 1.2}{0.99985} \quad (6)^{**}$$

**Note :** **Equation 1 can be used to convert brix to density at the temperature of polarisation reading (Inlet juice temperature). This value may then be converted to Apparent Density by the relationship given in Equation 6 and used to calculate the 'pol factor' for determining Pol per cent Juice.**

* Proc. 21st Session ICUMSA, 1994, 332 - 334
** ICUMSA Methods Book, 1994, Specification Standard SPS - 4 (1994)

# APPENDIX 3

## SYSTEM CONFIGURATIONS

### A Instrument Tubing Size, Pumping Rate and Sample Volume

Pumping Rate: Experimental observations suggest that a Reynolds Number of 600 in each tube of the vibrating tube density measuring instrument allows a sufficient flow rate to avoid blockage of the instrument when measuring cane juice solutions containing typically high soil concentrations. For configurations other than that of Section 4.1, the flow rate for each measuring tube may be determined from the relationship:

Flow rate per tube (mL/min) = 45 x Tube ID (mm) (1)

Sample Volume: The sample volume of 400 mL specified in Section 5.3.2 and subsequent sections is based on a measuring instrument with two vibrating tubes each 458.22 mm in length and 5.23 mm internal diameter set up with the approximate configuration described in Appendix 1. The required sample volume is a function of the complete system geometry (from the sampling tube to the end of the measuring tubing) and pumping rate. For increasing flows steady state in density reading is achieved in a shorter time but larger sample volumes are required.

If there is a significant alteration in the configuration or change to the instrument in use, the analyst should redetermine the sample volume as follows:

Flush the meter with water at high flow rate to purge any entrapped air from the system.

Draw a pure sucrose solution, approximately 16° to 20° Bx, through the meter at the rate determined by Equation (1) above. Record the density readings every 5 seconds and determine the time to reach stability. Record the computed brix and corresponding pumping times.

The required sample volume is determined from the estimated time at which the computed brix is constant ± 0.05° Bx.

Note the volume, the pumping rate and the time.
(Volume = Pumping Rate x Time)

### B Settling Vessel Selection

Settling vessel dimensions and filled height may be determined mathematically.

Given the required sample volume (v mL), and settling vessel diameter (D mm) and height (H mm), the vessel should be filled to no less than:

$$h = H - 20 \times \left( \frac{2.9504 \times H}{1 + 0.062529 \times H} - 37.644 \right) + \frac{1274 \times (v + 20)}{D^2} + 15$$

where the relationship 2.9504 x H /(1+(0.062529 x H))] -37.644 is the mean settling rate (mm/min) of a column of liquid of height H. This relationship was empirically determined. It resulted from work which produced a mathematical relationship linking filled height and mean settling rate over 20 minutes for juices that would be readable by spindle and not influenced by mud.

A mathematical relationship links the required positioning of sampling holes above the bottom of the settling vessel with sample volume and settling vessel diameter:

$$\text{Sampling point (mm above vessel base)} = h - \frac{1274 \times (v + 20)}{D^2} - 10$$

# APPENDIX 4

## SUPPLEMENTARY NOTES

These notes are designed to provide supplementary information for the user of the vibrating tube instruments.

- This technique was developed in the SRDC project BS133S. Further background and detail may be found in that report.

- Commercial vibrating tube density meters typically have built in conversion equations for calculation of brix from density and temperature data. It is essential that the ICUMSA relationships specified in Method 3A are used in all computations associated with these instruments and not the instrument's relationships.

- The 'Sensor' temperature is used for brix computations and not a juice temperature.

- The temperature of the juice in the settling vessel is used to calculate the apparent density of the juice for pol calculations. This entity can be either computed by the relationships in Appendix 2 of Method 3A or obtained from the Laboratory Manual, Tables I and XVI working back from the known brix.

- BSES provides certification when assessing the instrument's suitability for cane payment analysis that the instrument's internal thermometer and the other juice thermometers are correct to $\pm 1$°C. Thereafter, a temperature check should be undertaken periodically and at least once per year prior to the crushing season or if a suspect reading is obtained from any of the thermometers. The check can be accomplished by switching the instrument off, disabling the juice heat exchanger and cooling fans and circulating water through the instrument for 45 minutes to achieve steady state. Note the water temperature using a BSES calibrated thermometer. After steady state conditions are assured, switch the instrument back on with the water still circulating and immediately note the sensor and settling vessel juice temperatures and, if installed, the juice temperature after the heat exchanger. They each should be within $\pm 1$°C of the reference thermometer.

- The internal juice heat exchanger incorporated in some instruments has been designed to provide approximately 3°C of heating. Two operating protocols are recommended to cover juices that are hotter or colder than the ambient laboratory temperature respectively. For hotter juices, firstly estimate the maximum juice temperature expected (X)°C. Set the sensor temperature set point to (X-0.5)°C or (Y)°C. Set the juice heat exchanger set point to (Y-0.5)°C. This configuration is suitable for juices presented for analysis in the range X°C to (X-4)°C. For colder juices, set the heat exchanger set point 2°C above the expected maximum juice temperature and the sensor set point a maximum of 1°C above the juice temperature. A tolerance of 0.5°C is allowable on these limits.

- If a change is made to the sensor temperature set point, a stabilisation period of at least 30 minutes should be allowed before resuming analyses. If a change of greater than 2°C is made, then recirculate water through the instrument at a temperature approximating the new set point during the stabilisation period.

- The instrument can tolerate only limited air ingress into the measurement cell. Method 3A seeks to minimise this occurrence. The instrument reading should be recorded with stationary juice flow and only when the reading has stabilised. If air enters the instrument it can be removed by pumping approximately 1400 mL of water through the instrument at high volumetric flow rate (about 1500 mL/minute). This purge volume can be reduced by pumping for short periods (3 to 5 seconds) with flow reverses. A stable, 'sensible' reading indicates the air has been purged from the system.

- The instrument manufacturer's calibration procedure must be followed rigorously. For example, in the case of the Rosemount instrument:

  (a) where the juice temperature is controlled by an internal heat exchanger, set the sensor temperature to a typical operating level, (Y)°C, and the heat exchanger outlet set point to (Y-0.5)°C. Calibration solution temperatures should be in the range (Y-2) $\pm 0.5$°C. The latter lies at a median inlet juice temperature for the conditions described (see above). Nitric acid calibration checks should maintain a similar relativity of sensor and juice heat exchanger set points and the temperature of the acid.
  (b) the lower calibration constant must only be inserted when the sensor contains the lower density calibration fluid.
  (c) the higher calibration constant must only be inserted when the sensor contains the higher density calibration fluid.
  (d) the higher calibration constant must not be adjusted without firstly proceeding through the calibration sequence for the lower constant.

Failure to follow the manufacturer's procedures will result in the instrument determining brix incorrectly.

The instrument can be returned to correct status by a basic calibration using air and water and following the procedures provided in the instrument manufacturer's operating manual.

Proceed with a routine calibration at 16°Bx and 24°Bx and validation at 20°Bx.

- Depending on local environmental factors eg soil types, cane varieties etc, some fouling of the inside walls of the measuring tubes may occur over time.

The operating principle of the instrument requires a constant mass of the sensor tubes. Variation in this mass should be caused solely by the liquid under test. In this way, the density reading is a function of the mass of juice in the measuring tubes. At some mills there may be a gradual build up of scale on the tube walls following a calibration. The density as determined by the meter will be affected. The mass of scale will eventually reach a critical level at which the nitric acid calibration check will show the instrument to be outside the calibration limits.

Include a regular cleaning program as follows:

(1) Following a routine water flush, recirculate a caustic solution through the meter at a high flow rate for approximately one minute. Reversing the direction of flow occasionally helps to dislodge stubborn scale. Flush with water. The strength of the caustic solution used will depend on the degree of fouling, but can be as high as 50% if needed without having corrosive effects on the sensor tubing.
(2) When time permits eg during extended mill stops, leave the tubes filled with the caustic solution for two hours and then carry out flushing as in (1).

**Note**: The impurities in NaOH include chloride ions that have a corrosive effect on 316 stainless steel if present in sufficient concentration. The upper limit is a function of temperature. The following table may be used as a guide in choosing the grade of NaOH used. The NaOH may be re-used.

| Temperature (°C) | Maximum Chloride Ion Concentration (ppm) in contact with 316 Stainless Steel |
|---|---|
| 20 | 200 |
| 30 | 90 |
| 40 | 50 |
| 50 | 20 |

# METHOD 4A
# FIBRE - DETERMINATION IN CANE BY SRI CAN FIBRE MACHINE

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian sugar industry. The method is applicable to the determination of fibre in sugar cane. Analytical results derived from this method are used in the calculation of both brix and pol in cane.

**2.0 PRINCIPLE**

Fibre in cane is directly determined by washing out the juice and soluble solids with water and drying and weighing the remaining fibre.

A representative sample of the prepared, shredded cane is collected and reduced by coning and quartering. The sample is comminuted in a cutter-grinder, and mixed again.

A subsample is weighed and lightly compressed in a tapered cylindrical container fitted with a base gauze below the subsample and perforated screen on top. It is then attached to the washing section of the can fibre machine. Warm water (about 40°C) is recirculated through the sample for 20 minutes to form the sample into a filter bed which retains all solids that would otherwise be washed from the sample. The water is then run to waste. Over the next 45 minutes hot water (90°C) is circulated through the sample. A purge of fresh hot water (1.5 L/minute) enters the system and the resulting overflow water is dumped to waste. At the end of the 45 minute period the wash water, now effectively clear of soluble solids, is dumped to waste and the sample container relocated to the drying section of the machine. The sample is dried by passing a controlled flow of air at 105°C through the sample for approximately one hour until constant weight is achieved.

**3.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

3.1 **Cutter-grinder**

*Safety*

The cutter-grinder should be used with care at all times. Display instructions beside the machine. Guard the motor.

**ENSURE THE MACHINE HAS STOPPED AND CONTROLS ARE ISOLATED BEFORE OPENING THE COLLECTION BOX.**

**Use the plunger, not hands, to feed cane.**

Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.

3.2 **SRI Can Fibre Machine**

The machine is self contained comprising washing, drying and control sections. The machine requires two water supplies - a 'cold' supply at 20 ± 15°C and a supply from a hot water system at about 60°C. In addition the machine requires a high pressure, clean air supply, to dry the fibre. Input electrical power is 20 amp 3-phase with neutral and earth.

Schematic diagrams of the machine and sample container are shown in Figures 1 and 2.

Installation and regular maintenance procedures as recommended in the instructions supplied by SRI must be followed.

3.3 **Mixing trays, metal**

NOTE: The cutter-grinder and mixing trays must be located in an area sheltered from sun, wind, rain and steam.

3.4 **Balance** to weigh 1.5 kg to 0.1 g complying with legal metrology requirements.

3.5 **Small brush.**

**4.0 SAMPLING**

4.1 Sample approximately 20 kg of prepared cane. Samples collected should be representative of the overall cane supply being sampled.

4.2 Level the sample immediately and transfer about 2 kg (eg 3 large random handfuls through the depth of prepared cane) to a bin with a well fitted lid. If necessary a small plastic bucket (with lid) or plastic bag may be used to transfer the sample from the sampling point to the sample storage location.

4.3 For composite samples, transfer the sample to a mixing tray. Quickly but carefully break up conglomerates of prepared cane to achieve representative mixing. Reduce the sample size to 2-3 kg by coning and quartering,

4.4 Close up the clean, dry cutter-grinder and switch it on. "Wet condition" the machine by passing some of the unwanted portion of the sample through it. Switch the machine off and isolate the motor. **WHEN THE CUTTER HAS STOPPED TURNING — AND NOT BEFORE** - open the collection box and remove the fibrated cane. Do not open the machine.

4.5 Close the box, restart the motor and pass the mixed sample (4.3) through it. Use a suitable device (ie plunger) to push all the cane through the blades. On no account should the hand be pushed past the outer confines of the machine guard.

Switch the motor off and isolate the motor. **WHEN THE CUTTER HAS STOPPED TURNING — AND NOT BEFORE** —open the machine and collection box and remove the fibrated material by hand, transferring it to a closed plastic bucket, or other suitable container. Any fibrated material adhering to the collection box or discharge chute is included in the sample. Any cane lying above the cutting disc is not to be included in the sample, but must be discarded before the machine is wet conditioned with the next sample.

4.6 **Mixing**

Transfer the freshly-fibrated cane sample to the mixing tray located in a convenient dry place sheltered from sun, wind and rain and away from steam.

Rapidly mix the fibrated cane. Break up large conglomerates of fibre. Mix again and spread the fibrated cane evenly over the bottom of the tray to a depth of about 5 cm.

Note: It is important that the sample is thoroughly but quickly mixed, since it is subject to loss of weight by evaporation while it is exposed to the atmosphere. Take no longer than five minutes for this operation.

## 5.0 PROCEDURE

### A. Sample Preparation

5.1 Transfer up to 500g of freshly-fibrated prepared cane from the mixed sample to a clean dry tared-off container, or to a tared fibre can (5.3), by 'pinching out' sections of the layer at random. It is important that the full depth of the layer is sampled, and a portion of the floor of the mixing tray is left bare at each point of sampling.

5.2 Weigh sample to 0.1 g and record ($m_1$).

5.3 Transfer all the weighed sample carefully to a clean dry cylindrical sample container fitted with a 120 mesh base gauze. Gently press around the edges to form a slight cone. The sample must be evenly placed in the container and not unduly compacted as this will cause abnormally high pressures during the washing cycles.

NOTE: Insert the base gauze with the support ring up, as illustrated in Figure 2.

5.4 Place a perforated screen on top of the fibrated cane in the container as shown in Figure 2. The container is now ready to be placed in the washing section of the can fibre machine.

5.5 If a number of samples have been mixed at the same time, the ones not immediately analysed can be weighed into fibre cans, placed in sealed plastic bags and stored in a refrigerator until analysed.

### B. Washing

5.6 The machine should be flushed with clean water at the start of each day. Follow the instructions for flushing the machine set out in the instruction manual.

5.7 Carefully position and clamp the full sample container in the right hand sample position. Over tightening will result in seal deterioration and leaks.

5.8 To commence the washing cycle press the "**ATTACH SAMPLE"** button if the previous operation was a flush cycle. If it was not a flush cycle depress the **"RECIRC"** button to commence the washing cycle.

5.9 Completion of the washing cycle (after approximately 65 minutes) is indicated by an audible alarm and an illuminated **"FINISH WASH"** light. Press the "**RESET WASH"** button and remove the sample container for drying.

**Allow the container to sit, preferably on a slight angle, for approximately 5 minutes to allow excess water to drain before re-attachment to the left hand side.**

### C. Drying

5.10 Carefully position and clamp the sample container in the left hand side of the machine for drying.

5.11 Press the **"INITIATE DRY"** button to commence the drying cycle.

5.12 Completion of the drying cycle (after approximately 45 minutes) is indicated by an audible alarm and an illuminated "**FINISH DRY"** light. Press the "**RESET DRY"** button and remove the sample container from the machine. Immediately weigh the sample and container to 0.1 g and record. Once weighed, reposition the container in the machine.

5.13 The drying cycle is then restarted for a further 15 minutes by pressing the **"CHECK DRY"** button. When the alarm sounds press the "**RESET DRY"** button and remove the sample container from the machine. Immediately weigh the sample container to 0.1 g and record. If the weight is within ± 0.1 g of the previous weighing no further drying is required. If not, repeat this step (5.13) until consecutive weighings are in agreement and record ($m_2$).

5.14 When the sample is dry and weighing is complete, remove the top perforated screen, gently tap the container to remove the fibrous plug and brush any adhering fibre from the container and gauze. While the container is still hot and dry, weigh the container, gauze and perforated screen to 0.1 g and record ($m_3$).

5.15 Wash the can and gauzes thoroughly to remove all traces of fibre before re-use, and re-dry.

## 6.0 CHECK OPERATIONS

Routine checks of the machine's functions must be carried out as recommended in the instructions supplied by SRI with the machine.

## 7.0 CALCULATIONS

7.1 Calculate the % fibre in cane (mass/mass) to two decimal places.

Percent fibre in cane

$$= \frac{100}{1} \times \frac{\text{mass dry fibre}}{\text{mass fibrated cane}}$$

$$= \frac{100}{1} \frac{[m_2 - m_3]}{m_1}$$

where:
$m_1$ = mass of wet fibrated cane (5.2)
$m_2$ = mass of dry fibre and hot container (5.13)
$m_3$ = mass of hot container (5.14)
**Round and record the result to the first decimal.**
When the second decimal is 5, round the result to the nearest even first decimal.

## 8.0 PRECISION

| Sample | Concentration range % (m/m) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Prepared Cane | 8-20 | 0.29 | N.A. |

## 9.0 REFERENCES


1. Bureau of Sugar Experiment Stations (1991). The Standard Laboratory Manual for Australian Sugar Mills. Volume 2. Analytical Methods and Tables. Method No 4 BSES Publications, Brisbane.
2. Loughran J G, Ivin P C, Vidler T L, MacCarthy W N (1988). A new method and apparatus for the determination of fibre in cane. Sugar Research Institute Technical Report No 200.
3. Sugar Research Institute (1994). Instructions for the Use of 500 gram Can Fibre Machine.

[WA10001.TIF]

Figure 1. Functional items of the SRI Cane Fibre Machine

[WA10002.TIF]

DIMENSIONS ARE REFERENCED
TO THE INSIDE OF THE CONTAINER

ITEMS
DESCRIPTION

1,
Perforated Screen
Holes 0.2 mm Dia.
1.6 mm Pitch
Material: Brass or Copper

2.
Container
Material: Stainless Steel
16 Gauge (Grade 301)

3.
Cane Sample

4.
Base Gauze 120 Mesh/Inch
Material: Brass or Copper
Supported at Outer Edge
by Copper Ring

5.
Drilled Base
Area of Holes - 2800 sq mm
Max. Hole Size 5 mm Dia.

Figure 2. Physical details of the sample container

# METHOD 4B
# FIBRE — DETERMINATION IN CANE BY NEAR INFRARED REFLECTANCE

## 1.0 SCOPE AND FIELD OF APPLICATION

This Level 3 method is applicable to the determination of fibre in sugarcane. Analytical results derived from this method can be used in the calculation of both brix and pol in cane.

## 2.0 PRINCIPLE OF METHOD

A scanning apparatus of a near infrared spectrophotometer is sealed at a fixed distance as near as possible from a homogeneous bed of prepared cane. Prepared cane passing the instrument is scanned through a heat treated and toughened glass window installed in the feed chute of No. 1 mill or other suitable location. Spectra are measured by the near infrared spectrophotometer. A number of spectra are averaged and a calibration equation applied to the spectra to estimate the percent fibre in cane. The calibration equation is a statistical relationship between spectral data and fibre % cane values as determined by Method 4A.

## 3.0 APPARATUS

**As most of the apparatus operates on-line continuously, visual checking for damage should be routine and appropriate to the operation of the apparatus. Particular attention is to be shown to glasses.**

3.1 **Near infrared spectrophotometer system** capable of scanning prepared sugarcane to produce NIR reflectance spectra of that cane with predictive results to a standard of performance not less than that identified in this method.

3.2 **A database** containing NIR spectra and a calibration equation linking reflectance characteristics by wavelength with fibre % cane.

3.3 **A computer** for quantifying fibre % cane by application of the calibration equation to the acquired spectrum from the sample and for controlling the operation of the instrument in association with the mill computer network.

3.4 Other apparatus as described in Method 4A.

## 4.0 CALIBRATION

Development of calibration equations for prepared cane involves several processes, each of which is critical to the overall calibration accuracy. The methodology is that prescribed in the instrument manual by the manufacturer of the NIR analysis system in use. The system is to be operated to manufacturer's standards.

4.1 **Sampling for calibration and validation**

The accuracy of this step determines the minimum uncertainty which can be expected from the calibration. It is essential that corresponding samples are used for the NIR spectral data and the laboratory analysis by Method 4A.

4.2 **Acquisition of Spectra**

Correct presentation of the prepared cane to the scanning apparatus is the dominant consideration. There must be minimal air voids in the scanned material. The signal to noise ratio associated with the spectral result must be maximised.

4.3 **Population Structuring**

To produce a robust calibration database and prediction equation, samples of cane covering the entire fibre range need to be included and should be equally represented in the calibration.

4.4 **Equation Development**

The aim of the calibration process is to produce equations that are insensitive to spectral variation not related to the composition of the prepared cane being scanned. Success in this regard will result in equations which are not influenced by such events as replacement of viewing glasses, glass wear, instrument hardware changes ie detectors, lamps, use of a different instrument and degree of cane preparation.

Equations that demonstrate insensitivity in this manner are considered to be global equations.

NOTE: A suggested minimum number of samples is 200.

4.5 **Statistical analysis and validation of matched populations**

Principal component analysis and Mahalanobis nearness test are used to determine the population boundaries of the calibration set which is necessary to define the spectra which are represented by the calibration equation. The equations are validated, firstly, by applying the equation to the known samples not included in the calibration set and then in an on line situation with spectra that are not associated with the calibration process.

## 5.0 ROUTINE OPERATION OF THE NIR SYSTEM

The system should be operated in accordance with the instrument manual descriptions and specifications.

5.1 **Description of the system**

The system uses a scanning NIR instrument with a sensor reading through a 'window' into the bed of prepared cane. The window is located so as to expose a representative sample of prepared cane and is situated to give the highest probability of scanning a continuous feed of lightly compressed prepared cane, with little or no void volume.

The sensor signal is interlocked with other necessary signals from the sample tracking system so that the scanning operation can be controlled to be either on or off:

(a) should the mill stop or the cane supply be interrupted, and also when

(b) the start and end of samples are identified.

The scanning of a sample should not start if the height of prepared cane above the sensor is not sufficient to ensure little or no void volume.

The spectra obtained from the scans are evaluated using the instrument supplier's criteria for their suitability and if acceptable are averaged in the manner prescribed for the instrument. The fibre is calculated using the prediction equation. Instrument manuals identify the internal checks that the result must pass before it is recorded for use.

5.2 **NIR System Checks**

All elements of the operation should be routinely checked including, particularly:

5.2.1 **Sample Tracking System**

The NIR system is synchronised with the same tracking system as the juice sampling system to identify individual rakes of cane.

Checks that the NIR computer system is receiving the correct sample information should be done on a regular basis.

5.2.2 **NIR Installation**

Internal checks should be run on NIR instrument performance. These checks will be as specified by the instrument supplier.

5.2.3 **Sampling Procedures**

All aspects of sampling of fibre should be checked, including the checks specified in Method 4A.

5.2.4 **Can Fibre Machine (CFM)**

Operation of the CFM should be checked as specified in Method 4A.

5.3 **Hygiene and Installation Checks**

The viewing glass must be clean and not damaged or show signs of excessive wear. There must be no obstructions between the scanning head and the window.

## 6.0 VALIDATION CHECKS OF THE SYSTEM

The sampling of a prepared cane parcel for can fibre measurement and on line NIR prediction of the parcel must be conducted in such manner that the two are strictly corresponding. The fibre of the parcel, determined by NIR is compared with the result obtained by Method 4A for a sample from the same parcel.

The system is validated by the completion of a number of checks. The samples taken over time should be representative of the range of fibre levels. The minimum number of system validations is three checks each 24 hours.

Sampling is commenced when cane uncontaminated by the previous parcel and matching that scanned or being scanned by the NIR spectrophotometer is at the sampling door. Automatic sampling is desirable, however, when sampling manually, samples are obtained by taking a full depth drop from the fibre door and immediately transferring two handfuls at random from the drop to a suitable container. The drop is cleared from the sampling table as quickly as possible and another drop taken and sampled. It is expected that a 'drop' sample can be taken each 30 seconds or better. The size of the handfuls taken should be consistent throughout a parcel and selected, on the basis of experience, to result in approximately 10 kg of sample from each parcel. Sampling stops when the parcel has passed the sample point and before contamination from the next parcel. A minimum of 15 'drop' samples shall have been obtained. The identity of the sample or samples should be recorded and the NIR instrument confirmed to have a valid result for the sample or samples before proceeding to the next phase.

At the end of the sampling period, transfer the composite (10 kg) sample to a suitable mixing tray, mix, sub sample and fibrate according to Method 4A. The fibrated sample is analysed in duplicate using Method 4A.

Conventional Skewhart control charts (2, 3) are used to record the performance of the validation process. The control charts should cover the mean difference between the NIR and can fibre results for three consecutive samples and the range of the differences for the same three samples. Action and warning limits are set in the accepted manner, representing values that should only be exceeded once in 1000 or 0.1% of the time and 25 times in 1000 or 2.5% of the time respectively. The standard error of prediction specified in section 7.0 is used in the derivation of the control limits. A deviation outside the 'action' limits of the control chart should be investigated immediately. If there are three consecutive deviations beyond the warning limit on the same side, the system should be investigated, as it should if there is repeated high or low bias. These investigations may involve the system checks described in section 5.2 and calculation of the SEP over the last 12 validation samples.

The NIR prediction of fibre by this Method is invalid if standard error of prediction (SEP) over the last 12 validation samples is greater than the SEP reported in this Method.

All validation data should be retained.

## 7.0 PRECISION

On line operation:

| | |
|---|---|
| Standard error of prediction | 1.00 units of fibre |

## 8.0 REFERENCES

1. Staunton, S.P. et al (1999). On-line cane analysis by near infrared spectroscopy. Proc. Aust. Soc. Sugar Cane Technol. 21: in print
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# METHOD 5
## SAMPLING AND ANALYSIS FOR EVALUATING MILLING TRAIN PERFORMANCE

### 1.0 INTRODUCTION

To evaluate milling train performance, there is a need to summarise the sampling and analysis procedures required. This method gives details of these procedures and discusses the derivation of milling performance parameters from the bagasse and cane data.

No attempt is made to cover specific requirements for evaluating diffuser performance. However, a great deal of information given here is directly applicable.

Milling train sample analysis is carried out for two different purposes: factory control and extraction performance analysis. Accurate factory control requires that prepared cane and final bagasse be composited and analysed over the full shift production regardless of whether milling performance was satisfactory. In this way, the estimate of pol lost in bagasse represents average shift operating conditions. The same shift final bagasse composite may not represent actual milling performance under steady conditions.

Milling train performance implies a more detailed examination of the performance of each element in the extraction train. This performance can be assessed over a short time period taken for one single rake of cane or over a longer period such as a shift. In most sugar factories milling train performance is assessed over a shift. This produces an averaged estimate of the extraction station performance and gives a good indication of milling train efficiency over a week. This is a reasonable approach providing all calculations are based on the results of analyses carried out on carefully preserved samples of prepared cane and mill bagasses. These samples must all be taken with an appropriate time interval between milling units so that relativity is maintained with the one particular rake or parcel of shredded cane chosen. Deviation from these procedures reduces the value of the evaluation. For example, if the fibre per cent cane value is taken from the previous shift then this estimate of milling performance may be inaccurate. Full disintegrator analysis provides all relevant analytical results for each sample.

Since preservation of samples may present problems and sampling of prepared cane and bagasses could be mismatched, the short period test may be preferable. With short period tests there are no preservation worries and analyses are related to the same unique parcel of cane. However, in this case, at least two, and preferably more, people are required to handle adequately the matching and collection of samples along the milling train.

### 2.0 COLLECTION AND PRESERVATION OF SAMPLES

2.1 *Introduction*

The procedure for collecting and preserving prepared cane or bagasse samples is basically similar for both short and long period tests although, with short period tests, care is required to ensure that samples are taken from the same parcel of cane as it proceeds through the train. With long period tests, preservation is important. A short period test should be of at least 15 and preferably 30 minutes duration. There should be no mill stops in a short period test.

Aspects of the testing procedure for both short term and shift-based analyses are covered in the following.

2.2 *Elapsed Time Between Tip and Mills*

For the short period test, it is important to know the time taken for a parcel of cane to travel from the tip to each of the mills to be sampled. A coloured dye (e.g. ICI Edicol Poinceau 4R) can be used as a marker so that the travel time can be measured. Handful-size plastic bags of the dye are easy to throw into the cane. Generally the dye will need 'boosting' with another bag after two mills. The dye is a strong colorant and can be very messy if handled carelessly.

2.3 *Prepared Cane Sample*

This is easily taken from the prepared cane elevator using the sampling equipment already installed. Care should be taken to ensure that the full depth of bed of prepared cane is sampled over the full width of the sample hatch opening. The sample is then spread evenly over the collection tray and grab samples taken representatively from the tray through the complete thickness and over the full area of the layer to obtain the sample size required.

Depending upon the frequency of sampling, the subsample size is gauged so that a composite sample weight of 15-20 kg has accumulated at the end of the test period.

2.4 *Bagasse Sampling*

For the short period test, the elapsed times obtained from the dye test are used to determine when the first sample of bagasse should be taken from each mill after the test has started. Up to ten samples should be taken from each point spread evenly over the duration of the test. Samples should be taken from each mill in sequence, as closely as possible from the same parcel of cane as it moves down the train.

In the shift period test, samples should be taken at intervals of no longer than one hour. Generally, no effort is made to ensure that the prepared cane and bagasse samples come from the same parcel of cane although it is preferable that they do.

Every effort should be made to obtain bagasse samples which are representative of the total bagasse production. Thus bagasse should be collected in sequence from the left side, centre and right side of the bagasse blanket and the tongs used for sampling should penetrate the blanket of bagasse to the plate below.

Subsample sizes should be of the order of 1.5 kg for cane graduating down to about 1.0 kg for final bagasse. Subsampling on site may be necessary to achieve these

weights. A total composite should weigh about 10 to 15 kg depending upon its origin.

2.5 *Back Roller Juices*

If brix per cent bagasse is required and a precision refractometer is not available for the analysis of the disintegrated bagasse extracts, an estimate of bagasse extract purity can be obtained from back roller juice. This analysis is carried out by some mills. Although not strictly correct, this purity value can then be taken as an approximate estimate of the residual juice purity of the bagasse in later calculations (Section 3.4).

2.6 *Bagasse and Cane Sample Preservation*

The samples should be stored in a sealed container to prevent loss of moisture. Unless the samples are to be analysed within an hour or so of collection, steps should be taken to preserve the sample to prevent degradation of the pol by microbiological action.

As chloroform is no longer a permissible preservative, two other procedures are recommended. If storage up to a limit of 24 hours is required, toluene can be added at a rate of 1 mL per 1 000 g sample and the sample should be stored in a sealed plastic bag at a maximum temperature of 4 °C. Alternatively, the sample should be quickly frozen below — 20 °C within a sealed plastic bag, sized so that the thickness of the bagasse is not greater than about 80 mm. Samples in thick overfilled bags take too long to freeze and defrost and may then suffer localised deterioration. Sample bags should not be placed on top of one another until frozen.

If sample composite bins are left along the milling train under hot moist conditions for over four hours with no preservative added, sample deterioration will occur. Satisfactory analytical results can be obtained only by immediately preserving each subsample.

The composite sample will not be obtained until all the subsamples have been collected over the required period. Special care should be taken to bring the sealed subsamples quickly back to ambient temperature prior to the mixing together to obtain the composite sample, otherwise deterioration and condensation can affect the analysis. Manipulation of the sample within the sealed bag will be necessary at regular intervals during the defrost period to break up lumps and help heat transfer. Great care must be taken with frozen samples to prevent extra moisture condensing on the bagasse and thereby affecting the analysis.

For a shift composite sample, the only method that has been rigorously established as giving an accurate result involves taking every sample back to a refrigerator (if toluene is added to each sample) or to a freezer. Placing each sample in a bin at ambient temperature for the duration of the shift is suspect due to the probability of microbial degradation especially under warm, humid conditions.

## 3.0 ANALYSIS OF CANE AND BAGASSE

3.1 *Preparation of Sample*

The possibility of obtaining some separation of large or small particles during collection or mixing must be recognised and suitable techniques (Section 3.2) must be employed to avoid any bias.

Shredded cane is often sufficiently well broken and divided to require little further attention but any long fibrous pieces (or whole stick portions if they should occur) may be cut up with a cane knife. Some cane varieties which are long fibred will not disintegrate satisfactorily if long pieces are presented to the machine. These tend to wrap around the blades or shaft in the first few seconds after start-up. Long thick pieces also cause errors in moisture measurement. Sections of whole stick which have come through the shredder unbroken may be rejected if they comprise not more than five per cent of the total sample weight.

Bagasse samples should not require further preparation before disintegration.

3.2 *Mixing of Sample*

The following method ensures good mixing and keeps evaporation to a minimum. Two people are required to carry out the operation efficiently. It must be carried out quickly on a clean non-absorbing surface in a sheltered, draught-free area.

The sample is heaped in a cone on the mixing surface and then halved by the two people leaving a free space for mixing between the halves. Each person then spreads their sample half in portions evenly over the cone of a new heap being formed between them. This heap is then rotated by approximately 90 degrees and then halved again.

The heaping and heap rotation is repeated followed by a final halving and heaping. This third mixed heap is then transferred 'en masse' into a suitable container and sealed with a lid. It is important to avoid overhandling the material as this may cause unnecessary evaporation or separation of fines. Subsamples for analysis may then be taken at random from the mixed sample with particular care that there is no bias towards selection of fine or coarse particles.

3.3 *Disintegration*

The sample of bagasse (or cane) is introduced into the disintegrator can, followed by a weighed quantity of water. The mixture is then disintegrated for 30 minutes for bagasse and for 40 minutes for prepared cane. Typical quantities are shown in Table A within this method for SRI type disintegrators. Some disintegrators are designed to handle larger volumes (Refer method for Pol in Bagasse where 9 kg water is used with 0.9 kg of bagasse).

In Table A within this method the amount of water used is a constant 6 kg. The amount of cane or bagasse used is intended to supply approximately 300 g of fibre to each disintegrator sample. This results in a thinner slurry for cane (with its higher natural moisture) and a heavier slurry for the final bagasse (lower moisture). The higher fibre/total moisture ratio for final bagasse is desirable so that a reasonable pol reading can be obtained. It is inadvisable to go above this loading because of excessive heating and lack of mobility of the slurry.

Certain models of the Jeffco brand of disintegrator require a lower total mass but the same ratio of sample to water.

On completion of the disintegration, a quantity of the slurry is removed and strained through a fine wire gauze with an opening of approximately 0.5 mm.

Approximately 250 mL of this strained extract is collected in a stoppered flask and cooled quickly to room temperature. Pol and brix are determined on this solution.

The following points should be noted about disintegrator analysis:

(i) In the absence of a large capacity single pan balance for weighing the water, the use of a specially constructed fixed volume container with an overflow side-arm is acceptable. This is adjusted to deliver the required mass of water and should be check-calibrated at regular intervals.

(ii) To minimise evaporation losses and possible thermal degradation of the sample, water cooling should be provided for the disintegrator can.

(iii) Disintegrator blades should be kept sharp, and 'touched up' with a file after every third or fourth disintegration.

(iv) The original Sugar Research Institute design (Foster, 1954) is used by various mills. Good disintegration is obtained with three 150 mm blades rotating at approximately 5800 rev/min. The blades should be spaced at 12.5 mm intervals from the shaft end. The shaft end should be about 3 mm from the bottom of the can.

(v) The Jeffco cane disintegrator fitted with 14 litre capacity bowl with internal baffles is also satisfactory. This should have a top spinner blade in addition to the three cutter blades.

(vi) A check should be made at intervals to ensure that the disintegration time is adequate. A minimum volume of slurry sample should be taken at say 30, 35, 40 and 45 minutes after start of disintegration for analysis of pol and refractometer brix. These results should indicate whether the disintegration is complete in the standard time period and whether the machine is in adequate condition.

3.4 *Brix Determination*

Approximately 100 mL of the strained disintegrator extract is shaken in a stoppered flask with two heaped teaspoons (six to seven grams) of dry Supercel. Filtration is then made through an 11 cm paper in a 65 mm diameter funnel into a 100 mL Erlenmeyer flask. After running the first 10 mL of filtrate to waste it may be necessary to return cloudy filtrate to the paper until the runnings are clear. When 15-20 mL of the clear filtrate has been collected, the refractometer brix can be determined. Either the Bellingham and Stanley or Zeiss dipping type precision refractometer, each with jacketed prism, is satisfactory for this purpose. Regular checks on the standardisation should be carried out, either with distilled water or the calibration plates provided. A determination of the refractometer brix of the water used in disintegration must also be made and the brix of the disintegrator extract is corrected accordingly:

Brix of extract (corrected) = Measured brix of extract — brix of water.

If a suitable precision refractometer is not available, then brix can be inferred from pol using the purity of the back roller juice. This assumes that the bagasse purity is approximately equal to the back roller juice purity.

3.5 *Pol Determination*

Approximately 150 mL of the strained disintegrator extract is clarified with a minimum quantity of dry lead and filtered. The usual precautions of covering the filter funnel and rejecting the first 15-20 mL of filtrate are observed. A polarimeter is then used to obtain the pol reading for the extract. The polarimeter tubes used are normally 200 mm long in automatic polarimeters and 400 mm long in manual polarimeters. This difference must be taken into account in calculations.

3.6 *Moisture Determinations*

Spencer type drying ovens are used to determine the moisture content, with sample cans initially containing between 100 g (standard Spencer oven) and 1000 g (SRI type oven) of moist bagasse or prepared cane. Reduction of sample size reduces the available accuracy of the determination. Drying is carried out with air at 105 to 115 °C to constant weight. Usually, the initial weight is measured for SRI type ovens after one hour of drying, with check weights carried out at 15 minute intervals thereafter until two successive weighings agree within 1 g. For other oven types, cans are weighed after three hours and then at hourly intervals. Underdrying not only results in incorrect moisture level determination but also increases the calculated fibre level if fibre content is determined by an indirect (i.e. difference) method (see Section 3.7).

3.7 *Calculation of Pol, Brix and Fibre Level*

*Definitions —*

B = Brix % cane (or bagasse)
P = Pol of cane (or bagasse)
M = Moisture % cane (or bagasse)
F = Fibre % cane (or bagasse)
b = Brix of disintegrator extract (°bx)
Y = Mass cane (or bagasse) for analysis (g)
Z = Mass added water (g)
d = Apparent density of disintegrator extract (g/mL)
Q = Purity of back roller juice (assumed equal to purity of bagasse extract)
R = Pol reading of extract in 200 mm tube (°Z)
If a 400 mm tube is used, $R = \frac{\text{reading}}{2}$

An assumption that fibre contains 25 per cent hygroscopic moisture is made.

Then

$$B = \frac{b(Z - 0.25Y + 0.0125\ YM)}{Y(1 - 0.0125b)}$$

$$F = 100 - B - M$$

$$P = \frac{R \times 0.26\,(Z + Y - 0.0125\ FY)}{d\ Y}$$

If accurate brixes of extract for final bagasse cannot be measured, an alternative formula can be used which relies on the assumption that the specific gravity of the extract is 1.000 and that the purity of the residual juice in bagasse is equivalent to that of the back roller juice.

$$P = \frac{R\ 0.26(Z - 0.25Y + 0.0125\ MY)}{Y(1 - (0.16R)/Q)}$$

A relationship for all bagasses, based on a 10:1 ratio of water to bagasse in the disintegration, assuming disintegrator fluid purities of 80 for first bagasse and

75 for all other bagasses and a hygroscopic water of 25% on dry fibre, is given in Table VII. This Table is a convenient aid to the calculation of results for control laboratory purposes.

3.8 *Pol in Open Cells*

Although the determination of pol in open cells is normally done on prepared cane, it is sometimes carried out on bagasse. The following method is applicable to prepared cane. Table A within this method describes the variations needed for bagasses. A 15 litre bottleneck can, used for the cold leaching step, is shown in Figure 1, together with details of supply and construction. Cane (1 000 g) and water (10 000 g) are placed in the container and, after sealing, the contents are rotated on a jar roller at 70 r/min for 10 minutes ± 5 seconds. The extract is immediately strained through a fine wire gauze funnel to avoid further extraction. The pol reading, x, is then determined on the extract.

For the wet disintegrator, cane (2 000 g) and water (6 000 g) are weighed out, placed in the disintegrator and the soluble solids extracted for 40 ± 1 minutes. The pol reading, p, of the extract is then determined. The ratio, r, is given by:

$$r = \frac{\text{pol reading for tumbler}}{\text{pol reading for disintegrator}} = \frac{x}{p}$$

and the value of % pol in open cells, K, is given by the formula:

$$K = \frac{1000r}{3.838 - .838r}$$

As mentioned in Section 3.3, it may be necessary to deviate from the recommended quantities in Table A. In this case, the following general formula can be used for the determination of per cent pol in open cells (K):

$$K = \frac{100rW_T}{C_T(1 - r)(1 - 1.25F/100) + W_DC_T/C_D}$$

where $W_T$ is weight of water added in tumbler (g),
$W_D$ is weight of water added in disintegrator (g),
$C_T$ is weight of cane or bagasse added in tumbler (g),
$C_D$ is weight of cane or bagasse added in disintegrator (g), and
F is fibre per cent cane or bagasse.

3.9 *Precision of Analysis*

Table B within this method gives information on the precision of methods of cane analysis, obtained from statistical analyses of variance of test data gained from duplicate measurements on separate samples of cane. As only a limited number of samples were measured, the precision estimates should only be used as a guide. Sample size may have a marked effect on the precision of an analytical determination. The following test illustrates the need to select the largest size possible for mill samples.

Five separate samples of prepared cane were analysed for moisture content with three different sizes of subsamples taken in replicate.

A Spencer type bagasse oven was used to dry 1 000 g and 500 g subsamples, each in quadruplicate. A like number of 50 g subsamples were dried in sealable cans in a laboratory oven. The results for each sample weight were then treated with a statistical one-way analysis of variance to determine the individual precision of the moisture determination. The estimated precision obtained for the 1 000, 500 and 50 g sample weights were 0.24, 0.40 and 0.46 per cent relative standard deviation respectively.

## 4.0 SUMMARY

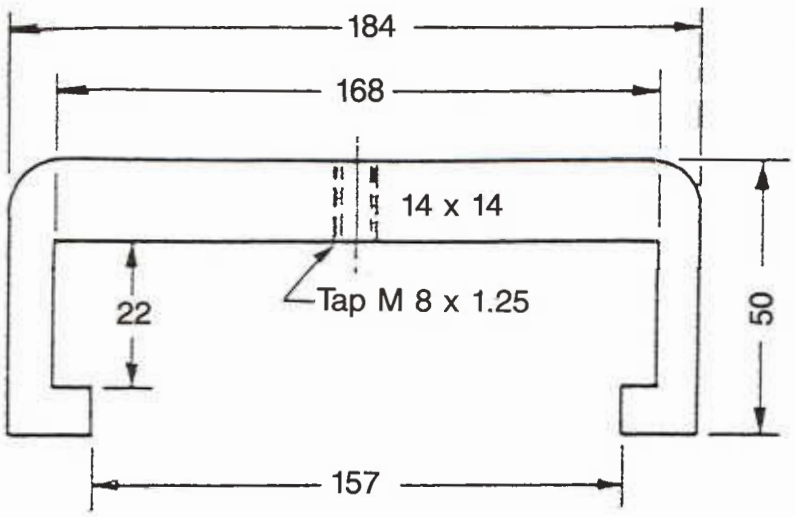
Measurements conducted over a short period of time with samples from the one rake of cane require more intensive analyst involvement but avoid the problems of preservation and ensure that all samples are matched with each other.

The alternative evaluation carried out over the period of a shift may produce a better indication of milling performance but only if the samples are taken when milling conditions are settled.

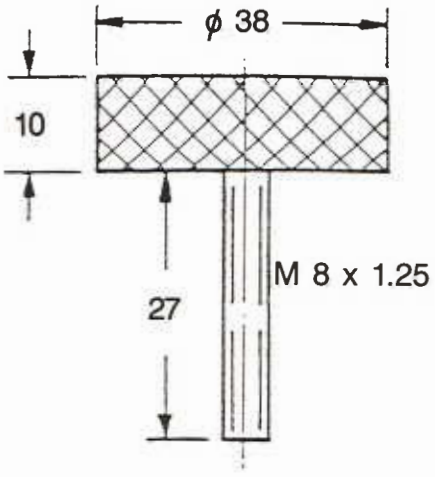
In this case, the bagasse sampling has been selective and the final mill bagasse sample is unlikely to be representative of the bagasse sent to the boiler station and thus may give a result which is misleading for factory control purposes.

## 5.0 REFERENCES

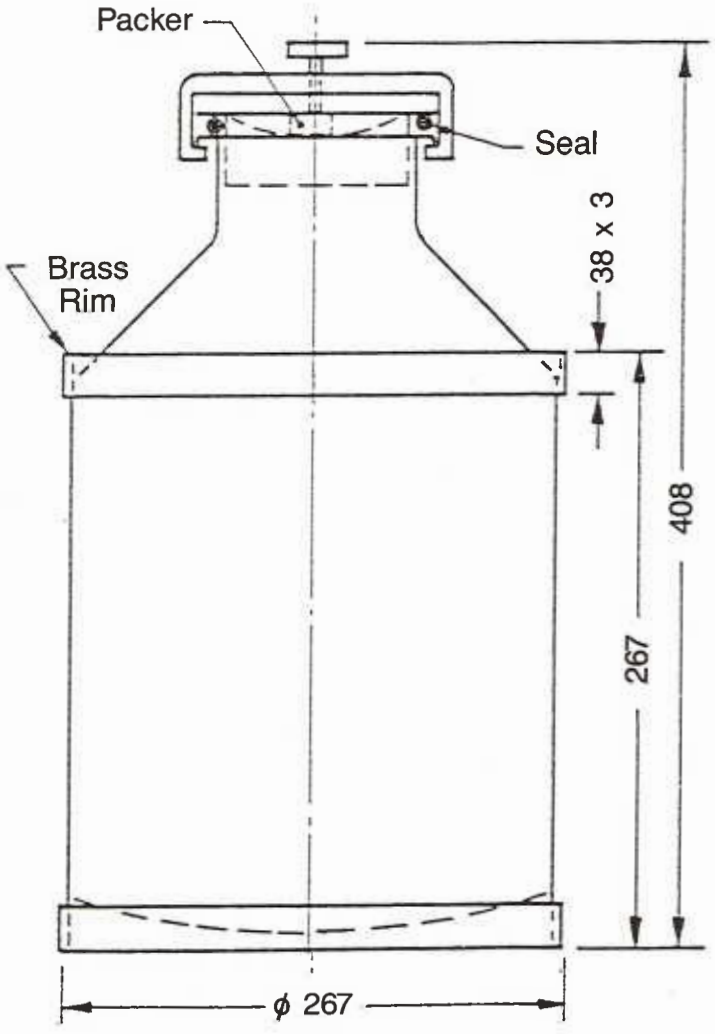
Foster, D.H. (1955). The determination of pol in bagasse. Proc. Qd Soc. Sugar Cane Technol. 279-283.

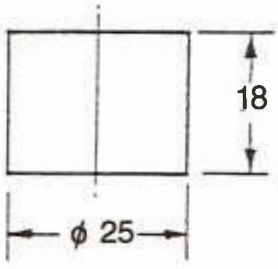



Holding Down Bracket Material:
14 x 14 Mild Steel




Tightening Screw Material:
Mild Steel




Packer Material: Brass

These measurements may need to be modified to suit the latest design of can.

Materials required:

1 only 15 litre Bottleneck Can
(Milking Machine Supplies,
6 Light Street,
Fortitude Valley, Qld.
Phone: (07) 252 2095

1 only O-ring, BS436
O.D. 161.9 I.D. 149.2
(Bearing Service Centre)

Figure 1. Modification of 15 litre bottleneck can for pol in open cell determination

TABLE A. ANALYSIS AND CALCULATION OF POL IN OPEN CELLS

| Sample | Disintegrator | Tumbler | Formula |
|---|---|---|---|
| Shredded cane | 2 000 g + 6 kg water | 1 000 g + 10 kg water | $K = \frac{1\,000\,r}{3.838 - .838r}$ * |
| No. 1 Mill Bagasse | 1 000 g + 6 kg water | 500 g + 10 kg water | $K = \frac{2\,000r}{7 - r - \frac{1.25F}{100}(1 - r)}$ |
| Nos. 2 and 3 Mills Bagasse | 800 g + 6 kg water | 400 g + 10 kg water | $K = \frac{2\,500\,r}{8.5 - r - \frac{1.25F}{100}(1 - r)}$ |
| Nos. 4, 5 and 6 Mills Bagasse | 600 g + 6 kg water | 300 g + 10 kg water | $K = \frac{3\,333r}{11 - r - \frac{1.25F}{100}(1 - r)}$ |

*Formula for shredded cane generally used in this form. Fibre content of 13.0% is assumed. Calculated value with ±0.5 units over range 11 to 15% fibre.

r = Ratio of pol readings
K = Per cent pol in open cells
F = Fibre per cent cane or bagasse

TABLE B. ESTIMATED PRECISION OF METHODS OF CANE ANALYSIS

| Analysis | No. of samples | Mean value | Standard deviation | Relative standard deviation (%)[1] | Repeatability[2] |
|---|---|---|---|---|---|
| Pol % cane | 15 | 18.30 | 0.062 | 0.34 | 0.18 |
| Brix % cane | 15 | 19.85 | 0.14 | 0.71 | 0.40 |
| Moisture % cane | 6 | 71.6 | 0.27 | 0.38 | 0.76 |
| P.O.C. (%) | 10 | 82.1 | 1.1 | 1.4 | 3.2 |
| Fibre % cane, by bag | 14 | 14.1 | 0.17 | 1.2 | 0.49 |
| Fibre % cane, by disintegrator[3] | — | — | 0.30 | — | 0.86 |

[1] The standard deviation (s), expressed as a percentage of the mean. [2] Repeatability = $2\sqrt{2}\,s$
[3] Inferred from variances of brix % cane and moisture % cane with relationship F = 100 — B — M.

# METHOD 6
# POL — DETERMINATION IN BAGASSE BY WET DISINTEGRATION

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian sugar industry. It applies to bagasse from all mills in a crushing train.

Pol in bagasse is used to determine the extraction of sucrose from cane by successive mills and is an indication of the performance of the crushing station. Adjustments to mill settings are based on pol losses calculated from the results.

**2.0 PRINCIPLE OF METHOD**

A weighed sample of bagasse is mixed with a measured volume of water and disintegrated in a high speed disintegrator. The fibre cells are broken, releasing the sucrose content. The disintegrated slurry is screened and the liquor obtained cooled and clarified by adding basic lead acetate. The pol of the clarified solution is read in a 200 mm polarimeter tube in a sugar polarimeter. Per cent pol in bagasse is read from a Table.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Wet disintegrator.** This is essentially a high speed (5,800 r/min) wet blender. Either a commercially available unit such as the Jeffco wet disintegrator or a specially fabricated unit is suitable.

The disintegrator is fitted with 3 blades, each 150 mm long × 32 mm wide × 1.5 mm thick. The blades are fitted at the end of the shaft with spacing nuts (13 mm) between each blade. Each blade should be at an angle of 60° to the one below it, to balance the shaft and avoid vibrations. The shaft should end about 3 mm above the bottom of the drum. The blades should be sharpened regularly with a file.

NOTE: For more accurate analysis, e.g. investigational work, thinner, 0.8 mm blades should be used to give better extraction.

3.2 **Wet disintegrator drum,** 20 L capacity, with lid and rubber seal, and fitted with a water-cooled baffled jacket.

*Safety*

The machine must never be operated without the drum in position. Nuts and blades should be inspected regularly, tightened if necessary, and replaced if worn. When removing or placing the drum into position, make sure that the power isolator switch, as well as the ON/OFF switch for the motor, is turned OFF. No work should be done on the disintegrator unless these switches are OFF and a DO NOT START tag placed on the isolator switch.

A hinged guard to fit around the 20 L drum should be provided in case of blade failure.

3.3 **Water dispenser** 9 kg ± 50 g capacity. The accuracy should be checked weekly by weighing the water discharged.

3.4 **Cooling bath,** with running cold water.

3.5 **Sugar Polarimeter.**

3.6 **Polarimeter tube,** 200 mm long for automatic polarimeters

3.7 **Balance** to weigh 900 ± 10 g, plus container.

**4.0 REAGENTS**

4.1 **Basic lead acetate powder (dry lead) or basic lead acetate solution (wet lead).**

**5.0 TREATMENT OF SAMPLE**

Intermediate mill bagasse samples are placed in an airtight plastic bag and taken to the laboratory for immediate analysis. Frequency of sampling of intermediate mill bagasse may vary from mill to mill.

Final mill bagasse is sampled hourly. The sample is placed in an airtight plastic bag and taken to the laboratory where 2-3 drops of toluene are added (as a preservative). The bag is sealed and the sample stored in the laboratory freezer until required for analysis. Near the end of each shift the hourly samples are removed from the freezer, completely thawed and thoroughly mixed together to form a composite from which the sample to be analysed is taken.

**6.0 PROCEDURE**

6.1 Weigh out 900 ± 10 g of bagasse and place into the 20 litre wet disintegrator drum. Place the drum and its contents in position on the wet disintegrator.

NOTE: A larger sample may be needed with some varieties (e.g. varieties with long fibres and those that tend to break into small sections) to ensure a reasonable fibre to water ratio.

6.2 Add 9 kg ± 50 g of water. This water is added volumetrically using the 9 litre water dispenser (3.3).

6.3 Fit the lid on the drum carefully and ensure the rubber seal is correctly positioned and firmly clamped. Turn on the cold water and ensure an adequate water flow. Run wet disintegrator for 30 minutes ± 30 seconds.

6.4 Turn off machine, making sure to turn OFF isolator switch, unclamp and remove lid. Turn off the cooling water and remove hoses from the couplings. Remove drum and pour 500 mL of slurry through a coarse sieve to remove bagasse fibre into a clean, dry jar. Cover with lid and cool to room temperature in a cooling bath.

6.5 Transfer about 150 mL of the cool liquor to a dry 250 mL boiling flask. Immediately add the minimum quantity of dry lead (or wet lead) needed to clarify the juice. Mix well and allow to stand for 2 minutes.

6.6 Filter the solution through a Whatman 15 cm No. 91 filter paper in a covered filter funnel. Discard the first 10-15 mL and collect about 100 mL of clear filtrate.

6.7 Rinse twice then fill the polarimeter tube with water. Read the water reading of the polarimeter and record to 0.01°Z (average of 4 readings).

NOTE: With automatic polarimeters allow the reading of the water filled tube to stabilise and then re-set to zero.

6.8 Rinse twice and fill the polarimeter tube with the clear filtrate. Read the pol in a sugar polarimeter and record the results to 0.01°Z (average of 4 readings).

## 7.0 CALCULATIONS

7.1 **Record** the per cent moisture in bagasse obtained by the method, "Moisture — Determination in bagasse and cane by drying".

7.2 **Correct** the pol reading of the liquor —

Corrected pol reading = pol reading (6.8) — water reading (6.7)

7.3 **Per cent pol in bagasse** is obtained from Table VII. **Table VII** gives the pol in bagasse according to the corrected polarimeter reading, (in a 200 mm pol tube) and the per cent water in the bagasse sample.

NOTE: (i) If the pol was read in a 400 mm tube, then divide the corrected pol reading (7.4) by 2, before obtaining % pol in bagasse from Table VII.

(ii) If the sample to water ratio was not 1 in 10 (see Note 6.1), use the relationship given in Table VII to calculate % pol in bagasse.

7.4 Record the result as % pol in bagasse to 0.01.

## 8.0 PRECISION

The precision of the method has not been determined. The expected range of results is 1.0% - 15.0% pol in bagasse.

## 9.0 REFERENCE

Foster, D.H., (1955). The Determination of Pol in Bagasse. Proc. Qd Soc. Sugar Cane Technol., 22nd Conf. 279-283.

# METHOD 7
## MOISTURE — DETERMINATION IN BAGASSE AND CANE BY DRYING

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The moisture content in bagasse is used in calculations of crushing station extraction efficiencies and pol losses. The result is needed also for the determination of pol in bagasse. The method can be used also to directly determine moisture in cane (which has been fibrated as in the method for fibre analyses).

**2.0 PRINCIPLE OF METHOD**

Per cent moisture in bagasse is the loss of mass after drying a test portion of the bagasse to constant weight in a stream of hot air in a special oven at 110 °C ± 5 °C. The determination of water and of pol in bagasse are usually done together on sub-samples of the same sample.

**3.0 APPARATUS**

3.1 **Drying oven,** either a modified Spencer oven or an SRI type oven capable of being controlled at 110 °C ± 5 °C

3.2 **Drying Cans,** aluminium.

3.2.1 For Spencer type ovens, 100 mm dia x 200 mm high, fitted with a 150 micron gauze at the bottom.

3.2.2 For SRI type ovens, drying can to hold 500-1000 g bagasse, fitted with 120 micron gauze at top and 36 micron gauze at bottom.

**3.3 Stiff Brush,** for cleaning the gauze in the drying can.

**4.0 SAMPLE PREPARATION**

4.1 The bagasse must be sampled through the full depth of the bagasse mat and care must be taken to ensure the sample is representative.

4.2 For moisture in cane, a representative sample of prepared cane is collected from the cane elevator and stored in a sealed container. The sample is prepared for analysis by reduction in a cutter grinder or hammer mill as in Method 4, "Fibre Determination in Sugar Cane".

**4.3** The sample is thoroughly mixed and subsampled. Large samples can be sub-sampled by coning and quartering. Sub-sampling must be done quickly to avoid evaporation losses. A sub-sample of about 3 kg is taken and placed in a sealed plastic bag.

4.4 If the sub-sample is to be stored for any time add 2-3 drops of toluene, seal the bag and store in a freezer. The sample should be removed from the freezer, completely thawed and re-mixed before analysis.

**5.0 PROCEDURE**

5.1 Select a clean, dry and empty drying can.

5.2 Discard the top layer (3 cm) of the sample and weigh a sample of bagasse into the can, 100 ± 0.1 g sample for the Spencer oven, 1,000 ± 1 g for the SRI oven. Record the mass of can + sample, $m_j$.

NOTE: With some Spencer type ovens a larger 400 ± 0.1 g sample can be used and is preferred.

5.3 Place the can plus sample in the drying oven and switch the oven on.

5.4 Check that the temperature is controlled at the recommended temperature (3.1), particularly over the last stages of drying.

5.5 After three hours drying, switch off the oven, remove the can and sample and weigh while still hot. Record the mass of the can plus dried sample.

5.6 Replace the can in the oven and dry for a further 1 hour. Re-weigh the can plus dry sample, while still hot and record the weight.

5.7 Repeat step 5.6, until the can plus dried sample reaches constant mass. This is when the difference between two weighings is <0.1% of the total weight. Record the final mass of the can plus dried sample, $m_2$.

***Safety***

Heat resistant gloves should be worn while handling the hot drying can.

5.S Immediately empty the can, brush out residual dried bagasse from the can and gauze. Weigh the empty can while still hot and record the mass, $m_3$.

NOTE: For convenience a standard drying time can be measured for each type of bagasse. With all the cans in the oven filled with one type of bagasse, determine the time taken for the samples to reach constant mass. The standard drying time will be this time plus 1 hour.

**6.1) CALCULATIONS**

**Calculate** the per cent (m/m) moisture in the sample

$$\% \text{ moisture} = \frac{[m_1 - m_2]}{[m_1 - m_3]} \times \frac{100}{1}$$

where: $m_1$ = mass drying can + wet bagasse (5.2)

$m_2$ = mass drying can + dried bagasse (5.7)

$m_3$ = mass of empty drying can (5.8)

**Record** the result as % (m/m) moisture in the sample to 0.1%

**7.0 PRECISION**

The expected range of results 35-65% (m/m) moisture in bagasse.

The precision of the method has not been determined.

# METHOD 8
## TOTAL AND SOLUBLE PHOSPHATE — DETERMINATION IN CANE JUICE

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian sugar industry. The method applies to the determination of total, that is, acid-soluble inorganic phosphate and soluble, that is, water soluble inorganic phosphate.

The concentrations of phosphate in the juice, before and after clarification, give an indication of clarifier performance.

**2.0 PRINCIPLE OF METHOD**

If total phosphate is being determined, the pH of the juice is adjusted to 4.0 by adding sulphuric acid. Soluble phosphate is determined at the natural pH of the juice.

An aliquot of the juice is filtered under vacuum with acid-washed Supercel. Acid-molybdate, amidol and sodium metabisulphite solutions are added to an aliquot of the filtrate and phosphate is measured as the absorbance, at a wavelength of 660 nm, of the blue colouration which develops. Interference from low concentration of organic phosphates and from anions and cations found in sugar is not significant.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Spectrophotometer** suitable for the measurement of absorbance in the region of 660 nm with matched 2 cm cells.

3.2 **Bulb pipettes** — Class A (AS 2166) — 2 mL, 3 mL, 5 mL, 8 mL, 10 mL

3.3 **Graduated straight pipette** Class A (AS 2167) — 10 mL

3.4 **Automatic dispenser,** 10 mL

**4.0 REAGENTS**

During the analysis, unless otherwise stated, use only analytical grade reagents and only distilled water.

4.1 **Acid-molybdate reagent.** Dissolve 16.6 ± 0.1 g ammonium molybdate tetrahydrate in about 600 mL of distilled water in a 1 litre beaker. Add 96 ± 1 mL of concentrated sulphuric acid. Add the acid slowly with stirring to avoid the generation of excessive heat. Cool, transfer to a 1 litre volumetric flask, make to the mark, and mix well.

*Safety*

Sulphuric acid is very corrosive. Safety glasses and gloves should be used when handling the acid. It must not be pipetted by mouth.

If in contact with skin, remove all contaminated clothing and wash affected area with copious quantities of water. If ingested administer large quantities of aluminium hydroxide or milk of magnesia (magnesium hydroxide). Do not induce vomiting.

4.2 **Acid reagent.** Add 96 ± 1 mL of concentrated sulphuric acid ($\rho_{20}$ = 1.84 g/mL) to about 600 mL of distilled water in a 1 litre beaker, slowly as in 4.1. Cool, transfer to a 1 litre volumetric flask, make to the mark, and mix well.

4.3 **Amidol reagent.** Dissolve 1.00 ± 0.01 g of amidol (2, 4-diaminophenol hydrochloride) and 20 ± 1 g of sodium metabisulphite in distilled water in a 100 mL volumetric flask, make to the mark and mix well. Filter under vacuum through a Whatman No. 3 filter paper with a level teaspoon of acid-washed Supercel (4.4). Store in a dark bottle in a refrigerator, and discard after one week.

*Safety*

Amidol/sodium metabisulphite reagent and its intermediate oxidation products are toxic and have a pungent odour. They may produce bronchial asthma and hence should not be inhaled. Contact with the skin may give rise to an irritation leading to dermatitis; the reagent should be washed off the skin immediately. Use gloves when handling. Do not pipette by mouth.

4.4 **Acid-washed Supercel.** Add 50 ± 5 g of Supercel to 1 litre of distilled water. Add 50 ± 5 mL of concentrated hydrochloric acid and stir for 5 minutes. Filter and wash Supercel cake free of acid with distilled water. Test washings with litmus paper. Dry Supercel for 6 hours at 90-100 °C and store in a closed container.

*Safety*

Concentrated hydrochloric acid is very corrosive and gives off pungent, toxic fumes. It must be handled using safety glasses and gloves in a well ventilated atmosphere. It should not be pipetted by mouth.

First aid is the same as for sulphuric acid (4.1).

4.5 **Standard phosphate stock solution,** 1.0 mg P/mL. Dry about 1.5 g of potassium dihydrogen phosphate for 1 hour at 110 °C. Weigh out 1.0984 ± 0.0001 g of the dried salt, dissolve in freshly distilled water and make up to 250 mL in a volumetric flask. Do not keep for longer than one month.

4.6 **Working standard phosphate solution,** 0.01 mg P/mL. Dilute 10.00 ± 0.05 mL of the standard phosphate stock solution to 1 litre in a volumetric flask, and mix thoroughly.

**5.0 STANDARDISATION OF METHOD**

5.1 Using Class A pipettes, pipette aliquots of 0, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00 mL of working solution (4.6) into separate 50 mL volumetric flasks. These solutions are equivalent to 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 mg phosphate.

5.2 To each flask add 2 drops of acid reagent (4.2). Adjust the volume to about 30 mL with distilled water. Add 10.0 ± 0.2 mL of acid-molybdate reagent (4.1) and 4.0 ± 0.2 mL of amidol reagent (4.3). Make each flask to 50 mL with distilled water, stopper and mix well.

5.3 Allow the solutions to stand for at least 10 minutes but not more than 30 minutes.

NOTE: If the temperature of the solutions is above 25 °C, do not allow to stand for more than 15 minutes.

5.4 Read the absorbance of each standard solution in a spectrophotometer at 660 nm, using a 2 cm cell with

water as the reference solution. Record the absorbance to 0.001 units.

As a guide to performance of the method, the 0.06 mg P standard measured at 660 nm in a 2 cm cell should read 0.280 absorbance. The reading for the zero P standard in a 2 cm cell against distilled water should not exceed 0.001 absorbance.

5.5 Plot the absorbance of each standard solution against the concentration of phosphate.

If two or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular phosphate concentration, the standardisation must be repeated.

The calibration graph should be constructed using the mean of independent standardisations by at least two analysts. Each set of results and the mean must comply with the limits above. In addition the slopes of the individual lines must not differ by more than 2%.

Each time a new batch of reagents is prepared, check the calibration at the zero point (reagent blank) and at one other point by repeating steps 5.2 to 5.4. If the results do not lie within the limits set out above repeat the calibration.

## 6.0 PROCEDURE

A. PREPARATION OF TEST SOLUTION

6.1 If the final result is to be expressed on solids (mg/kg) then determine the brix of the sample at 20 °C and record to 0.1 °bx.

6.2 For total phosphate only, transfer about 100 mL of the juice to a 150 mL beaker. While stirring on a magnetic stirrer, add sufficient acid reagent (4.2) to adjust the pH to 4.0 ± 0.5.

NOTE: The analysis must be completed within 1 hour of adding acid.

6.3 For both total and soluble phosphate, filter under vacuum through a Whatman No. 3 filter paper. Precoat the filter with a slurry consisting of a level teaspoon of acid washed Supercel (4.4) with about 10 mL of the solution. Use the filtrate from this precoat to rinse the filter flask.

6.4 Pipette an aliquot (V) of the sample into each of two 50 mL volumetric flasks.

For mixed juice, take 2.00 ± 0.02 mL.

For clarified juice, take 20.00 ± 0.06 mL

NOTE: The aliquot (V) of the test solution should be selected to give a reading of between 0.10 and 0.40 absorbance.

6.5 To one flask add 10.0 ± 0.2 mL of acid-molybdate reagent (4.1) and 4.0 ± 0.2 mL of amidol reagent (4.3). Make up to 50 mL with distilled water, stopper and mix well. Stand for at least 10 minutes, but not more than 30 minutes. This is the test solution.

NOTE: If the temperature of the solution is above 25 °C, read between 10 and 15 minutes.

B. PREPARATION OF BLANK SOLUTION

6.6 To the second 50 mL flask (6.5) add 10.0 ± 0.2 mL of acid reagent (4.2). Make up to 50 mL with distilled water, stopper and shake. Stand for at least 10 minutes, but not more than 30 minutes.

C. MEASUREMENT OF ABSORBANCE

6.7 Read the absorbance of the test solution (6.5) in a spectrophotometer at 660 nm, in a 2 cm cell using the corresponding test blank solution (6.6) as the reference solution. Record the absorbance to 0.001 units.

## 7.0 CALCULATIONS

7.1 **Read** from the standard graph "mg phosphate" in the test solution.

7.2 **Calculate** the phosphate (mg/L) on juice —

$$P\ (mg/L) = \frac{\text{mg P from graph}}{1} \times \frac{1000}{V}$$

where V is the sample aliquot (6.4)

or calculate P (mg/kg) on solids —

$$P\ (mg/kg) = \frac{\text{mg P from graph}}{1} \times \frac{10^5}{V} \times \frac{1}{\text{density} \times \text{brix}}$$

The density at 20 °C corresponding to the measured brix of the juice (6.1) is found from Table XII or Table XV.

**Table XII** gives "Degrees Brix, Density and Concentration of Sucrose Solutions at 20 °C corresponding to Refractive Index Reading".

**Table XV** gives "Densities of Solutions of Cane Sugar at 20 °C in g/mL".

## 8.0 PRECISION

| Sample | Concentration Range | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Mixed Juice or Clarified Juice | 5-150 (mg/L) on juice 25-850 (mg/kg) on solids | 3 (mg/L) | 6 (mg/L) |

## 9.0 REFERENCE

Allen, R. J. L., Biochem, J.; Vol. 34 (1940).

# METHOD 9
## STARCH — DETERMINATION IN CANE JUICES

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian sugar industry. The method measures a substance (or group of substances) which is basically starch or derived from starch. The method gives a relative measure of the starch content in cane juices. Cane starch can be occluded in the raw sugar crystal and affects the filtration rate of liquor from the refinery carbonatation process.

**2.0 PRINCIPLE OF METHOD**

The juice is sampled and a preservative added immediately to inhibit naturally occurring starch-degrading enzymes. The brix is measured and the pH adjusted to 6.0. An aliquot of the juice is added to a boiling solution of uranyl acetate and calcium chloride to precipitate protein and other organic impurities. The solution is boiled and then digested at 95-100 °C to solubilise starch, and then filtered under vacuum with Celite filter aid.

The filtrate is diluted and potassium iodide/iodate solution is added to an aliquot to form the blue starch iodide complex. The absorbance of this complex is read in a spectrophotometer at 700 nm. At this wavelength the effect on absorbance by the impurities in the cane juice is minimal.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Spectrophotometer** suitable for the measurement of absorbance at 700 nm with a set of matched 2 cm cells.

3,2 **Water bath** capable of being controlled at 95-100 °C.

3.3 **Graduated straight pipette** with a wide delivery tip opening (3 mm internal dia).

3.1 **Volumetric flask —** Class A (AS 2164), 100 **mL**

3.5 **Bulb pipettes —** Class A (AS 2166), 2 **mL,** 5 **mL,** 10 **mL,** 15 **mL,** 20 **mL**

3,6 **Automatic pipette,** 5 **mL** and 25 **mL** capacity.

**4.0 REAGENTS**

During the analysis, unless otherwise stated, use only reagents of analytical grade and only distilled water.

4.1 **Calcium Chloride Solution 40Vo (m/m).** Dissolve 53.0 ± 0.1 g of calcium chloride dihydrate in distilled water and dilute to 100.0 ± 0.1 g with distilled water. As calcium chloride is highly deliquescent, stocks should not be exposed to air. The strength of the calcium chloride solution must be checked and, if necessary, adjusted to 40.0 ± 0.3Vo (m/m) with distilled water or with calcium chloride dihydrate (approximately 75.5Vo CaCL).

The solution strength can be checked by measuring its specific gravity. The specific gravity of 40.0Vo m/m calcium chloride solution is 1.3942 at 24 °C. Within the temperature range 20-30 °C it decreases on an average by 0.0007 per degree above 24 °C and increases by the same amount per degree below 24 °C. In the region of 40Vo (m/m), a change of O.lVo in calcium chloride concentration produces a change of 0.001 in specific gravity,

Specific gravity may be measured by pycnometer, hydrometer or other instrument capable of the accuracy required. A convenient method is as follows:

(i) Allow stoppered containers of calcium chloride solution and distilled water to stand for a number of hours (e.g. overnight) so that both reagents come to ambient temperature.

(ii) Weigh a clean, dry 100 mL volumetric flask to 0.02 g. Fill to the mark with calcium chloride solution and weigh again to 0.02 g. Note the temperature of the solution to 0.1 °C.

(iii) Rinse out the flask and fill to the mark with distilled water at the same temperature as the calcium chloride solution, and weigh to 0.02 g. From the weights of the solution and water, and the temperature, calculate the specific gravity to 0.001.

4,2 **Acetic Acid (1 mol/L approx.).** Dilute 57 ± 1 mL of glacial acetic ($\rho 20$ = 1.049 g/mL) acid to one litre with distilled water.

4,3 **Acetic Acid (0.1 mol/L approx.).** Dilute 10.0 ± 0.2 mL 1 mol/L acetic acid (4.2) to 100 mL with distilled water.

4.4 **Calcium Chloride/Acetic Acid Reagent.** Using a pH meter and stirrer, adjust the pH of a 100 mL aliquot of calcium chloride solution (4.1) to pH 3.0 ± 0.1 with 0.1 mol/L acetic acid (4.3). Because of the large salt concentration, allow sufficient time for the pH meter to equilibrate.

Adjust the pH of the bulk of the calcium chloride solution in the same proportion. Although the pH of a calcium chloride/acetic acid solution tends to alter on standing, do not readjust to pH 3.0 before using.

4,5 **Uranyl Acetate Solution 2.5 g/100 mL.** Dissolve 2.50 ± 0.01 g of uranyl acetate in distilled water and make to 100 mL. If necessary add not more than ten drops of glacial acetic acid per 100 mL of solution to aid solution. This reagent should be discarded after 3 months.

***Safety***

Uranyl acetate is highly toxic. If ingested, induce vomiting with lOVo aqueous magnesium sulphate, followed by milk or egg white in cold water.

4.6 **Potassium Iodate Solution, 0.0017 mol/L.** Dry about 0.5 g potassium iodate at 105-110 °C for 1 hour. Dissolve 0.3566 ± 0.0002 g of the dried reagent in distilled water and make to 1 litre. Store in the dark in a brown, glass-stoppered bottle.

4.7 **Potassium Iodide, 10 g/100 mL.** Dissolve 10.0 ± 0.1 g of potassium iodide in distilled water and dilute to 100 mL. Store in the dark in a brown, glass-stoppered bottle. Discard the solution if it becomes yellow.

4,8 **Potassium Iodide/Potassium Iodate Reagent.** This reagent must be prepared on the day it is to be used. Mix 10.0 ± 0.5 mL of potassium iodide solution with 90.0 ± 0.5 mL of distilled water. To this solution add

100.0 ± 0.5 mL of potassium iodate reagent. Mix the reagents and keep in a brown, glass-stoppered bottle. This reagent must be discarded after 1 day.

4.9 **Standard Starch Solution, 1000 mg/L.** Use an approved batch of B.D.H. Laboratory Reagent Potato Starch, supplied by CSR Central Laboratory. Determine its moisture content, correct to 3 decimal places, by drying about 2 g (weighed to 0.0001 g) at 105-110 °C for 2 hours.

4.9.1 Weigh into a 25 mL beaker $\frac{(1.0000 \times 100)}{(100 - \%H2O)}$ ± 0.0002 g of fresh starch, i.e. equivalent to 1.0000 ± 0.0002 g of anhydrous starch.

4.9.2 To the weighed quantity of starch, add 5 mL of cold distilled water and mix with a glass rod. Before the starch settles, transfer the mixture quantitatively to 500 mL of boiling water in a 1 litre conical flask so that no slurry touches the wall of the flask.

4.9.3 With at least three additional 5 mL portions of distilled water, transfer all the starch to the flask. This operation should be completed within 1 minute.

4.9.4 Boil the starch for 3 minutes ± 10 seconds, timed from the moment the first 5 mL of starch slurry enters the boiling water.

4.9.5 Rinse a 1 litre volumetric flask with hot distilled water. Quantitatively transfer the hot solution through a glass funnel to the 1 litre volumetric flask. Wash the conical flask at least twice with hot distilled water, by adding the water to the 25 mL beaker (4.9.1.) and then transferring it to the conical flask. Continue washing the conical flask with hot distilled water and transferring to the volumetric flask until the latter is filled to approximately 900 mL.

4.9.6 Swirl to mix the flask contents and cool under running water to room temperature. Make the solution to 1 litre, stopper and mix well. Store in a refrigerator.

NOTE: The solution will keep for one week.

4.10 **Working Standard Starch Solution, 200 mg/L.** Using a Class A bulb pipette, pipette 50.00 mL of the 1000 mg/L standard starch solution (4.9) into a 250 mL volumetric flask. Dilute to 250 mL with distilled water and mix well.

This reagent will not keep and must be prepared on the day the standardisation is carried out.

4.11 **Starch Free Sucrose.** Use only sugar, tested to be free of starch. Suitable starch free sugar is available from CSR Central Laboratory.

4.12 **Mercury (II) Chloride Solution, 0.5 g/100 mL.**

***Safety***

Mercury (II) chloride is highly toxic. Gloves should be worn when handling the reagent. It should not be pipetted by mouth.

If in contact with the skin, remove all contaminated clothing, etc. and wash affected area with copious quantities of water.

If ingested, quickly administer a large quantity of egg white in water or medical charcoal in suspension and induce vomiting with 10% magnesium sulphate. Follow with milk or egg white in water. Obtain medical attention immediately. Speed is essential since this poison is fast acting and extremely dangerous.

4,13 **Celite 560 filter aid.**

4,14 **Hydrochloric Acid —** 1 mol/L approximately.

4.15 **Sodium Hydroxide —** 1 mol/L approximately.

**4.16 Blank Sugar Reagent.** Mix 10.0 ± 0.1 mL of an 8 g/50 mL solution of sucrose (4.11) in water with 20.0 ± 0.2 mL of calcium chloride-acetic acid (4.4) and 2.0 ± 0.2 mL uranyl acetate reagent (4.5) in a 50 ml volumetric flask. Heat in a water bath at 95-100 °C for 15 ± 1 minutes, cool, make to the mark, stopper and mix well.

**4.17 Sucrose Solution, 30 g/100 mL.**

## 5.0 STANDARDISATION OF METHOD

A. PREPARATION OF STANDARDS

5.1 Pipette, using class A bulb pipettes 0, 2.00, 5.00, 10.00, 15.00 and 20.00 mL aliquots of the 200 mg/L working standard starch solution (4.10) respectively into six 100 mL volumetric flasks.

The final concentration in the flasks will be 0, 4, 10, 20, 30 and 40 mg/L of starch.

5.2 To each flask add 10 ± 0.1 mL of 30% sucrose solution (4.17), 40.0 ± 0.2 mL of calcium chloride/acetic acid reagent (4.4) and 4.0 ± 0.2 mL of uranyl acetate solution (4.5). Dilute each flask to about 80 mL with water.

5.3 Mix by swirling and place the flasks in a boiling water bath at 95-100 °C for 15 ± 1 minutes. Swirl each flask at 5 and 10 minutes after placing in the bath.

5.4 After 15 minutes remove the flasks and cool in a water bath to room temperature. Dilute each flask to the mark with distilled water, stopper and mix well.

B. MEASUREMENT OF ABSORBANCE

5.5 Pipette 15.0 ± 0.02 mL of each of the standard solutions into a 25 mL volumetric flask.

5.6 Add to each flask 2.50 ± 0.02 mL of 1 mol/L acetic acid (4.2), and 5.00 ± 0.02 mL of the potassium iodide/iodate reagent (4.8). Mix by swirling and make to the mark with water. Mix well.

5.7 Immediately read the absorbance of the each standard solution in a 2 cm cell, in a spectrophotometer at 700 nm against distilled water as the reference. Record the absorbance to 0.001.

NOTE: (i) The absorbance of the test solution must be read between two and five minutes after adding the iodide-iodate reagent to the test solution.

(ii) The reading of the 0 mg/kg starch standard should not exceed 0.010 absorbance for a 2 cm cell. As a guide the reading for the 20 mg/L starch standard should be about 0.250 absorbance in a 2 cm cell at 700 nm.

C. PREPARATION OF GRAPH

5.8 Plot mg/L starch (5.1) against absorbance (5.7). A straight line must be obtained for the graph. If three or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular starch concentration, the standardisation must be repeated.

5.9 The graph used by any laboratory for routine analysis should be constructed using the mean of independent standardisations by at least two analysts. Each set of

results and the mean must comply with the 5% limits above (5.8). In addition, the slopes of the individual lines must not differ by more than 5%.

Each time a new batch of reagents is prepared, check the calibration at the zero point and at one other point by repeating steps 5.1 to 5.7.

## 6*0 TREATMENT OF SAMPLE

A. PREPARATION OF TEST SOLUTION

When the juice is sampled, immediately add two drops (0.1 mL) of the 0.5*% mercury (II) chloride solution (4.12) per 100 mL of juice. Cool the juice as quickly as possible to 1-6 °C, in a refrigerator.

NOTE: Mercury (II) chloride is added to the juice to inhibit natural enzymes and to minimise loss of starch. With this treatment and storage at 1-6 °C, starch levels should remain constant for at least 24 hours. On no account should juice be frozen for storage.

## 7J PROCEDURE

7,1 Warm the sample to room temperature by placing the container in a water bath or under running tap water. Measure and record the brix to 0.1°bx.

7.2 Adjust the pH to 6.0 against suitable pH indicator paper, with 1 mol/L sodium hydroxide or 1 mol/L hydrochloric acid. (First expressed juice generally takes 2-6 drops 1 mol/L sodium hydroxide).

7.3 Add to a 100 mL conical flask, 20.0 ± 0.2 mL of calcium chloride/acetic acid reagent (4.4) and 2.0 ± 0.2 mL of uranyl acetate solution (4.5). Mix well.

7.4 Bring the solution to boiling and using a 10 mL calibrated pipette with a wide delivery tip (3.3), pipette 10 mL of the juice sample into the boiling solution. Add the first 1-2 mL of juice dropwise to minimise frothing, then quickly add the remainder of the solution.

7,5 Continue boiling the solution for 1 minute ± 10 seconds after adding the juice sample.

7.6 Swirl to mix the solution and transfer the flask to a water bath at 95-100 °C for 15 ± 1 minutes. Mix by swirling after 5 and 10 minutes.

7.7 After 15 minutes, remove the flask from the bath and immediately add about 0.5 g Celite 560 (4.13). Mix well.

7.8 Without delay, filter under vacuum through a 5.5 cm Whatman No. 541 filter paper into a 250 mL Buchner flask. Filter to dryness.

7.9 Wash down the sides of the conical flask with 10 mL of hot water and again filter to dryness through the No. 541 paper.

7.10 Transfer the filtrate into a 50 mL volumetric flask. Rinse the Buchner flask with 1x5 mL of hot water and add the washings to the 50 mL volumetric flask.

7.11 Cool the flask to room temperature and dilute to volume with water. Mix well.

B. MEASUREMENT OF ABSORBANCE

7.12 Pipette 15.00 ± 0.02 mL of the filtrate into each of two 25 mL volumetric flasks.

(a) sample blank solution

(b) sample test solution

7.13 Add to each flask, 2.50 ± 0.02 mL of 1 mol/L acetic acid (4.2). Mix by swirling.

7.14 Make the sample blank solution (7.12a) to the mark with water. Stopper and mix well.

7.15 To the sample test solution (7.12b) add 5.00 ± 0.02 mL of potassium iodide/iodate reagent (4.8). Mix by swirling and make to the mark with water. Stopper and mix well.

7.16 Read the absorbance of the sample test solution (7.15) in 2 cm cells in a spectrophotometer at 700 nm, against the sample blank (7.14) as the reference solution. Record the absorbance to 0.001 units.

NOTE: (i) The readings must be made between two and five minutes after the addition of the potassium iodide-iodate reagent to the sample test solution.

(ii) If the absorbance reading of the test solution exceeds 0.700 repeat step 7.12, taking a 5 mL aliquot of the filtrate. Add 10.0 ± 0.2 mL of the blank sugar reagent (4.16) and proceed as in steps 7.13-7.16.

## 8.0 CALCULATIONS

8.1 **Read** the concentration **in** g/mL of the sample solution at 20 °C using Table XII or XV.

**Table XII** gives the Brix, density, and concentration in g/mL of sucrose solutions corresponding to refractive index at 20 °C.

**Table XV** gives the density in g/mL of sucrose solutions corresponding to Brix at 20 °C.

8.2 **Read the mg/L starch** in the test solution from the calibration graph.

8.3 **Calculate mg/kg** of starch (on solids).

$$\text{mg/kg starch} = \frac{\text{mg/L from graph}}{1000} \times \frac{50}{10} \times \frac{1000}{\text{cone. (g/mL at 20°C)}} \times \frac{15}{V}$$

where V is the aliquot taken (7.12), normally 15.0 mL

For a 15 mL aliquot, mg/kg starch

$$= \frac{\text{mg/L from graph}}{\text{cone. (g/mL)}} \times \frac{5}{1}$$

## 9.9 PRECISION

| Sample | Concentration Range mg/kg Starch | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Cane Juice | 100-1500 | 10 mg/kg | 25 mg/kg |

## 10.0 REFERENCES


10.1 Whistler, R. L. (ed); Methods in Carbohydrate Chemistry; Vol. 4 (Starch); Academic Press (1964).

10.2 Balch, R. T.; The Sugar Journal; Vol. 15, No. 8 (1953), p. 11.

10.3 Alexander, J. B.; South African Sugar Journal; Sept. 1954, p. 617.

10.4 Wei Chen & Mei-Wei Chen; Taiwan Sugar Journal; Jan-Mar 1965, p. 27.

# METHOD 10
## TURBIDITY — DETERMINATION IN CLARIFIED JUICE

### 1.0 SCOPE AND FIELD OF APPLICATION
This method is recommended for use within the Australian Sugar Industry. The method is applicable to all clarified cane sugar juices and is indicative of the efficiency of the clarification operation.

### 2.0 DEFINITION
**Turbidity index,** s, is a measure of "turbidity" or degree of light scattering —

$$s = \frac{A}{b}$$

where: A is the absorbance measured at a wavelength (900 nm) where the effect of light absorption can be assumed to be zero.
b is the cell length

Because the Turbidity Index is generally a small number, turbidity (S) is expressed as S = 100s. Therefore turbidity, S = 100A where A is measured at 900 nm in a 1 cm cell.

### 3.0 PRINCIPLE OF METHOD
The test sample is cooled to room temperature and the absorbance in a 1 cm cell read in a spectrophotometer at 900 nm against water as the reference.

### 4.0 APPARATUS
**4.1** **Spectrophotometer** suitable for the measurement of absorbance at 900 nm with matched 1 cm cells. The spectrophotometer should comply with the following specification:

- spectral band pass 10 nm or less
- wavelength reproducibility ± 0.5 nm
- absorbance reproducibility ± 0.003 at 1.0 abs

### 5.0 TREATMENT OF SAMPLE
Flush the sample pipe and rinse the sample container 2-3 times with the hot juice immediately before taking a sample. The sample will be hot, and should be cooled under running cold water to room temperature, as soon as possible. The sample should be analysed as soon as possible but, if necessary, may be stored in a sealed container in a refrigerator for up to 12 hours.

### 6.0 PROCEDURE
6.1 Select a pair of matched 1 cm cells. Rinse twice and fill one cell with the cooled test sample. Fill the other cell with distilled water.

**6.2** Read the absorbance of the test solution in the spectrophotometer at 900 nm, against the waterfilled cell as the reference. Record the reading to the nearest 0.001 absorbance.

### 7.0 CALCULATION
**Calculate** clarified juice Turbidity

Thrbidity, S = 100 x Absorbance

### 8.0 PRECISION
The expected range of results is 0-100
The 95% repeatability, r is 0.3 units

# METHOD 11
## POL — DETERMINATION IN FILTER CAKE

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The method gives an estimate of pol (% cane sugar) in the wet filter cake from rotary vacuum filters. The result is used to assess filter performance and the weight of pol lost is used in compiling the mill chemical balance.

**2.0 PRINCIPLE OF METHOD**

The method has three steps:

- Dilution of a 50 g test portion to 200 mL with water.
- Clarification with basic lead acetate followed by filtration.
- Reading the pol of the clarified solution in a polarimeter.

An arbitrary adjustment is made to the weight of the test portion to correct for the presence of insoluble solids and therefore only 50 g of sample per 200 mL is used instead of 52 g (or 1 normal weight).

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Polarimeter tube,** 200 mm long for automatic polarimeters,
400 mm long for visual polarimeters.

3.2 **Volumetric flask,** Kohlrausch, 200 mL.

3.3 **Sugar Polarimeter** (26 g normal weight).

**4.0 REAGENTS**

Unless otherwise stated use only analytical grade reagents and only distilled water.

4.1 **Basic lead acetate** (dry lead), or basic lead acetate solution (wet lead).

**5.0 TREATMENT OF SAMPLE**

Vacuum filter cake is best sampled from the mud conveyor just prior to the mud hopper. Samples should be taken hourly and placed in a suitable sealed container. Samples from separate filters may be obtained using a metal tray to either sample the entire length or only a section of the filter. These samples should be taken hourly and placed in a sealed container. Each shift, the composite samples are taken to the laboratory, mixed thoroughly and analysed.

**6.0 PROCEDURE**

6.1 Weigh 50 ± 0.1 g of sample into a basin. Make a slurry with water and wash quantitatively into a 200 mL Kohlrausch flask (3.2) with water. Dilute to about 150 mL with water and mix well.

6.2 Add sufficient wet lead (or dry lead) to the flask to clarify the solution. Mix well.

6.3 Make exactly to the mark with water using a fine dropper. Stopper the flask, mix well and allow to stand for 5 minutes.

6.4 Filter through a Whatman 15 cm No. 91 filter paper in a covered filter funnel. Discard the first 10-15 mL and collect about 100 mL of clear filtrate.

6.5 Rinse twice and fill a 200 mm pol tube with water. Read the water tube in the polarimeter. Take 4 readings and record the mean water reading to 0.01°Z.

NOTE: With automatic polarimeters allow the reading of the water-filled tube to stabilise and then re-set to zero.

6.6 Rinse the 200 mm pol tube with the filtrate (6.4) three times then fill. Read the pol of the sample in a polarimeter. Take 4 readings and record the mean reading to 0.01°Z.

**7.0 CALCULATIONS**

7.1 **Correct** the pol reading of the sample (6.6) for the water reading (6.5).

Corrected Pol reading = pol reading (6.6) — water reading (6.5)

% Pol in filter cake = corrected pol reading (for a 200 mm pol tube)

$$\% \text{ Pol in filter cake} = \frac{\text{corrected pol reading}}{2}$$

(for a 400 mm pol tube)

7.2 Record results as % pol in wet filter cake to 0.01.

**8.0 PRECISION**

The expected range of results is 1.0 - 5.0% pol on wet filter cake. The precision of the method has not been determined.

# METHOD 12
## MOISTURE — DETERMINATION IN FILTER CAKE

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The determination of the % moisture in filter cake, in conjunction with a % pol and % fibre in filter cake, is used to determine filter efficiency.

**2.0 PRINCIPLE OF METHOD**

The per cent water in filter cake is defined as the percentage loss in mass of a test portion dried in an oven overnight at 100 °C ± 2 °C.

**3.0 APPARATUS**

3.1 **Evaporating basin,** 150 mm dia.

3.2 **Drying oven,** forced draft capable of being controlled at 100 °C ± 2 °C.

**4.0 PROCEDURE**

4.1 Collect the sample of filter cake and place in a suitable air-tight container. Mix the sample well and take a sub-sample of about 25 g for the analysis.

4.2 Weigh a clean, dry evaporating basin (3.1) and record the mass of the basin ($m_1$), to 0.1 g.

4.3 Weigh 10.0 g ± 0.5 g of sample into the evaporating basin. Record the mass of the basin plus wet sample ($m_2$), to 0.1 g.

4.4 Spread the sample in the basin to expose the maximum surface area of sample. Do not compress the sample.

4.5 Dry the sample plus basin in the air oven overnight at 100 °C ± 2 °C until constant weight is achieved.

4.6 After drying, remove the sample from the oven and place in a desiccator with fresh silica gel, to cool for 45-60 minutes.

4.7 Weigh the basin plus dried sample. Record the mass of the basin plus dried sample ($m_3$), to 0.1 g.

**5.0 CALCULATION**

**Calculate** moisture % (m/m) in mill mud.

$$\% \text{ moisture} = \frac{[m_2 - m_3]}{[m_2 - m_1]} \times \frac{100}{1}$$

where: $m_1$ = mass dry basin (4.2)
$m_2$ = mass basin + wet filter cake (4.3)
$m_3$ = mass basin + dry filter cake (4.7)

**Record** the result as moisture % (m/m) on filter cake to 0.1%.

**6.0 PRECISION**

The expected range of results is 70-80% moisture on filter cake.

The precision of the method has not been determined.

# METHOD 13
## FIBRE — DETERMINATION IN JUICE AND MUD STREAMS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry.

The results from these analyses are used to assess juice screen, clarifier and filter station performance.

**2.0 PRINCIPLE OF METHOD**

Fibre is determined after filtering the sample through a 75 micron screen and washing with water to remove mud solids and soluble solids. A decantation step removes any sand present. The filtered sample is dried.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Fibre can,** either rolled galvanised iron or stainless steel, approximately 100 mm diameter and 150 mm deep with 75 micron stainless steel screen in the bottom.

3.2 **High pressure fish-tail water jet.** This can be achieved by clamping the end of a rubber hose attached to the tap.

3.3 **Drying oven,** forced draft, capable of being controlled at 100 °C ± 2 °C.

**4.0 PROCEDURE**

4.1 Weigh out the appropriate quantity of sample as shown in the following Table. Record the mass of sample ($m_1$) to 0.1 g.

| Product | Sample quantity | Sample beaker |
|---|---|---|
| Mixed juice | 2000 g | 4000 mL |
| Primary mud | 200 g | 500 mL |
| Filter feed | 200 g | 500 mL |
| Filter cake | 50 g | 500 mL |

4.2 Transfer portion of the sample to the can and wash the mud solids and soluble solids through the 75 micron gauze with a fish-tail jet of high pressure water. Hold the jet fairly close to the bottom of the can to achieve the maximum washing effect and also to avoid loss of fibre by splashing. If the water level builds up in the can after initial washing, gently swirl the can to assist liquid drainage.

4.3 Repeat the above steps until the sample beaker is empty. Rinse the beaker with water to wash all remaining sample into the can. Continue washing until the clarity of the filtrate is comparable with that of the water source.

4.4 A decantation step is used to remove any sand from the fibre mat. Upend the can and wash all the contents into a 1000 mL beaker, making the final volume to about 500 mL. Swirl the contents of the beaker. The sand settles quickly to the bottom. Return the supernatant containing suspended fibre to the can. Rinse the beaker with water and return any remaining fibre to the can. Allow the can to drain.

4.5 Dry the fibre in the can in an air oven overnight at 100 °C ± 2 °C until constant weight is achieved.

4.6 While hot, weigh the sample and can. Record the mass of the can and dried fibre ($m_2$) to 0.1 g.

4.7 Empty the fibre out of the can, using a brush to remove all traces of fibre. Weigh the empty can while hot, and record the mass ($m_3$) to 0.1 g.

**5.0 CALCULATION**

% Fibre in Mixed Juice, Filter Cake, Primary Mud and Filter Feed.

5.1

$$\text{Fibre \% (m/m)} = \frac{(m_2 - m_3)}{m_1} \times \frac{100}{1}$$

where: $m_1$ = mass of sample (4.1)
$m_2$ = mass of can + dried fibre (4.6)
$m_3$ = mass of fibre can (4.7)

**6.0 PRECISION**

The precision of these methods has not been determined. The expected range of results is:

| | |
|---|---|
| Mixed Juice: Unscreened | 0-2% |
| Screened | 0-0.5% |
| Primary Mud: | 1-4% |
| Filter Feed: | 1-6% |
| Filter Cake: | 4-8% |

# METHOD 14

## TOTAL INSOLUBLE SOLIDS AND MUD SOLIDS DETERMINATION IN JUICE AND MUD STREAMS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry.

The results from these analyses are used to assess clarifier and rotary vacuum filter performance.

**2.0 PRINCIPLE OF METHOD**

The analyses consist of physical separations. For all streams except filter cake, total insoluble solids are determined by vacuum filtration of a flocculated test portion of the sample through a Whatman No. 54 filter paper. The soluble solids are removed completely by two series of dilution, settling and decantation steps, prior to vacuum filtration and washing of the formed cake. The filtered sample is dried.

For filter cake, soluble solids are calculated from per cent pol and an assumed juice purity in the cake. Insoluble solids are the difference between total solids (100 – moisture) and soluble solids.

For all streams, mud solids is the difference between per cent total insoluble solids and per cent fibre. The fibre level in filtrate is negligible and only insoluble solids are reported.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Drying oven**, forced draft, capable of being controlled at 100 °C ± 2 °C.

**4.0 PROCEDURE**

4.1 Dry a 27 cm Whatman No. 54 filter paper in the oven at 100 °C ± 2 °C for 3 hours. Weigh the dried filter paper and record the mass ($m_1$) to 0.1 g.

4.2 Weigh out the appropriate quantity of sample as shown in the following Table. Record the mass of sample ($m_2$) to 0.1 g.

| Product | Sample quantity | Sample beaker |
|---|---|---|
| Mixed juice | 500 g | 1000 mL |
| Primary mud | 200 g | 500 mL |
| Filter feed | 200 g | 500 mL |
| Filtrate | 500 g | 1000 mL |

4.3 Transfer the sample to a 5 litre beaker, using hot water to wash any residual sample into this beaker. Make the final volume up to about 4.5 litres. Avoid aerating the insoluble solids by pouring the water slowly down the wall of the beaker.

4.4 For mixed juice and filtrate, heat the suspension until just boiling, with stirring to prevent bumping. For mixed juice, add sufficient factory saccharate with stirring to raise the pH of the liquid to within the range 7.5-8.0.

4.5 Add about 5 mL of 0.1% factory flocculant solution to the beaker and stir in gently until flocculation occurs. This allows the flocculated material to settle. Any floating particles will sink if gently disturbed with a spatula. The solids should compact to about 30% of the original volume. Carefully decant off as much clear liquid as possible without losing solids.

4.6 Repeat steps 4.3-4.5

4.7 Place the pre-dried filter paper from step 4.1 in a 15 cm Buchner funnel so as to form a cup. Connect to the flask and apply vacuum. Rinse the edges of the filter paper using a wash bottle and pour the settled solids into the funnel. Wash all remaining solids into the funnel.

4.8 When the surplus liquid has filtered, flood the cake with hot water to a depth of about 1 cm and vacuum filter until the surplus liquid is removed. Dry the filter cake as much as possible by suction.

4.9 Dry the filter paper plus contents in the air oven overnight at 100 °C ± 2 °C until constant weight is achieved. Weigh the dried cake and paper and record the mass ($m_3$) to 0.1 g.

**5.0 CALCULATION**

% Total Insoluble Solids in Mixed Juice, Primary Mud, Filter Feed and Filtrate.

$$\text{Total insoluble solids \% (m/m)} = \frac{(m_3 - m_1)}{m_2} \times \frac{100}{1}$$

$m_1$ = Mass of dried filter paper (4.1)
$m_2$ = Mass of sample (4.2)
$m_3$ = Mass of dried cake + filter paper (4.9)

Report the result as total insoluble solids % (m/m) to 0.1.

**6.0 CALCULATION**

% Total Insolubles in Filter Cake

6.1 Determine % pol in filter cake (see Method 11).

6.2 Determine % moisture in filter cake (see Method 12).

6.3 Calculate total insoluble solids % (m/m) in filter cake.

% total insoluble solids = % total solids — % soluble solids.

where: % total solids = 100 — % moisture
% soluble solids = % pol/0.8

where: purity of the juice in the mud is assumed to be 80%. Report the result as total insoluble solids % (m/m) to 0.1.

**7.0 CALCULATION**

% Mud Solids

7.1 Determine % fibre (see Method 13).

Mud solids % (m/m) = % total insoluble solids — % fibre.

Report the result as mud solids % (m/m) to 0.1

## 8.0 PRECISION

The precision of these methods has not been determined.

The expected range of results is:

| | | |
|---|---|---|
| Mixed Juice: | Total insoluble solids | 0-2% |
| | Mud solids | 0-2% |
| Primary Mud: | Total insoluble solids | 4-12% |
| | Mud solids | 3-12% |
| Filter Feed: | Total insoluble solids | 4-12% |
| | Mud solids | 3-12% |
| Filtrate: | Total insoluble solids | 0-3% |
| Filter Cake: | Total insoluble solids | 12-30% |
| | Mud solids | 8-20% |

# METHOD 15
## POL — DETERMINATION IN PAN PRODUCTS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry.

The method is used mainly for factory control purposes. However it should be noted that pol, as a measure of sucrose, becomes less accurate as the purity of the product decreases.

**2.0 PRINCIPLE OF METHOD**

A test portion of the product is weighed, diluted with water and clarified by adding basic lead acetate powder, followed by filtration. The pol of the clarified solution is read in a sugar polarimeter.

Low purity products need relatively large quantities of basic lead acetate for clarification. This can cause overleading and inflate the pol reading. Acetic acid is therefore added to release fructose from any lead fructose compounds formed.

If a product cannot be clarified by basic lead acetate powder, a solution of lead nitrate/sodium hydroxide (Herles' Reagent) can be used.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Sugar polarimeter** (26 g normal weight).

3.2 **Polarimeter tube,** 200 mm.

3.3 **Volumetric flasks,** Class A (AS 2164) 100 mL and 250 mL.

3.4 **Pipette,** Class A (AS 2166) 50 mL.

3.5 **Volumetric flask,** 50-55 mL.

**4.0 REAGENTS**

4.1 **Basic lead acetate powder** (dry lead).

4.2 **Acetic acid,** $\rho_{20}$ = 1.050 g/mL) diluted 2 + 8 with water.

**5.0 PROCEDURE**

5.1 For syrups, weigh 26.0 ± 0.1 g into a 100 mL volumetric flask. Dissolve in about 50 mL water, then dilute to volume. Proceed as in steps 5.6 - 5.8.

5.2 For other products, weigh 50 ± 1 g of product into a tared 250 mL beaker. Record the mass of the samples to 0.1 g. Add approx. 30 mL of hot (60°C) water and dissolve the sample carefully by stirring.

5.3 Add water until the total mass is double the mass of the original sample to the nearest 0.1 g. Mix well by stirring and cool in a water bath to room temperature.

5.4 Weigh the required amount of the sample into a 100 mL or 250 mL volumetric flask (as specified in the Table below).

| Product | Mass of Sample g | Final Volume mL | Mass of Dry Lead g |
|---|---|---|---|
| Syrup | 26 straight syrup | 100 | 0.5 |
| A and B Massecuite | 26 of 1:1 dilution | 100 | 2.0 |
| A and B Molasses | 26 of 1:1 dilution | 100 | 3.0 |
| Magma | 26 of 1:1 dilution | 100 | 2.0 |
| C Massecuite | 52 of 1:1 dilution | 250 | 5.0 |
| Final Molasses | 52 of 1:1 dilution | 250 | 8.0 |

5.5 Dilute to volume with water. Defroth if necessary with an alcohol blower before the final adjustment to the mark. Stopper and mix well.

5.6 Transfer to a clean dry 500 mL beaker and add the prescribed amount of dry lead (refer to 5.4). Mix well by stirring.

5.7 Filter through a Whatman 15 cm No. 91 filter paper in a covered stemless filter funnel, into a filter glass. Discard the first 10-15 mL and collect the clear filtrate.

5.8 Rinse twice and fill a 200 mm pol tube with water. Read the water tube in the polarimeter. Take 4 readings and record the mean water reading to 0.01°Z.

NOTE: With automatic polarimeters allow the reading of the water-filled tube to stabilise and then re-set to zero.

5.9 Rinse twice and fill a 200 mm polarimeter tube with the clear filtrate. Determine the pol reading of the filled tube in a sugar polarimeter. Take 4 readings and record the average pol reading to 0.01°Z.

5.10 **For C Massecuite and Final Molasses only.**

Pipette with a Class A pipette 50.0 mL of filtrate (5.7) into a 50-55 mL volumetric flask. Add 2.00 ± 0.02 mL of diluted acetic acid (4.2). Dilute to 55 mL with water. Fill a 200 mm polarimeter tube and read the pol of the solution as in 5.8 and 5.9. Record the average pol reading to 0.01°Z.

NOTE: If the sample cannot be clarified by dry lead, a solution of lead nitrate/sodium hydroxide can be used (Herles' Reagent). Refer to the Method No. 2 "Pol — Determination in Juice".

**6.0 CALCULATIONS**

6.1 **Syrup** — 1 normal weight of straight syrup/100 mL.

pol % sample = pol reading (5.9)

6.2 **A & B Massecuite**, **A & B Molasses**, **Magma** — 1 normal weight of 1:1 dilution of sample/100 mL

pol % sample = pol reading (5.9) × 2

6.3 **C Massecuite**, **Final Molasses** — 2 normal weights of 1:1 dilution of sample/250 mL

pol % sample = pol reading (5.10) × 2.75

**Record** the result to the nearest 0.1% pol

6.4 **Calculation where a more precise result is required:**

6.4.1 Record
- — the scale correction SC for the polarimeter. [Refer to Method No. 1 "Calibration of Polarimeter by Quartz Plate Check"]
- — the temperature of making to the mark, $t_m$ (5.5)
- — the temperature of reading the polarimeter, $t_r$ (5.9 or 5.10)

6.4.2 Calculate
- — proportional scale correction, $SC_p$
- — correction for temperature of making to the mark, $C_{tm}$
- — correction for temperature of reading, $C_{tr}$

[Refer to Method No. 30 "Polarisation — Determination in Raw Sugar using Visual or Automatic Polarimeters" for the procedure of calculating these corrections]

6.4.3 Calculate the corrected pol reading

Corrected pol reading —

$= P - P_o + SC_p + C_{tr} + C_{tm}$

where:

P = pol reading of the filtrate (5.9 or 5.10)

$P_o$ = pol reading of the water filled tube (5.8)

$SC_p$ = proportional scale correction

$C_{tr}$ = correction for temperature of reading

$C_{tm}$ = correction for temperature of making to the mark

6.4.4 Use the corrected pol reading to calculate pol % sample in 6.1, 6.2 or 6.3.

## 7.0 PRECISION

The expected range of results is 30-90% pol in sample. The precision of the method has not been determined.

# METHOD 16

## BRIX (TOTAL SOLIDS) — DETERMINATION IN MOLASSES AND CANE INVERT BY HYDROMETER

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian Sugar Industry. It is used for process control in the factory and for payment analyses for molasses and cane invert sold overseas.

This method is often used as a measure of total dissolved solids in low grade products but becomes less accurate as the purity decreases from syrup to final molasses.

**2.0 PRINCIPLE OF METHOD**

The test sample is diluted 1:1 by weight, with water, cooled to room temperature and the brix measured by hydrometer (brix spindle).

The contraction in volume associated with the dilution prevents a true measurement of the brix of the original material.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Brix Spindles** (Hydrometers), range 30-40 and 40-50 °bx, at 20 °C, calibrated to 0.1 °bx.

3.2 **Brix cylinder,** metal or glass, at least 75 mm longer than the brix spindle.

3.3 **Cold water bath.**

3.4 **Balance** to weigh 1000 ± 0.1 g.

**4.0 PROCEDURE**

NOTE: Store the sample in a sealed container, below 30 °C, to minimise evaporation.

4.1 Weigh into a tared 1L conical flask 450 ± 10 g of molasses or cane invert. Record the weight to 0.1 g.

4.2 Add to — Molasses: approx. 300 mL hot (60 °C) water
Cane Invert: approx 300 mL cold water

Dissolve the sample completely by stirring carefully with a brass rod fitted with a rubber policeman. Rinse the rod with water into the dissolved sample.

4.3 Add water until the weight of the solution is double the weight of the original sample, to the nearest 0.1 g. Stopper the flask and mix well.

4.4 Stand the diluted solution in a running water bath until the solution cools to room temperature.

4.5 Mix the test solution well by swirling then rinse the brix cylinder (3.2) with a portion of the solution. Immediately fill the cylinder completely with the solution.

4.6 Allow the solution in the brix cylinder to stand for 5 minutes to allow air bubbles and froth to rise to the surface, then pour in more solution to displace all the froth.

4.7 Stand the solution for a further 15 minutes and then remove any traces of froth.

4.8 Select a clean dry brix spindle. Hold the spindle in the midstem, between thumb and index finger and lower it carefully into the solution until the flotation level is reached. Do not wet the stem any further than is necessary to form a natural meniscus.

4.9 Withdraw the spindle, and then gently refloat it to the same level as in 4.8. The stem should not be wet more than two graduations above the liquid level.

4.10 When the spindle has settled, read the brix of the solution. Take the reading (with the eye level with the juice surface), by estimating the point at which the surface would cut the scale. Record the brix to 0.1°.

NOTE: Insoluble solids from molasses samples may settle out in the brix cylinder. A serious error will result if the bottom of the spindle enters this layer. A glass cylinder can be used to ensure the spindle is floating above the insoluble layer.

4.11 Measure the temperature of the test solution with a 10-35 °C thermometer. Record the temperature to 0.1 °C.

**5.0 CALCULATIONS**

5.1 **Determine** the temperature correction (to 20 °C) from Table I.

**Table I** gives Temperature Corrections to readings of brix hydrometers (calibrated at 20 °C).

5.2 **Calculate** the brix reading of the diluted sample, corrected to 20 °C:

brix at 20 °C = brix reading + temperature correction

5.3 **Calculate** the brix of the original sample:

brix of sample = 2 × corrected brix of diluted sample (5.2)

5.4 **Report** the result as degrees brix (or % total solids) at 20 °C to the nearest 0.1.

**6.0 PRECISION**

| Sample | Concentration Range °bx | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Molasses | 65 - 95 | 0.5 | not determined |
| Cane Invert | 80 - 90 | 0.5 | not determined |

# METHOD 17
## BRIX (TOTAL SOLIDS) — DETERMINATION IN SUGAR SOLUTIONS BY REFRACTOMETER

**1.0 SCOPE AND FIELD OF APPLICATION**

This Level 3 method has been adopted by the Australian sugar industry.

For the purpose of cane payment refer to Note 3 – Conventions at the front of this manual.

The brix, determined by refractometer, gives an approximate measure of the total solids content of an impure sugar solution. It applies to solutions up to a maximum 85% total solids. The approximation becomes increasingly inaccurate as the purity of the solution decreases and care must be taken when interpreting the brix of low purity products, determined by this method.

**2.0 PRINCIPLE OF METHOD**

The refractive index of sugar solutions is determined using a sugar refractometer. In the Abbé type refractometer the refractive index is measured by observing the critical angle of a thin film of the sample held between two glass prisms. The reading is temperature dependent and is corrected to 20°C by referring to temperature correction tables.

**3.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

3.1 **Abbé type refractometer**, with suitable light source and thermometer.

3.2 **Stemless filter funnel**, glass or plastic, 7.5 cm diameter.

3.3 **Filter paper,** Whatman No. 91 or equivalent (15 cm).

**4.0 CALIBRATION**

4.1 **Calibration of Refractometer Scale**

All refractometers used for first expressed juice analysis for cane payment purposes in the Australian sugar industry must be certified by an appropriately accredited NATA laboratory. The refractometer is calibrated using distilled water and a lithium fluoride prism of refractive index 1.3921 (36°Bx). The refractometer scale calibration should be checked regularly against liquid traceable standards. The refractive index of distilled water at various temperatures is given in Table X.

4.2 For the calibration of the refractometer refer to the manufacturer's instruction manual.

**5.0 PROCEDURE**

5.1 Connect the prism water jackets to a water supply at ambient temperature and ensure that the water is running steadily. Position the illuminating source and turn it on.

5.2 Clean the prisms with a damp tissue using a dabbing action. Do not rub the prisms, as they scratch very easily. Dry the prisms thoroughly with a dry tissue.

5.3 Place one or two drops of distilled water on the lower prism with a plastic rod and then close the prisms to finger tightness only. The prisms become optically active if strained. Allow sufficient time for the temperature to equilibrate if the sample is not at the same temperature as the refractometer.

5.4 View the field through the telescope and adjust the dispersion compensator at the lower end of the telescope until the light and dark portions of the field are divided by a sharply focussed horizontal line. Adjust the scale until this line is set at the intersection of the cross hairs of the eyepiece. Take three readings, approaching the cross hairs alternately from above and below. Record the refractive index readings to 0.0001 and average. Record the temperature to 0.1°C.

5.5 Open the prisms and dry by dabbing with a fresh tissue. Place a drop of the sample to be analysed on the lower prism with a plastic rod and close the prisms quickly to finger tightness to avoid evaporation of the sample. Obtain the refractive index and temperature of reading as set out in 5.3 and 5.4.

NOTE: First expressed juice and other milling train juices should be filtered prior to reading. This provides a better field and also prevents damage to the prisms from particulate matter in the raw juice.

5.6 Open the prisms and wash away all traces of sugar solution using distilled water from a wash bottle. Clean and dry the prisms by dabbing gently with a fresh tissue. Leave a folded tissue between the prisms when the refractometer is not in use.

**6.0 CALCULATION**

A: FOR REFRACTIVE INDEX SCALE

6.1 **Calculate** the refractometer scale correction.

Scale Correction = R.I. of water (Table X) — observed R.I. of water (5.4).

Record the scale correction to 0.0001.

6.2 **Obtain** the temperature correction for the sugar solution from Table XI.

**Table XI** gives temperature corrections to be applied to refractometer readings of sucrose solutions of various concentrations to obtain the Refractive Index at 20°C.

6.3 **Calculate the R.I** at 20°C of the test sample by adding the scale correction and adding or subtracting the temperature correction as required.

R.I. (20°C) = R.I. (t°C) + scale correction ± temperature correction.

Record the refractive index of the test sample to 0.0001.

6.4 **Determine** the brix (or % total solids) of the test sample from Table XII.

**Table XII** gives brix, true densities and concentration of sucrose solutions at 20°C, corresponding to refractive index reading.

B: FOR BRIX SCALE

NOTE: Unless a refractometer has suffered physical damage or been tampered with it is rare for the scale setting to change. An instrument which is calibrated by an appropriately accredited laboratory should have a negligible scale correction (4.1) and when calculating the brix at 20°C it is assumed to be zero.

6.5 **Obtain** the temperature correction for the refractometer reading from Table IX.
Table IX gives "Temperature Corrections for the Abbé Refractometer calibrated at 20°C".

6.6 Calculate the brix at 20°C by adding or subtracting the temperature correction as required.
Brix at 20°C = brix reading (t°C) ± temperature correction.

6.7 Record the result to one fifth of a scale division.

**7.0 PRECISION [VALIDATION REQUIRED]**

| Sample | Concentration °brix | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Sugar Solutions | 0 – 85 | 0.2° Bx | not determined |

**8.0 REFERENCES [VALIDATION REQUIRED]**

8.1 Sugar Analysis — ICUMSA Methods, Ed. F. Schneider; ICUMSA (1979); p.177.

8.2 ICUMSA, Proceedings of the Seventeenth Session (1978), Subject 12.

8.3 Brown, C.A. and Zerban, F.W., Physical and Chemical Methods of Sugar Analysis, Ed. 3; (1941).

8.4 ICUMSA, Proceedings of 19th Session (1986).

8.5 ICUMSA, Proceedings of 21st Session (1994), General Subject 4.

8.6 ICUMSA Methods Book (1994).

# METHOD 18
## SUCROSE — DETERMINATION IN MILL PRODUCTS BY DOUBLE POLARISATION

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. It is based on the Jackson and Gillis Method 2.

The double polarisation gives a more accurate estimate of sucrose than does single (plain) polarisation for products with reducing sugar content above 1%. However it is not an absolute method.

**2.0 PRINCIPLE OF METHOD**

A solution of the sample is clarified with basic lead acetate, and the pol of the clarified solution determined before and after the sucrose in the solution is inverted by hydrochloric acid, to glucose and fructose. The percentage sucrose in the sample is calculated from the change in polarisation. This procedure eliminates interference from other substances in the sample, that are optically active but are not inverted by hydrochloric acid. In low grade products, oligosaccharides may be hydrolysed to reducing sugars causing significant errors.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Volumetric flasks,** Class A (AS 2164), 100 mL and 250 mL.

3.2 **Bulb pipette,** Class A (AS 2166), 5 mL.

3.3 **Polarimeter tube,** 200 mm, centre filling (glass or glass lined).

3.4 **Cooling bath.**

3.5 **Hot water bath,** capable of being controlled at 68.0 ± 0.5 °C.

3.6 **Sugar polarimeter** (26 g normal weight).

**4.0 REAGENTS**

Unless otherwise specified, use only analytical grade reagents and only distilled water.

4.1 **Basic lead acetate powder** (dry lead).

4.2 **Hydrochloric Acid** (6.34 mol/L), $\rho_{20}$ = 1.103 g/mL. Dilute 630 ± 5 mL of hydrochloric acid ($\rho_{20}$ = 1.17 g/mL), to 1L with water and adjust to exactly 6.34 mol/L, after titrating 5 mL with 1 mol/L NaOH using methyl orange indicator.

4.3 **Ammonia solution.** Dilute 300 mL of $NH_4OH$, ($\rho_{20}$ = 0.90 g/mL), to 1L with water.

4.4 **Ammonium chloride** ($NH_4Cl$) 226 g/L solution.

4.5 **Supercel.**

4.6 **Norit decolourising carbon,** (SX11).

4.7 **Whatman No. 91** filter paper, 15.0 cm. dia.

**5.0 PROCEDURE**

A. CLARIFICATION

5.1 Weigh the product to 0.01 g into a tared basin.
Juices: 65.00 g (one normal weight/250 mL)
Mill Products: 32.50 g (half normal weight/250 mL)

5.2 Transfer the sample to a 250 mL Class A volumetric flask with hot water. Dissolve the sample completely by swirling and cool quickly to room temperature in a cold water bath. Fill the flask to within a few mm of the mark, defroth with an alcohol blower and adjust exactly to the mark. Stopper and mix well.

5.3 Add approximately 8-12 g of dry lead for molasses and less for products of higher purity. Stopper the flask, mix well and allow to stand for 5-10 minutes.
For molasses it is more convenient to place the dry lead in a 400 mL beaker and, using a rubber tipped glass rod, make a thick, smooth slurry with a few mL of the solution. Then, add the remainder of the solution, with continuous stirring and allow to stand for 5-10 minutes. Cover the beaker to minimise evaporation.

5.4 If after standing, the clarified solution appears too dark to read in a polarimeter after a 50-50 dilution, add more lead acetate. However the total amount of dry lead must be kept to a minimum.

5.5 Filter the solution through a 15 cm Whatman 91 filter paper in a stemless funnel, into a filter glass. Place a cover slip over the filter funnel to minimise evaporation. Discard the first 10-15 mL then collect a total of 120 mL of the clear filtrate.

NOTE: To speed filtration, filter the solution through two filter funnels and combine the filtrate.

B. PREPARATION OF PLAIN AND INVERT SOLUTIONS

5.6 Determine the volume, to 0.1 mL, of $NH_4OH$ solution (4.3) required to neutralise 10.0 ± 0.1 mL of 6.34 mol/L HCl (4.2) by titration, using methyl orange as indicator.

5.7 **Plain Solution.** Pipette 50.00 ± 0.05 mL of filtrate (5.5) into a 100 mL Class A flask and add 15.0 ± 0.1 mL $NH_4Cl$ solution (4.4). Mix the contents by swirling and make to the mark with water. Filter using Supercel if necessary, through a Whatman 91 filter paper in a covered filter funnel. Discard the first 10 mL filtrate. If the filtrate is too dark to read on the polarimeter, discard and repeat the analysis using additional dry lead for clarification.

5.8 **Invert Solution.**

5.8.1 Pipette 50.00 ± 0.05 mL of filtrate (5.5) into a 100 mL Class A flask and run in 10.0 ± 0.1 mL 6.34 mol/L HCl from a burette.

NOTE: If a heavy precipitate of lead chloride forms, the solution has been over-leaded. For the analysis of other samples of this product add a smaller amount of dry lead.

5.8.2 Add 20 ± 1 mL of water from a measuring cylinder and mix. Place the flask in a water bath at 68.0 ± 0.5 °C for 10 min. ± 10 sec. agitating the flask for the first 3 minutes.

5.8.3 Cool the solution to room temperature in a cold water bath and add the determined volume of $NH_4OH$ solution (5.6) slowly, and with constant swirling of flask. Again cool to room

temperature in a water bath. Make to mark with water and mix well.

5.8.4 If the solution appears too dark, add a small quantity (enough to cover a 5 cent coin) of Norit (4.6) and mix. Allow to stand for 5 minutes.

5.8.5 If a precipitate forms and/or if Norit has been added, filter using Supercel if necessary, through a Whatman 91 filter paper in a covered filter funnel. Discard the first 10 mL of filtrate.

C. POLARISATION

5.9 Record the scale correction, SC, of the polarimeter. Refer to Method No. 1 "Calibration of Polarimeter by Quartz Plate Check".

5.10 Rinse twice and fill a clean 200 mm pol tube (3.3) with the plain solution (5.7). Check to ensure that there are no bubbles.

5.11 Determine the pol reading of the filled tube in a sugar polarimeter. Take 4 readings. Average all readings and record the mean *plain solution polarisation*, p, to 0.01 °Z.

5.12 Record the *temperature of reading of the plain solution*, $t_p$, in the polarimeter tube to 0.1 °C.

5.13 Empty and drain the tube. Rinse twice and fill the same pol tube with invert solution (5.8.4).

5.14 Determine the pol reading of the invert solution following the same procedures in 5.11. Place the polarimeter tube in the polarimeter in exactly the same manner and position as in 5.11.
Average all the readings and record the *mean invert solution polarisation*, i, to 0.01 °Z.

NOTE: The readings of the invert solution will be on the negative scale of the polarimeter.

5.15 Record the *temperature of reading of the invert solution*, $t_i$ in the polarimeter tube to 0.1 °C.

NOTE: If the same polarisation tube is used for both solutions the "zero" correction cancels out and need not be determined.

## 6.0 CALCULATIONS

6.1 **Subtract** the *invert solution polarisation*, i, (5.14), from the *plain solution polarisation*, p, (5.11)
i.e. (p — i)

6.2 **Calculate** the proportional scale correction, SCp

$$SC_p = \frac{(p - i)}{Q_p} \times SC$$

where: $Q_p$ is the certified value of the quartz plate
SC is the scale correction for the quartz plate reading

6.3 **Add** the proportional scale correction, $SC_p$ to (p — i)
$(p - i)^* = (p - i) + SC_p$

6.4 **Correct** (p — i) to normal strength $\left[\frac{1}{\text{normality}}\right]$
and apply dilution factor (multiply by 2).
If 1/2 normal solution (i.e. 32.5 g/250 mL),
P — I = 4 (p — i)*
If 1 normal solution (i.e. 65 g/250 mL),
P — I = 2 (p — i)*

6.5 **Multiply** P — I by the original normality of the solution and determine the factor corresponding to this value from Table IV.
**Table IV** gives factors for the calculation of sucrose according to Jackson and Gillis, Method 2.

6.6 **Calculate** the % Sucrose in the sample

$$\%\ \text{Sucrose} = \frac{100 \times [P - I]}{\text{Factor} - 0.53\ [t_i - 20]}$$

where $t_i$ = temperature of reading of the invert solution (5.15)

NOTE: The temperature coefficient of Jackson and Gillis is 0.53, comprising 0.03 for sucrose and 0.50 for invert sugar.

**Record** the result as % sucrose to 0.01%

6.7 The calculation assumes that both the plain and invert solutions have the same temperature at reading. Where this difference is greater than 1 °C the plain pol reading p, (5.11) should be corrected to the temperature of the invert solution from Table V.
**Table V** gives temperature corrections for plain polarisation reading in the determination of sucrose by double polarisation.

6.8 **% Sucrose** may be determined directly from (P — I) values listed in Table VI.
**Table VI** gives % Sucrose corresponding to various values of (P — I) for half normal solutions at various temperatures.
This table should only be used when expressing the result to 0.1% gives sufficient accuracy.

## 7.0 PRECISION

| Sample | Concentration Range % Sucrose | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Pan Products | 10 — 90 | 0.8% | 1.4% |

## 8.0 REFERENCES

(1) Clerget, T., Ann. Chim. Phys., Vol. 26 (1849), p. 175.

(2) Jackson, R.F. and Gillis, C.L., U.S. Bureau of Standards, Scientific Paper No. 375 (1920).

(3) Brown, C.A. and Zerban, F.W., Physical and Chemical Methods of Sugar Analysis, Ed. 3, Wiley (1955), pp. 321-5.

(4) Wilson, R.A.M., International Sugar Journal, Vol. 67 (1965), p. 235.

# METHOD 19
## TOTAL SOLIDS (DRY SUBSTANCE) — DETERMINATION IN MILL PRODUCTS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The method is used to determine water and/or total solids in factory process products of more than 30° brix.

This is an empirical method which assumes that all the water in the sample is removed by the drying process and that the loss of mass is due only to the removal of this moisture.

**2.0 PRINCIPLE**

A test portion of the sample is diluted to approximately 30° brix in a weighing tube. The solution is soaked into a coiled strip of filter paper to increase its surface area. This assists water evaporation and avoids formation of a protective skin. The sample is then dried at atmospheric pressure for 6 hours in an oven at 98 °C or in a vacuum oven at 65 °C for 18 hours at a pressure not exceeding 50 mm mercury. The loss of mass after drying represents the water in the sample. It should be pointed out that the reference method is that based on the vacuum oven.

Some sugars, especially fructose, begin to decompose in the temperature range 70-100 °C, producing water and other volatile products which contribute to the loss of mass and can cause high moisture (low total solids) values.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Desiccator,** with self indicating silica gel.

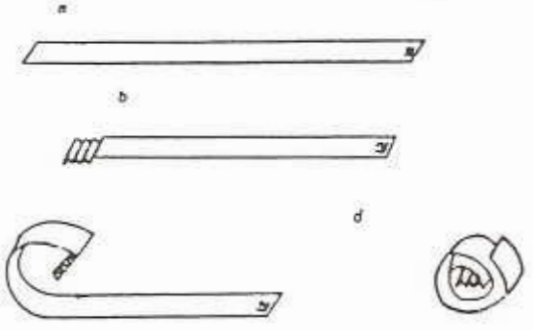
3.2 **Drying oven,** forced draft, capable of being controlled at 98 ± 2 °C or **vacuum oven** capable of being controlled at 60 ± 2 °C.

3.3 **Weighing bottle,** glass or metal, with lid, 70 mm × 35 mm.

3.4 **Filter paper strip,** 600 mm long x 50 mm wide. For example, suitable strips may be cut from Whatman No. 4 chromatography paper sheets.

**4.0 PROCEDURE**

4.1 Roll a filter paper strip (3.4) into a coil, mark the number of the weighing bottle (3.3) on the paper with a pencil and place the paper in the weighing bottle. The paper coil is best made by making several pleats (about 5 mm) in the end of the strip and then, using these as the axis, coil the rest of the strip.




4.2 Place the weighing bottle containing the paper, in the drying oven with the lid alongside, and dry for 3-6 hours at 98 ± 2 °C or in a vacuum oven and dry for 6 hours at 60 ± 2 °C. Cool the tube and paper, with the lid fitted, in a desiccator for 45-60 minutes.

4.3 Weigh the bottle and dried paper with the lid on to 0.0001 g ($m_1$). Store the bottle with lid in place in a desiccator.

4.4 Remove the paper from the tube and weigh empty tube and lid to 0.0001 g ($m_2$).

4.5 Stir the sample to be analysed thoroughly with a brass rod, removing surplus material from the rod by wiping it on the side of the sample container.

4.6 Transfer the required amount of sample to within 0.1 g to the weighing bottle (see 4.7). Weigh the bottle and lid and sample to 0.0001 g ($m_3$).

When transferring the sample to the bottle, move the rod carefully down the centre of the bottle, to avoid getting any sample on the walls of the bottle where it is difficult to dissolve. If this occurs and it cannot readily be removed with a tissue or glass towel the bottle should be discarded and a fresh start made with a clean bottle.

4.7 Dilute the sample to approximately 30° bx by adding the required volume of distilled water (to within 0.1 mL) with a barrel pipette.

| Approximate % solids in product | Mass of product to be taken (g) | Volume of water to be added (mL) |
|---|---|---|
| 100 | 2.0 | 4.5 |
| 90 | 2.2 | 4.3 |
| 80 | 2.5 | 4.0 |
| 70 | 2.9 | 3.6 |
| 60 | 3.3 | 3.2 |
| 50 | 4.0 | 2.5 |
| 40 | 5.0 | 1.5 |
| 30 | 6.7 | 0.0 |
| Less than 30 | 7.0 | 0.0 |

4.8 Boil water in a 1000 mL boiling flask with 7.5 cm diameter glass funnel placed in the neck of the flask.

4.9 Dissolve the sample by steam heating the bottle in the glass funnel (4.8). Solution is completed by gently swirling the heated bottle. Do not use a bunsen flame or hot plate to heat the bottle since these may cause decomposition of the sample. Care must be taken to ensure that all the sample has dissolved, particularly in the case of massecuites and molasses. Do not heat any longer than is necessary to obtain dissolution.

4.10 Place the loosely rolled filter paper into the bottle and incline bottle about 60° from the vertical. Slowly rotate the bottle to assist absorption of all the solution. Allow the bottle to stand for 10 minutes and check that all the solution has been absorbed.

NOTE: Check that the number on the paper and that on the bottle coincide.

4.11 Place the bottle and contents with lid alongside, in the drying oven and dry for 6 hours ± 10 minutes at 98 ± 2°C or dry in a vacuum oven at a pressure not exceeding 50 mm mercury for 18 hours at 65 ± 2°C. After drying, fit the lid on the bottle and cool in desiccator for 45-60 minutes.

4.12 Weigh dry bottle, paper and solids, with lid on, to 0.0001 g ($m_4$).

## 5.0 CALCULATIONS

5.1 Calculate % total solids.

$$\% \text{ total solids} = \frac{\text{mass of dry solids}}{\text{mass of sample}} \times \frac{100}{1}$$

$$= \frac{(m_4 - m_1)}{(m_3 - m_2)} \times \frac{100}{1}$$

where: $m_1$ = mass of weighing bottle + lid + paper (4.3)

$m_2$ = mass of weighing bottle + lid (4.4)

$m_3$ = mass of weighing bottle + lid + sample (4.6)

$m_4$ = mass of weighing bottle + lid + paper + dried sample (4.12)

Record the result as % (m/m) total solids to the nearest 0.1%

5.2 **Calculate** % moisture

% moisture = 100 − % total solids

Record the result as % (m/m) moisture to the nearest 0.1%.

## 6.0 PRECISION

| Sample | Concentration Range % (m/m) moisture | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Factory products (>30° bx) | 5 - 90 | 0.4% | 1.0% |

## 7.0 REFERENCE

Jossé, A.; Bull. Assoc. Chem. Suc.; Vol. 10 (1893), p. 656.

# METHOD 20

## REDUCING SUGARS — DETERMINATION IN MILL JUICES, CLARIFIED JUICES AND PAN PRODUCTS BY THE CONSTANT VOLUME MODIFICATION OF THE LANE AND EYNON METHOD

### 1.0 SCOPE AND FIELD OF APPLICATION

The method has been officially adopted by the ICUMSA and will supersede the previous classical procedure of the Lane and Eynon Method.

The reducing sugar concentration in the test sample should lie within the range of 250 to 400 mg/100 mL. Samples with levels outside this range are either diluted, or standard invert solution is added, prior to analysis. In the latter instance, the concentration in the original test solution is determined by subtraction of the added invert from the total reducing sugars found.

### 2.0 PRINCIPLE OF METHOD

The principle of this constant volume modification is the same as for the original method of Lane and Eynon, in which the main part of the test solution is added to a definite volume of a strongly alkaline cupric-complex salt solution, Fehling's solution, which is then boiled.

The remainder of the test solution is then added, until, at the end-point, the cupric ions are completely reduced to cuprous oxide and the blue colour of the solution disappears. The sharpness of the end-point is improved by the use of an indicator, methylene blue, which is decolourised in the presence of a minute excess of reducing sugars.

The constant volume modification differs only in the fact that at the end-point, the final volume, and consequently the concentration of the Fehling's solution in the reaction flask, has been kept constant by the prior addition of a predetermined amount of water. For this reason the titration always corresponds to the same amount of invert sugars and allows the use of a simple formula instead of tables. As sucrose will be present, and be partially converted to reducing sugars due to the high pH and temperature of the reaction mixture, the result must be multiplied by a sucrose correction factor. The experimental conditions, including volume and concentration of Fehling's solution, time of boiling and final volume of reaction mixture, are strictly defined.

Therefore, for accurate work, three titrations are necessary, one preliminary test run to determine the volume of added water required and two titrations which fulfil the requirement that all but the last 1 mL of test solution is added before heating.

### 3.0 APPARATUS

Ordinary laboratory apparatus and

3.1 **Burette**, 50 mL, AS 2165, graduated in 0.1 mL for the sugar solution. The burette should have a pinch-cock instead of a glass tap and a bent outlet tube in order to keep the graduated section of the burette out of the steam while additions are made to the boiling mixture.

3.2 **Bulb pipettes**, 15, 20 and 25 mL, Class A (AS2166).

3.3 **Heat resistant plate** (about 15 cm x 15 cm) with a 5 cm diameter hole.

3.4 **Anti-bumping granules** (pumice).

3.5 **Boiling flasks** (florence flask) 200/250 mL, flat bottom.

3.6 **Volumetric flask**, 100 mL A class (AS 2164).

3.7 **Stainless steel gauze strainer** about 5 cm dia., 100 mesh.

3.8 **Stopwatch** with 60 second sweep.

3.9 **Bunsen burner**.

### 4.0 REAGENTS

4.1 Fehling's Solutions (Soxhlet's Modification)

Mixed Fehling's solution does not keep indefinitely and the ingredients are therefore dissolved in two separate solutions, A and B, which are mixed together immediately before use. This mixing is done by adding a volume of solution A to an exactly equal volume of solution B. It is essential that the mixture be carried out in this order otherwise the precipitate of cupric hydroxide initially formed may not redissolve completely.

Solution A: cupric sulphate petahydrate, AR (69.28 ± 0.01 g) is dissolved and diluted to 1 L in distilled water.

Solution B: sodium potassium tartrate tetrahydrate, AR (346 ± 1 g) and sodium hydroxide AR (100 ± 0.5 g) are dissolved in distilled water and diluted to 1 L.

4.2 Standard Invert Sugar Solution, 1 g Invert Sugar/ 100mL:

Stock Solution

4.2.1 Weigh 9.500 ± 0.001 g of sucrose and transfer quantitatively to 1 L volumetric flask with 100 ± 5 mL of water. Swirl to dissolve.

4.2.2 While swirling gently, add 5 mL of hydrochloric acid, ($\rho_{20}$ = 1.17 g/mL). Cover the flask mouth with a small beaker and stand for 3-4 days at 20-25 °C for complete inversion of the sucrose.

4.2.3 Dilute the invert solution to about 800 mL with water. Dissolve 2.0 ± 0.1 g benzoic acid in hot water, cool and add to the invert solution. Dilute to exactly 1L and mix well. Stored in a stoppered bottle, this solution will keep for several weeks.

4.3 Neutral Standard Invert, 0.25 g/100 mL Solution

Pipette 50mL of 1% invert solution (4.2) into a 200 mL volumetric flask. Add 2 drops phenolphthalein solution and while swirling gently add 1 mol/L NaOH solution from a burette until the solution is just pink. Approximately 2.5 mL of 1 mol/L NaOH will be needed.

Add 1-2 drops of 0.5 mol/L HCl to just discharge the pink colour. Dilute the solution to exactly 200 mL with water and mix well.

4.4 Methylene Blue Indicator, 1 g/100 mL

Pure methylene blue (1.0 ± 0.05 g) is dissolved and diluted to 100 mL using distilled water, and then filtered if necessary.

## 5.0 PROCEDURE

5.1 Standardisation of the Fehling's Solution

The copper content of the Fehling's solution, prepared as described above (4.1), varies slightly from one solution to another and must therefore be adjusted. To 20 ± 0.06 mL of Fehling's solution are added 15 ± 0.05 mL distilled water and 39.0 ± 0.1 mL (by burette) of invert sugar solution (0.25 g/100 mL). Using the procedure of section 5.4 from the second paragraph onwards, the titration is completed with the invert sugar solution (0.25 g/100 mL). The total volume of invert sugar solution required should be 40 ± 0.1 mL if the Fehling's solution contains the correct amount of copper (equivalent to 100 mg invert sugar). The Fehling's solution should be adjusted as necessary by the addition of a calculated amount of cupric sulphate or water, followed by thorough mixing. Another standardisation should be carried out after any such adjustment.

5.2 Preparation of the Test Solution

(a) Mill Juice and Clarified Juice

The concentration of the test solution should, if necessary, be adjusted to fall between 250 and 400 mg invert sugar/100 mL.

(b) Pan Products

The dilution required for sugar mill products must be ascertained by a trial and error basis to produce test solutions with a reducing sugar concentration with the range of 250 to 400 mg/100 mL.

Calcium present in molasses forms a complex with glucose and fructose, resulting in slower reaction rates and apparently low reducing sugar content. The removal of calcium is therefore achieved by adding a complexing agent such as EDTA which forms a stronger complex with calcium than do the hexoses. An effective decrease in colour and an improved end-point are obtained. 4 mL of EDTA solution (4 ± 0.01 g/100 mL ethylene diamine tetra acetic acid, disodium salt) per g of cane molasses is recommended.

The required volume of EDTA solution is added to the subsample of pan product once it is dissolved but prior to making to the mark with distilled water.

5.2.1 For juices, where a volume is taken, determine the brix to 0.1 °bx by spindle or refractometer.

5.2.2 Where the invert sugar concentration of the test solution falls below 250 mg/100 mL, additional invert in the form of a known volume of standard invert (e.g., 1.0 g/100 mL) should be added to bring the resultant diluted sample up to a final invert concentration within the desired range.

5.3 Preliminary Test

A preliminary test should be carried out to ascertain the volume of water to be added to the 20 mL of Fehling's solution in order to obtain a final total volume of 75 mL when the end-point of the titration is reached.

The following mixture is prepared:

20 ± 0.06 mL of Fehling's solution,

25 ± 0.06 mL of the test solution, and

15 ± 0.5 mL of distilled water.

The last two additions correspond to the 40 mL of dilute invert sugar solution used in the calibration procedure. The mixture is then titrated with additional test solution using the procedure of the following section (5.4) from the second paragraph. The bulk of the test solution should be added as early as possible during the initial warm up before the boiling period. If the reddish colour of the boiling solution persists after the addition of the methylene blue indicator, this indicates that the test solution must be discarded and a less concentrated solution used.

If more than 50 mL of test solution, added to 20 mL of the Fehling's solution, are required to obtain the reddish colour, this indicates that a more concentrated solution should be used.

The volume of water to be added is calculated as 75 mL (total) — 20 mL (Fehling's solution) — volume (mL) of test solution = volume (mL) of water to be added.

5.4 Titration

Mixed Fehling's solution (4.1) (20 ± 0.06 mL) is pipetted into a glass flask (3.5); the volume of distilled water indicated by the preliminary test is then added. The burette (3.1) is rinsed and filled with the test solution. The whole volume of test solution required in the preliminary test less 1 mL is run into the flask. A few fragments of pumice (3.4) are added and the contents of the flask are well mixed by gentle swirling. The flask is placed on a wire gauze (3.7) over a bunsen flame and heated to boiling (within approximately 2 minutes 30 seconds). (See 5.5).

The liquid is kept boiling moderately fast for precisely 2 minutes and then 3 or 4 drops of methylene blue indicator (4.4) are added directly into the boiling mixture. The mixture should assume a distinctly blue colour.

The titration is completed in 1 minute ± 5 seconds by the further addition of small increments, initially of 0.2 mL, then 0.1 mL and finally of single drops until the end-point is reached. It is indicated by the complete disappearance of the blue colour of the indicator and the appearance of the reddish colour due to the precipitated cuprous oxide.

5.5 Precautions

The titration should be completed in 3 minutes ± 5 seconds from the commencement of boiling.

The heating device* used for boiling the reaction mixture during the titration is of prime importance when accurate results are to be guaranteed. During the whole time, the flask should remain on the wire gauze and boil at a moderate rate. The continuous emission of steam from the neck prevents atmospheric oxidation of the Fehling's solution or of the indicator. During the additions of sugar solution to the boiling liquid, the main burette tube (3.1) must be kept out of the steam outlet while the jet is brought over the mouth of the flask.

* An electric heater called the "United Molasses Combined Heater / Illuminator" is recommended for this purpose and is available from R&L Slaughter Ltd., 162 Balgores Lane, Gidea Park, Romford, Essex, U.K.

## 6.0 CALCULATIONS

6.1 Where a measured volume of sample is taken, calculate the weight of sample per 100 mL based upon the density (g/mL) obtained from Table XV, using the brix value measured in 5.2.

6.2 If necessary, calculate the amount of sucrose per 100 mL titrating solution from the weight of sample and its approximate percentage sucrose. If a measured volume of sample is taken, estimate the sucrose content by using the density obtained from Table XV using the brix value (from 5.2) and assumed purity, e.g. if assumed purity = 80% and brix = 20%. Then density = 1.081 g/mL (Table XV). And —

$$\text{Sucrose (g per 100 mL)} = \frac{8}{100} \times 20 \times \frac{1}{1.081}$$

6.3 The reducing sugar content is given by the formula:

$$\text{Reducing sugar, (\%)} = \frac{1000f}{V_t C_t} - \frac{C_s V_s}{C_t}$$

Where $C_t$ is concentration (g/100 mL) of sample in the test solution, $V_t$ is the volume (mL) of the test solution used in the titration, $C_s$ is the concentration (g/100 mL) of the standard invert added, if necessary, $V_s$ is the volume (mL) of any invert added in the test solution and f is the correction factor deduced from the Emmerich Table (Table 1) based on the amount of sucrose present.

The amount of sucrose present in the mixture (g) is equal to sucrose (g per 100 mL) in the titrating mixture x 0.01 $V_t$.

When no invert is added, the second term is zero and

$$\text{Reducing sugar, (\%)} = \frac{1000f}{V_t C_t}$$

For amounts of sucrose intermediate between two consecutive figures in Table 1, the correction factor is obtained by interpolation or from the formula:

$$f = 1 - 0.0292\ S^{2/3}$$

where: S = approximate sucrose content in test solution.

## 7.0 RANGE AND PRECISION

The expected range of results is —

Direct method 0.6 to 100%

Added invert 0.05 to 0.6%

The precision that should normally be obtained by following the direct procedure is:

Standard deviation for one analyst in one laboratory: 0.1%

Standard deviation for different analysts in different laboratories: 0.5%

95% repeatability*: 0.3%

95% reproducibility*: 1.4%

## 8.0 REFERENCES

Pieck, R. Subject 14: Reducing Sugars. Proc. 17th Session ICUMSA, 1978, 190-211.

Laursen, J. Subject 14: Reducing Sugars. Proc. 18th Session ICUMSA, 1986 232-267.

* These figures are to be used when there are two and only two, determinations.

**TABLE 1**
**SUCROSE CORRECTION TO BE APPLIED IN LANE AND EYNON CONSTANT VOLUME METHOD (ACCORDING TO PIECK, ICUMSA, 17TH SESSION (1978) SUBJECT 14, TABLE 4, 194)**

| Sucrose in boiling mixture (g) | Correction factor (f) | Sucrose in boiling mixture (g) | Correction factor (f) |
|---|---|---|---|
| 0.0 | 1.000 | | |
| 0.5 | 0.982 | | |
| 1.0 | 0.971 | 6.0 | 0.904 |
| 2.0 | 0.954 | 7.0 | 0.893 |
| 3.0 | 0.939 | 8.0 | 0.883 |
| 4.0 | 0.926 | 9.0 | 0.874 |
| 5.0 | 0.915 | 10.0 | 0.864 |

These corrections are being investigated and may undergo slight changes in the near future.

# METHOD 21

## REDUCING SUGARS — DETERMINATION IN MILL PRODUCTS BY THE LANE AND EYNON METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is used in the Australian sugar industry for the determination of reducing sugars in factory process materials. The results obtained are used for process control.

**2.0 PRINCIPLE OF METHOD**

An appropriate amount of the product, based on the expected reducing sugars and sucrose contents is weighed and dissolved in water. The reducing sugars are determined by the Lane and Eynon titration.

**3.0 APPARATUS**

Ordinary laboratory apparatus and

3.1 **Bulb pipette,** 5 mL Class A. (AS 2166)

3.2 **Bulb pipette,** 10 mL Class A. (AS 2166)

3.3 **Burette,** 50 mL Class A, (AS 2165) graduated in 0.1 mL for the sugar solution. The burette tap is replaced by a rubber tube and pinch-cock, connected to a bent outlet tube in order to keep the graduated section of the burette out of the steam while additions are made to the boiling mixture.

3.4 **Heat resistant plate** (about 15 cm x 15 cm) with a 5 cm diameter hole.

3.5 **Anti-bumping granules.**

3.6 **Boiling flasks** (florence flask) 200/250 mL, flat bottom.

3.7 **Volumetric flask,** 100 mL A-class. (AS 2164).

3.8 **Stainless steel gauze strainer** about 5 cm dia., 100 mesh.

3.9 **Stop-watch** with 60 second sweep.

3.10 **Bunsen burner.**

**4.0 REAGENTS**

During the analysis unless otherwise stated, use only reagents of analytical grade and only distilled water.

4.1 **Fehling's Solution A.**

4.2 **Fehling's Solution B.**

4.3 **Methylene Blue,** 1 g/100 mL solution in water.

4.4 **Standard Invert Solution.**

4.5 **Differential Invert Solution.**

NOTE: Directions for preparing the above reagents are given in Method 32 "Reducing Sugars-Determination in raw sugar by the Lane and Eynon Method".

4.6 **Preserving solution for mill juices.** Dissolve 50 g of anhydrous sodium carbonate in water, add 50 mL of formalin (37 g/100 g formaldehyde), dilute to 1 litre and mix.

4.7 **EDTA,** 4.00 ±.01 g/100 mL solution in water of ethylenediaminetetra-acetic acid, di-sodium salt.

**5.0 TREATMENT OF SAMPLE**

5.1 When sampling mill juices which are to be composited and stored before analysis, immediately add 1.0 mL preserving solution (4.6) for each 10.0 mL juice. Composite the samples, mix well and store in a sealed bottle at room temperature.

**6.0 PROCEDURE**

A. STRAIGHT METHOD (for products with >0.6% RS)

6.1 Weigh out the appropriate quantity of product indicated in Table XXXI. For juices, using a measuring cylinder take 20, 30, 40 or 50 mL of the preserved sample as required.

Determine the brix of the juice sample to 0.1° by spindle or refractometer.

6.2 Wash the sample into a 100 mL volumetric flask. Hot water may be needed for some products.

NOTE: For invert syrup, prepare 1L of solution. For molasses, prepare 200 mL.

6.3 Dissolve the sample by shaking and cool to room temperature. Add water to fill the bulb of the flask and mix well. Make to the mark, stopper and mix well.

6.4 Using a 5 mL class 'A' pipette, add 5.00 mL of Fehling's A solution to a 200 mL boiling flask. Using a safety pipette, add 5.0 ± 0.1 mL of Fehling's B solution and swirl to complete the preparation of the alkaline copper solution. Fehling's B should be added just before the titration is to be done.

6.5 Add a few pieces of anti-bumping granules to the boiling flask to prevent bumping.

6.6 Rinse (about 10 mL) and fill a clean 50 mL Class A burette with titrating solution (6.3). Squeeze the rubber tubing above the pinch-cock to remove air-bubbles; open the pinch-cock and incline the jet to remove the remaining air-bubbles. Adjust the level of solution to the zero mark.

6.7 Add 15 mL of the solution from the burette to the alkaline copper solution (6.4), swirl, and place the flask on a heat resistant plate (3.4) over a bunsen burner.

6.8 Bring the mixture to the boil, making sure that the flame does not impinge on the flask above the level of the solution.

6.9 Start the stopwatch when the mixture commences to boil vigorously and adjust the flame to give moderate even boiling.

6.10 After boiling for 15 secs, if the solution is still blue, add rapidly successive small portions of solution from the burette (but without interrupting boiling) until the reddish colour shows that the end-point is near. With experience this can be judged to within 2-3 mL. This estimated end-point should be reached within the first minute of boiling.

6.11 After 2 minutes of boiling add 3 drops of methylene blue indicator (4.3).

6.12 Continue titrating adding a few drops at a time, allowing 5-10 secs between each portion, until the blue colour is completely discharged, and note the final burette reading. The total boiling time should be 3.0 ± 0.5 min. and boiling should not be interrupted during the titration (to prevent air entering the flask and oxidising the contents).

6.13 For routine work, the above incremental method gives satisfactory results, provided that no more than 3 mL

of solution is needed to complete the titration after step 6.10. For accuracy a second titration is required.

6.14 Prepare another alkaline copper solution (6.4), add all but 1-2 mL of the required amount of titrating solution from the burette, and repeat steps 6.8 and 6.9.

6.15 Continue boiling for 2 minutes before adding the methylene blue indicator and complete the titration as before. The total boiling time should be kept as close as practicable to 3 minutes (but no more than 3½ minutes). Record the end-point to nearest 0.1 mL. The end point must lie between 17 and 35 mL.

B. 1ST DIFFERENTIAL METHOD (for products with 0.05-0.6% RS)

6.16 Weigh to ± 0.01 g a quantity of the product that will contain 25 g of sucrose.

6.17 Wash the sample into a 100 mL Class 'A' volumetric flask. Hot water may be needed for some products. Add water to 50-60 mL and dissolve by shaking.

6.18 Using a 10 mL Class 'A' bulb pipette, add 10.00 mL neutralised differential invert solution (4.5), add water to fill the bulb of the flask, swirl, make to the mark, stopper and shake well. This is the test solution.

6.19 Prepare a "blank" solution by weighing out 25.00 ± 0.01 g standard sugar and repeating steps 6.17 and 6.18.

6.20 Titrate the test solution (6.18) as described in steps 6.4 to 6.15 and record the end point to 0.1 mL.

6.21 Titrate the blank (6.19) as described in steps 6.4 to 6.15 but run in 31 mL solution for the first titration. (The end-point should lie between 32 and 36 mL). Record the end-point to 0.1 mL.

C. 2ND DIFFERENTIAL METHOD (for sweetwaters, etc.)

6.22 Weigh a quantity of the sample containing not more than 0.2 g RS into a 200 mL volumetric flask.

6.23 Pipette 25.0 mL of neutralised differential invert solution (4.5) into the flask. Add 4.0 mL of EDTA solution (4.7) for each gram of product if it is necessary to mask calcium salts.

6.24 Make to the mark with water and mix well. Filter through a Whatman No. 31 filter paper. This is the test solution.

6.25 Prepare a blank solution with 25.0 ± 0.1 mL of neutralised differential invert solution and water in a second 200 mL volumetric flask. Add the same volume of EDTA if this reagent was used in (6.23).

6.26 Titrate the test solution as in 6.4 to 6.15. Record the end point to 0.1 mL.

6.27 Titrate the blank solution as in 6.4 to 6.15. Record the end point to 0.1 mL.

## 7.0 CALCULATIONS

7.1 **Table XXXI** gives the weight of product to be weighed out per 100 mL of titrating solution for the determination of RS in products of various sucrose and RS content.

7.2 If necessary, calculate the amount of sucrose per 100 mL of titrating solution from the weight of sample and the approximate % sucrose. If a measured volume of sample is taken, calculate the amount of sucrose from the density at 20 °C (Table XII or XV) and the assumed purity. Make allowance for any dilution.

7.3 Determine the grams RS per 100 mL of titrating solution corresponding to the end point of the titration from Tables XXXII or XXXIII, (e.g. 0 g sucrose for 2nd differential method).

7.4 For 1st differential method read grams RS in 100 mL blank solution under the 25 g sucrose column of Table XXXII.

For 2nd differential method read grams of RS in 100 mL blank solution under the 0 g sucrose column of Table XXXIII.

7.5 Calculate the % RS in product.

For straight method:

$$\% \text{ RS} = \frac{100 \times \text{g RS per 100 mL titrating solution}}{\text{g sample per 100 mL titrating solution}}$$

For differential methods:

$$\% \text{ RS} = \frac{100 \times [\text{g RS per 100 mL titrating solution} - \text{g RS per 100 mL blank}]}{\text{g sample per 100 mL titrating solution}}$$

7.6 **Record** the result as % RS in product to

Juices 0.01%

Mill products 0.1%

## 8.0 PRECISION

| Sample | Concentration range (percent reducing sugar) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Molasses | 5-20 | 0.30 | 1.40 |

# METHOD 22

## TOTAL SUGARS (AFTER INVERSION) — DETERMINATION IN MOLASSES AND CANE INVERT BY THE CONSTANT VOLUME MODIFICATION OF THE LANE AND EYNON METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian Sugar Industry. It is the method specified under the Australian Molasses Pool contract and is based on the method published by the United Molasses Co. Ltd., London, (U.M.C. Method).

**2.0 PRINCIPLE OF METHOD**

The test sample is diluted to a fixed concentration and sucrose in the sample hydrolysed (inverted) with hydrochloric acid at 60 °C under carefully controlled conditions. The total reducing sugars in the inverted solution are determined by the constant volume modification of the Lane and Eynon titration. EDTA solution is added to the inverted solution to suppress interference from calcium.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Bulb pipette,** Class A (AS 2166) — 5 mL, 20 mL, 25 mL, 50 mL

3.2 **Burette,** 50 mL **Class** A, (AS 2165) graduated in 0.1 mL for the sugar solution. The burette tap is replaced by a rubber tube and pinch-cock and connected to a bent outlet tube in order to keep the graduated section of the burette out of the steam while additions are made to the boiling mixture.

3.3 **Heat resistant plate** (about 15 cm x 15 cm) with a 5 cm diameter hole.

3.4 **Anti-bumping granules.**

3.5 **Boiling flasks** (florence flask) flat bottom, 250 mL.

3.6 **Volumetric flask,** A-class (AS 2164) — 100 mL, 200 mL, 250 mL, 1000 mL

3.7 **Stop watch** with 60 second sweep.

3.8 **Bunsen burner.**

3.9 **Water bath,** maintained at 60 ± 1°C.

**4.0 REAGENTS**

Unless otherwise specified use only analytical grade reagents and only distilled water.

4.1 **Fehling's Solution A.** A solution of 69.28 ± 0.05 g of $CuSO_4.5H_2O$ in 1 litre of water.

4.2 **Fehling's Solution B.** Dissolve 346 ± 1 g sodium potassium tartrate in distilled water. Add that volume of standardised 5 mol/L sodium hydroxide solution which contains 100.0 ± 0.5 g NaOH. Dilute the solution to 1 litre.

*Safety*

This reagent is strongly caustic. It should not be pipetted by mouth. If in contact with skin, remove all contaminated clothing and wash the affected area with copious quantities of water. If ingested administer large quantities of 1% acetic acid. Do not induce vomiting.

4.3 **Methylene Blue Solution** — 1 g/100 mL solution in water.

4.4 **Standard Invert** — 10 g/litre solution.

4.4.1 Weigh 9.500 ± .001 g of sucrose and transfer quantitatively to 1L volumetric flask with 100 ± 5 mL of water. Swirl to dissolve.

4.4.2 While swirling gently, add 5 mL of hydrochloric acid, $\rho_{20}$ = 1.18 g/mL. Cover the flask mouth with a small beaker and stand for 3-4 days at 20-25 °C for complete inversion of the sucrose.

4.4.3 Dilute the invert solution to about 800 mL with water. Dissolve 2.0 ± 0.1 g benzoic acid in hot water, cool and add to the invert solution. Dilute to exactly 1L and mix well. Stored in a stoppered bottle, this solution will keep for several weeks.

4.5 **Neutral Standard Invert,** 0.25 g/100 mL Solution

Pipette 50 mL of 1% standard invert solution (4.4) into a 200 mL volumetric flask. Add 2 drops phenolphthalein solution and while swirling gently add 1 mol/L NaOH solution from a burette until the solution is just pink. Approximately 2.5 mL of 1 mol/L NaOH will be needed.

Add 1-2 drops of 0.5 mol/L HCl to just discharge the pink colour. Dilute the solution to exactly 200 mL with water and mix well.

4.6 **Sodium Hydroxide,** approx. 2 mol/L solution.

4.7 **Hydrochloric Acid,** approx. 0.5 mol/L solution.

4.8 **Phenolphthalein,** 1 g/100 mL solution in alcohol.

4.9 **Hydrochloric Acid,** 6.34 mol/L solution $\rho_{20}$ = 1.103 g/mL. Dilute 630 ± 5 mL of hydrochloric acid, $\rho_{20}$ = 1.18 g/mL, to 1L with water and adjust to exactly 6.34 mol/L after titrating 5 mL with 1 mol/L NaOH using methyl orange indicator.

4.10 **Di-Sodium Salt of EDTA,** 4 g/100 mL solution.

**5.0 STANDARDISATION OF FEHLING'S SOLUTION A**

5.1 Using Class A pipettes with safety bulb, pipette 50 mL of Fehling's A (4.1) followed by 50 mL of Fehling's B (4.2) into a clean, dry beaker. Mix by swirling.

5.2 Pipette 20 mL of the mixed Fehling's solution into a boiling flask. Add 15 mL water from the burette.

5.3 Rinse and then fill the burette (3.2) with standard invert solution (4.4). Add 39 mL of standard invert to the boiling flask, giving a total volume of 74 mL. Add a few pieces of anti-bumping granules.

5.4 Place the flask on a heat resistant plate (3.3) over the bunsen burner. Bring the solution to the boil and adjust the flame to give even moderate boiling.

5.5 Start the stop watch when the solution begins to boil and allow to boil for exactly 2 minutes. Add 4 drops of methylene blue solution and continue to titrate the boiling solution with standard invert from the burette.

5.6 Add a few drops at a time, allowing 5-10 secs between each addition, until the blue colour is completely discharged. The total boiling time should be 3 min. ± 5 secs and boiling should not be interrupted during the titration (to prevent air entering the flask and oxidising the contents).

Record the total titre to 0.05 mL.

5.7 If the Fehling's solution is the correct strength then 20 mL of this solution should require 40 mL of standard invert solution giving a total solution volume of 75 mL before commencing boiling.

5.8 If the titre is less than 40 mL then the Fehling's A solution is deficient in copper. Therefore add $\left[1 - \frac{\text{Titre}}{40.0}\right] \times 69.28$ g $CuSO_4.5H_2O$ to each litre of Fehling's A.
Dissolve 105% of the calculated amount in the solution and titrate again. In this way the strength of the Fehling's A will finally be adjusted by dilution, the simpler procedure.

5.9 If the titre is more than 40 mL the Fehling's A is too strong and each titre must be diluted by an amount equal to $\left[\frac{\text{Titre}}{40.0} - 1\right] \times 100$ mL water.
Add 95% of the calculated volume of water and titrate again.

5.10 When the Fehling's A solution is the correct strength 20 mL of mixed Fehling's solution (5.1) is equivalent to 100 mg of invert sugar.
If the titre is between 39.90 and 40.10 mL then a correction factor can be calculated and applied —

$$F = \frac{\text{standard titre}}{40.0}$$

## 6.0 PROCEDURE

6.1 Prepare a 5% solution of molasses or cane invert. Weigh 10.00 ± 0.01 g of the sample into a 250 mL beaker. Dissolve in approximately 50 mL of warm water (cold water for cane invert), cool and transfer quantitatively to a 200 mL volumetric flask. Dilute to exactly 200 mL with water and mix well.

6.2 Prepare a 0.5% solution by pipetting with a Class A pipette, 25 mL of the 5% solution into a 250 mL volumetric flask.

6.3 Add, while swirling the solution, 5.00 ± 0.05 mL of 6.34 mol/L HCl (4.9) using an automatic dispenser.

6.4 Immerse the flask in the water bath at 60°C. Swirl gently for 3-4 minutes to raise the temperature of the solution as quickly as possible. Leave the flask in the bath for a total of 15 minutes.

6.5 Remove the flask from the hot water bath and cool rapidly under running cold water.

6.6 Add 95-100 mL water and 3 drops of phenolphthalein solution. Add sufficient 2 mol/L NaOH solution till the solution is just pink and then discharge the colour by adding a few drops of 0.5 mol/L HCl.

6.7 Add 4.0 mL EDTA solution (4.10) mix well and dilute to volume with water. Mix well.

PRELIMINARY TITRATION

A preliminary titration is made to find the volume of water to be added to 20 mL of mixed Fehling's solution to obtain a final total volume of 75 mL at the end-point of the titration.

6.8 Pipette with a Class A pipette, 20 mL of mixed Fehling's solution (5.1) into a boiling flask.

6.9 Rinse and then fill the burette (3.2) with the inverted test solution (6.7). Add 25 mL of the solution to the boiling flask. Add a few pieces of anti-bumping granules and 1 drop paraffin oil to prevent frothing.

6.10 Place the flask on a heat resistant plate (3.3) over the bunsen burner. Bring the solution to the boil and adjust the flame to give even moderate boiling.

6.11 Start the stop watch when the solution begins to boil and allow to boil for exactly 2 minutes. Add 4 drops of methylene blue solution and continue to titrate with the test solution from the burette.

6.12 Add a few drops at a time, allowing 5-10 secs. between each addition, until the blue colour is completely discharged. The total boiling time should be 3 mins. ± 5 secs. Record the titre to 0.05 mL.

TITRATION

6.13 Carry out a further titration but this time add to the boiling flask —
- 20 mL of mixed Fehling's solution
- the volume of test solution from the first titration (6.12) less 1 mL
- sufficient water, from a burette to give a total volume of 74 mL

Carry out the titration as in steps 6.10-6.12. Record the titre to 0.05 mL.

6.14 Carry out a third titration, repeating step 6.13. The two titrations (6.13 and 6.14) must agree to within 0.2 mL or the titrations must be repeated.

## 7.0 CALCULATIONS

**Calculate** the per cent total sugars (as invert) in the sample —

$$\%\ \text{Total Sugars} = \frac{1000 \times F}{C \times T}$$

where: T = the mean titre (6.14)
C = concentration of the sample (6.2), usually 0.5 g/100 mL
F = standardisation factor (5.10)

## 7.0 PRECISION

The expected range of results is:

| | |
|---|---|
| Molasses | 45-60% Total Sugars |
| Cane Invert | 78-81% Total Sugars |

The precision of the method has not been determined.

# METHOD 23

## SUPERSATURATION AND SATURATION CONDITIONS — DETERMINATION IN PAN PRODUCTS

### 1.0 DEFINITIONS

The determination of the degree of supersaturation of molasses is of considerable importance in the study of pan boiling and crystallization. The explanation of the theory associated with the determination of coefficient of supersaturation involves the use of terms which are defined as follows:

(a) Concentration is the percentage ratio by mass of solute to solvent in the solution under consideration. For molasses this corresponds to the percentage ratio of sucrose to water and must be determined analytically.

$$\text{Concentration} = \frac{\%\ \text{sucrose in molasses}}{100 - \%\ \text{dry substance}} \times 100$$

(b) Saturation is the condition in which the quantity of solute (sucrose) is the maximum that can be dissolved in the solvent (water). When less than this quantity is dissolved, the solution is referred to as unsaturated (or undersaturated). If water is evaporated from a saturated solution or a saturated solution is cooled, then more sucrose is in solution than would exist at equilibrium and the solution is referred to as supersaturated. Although the condition of supersaturation is thermodynamically unstable, the tendency to revert to the equilibrium saturated condition by crystallization is sometimes very feeble.

(c) Solubility is the concentration of solute in the solvent at saturation. The solubility of sucrose in water increases with increasing temperature in a non-linear relationship which has been determined experimentally. There are various ways of expressing the solubility, with grams of sucrose per 100 grams of water (Table XIII) being the most appropriate for the calculation of supersaturation coefficient. The solubility of sucrose in water is also influenced by the presence of soluble impurities, the effect varying with the quantity and type of impurity. Some impurities give sucrose an enhanced solubility while others cause solubility depression. The overall effect of the impurities present in cane molasses is usually to lower the solubility of sucrose.

(d) Solubility coefficient is the ratio of the solubility of sucrose in the impure liquid phase of a sample to the solubility of sucrose in pure water, at the same temperature. As such, it quantifies the overall effect of the soluble impurities on the sucrose solubility, as discussed above.

$$\text{S.C.} = \frac{\text{S/W ratio in molasses saturated at T°C}}{\text{S/W ratio in pure water saturated at T°C}}$$
$$= \text{f(T,I/W, impurity constituents and concentrations)}$$

where:

I/W = mass ratio of impurities to water in the molasses

(e) Supersaturation coefficient, usually abbreviated to supersaturation or S.S., is the ratio of the actual sucrose concentration in a sample to the solubility of sucrose in the liquid phase in the sample, at the same temperature and the same impurity to water ratio (I/W).

$$\text{S.S.} = \frac{\text{actual S/W ratio in the molasses}}{\text{S/W ratio in saturated molasses at the same T and I/W ratio.}}$$
$$= \frac{\text{actual S/W ratio in molasses at T °C}}{\text{S/W ratio in saturated pure water at T °C}} \times \text{S.C.}$$

where S.C. is determined for the molasses at the same T and I/W ratio.

The supersaturation coefficient is the fundamental driving force for crystal growth and accordingly is very important in any crystallization studies. However, the determination of S.S. is difficult and some approaches are inappropriate. For example, stirring a supersaturated crystal sucrose-molasses mixture at constant temperature will not achieve a saturated condition by crystallization, in a finite time. This results from the fact that, although growth rate is linearly related to supersaturation, there exists a null zone at low supersaturation values in which crystal growth rates are extremely low. Thus any attempts to achieve a saturated condition must approach from an unsaturated condition, i.e. by dissolution, for which no such null zone exists.

### 2.0 MEASUREMENT OF SATURATION CONDITIONS

The measurement of the saturation conditions of a molasses sample is generally undertaken using one of two available methods.

1. The first is the saturation cell method in which the molasses sample under test, pre-mixed with fine seed crystal, is heated until dissolution commences. The temperature at which this occurs, called the saturation temperature, is noted. Originally the onset of dissolution was observed through a microscope (saturascope apparatus). The technique has been automated to some extent by the Sugar Research Institute by incorporating a photocell to monitor the change in light transmission that occurs at the start of dissolution. At present this version of the saturation temperature apparatus is not available commercially.

   The saturation cell method provides a measure of the saturation temperature and from this, and

the analysis of the test sample, the solubility coefficient at the saturation temperature can be estimated. This is useful for many crystallization applications but is less suitable for fundamental research into the solubility of molasses where saturation measurements at different temperatures are required.

2. The second is the equilibration method where the test molasses is brought to saturation by tumbling at constant temperature from an initially undersaturated condition in the presence of excess sugar crystals. At the end of the equilibration time, the remaining crystals are observed under a microscope to check that the edges have been rounded, confirming that an initial undersaturated condition existed. A sample of the equilibrated molasses, obtained by pressure filtration, is analysed, allowing the solubility coefficient to be determined. Unfortunately, the application of this technique is limited to an impurity/water ratio of about 3.0 or less because of the tendency of molasses to degrade (and gasify) if held for extended periods above about 65 °C.

## 3.0 CALCULATION OF SUPERSATURATION

The case of a supersaturated solution of sucrose in pure water is very simple. If the solution has a temperature $T_1$ and on heating is found to be saturated at $T_2$, then the actual concentration of sucrose present is that corresponding to the solubility of sucrose at $T_2$ which can be obtained from appropriate tables. The concentration of sucrose required to saturate the solution at its initial temperature $T_1$ is merely its solubility at $T_1$ from the same table.

$$\text{Thus S.S. at } T_1 = \frac{\text{sucrose solubility at } T_2}{\text{sucrose solubility at } T_1}$$

For impure solutions such as syrups and molasses, the situation is complicated by the effect that the impurities have on the sucrose solubility. Consider a supersaturated molasses sample at temperature $T_1$. Then:

$$\text{S/W actual} = \text{sucrose solubility at } T_1 \times \text{S.C. at } T_1 \times \text{S.S.}$$

If this material is heated to its saturation temperature $T_2$, then the concentration is unchanged but the supersaturation has dropped to unity. Therefore at $T_2$:

$$\text{S/W actual} = \text{sucrose solubility at } T_2 \times \text{S.C. at } T_2 \times 1$$

Equating these and rearranging —

$$\text{S.S. at } T_1 = \frac{\text{sucrose solubility at } T_2 \times \text{S.C. at } T_2}{\text{sucrose solubility at } T_1 \times \text{S.C. at } T_1}$$

where the solubility coefficients at the two temperatures are determined at the same I/W ratio. Generally it is difficult to determine the supersaturation coefficient accurately because the values of the solubility coefficient are unknown and would require determinations by the equilibration method. As a first approximation, depending on the range of temperatures involved, it may be feasible to assume the solubility coefficients at the two temperatures are equal. This simplifying assumption then gives:

$$\text{S.S. at } T_1 = \frac{\text{sucrose solubility at } T_2}{\text{sucrose solubility at } T_1}$$

However, for cane molasses, the solubility coefficient is not independent of temperature, so care is required as the simplifying assumption made above may give rise to serious errors.

# METHOD 24
## VISCOSITY DETERMINATION IN PAN PRODUCTS

### 1.0 GENERAL

Molasses and massecuites generally exhibit non-Newtonian flow properties with the viscosity of these materials decreasing as higher rates of shear are applied. This is known as pseudoplastic flow behaviour.

Except for routine comparative analysis where a consistent rate of shear is encountered, it is not sufficient to define a single viscosity value for such materials but the viscosity must be determined over the shear rate range of interest for the process.

### 2.0 MODEL OF RHEOLOGICAL BEHAVIOUR

The flow behaviour of a material under a shear force is generally presented as a plot of shear stress against shear rate (flow curve). For Newtonian fluids, a constant proportionality exists between the shear stress $\tau$ and the shear rate $\gamma$ in laminar flow viz.

$$\tau = \mu\gamma$$

where $\mu$ = viscosity of the fluid (S.I. units, Pa.s)

For non-Newtonian materials the velocity gradient changes shape at the interface of the fluid and the shear inducing element so that the viscosity is no longer constant but depends on the shear stress/shear rate. An absolute measurement of the viscosity of the non-Newtonian solution requires that a correction for the change in shear rate is made.

$$\mu = \tau/\gamma_{corr}$$

where $\mu$ = viscosity (absolute viscosity)
$\tau$ = true shear stress at the interface
$\gamma_{corr}$ = corrected shear rate at the interface.

If no correction is made for the change in the shear rate profile due to the non-Newtonian behaviour, an apparent viscosity ($\mu_{app}$) is determined:

$$\mu_{app} = \frac{\tau}{\gamma_{uncorr}}$$

where $\gamma_{uncorr}$ = shear rate induced in a Newtonian fluid for the same relative motion (rotational speed, average velocity).

For measurements in simple commercial viscometers, this is the viscosity value determined using the supplied calibration factors.

In the usual practical range of shear rate for pipeline flow in factories, massecuites and molasses generally show pseudoplastic flow behavious. In common with other such materials, a "Power Law" model usually adequately describes their non-Newtonian behaviour, i.e.

$$t = K\gamma_{corr}^{n}$$

and $$\mu = \tau/\gamma_{corr} = K\gamma_{corr}^{n-1}$$

where K is the consistency; the higher the value of K the more viscous the material (S.I. units $Ns^{-n}m^{-2}$)

and n is the flow behaviour index; a measure of the degree of the non-Newtonian behaviour. For pseudoplastic fluids, n lies between zero and unity with values further removed from unity indicating a more pronounced non-Newtonian behaviour.

Thus, for such materials, the consistency and flow behaviour index should be specified rather than defining a single viscosity value. Figure 1 shows a typical viscosity versus shear rate relationship for a pseudoplastic material.

Because the flow behaviour index may vary over a wide range of shear rate then, whenever possible, the viscosity determinations should be made at the shear rate range of interest for the process. If extrapolation of data to a different shear range is necessary for design or process calculations, care should be exercised that significant errors are not introduced.

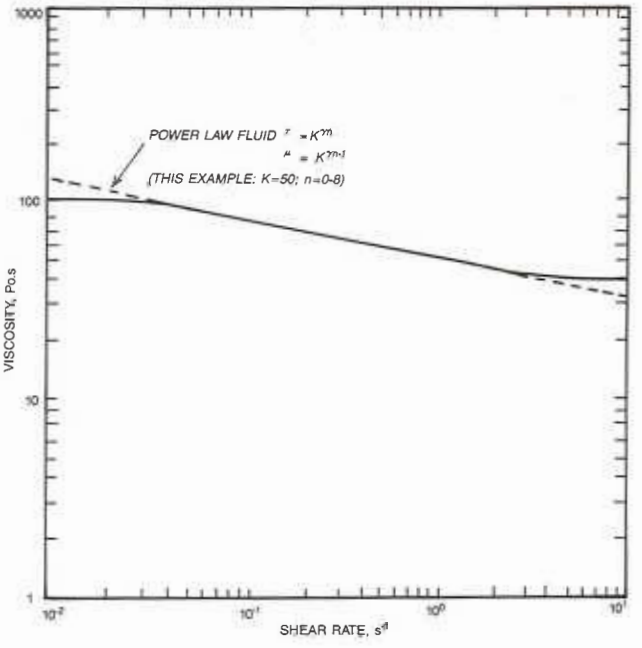



Figure 1. Viscosity as a Function of Shear Rate for a Material showing Pseudoplastic Flow Behaviour.

### 3.0 VISCOSITY MEASUREMENTS

To adequately measure the flow behaviour of molasses or massecuites, a viscometer is required in which the actual shear stress and shear rate can be calculated and with which operation over a range of shear rates is possible.

Two viscometers that provide a well defined shear field and are therefore suitable for this purpose are the rotational viscometer and the pipeline viscometer. Both of these types of viscometer have proved useful for rheological studies of pan products.

### 4.0 ROTATIONAL VISCOMETER

With the rotational viscometer, a controlled speed electric motor is used to apply a known rate of rotation

to the measuring element and the torque resulting from viscous drag on the element is measured. By providing the instrument with a range of rotational speeds, measurements may be made over a range of shear rates. Measuring geometries which are suitable for use in non-Newtonian fluids include the coaxial cylinder, the cone-and-plate, and the parallel plate design. The shear stress can be calculated from the measured torque and the dimensions of the rotating element and is independent of whether the sample is a Newtonian or non-Newtonian fluid. The equation for the shear rate is a function of the geometry of the measuring elements and does depend on the nature of the flow behaviour of the fluid. From the measurement of these quantities the consistency and the flow index for a Power Law fluid can be determined.

Several commercial designs of rotational viscometers are available from instrument suppliers.

## 5.0 PIPELINE VISCOMETER

With the efflux type pipeline viscometer, the sample is maintained in a reservoir and gas pressure is applied to drive the material through a horizontal or vertical tube of accurately known dimensions. A schematic arrangement of a pipeline viscometer is shown in Figure 2.

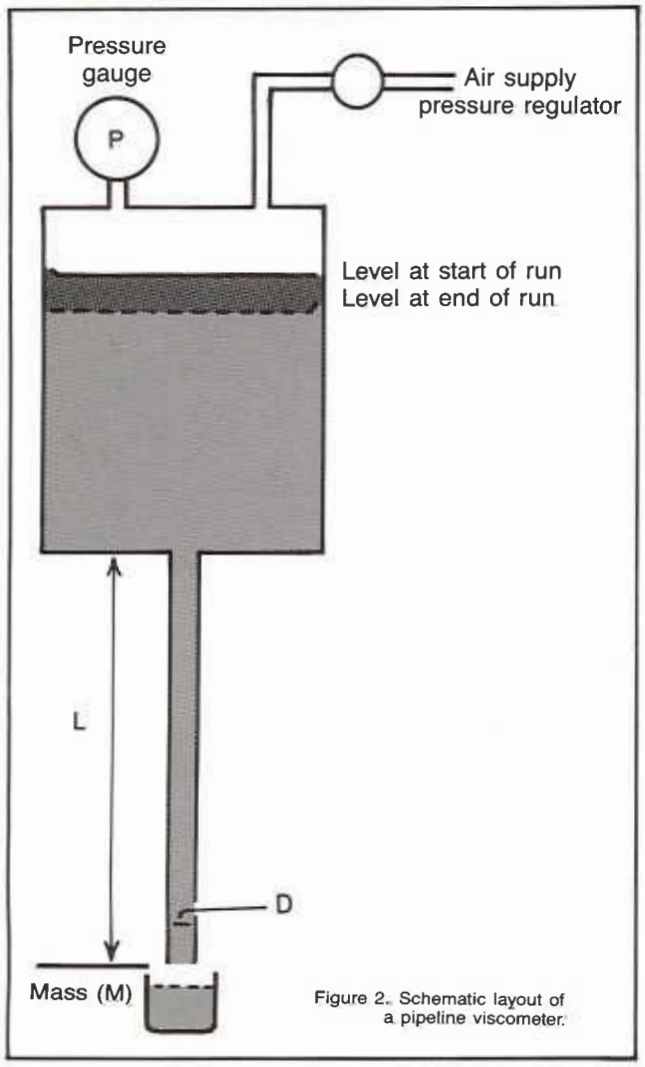



Figure 2. Schematic layout of a pipeline viscometer.

A series of measurements of the efflux rate of the material from the tube at different applied pressures allows measurements of the wall shear stress to be made over a range of shear rates. Usually between five and seven measurements in the shear rate range of interest are obtained on a single sample.

The true shear stress and shear rate at the wall of the tube can be calculated from the total pressure differential, the tube dimensions, and the average velocity of the fluid through the tube and from these the consistency and flow index for a "Power Law" fluid can be determines.

Systematic errors can be introduced through end and wall effects unless care is taken with the design of the viscometer. An instrument of a standardized design with specified dimensions to minimize such errors is required. For this, the Sugar Research Institute and BSES have jointly developed a 'standard' design of pipeline viscometer recommended for use in Australian sugar mills.

Notes on the construction, test procedure and data analysis are available from either organization.

## 6.0 VISCOSITY OF MOLASSES AND MASSECUITE

The rotating cylinder method has been adopted by ICUMSA as an Official Method for determining the viscosity of molasses. Various types of rotating cylinder viscometers are available and among these the coaxial cylinder viscometers are well suited. These are particularly useful for determinations when only small volumes of samples are available, e.g. as obtained from pressure filtration of massecuites. The pipeline viscometer has also been adopted by ICUMSA as an alternative technique for measuring the viscosity of molasses.

When viscosity determinations on molasses are undertaken, pertinent variables such as temperature, concentration, purity and presence of entrapped gases should be recorded.

The measurement of the flow behaviour of massecuite is more complex than that of molasses due to the effects of the solid phase. The pipeline method is being assessed by ICUMSA as a suitable technique for determining the rheological properties of massecuites. At present it is regarded as the most appropriate method for a detailed assessment of massecuite viscosities. Unfortunately, the technique is time consuming (e.g. one to two hours per sample) and for greater convenience and speed of measurement the rotational cylinder viscometer is useful for comparative assessments. This version of the rotational viscometer comprises a cylinder of small diameter rotating in a large volume of sample, e.g. 600 mL low form beaker. Besides the properties of the mother molasses, the massecuite viscosity is influenced by the crystal content and to a lesser extent by the mean size and spread of the crystal population.

# METHOD 25
## FILTRABILITY — DETERMINATION IN PRODUCTS OTHER THAN RAW SUGAR

**1.0 SCOPE AND FIELD OF APPLICATION**

This method can be used to provide comparisons between similar factory products and can be used to assist in factory process control and trouble-shooting.

**2.0 DEFINITION**

The filtrability of a product is the ratio of the filtration rate of a solution of the product, adjusted by the addition of refined sugar to approximately raw sugar purity, to that of a solution of pure sugar of the same concentration, filtered under identical conditions.

**3.0 PRINCIPLE OF THE METHOD**

The method is very similar to that used for determination of the filtrability of raw sugars.

A solution of the product is made up to 60.0 ± 0.5 °bx and a purity similar to that of raw sugars by the addition of water and refined sugar. Celite 505 and buffer are added to this solution to bring the pH to between 8.5 and 9.1. The solution is filtered under constant pressure. Filtrate is initially run to waste for two minutes, to allow a uniform Celite filter cake to form. The filtrate is then collected over a precisely timed five minute period, weighed and expressed as a percentage of the weight of pure sucrose solution which would be collected under identical conditions of density, pH, temperature and pressure.

The per cent filtrability is defined at 20 ± 1°C. At temperatures above 20°C, a lower filtrability is obtained and at temperatures below 20°C the filtrability is higher. If a test is done at a temperature other than 20 ± 1°C, the result can be expressed only as "filtrability at t°C".

**4.0 APPARATUS**

Ordinary laboratory glassware and apparatus as for Method No. 36, "Filtrability of Raw Sugar by the Celite Method".

**5.0 REAGENTS**

5.1 Standard Filter Aid — Celite 505 standardised at CSR Central Laboratory.

5.2 Filtrability Buffer

5.2.1 Prepare 1L of 50% (m/m) solution of glycerol in water.

5.2.2 Dissolve 15.0 ± 0.1 g of calcium acetate in 200-300 mL of 50% glycerol solution in a 500 mL beaker.

5.2.3 Dissolve 400.0 ± 0.5 g of diethanolamine in 200-300 mL of 50% glycerol solution in a 500 mL beaker.

5.2.4 Transfer the calcium acetate solution and the diethanolamine solution to a 1L volumetric flask, rinsing both beakers with 50% glycerol solution.

5.2.5 Make up to 1L with 50% glycerol solution, mix well and allow to stand overnight. Add a teaspoon of Celite 505 and filter in the pressure filter through a No. 54 filter paper. The filtered solution can be stored for 6 months.

5.2.6 The pH of the test solution should be greater than 10.5.

*Safety*

Diethanolamine is a mild eye and skin irritant and wearing safety glasses and rubber gloves is recommended.

5.3 Sucrose or high purity refined sugar.

5.4 Filter paper — Whatman Grade 54, 5.5 cm diameter. The filter papers are selected from the one 'making' and should have a mean Gurley time of 7.6 seconds (air flow measured by Gurley Densometer). Suitable selected Whatman 54 filter papers can be obtained from CSR Limited, Central Laboratory.

**6.0 STANDARDISATION OF CELITE 505 FILTER AID**

The Celite 505 to be used for the test is standardised at Central Laboratory. With each batch of standardised Celite 505, Central Laboratory issues a reference Table (No. XVII). The Table gives the weights of pure syrup, filtered between 2 and 7 minutes after application of pressure at various temperatures, under the standard condition of the test. Each Table is specific to a particular batch of Celite 505.

**7.0 PROCEDURE**

7.1 For samples of clarified juice, syrup and molasses, measure the brix of the solution (b).

7.2 Weigh out the appropriate quantity of sample as shown in Table I.

7.3 Weigh out and add the amounts of refined sugar and water obtained from Table I.

**TABLE I**
**QUANTITIES REQUIRED FOR CELITE FILTRABILITY OF FACTORY PRODUCTS**

| Product | Sample Mass (g) | Refined Sugar (g) | Water (g) | Celite (g) | Buffer (mL) |
|---|---|---|---|---|---|
| ESJ | 200.0 | 5(60−b) | 0 | 0.03 (100−b)c | 3.2 |
| Syrup | $\frac{2440}{b}$ | 150.0 | $116.3 - \frac{24.4\,(100-b)}{b}$ | 1.744c | 1.5 |
| A & B Massecuites | 50.0 | 250.0 | 193.4 | 2.95 c | 3.5 |
| A & B Molasses | $\frac{1300}{b}$ | 350.0 | $242 - 13\left(\frac{100-b}{b}\right)$ | 3.63 c | 2.8 |
| Magma | 50.0 | 250.0 | 186.7 | 2.92 c | 2.5 |
| C Sugar | 50.0 | 250.0 | 198 | 3.0 c | 2.2 |

NOTE: b = brix of sample
c = addition rate of Celite (% on solids)

7.4 From Table XVII, obtain the addition rate of the batch of Celite 505 in current use (C% on solids). Weigh out and add the amount of Celite shown in Table I.

7.5 Dissolve the sugar without heating, using an electric stirrer at 900 ± 100 r/min.

7.6 With the stirrer still going, add the appropriate volume of special buffer to the solution down the side of the beaker (see Table I). Mix for 2 minutes ± 10 seconds after addition of all buffer. Switch the stirrer off and remove from the solution. Cover the beaker and allow to stand for 15 minutes ± 30 seconds.

NOTE: The buffer used for these products is *not* the same as used for raw sugars (see 5.2).

7.7 Filter the sample in the same manner as for raw sugars.

NOTE: For a guide to the precautions and procedure in the above steps refer to Method No. 36 "Filtrability — Determination in Raw Sugar by the Celite Method".

## 8.0 CALCULATIONS

8.1 Calculate the per cent filtrability of the sample as for raw sugars.

## 9.0 PRECISION

No information is available on the precision of the method. The results cannot be used to predict the filtrabilities of raw sugars made from the products or of other factory products. However, the information can give a useful guide to filtrability levels.

# METHOD 26
## ASH — DETERMINATION IN SUGAR PRODUCTS BY THE SINGLE SULPHATION METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This is a Level 1 method for use within the Australian sugar industry.

**2.0 DEFINITION**

Ash is inorganic matter. It is composed mainly of chlorides, sulphates, nitrates, carbonates and phosphates of K, Ca, Mg, Na, Fe and Al, as well as salts of numerous organic acids. Silica is also usually present.

**3.0 PRINCIPLE OF METHOD**

This is a gravimetric method. The sample is weighed into a platinum crucible, evaporated if necessary, and moistened by dropwise addition of concentrated sulphuric acid. Charring commences at this stage, and is accelerated by slow heating. The charred sample is ignited in a muffle furnace at a temperature of 525 ± 25°C.

The sulphation of the sugar prior to ignition assists in obtaining an ash that is free of carbon at the low ignition temperature used. After sulphation, the cations are recovered as sulphates together with phosphates and silicates.

The sulphates are less volatile and less hygroscopic. Because sulphated ash is heavier than the ash obtained by direct incineration, a 10% deduction is applied to sulphated ash determinations.

**4.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

Ordinary laboratory apparatus and

4.1 **Muffle furnace** capable of being controlled at 525 ± 25°C and having provision for a flow of air through the heated cavity.

4.2 **Platinum crucibles** of a suitable size to take the required quantity of sample in a layer about 10 mm thick.

4.3 **Automatic pipette** or burette for concentrated sulphuric acid.

4.4 **Aluminium or steel block**, approx. 150 mm x 150 mm x 25 mm.

4.5 **Tongs**, stainless steel, about 400 mm long with insulated handles.

4.6 **Wire**, platinum or stainless steel, approx. 100 mm long.

4.7 **Tweezers**, rubber tipped.

4.8 **Stainless steel tray** (approx. 150 mm x 150 mm x 40 mm) containing a ceramic tile and fitted with a lid.

4.9 **Soft brush**, approximately 5 mm diameter.

4.10 **Balance** to weigh to 0.0001 g.

4.11 **Fume cupboard.**

**5.0 REAGENTS**

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

5.1 **Concentrated Sulphuric Acid** $\rho_{20}$ = 1.84 g/mL.

This must be free of mineral impurities. Before use, each bottle of acid should be checked as follows:

With a measuring cylinder, transfer 25.0 ± 0.5 mL of the acid into a clean tared platinum crucible. Evaporate slowly to avoid spitting and then ignite the residue in the muffle furnace at 525 ± 25°C. The acid is suitable if the residue, on ignition, is less than 0.0025 g, i.e. 0.0001 g/mL.

*Safety*

Concentrated sulphuric acid is very corrosive. Safety glasses and gloves must be used when handling the acid. It must not be pipetted by mouth.

If in contact with skin, remove all contaminated clothing etc. and wash affected area with copious quantities of water.

If ingested, administer large quantities of aluminium hydroxide gel or milk of magnesia (magnesium hydroxide). Do not induce vomiting.

Steps 7.4 and 7.5 should be done behind a perspex safety shield and the analyst must wear eye protection, e.g. safety glasses.

**6.0 TREATMENT OF SAMPLE**

6.1 **Juice**. Allow juice samples to stand for 1 hour to allow suspended matter to settle and then decant.

**7.0 PROCEDURE**

7.1 Switch on the muffle furnace at least an hour before use and adjust the temperature to 525 ± 25°C.

7.2 Weigh into a clean dry platinum crucible the appropriate amount of sample as follows.

| | | |
|---|---|---|
| Cane Juice | : | 25.0 ± 0.5 g |
| Raw Sugar | : | 5.0 ± 0.5 g |
| Syrup/Liquor | : | 5 - 10 g |
| A, B and C molasses | : | 3.0 ± 0.5 g |

Record the mass of the crucible plus sample to 0.0001 g ($m_1$).

NOTE: Ash from juice, intermediate mill products and molasses is generally difficult to brush out of the platinum crucible after ignition and so for these products the empty platinum crucible is weighed to 0.0001 g and recorded ($m_3$).

7.3 **For juice samples only**. Evaporate the sample in the crucible, to dryness in an oven at about 100°C, and allow to cool before adding sulphuric acid.

7.4 **For all samples.** Moisten the sugar in the crucible with 2.0 ± 0.1 mL of concentrated sulphuric acid (5.1), adding slowly from a burette or automatic pipetter. Distribute the acid evenly over the sample. This operation, and the subsequent charring and incineration steps, should be carried out in a fume cupboard.

7.5 Place the crucible on a pipeclay triangle on a tripod, and heat slowly with a bunsen until charring is complete, i.e. no further swelling occurs. To prevent spillage during charring, tilt the crucible with tongs. Avoid impinging the reducing section of the flame onto the platinum crucible.

If gas burners are not available, adequate charring may be achieved by burning off at the mouth of the muffle furnace (or on a hot plate). Care should be taken to do this slowly to avoid spillage, as charring is sometimes violent.

7.6 Transfer the crucible and its contents to the muffle furnace using long tongs and incinerate for 75-90 minutes, or until all the carbon has been burnt. The temperature of the muffle should be 525 ± 25°C. If the temperature is above 550°C, the ash may fuse, and if below 500°C the time required for complete incineration is too long. The final ash should have an even white appearance with no dark areas of unburnt carbon.

7.7 Remove the crucible from the muffle and check for unburnt carbon by breaking up the ash residue with a platinum or stainless steel wire. If particles of carbon are present, replace the crucible in the muffle until completely incinerated.

Allow the platinum crucible to cool on a steel or aluminium block to room temperature, then place on a ceramic tile in a covered, stainless steel tray for fifteen minutes. Covering of the crucible is important to prevent loss of ash caused by draughts.

7.8 Using rubber tipped tweezers, transfer the crucible to the balance and weigh to 0.0001 g and record ($m_2$). Brush out the ash residue, and for all samples except juice, intermediate mill products and molasses weigh to 0.0001 g and record ($m_3$).

## 8.0 CALCULATIONS

Calculate the % ash in sugar or sugar product

$$\%\ \text{ash} = \frac{9}{10} \times \frac{\text{mass of ash residue}}{\text{mass of sample taken}} \times \frac{100}{1}$$

$$= 90 \times \frac{(m_2 - m_3)}{(m_1 - m_3)}$$

where: $m_1$ = wt. of crucible + sample (7.2)
$m_2$ = wt. of crucible + ash (7.8)
$m_3$ = wt. of empty crucible (7.2 or 7.8)

Report result as percent ash to 0.01 for sugars and juices and to 0.10 for mill products.

## 9.0 PRECISION

| Sample | Concentration Range % Ash (m/m) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 0.20 — 0.60 | 0.03 | 0.06 |
| 1st Expressed Juice | 0.3 — 0.6 | 0.30 | 0.50 |
| Molasses (wet basis) | 9 — 15 | 0.80 | 1.60 |

## 10.0 REFERENCES


10.1 Browne, C.A. and Zerban, EW.; Physical and Chemical Methods of Sugar Analysis; Ed. 3; Wiley (1948), p. 1021. Refer: Scheibler, C.; Rubenzuckerind; Vol. 14 (1864), p. 188.

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10.7 ICUMSA Methods Book (1994).

10.8 ICUMSA 1998 Report, Subject 16, Ash, Appendix 2, Collaborative Testing.

# METHOD 27

## ASH — DETERMINATION IN SUGAR PRODUCTS BY THE DOUBLE SULPHATION METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. It is based on the official ICUMSA method, except that the second incineration is done at 550°C instead of 650°. The method is applicable to all sugar products.

The determination of ash is used by sugar mills for process control purposes.

**2.0 PRINCIPLE OF METHOD**

This is a gravimetric method. Sulphuric acid is added to the test portion in a platinum crucible which is then heated gently to completely char the sample. The charred sample is incinerated in a furnace at 550°C until all organic matter is destroyed, cooled, further sulphuric acid added, followed by a second incineration at 550°. Ash is calculated as the percent loss of mass after the two incinerations, (without any deduction for the sulphation).

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Muffle Furnace** capable of being controlled at 550°C and having provision for a flow of air through the heated cavity.

3.2 **Platinum crucible** of a suitable size to take the required quantity of sample in a layer about 10 mm thick.

3.3 **Automatic pipette** or burette for sulphuric acid (4.1).

**4.0 REAGENTS**

4.1 **Sulphuric acid** $\rho_{20}$ = 1.84g/mL.

*Safety*

Concentrated sulphuric acid is very corrosive. Safety glasses and gloves must be used when handling the acid. It must not be pipetted by mouth. If in contact with skin, remove all contaminated clothing etc. and wash affected area with copious quantities of water. If ingested, administer large quantities of aluminium hydroxide gel or milk of magnesia (magnesium hydroxide). Do not induce vomitting.

**5.0 TREATMENT OF SAMPLE**

5.1 **Juice.** Stand samples for 1 hour to allow suspended matter to settle and then decant.

**6.0 PROCEDURE**

6.1 Weigh a clean dry platinum crucible to 0.0001 g and record the mass ($m_1$). Weigh the appropriate amount of sample into the crucible.

| | |
|---|---|
| Cane Juice | : 30.0 ± 0.5 g |
| Raw Sugar | : 5.0 ± 0.5 g |
| Liquor | : 5.0 ± 0.5 g |
| Syrup and Molasses | : 3.0 ± 0.2 g |

Record the mass of the crucible plus sample to 0.0001 g ($m_2$).

6.2 **For juice samples only.** Evaporate the sample in the crucible, to near dryness in an oven at about 95°C, before adding sulphuric acid.

6.3 **For all samples.** Add 1.0 mL of sulphuric acid, dropwise over the total surface of the sample. This operation, and the subsequent charring and incineration steps should be carried out in a fume cupboard. The analyst should wear safety glasses.

6.4 Heat the crucible slowly on a hot plate or over a bunsen burner until the test portion is completely charred.

6.5 Transfer the crucible and its contents to the muffle furnace and incinerate at 550°C until no trace of unburnt carbon is visible. This usually takes 2-3 hours.

6.6 Remove the crucible from the muffle and allow to cool.

6.7 Add 1.0 mL of sulphuric acid to wet the residue, allow this to evaporate at the mouth of the muffle furnace and incinerate at 550°C for 2 hours (or to constant weight).

6.8 Remove the platinum crucible from the muffle and place in a desiccator and allow to cool to room temperature.

6.9 Weigh the crucible plus ash to 0.0001 g and record the mass ($m_3$).

**7.0 CALCULATIONS**

Calculate the % ash in sugar

$$\%\ \text{ash} = \frac{\text{mass of ash residue}}{\text{mass of sample taken}} \times \frac{100}{1}$$

$$\frac{(m_3 - m_1)}{(m_2 - m_1)} \times \frac{100}{1}$$

where: $m_1$ = mass of empty crubicle (6.1)

$m_2$ = mass of crucible + sample (6.1)

$m_3$ = mass of crucible + ash (6.9)

Report result as per cent ash to 0.01% for sugars and juices and to 0.1% for mill syrups and molasses.

**8.0 PRECISION**

| Sample | Concentration Range % Ash | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 0.20-0.60 | 0.03 | 0.06 |
| 1st Expressed Juice | 0.3-0.6 | 0.30 | 0.50 |
| Molasses (wet basis) | 9-15 | 0.80 | 1.60 |

**9.0 REFERENCES**

ICUMSA, Proceedings of Eighteenth Session, 1982; Subject 16.

# METHOD 28
## DEXTRAN — DETERMINATION IN RAW SUGAR AND SUGAR PRODUCTS BY THE ALCOHOL HAZE METHOD

### 1.0 SCOPE AND FIELD OF APPLICATION

This Level 2 method has been adopted by the Australian sugar industry. Dextran, for the purpose of this analysis, is a high molecular weight and predominantly straight chained glucose polymer with a majority of $\alpha$1-6 glucosidic linkages formed by the action of certain species of bacteria on sucrose during cane and juice storage.

High dextran concentrations in juice or raw sugar cause severe processing problems in mills and refineries.

This method is applicable to cane juice, raw sugar and process materials of 80 purity or greater.

### 2.0 PRINCIPLE OF METHOD

The test sample is dissolved in water. Soluble starch is destroyed by incubation with a suitable enzyme. Inorganic salts are removed by treatment with mixed ion-exchange resins. Protein is removed by precipitation with trichloroacetic acid followed by filtration with acid washed Supercel.

The dextran haze is produced by diluting an aliquot of the treated, filtered solution, to twice the aliquot volume by the addition of ethanol. The turbidity of the dextran haze is measured by reading in a spectrophotometer at a wavelength of 720 nm.

The method is standardised against Pharmacia Dextran T500.

### 3.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

Ordinary laboratory apparatus and glassware and

3.1 **Spectrophotometer** suitable for the measurement of absorbance at 720 nm with matched 2 cm cells. The spectrophotometer should comply with the following specifications:

- spectral band pass 10 nm or less
- wavelength reproducibility ± 0.5 nm
- absorbance reproducibility ± 0.003 at 1.0 abs

3.2 **Water bath** capable of operating at 85-95°C.

3.3 **Flask shaker.**

3.4 **Balance**, to weigh to 0.0001 g.

3.5 **Stopwatch**.

3.6 **Volumetric flasks**, Class A (AS 2164) — 100 mL, 25 mL.

3.7 **Bulb pipettes**, Class A (AS 2166) — 1 mL, 2 mL, 5 mL, 10 mL.

3.8 **Graduated** straight pipettes, Class A (AS 2167) — 2 mL, 5 mL, 10 mL.

3.9 **Measuring cylinder**, 100 mL with plastic stopper.

3.10 **Funnel** fitted with 150 $\mu$m mesh screen to fit into measuring cylinder.

3.11 **Automatic dispenser** or safety pipette, 10 mL.

3.12 **Buchner funnel**, 5.5 cm and 250 mL Buchner flask.

3.13 **Burette**, 50 mL capacity or suitable automatic burette.

### 4.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

4.1 **Standard dextran**, 1 mg/mL solution. Use Pharmacia Dextran T500. Determine its moisture content, in duplicate, correct to 3 decimal places by drying approximately 2 g of the solid at 105°C in an oven for three hours. Record the weights to 0.0001 g. Individual determinations must be within 5% of the mean of the measured value.

Weigh a quantity of the undried dextran that contains 0.1000 ± 0.0001 g of anhydrous dextran, ie

$$\text{weigh out } \frac{0.1000 \times 100}{(100 - \%\text{ water in dextran})}$$

of undried dextran into a 100 mL beaker.

Dissolve the dextran by adding 1-2 mL water to form a slurry. Allow the particles to become uniformly hydrated by standing and occasionally stirring. Add water gradually to avoid having an excess present while gel masses remain. When about 25 mL has been added and no gel is present, wash the slurry into a 100 mL flask with water to a volume of about 80 mL. Place the flask in a boiling water bath for 30 min. Cool to room temperature and make to the mark (100 mL) with water. Prepare standard dextran solution daily. Do not store overnight.

4.2 **Trichloroacetic acid (TCA)**, 10% (w/v). Dissolve 10.0 ± 0.1 g of trichloroacetic acid in distilled water and dilute to 100 mL in a volumetric flask. This reagent will keep for two weeks, stored under refrigeration in a dark brown bottle.

*Safety*

Trichloroacetic acid at this concentration attacks protein and should not be allowed to come in contact with any part of the body, particularly the tongue. Under no circumstances pipette TCA by mouth. Do not store TCA in plastics. If in contact with skin, remove all contaminated clothing etc. and wash affected area with copious quantities of water. Safety glasses and gloves must be worn while transferring the solid chemical, dissolving it or pipetting the solution. Label all containers and solutions of trichloroacetic acid with "CAUSES SEVERE BURNS" and "SERIOUS RISK OF POISONING BY INHALATION, SWALLOWING OR SKIN CONTACT".

If ingested, administer large quantities of aluminium hydroxide gel or milk of magnesia (magnesium hydroxide). Do not induce vomiting.

4.3 **Denatured absolute alcohol**. Store in an airtight container.

*Safety*

Alcohol is highly flammable. The methanol used for denaturing adds substantially to its toxicity. Avoid breathing its vapours. It reacts with varying degrees of violence with a wide range of oxidants. DAA's flash point is in the range 10-12°C.

Alcohol fires may be extinguished with water spray, dry powder, carbon dioxide or vaporising liquids. Label all containers of DAA "HIGHLY FLAMMABLE"

4.4 **Dextran and starch-free sucrose** (standard sugar). Use only pure refined sugar which meets the following requirements. Check the haze which develops when 12.0 mL of a 20% (w/v) solution of this standard sugar (4.6), plus 0.5 mL of TCA reagent (4.2), are diluted to 25 mL with denatured absolute alcohol (4.3), according to the method procedure Absorbance should not exceed 0.003 (in a 2 cm cell) at 720 nm.

4.5 **Sucrose**, 50% (w/v) solution. Dissolve 50.0 ± 0.1 g of standard sugar (4.4) in distilled water and dilute to 100 mL. This solution should be made up freshly as required.

4.6 **Sucrose**, 20% (w/v) solution. Dissolve 20.0 ± 0.5 g of standard sugar (4.4) in distilled water and dilute to 100 mL in a volumetric flask. This solution should be made up freshly as required.

4.7 **Acid-washed Supercel**. Supercel (50 ± 5 g) is added to 1 L distilled water; concentrated hydrochloric acid (50 ± 5 mL) is added and the mixture stirred for 5 mins. After filtration the Supercel is washed free from acid with distilled water, testing the washings with litmus paper. The washed Supercel is dried for 6 hours at 96-100°C and stored in a closed container.

4.8 **Starch removing enzyme.** Use a heat stable alpha amylase such as Termamyl 120 L (Novo Nordisk, Denmark) or Spezyme AA (Genecor International, The Netherlands). These enzymes will keep for 12 months if stored to manufacturer's instructions.

4.9 **Ion exchange resin mixture.** The recommended resin is Amberlite UP604 mixed bed resin supplied by U.S. Filter (Australia) Pty. Ltd., Sydney. This resin is supplied wet and should be washed by shaking with at least twice its weight of distilled water, drained dry, then washed briefly by shaking with an equal weight of acetone for no longer than two minutes, and the solvent immediately removed as before. Air dry after pressing between filter papers or oven dry at low temperature, approximately 30°C.

4.10 **Formaldehyde Solution**, 37-41% (w/v)

*Safety*

The liquid and the vapour irritate the eyes severely. Formaldehyde solution in contact with the skin has a hardening or tanning effect and causes irritation. Severe abdominal pains with nausea and vomiting and possibly loss of consciousness follow ingestion. The vapour irritates all parts of the respiratory system.

High concentration of vapour inhaled for long periods may cause laryngitis, bronchitis or bronchial pneumonia and prolonged exposure may cause conjunctivitis. If in contact with the skin for long periods, it will cause cracking of skin and ulceration, particularly around the fingernails.

The laboratory should be well ventilated to ensure that its concentration in the air is kept below 3 mg $m^{-3}$, the maximum allowable concentration for safe working over prolonged periods.

A 37% solution has a flash point of 50°C. Its fire can be extinguished with water spray, dry powder, carbon dioxide or vaporising liquid. Safety glasses and gloves must be worn while handling this solution. Label all containers of formaldehyde solution "FLAMMABLE", "SERIOUS RISK OF POISONING BY INHALATION OR SWALLOWING", "CAUSES BURNS" and "IRRITATING TO SKIN, EYES AND RESPIRATORY SYSTEM".

## 5.0 STANDARDISATION OF METHOD

A. PREPARATION OF STANDARDS

5.1 **Working standards.** In eleven 25 mL, A-class volumetric flasks prepare the standard solutions shown in Table 1.

5.2 Into each flask pipette 0.5 ± 0.05 mL (graduated pipette) of the TCA 10% (w/v) solution (4.2). Do not pipette by mouth — a safety bulb must be used.

5.3 Add 5.00 ± 0.05 mL of 50% (w/v) sucrose solution (4.5) to each flask.

5.4 Using A-class bulb or graduated pipettes, add respectively to the first ten flasks, aliquots of the standard dextran solution (4.1) in accordance with Table 1.

5.5 Using a 10 mL graduated pipette add respectively to the 10 flasks aliquots of distilled water according to Table 1, to make a total volume of 12.5 mL.

5.6 Make flask number 11 to the mark using distilled water and mix by shaking. This is the blank solution.

5.7 Add denatured absolute alcohol (4.3) slowly from a burette to the 25 mL mark of the first flask while gently swirling the flask. The time for the alcohol addition should be between 30 and 60 secs. Mix the contents of the flask by inverting gently three times.

**TABLE 1**
**STANDARD DEXTRAN SOLUTIONS**

| Flask No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 100 g/L TCA solution (4.2) (mL) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| 500 g/L sucrose solution (4.5) (mL) | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Standard Dextran solution (4.1) (mL) | 0 | 0.2 | 0.4 | 0.6 | 1.0 | 1.5 | 2.0 | 3.0 | 5.0 | 7.0 | 0 |
| Distilled Water (mL) | 7.0 | 6.8 | 6.6 | 6.4 | 6.0 | 5.5 | 5.0 | 4.0 | 2.0 | 0 | 19.5 |
| Dextran Content (mL) | 0 | 8 | 16 | 24 | 40 | 60 | 80 | 120 | 200 | 280 | BLANK |

Start the stopwatch immediately after the mixing step is complete.

B. MEASUREMENT OF ABSORBANCE

5.8 Determine the cell corrections of a pair of matched 2 cm cells using distilled water.

5.9 Approximately 15 minutes after the completion of the mixing step (5.7), rinse a 2 cm cell twice with the blank solution, and then fill the cell.

5.10 In a similar way, rinse and fill another 2 cm cell with one of the standard solutions (5.5). Clean the optical faces of the cells with a tissue.

5.11 At 20 minutes ± 10 seconds after completion of the mixing step (5.7) read and record to 0.001 the absorbance of the test solution against that of the blank solution at 720 nm.

5.12 Repeat steps 5.7, 5.10, and 5.11 for each concentration of dextran involved in the standardisation. It is not necessary to refill the blank solution cell (5.9) for each determination.

C. PREPARATION OF CALIBRATION GRAPH

5.13 Apply the cell correction (5.8) to the absorbance of each standard solution.

5.14 Plot "dextran mg/L" against "absorbance". Points representing 60 to 280 mg/L dextran should be approximately on a straight line. The plot of low dextran concentrations (0 to 60 mg/L) is however non-linear and this section of the calibration graph should appear as a gradual curve.

5.15 The graph used by any laboratory for routine analysis should be constructed using the mean of independent standardisations by at least two analysts. Individual points must be within 5% of the mean line of best fit.

NOTE: As a guide to performance of the method, the 80 mg/L dextran standard measured at 720 nm in a 2 cm cell should give an absorbance of about 0.230.

**6.0 TREATMENT OF SAMPLE** — Cane Juice

Raw cane juices require special treatment. Unless the juice is to be analysed within 30 minutes of collection, preservation is necessary. If the juice is to be stored for longer than 30 minutes prior to analysis, the following preservation step should be carried out immediately after collection of the sample.

Add 0.5 mL formaldehyde solution (4.10) to each 100 mL of juice, stir and store in a closed vessel in a refrigerator (do not freeze the juice). Juices preserved by this method will keep for 24 hours. If refrigerated storage is not available, the juice, preserved as above, should not be stored for longer than 8 hours. Use this method of storage only as a last resort.

**7.0 PROCEDURE**

A. PREPARATION OF TEST SOLUTIONS — Raw Sugar and Process Materials

7.1 (a) Weigh out 40.0 ± 0.1 g of raw sugar, transfer to a 100 mL flask, dissolve and make to the mark with distilled water, stopper and mix well.

(b) Prepare 100 mL of approximately 40°Bx solution of process material and measure the refractive index.

7.2 Using a 50 mL measuring cylinder, transfer 50 mL of the solution into a 100 mL conical flask. Add 0.1 mL of the enzyme (4.8). Mix the contents well and stopper the flask.

7.3 Place the flask in a water bath at 55 ± 5°C for 15 ± 2 mins. Agitate every 5 minutes.

7.4 At the end of 15 minutes cool to room temperature and add mixed ion exchange resins; 1 g for raw sugars and 10 g for process materials.

7.5 Shake the flask for 30 ± 2 minutes on a flask shaker.

7.6 Pour the mixture through a funnel containing a 150 μm gauze into a 100 mL measuring cylinder which can be stoppered.

7.7 With distilled water, rinse the flask thoroughly, through the funnel into the measuring cylinder.

7.8 Dilute the sample and washings to 100 mL, then add 10 ± 1 mL TCA reagent (4.2). Do not pipette by mouth. A safety bulb must be used.

7.9 Add two heaped teaspoonfuls (about 6-8 g) of acid-washed Supercel (4.7). Stopper and mix well. Proceed according to steps 7.15 to 7.23.

B. PREPARATION OF TEST SOLUTION — Cane Juice

7.10 If the juice has been refrigerated, bring it rapidly to room temperature with running cold water. Hot water must not be used for this purpose because some solubilisation of starch may occur.

7.11 Measure and record, corrected to 20°C, the brix of the juice to 0.1° brix.

7.12 Shake 60 ± 1 mL juice with 2.0 ± 0.2 g mixed resin (4.9) for 10 ± 1 minutes and filter through 150 μm gauze. Transfer 50 ± 1 mL (cylinder) of juice to a 100 mL beaker.

7.13 Add 10.0 ± 0.1 mL TCA reagent (4.2). Do not pipette by mouth. A safety bulb must be used.

7.14 Add two heaped teaspoons of acid-washed Supercel (4.7), stopper and mix well. Proceed according to steps 7.15-7.23.

C. FILTRATION AND PRODUCTION OF HAZE

7.15 Filter mixture (7.9 or 7.14) through a 5.5 cm Buchner funnel (Whatman No. 5 filter paper), using the first 10 to 15 mL of filtrate to rinse the funnel and flask.

7.16 Using a 25 mL graduated pipette, add 12.5 mL of the filtrate to each of two clean, dry 25 mL volumetric flasks.

7.17 Add denatured absolute alcohol (4.3) slowly from a burette to the 25 mL mark of one flask, gently swirling the flask. The time for the alcohol addition should be between 30 and 60 secs. Mix the contents of the flask by inverting gently three times. Start the stopwatch immediately the mixing step is complete.

NOTE: (i) Alcohol must be added within 20 minutes of commencement of step 7.8 (sugars etc.) or 7.14 (juices).

(ii) Vigorous shaking of the flask may cause coagulation of the dextran haze, and must be avoided.

7.18 To the other flask add distilled water to the 25 mL mark and mix. This is the test blank.

D. MEASUREMENT OF ABSORBANCE

7.19 Determine the cell corrections of a pair of matched 2 cm cells using distilled water.

7.20 Approximately 15 minutes after the completion of the mixing step (7.17), rinse a 2 cm cell twice with the blank solution, and then fill the cell.

7.21 In a similar way, rinse and fill the other 2 cm cell with the test solution. Clean the optical faces of the cells with a tissue.

7.22 At 20 minutes ± 10 seconds after the completion of the mixing step for the test solution (7.17), read and record to 0.001 the absorbance of the test solution against that of the blank solution at 720 nm.

NOTE: If the dextran haze reading is beyond the calibration limits of the standard graph, smaller aliquots of the filtrate (7.16) should be taken. Dilute the aliquot in each 25 mL flask to a total volume of 12.5 mL by the addition of a 20% (w/v) sucrose solution (4.6). Proceed as from step 7.16.

7.23 Immediately after reading, visually inspect the contents of the test solution cell to check for flocculation. If the haze has flocculated, repeat the analysis.

## 8.0 CALCULATIONS

8.1 Apply the cell correction (7.19) to the observed absorbance.

8.2 Obtain from the standardisation graph "mg/L dextran in the test solution" corresponding to the corrected observed absorbance.

8.3 For process materials use Table XII or XV to determine the concentration in g/mL of the sugar solution from the appropriate brix measurement (7.1).

**Table XII** gives the degrees brix, density and concentration in g/mL of sucrose solutions at 20°C, corresponding to refractive index readings.

**Table XV** gives the density and concentration in g/mL of sucrose solutions at 20°C, corresponding to degrees brix.

8.4 Calculate dextran in sample mg/kg.

$$\text{Dextran (mg/kg)} = \frac{\text{mg/L dextran} \times y^* \times 0.5}{\text{conc. (g/mL)} \times \text{aliquot}}$$

* = 110 for raw sugars and process materials.

y* = 60 for raw cane juices

aliquot = volume pipetted in step 7.16 (usually 12.5 mL)

8.5 Record the results as mg/kg dextran in the sample to the nearest 1 mg/kg.

## 9.0 PRECISION

| Sample | Concentration Range mg/kg Dextran | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 0 - 1,000 | 20.8 | Not determined |
| Cane Juice | 0 - 12,000 | Not determined | Not determined |
| Process Materials | 0 - 1,000 | Not determined | Not determined |

## 12.0 REFERENCES

12.1 Nicholson R.I., and Horsley M.; J. Ag. and Food Chem.; Vol. 7, No. 9 (1959), p. 64.

12.2 ICUMSA Methods Book (1994).

12.3 ICUMSA. Proceedings of 21st Session (1994). General Subject 1.

# METHOD 29
## PHENOLIC ACIDS — DETERMINATION IN RAW SUGAR, SYRUPS AND CANE JUICE

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry.

Phenolic and amino acids are the two major groups of colour precursors in cane sugar. Phenolic acids form coloured complexes with iron and copper, and some are readily oxidised to form coloured polymers. The concentration of phenolic acids therefore relates to the tendency of the sugar to darken in storage.

The phenolic acids found in sugar cane are hydroxy derivatives of benzoic acid or cinnamic acid.

**2.0 PRINCIPLE OF METHOD**

A known quantity of the sample in water is reacted with E.D.T.A., sodium nitrite and acidified sulphanilic acid. The intensity of the yellow colour developed after 60 minutes is measured in a spectrophotometer at 420 nm.

Gallic acid is used as the standard. Gallic acid and the phenolic acids in sugar have varying sensitivity to the diazo reaction, and some flavonoids respond to it also. This analysis therefore indicates only relative levels of phenolic acids in different samples.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Volumetric flasks**, Class A, (AS2164), 500 mL, 100 mL, 50 mL.

3.2 **Bulb pipettes**, Class A, (AS2166), 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL.

3.3 **Buchner funnel**, 5.5 cm with 250 mL buchner flask.

3.4 **Automatic pipette**, or dispenser to deliver 10.0 ± 0.2 mL.

3.5 **Spectrophotometer**, with matched 2.0 cm cells.

3.6 **Balance** to weight 100.00 ± 0.02 g.

**4.0 REAGENTS**

During the analysis, unless otherwise stated, use only reagents of analytical grade and only distilled water.

4.1 **E.D.T.A., 0.01 mol/L Solution**. Weigh 3.72 g of E.D.T.A. (di-sodium salt) and dissolve in water in a 1L volumetric flask.

4.2 **Sulphuric acid, 0.5 mol/L**. Prepare from a commercial concentrated volumetric solution supplied in a sealed ampoule.

4.3 **Sulphanilic acid**, 0.8% (w/v) solution. Dissolve 0.800 ± 0.001 g of sulphanilic acid in about 50 mL of hot water in a 150 mL beaker; add 10 mL of 0.5 mol/L sulphuric acid (4.2). Mix to dissolve, transfer to a 100 mL volumetric flask, cool and dilute to volume, mix well by shaking.

This reagent will not keep and a fresh solution must be made up daily or when required.

4.4 **Sodium Nitrite**, 4% (w/v) solution. Dissolve 1.00 ± 0.01 g in 25 mL water. Prepare a fresh solution every 2 hours.

4.5 Gallic acid standard solution, 200 mg/L. Weigh 0.1000 ± 0.0001 g of gallic acid into a 500 mL Class A volumetric flask. Dissolve in distilled water and make to the mark. Stopper and mix well.

**5.0 STANDARDISATION OF METHOD**

The absorbance of the coloured complex varies with sugar concentration and it is necessary to prepare a separate calibration curve for juices which omits addition of sucrose. For juices, omit step 5.1.

5.1 Weigh 8.00 ± 0.02 g of sucrose into each of seven 100 mL Class A volumetric flasks. Add about 50 mL water to each flask and dissolve the sugar using a shaker.

5.2 Using Class A bulb pipettes, pipette 0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mL of Gallic acid standard solution (4.5) respectively into the 100 mL flasks. Make each flask to the mark with water, stopper and mix well.

The solutions contain 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 mg of gallic acid per 100 mL.

5.3 With a Class A pipette, pipette 10.0 mL of each of the gallic acid solutions (5.2) into each of two 50 mL volumetric flasks [(a) standard blank (b) standard solution].

5.4 With an automatic pipette add 10.0 ± 0.2 mL, E.D.T.A. solution (4.1) to each flask.

5.5 Dilute the standard blank to the mark with water. Mix well.

5.6 Pipette 4.00 ± 0.02 mL sulphanilic acid solution (4.3) and 2.0 ± 0.1 mL of sodium nitrite solution (4.4) into the standard solutions. Mix by swirling after each addition. Dilute each solution to the mark with water and mix well. Allow the solutions to stand for 60 ± 5 minutes after the addition of sodium nitrite solution.

NOTE: For a large number of samples, add the nitrite to successive samples at 3-5 minute intervals to allow time to rinse and refill the blank and standard cells between absorbance readings.

5.7 Read the absorbance of the standard solutions and the standard blanks in 2.0 cm cells in a spectrophotometer at 420 nm with water as the reference. Record the absorbance to 0.001 units.

Subtract the absorbance obtained for the blank from the absorbance obtained for the standard solution to give the true absorbance for each standard.

As a guide to performance of the method, the 0.8 mg gallic acid standard measured at 420 nm in 2.0 cm cells should read about 0.290 absorbance.

5.8 Plot mg/100 mL phenolic acids (as gallic acid) against absorbance for each standard solution. If 2 or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular gallic acid concentration, the standardisation must be repeated.

The slopes of independent calibration lines should not differ by more than 2%.

## 6.0 PROCEDURE

### (a) Raw Sugar and Syrups

6.1(a) Weigh — for raw sugar 8.00 ± 0.02 g
— for liquor the equivalent of 8.00 g of solids
Transfer to a 100 mL volumetric flask, dissolve in water and make to the mark. Mix well.

6.2(a) Filter the solution through a Whatman No. 5, 5.5 cm paper in a buchner funnel.

6.3(a) Pipette 10.00 ± 0.02 mL of the filtrate into each of two 50 mL volumetric flasks. Mark one flask as 'test solution', and the other flask as 'test blank'.
Continue at step 6.4.

### (b) Juices

6.1(b) Take 30 mL juice, add about 3 g Supercel, mix and filter on a buchner funnel using a No. 1 or other suitable paper.

6.2(b) Determine the refractive index of the filtered juice and hence °bx and concentration (g sucrose/mL). With a Class A pipette, pipette 3 mL of filtered juice into a 50 mL Class A volumetric flask and make up to the mark.

6.3(b) Pipette 10 mL ± 0.02 mL of the diluted juice into two 50 mL volumetric flasks. Mark one flask as 'test solution' and the other flask as 'test blank'.

6.4 With an automatic pipette add 10.0 ± 0.2 mL E.D.T.A. solution (4.1) to both flasks.

6.5 Dilute the 'test blank' to the mark with water. Mix well.

6.6 Pipette 4.00 ± 0.02 mL of sulphanilic acid solution (4.3) and 2.00 ± 0.01 mL of sodium nitrite solution (4.4), into the test solution. Mix by swirling after each addition. Dilute to the mark and mix well.
Allow the solution to stand for 60 ± 5 minutes after adding sodium nitrite solution for full colour development.

6.7 Read the absorbance of the test solutions and the test blanks in a spectrophotometer set at 420 nm, in 2 cm cells with water (6.5) as a reference. Record the absorbance to 0.001 units.
Subtract the absorbance obtained for the test blank from the absorbance obtained for the test solution to give the absorbance due to phenolic acids.

## 7.0 CALCULATIONS

7.1 Read from the standard graph the mg phenolic acids in 100 mL of the sample solution.

7.2(a) For raw sugar or syrup samples, calculate phenolic acids, mg/kg, in the sample using:

$$\text{phenolic acids, mg/kg} = \frac{\text{mg phenolic acid/100 mL (from graph)}}{\text{m}} \times 1000$$

where: m is the sample mass (g)

phenolic acids, mg/kg = mg phenolic acid (from graph) x 125, for an 8.0 g sample

7.2(b) For juice samples, calculate phenolic acids, mg/kg, on sugar solids, using:

$$\text{phenolic acids, } (\mu\text{ g/mL}) \text{ in the juice} = \frac{\text{(mg phenolic acid/100 mL from graph)}}{2 \times 3} \times 1000$$

$$\text{mg/kg phenolics, on sugar solids} = \frac{\text{g phenolic acids/mL juice}}{\text{g sucrose/mL juice}}$$

## 8.0 PRECISION

| Sample | Concentration Range (mg/kg) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 0-250 | Not determined | Not determined |

# METHOD 30
# POLARISATION — DETERMINATION IN RAW SUGAR USING AUTOMATIC POLARIMETERS

## 1.0 SCOPE AND FIELD OF APPLICATION

This Level 1 method has been adopted by the Australian sugar industry for the determination of the polarisation of raw sugar. It is not materially different to ICUMSA method GS-1/2/3-1 (1994).
Sucrose is the major component of raw sugar and the polarisation (pol) of a raw sugar solution gives a commercial estimate of the sucrose content.
This method is applicable to all raw sugars.

## 2.0 DEFINITIONS

2.1 **The normal sugar solution** is defined by ICUMSA as 26.0160 g of pure sucrose weighed in vacuo and dissolved in pure water at 20.00°C to 100.00 mL. This corresponds to a concentration of 26.0000 g of sucrose, weighed with brass weights in air, under normal conditions [101 kPa pressure, 20°C, 50% relative humidity] in 100.00 mL of solution at 20.0°C.

2.2 ICUMSA - 19th Session 1986, re-defined the "**International Sugar Scale**". The new definition came into effect in July 1988.
In the new definition the rotation of the normal sugar solution in a 200.000 mm tube at the mercury wavelength, 546.2271 nm (in vacuo), at 20.00°C is 40.777° which is the **100°Z** point of the International Sugar Scale [replacing the previously defined 100°S point].

## 3.0 PRINCIPLE OF METHOD

This is a physical analysis involving three steps. A normal solution of raw sugar is prepared. The solution is clarified by adding a solution of basic lead acetate, (which coagulates colloidal impurities and removes some colourant matter), followed by filtration. The pol of the clarified solution is read in a tube of standard length in a sugar polarimeter.

## 4.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**
Ordinary laboratory apparatus and glassware, and

4.1 **Balance** to weigh rapidly 26 g, plus container, to ± 0.002 g.

4.2 **Volumetric Flasks (S-flask)**. Flasks used for raw sugar polarisation should conform with the specification set out by ICUMSA in Subject 2, 1978 in respect of "ICUMSA Special Flasks". That is a 100 mL S-flask will be used which has an accuracy of 100.00 ± 0.02 mL.

4.3 **Sugar Polarimeters**. All sugar polarimeters used in the Australian sugar industry must meet legal metrology requirements.
Circle polarimeters or quartz wedge polarimeters (saccharimeters) may be used. However, temperature correction tables may be different for each type of polarimeter (14.5, 14.9).

4.4 **Quartz Control Plates** (Nominal value between 95°Z and 105°Z). The design, material, workmanship, dimensions and properties of the quartz plates shall be of the standard laid down by ICUMSA (1994) SPS-1, p7.5.
Quartz control plates shall be standard plates which meet legal metrology requirements.

4.5 **Long stem, corrosion resistant metal funnel**, approximately 10 cm diameter, in stand.

4.6 **Stemless glass or plastic filter funnel**, 7.5 cm diameter, 100 mL capacity.

4.7 **Filter glasses** or conical beakers, 300 mL capacity.

4.8 **Polarimeter tube**, 200 mm long, and cover glasses, conforming to specifications given in ICUMSA (1994) SPS-1, p7.3-7.4. Polarimeter tubes should be certified by an appropriately accredited laboratory.

4.9 **Auto dispenser 0-2 mL or safety burette**, 50 mL capacity, fitted with $CO_2$ absorption tubes.

4.10 **Hypodermic syringe**, 5 mL capacity.

4.11 **Black clips** for "S" flasks.

4.12 **Constant temperature bath** (not essential) - capable of being controlled at the ambient room temperature ± 1°C.

4.13 **Alcohol blower**.

4.14 **Thermometers**, length 20 cm, 10-35°C, capable of being read to 0.1°C.

4.15 **Whatman No. 91 filter paper** (15 cm) or equivalent with moisture content in range 6-8% water, determined by drying it for 3 hours at 100°C. If necessary store the papers in containers, in which the relative humidity is such that the equilibrium moisture content of the papers is in the range 6-8%.

## 5.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

5.1 **Basic Lead Acetate Solution** (Wet Lead).

The reagent should meet the following specifications:

Basic lead content: 10.0 ± 0.2 g PbO/100 mL

Total lead content: 24.4 ± 0.2 g PbO/100 mL

(equivalent to a specific gravity of 1.240 ± 0.002)

Basicity: 41.0 ± 1.2%

Each batch of wet lead should be stored in airtight containers, labelled with the batch number, date and specification.

Wet lead working solution is normally dispensed by a safety burette fitted with $CO_2$ absorption tubes. $CO_2$ absorption cartridges should be included in all lines open to the air to remove carbon dioxide.

5.2 **Distilled Water**

Check the distilled water supply daily by a thymol test for the presence of sugar. If the sugar content exceeds 5 mg/kg, then it must not be used for polarisation analysis.

## 6.0 CHECKING THE CALIBRATION OF THE POLARIMETER

There are two recommended procedures for checking the calibration of the polarimeter and calculating the polarisation of test solutions corrected to 20°C. *Procedure 1* requires that with each batch of test solutions a calibration with a quartz plate is carried out at the same time. This procedure assumes that the polarimeter is operating at a constant but unmeasured instrument temperature. *Procedure 2* requires that quartz plate readings be carried out periodically but not necessarily at the same time as the test solutions are read. Correction for the calibration of the polarimeter different to the quartz plate value is achieved by application of a scale correction. (See Method 1.)

## 7.0 TREATMENT OF SAMPLE

If the sugar has not been freshly mixed, or there is reason to suspect that the sample container is not airtight, the top 1-2 cm of sample should be discarded immediately before weighing.

## 8.0 PROCEDURE

A. SOLUTION PREPARATION

8.1 Weigh slightly more than 26 grams of sample into a tared basin and reseal the sample container immediately. Quickly adjust the weight of the sample to 26.000 ± 0.002 g.

8.2 Place a 100 mL 'S' flask (4.2) under a long stem funnel. The end of the funnel stem should be in the centre of the flask bulb, and there should be a gap of not less than 5 mm between the top of the flask and the funnel apex, to allow air to escape.

Wash the sugar into the flask with distilled water, rinse the tare basin (both sides), funnel mouth and the outside of the funnel stem as it is withdrawn from the flask.

8.3 Fill the flask with distilled water to a volume not exceeding 70 mL.

Dissolve the sugar by swirling or by use of a mechanical shaker. If a mechanical shaker is used it may be necessary to fill the flask to less than 70 mL to prevent splashing or air entrainment. Hold the flask to the light and check that all sugar is dissolved.

8.4 Add distilled water, if necessary, to bring the volume to 70-80 mL.

8.5 Run about 2 mL of wet lead to waste from the burette to remove any solution that may have absorbed $CO_2$ from the air.

8.6 Add wet lead according to the expected polarisation of the raw sugar: below or equal to 99.30°Z use 1.00 ± 0.05 mL and above 99.30°Z use 0.50 ± 0.05 mL. For VLC use 0.10 ± 0.05 mL.

8.7 Add distilled water, swirling continuously until the bulb of the flask is full.

8.8 *If laboratory has a controlled temperature bath* (for air conditioned laboratories only), place the flask in the bath for at least 15 minutes. The temperature of the bath should be controlled to within ± 1 °C of the laboratory temperature.

Remove the flask from the water bath, quickly wipe dry and place the flask on the bench.

*If a cooling bath is not used*, stand the flask on the bench for at least 15 minutes to allow it to attain room temperature.

**NOTE:** Always handle the flask by the top of the neck to avoid warming the solution.

8.9 Add distilled water, slowly running it down the inside of the neck of the flask (to minimise aeration) until the level is about 1 mm below the calibration mark. Ensure that the whole of the inside of the neck is washed.

8.10 Incline the flask and tap with the finger to dislodge bubbles, then defroth the solution with an alcohol blower and dry with a clean roll of filter paper inside the neck of the flask to within a few millimetres of the mark.

8.11 Place a black clip on the neck of the flask about 1 mm below the mark, to throw a shadow on the meniscus. Hold the flask vertically by the top of the neck with the calibration mark at eye level. Add distilled water dropwise from a hypodermic syringe until the bottom of the meniscus and the top of the calibration mark just coincide (the flask should be viewed against a well-lit background).
If fine bagasse or fibre particles are present, flick on to the side of the neck momentarily so that the true position of the meniscus can be seen. Again dry the inside of the neck of the flask using a clean roll of filter paper.

8.12 Seal the flask with a clean, dry stopper and mix thoroughly by inverting and shaking several times.

8.13 Insert a 10-35°C clean, dry thermometer into the flask with the bulb of the thermometer in the middle of the flask bulb. Record the *temperature of making to the mark* ($t_m$) to the nearest 0.1°C. Reseal the flask. Clean and dry the thermometer.

8.14 Stand the solution for 5 minutes to allow the precipitate to settle.

B. FILTRATION

8.15 Fold a Whatman 91 filter paper (or equivalent) and place in a clean, dry, stemless glass or plastic funnel. Do not use a fluted paper or filter aid. Place the funnel containing the filter paper on a waste filter glass.

8.16 Fill the filter paper by decanting the solution, ensuring that the paper is fully wetted but not overfilled. Immediately cover the filter funnel with a cover slip. Allow approximately 10 mL of solution to run into the waste filter glass and then transfer the filter funnel to a clean, dry filter glass. The filtrate should be clear and bright. If the filtrate in the filter glass is not clear and bright, allow a further 10 mL to filter, then transfer the funnel to another clean dry filter glass.
If the filtrate is still not clear, discard and repeat the preparation.
**NOTE:** To minimise evaporation, the solution in the filter paper must not be replenished, and must be kept out of draughts and direct sunlight.

8.17 Collect approximately 50 mL of the filtrate (sufficient to rinse and fill a polarimeter tube), remove the filter funnel and place the cover slip on the filter glass.
**NOTE:** To minimise evaporation the solution must be read as soon as possible after sufficient filtrate has been collected.

C. POLARISATION — FILLING TUBE

**NOTE**: Polarimeter tubes should be held by the metal flanges, not the glass, as heat from the hand can cause the temperature of the solution in the tube to rise.

8.18 Assemble one end of the tube with a cover glass, rubber washer, and metal cap in that order. Screw the cap on only as tightly as necessary to prevent leakage.

8.19 Hold the tube by the metal flange and remove the cover slip from the filter glass. Pour the solution into the tube taking care that no solution spills onto the outside of the tube.
**NOTE:** If the outside of the tube becomes wet it must be dried immediately as evaporation from the outside of the tube can cool the solution in the tube.

8.20 About two thirds fill the tube with filtrate, seal the open end with a finger, invert, and then empty to waste. Shake the tube during the draining. Repeat this procedure twice.

8.21 Fill the tube and top the solution up to form a liquid bead above the end of the tube. Slide on a cover glass, taking care to avoid trapping an air bubble. Screw on the cap, containing a rubber washer, only as tightly as necessary to prevent leakage.
**NOTE:** If a cap is screwed on too tightly, strain can make the cover glass optically active and cause errors in the polarimeter readings.

8.22 Hold the polarimeter tube by the metal flanges, end-on to the light, and check that the solution is not cloudy, or too dark, and that there are no air bubbles or striations.
If there are air bubbles present, unscrew the cap and top up the solution in the tube. If there are striations present, re-rinse and fill the tube. If the solution is cloudy or too dark, discard the solution and repeat the whole analysis.

8.23 Dry the outside of the tube to prevent cooling through evaporation. Place the tube in a tray near the polarimeter.
**NOTE:** Keep pol tubes away from sources of heat or cold. In particular, do not place pol tubes near the air outlet vent of automatic polarimeters.

## 9.0 READING THE POLARIMETER — PROCEDURE 1

Procedure 1 requires that with each batch of test solutions a standardisation with a quartz plate is carried out at the same time. This procedure assumes that the polarimeter is operating at a constant but unmeasured instrument temperature.

9.1 Turn on the polarimeter at least 45 minutes before readings are to be made.

9.2 Stand the quartz plate in a stand adjacent to the polarimeter (but away from any source of heat). Fix a thermometer (4.14) adjacent to the quartz plate to measure the quartz plate temperature allow the quartz plate to equilibrate to ambient temperature for at least 30 minutes.

9.3 Record the *temperature of the quartz plate*, $t_q$, to 0.1°C. Allow the reading of the empty polarimeter to stabilise and reset the zero.

9.4 Place the quartz plate in the trough of the polarimeter with a minimum of handling. Close the lid without jarring.

9.5 Allow the polarimeter reading to stabilise and record the reading. Obtain four readings rotating the quartz plate through 45° between each reading. Ensure the lid is closed before reading. Average the four readings and record to the nearest 0.01°Z. This is the *quartz plate reading*, $Q_{tq}$, at temperature $t_q$.

9.6 Remove the quartz plate and close the lid.

9.7 Place the filled tube (8.23) in the trough of the polarimeter and close the lid without jarring.

9.8 Allow the polarimeter reading to stabilise and record the reading. Obtain four readings, rotating the tube 45° between each reading. Average the four readings and record to the nearest 0.01°Z. This is the *polarisation reading of the test solution*, $P_{tr}$, at temperature, $t_r$.

9.9 Remove the tube from the trough, close the lid and check that the polarimeter returns to zero ± 0.02°Z. If not, repeat steps 9.3 to 9.7.

**NOTE:** If after the second set of readings the zero still does not return to ± 0.02°z, a fault in the instrument is indicated. The instrument should be checked by a qualified service technician / servicing licensee.

9.10 Hold the tube vertically by the bottom metal flange, unscrew the cap and remove the cover glass. Flick the tube such that small portions of the solution are discarded until the level of solution is about 2 cm below the top of the metal flange. Insert a clean, dry 10-35°C thermometer into the tube so that the bulb is near the centre of the tube. Rotate the thermometer between finger and thumb for a few seconds until the mercury column is steady. Record the *temperature of reading*, $t_r$, to the nearest 0.1°C. Do not handle the tube by the glass before reading the temperature. Wash and dry the thermometer after each temperature measurement.

## 10.0 CALCULATION (FOR READING POLARIMETER BY PROCEDURE 1)

10.1 The following readings have been recorded:

$Q_{tq}$ — the quartz plate reading at temperature $t_q$ (9.5).

$t_q$ — the temperature of the quartz plate at the time of reading (9.3).

$t_m$ — the temperature of making to the mark (8.13).

$t_r$ — the temperature of reading (9.10).

$P_{tr}$ — the polarisation of the test solution at temperature $t_r$ (9.8).

$Q_{20}$ — the certified value of the quartz plate at 20°C, for the type of polarimeter used.

10.2 Determine $C_{tq}$, the correction to the quartz plate polarisation for the temperature of the quartz plate, from Table XXVI.

10.3 Determine $C_{tm}$, the correction to polarisation for the temperature of making to the mark, from Table XXVII.

10.4 Determine $C_{tr}$, the correction to polarisation for the temperature of reading, from Table XXVIII.

10.5 Calculate $P_{20}$, the polarisation at 20°C, from

$$P_{20} = P_{tr} + Q_{20} - Q_{tq} - C_{tq} + C_{tm} + C_{tr}$$

10.6 Report the result to the nearest 0.01°Z.

## 11.0 READING THE POLARIMETER — PROCEDURE 2

**NOTE:** Procedure 2 requires that quartz plate readings are carried out periodically but not necessarily at the same time as the test solution is read. The polarimeter is calibrated by applying a scale correction determined by the procedure described in Method 1. A correction for the polarimeter temperature is required for quartz wedge polarimeters or circle polarimeters with a white light source and interference filter.

11.1 Turn the polarimeter on at least 45 minutes before readings are to be made.

11.2 Allow the polarimeter reading to stabilise then reset the display to zero. Place the filled tube (8.23) in the trough of the polarimeter and close the lid without jarring.

11.3 Record the *polarimeter temperature*, $t_p$, to 0.1°C each time a tube is placed in the polarimeter.

The thermometer (4.14) to measure the polarimeter temperature should be fixed vertically close to the polarimeter, but away from any source of heat.

11.4 Allow the polarimeter reading to stabilise and record the reading. Obtain four readings, rotating the tube 45° between each reading. If all four readings are not within a range of 0.02°Z, discard and take another set of four readings. Ensure the lid is closed before each reading. Average the four readings and record to the nearest 0.01°Z. This is the *polarisation reading of the test solution*, $P_{tr}$, at temperature, $t_r$.

11.5 Remove the tube from the trough, close the lid and check that the polarimeter returns to zero ± 0.02°Z. If not, repeat steps in 11.2 to section 11.5.

**NOTE:** If after the second set of readings, the zero still does not return to ± 0.02°z, a fault in the instrument is indicated. The instrument should be checked by a qualified service technician / servicing licensee.

11.6 Hold the tube vertically by the bottom metal flange, unscrew the cap and remove the cover glass. Flick the tube so that small portions of the solution are discarded until the level of solution is about 2 cm below the top of the metal flange Insert a clean, dry, 10-35°C short stemmed thermometer into the tube so that the bulb is near the centre of the tube. Rotate the thermometer between finger and thumb for a few seconds until the mercury column is steady. Record the *temperature of reading*, $t_r$, to the nearest 0.1°C. Do not handle the tube by the glass before reading the temperature. Wash and dry the thermometer after each temperature measurement.

11.7 To obtain the *scale correction*, SC, see Method 1.

## 12.0 CALCULATIONS (FOR READING THE POLARIMETER BY PROCEDURE 2)

12.1 The following readings have been recorded:

$t_m$ — the temperature of making to the mark (8.13).

$t_r$ — the temperature of reading (11.6).

$t_p$ - the polarimeter temperature (11.3)

SC — the polarimeter scale correction (Method 1).

$P_{tr}$ — the polarisation of the test solution at temperature $t_r$ (11.4).

12.2 Determine $C_{tm}$, the correction to polarisation for the temperature of making to the mark, from Table XXVII.

12.3 Determine $C_{tr}$, the correction to polarisation for the temperature of reading, from Table XXVIII.

12.4 Determine $C_{tp}$, the correction to polarisation for the polarimeter temperature, from Table XXIX (for quartz wedge polarimeters or circle polarimeters with interference filters).

12.5 Calculate $P_{20}$, the polarisation at 20°C from

$$P_{20} = P_{tr} + SC + C_{tm} + C_{tr} + C_{tp}$$

12.6 Report the result to the nearest 0.01°Z.

## 13.0 RANGE AND PRECISION

| Concentration Range 'Degrees Z' | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|
| 96.00 - 100.00 | 0.147 | 0.251 |

## 14.0 REFERENCES


14.1 ICUMSA; Proceedings of Thirteenth Session (1962), Subject 11.

14.2 ICUMSA; Proceedings of Fourteenth Session (1966), Subject 11.14.3 ICUMSA; Proceedings of Fifteenth Session (1970), Subject 11.

14.4 ICUMSA; Proceedings of Sixteenth Session (1974), Subject 11.

14.5 ICUMSA; Proceedings of Seventeenth Session (1978), Subject 11.

14.6 ICUMSA; Proceedings of Eighteenth Session (1982), Subject 11.

14.7 ICUMSA; Proceedings of Nineteenth Session (1986), Subject 11.

14.8 Internat. Sug. J., *59* (1957), p. 156.

14.9 Internat. Sug. J., *82* (1978), p. 263.

14.10 Internat. Sug. J., *67* (1965), p. 234 and 265.

14.11 Sugar Analysis. Official and Tentative Methods Recommended by ICUMSA Ed. F. Schneider (1979), p. 2.

14.12 ICUMSA. Proceedings of 20th Session (1990), Subject 11.

14.13 ICUMSA, Proceedings of 21st Session (1994), General Subject 1 & Subject 4.

14.14 ICUMSA Methods Book (1994), SPS-1.

14.15 Zuckerind., 113, 512-514 (1998).

# METHOD 31
# MOISTURE — DETERMINATION IN RAW SUGAR BY DRYING AT ATMOSPHERIC PRESSURE

**1.0 SCOPE AND FIELD OF APPLICATION**

This Level 2 method has been adopted by the Australian sugar industry. Moisture is defined as the loss of mass of the sample when it is heated for five hours at 96-100°C.

**2.0 PRINCIPLE OF METHOD**

The test portion is dried in a laboratory oven at 96-100°C and the loss of mass is recorded.

This analysis *assumes* that all the water in the sample is removed by the drying process and that the loss of mass is due only to the removal of this water. The temperature of 96-100°C is used to minimise any decomposition of reducing sugars.

**3.0 APPARATUS**

**All apparatus should be visually checked for damage prior to use.**

Ordinary Laboratory apparatus and

3.1 **Laboratory drying oven** capable of being controlled at 98 ± 2°C. A forced draft (fan) oven is preferred.

3.2 **Drying basin**, of metal or glass of a suitable size to take the required (5 g) quantity of sample in a layer about 10 mm thick.

3.3 **Lid** to fit over basins.

3.4 **Desiccator** with active, self-indicating silica gel (silica gel can be reactivated by heating in an oven at 110-150°C until the dark blue colour is restored, or by heating for a few minutes in a microwave oven).

3.5 **Analytical balance** to weigh to 0.0001 g.

**4.0 PROCEDURE**

4.1 Weigh a clean, dry, empty drying basin and lid to the nearest 0.0001 g. Record the mass of the basin and lid ($m_1$).

4.2 Add 5 ± 0.5 g raw sugar to the basin and place lid on top. Weigh basin and lid and sugar to the nearest 0.0001 g and record the mass ($m_2$).

4.3 Heat basin and sugar with lid removed, in the oven for 5 hours ± 10 minutes at 96-100°C. Basins must be at least 3 cm from the sides of the oven, 10 cm from the top, and 10 cm away from air vents. Avoid opening the door of the oven during drying.

4.4 Place the lid on the basin and remove from the oven and cool in a desiccator for 30 ± 5 minutes.

4.5 Reweigh basin, lid and sugar to the nearest 0.0001 g. Record the mass ($m_3$). This operation should be completed within half a minute of removing the basin from the desiccator.

**5.0 CALCULATIONS**

Calculate the % moisture in the sugar.

$$\% \text{ moisture} = \frac{100}{1} \times \frac{\text{loss of mass}}{\text{mass of test sample}}$$

$$= 100 \times \frac{m_2 - m_3}{m_2 - m_1}$$

where:

$m_1$ = mass of the empty basin + lid (4.1)

$m_2$ = mass of basin + lid + sample (4.2)

$m_3$ = mass of basin + lid + dry sample (4.5)

Report the result as per cent moisture to 0.01%.

## 6.0 PRECISION

| Sample | Concentration Range % Moisture (m/m) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 0.1 - 0.7 | 0.040 | not determined |

## 7.0 REFERENCES


7.1 Plews, R.W. (Ed.): Analytical Methods Used in Sugar Refining; Elsevier (1970).
7.2 Schneider, F. (Ed.): Sugar Analysis ICUMSA Methods; ICUMSA (1979).
7.3 ICUMSA Methods Book (1994).
7.4 ICUMSA, Proceedings of Twenty-first Session (1994), Subject 5.

# METHOD 32
## REDUCING SUGARS - DETERMINATION IN RAW SUGAR BY THE LANE AND EYNON METHOD

### 1.0 SCOPE AND FIELD OF APPLICATION

This Level 2 method has been adopted by the Australian sugar industry.

It is assumed for this method that the reducing sugars are glucose and fructose.

### 2.0 PRINCIPLE OF METHOD

Reducing sugars reduce copper (II) salts, in alkaline solution, to a red precipitate of copper (I) oxide. In the Lane and Eynon method, a 10 mL volume of the copper solution (Fehling's solution) is titrated with the sugar solution until all the copper is reduced.

The Fehling's solution is prepared immediately before use by mixing equal volumes of Fehling's A (copper sulphate solution) and Fehling's B (potassium tartrate/sodium hydroxide solution). The bulk of the test solution is added to the Fehling's solution which is then boiled, and the final amount of the test solution is added to the boiling solution until the copper (II) ions are completely reduced. Methylene blue is added near the end-point as an internal indicator. Reaction time is standardised at 3 ± 0.5 minutes from the start of boiling.

For accurate work at least two titrations are done: one preliminary titration to find the approximate endpoint; then one or more titrations in which all except 1-2 mL of the sugar solution is added before boiling.

The amount of reducing sugars which corresponds to the fixed volume of Fehling's solution varies with the total volume of the reaction mixture and also with the concentration of sucrose (some sucrose is converted to reducing sugars at the high pH). A table is therefore used to calculate the amount of reducing sugars in the test solution.

When the amount of reducing sugars in the test solution is less than 0.6%, a known volume of standard reducing sugar solution is added so that the titration volume remains in the standard range. This procedure is referred to as the first differential method.

### 3.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

Ordinary laboratory apparatus.

3.1 **Bulb pipettes,** Class A. (AS 2166) — 5 mL, 10 mL.

3.2 **Burette**, 50 mL Class A, (AS 2165) graduated in 0.1 mL for the sugar solution. The burette tap is replaced by a rubber tube and pinch-cock, connected to a bent outlet tube in order to keep the graduated section of the burette out of the steam while additions are made to the boiling mixture.

3.3 **Heat resistant plate**, (about 15 cm x 15 cm) with a 5 cm diameter hole.

3.4 **Anti-bumping granules**.

3.5 **Boiling flasks**, (florence flask) 250 mL.

3.6 **Volumetric flask**, Class A. (AS 2164) — 100 mL, 1000 mL.

3.7 **Stainless steel gauze** strainer, about 5 cm dia., 150 micron.

3.8 **Stop-watch**, digital.

3.9 **Bunsen burner**.

3.10 **Balance** weighing to 0.001 g.

### 4.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

4.1 **Fehling's Solution A**. For each litre of solution, dissolve 69.28 ± 0.05 g of cupric sulphate pentahydrate in distilled water. Add about 10 drops of concentrated sulphuric acid and dilute to 950 mL. (The sulphuric acid retards the formation of moulds which can grow in copper sulphate solutions during long storage).

4.2 **Fehling's Solution B**. Dissolve 346 ± 1 g sodium potassium tartrate in distilled water. Add that volume of standardised 5 mol/L sodium hydroxide solution (4.11) which contains 100.0 ± 0.5 g NaOH. Dilute the solution to 1L.

*Safety*

This reagent is strongly caustic. It should not be pipetted by mouth. If in contact with skin, remove all contaminated clothing and wash the affected area with copious quantities of water. If ingested, administer large quantities of 1% acetic acid. Do not induce vomiting.

4.3 **Methylene Blue**, 1 g/100 mL solution in water.

4.4 **Standard Invert Solution**. This solution is used for standardising Fehling's Solution A and contains 0.2048 gram of invert sugar per 100 mL.

4.4.1 Determine the water content of the standard sugar (sucrose) to be used. Take a weight of the undried sugar which will contain 1.946 ± 0.001 g of dry substance and dissolve in about 40 mL distilled water in a 100 mL flask, i.e.

$$\text{weight sugar} = \frac{1.946 \times 100}{(100 - \%H_2O)} \pm 0.001\text{g}$$

4.4.2 Add 3.0 mL of 10 mol/L HCl (4.8) using a 10 mL safety pipette and mix. Heat in a water bath at 68.0 ± 0.5 °C for 10 minutes ± 10 secs. During the first three minutes agitate the flask continuously. Cool and wash the solution into a one litre volumetric flask.

4.4.3 Neutralise the solution with solid sodium carbonate, using a pH 7.0 indicator paper. Dilute to the mark and shake well. This solution must be freshly prepared for each set of titrations of Fehling's Solution A during standardisation.

4.5 **Differential Invert Solution** (for the differential method)

4.5.1 Dissolve 12.0 ± 0.1 g of standard sugar in about 40 mL of distilled water and invert as described above (4.4.2). Cool, add 0.5 ± 0.1 mL 1% phenolphthalein solution, dilute to 1 litre without neutralising and store. The solution will keep for several weeks if not neutralised as the acid prevents the growth of

bacteria. The actual concentration of invert sugar in this solution is not critical, as a blank test is carried out each time it is used.

4.5.2 When required, neutralise with solid sodium carbonate, sufficient of the differential invert solution for the number of titrations to be performed, (each titration requires 10 mL). When correctly neutralised the solution should be faintly pink.
If too much $Na_2CO_3$ is added, add acid invert solution from the stock until correct colour is obtained. Store the neutralised solution in a stoppered reagent bottle.

4.6 **Sucrose** (or standard sugar).

4.7 **Sodium Carbonate**.

4.8 **Hydrochloric Acid** $\rho_{20}$ = 1.17 g/mL.

4.9 **Sulphuric Acid** $\rho_{20}$ = 1.84 g/mL.

4.10 **Phenolphthalein**, 1 g/100 mL solution in alcohol.

4.11 **Sodium hydroxide** 5 mol/L solution. The concentration of this solution must be determined by standardisation so that it is known within 0.02 mol/L.

## 5.0 STANDARDISATION OF FEHLING'S SOLUTION A

The strength of Fehling's Solution A is determined by titration against standard invert solution (4.4). The procedure must be identical to that described in the procedure (7.3 to 7.12). The amount of invert required should be 25.00 ± 0.05 mL; adjustments to the Fehling's Solution A must be made until this is obtained.

5.1 If the Fehling's Solution A is too strong, i.e. end point more than 25 mL, each litre must be diluted by an amount equal to
1000 x [(end point/25.0) - 1.0] mL of water.
Add 95% of the required volume and titrate again.

5.2 If the Fehling's Solution A is too weak, i.e. end point less than 25 mL, each litre must be strengthened by the addition of
69.28 x [1.0 - (end point/25.0)] g of cupric sulphate.
Dissolve 105% of the calculated amount in a portion of the solution, add to the bulk of solution and titrate again. In this way, the strength of the Fehling's Solution A will be finally adjusted by dilution, the simpler procedure. The stock solution should be thoroughly mixed after each adjustment.

## 6.0 STANDARDISATION OF METHOD

The copper-invert sugar equivalents, using various concentrations of sucrose, were established by Lane and Eynon. Provided the conditions specified in their procedure are carefully followed, no separate standardisation is required. Tables XXX and XXXIII are based on the original Lane and Eynon standardisation.

## 7.0 PROCEDURE

A. STRAIGHT METHOD (for raw sugar with >0.6%RS)

7.1 Weigh out 25.0 ± 0.1 g of raw sugar.
Wash the sample into a 100 mL, Class A volumetric flask, add distilled water to 50-60 mL and dissolve by shaking.

7.2 Add distilled water to fill the bulb of the flask and mix well. Make to the mark with distilled water and shake well.

7.3 Using a 5 mL Class A pipette, add 5.00 mL of Fehling's Solution A to a 200 mL boiling flask (3.6). Using a safety pipette add 5.0 ± 0.1 mL of Fehling's Solution B and swirl, to complete the preparation of the alkaline copper solution. Fehling's B should be added just before the titration is to be done.
Add a few pieces of anti-bumping granules to the boiling flask to prevent bumping.

7.4 Rinse (about 10 mL) and fill a clean 50 mL Class A burette (3.3) with titrating solution (7.1). Squeeze the rubber tubing above the pinchcock to remove air-bubbles; open the pinchcock and incline the jet to remove the remaining air-bubbles. Adjust the level of solution to the zero mark.

7.5 Add 15 mL of the solution from the burette to the alkaline copper solution (7.3), swirl, and place the flask on a heat resistant plate (3.4) over a bunsen burner (3.10).

7.6 Bring the mixture to the boil, making sure that the flame does not impinge on the flask above the level of the solution.

7.7 Start the stopwatch when the mixture commences to boil vigorously and adjust the flame to give moderate, even boiling.

7.8 After boiling for 15 secs, if the solution is still blue, add rapidly successive small portions of solution from the burette (but without interrupting boiling) until the reddish colour shows that the end-point is near. With experience this can be judged to within 2-3 mL. This estimated end-point should be reached within the first minute of boiling.

7.9 After 2 minutes of boiling, add 3 drops of methylene blue indicator (4.3).

7.10 Continue titrating, adding a few drops at a time, allowing 5-10 secs between each portion, until the blue colour is completely discharged, and note the final burette reading. The total boiling time should be 3.0 ± 0.5 min. and boiling should not be interrupted during the titration (to prevent air entering the flask and oxidising the contents). For routine work, the above incremental method gives satisfactory results, provided that no more than 3 mL of solution is needed to complete the titration after step 7.8. For accuracy, a second titration is required.

7.11 Prepare another alkaline copper solution (7.3), add all but 1-2 mL of the required amount of titrating solution from the burette, and repeat steps 7.6 and 7.7.

7.12 Continue boiling for 2 minutes before adding the methylene blue indicator and complete the titration as before. The total boiling time should be kept as close as practicable to 3 minutes (but no more than 3.5 minutes). Record the end-point to nearest 0.1 mL. The end point must lie between 17 and 35 mL.

B. 1ST DIFFERENTIAL METHOD (for sugar with 0.02-0.6 % RS)

7.13 For raw sugar, weigh out 25.0 ± 0.1 g. For other products, weigh a suitable quantity that will contain 25 g of sucrose.

Wash the sample into a 100 mL Class A volumetric flask, add distilled water to 50-60 mL and dissolve by shaking.

7.14 Using a 10 mL Class A bulb pipette, add 10.00 mL neutralised differential invert solution (4.5): Add distilled water to fill the bulb of the flask, swirl, make to the mark, and shake well.

7.15 Prepare a "blank" solution by weighing out 25.00 ± 0.01 g standard sugar and repeating steps 7.13 and 7.14.

7.16 Titrate the unknown (7.14) as described in steps 7.3 to 7.12 and record the end point to 0.1 mL.

7.17 Titrate the blank (7.15), as described in steps 7.3 to 7.12, but run in 31 mL solution for the first titration. (The end-point should lie between 32 and 36 mL). Record the end-point to 0.1 mL.

## 8.0 CALCULATIONS

8.1 **Table XXXII** gives the grams of Reducing Sugars per 100 mL of titrating solution corresponding to the titration (7.12 or 7.17) for a sucrose concentration of 25 g/100 mL.

8.2 **Calculate** the % RS in the sample.

**For the straight method:**

$$\%\,RS = \frac{100 \times g\ RS \text{ per } 100\,mL \text{ titrating solution}}{\text{mass sample per } 100\,mL \text{ titrating solution}}$$

**For the differential method:**

$$\%RS = \frac{100 \times [g\ RS \text{ per } 100\,mL \text{ titrating solution} - g\ RS \text{ per } 100\,mL \text{ blank}]}{\text{mass sample per } 100\,mL \text{ titrating solution}}$$

where: mass sample/100 mL titrating solution for raw and refined sugar = 25 g

**Report** the results as % RS in raw sugar to 0.01%

## 9.0 PRECISION

| Sample | Concentration range per cent reducing sugar | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 0.60 - 1.0 (straight method) | 0.030 | Not determined |
| Raw and Refined Sugar | 0.02-0.60 (differential method) | 0.030 | Not determined |

## 10.0 REFERENCES


10.1 De Whalley, H.C.S., ICUMSA Methods of Sugar Analysis; Elsevier (1964).

10.2 Browne, C.A. and Zerban, EW.; Physical and Chemical Methods of Sugar Analysis; Ed. 3; Wiley (1955).

10.3 Honig, P. (Ed.); Principles of Sugar Technology; Vol, 1, Ed. 1., Elsevier (1953).

10.4 Lane, J.H. and Eynon, L.; J.Soc. Chem. Ind.; Vol. 42, (1923).

10.5 Lane, J.H. and Eynon, L.; J.Soc.Chem. Ind.; Vol. 50, (1931).

10.6 ICUMSA 21st Session Proceedings (1994), Subject 15.

10.7 ICUMSA Methods Book (1994).

# METHOD 33
# TOTAL COLOUR (COLOUR INDEX) – DETERMINATION IN RAW SUGAR

## 1.0 SCOPE AND FIELD OF APPLICATION

This Level 1 method has been adopted by the Australian sugar industry.

It is used for the determination of sugar solution colour.

The method can be applied to raw sugars, affined raw sugars and very low colour sugars provided that a filtered solution can be prepared by the procedure specified in the method. The method is designed for sugars in the solution colour range of 100 to 16 000 ICUMSA Units (IU).

## 2.0 DEFINITIONS

2.1 **Transmittance of a solution.** If $I_1$ represents the radiant energy incident upon the first surface of the solution and $I_2$ represents the radiant energy leaving the second surface of the solution. Then:

$$T = \frac{I_2}{I_1} = \text{transmittance of the solution}$$

($100T$ = percentage transmittance)

2.2 **Transmittancy.** Let $T_{soln}$ represent the transmittance of a cell containing the solution and let $T_{solv}$ represent the transmittance of the same or duplicate cell containing the pure solvent. Then:

$$T_s = \frac{T_{soln}}{T_{solv}} = \text{transmittancy of the solution}$$

2.3 **Absorbancy (extinction).**

$$A_S = -\log_{10} T_S = \text{absorbancy of the solution}$$

2.4 **Absorbancy index (extinction index).** Let $b$ represent the length, (cm), of the absorbing path between the boundary layers of the solution and let $c$ represent the concentration, (g/mL), of the sugar solution. Then:

$$a_s = \frac{A_s}{bc} = \text{absorbancy index of the solution}$$

2.5 **ICUMSA Colour.** The value of the absorbancy index multiplied by 1000 is reported as ICUMSA Colour at 7pH and a wavelength of 420 nm. The resulting values are designated as ICUMSA Units (IU).

## 3.0 PRINCIPLE OF METHOD

Sugar is dissolved in water and MOPS buffer pH 7.0, is added; the solution is then filtered through a membrane filter to remove turbidity. The absorbancy of the filtered solution is measured at a wavelength of 420 nm and the solution colour is calculated.

The solution concentration and cell length are chosen to give a transmittancy in the preferred range of 15% to 80%, equivalent to an absorbancy range of 0.8 to 0.1.

The membrane brand and type is specified, and must be adhered to, as different membrane types have been shown to give different results.

## 4.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

4.1 **Spectrophotometer** suitable for the measurement of absorbance at a wavelength of 420 nm, with a spectral band pass 10 nm or less, wavelength reproducibility ± 0.5 nm and absorbance reproducibility ± 0.0003 at 1.0 abs.

4.2 **Matched optical cells** – length 1.0, 2.0, 4.0, 5.0 ± 0.001 cm. For very low colour sugars 4.0 cm cells are appropriate.

4.3 **Membrane filter units** – Millipore Millex-HA, pore size 0.45 µm, diameter 25 mm. These are designed for use with a syringe and have a female Luer-Lok inlet connection. The membrane is made of mixed cellulose esters.

NOTE: Pore size as determined by 'bubble point' testing.

4.4 **Membrane pre-filter units** – Cellulose ester membrane, with pore size 0.80 µm, diameter 25 mm, designed for use with a syringe and having a female Luer-Lok inlet connection and male Luer outlet.

NOTE: Suitable prefilter units include Sartorius Minisart 16592 and Millipore Millex AA.

4.5 **Plastic syringes** – Capacity 10 mL – 50 mL, with male Luer-Lok outlet.

NOTE: Use the smallest syringe that provides sufficient volume of solution for rinsing and filling the optical cell, to minimise the plunger force.

4.6 **pH meter** – to measure to 0.01 pH.

4.7 **Magnetic stirrer**.

4.8 **Ultrasonic bath** – for de-aeration of the filtered sugar solution.

4.9 **Laboratory balance** – readable to 0.01 g.

4.10 **Volumetric flasks,** Class A (AS 2164) - 100 mL.

4.11 **Bulb Pipette**, Class A (AS 2166) – 20mL.

## 5.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity.**

5.1 **MOPS buffer solution, 0.2M.** Dissolve 41.8 ± 0.1 g MOPS (3-(N-morpholino)propanesulfonic acid) in 800 mL distilled water. Clean and dry the electrode of the pH meter and immerse it in the MOPS solution. Adjust the solution pH to 7.00 ± 0.01 by adding approx. 1 mol/L sodium hydroxide solution. Stir the solution continuously with a magnetic stirrer while the pH is being adjusted. (About 80 mL of sodium hydroxide solution may be required.) Remove the pH electrode. Make the solution to 1L.

**Store this solution in a refrigerator, and discard after two weeks.**

## 6.0 PROCEDURE

A. SAMPLE PREPARATION

6.1 Mix the sample of sugar thoroughly. Weigh the quantity of sugar shown in the tabulation overleaf (chosen according to the cell length and sugar colour to give a transmittancy in the preferred range, ie for very low colour sugars – 4 or 5 cm cells and 20 g/100 mL concentration) and wash with water into a 100 mL

volumetric flask, dissolve by swirling at room temperature and add 10 ± 0.1 mL MOPS buffer solution. Make to the mark with distilled water.

6.2 Prepare the reference solution by adding 10 ± 0.1 mL MOPS buffer solution to a 100 mL volumetric flask, and making to the mark with distilled water.

B. SAMPLE FILTRATION

6.3 Connect a pre-filter unit (4.4) to the syringe (4.5), then connect the 0.45 µm membrane filter (4.3) to the pre-filter unit. Filter sufficient of the sample solution into a clean, dry sample beaker. Change filters if solutions are slow filtering to obtain the necessary volume. Do not filter the last few drops of the solution, to avoid introducing bubbles into the filtered solution.

6.4 Similarly filter the reference solution (6.2).

NOTE: If necessary de-aerate the filtered solution by immersing the sample beaker in an ultrasonic bath for 3 minutes, or by covering the beaker, and allowing it to stand for 1 hour.

Aliquot of sugar for colour measurement

| Cell length (*b*) cm | ICUMSA Colour Range IU | Conc (*c*) g/mL | Sugar aliquot g |
|---|---|---|---|
| 5 | 100 – 800 | 0.2 | 20 ± 0.04 |
| | 200 – 1600 | 0.1 | 10 ± 0.02 |
| | 400 – 3200 | 0.05 | 5 ± 0.01 |
| 4 | 125 – 1000 | 0.2 | 20 ± 0.04 |
| | 250 – 2000 | 0.1 | 10 ± 0.02 |
| | 500 – 4000 | 0.05 | 5 ± 0.01 |
| 2 | 250 – 2000 | 0.2 | 20 ± 0.04 |
| | 500 – 4000 | 0.1 | 10 ± 0.02 |
| | 1000 – 8000 | 0.05 | 5 ± 0.01 |
| 1 | 500 – 4000 | 0.2 | 20 ± 0.04 |
| | 1000 – 8000 | 0.1 | 10 ± 0.02 |
| | 2000 – 16000 | 0.05 | 5 ± 0.01 |

Note: It is permissible to use smaller volumetric flasks, adjusting the sugar aliquot and buffer volume in proportion, providing that the sugar aliquot is not less than 5 g.

C. MEASUREMENT OF ABSORBANCE

6.5 Determine the cell correction at 420 nm on a matched pair of appropriately sized cells (4.2), using the reference solution (6.4).

6.6 Read the absorbance of the sample test solution using the reference solution as the blank.
Record the absorbance of the sample test solution at 420 nm to 0.001 units.

## 7.0 CALCULATIONS

7.1 Calculate the concentration in g/mL of the test sugar solution (6.1).

7.2 Apply any cell correction (6.5) to the absorbance at 420 nm (6.6).

7.3 Calculate the absorbancy index of the test sugar solution:

$$a_S = \frac{A_S}{bc}$$ where $A_S$ = absorbancy of the solution at 420 nm
$b$ = cell length (cm)
$c$ = concentration (g/mL) of the sugar solution

7.4 The ICUMSA colour is given by:

$$\text{ICUMSA Colour} = a_s \times 1\,000 \text{ IU}$$

For results over 1 000 IU, express results to the nearest 10 IU; for results under 1 000 IU, express results to the nearest 1 IU.

## 8.0 RANGE AND PRECISION

| Sample | Concentration Range Colour Units | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Very low colour sugar | 100-400 | 25 | 69 |
| Very high pol sugar | 400-1200 | 90 | 200 |
| High pol sugar | 1200-2500 | 90 | 350 |
| Low pol sugar | 2500-5000 | 150 | 530 |

## 9.0 REFERENCES

9.1 Schneider F, ed. (1979): Sugar Analysis: ICUMSA Methods, 125–126.

9.2 Nguyen H H, Rowe G S, McCunnie K A, and Player M R (1996): "Rapid analytical methods for colour and ash in raw sugar", Proc 18th Conf Aust Soc Sugar Cane Technol, 341–349, reprinted in Sugar Journal (May 1997) 25–28.

9.3 Nguyen H H, Rowe G S, McCunnie K A, and Player M R (1997): "Rapid analytical methods for colour and ash in raw sugar – Results of 1996 season", Proc 19th Conf Aust Soc Sugar Cane Technol, 387–3954.

9.4 Referees Report GS1, Proceedings ICUMSA 22nd Session, 1998.

# METHOD 34
## AFFINED COLOUR — DETERMINATION IN RAW SUGAR

### 1.0 SCOPE AND FIELD OF APPLICATION

This method is used by CSR Central Laboratory to assess the affining quality of raw sugars. It follows closely the method developed by the Amstar Corporation and included in their general contract provisions. Similarly based procedures are included in nearly all USA raw sugar contracts.

Affined colour represents the colour to be expected after a raw sugar has passed through a refinery affination station. It is the sum of the colour in the residual film of syrup coating which has not been possible to remove under the standard conditions of the test and the colour included in the raw sugar crystal itself. Affined sugar colour represents the colour load to be expected in the main course of a refinery.

The first part of this test can be used to prepare a raw sugar for analysis of any affined sugar parameter.

The method, which is applicable to all raw sugars, consists of two parts:

(i) Removal of that part of the syrup coating the raw sugar crystal that is likely to be removed during refinery affination.

(ii) Determining the colour of the affined sugar after dissolving it in water.

### 2.0 PRINCIPLE OF METHOD

The sugar is mixed with 64.0 °bx high purity liquid sugar and the resultant mixture (magma) centrifuged. The affined sugar is spread in a thin layer to dry and the dried, affined sugar is stored for the determination of colour (or any other affined sugar parameter). The colour of the affined sugar is measured (after dissolving in water and adjusting the pH) as the absorbance at 420 nm, corrected for turbidity measured at 720 nm.

### 3.0 APPARATUS

Ordinary laboratory apparatus and glassware and

3.1 **Centrifuge,** fitted with 200-230 mm dia basket, faced with 500 $\mu$m mesh metal screen, capable of operating at 3000 r/min.

3.2 **Mixer,** with flat beaters and approx. 2L bowl (Kitchen-Aid-Model K5, with No. K-5-A-B flat beaters, from Hobart Manuf. Co. Troy, Ohio, USA) or similar mixer.

3.3 **Dispensing burette,** or peristaltic pump, to deliver liquid sugar at constant rate of 84-85 mL/minute.

3.4 **Balance,** to weigh 1000 ± 5 g.

3.5 **Sheets glazed paper,** about 1 m².

### 4.0 REAGENTS

4.1 **Liquid Sugar,** 64.0 °bx. Prepare from high quality refined sugar or from superfine liquid sugar. Adjust to 64.0 °bx at 20 °C.

### 5.0 PROCEDURE

A. AFFINATION

5.1 Weigh 1000 ± 5 g of well mixed raw sugar into the mixer bowl (3.2). Turn the mixer on to low speed (No. 1 setting approximately 100 r/min).

5.2 With a dispensing pipette or pump (3.3), add 380 mL of 64 °bx liquid sugar at a uniform rate over 4.5 minutes (84-85 mL/minute).

5.3 Continue to mix the raw sugar and liquid sugar for one more minute. The total mixing time from the commencement of adding liquid sugar is 5.5 minutes.

5.4 Immediately transfer the entire magma (raw sugar and liquid sugar mixture) to the centrifuge basket (3.1).

5.5 Bring the centrifuge up to 3000 r/min in 15 seconds and spin at 3000 r/min for exactly two minutes.

5.6 Remove the sugar from the centrifuge basket and spread on the glazed paper in a layer not to exceed 5 mm thick.

5.7 Allow the sugar to dry (in a clean dust free area). Mix the sugar periodically by hand, with a flat spatula, so that the sample dries uniformly and is well mixed.

5.8 Store the dry affined sugar in a sealed heavy-duty plastic bag for subsequent determination of colour or other parameters.

B. COLOUR DETERMINATION

Measure the colour of the affined sugar according to the procedure described in the Method No. 33 "Total Colour (Colour Index) — Determination in Raw Sugar".

### 6.0 CALCULATION

**Follow** the procedure in Method No. 33 and calculate the colour index of the affined sugar.

**Record** the colour index of the affined sugar to the nearest 10 colour units.

### 7.0 PRECISION

| Sample | Concentration Range (Colour Units) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 500-1500 | 96 | Not Determined |

### 8.0 REFERENCE

Method No. 33, "Total Colour (Colour Index) — Determination in Raw Sugar".

# METHOD 35
# AMINO NITROGEN – DETERMINATION IN RAW AND REFINED SUGAR, MILL AND REFINERY LIQUORS AND OTHER PRODUCTS

## 1.0 SCOPE AND FIELD OF APPLICATION

This Level 3 method is recommended for use within the Australian sugar industry. It is not materially different to CSR Ltd. A & M Specifications Method 06 (1995).

This method is applicable to all raw sugars and liquors. It can be used for refined sugar, refinery products and other mill products provided the sample weight taken for analysis is modified accordingly.

The ninhydrin/hydrindantin method is sensitive to other organic nitrogen compounds, so the analysis must not be regarded as an absolute measure of amino acid content. However, it measures all the major amino acids likely to be present in raw sugar and its liquors with adequate accuracy, and gives results which assist in the prediction of the likelihood of colour formation in raw sugars.

## 2.0 PRINCIPLE OF METHOD

Ninhydrin reacts with amino-acids and ammonium ions to give a blue-violet colour which can be extracted into an aqueous solution of isopropanol. The absorbance of this solution is measured at 570 nm.

Standard solutions are prepared with L-aspartic acid because asparagine and aspartic acid are the major amino acids occurring in raw sugar.

## 3.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

Ordinary laboratory apparatus and glassware and

3.1 **Volumetric flasks**, Class A, (AS 2164), 100 mL.

3.2 **Test tubes**, pyrex, calibrated to 15 mL with stoppers (Quickfit type, B19/26 neck).

3.3 **Metal test-tube rack.**

3.4 **Boiling water bath.**

3.5 **Bulb pipettes**, Class A, (AS 2166) 1.0 mL, 2.0 mL, 3.0 mL, 4.0 mL, 5.0 mL.

3.6 **Automatic pipette** to deliver 1.0 ± 0.1 mL.

3.7 **Balance** with a discrimination of 0.0001 g.

3.8 **Spectrophotometer** with matched 1.0 cm cells.

3.9 **Stop watch.**

## 4.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

4.1 **Acetate Buffer Solution** (pH 5.5). Weigh out 54.4 g of sodium acetate trihydrate into a 100 mL volumetric flask and add 40 mL water. Heat in a hot bath and shake until dissolved. Cool, and add 10 mL of glacial acetic acid, mix, make to mark, and shake well. Check the pH of the solution which should be 5.51 ± 0.03. If necessary adjust pH using glacial acetic acid or NaOH (0.1 g of NaOH is approximately equivalent to 0.04 pH units). This solution keeps indefinitely under normal refrigerated conditions (approx. 4°C).

4.2 **2-methoxyethanol, (Methyl Cellosolve)** peroxide free.

*Safety*

2-methoxyethanol is a suspected carcinogen. Safety gloves must be worn at all times.

4.3 **Ninhydrin/Hydrindantin Solution.** For the preparation of 400 mL volume, weigh out 8.0 g ninhydrin powder and 1.2 g hydrindantin powder and dissolve in 300 mL of 2-methoxyethanol (4.2). Add 100 mL of buffer (4.1). Transfer immediately to a clean dry brown reagent bottle previously purged with nitrogen or carbon dioxide gas. Insert the automatic pipette (3.6) and carefully add 25 mL of laboratory grade paraffin oil to form a layer over the liquid surface. Cover the bottle with aluminium foil and store in a refrigerator.

The reagent must not be placed in direct sunlight when being used.

This reagent must be discarded after 3 weeks.

Before using the reagent run a little to waste to clear the delivery tip of the automatic dispenser.

*Safety*

Ninhydrin is a poison and irritates the skin. It is a suspected carcinogen. Safety gloves must be worn at all times. The addition of 2-methoxyethanol to the ninhydrin/hydrindantin powders should be carried out in a fume cupboard.

4.4 **Propan-2-ol Solution** (50% v/v). Dilute 50 mL of Propan-2-ol to 100 mL with water.

4.5 **Standard Solution of L-Aspartic Acid** (100 mg/L of nitrogen). Weigh out 0.0951 ± 0.0001g of L-Aspartic acid. Wash into a 100 mL volumetric flask, dissolve and make to the mark with distilled water. This solution must be prepared freshly before use.

## 5.0 STANDARDISATION

5.1 **Working Standards.** With Class A bulb pipettes (3.5) pipette aliquots of the standard L-Aspartic acid solution into separate 100 mL volumetric flasks (3.1) as shown in the following table. Use a 0.1 mL Class A graduated barrel pipette for the 0.5 mL aliquot. Make each flask to the mark with distilled water and mix well.

| Volume of Standard L-Aspartic Acid Solution (4.5) | Amino Nitrogen in Working Standards (expressed as Amino Nitrogen in Aspartic Acid) | Concentration of Amino Nitrogen in 15 mL test solution |
|---|---|---|
| mL | mg N/L | mg N/15 mL |
| 0.5 | 0.5 | 0.005 |
| 1.0 | 1.0 | 0.01 |
| 2.0 | 2.0 | 0.02 |
| 3.0 | 3.0 | 0.03 |
| 4.0 | 4.0 | 0.04 |
| 5.0 | 5.0 | 0.05 |

5.2 Work with sets of six test tubes at a time; two marked for blanks and two appropriately marked for each of the standard dilutions.
Pipette 1.00 ± 0.01 mL of distilled water in each of two test tubes for blanks and 1.00 ± 0.1 mL of each of two standard solutions into each pair of remaining test tubes.

5.3 Using an automatic pipette (3.6) add 1.0 ± 0.1 mL of ninhydrin/hydrindantin solution (4.3) to each test tube and mix by swirling. Avoid excess splashing of reagent on the sides of the test tube.

5.4 Plug the necks of the test tubes loosely with cotton wool and place them in the metal test tube rack.

5.5 Place the rack containing the test tubes into a boiling water bath for 15 minutes ± 10 seconds. Ensure the water level is higher than the level of solutions in the test tubes.

5.6 Remove from the bath and immediately dilute with 50% v/v isopropanol solution (4.4) to about 5 mm below the 15 mL mark. Stopper the tubes and shake vigorously for 15 seconds.

5.7 Cool in cold water for 15 minutes then on the bench out of sunlight for 30 minutes. Dilute each solution to the 15 mL mark with 50% v/v isopropanol solution (4.4) and mix again, for 15 seconds. The solutions must be read within the next 30 minutes.

5.8 Read the absorbance of each standard blank and each standard solution in a spectrophotometer at 570 nm in matched 1.0 cm cells using water as the reference solution. Record the absorbance to 0.001 units.

5.9 Subtract the average of the absorbance of the 2 standard blanks from each of the standard solutions.

5.10 Plot the absorbance of each standard solution against the concentration of nitrogen in mg/L.
As a guide the absorbance of the 2.0 mg/L nitrogen standard should read about 0.195 absorbance units.

5.11 The calibration line should be established from duplicate results by two independant analysts.
The standardisation is repeated if:

- two or more points lie off the line of best fit by more than 5% of the absorbance value at the particular point;
- the line of best fit does not pass within 0.003 absorbance units from the origin;
- the slope of the calibration line obtained by individual analysts differs by more than 2%.

## 6.0 PROCEDURE

6.1 Weigh out 5.00 ± 0.02g for a high pol sugar
3.00 ± 0.02g for a low pol sugar
0.50 ± 0.02g for a mill liquor
15.00 ± 0.02g for refinery raw liquor
30.00 ± 0.02g for refinery fine liquor.
Measure and record the brix of the liquor.

6.2 Wash the sample into 100 mL volumetric flask and completely dissolve the sample in approximately 60 mL of distilled water. Make the solution to the mark, stopper the flask and mix well.

**NOTE:** One standard solution, ie 3 mg N/L, should be checked with each batch of samples analysed. If different by more than 5% of the calibration absorbance value, prepare a new ninhydrin/hydrindantin solution and repeat the procedure.

6.3 Pipette 1.00 ± 0.01 mL of distilled water into two test tubes. These are the test blanks.

6.4 Pipette 1.00 ± 0.01 mL of the test solution (6.2) into a test tube.

6.5 Using an automatic pipette add 1.0 ± 0.1 mL of ninhydrin/hydrindantin solution to each test tube. Mix by swirling. Avoid splashing the reagent on the walls of the test tubes.

6.6 Plug the necks of the test tubes loosely with cotton wool and place them in the test tube rack.

6.7 Place the rack with test tubes into a boiling water bath for 15 minutes ± 10 seconds. Ensure that the water level in the bath is higher than the level of the solutions in the test tubes.

6.8 Remove from the water bath and immediately dilute each solution with 50% v/v isopropanol solution (4.4) to 5 mm below the 15 mL mark. Stopper and shake vigorously for 15 seconds in pairs. Cool in a cold water bath for 15 minutes, then on the bench out of sunlight for 30 minutes.

6.9 Make to 15 mL mark with 50% v/v isopropanol solution and mix, again for 15 seconds. The solutions must be read within the next 30 minutes.

6.10 Read the absorbance of the blanks and test solutions in a spectrophotometer at 570 nm in matched 1.0 cm cells using water as a reference solution.

## 7.0 CALCULATIONS

7.1 Average the two blank absorbance readings and subtract the average blank absorbance from the absorbance of each test solution.

7.2 Read the mg N in 15 mL test solution directly from the calibration graph.

7.3 The amino nitrogen content in the sample mg/kg (expressed as Amino Nitrogen in L-Aspartic acid) is given by :

$$\text{Amino Nitrogen (mg/kg)} = \frac{\text{mg/L N, in 15 mL test solution}}{\text{sample weight}} \times 100$$

7.4 Record the mg/kg amino nitrogen, expressed as amino nitrogen in L-Aspartic acid to the nearest whole number.

## 8.0 PRECISION

| Sample | Concentration Range mg/kg N (as Amino Nitrogen in L-Aspartic Acid) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw Sugar | 20-200 | 5.5 | Not determined |

## 9.0 REFERENCES

9.1 ICUMSA, Report of the Proceedings 14th Session (1966): Subj. 18.

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# METHOD 36
## FILTRABILITY — DETERMINATION IN RAW SUGAR BY THE CELITE METHOD

### 1.0 SCOPE AND FIELD OF APPLICATION
This Level 1 method has been adopted by the Australian sugar industry.
The filtrability result of a raw sugar reflects the efficiency of the mill clarification process and is broadly indicative of refinery carbonatation/filtration performance.

### 2.0 DEFINITION
The filtrability value of a raw sugar is the percentage ratio of the filtration rate of a solution of the raw sugar with that of a solution of pure sugar of the same concentration, filtered under identical conditions.

### 3.0 PRINCIPLE OF METHOD
A raw sugar solution is made up to 60.0 ± 0.5° Bx, and Celite 505 and buffer are added to this solution. The buffer brings the pH of the solution to between 8.5 and 9.1. The solution is filtered under constant pressure of 345 kPa. Filtrate is initially run to waste for two minutes, to allow a uniform Celite filter cake to form. The filtrate is then collected over a precisely timed five minute period, weighed and expressed as a percentage of the weight of pure sucrose solution which would be collected under identical conditions of density, pH, temperature and pressure.
The per cent filtrability is defined at 20 ± 1°C. At temperatures above 20°C, a lower filtrability is obtained, and at temperatures below 20°C the filtrability is higher. If a test is done at a temperature other than 20 ± 1°C, the result can be expressed only as "filtrability at t°C".

### 4.0 APPARATUS
**All apparatus should be visually checked for damage prior to use.**
Ordinary laboratory apparatus and glassware and

4.1 **Pressure filter**, of brass or stainless steel including cylinder body, screw on top with air hose connection, screw on base, filter disc, retaining ring and rubber gaskets (Figure 1).

4.2 **Support stand** and locking ring.

4.3 **Variable speed stirrer**, capable of being controlled at 900 ± 100 r/min with either three blade impeller (38 mm diameter with 12 mm square blades, pitch 20°) or 35 mm propeller type blade (11 mm width, pitch 20°).

4.4 **Compressed air** or nitrogen supply, with reducing valve, regulator, safety relief valve, three-way valve and Bourdon type pressure gauge with a range of 0-700 kPa.

4.5 **Balance**, with a capacity of 1000 g and a resolution of 0.01 g.

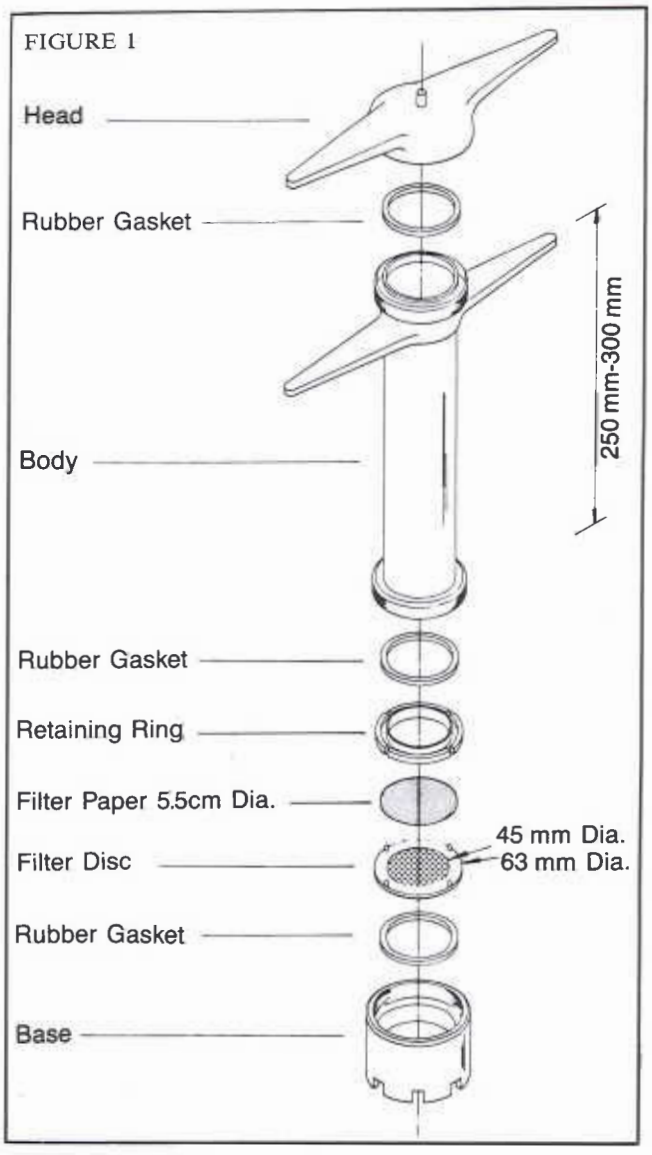
4.6 **Stopwatch**, digital.

4.7 **Thermometer**, 200-400 mm long, 10-35°C, reading to 0.2°C.

4.8 **Pipette**, 5.0 ± 0.1 mL [graduated straight pipette or automatic pipette].

4.9 **Dispenser**, to deliver 99.4 ± 0.1 mL.

FIGURE 1




### 5.0 REAGENTS
**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

5.1 **Filter Aid - Celite 505**. For the purpose of raw sugar quality analysis in Queensland, suitable standardised Celite 505 can be obtained from the laboratory contracted to perform raw sugar analysis by Queensland Sugar Limited. It is accompanied by a table (Table XVII) which gives the mass of pure syrup filtered between 2 and 7 minutes after application of pressure, under the standard condition of the test. Each table is specific to a particular batch of Celite 505.

5.2 **Filtrability Buffer**

5.2.1 Prepare 1L of a 50% (m/m) solution of glycerol in water.

5.2.2 Dissolve 15.0 ± 0.1 g of calcium acetate in 200-300 mL of 50% glycerol solution in a 500 mL beaker.

5.2.3 Dissolve 400.0 ± 0.5 g of triethanolamine in 200-300 mL of 50% glycerol solution in a 500 mL beaker.5.2.4 Transfer the calcium acetate solution and the triethanolamine solution to a 1L volumetric flask, rinsing both beakers with 50% glycerol solution

5.2.5 Make up to 1L with 50% glycerol solution, mix well and allow to stand overnight. Add a teaspoon of Celite 505 and filter in a Buchner filter through a No. 54 filter paper. The filtered solution can be stored for 6 months.

5.2.6 The pH of the test solution should be greater than 10.5 pH.

*Safety*. Triethanolamine is a mild eye and skin irritant and wearing safety glasses and rubber gloves is recommended.

5.3 **Sucrose**, or high purity refined sugar certified starch free.

5.4 **Filter paper**, Whatman Grade 54, 5.5 cm diameter. The filter papers are selected from the one "making" and should have a mean Gurley time of 7.6 seconds [air flow measured by Gurley Densometer]. For the purpose of raw sugar quality analysis in Queensland, suitable selected Whatman 54 filter papers can be obtained from the laboratory contracted to perform raw sugar analysis by Queensland Sugar Limited.

## 6.0 CALIBRATION OF FILTER DISC

The filter disc calibration is checked by measuring the filtration rate of a pure sugar syrup (60.0° Bx), filtered with the Celite under the same conditions as the test.

6.1 Prepare a 60.0° Bx solution of starch-free refined sugar (5.3) by dissolving 600 ± 5 g of refined sugar in water and diluting to 1000g.

6.2 Add 15 g of Celite 505 (2.5% by weight on solids) and mix well. Filter the syrup through a fresh Whatman 54 filter paper (in a Buchner funnel, under vacuum or in a pressure filter). Discard the first 40-50 mL of filtrate.

6.3 Transfer the filtered syrup to a 1L conical flask and mix well. Check the brix by refractometer. Adjust the syrup to 60.0 ± 0.1° Bx with distilled water or refined sugar. Stopper the flask and mix well.

6.4 Weigh 333 g of the 60.0° Bx pure sugar syrup (6.3) into a 500 mL beaker, i.e. 200 ± 0.1 g solids.

6.5 From Table XVII, determine the correct amount of Celite 505 to be added to 200 g solids of pure sugar syrup *for the particular batch of Celite 505.*

6.6 Weigh the correct weight of Celite 505 into the 200 g solids of pure sugar. Stir the mixture with an electric stirrer at 900 ± 100 r/min (see 7.4).

6.7 Add, while stirring, 1.00 ± 0.02 mL filtrability buffer (5.2) with a graduated pipette. Continue stirring for 2 minutes ± 10 seconds after adding buffer. Cover and allow to stand for 15 minutes ± 30 seconds.

6.8 Filter the syrup following the procedure outlined in sections 7.6-7.13.

6.9 The mass of filtrate should be within 4% of the mass given in Table XVII for the particular batch of Celite.

NOTE: If the solution blows through before the completion of 7 minutes filtration repeat from step 6.4 using an increased quantity of pure sugar syrup (additional Celite will also be required to maintain the correct percentage on solids for the particular batch of Celite).

## 7.0 PROCEDURE

A. PREPARATION OF RAW SUGAR SOLUTION

7.1 Weigh out in a 250 mL beaker, the appropriate amount of sugar from the following Table:

| % Water in Sugar | Mass of Sample ± 0.05 g | |
|---|---|---|
| | Average filtrability sugars | High filtrability sugars |
| 0.0-0.2 | 149.00 | 198.70 |
| 0.2-0.5 | 150.00 | 200.00 |
| 0.5-0.8 | 151.60 | 201.50 |
| 0.8-1.0 | 152.60 | 202.70 |

7.2 Add 99.4 ± 0.1 mL at 20°C (equivalent to 99.1 ± 0.1 g) distilled water from an automatic pipette or by weighing. For high filtrability sugars, add 132.5 ± 0.1 mL at 20°C (equivalent to 132.1 ± 0.1 g) distilled water.

7.3 From Table XVII, determine the correct mass of Celite 505 to be added. Add in the dry form, the correct mass of Celite 505 to the sugar in the 250 mL beaker.

NOTE (1) Table XVII is applicable to a specific batch of standardised Celite 505 and should only be used with that batch.

(2) Before use, mix the Celite by gently tumbling in the bottle.

7.4 Dissolve the sugar without heating, using an electric stirrer rotating at 900 ± 100 r/min. *Check visually to ensure that all crystals are dissolved.* This usually requires 20-30 minutes stirring although low filtrability sugars may take longer to dissolve.

If the stirring time exceeds 45 minutes, the analysis should be repeated.

NOTE: To avoid aerating the solution, the stirrer shaft must be vertical and centred in the vessel. The base of the shaft should be 1 cm above the bottom of the vessel. To minimise grinding of Celite 505 particles, stirrer speed must be 900 ± 100 r/min, and stirring time (before addition of buffer) should be less than 45 minutes.

7.5 With the stirrer still going, add 2.00 ± 0.02 mL standard buffer (5.2) (down the side of the beaker to prevent aeration), or 3.00 ± 0.3 mL for high filtrability sugars, to the solution. Mix for 2 minutes ± 10 seconds after addition of all buffer. Switch the stirrer off and remove from the solution. Cover the beaker and allow to stand for 15 minutes ± 30 seconds.

B. FILTRATION

7.6 Assemble the filter in the order: base, rubber gasket, filter disc, filter paper, retaining ring, second rubber gasket. Screw the assembled end on to the body of the filter. See Figure 1.

7.7 Tare 150 mL beaker to 0.1 g and record mass ($m_1$).

7.8 At the end of the 15 minutes ± 30 seconds standing time, stir solution (7.5), using electric stirrer, for 1

minute ± 10 seconds. Switch off the stirrer and remove from the solution. Transfer all the solution to the filter.

7.9 Screw the top on to the filter body, place in stand and clamp tightly, connect pressure hose, and apply pressure of 345 ± 3.5 kPa (50.0 ± 0.5 psig). Start stopwatch immediately pressure is applied.7.10

Run filtrate to waste for 2 minutes ± 1 second then collect the filtrate in the previously tared 150 mL beaker (7.7) for the next 5 minutes ± 1 second.

7.11 At 7 minutes ± 1 second after application of pressure, remove beaker.

7.12 Weigh beaker and contents to 0.1 g and record the mass ($m_2$). Read the temperature of the filtrate to 0.1°C and record (T).

7.13 Turn off compressed gas, release pressure from filter, remove air hose, unclamp filter from stand, and open it. Wash and dry the filter, and reassemble it for the next run.

## 8.0 CALCULATIONS

8.1 **Calculate** the mass of filtrate delivered during the interval from 2 to 7 minutes after the application of pressure:

Weight filtrate = $m_2 - m_1$

where: $m_1$ = mass beaker (7.7)

$m_2$ = mass beaker and filtrate (7.12)

8.2 **Determine** the weight of pure 60° Bx sugar syrup that would have filtered between 2 and 7 minutes after application of the pressure at the temperature T, from Table XVII.

**Table XVII** gives mass of pure sugar syrup filtered (between 2 and 7 minutes after application of pressure) at various temperatures, under the standard conditions of the filtrability test.

NOTE: The table is applicable to specific batches of Standard Celite 505. It should only be used in conjunction with the appropriate batch.

8.3 Calculate the mass of raw sugar syrup filtered in the 2-7 minute interval as a percentage of the weight of pure sugar syrup that would have been filtered in the same time and at the sample temperature.

% Filtrability

$$= \frac{\text{mass filtrate test solution at Temp. T}}{\text{mass filtrate pure sucrose at Temp. T}} \times \frac{100}{1}$$

**Record** the result to 0.1% filtrability.

## 9.0 PRECISION

| Sample | Concentration Range (% Filtrability) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 0 - 100 | 3.3 | 6.0 |

# METHOD 37
## STARCH — DETERMINATION IN RAW SUGAR

### 1.0 SCOPE AND FIELD OF APPLICATION

This Level 1 method has been adopted by the Australian sugar industry.

Cane starch is composed of amylose and amylopectin with amylose being the fraction that forms a blue colour with iodine. *Cane starch* can be occluded in the raw sugar crystal and affects the filtration rate of liquor from the refinery carbonatation process. The amylose fraction is responsible for this effect.

### 2.0 PRINCIPLE OF METHOD

The sugar is dissolved in water and the solution is digested with hot calcium chloride/acetic acid to solubilise any starch present. Potassium iodide/iodate solution is added to form the blue starch-iodine complex. The absorbance of this complex is read in a spectrophotometer at 700 nm. At this wavelength, the effect on the absorbance of the impurities in raw sugar is minimal.

### 3.0 APPARATUS

**All apparatus should be visually checked for damage prior to use.**

Ordinary laboratory apparatus and glassware

3.1 **Spectrophotometer** suitable for the measurement of absorbance at 700 nm with a set of matched 2 cm cells.

3.2 **Water bath** capable of being controlled at 95-100°C.

3.3 **Flask shaker**.

3.4 **Volumetric flask**, with wide neck (Kohlrausch), 50 mL capacity.

3.5 **Bulb pipettes**, Class A (AS 2166), 1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 10 mL, 15 mL, 20 mL

3.6 **Automatic dispenser**, 15 mL

3.7 **Balance**, to weigh up to 100.00 ± 0.01g.

### 4.0 REAGENTS

**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity.**

4.1 **Calcium Chloride Solution 40% (m/m)**. Dissolve 53.0 ± 0.1 g of calcium chloride dihydrate in distilled water and dilute to 100.0 ± 0.1 g with distilled water. As calcium chloride is highly deliquescent, stocks should not be exposed to air. The strength of the calcium chloride solution must be checked and, if necessary, adjusted to 40.0 ± 0.3% (m/m) with distilled water or with calcium chloride dihydrate (approximately 75.5% $CaCl_2$).

The solution strength can most readily be checked by measuring its specific gravity. The specific gravity of 40% m/m calcium chloride solution is 1.3942 at 24°C. Within the temperature range 20-30°C, it decreases on an average by 0.0007 per degree above 24°C and increases by the same amount per degree below 24°C. In the region of 40% (m/m), a change of 0.1% in calcium chloride concentration produces a change of 0.001 in specific gravity.

Specific gravity may be measured by pycnometer, hydrometer or other instrument capable of the accuracy required. A convenient method is as follows:

(i) allow stoppered containers of calcium chloride solution and distilled water to stand for a number of hours (e.g. overnight) so that both reagents come to ambient temperature.

(ii) Weigh a clean, dry 100 mL volumetric flask to 0.02 g. Fill to the mark with calcium chloride solution and weigh again to 0.02 g. Note the temperature of the solution to 0.1°C.

(iii) Rinse out the flask and fill to the mark with distilled water at the same temperature as the calcium chloride solution, and weigh to 0.02 g. From the masses of the solution and water, and the temperature, calculate the specific gravity to 0.001.

4.2 **Acetic Acid (1 mol/L approx.)**. Dilute 57 ± 1 mL of glacial acetic acid ($\rho_{20}$= 1.049 g/mL) to one litre with distilled water.

4.3 **Acetic Acid (0.033 mol/L approx.)**. Dilute 3.3 ± 0.03 mL of 1 mol/L acetic acid (4.2) to 100 mL with distilled water.

4.4 **Calcium Chloride-Acetic Acid Reagent**. Using a pH meter and stirrer, adjust the pH of a 100 mL aliquot of calcium chloride solution (4.1) to pH 3.0 ± 0.1 with 0.033 mol/L acetic acid. Because of the large salt concentration, allow sufficient time for the pH meter to equilibrate.

Adjust the pH of the bulk of the calcium chloride solution in the same proportion. Although the pH of a calcium chloride/acetic acid solution tends to alter on standing, do not readjust to pH 3.0 before using.

4.5 **Potassium Iodate Solution (0.0017 mol/L)**. Dry about 0.5 g potassium iodate at 105-110°C for 1 hour. Dissolve 0.3566 ± 0.0002 g of the dried reagent in distilled water and make to 1 litre. Store in the dark in a brown, glass-stoppered bottle.

4.6 **Potassium Iodide 10% (m/v)**. Dissolve 10.0 ± 0.1 g of potassium iodide in distilled water and dilute to 100 mL. Store in the dark in a brown, glass-stoppered bottle. Discard the solution if it becomes yellow.

4.7 **Potassium Iodide-Potassium Iodate Reagent**. This reagent must be prepared on the day it is to be used. Mix 10.0 ± 0.5 mL of potassium iodide solution (4.6), with 90.0 ± 0.5 mL of distilled water. To this solution add 100.0 ± 0.5 mL of potassium iodate reagent. Mix the reagents and keep in a brown, glass-stoppered bottle. This reagent must be discarded after 1 day.

4.8 **Standard Starch Solution (900 mg/L)**. Use an approved batch of B.D.H. Laboratory Reagent Potato Starch. Determine its moisture content, correct to 3 decimal places, by drying about 2 g (weighed to 0.0001 g) at 105-110°C for 2 hours.

4.8.1 Weigh into a 25 mL beaker $\frac{(0.9000 \times 100)}{(100 - \%H_2O)}$ ± 0.0001 g of fresh starch, i.e. equivalent to 0.9000 ± 0.0002 g of anhydrous starch.

4.8.2 To the weighed quantity of starch, add 5 mL of cold distilled water and mix with a glass rod. Before the starch settles, transfer the mixture quantitatively to 500 mL of boiling water in a litre conical flask so that no slurry touches the wall of the flask.

4.8.3 With at least three additional 5 mL portions of distilled water, transfer all the starch to the flask. This operation should be completed within 1 minute.

4.8.4 Boil the starch for 3 minutes ± 10 seconds, timed from the moment the first 5 mL of starch slurry enters the boiling water.

4.8.5 Rinse a 1 litre volumetric flask with hot distilled water. Quantitatively transfer the hot solution through a glass funnel to the 1 litre volumetric flask. Wash the conical flask at least twice with hot distilled water, by adding the water to the 25 mL beaker (4.8.1) and then transferring it to the conical flask. Continue washing the conical flask with hot distilled water and transferring to the volumetric flask until the latter is filled to approximately 900 mL.

4.8.6 Swirl to mix the flask contents and cool under running water to room temperature. Make the solution to 1 litre, stopper and mix well. Store in a refrigerator.

NOTE: The solution will keep for one week, but if possible, the standardisation should be done on the same day as the solution is prepared, to eliminate all possibility of its deterioration.

4.9 **Standard Starch Solution (180 mg/L)**. Using a Class A bulb pipette, pipette 20.00 mL of the 900 mg/L standard starch solution (4.8) into a 100 mL volumetric flask. Dilute to 100 mL with distilled water. Mix well by inverting and shaking.

This reagent will not keep and must be prepared on the day the standardisation is carried out.

4.10 **Starch Free Sucrose**. Use only sugar, tested to be free of starch.

## 5.0 STANDARDISATION OF METHOD

A. PREPARATION OF STANDARDS

5.1 Weigh 3.60 ± .02 g of starch-free sucrose (4.10) into each of six 50 mL Kohlrausch volumetric flasks (3.4).

5.2 Pipette, using Class A bulb pipettes 0, 1.00, 2.00, 3.00, 4.00, 5.00 mL aliquots of the 180 mg/L standard starch solution (4.9) respectively into each of the six flasks. The final solutions in the flasks correspond to 0, 50, 100, 150, 200 and 250 mg/kg of starch in sugar.

5.3 Pipette, using a 25 ml graduated pipette, respectively into each of the six flasks 7.0 ± 0.1, 6.0 ± 0.1, 5.0 ± 0.1, 4.0 ± 0.1,3.0 ± 0.1, and 2.0 ± 0.1 mL of distilled water to make the liquid volume in each flask to 7.0 mL. Swirl each flask to completely dissolve the sugar.

5.4 Pipette, using an automatic dispenser or a 25 mL graduated pipette 15.0 ± 0.1 mL of the calcium chloride acetic acid reagent (4.4) into each flask. Mix well.

5.5 Stopper each flask loosely and place in a boiling water bath (95-100°C) for 15 ± 1 minute. Commence timing from the moment the flasks are placed in the bath. Swirl each flask about 5 and 10 minutes after placing in the bath to aid dissolution of the starch.

NOTE: The flasks must be placed in the heating bath within 30 minutes after adding water to dissolve the sugar (5.3)

5.6 After 15 minutes, remove the flasks from the heating bath and cool to room temperature in a running water bath.

5.7 To each flask, add 15.00 ± 0.05 mL of 0.033 mol/L acetic acid (4.3). Use a 15 mL bulb pipette or an automatic pipette. Mix well.

B. MEASUREMENT OF ABSORBANCE

5.8 Determine the cell corrections for a pair of matched 2 cm cells using distilled water.

5.9 Add to the 0 mg/kg test solution 10.00 ± 0.05 mL of the potassium iodide-potassium iodate reagent (4.7) using a 10 mL barrel pipette or automatic dispenser. Mix well and make to the mark with distilled water. Stopper the flask and mix well.

5.10 *Immediately* rinse twice and fill a 2 cm cell with the 0 mg/kg test solution. Read the absorbance of the solution in a spectrophotometer at 700 nm against distilled water as the reference. Correct the absorbance reading for any cell correction (5.8). Record the absorbance to 0.001 units.

NOTE: The absorbance of the test solution must be read between two and five minutes after adding the iodide-iodate reagent to the test solution.

5.11 Repeat steps 5.9 and 5.10 for each concentration of starch involved in the standardisation (5.3).

NOTE: The reading of the 0 mg/kg starch standard should not exceed 0.010 absorbance for a 2 cm cell. As a guide the reading for the 200 mg/kg starch standard should be about 0.320 absorbance for a 2 cm cell at 700 nm.

C. PREPARATION OF GRAPH

5.12 Plot "mg/kg starch in sugar" (5.2) against "absorbance".

5.13 A straight line must be obtained for the graph. If three or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular starch concentration, the standardisation must be repeated.

5.14 The graph used by any laboratory for routine analysis should be constructed using the mean of independent standardisations by at least two analysts. Each set of results and the mean must comply with the 5% limits above (5.13). In addition, the slopes of the individual lines must not differ by more than 5%.

## 6.0 PROCEDURE

A. PREPARATION OF TEST SOLUTIONS

6.1 Weigh 3.60 ± 0.02 g of raw sugar into each of two 50 mL Kohlrausch volumetric flasks:

(a) sample blank solution
(b) sample test solution

6.2 Using an automatic dispenser or 25 mL graduated pipette add 7.0 ± 0.1 mL of distilled water to each flask. Swirl the flasks to completely dissolve the sugar.

6.3 Add to each flask 15.00 ± 0.05 mL of calcium chloride/acetic acid reagent (4.4) using an automatic dispenser or a 15 mL bulb pipette. Mix well.

6.4 Stopper each flask loosely and place in boiling water bath at 95-100°C for 15 ± 1 minutes. Commence timing from the moment the flask is placed in the bath. Swirl each flask after 5 and 10 minutes, to aid dissolution of starch.

NOTE: The flasks must be placed in the boiling water bath within 30 minutes of adding water to dissolve the sugar (6.2).

6.5 Remove the flasks from the bath and cool in a running water bath to room temperature.

6.6 To each flask, add 15.00 ± 0.05 mL of 0.033 mol/L acetic acid (4.3). Use a 15 mL bulb pipette or automatic dispenser. Mix well.

6.7 Make the sample blank solution (6.1(a)) to the mark with distilled water and mix well.

6.8 Add 10.00 ± 0.04 mL of potassium iodide-iodate reagent (4.7) to the sample test solution (6.2(b)), and make to the mark with distilled water. Stopper the flask and mix well.

6.9 Read the absorbance of the sample test solution (6.8) in a 2 cm cell in a spectrophotometer at 700 nm, against the sample blank (6.7) as the reference solution. Record the absorbance to 0.001 units.

NOTE: The readings must be made between two and five minutes after the addition of the potassium iodide-iodate reagent to the sample test solution.

## 7.0 CALCULATIONS

**Read the mg/kg starch** in sugar directly from the calibration graph (5.12).

**Record** the result to 1 mg/kg starch in sugar.

## 8.0 PRECISION

| Sample | Concentration Range (mg/kg Starch) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 0 - 300 | 8.3 | 23 |

## 9.0 REFERENCES

9.1 Alexander J.B., South African Sugar Journal (1954).

9.2 Whistler R.L., Methods in Carbohydrate Chemistry, Academic Press, Vol 4 (1964).

# METHOD 38

## ACID-SOLUBLE PHOSPHATE — DETERMINATION IN RAW SUGAR BY THE AMIDOL METHOD

### 1.0 SCOPE AND FIELD OF APPLICATION

This method has been adopted officially within the Australian Sugar Industry.

The phosphate content of cane juice is an important factor in efficient clarification. The phosphate result for a raw sugar is indicative of the efficiency of the mill clarification process.

### 2.0 PRINCIPLE OF METHOD

The sugar is dissolved in distilled water and the pH adjusted to between 3 and 4 with dilute sulphuric acid. The solution is filtered with Celite. Acid molybdate, amidol and sodium metabisulphite solutions are added to an aliquot of the filtrate and phosphate is measured as the absorbance, at a wavelength of 660 nm, of the blue coloration which develops. Interference from low concentrations of organic phosphates and from anions and cations found in sugar are not significant.

### 3.0 APPARATUS

Ordinary laboratory apparatus and glassware and

3.1 **Spectrophotometer**, suitable for the measurement of absorbance at 660 nm with matched 2 cm cells.

3.2 **Bulb pipettes**, Class A (AS 2166) — 1 mL, 2 mL, 3 mL, 5 mL, 10 mL.

3.3 **Graduated straight pipette**, Class A (AS 2167) — 10 mL

3.4 **Automatic dispenser**, 10 mL

### 4.0 REAGENTS

During the analysis, unless otherwise stated, use only analytical grade reagents and only distilled water.

4.1 **Acid molybdate reagent**. Dissolve 16.6 ± 0.1 g ammonium molybdate tetrahydrate in about 600 mL of distilled water in a 1 litre beaker. Add 96 ± 1 mL of concentrated sulphuric acid. Add the acid slowly with stirring to avoid the generation of excessive heat. Cool, transfer to a 1 litre volumeric flask, make to the mark, and mix well.

*Safety*

Sulphuric acid is very corrosive. Safety glasses and gloves should be used when handling the acid. It must not be pipetted by mouth.

If in contact with skin, remove all contaminated clothing and wash affected area with copious quantities of water. If ingested, administer large quantities of aluminium hydroxide or milk of magnesia (magnesium hydroxide). Do not induce vomiting.

4.2 **Acid reagent**. Add 96 ± 1 mL of concentrated sulphuric acid ($\rho_{20}$ 1.84 g/mL) to about 600 mL of distilled water in a 1 litre beaker, slowly as in 4.1. Cool, transfer to a 1 litre volumetric flask, make to the mark, and mix well.

4.3 **Amidol reagent**. Dissolve 1.00 ± 0.01 g of amidol (2,4-diaminophenol hydrochloride) and 20 ± 1 g of sodium metabisulphite in distilled water in a 100 mL volumetric flask, make to the mark and mix well. Filter under vacuum through a Whatman No. 3 filter paper with a level teaspoon of acid washed Supercel (4.4).

Store in a dark bottle in a refrigerator and discard after one week.

*Safety*

Amidol-sodium metabisulphite reagent and its intermediate oxidation products are toxic and have a pungent odour. They may produce bronchial asthma, and hence should not be inhaled. Contact with the skin may give rise to an irritation leading to dermatitis; the reagent should be washed off the skin immediately. Use gloves when handling. Do not pipette by mouth.

4.4 **Acid washed Supercel**. Add 50 ± 5 g of Supercel to 1 litre of distilled water. Add 50 ± 5 mL of concentrated hydrochloric acid and stir for 5 minutes. Filter and wash Supercel cake free of acid with distilled water. Test washings with litmus paper. Dry Supercel for 6 hours at 90-100 °C and store in a closed container.

*Safety*

Concentrated hydrochloric acid is very corrosive and gives off pungent toxic fumes. It must be handled using safety glasses and gloves in a well ventilated atmosphere. It should not be pipetted by mouth.

First aid is the same as for sulphuric acid (4.1).

4.5 **Standard phosphate stock solution**, 1.0 mg P/mL. Dry about 1.5 g of potassium dihydrogen phosphate for 1 hour at 110 °C. Weigh out 1.0984 ± 0.0001 g of the dried salt, dissolve in freshly distilled water and make up to 250 mL in a volumetric flask. Do not keep for longer than one month.

4.6 **Working standard phosphate solution**, 0.01 mg P/mL. Dilute 10.00 ± 0.05 mL of the standard phosphate stock solution to 1 litre in a volumetric flask, and mix thoroughly.

### 5.0 STANDARDISATION OF METHOD

5.1 Using Class A pipettes, pipette aliquots of 0, 2.00, 4.00, 6.00, 8.00, 10.00, 12.00 mL of working solution (4.6) into separate 50 mL volumetric flasks. These solutions are equivalent to 0, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12 mg phosphate.

5.2 To each flask, add 2 drops of acid reagent (4.2). Adjust the volume to about 30 mL with distilled water. Add 10.0 ± 0.2 mL of acid molybdate reagent (4.1) and 4.0 ± 0.2 mL of amidol reagent (4.3). Make each flask to 50 mL with distilled water, stopper and mix well.

5.3 Allow the solutions to stand for at least 10 minutes but not more than 30 minutes.

NOTE: If the temperature of the solutions is above 25 °C, do not allow to stand for more than 15 minutes.

5.4 Read the absorbance of each standard solution in a spectrophotometer at 660 nm, in a 2 cm cell using water as the reference solution. Record the absorbance to 0.001 units.

As a guide to performance of the method, the 0.06 mg P standard measured at 660 nm in a 2 cm cell should read about 0.28 absorbance. The reading for the zero P standard in a 2 cm cell against distilled water should not exceed 0.001 absorbance.

5.5 Plot the absorbance of each standard solution against the concentration of phosphate.

If two or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular phosphate concentration, the standardisation must be repeated.

The calibration graph should be constructed using the mean of independent standardisations by at least two analysts. Each set of results and the mean must comply with the limits above. In addition, the slopes of the individual lines must not differ by more than 2%.

Each time a new batch of reagents is prepared, check the calibration at the 0 point (reagent blank) and at one other point by repeating steps 5.2 to 5.4. If the results do not lie within the limits set out above repeat the calibration.

## 6.0 PROCEDURE

A. PREPARATION OF TEST SOLUTION

6.1 Weigh out 20.0 ± 0.1 g of sugar, wash into 100 mL volumetric flask with distilled water, and dissolve.

6.2 Acidify with acid reagent (4.2). Add 2 drops for high pol sugars and 3 drops for low pol sugars. (This should reduce the pH to between 3 and 4). The remainder of the analysis should be completed within 1 hour of adding acid. Make to mark, stopper and mix well.

6.3 Filter under vacuum through a Whatman No. 3 filter paper. Precoat the filter with a slurry consisting of a level teaspoon of acid washed Supercel (4.4) with about 10 mL of the solution. Use the filtrate from this precoat to rinse the filter flask.

6.4 Transfer 20.0 ± 0.1 mL of the filtrate (6.3) into each of two 50 mL volumetric flasks.

6.5 To one flask, add 10.0 ± 0.2 mL of acid molybdate reagent (4.1) and 4.0 ± 0.2 mL of amidol reagent (4.3). Make up to 50 mL with distilled water, stopper and mix well. Stand for at least 10 minutes, but not more than 30 minutes.

NOTE: If the temperature of the solution is above 25 °C, read between 10 and 15 minutes.

B. PREPARATION OF BLANK SOLUTION

6.6 To the second 50 mL flask (6.5) add 10.0 ± 0.2 mL of acid reagent (4.2). Make up to 50 mL with distilled water, stopper and shake. Stand for at least 10 minutes, but not more than 30 minutes.

C. MEASUREMENT OF ABSORBANCE

6.7 Read the absorbance of the test solution (6.5) in a spectrophotometer at 660 nm, in a 2 cm cell using the corresponding test blank solution (6.6) as the reference solution. Record the absorbance to 0.001 units.

## 7.0 CALCULATIONS

7.1 **Read** from the standard graph "mg phosphate" in the test solution.

7.2 **Calculate** phosphate, (mg/kg) in the sample —

$$P\ (mg/kg) = \frac{\text{mg P from graph}}{1} \times \frac{100}{20} \times \frac{1}{m} \times 1000$$

where: m is the mass of sample taken, normally 20 g.

P (mg/kg) = 250 x mg P from graph (for a 20 g sample)

7.3 Express results as P, (mg/kg) to the nearest mg/kg.

## 8.0 PRECISION

| Sample | Concentration Range (mg/kg) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 0-30 | 2 | 3 |

## 9.0 REFERENCE

Allen, R.J.L., Biochem. J.; Vol. 34 (1940).

# METHOD 39
## PARTICLE SIZE AND SIZE DISTRIBUTION (GRIST) — DETERMINATION IN RAW SUGAR

### 1.0 SCOPE
This Level 1 method has been adopted by the Australian sugar industry. The size and uniformity of raw sugar crystals are important factors which affect washing and purging characteristics during refinery affination. Results from this method are influenced by crystal shape.

### 2.0 DEFINITIONS
**The % Fines** is the per cent by mass of crystals which pass through a 600 micron sieve.
**The Mean Aperture** (M.A.) of a sugar sample is that sieve opening through which 50% of the crystals, by mass, will pass.
**The Coefficient of Variation** (C.V.) is the standard deviation of the grain size of the sample, expressed as a percentage of the mean aperture.

### 3.0 PRINCIPLE OF METHOD
The raw sugar is washed with anhydrous methanol to remove the syrup film from the sugar crystals, followed by washing with iso-propanol to remove traces of methanol and any residual syrup. The washed crystals are dried in an oven at 80-90°C. The dried and washed sugar crystals are mechanically sieved through woven wire sieves and the separate fractions weighed.
The M.A.-C.V. terminology is a concise method of reporting the result of a sieving test on a sugar, eliminating the need to specify the sieves employed. Most samples of sugar have a normal size distribution and a plot of the cumulative percentages retained by a series of sieves, against sieve aperture will give a straight line on "arithmetic probability" paper.

### 4.0 APPARATUS
**All apparatus should be visually checked for damage prior to use.**

4.1 **Jar**, of metal or plastic, approximately 1L capacity and 100 mm dia. with tightly fitting lid.
4.2 **Rolling mill**, to rotate the jars at about 74 r/min.
4.3 **Metal lid** for jar, with centre cut out to form an opening of the same diameter as the jar. A 100 $\mu$m wire mesh screen is soldered across the opening to form a filter.
4.4 **Buchner flask**, about 1L capacity fitted with a large (100 mm dia.) plastic filter funnel.
4.5 **Measuring cylinder**, 250 mL.
4.6 **Drying tray**, stainless steel, approximately 150 mm x 150 mm x 15 mm.
4.7 **Laboratory drying oven**, capable of being controlled at 80-90°C, with a flame-proof rating, in a fume hood or vented to outside air.
4.8 **Sieve shaker**, Rotap (115 taps per minute) or Pascall Inclyno (300 vibrations per minute) or equivalent, with automatic timer set to 10 minutes ± 5 seconds.
4.9 **Set of woven**, wire test sieves, AS 1152, with the following nominal aperture sizes: 850 $\mu$m, 600 $\mu$m, 425 $\mu$m.
4.10 **Stiff-bristle brush**, for removing sugar crystals from sieve apertures.
4.11 **Soft-bristle brush**, to remove sugar from sieves and receiver.
4.12 **Sheet glazed paper,** about 400 mm x 400 mm.
4.13 **One pad** Mathematical probability paper, for M.A. - C.V. calculations.
4.14 **Balance**, to weigh 200 g to 0.01 g.

### 5.0 REAGENTS
**Refer to the MSDS for each reagent. Use only reagents of recognised analytical grade and distilled water or water of equivalent purity. Conduct of this method should be according to sound laboratory practice and relevant Workplace Health and Safety requirements.**

5.1 **Methanol,** (Technical, anhydrous, 99%) – $n_D^{20°}$ 1.329
*Safety*
*Methanol* is a dangerous cumulative poison if ingested, inhaled or absorbed through the skin. Symptoms of methanol poisoning are those of normal alcoholic intoxication accompanied by severe abdominal pain and visual disturbances. The symptoms may be delayed.
10 mL methanol is sufficient to cause permanent blindness and as little as 30 mL may be fatal.
If swallowed, administer large quantities of water or milk and induce vomiting. Follow with 2 tea-spoonfuls of sodium bicarbonate in water. Seek medical attention.
Alcohol vapours are inflammable so precautions must be taken to eliminate any ignition sources. The laboratory should be well ventilated to ensure that concentrations of methyl alcohol in the air are kept below 200 mg/m$^3$, the maximum allowable concentration for safe working over prolonged periods. The concentration of alcohol in the air can be determined by using a Drager test kit.

5.2 **Isopropanol**, (Technical, anhydrous, 99%) - $n_D^{20°}$ 1.377
*Safety*: Treat as for Methanol.

### 6.0 STANDARDISATION
Working sieves should be certified to comply with AS 1152. The sieves should be checked once each year against a reference set of sieves using sugar samples typical of the samples analysed in the laboratory.

### 7.0 PROCEDURE
A. PREPARATION OF WASHED SUGAR

7.1 Thoroughly mix the sample avoiding mechanical damage to the sugar crystals.
7.2 Weigh 115 ± 1 g of sample and transfer to a clean, dry jar (4.1).
7.3 Add 250 ± 10 mL of methanol (5.1), close the jar carefully with a tight fitting lid and rotate for 3 minutes ± 15 seconds on the jar rolling mill.
NOTE: Metal jars on the rollers should be separated by a piece of plastic or rubber to prevent sparks being struck.

7.4 Remove the lid and substitute the lid fitted with a wire screen (4.3). Invert the jar over the plastic funnel in the Buchner flask. Filter under vacuum to remove the alcohol. When filtration is complete remove the screen lid and carefully return any crystals adhering to the screen, back to the jar.

7.5 Repeat steps 7.3 and 7.4 using isopropanol (5.2) instead of methanol.

7.6 For Brand JA sugar, repeat steps 7.3 to 7.5.

7.7 Spread the alcohol wet sugar on a stainless steel tray and place this in the oven.

7.8 Continue drying the sugar in the oven until the crystals are dry, free-flowing and free of lumps (normally about 1-1.5 hours). Allow to cool to room temperature, and transfer to a suitable sealed container.

B. SIEVE ANALYSIS

7.9 Assemble the nest of sieves in the order, top to bottom: lid, 850 micron, 600 micron, 425 micron and receiver. The addition of a 1200 micron screen when significant quantities of large grain are present will result in more accurate MA - CV results.

7.10 Thoroughly mix the cooled sugar, *avoiding size segregation*, and weigh out 100.00 ± 0.01 g. Transfer the sugar to the top sieve of the nest of sieves.

7.11 Shake the nest of sieves on the mechanical shaker for 10 minutes ± 5 seconds.

7.12 Transfer the sugar crystals caught on the 850 micron sieve on to a sheet of glazed paper. Brush out the sieve with the stiff brush taking care not to damage the screen.

7.13 Transfer the crystals from the glazed paper to a tared 100 mL beaker. Weigh to 0.01 g to find the mass of crystals caught on the sieve, and record to 0.01 g as C850 (Caught 850 microns).

7.14 Repeat steps 7.12 and 7.13 for the crystals caught respectively on the 600 micron and 425 micron sieves and in the receiver. Record the mass of the fractions to 0.01 g as C600, C425, T425 (through 425 microns).

NOTE: Where % fines is the only result required from the grist analysis, the 850 and 425 microns sieves need not be used. The nest of sieves is then made up of the lid, 600 micron sieve, and receiver. The % fines is then directly given by the % caught in the receiver. When M.A. and C.V., alone, are required, the 850 and 600 micron sieves only, need to be used.

## 8.0 CALCULATIONS

8.1 **Per cent fines**

Per cent fines = % by mass through 600 microns

Record the results to 1 per cent fines.

8.2 **Full grist**

Add up all the masses (7.13, 7.14). If the total is 100.0 ± 0.2 g, round off the masses to one decimal place, so that the total is 100.0 g. If the total is outside the range, 100.0 ± 0.2 g, discard the results as unreliable, and repeat the analysis.

Convert the mass obtained to the "% caught" on each sieve expressed to 0.1% - this is the full grist, eg

| Sieve (μm) | Symbol | Mass (g) | Caught % | Cumulative % |
|---|---|---|---|---|
| 850 | C850 | 36.77 | 36.8 | 36.8 |
| 600 | C600 | 47.01 | 47.0 | 83.8 |
| 425 | C425 | 11.52 | 11.5 | 95.3 |
| — | T425 | 4.70 | 4.7 | 100.0 |

8.3 **Mean Aperture**. Convert the per cent caught by each sieve to cumulative percentages above each mesh size. Plot the values against the corresponding mesh aperture size (in mm) on arithmetic probability paper. Draw a straight line through all points with cumulative percentage in the range 10-90%. (or C850 and C600, if they are the only two sieves used).

From the graph:

Mean aperture (M.A.) = aperture size passing 50% of crystal

Report the mean aperture to the nearest 0.02 mm.

8.4 **Coefficient of Variation (C.V.)**

$$C.V. = \frac{\text{Size passing 16\% - Size passing 84\%}}{2 \times M.A.} \times \frac{100}{1}$$

Report the coefficient of variation to the nearest 0.2%.

## 9.0 PRECISION

The expected range for raw sugar is:

| | |
|---|---|
| % Fines | 0 to 50% |
| Mean Aperture | 0.3 to 1.5 mm |
| Coefficient of Variation | 10 to 50% |

The precision, for the percentage mass caught in each sieve, that should normally be obtained by following the procedure is:

| | |
|---|---|
| 95% repeatability r | 3.1% |
| 95% reproducibility R | 4.0% |

## 10.0 REFERENCES

10.1 Powers, H.E.C.; International Sugar Journal; Vol. 50 (1948).

10.2 Payne, J.H.; Sugar Cane Factory Analytical Control; Elsevier (1968), p. 85.

10.3 ICUMSA, Proceedings of 21st Session (1994), General Subject 1.

# METHOD 40
## MEAN ELONGATION — DETERMINATION IN RAW SUGAR

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian Sugar Industry for the measurement of the mean elongation of raw sugar crystals.

Crystal elongation is a raw sugar quality parameter. It usually results from processing cane juices with high dextran concentrations.

The shape, size and uniformity of raw sugar crystals are important sugar quality criteria as they affect purging characteristics during refinery affination.

**2.0 DEFINITIONS**

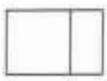
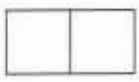
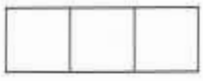
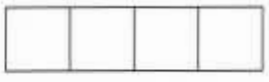
In crystallography, the terms 'length' and 'breadth' have specific well established meanings, namely the b and c axis dimensions respectively. The a:b:c axis ratio for a pure sucrose crystal is approximately 1.3:1.0:0.9, a b:c elongation of 1:1.1. Elongation can occur along the b axis or c axis with the latter being the most common.

However, given the difficulty in correctly recognising these axes in a routine laboratory situation, the above convention is not followed in this method.

For the purpose of this method, crystal elongation is taken as the ratio of the length of the crystal to the breadth of the crystal.

Mean elongation is the number average elongation of a minimum of 50 individual crystals.

**Classification**

| Crystal Shape | Elongation Classification | Category |
|---|---|---|
| | 1: 1.1 | 1 |
|  | 1: 1.5 | 2 |
|  | 1: 2.0 | 3 |
|  | 1: 3.0 | 4 |
|  | 1: 4.0 | 5 |

**3.0 PRINCIPLES OF METHOD**

A subsample of the raw sugar is washed with anhydrous methanol to remove the syrup film from the sugar crystals, followed by isopropanol to remove traces of methanol and any residual syrup. The washed crystals are dried in an oven at 80-90°C. The washed and dried sugar crystals are examined under a microscope and classified into five elongation categories. The mean elongation of the sugar is calculated from the number of crystals in each category of elongation.

**4.0 APPARATUS** (for analysis of one sample)

4.1 Jar, of metal or plastic, approximately 1L capacity and 100 mm diameter with tightly fitting lid.

4.2 Rolling mill to rotate the jar at approximately 74 r/min.

4.3 Metal or plastic lid for jar, with centre cut out and a 100 micron wire mesh screen permanently secured across the opening to form a filter.

4.4 Measuring cylinder, 250 mL capacity.

4.5 Buchner flask, about 1L capacity fitted with large plastic funnel.

4.6 Drying tray, stainless steel, approximately 150 mm × 150 mm × 15 mm.

4.7 Drying oven, controlled at 80-90 °C, with flame-proof rating.

4.8 Balance, to weigh 115 ± 1 g.

4.9 Projection microscope, objective magnification × 20 (or similar), with 10 mm measuring grid on viewing screen, or microscope with graticule in eye-piece.

4.10 Microscope slides, glass or plastic, approximately 100 mm × 100 mm.

**5.0 REAGENTS**

5.1 Methanol (Technical, anhydrous, 99%) – $n_D^{20^\circ}$ 1.329

*Safety*

*Methanol* is a dangerous cumulative poison if ingested, inhaled or absorbed through the skin. Symptoms of methanol poisoning are those of normal alcoholic intoxication accompanied by severe abdominal pain and visual disturbances. The symptoms may be delayed.

10 mL methanol is sufficient to cause permanent blindness and as little as 30 mL may be fatal.

If swallowed, administer large quantities of water or milk and induce vomiting. Follow with 2 teaspoonfuls of sodium bicarbonate in water. Seek medical attention.

Alcohol vapours are inflammable so precautions must be taken to eliminate any ignition sources. The laboratory should be well ventilated to ensure that concentrations of methyl alcohol in the air are kept below 200 mg/m³, the maximum allowable concentration for safe working over prolonged periods. The concentration of alcohol in the air can be determined by using a Drager test kit.

5.2 Isopropanol (Technical, anhydrous, 99%) – $n_D^{20^\circ}$ 1.377

*Safety.* Treat as for Methanol.

**6.0 PROCEDURE**

A. PREPARATION OF WASHED SUGAR

6.1 Thoroughly mix the sample to be measured, avoiding mechanical damage to the sugar crystals.

6.2 Weigh out 115 ± 1 g of sample and transfer to a clean, dry jar (4.1).

6.3 Add 250 ± 10 mL of methanol (5.1), close the jar carefully with a tight fitting lid and rotate for 3 minutes ± 15 seconds on the jar rolling mill.

NOTE: Metal jars on the rollers should be separated by a piece of plastic or rubber to prevent sparks being struck.

6.4 Remove the lid and substitute the lid fitted with a wire screen (4.3). Invert the jar over the plastic funnel in the Buchner flask. Filter under vacuum to remove the alcohol. When filtration is complete remove the screen lid and carefully return any crystals adhering to the screen, back to the jar.

6.5 Repeat steps 6.3 and 6.4, using isopropanol (5.2) instead of methanol.

6.6 For Brand JA sugar, repeat steps 6.3 to 6.5.

6.7 Spread the alcohol wet sugar on a stainless steel tray and place this in the oven.

6.8 Continue drying the sugar in the oven until the crystals are dry, free-flowing and free of lumps (normally about 1 hour). Allow to cool to room temperature, and transfer to a suitable sealed container.

B. EXAMINATION OF CRYSTALS

6.9 Thoroughly mix the cooled sugar, avoiding size segregation and crystal breakage.

6.10 Distribute approximately 0.5 g of the sugar evenly over the clean, dry glass slide so that individual crystals can be easily read.

6.11 Position the slide on the microscope stage so that a crystal's image is superimposed on the scale on the microscope viewing screen. Rotate the slide until the grid lines run parallel to the sides of the crystal.

If using a microscope fitted with a graticule in the eye-piece, rotate the eye-piece until the graticule lines are aligned with the sides of the crystal.

6.12 Estimate the elongation of each crystal and record a tick on the worksheet in the appropriate box for the particular category encountered, i.e. 1:1.1, 1:1.5, 1:2.0, 1:3.0, 1:4.0. For classification purposes, round upwards or downwards to the nearest classification.

NOTE: Refer to page 3 for example work sheet.

6.13 Repeat steps 6.11 and 6.12 until a minimum of 50 crystals are read.

6.14 Move the slide up and down and left to right systematically to avoid re-reading previously observed crystals.

NOTE: Do not attempt to measure broken crystals or conglomerates.

## 7.0 CALCULATIONS

7.1 *Mean Elongation*

$$ME = \frac{1.1 \times n_1 + 1.5 \times n_2 + 2.0 \times n_3 + 3.0 \times n_4 + 4.0 \times n_5}{n_1 + n_2 + n_3 + n_4 + n_5}$$

where: $n_1$ to $n_5$ = number of crystals observed in each category 1 to 5

## 8.0 PRECISION

| Sample | Expected Range | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Raw sugar | 1.1 - 4.0 | Not known | Not known |

## WORK SHEET
## CRYSTAL EXAMINATION — MEAN ELONGATION

Date: .................

Analyst: ..............

| Sample No. | No. of Crystals in each Category | | | | | | | | | | Sum Category Products (a) | Total Crystals (b) | M.E. (a/b) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1:1.1 | Sum | 1:1.5 | Sum | 1:2.0 | Sum | 1:3.0 | Sum | 1:4.0 | Sum | | | |
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Note (a) Sum of Category Products $= 1.1 \times n_1 + 1.5 \times n_2 + 2.0 \times n_3 + 3.0 \times n_4 + 4.0 \times n_5$
where $n_j$ = No. of crystals in category j

# METHOD 41

## ALKALINITY, TOTAL ALKALINITY AND CAUSTIC ALKALINITY — DETERMINATION IN WATER

### 1.0 SCOPE AND FIELD OF APPLICATION

This method is recommended for use within the Australian Sugar Industry. It is applicable to raw and treated waters and the results are used for the calculation of chemical dosages in the treatment of water supplies.

### 2.0 PRINCIPLE OF METHOD

Alkalinity is determined by titration with standard acid solution to a designated pH with a colour indicator. For this method the phenolphthalein alkalinity end-point (P) is at pH 8.3 and the total alkalinity end-point (M) is at pH 4.5 (methyl orange). These methods are not suited to boiler waters where the presence of organic salts may cause serious interference and therefore the methyl orange end-point becomes meaningless.

In the determination of caustic alkalinity [P($BaCl_2$)], carbonate ions, and most other organic salt ions, are removed by precipitation with barium chloride followed by titration with standard acid solution to pH 8.3 with phenolphthalein. This is the recommended method for the determination of caustic alkalinity in boiler waters (refer Laboratory Manual, Volume 1, p. 91) and caustic soda cleaning solutions.

NOTE: For a more precise determination, or when presence of colour or turbidity obscures the indicator end-points, or for boiler waters containing organic salts which interfere with the end-point, the potentiometric method described in Australian Standard 2449-1981 should be used.

### 3.0 REAGENTS

Unless otherwise stated, use only analytical grade reagents and only distilled water which has been freed of carbon dioxide by boiling for 15 minutes and cooled to room temperature immediately prior to use.

3.1 **Phenolphthalein indicator solution,** 1 g/100 mL. Dissolve 1 g phenolphthalein in 50 mL ethanol plus 50 mL distilled water.

3.2 **Methyl Orange indicator solution,** 500 mg/L. Dissolve 0.5 g methyl orange in 1L of distilled water.

3.3 **Mixed indicator solution.** Dissolve 0.02 g methyl red and 0.1 g bromcresol green in 50 mL ethanol plus 50 mL water.

3.4 **Sulphuric acid standard solution,** 0.01 mol/L. Prepare from a commercial concentrated volumetric solution.

NOTE: Commercial concentrated solutions may contain preservatives or other agents. For precise work standardise the sulphuric acid by titration against 0.025 mol/L standard sodium carbonate solution.

### 4.0 SAMPLING AND SAMPLES

4.1 **Sample containers.** Use borosilicate glass or polythene containers, prepared as follows :

(a) Wash the container and cap with detergent and tap water,
(b) Rinse thoroughly with tap water,
(c) Rinse twice with distilled water,
(d) Drain thoroughly and replace cap.

4.2 **Collection and preservation.** Rinse the container 2-3 times then completely fill with sample and seal. The determination should be performed immediately. If this is not possible store the sample at 4 °C and carry out the determination within 24 hours. As a check record the pH at sampling and again before analysis.

NOTE: Boiler waters collected under pressure should be cooled to near 20 °C while still under pressure to minimise the chance of losing dissolved gases.

### 5.0 PROCEDURE

A. ALKALINITY TO PHENOLPHTHALEIN, P

5.1 Pipette the sample aliquot (V) into a 250 mL conical flask keeping the tip of the pipette near the bottom of the flask. **For softened water** (boiler feed water) pipette 100 mL.

5.2 Add 3-4 drops of phenolphthalein indicator and if the sample turns pink, titrate over a white surface with 0.01 mol/L $H_2SO_4$ until the colour just disappears. Record the volume of $H_2SO_4$ used to 0.1 mL, ($V_1$).

B. ALKALINITY TO METHYL ORANGE, M (Total Alkalinity)

5.3 Add 5-6 drops of mixed indicator or 3-4 drops of methyl orange indicator to the test sample from 5.2 and continue titrating with standard 0.01 mol/L $H_2SO_4$. The colour change is from blue through grey to pink-grey for mixed indicator or yellow to orange for methyl orange. Record the total volume of $H_2SO_4$ used, (5.2 and 5.3) to 0.1 mL, ($V_2$).

C. ALKALINITY TO PHENOLPHTHALEIN AFTER ADDITION OF BARIUM CHLORIDE, P($BaCl_2$) (Caustic Alkalinity)

5.4 Measure 100 mL of the sample into a 250 mL conical flask. Add 3-4 drops phenolphthalein indicator followed by about 1 g of $BaCl_2$.

Swirl until dissolved and titrate over a white surface until the pink colour just disappears. Record the volume of $H_2SO_4$ used, to 0.1 mL, ($V_3$).

### 6.0 CALCULATION

6.1 **Calculate** the alkalinity to phenolphthalein, P

$$P = \frac{V_1}{V} \times 1000$$

where: V = volume of test sample, in mL (5.1)
$V_1$ = volume of standard acid used in titration to phenolphthalein end-point (5.2).

Record the result, expressed as mg/L $CaCO_3$ to 1 mg/L.

6.2 **Calculate** alkalinity to methyl orange, M

$$M = \frac{V_2}{V} \times 1000$$

where: V = volume of test sample, in mL (5.1)
$V_2$= volume of standard acid used in titration to methyl orange end-point in mL (5.2 + 5.3).

Record the result, as mg/L $CaCO_3$ to 1 mg/L.

6.3 **Calculate** alkalinity to phenolphthalein after addition of barium chloride, $P(BaCl_2)$

$$P(BaCl_2) = \frac{V_3 \times 1000}{V}$$

where V = volume of test sample in mL (5.4)
$V_3$= volume of standard acid used in titration to $P(BaCl_2)$ end-point (5.4)

Record the result, expressed as mg/L $CaCO_3$ to 1 mg/L.

## 7.0 CALCULATION OF ALKALINITY RELATIONSHIPS

**Calculate** the hydroxide, carbonate and bicarbonate alkalinities from the table. All alkalinities are expressed as mg/L $CaCO_3$.

**ALKALINITY RELATIONSHIPS**

| Results of titration | Hydroxide alkalinity as $CaCO_3$ | Carbonate alkalinity as $CaCO_3$ | Bicarbonate alkalinity as $CaCO_3$ |
|---|---|---|---|
| P = 0 | 0 | 0 | M |
| P < 0.5M | 0 | 2P | M — 2P |
| P = 0.5M | 0 | 2P | 0 |
| P > 0.5M | 2P — M | 2(M — P) | 0 |
| P = M | M | 0 | 0 |

These relationships do not apply to boiler waters containing organic salts which may cause significant interference.

## 8.0 PRECISION

No positive statement can be made about precision because of the wide variation in sample characteristics. The precision of titration is likely to be much greater than the uncertainties involved in sampling.
The expected range of results is:
Alkalinity to Methyl Orange (total alkalinity) —
10-500 mg/L CaCO3
Caustic alkalinity —
10-500 mg/L CaCO3

## 9.0 REFERENCES

(1) Laboratory Manual for Australian Sugar Mills (Vol I, 1984) p91.

(2) Australian Standard 2449-1981
Waters Determination of Alkalinity, Acidimetric Titration Method.

(3) Standard Methods of Examination of Water and Waste Water; 16th edition; American Public Health Association (1985).

(4) Australian Standard 2031.1-1986
Selection of Containers and Preservation of Water Samples for Chemical and Microbiological Analysis, Part 1, Chemical.

(5) Ivin, P.C. (1982). Condensate Quality and Sugar Mill Boiler Operation. Proc. Aust. Soc. Sugar Cane Technol. 205-212.

# METHOD 42
## CHLORIDE — DETERMINATION IN CAUSTIC (NaOH) CLEANING SOLUTIONS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. Chloride ions accelerate the dissolution of most metals and can cause corrosion or pitting in evaporation tubes.

**2.0 PRINCIPLE OF METHOD**

This is based on the standard Volhard's titrimetric procedure. A test sample of the cleaning solution is acidified with nitric acid. An excess of standardised silver nitrate is added to precipitate chloride ions. The excess silver nitrate is back-titrated with standard ammonium thiocyanate solution with ferric alum as an indicator.

**3.0 REAGENTS**

Unless otherwise stated, use only analytical grade reagents and only distilled water.

3.1 **Nitric acid**, $\rho_{20}$ = 1.41 g/mL.

3.2 **Nitrobenzene.**

3.3 **Ferric ammonium sulphate**, saturated solution.

3.4 **Ammonium thiocyanate solution**, 0.1 mol/L. Dissolve 8.5 g of ammonium thiocyanate in water and dilute to 1 litre in a volumetric flask. Standardise by titrating 25 mL of 0.1 mol/L silver nitrate, plus 2 mL nitric acid, $\rho_{20}$ = 1.41 g/mL, and 1 mL of ferric ammonium sulphate (3.3) with the ammonium thiocyanate solution. A white precipitate forms which as the end-point approaches becomes flocculent and settles easily. Shake vigorously after each addition of thiocyanate solution. At the end-point, 1 drop of thiocyanate solution produces a faint red-brown colour which does not disappear on shaking.

Molarity of ammonium thiocyanate solution

$$= \frac{25 \text{ x molarity of } Ag\,NO_3}{\text{volume ammonium thiocyanate titre}}$$

3.5 **Silver nitrate standard solution**, 0.1 mol/L. Dissolve 8.496 g $AgNO_3$ in water and dilute to 500 mL in a volumetric flask. Standardise by titrating 25 mL of 0.1 mol/L NaCl solution (3.6) plus 1 mL potassium chromate indicator, with the $AgNO_3$ solution (ref. to Method 43 "Chloride — Determination in Water"). Store in a brown bottle.

Molarity of silver nitrate solution

$$= \frac{25 \text{ x } 0.1}{\text{volume } AgNO_3 \text{ titre}}$$

3.6 **Sodium chloride standard solution**, 0.1 mol/L. Dissolve 2.923 g of NaCl (dried at 100-110 °C for 3 hours) in water and dilute to exactly 500 mL in a volumetric flask.

**4.0 PROCEDURE**

4.1 Shake the sample well, and with a bulb pipette fitted with a safety bulb, pipette 20 mL into a 1 litre conical flask containing approximately 50 mL of distilled water.

4.2 Carefully add 25 mL of concentrated nitric acid. Test the solution with litmus paper and add a further 1 mL aliquot of nitric acid until the litmus turns red.

4.3 Pipette into the solution 10.00 ± 0.02 mL of the standard silver nitrate solution (3.5). Add 1-2 mL nitrobenzene. Shake the flask to help coagulate any turbidity, then add 10 drops of ferric ammonium sulphate (3.3). This is the test solution.

NOTE: The volume of silver nitrate solution added may need to be varied to give a reasonable back-titration with ammonium thiocyanate.

4.4 Titrate, over a white background, with standard ammonium thiocyanate solution (3.4), with frequent shaking, until a faint red-brown colour persists for 30 secs. Record the volume of ammonium thiocyanate used, $V_1$, to 0.1 mL.

4.5 Repeat the titration (4.2-4.4) with 20 mL distilled water, instead of the test sample. This is the blank titration. Record the volume of ammonium thiocynate used, $V_b$, to 0.1 mL.

**5.0 CALCULATION**

5.1 Calculate chloride, (mg/L) in the test sample —

$$\text{Chloride (mg/L)} = \frac{(V_b - V_1) \text{ x } c \text{ x } 35450}{V}$$

where: V = mL of test sample (4.1)
$V_1$ = mL of ammonium thiocyanate to titrate test sample (4.4)
$V_b$ = mL of ammonium thiocyanate to titrate blank (4.5)
c = molarity of ammonium thiocyanate solution, mol/L (3.4)

**6.0 PRECISION**

The expected range of results is 200-8,000 mg/L chloride. The precision of the method has not been determined.

**7.0 REFERENCE**

(1) Anon. (1980). Standard Test Method TM36-1.7-1. Chloride (as NaCl) ICI Aust. Ltd., 27/10/80

(2) Ivin, P.C. and Doyle, C.D. (1978). Proc. Aust. Soc. of Sugar Cane Technol. 267-272.

# METHOD 43
## CHLORIDE — DETERMINATION IN WATER

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The presence of chloride ions indicates saline contamination in factory cooling water. Chlorides are relatively unreactive and the result can be used in a calculation of % blowdown.

**2.0 PRINCIPLE OF METHOD**

This method is based on the standard Mohr's filtration procedure.

The sample is adjusted to between 7 and 10 pH and titrated with silver nitrate in the presence of potassium chromate to indicate the end-point. Silver chloride is precipitated quantitatively before red silver chromate is formed.

Substances in the amounts normally found in boiler waters will not interfere. Sulphide, thiosulphate, and sulphite ions interfere and are removed by treatment with hydrogen peroxide.

**3.0 REAGENTS**

Unless otherwise stated, use only analytical grade reagents and only distilled or deionised water. (Distilled water that has been further treated by deionisation is preferred).

3.1 **Potassium chromate indicator solution.** Dissolve 50g $K_2CrO_4$ in a little water. Add $AgNO_3$ solution until a definite red precipitate is formed. Let stand 12 hours, filter and dilute to 1L with distilled water.

3.2 **Standard silver nitrate solution,** 0.02 mol/L. Dissolve 3.3980 g $AgNO_3$ in water and dilute to 1L. Standardize by titrating 25 mL 0.02 mol/L sodium chloride with the silver nitrate solution following the procedure in steps 5.1-5.5 below. Store in a brown bottle.

**Calculate** the concentration of the standard silver nitrate, c in moles per litre :

$$c = \frac{25 \times 0.02}{\text{Volume } AgNO_3 \text{ titre} - \text{Volume } AgNO_3 \text{ blank titre}}$$

3.3 **Standard sodium chloride solution,** 0.02 mol/L. Dissolve 1.1680 g NaCl (dried at 100-110 °C for 3 hours) in water and dilute to exactly 1L.

3.4 **Sulphuric acid solution,** 0.01 mol/L.

3.5 **Hydrogen peroxide,** 30%.

3.6 **Phenolphthalein solution,** 1 g/100 mL. Dissolve 1 g phenolphthalein in 50 mL ethanol plus 50 mL distilled water.

**4.0 SAMPLING AND SAMPLES**

4.1 **Sample bottles.** Use glass or polythene bottles prepared for sampling as follows —

(a) Wash the bottle and cap with detergent and tap water,
(b) Rinse thoroughly with tap water,
(c) Rinse twice with distilled water,
(d) Drain thoroughly and replace cap.

4.2 **Sampling.** Before sampling, thoroughly flush all distribution lines to ensure a representative sample. Rinse the sample bottle 2-3 times with the sample water then completely fill the container and replace cap. Boiler waters collected under pressure should be cooled to about 20 °C while still under pressure.

**5.0 PROCEDURE**

A. SAMPLE PREPARATION

5.1 Filter about 200 mL of the sample through a Whatman No. 41 paper. For boiler waters, pipette 50 mL of the filtered water into a 200 mL conical flask and add 50 mL of distilled water. For soft waters (boiler feed water), pipette 100 mL into a 250 mL conical flask.

5.2 If sulphite is present, add 1 mL $H_2O_2$ and stir for 1 minute.

B. TITRATION

5.3 Directly titrate samples in the pH range 7 to 10. Add 1-2 drops of phenolphthalein and adjust to pH 7-10 with 0.01 mol/L $H_2SO_4$.

5.4 Add 1.0 mL of $K_2CrO_4$ indicator solution. Titrate (over a white surface) with standard 0.02 mol/L $AgNO_3$ solution to a persistent pinkish-yellow end-point. Record the volume of $AgNO_3$ used, ($V_1$) to 0.1 mL.

5.5 Determine a reagent blank by titrating 100 mL distilled water as in 5.3-5.4 above. Record the volume of $AgNO_3$ used, ($V_b$) to 0.1 mL.

**6.0 CALCULATION**

6.1 Calculate chloride, (mg/L) in sample.

$$\text{Chloride (mg/L)} = \frac{(V_1 - V_b) \times c \times 35450}{V}$$

where: V = mL of test sample (5.1)
$V_1$ = mL of $AgNO_3$ to titrate test solution (5.4)
$V_b$ = mL of $AgNO_3$ to titrate blank (5.5)
c = Concentration (mol/L) of standard $AgNO_3$ solution (3.2)

Record the result as chloride in water to 1 mg/L.

**7.0 PRECISION**

| Sample | Concentration Range (mg/L) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Boiler water | 20-50 | 8 | 25 |
| Softened water | 100-50 | 8 | 25 |

**8.0 REFERENCES**

1. Standard Methods for the Examination of Water and Waste Water; 16th Edition; American Public Health Association (1985).

2. Australian Standard 2031.1-1986; Selection of Containers and Preservation of Water Samples for Chemical and Microbiological Analysis — Part 1 — Chemical.

# METHOD 44
## SOLUBLE PHOSPHATE — DETERMINATION IN WATER BY THE AMIDOL METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use in the Australian Sugar Industry. Phosphate is present in water in three main forms, orthophosphate (soluble phosphate), condensed phosphate and organophosphorus compounds. This procedure measures soluble phosphate only.

**2.0 PRINCIPLE OF METHOD**

The test sample is reacted with ammonium molybdate, under acidic conditions, to form phosphomolybdic acid which is reduced to molybdenum blue by amidol and sodium metabisulphite. The absorbance of the blue colour is measured in a spectrophotometer at 660 nm.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Spectrophotometer,** suitable for the measurement of absorbance at 660 nm with matched 2 cm cells.

3.2 **Automatic dispensers,** 4 mL and 10 mL capacities.

**4.0 REAGENTS**

Unless otherwise stated, use only analytical grade reagents and only distilled or deionised water.

4.1 Acid molybdate reagent.

4.2 Acid reagent

4.3 Amidol reagent

NOTE: Directions for preparing these reagents is given in the method: "Acid Soluble Phosphate — Determination in Raw Sugar by the Amidol Method".

**5.0 STANDARDISATION**

The method is standardised against a standard solution prepared from dried potassium dihydrogen phosphate. The calibration graph, covering the range 0 to 0.12 mg phosphate, prepared for Method No. 38 "Acid Soluble Phosphate — Determination in Raw Sugar by the Amidol Method" is also used with this method.

**6.0 SAMPLES AND SAMPLING**

6.1 **Sample containers.** Glass bottle with stopper which has been prepared by washing with hot, dilute hydrochloric acid and rinsing well with distilled water. Do not use detergent which may contain phosphate.

6.2 **Sampling.** Before sampling, thoroughly flush all distribution lines to ensure a representative sample. Rinse the container 2-3 times then completely fill with sample and seal. If the sample is to be stored for more than 4 hours add 1 mL of concentrated hydrochloric acid ($\rho_{20}$ = 1.18 g/mL), per litre.

**7.0 PROCEDURE**

NOTE: Wash all glassware with hot dilute HCl and rinse well with distilled water. Do not use detergents.

7.1 Pipette 5.0 mL of boiler water or 10.0 mL of softened water into each of two 50 mL volumetric flasks. Add 2-3 drops of phenolphthalein indicator and, if a pink colour develops, add acid reagent to discharge the colour.

7.2 To one flask, add with automatic dispensers 10 mL of acid molybdate reagent (4.1) and 4 mL of amidol reagent. Make to 50 mL with distilled water, stopper and mix well. This is the test solution.

7.3 To the second flask add 10 mL of acid reagent, dilute to 50 mL with distilled water, stopper and mix well. This is the blank solution.

7.4 After at least 10 minutes and no longer than 30 minutes read the absorbance of the test solution (7.2) in a 2 cm cell, in a spectrophotometer at 660 nm using the corresponding test blank solution (7.3) as the reference. Record the absorbance to 0.001 units.

**8.0 CALCULATION**

8.1 Read from the standard graph (5.0) mg of phosphate, P in the 50 mL of test solution (7.2).

8.2 Calculate soluble phosphate as P (mg/L) in the sample.

$$P(mg/L) = \frac{\text{mg P (from graph)}}{\text{sample aliquot}} \times 1000$$

8.3 Calculate soluble phosphate as $PO_4$ (mg/L)

$PO_4$ (mg/L) = P (mg/L) x 3.1

Record the result as soluble phosphate $PO_4$ (mg/L) to the nearest mg/L.

**9.0 PRECISION**

| Sample | Concentration Range $PO_4$ (mg/L) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Boiler water | 30 — 100 | 8 | Not determined |
| Softened water | 0 — 10 | Not determined | Not determined |

**10.0 REFERENCES**

10.1 British Standard Methods of Testing; Water used in Industry, Part 105. Soluble phosphate and organophosphorus compounds. BS 2690 : Part 105 : (1983).

10.2 Standard Methods for the Examination of Water and Waste Water; 16th Edition; American Public Health Association (1985).

# METHOD 45

## TOTAL DISSOLVED SOLIDS (TDS) — ESTIMATION IN WATER BY CONDUCTIVITY MEASUREMENT

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The results must be treated with some caution unless the conductivity meter is regularly standardised against solids by drying, as the relationship between conductivities and TDS will vary with differing composition of contaminants.

**2.0 PRINCIPLE OF METHOD**

The electrical conductivity of the test sample is measured under standard conditions using a conductivity meter calibrated in microsiemens per metre, S/m, (previously mhos/cm). Total dissolved solids is estimated by multiplying by an empirical factor. (Conductivity meters giving direct readout of total dissolved solids are available).

Total Dissolved Solids by Hydrometer, an alternative method, involves the use of a TDS hydrometer supplied by a commercial firm. Results from the hydrometer should also be checked regularly against solids by drying.

**3.0 APPARATUS**

3.1 **Conductivity meter,** with direct readout of total dissolved solids (mg/L) and with automatic temperature compensation.

**4.0 REAGENTS**

Unless otherwise stated use only analytical grade reagents. Distilled water which has been further purified by passing through a mixed bed deioniser is preferred. Conductivity of the distilled water should be less than 0.01 $\mu$S/m (1 $\mu$mho/cm).

4.1 **Phenolphthalein indicator** solution, 1 g/100 mL. Dissolve 1 g phenolphthalein in 50 mL ethanol plus 50 mL of distilled water.

4.2 Acetic acid $\rho_{20}$ = 1.06 g/mL.

**5.0 SAMPLES and SAMPLING**

5.1 Sample bottles. Use polythene bottles prepared for sampling as follows:

(a) Wash bottle and cap with detergent and tap water.
(b) Rinse thoroughly with tap water.
(c) Rinse twice with distilled water.
(d) Drain thoroughly and replace cap.

5.2 Sampling. Before sampling, thoroughly flush all distribution lines to ensure a representative sample. Rinse the sample bottle 2-3 times with the sample water then completely fill the container and replace cap. Boiler water collected under pressure should be cooled to about 20 °C while still under pressure. Conductivity should be measured within 4 hours of sampling.

**6.0 PROCEDURE**

6.1 Filter the cooled sample through a Whatman No. 1 filter paper.

6.2 Add 1-2 drops of phenolphthalein solution and if the water turns pink neutralise by adding acetic acid dropwise until the colour disappears.

6.3 Rinse and then fill the conductivity cell with sample.

6.4 Set the meter to read total dissolved solids and record the reading. (Refer to the operating instructions for the conductivity meter).

6.5 Record the total dissolved solids to 1 mg/L.

**7.0 PRECISION**

The expected range of results is 100-15,000 mg/L.
The precision of the method has not been determined.

# METHOD 46
# SULPHITE — DETERMINATION IN WATER

## 1.0 SCOPE AND FIELD OF APPLICATION

This method is recommended for use within the Australian Sugar Industry. Sulphite ions ($SO_3^{2-}$) may occur in boiler and boiler feed water treated with sulphite for dissolved oxygen control.

## 2.0 PRINCIPLE OF METHOD

A test sample of the water is acidified and titrated with standardised potassium iodide-iodate reagent. Free iodine liberated by the iodide-iodate reagent reacts with sulphite ions. When all the sulphite is oxidised an excess of free iodine occurs which forms a blue colour with water soluble starch indicator.

## 3.0 REAGENTS

Unless otherwise stated, use only analytical grade reagents and only distilled or deionised water.

3.1 **Standard potassium iodide-iodate** solution, 0.0125 mol/L. Dissolve 0.4458 g of anhydrous potassium iodate (dried for 4 hours at 120 °C), 4.35 g potassium iodide and 0.31 g sodium bicarbonate in distilled water and dilute to 1 litre.

3.2 **Starch indicator.** Use a commercial soluble starch indicator (e.g. Vitex).

## 4.0 SAMPLING AND SAMPLES

4.1 **Sample containers.** Use borosilicate glass containers prepared as follows :

(a) wash the container and cap with detergent and tap water,
(b) rinse thoroughly with tap water,
(c) rinse twice with distilled water,
(d) drain thoroughly and replace cap.

4.2 Before sampling, thoroughly flush all distribution lines to ensure a representative sample. Rinse the sample containers 2-3 times with the sample water then completely fill the containers and replace the cap. Take care to minimise contact with the air.

Boiler waters collected under pressure should be cooled to about 20 °C while still under pressure to minimise the loss of dissolved gases.

## 5.0 PROCEDURE

5.1 Measure 100 mL of sample into a 250 mL conical flask.

5.2 Add 0.5 mL of concentrated hydrochloric acid, $\rho_{20}$ = 1.16 g/mL. Use a graduated (barrel) pipette, with safety bulb.

5.3 Add, with a spatula, about 0.5 g of soluble starch indicator.

5.4 Titrate, over a white background, with potassium iodide-iodate reagent until a permanent blue tinge appears. Record the volume of iodide-iodate reagent used ($V_1$) to 0.1 mL.

5.5 Carry out a blank titration with 100 mL of distilled water following steps 5.2-5.4. Record the volume of iodide-iodate reagent used, $V_b$, to 0.1 mL.

## 6.0 CALCULATION

Calculate the sulphite in water as mg/L $SO_3^{2-}$

$$SO_3^{2-}\ (mg/L) = \frac{(V_1 - V_b) \times c \times 40\,000}{V}$$

where: V = mL sample taken
$V_1$ = mL iodide-iodate reagent to titrate sample (5.4)
$V_b$ = mL iodide-iodate reagent to titrate blank (5.5)
c = conc. (mol/L) of iodide-iodate reagent (3.1)

For a 100 mL sample aliquot

$SO_3^{2-}\ (mg/L) = (V_1 - V_b) \times 5$

Record the result as mg/L, $SO_3^{2-}$ to 1 mg/L

## 7.0 PRECISION

| Sample | Concentration Range $SO_3^{2-}$ (mg/L) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Boiler waters | 3-30 | 5 | 8 |

# METHOD 47

## TOTAL HARDNESS (CALCIUM AND MAGNESIUM) — DETERMINATION IN WATER BY EDTA TITRATION

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. Calcium and magnesium ions contribute to the formation of sludge and scale in water systems, and are removed by water treatment.

**2.0 PRINCIPLE OF METHOD**

The pH of the test sample is adjusted to a value of 10 by adding buffer solution, and the sample titrated with EDTA, using Eriochrome Black T indicator. The method measures the total content of calcium plus magnesium in the water.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Porcelain Basin** (evaporating basin), 25-30 cm dia.

3.2 **Magnetic stirrer**

**4.0 REAGENTS**

Unless otherwise stated, use only analytical grade reagents and only distilled or deionised water.

4.1 **Ammonium buffer solution,** dissolve 16.9 g ammonium chloride in 143 mL of concentrated ammonium hydroxide, $\rho_{20}$ = 0.88 g/mL. Add 1.25 g magnesium salt of EDTA and dilute to 250 mL with water.

If the magnesium salt of EDTA is unavailable, dissolve 1.129 g of disodium salt of EDTA and 0.780 g magnesium sulphate ($MgSO_4$ $7H_2O$) in 50 mL water. Add this solution to 16.9 g ammonium chloride and 143 mL concentrated ammonium hydroxide, $\rho_{20}$ = 0.88 g/mL. Mix well and dilute to 250 mL with distilled water.

Store the solution in a tightly sealed plastic bottle. Discard after 1 month.

4.2 **Eriochrome Black T** indicator. Dissolve 0.50 g eriochrome black in about 2 mL water, add 10 g sodium chloride and mix well. Dry for 1-2 hours at 100-105 °C. Add 90 g sodium chloride and grind until thoroughly mixed.

NOTE: Prepared dry indicator is available commercially (total hardness indicator tablets).

4.3 **Standard EDTA solution,** 0.01 mol/L. Dissolve 3.723 g EDTA in water and dilute to 1 litre in a volumetric flask. Store in a sealed polythene container. Standardise against standard calcium solution (4.4) by titrating 10.0 mL standard calcium solution, plus 80 mL distilled water, 2 mL ammonium buffer solution and 0.2 g of total hardness indicator with EDTA as described in steps 6.2-6.4 below.

Calculate the concentration, c of EDTA solution in moles per litre.

$$c = \frac{0.1}{\text{volume EDTA}}$$

4.4 **Standard Calcium Solution,** 1 mg/mL $CaCO_3$. Weigh 1.000 g anhydrous calcium carbonate into a 500 mL conical flask. Place a funnel in the neck of the flask and add 5 mol/L hydrochloric acid slowly until all the $CaCO_3$ is dissolved. Add about 200 mL water and boil 3-5 minutes to expel $CO_2$. Cool, add 2-3 drops methyl red indicator and adjust the pH to the intermediate orange colour by adding 5 mol/L HCl or 3 mol/L $NH_4OH$ as required. Transfer quantitatively to a 1L volumetric flask and dilute to volume with water.

**5.0 SAMPLING AND SAMPLES**

5.1 **Sample containers,** use polythene containers prepared as follows :

(a) Wash the container and cap with detergent and tap water,
(b) Rinse thoroughly with tap water,
(c) Rinse twice with distilled water,
(d) Drain thoroughly and replace cap.

5.2 **Collection and preservation.** Before sampling thoroughly flush all distribution lines to ensure a representative sample. Rinse the sample container 2-3 times with the sample then completely fill with sample and seal. Immediately store between 1 °C and 4 °C or acidify by adding 1 drop of concentrated nitric acid, $\rho_{20}$ = 1.42 g/mL.

**6.0 PROCEDURE**

6.1 With a measuring cylinder, transfer an aliquot (V) of the test sample to a porcelain dish

**For softened waters,** transfer 250 to 500 mL

**For raw waters,** transfer 50 to 100 mL

NOTE: The sample volume selected should require less than 15 mL of EDTA titrant and the titration should be completed within 5 minutes of adding buffer.

6.2 Add 2.0 mL of the ammonium buffer (4.1) for raw waters and 5.0 mL for softened waters. Mix well.

6.3 Add 0.2 g of total hardness indicator (4.2) or 0.4 g of indicator for softened waters. Mix well.

NOTE: For samples larger than 250 mL, increase the amounts of buffer and indicator proportionately.

6.4 Add EDTA (4.3) slowly with continuous stirring, until the last reddish tinge disappears [at the end-point the indicator changes from wine red to pale blue]. Record the volume of EDTA used, ($V_1$) to 0.1 mL.

**7.0 CALCULATION**

7.1 Calculate the total hardness of the sample expressed as mg/L $CaCO_3$.

$$\text{Hardness as } CaCO_3\text{(mg/L)} = \frac{V_1 \times c \times 100 \times 1000}{V}$$

where: V = mL of sample (6.1)
$V_1$ = mL of EDTA titrant (6.4)
c = concentration of standard EDTA solution, moles/L (4.3)

## 8.0 PRECISION

| Sample | Concentration Range (mg/L, as $CaCO_3$) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Softened water Boiler feed water | 0-50 | 8 | 40 |

## 9.0 REFERENCE

Standard methods for the Examination of Water and Waste Water; 16th Edition; American Public Health Association (1985).

# METHOD 48
## DISSOLVED OXYGEN (D.O.) — DETERMINATION IN WATER BY THE AZIDE MODIFICATION

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is recommended for use within the Australian Sugar Industry. The analysis for dissolved oxygen is a key test in water pollution and waste treatment process control. This procedure is used also to determine dissolved oxygen, before and after incubation, in the determination of biological oxygen demand (B.O.D).

**2.0 PRINCIPLE OF METHOD**

A divalent manganese solution, followed by a strongly alkaline solution, is added to the test sample. Dissolved oxygen oxidises an equivalent amount of the divalent manganous hydroxide precipitate to hydroxides of higher valency states. In the presence of iodide ions in acid solution, the oxidised manganese reverts to the divalent state, liberating iodine equivalent to the D.O. content. The iodine is titrated with standard thiosulphate solution.

Sodium azide is added if the sample contains more than 0.1 mg/L of nitrite N and not more than 1 mg/L of ferrous Fe. Other reducing or oxidising materials should be absent.

**3.0 REAGENTS**

3.1 **Manganous sulfate solution.** Dissolve 480 g $MnSO_4.4H_2O$, or 400 g $MnSO_4.2H_2O$, or 364 g $MnSO_4.H_2O$ in distilled water, filter, and dilute to 1 L. The $MnSO_4$ solution should not give a colour with starch when added to an acidified potassium iodide (KI) solution.

3.2 **Alkaline iodide-sodium azide solution.** Dissolve 500 g NaOH (or 700 g KOH) and 135 g NaI (or, 150 g KI) in water, dilute to about 950 mL and cool. Slowly with stirring add a solution of 10 g $NaN_3$ in 40 mL water. Store in a dark bottle.

This reagent should not give a colour with starch indicator when diluted and acidified.

*Safety*

Avoid contact with skin and eyes. Toxic if swallowed. Wear gloves and safety glasses. In cases of contact with skin or eyes wash with large quantities of cold water. If swallowed, give about 250 mL of cold water. Seek immediate medical advice. Contact with acids liberates a very toxic gas.

3.3 **Sulphuric acid,** concentrated $\rho_{20}$ = 1.84 g/mL.

*Safety*

Concentrated sulphuric acid is very corrosive. Safety glasses and gloves must be worn when handling the acid. It must not be pipetted by mouth.

3.4 **Sodium thiosulfate standard solution,** 0.025 mol/L. Dissolve 6.205 g $Na_2S_2O_3.5H_2O$ in water. Add 0.4 g solid NaOH and dilute to 1 L in a volumetric flask. Standardise against 0.0021 mol/L potassium bi-iodate solution (3.5). Dissolve about 2 g KI in 100-150 mL water in a conical flask. Add carefully, 2-3 drops of concentrated $H_2SO_4$ and 20.00 mL potassium bi-iodate solution. Titrate with sodium thiosulfate solution, to a pale straw yellow colour. Add starch indicator and continue titration to disappearance of blue colour.

Calculate the concentration of the sodium thiosulfate solution (c), in moles/L —

$$c = \frac{0.0021 \times 12 \times 20}{\text{volume } Na_2S_2O_3}$$

NOTE: It is convenient to adjust the concentration of thiosulfate solution to exactly 0.025 mol/L.

3.5 **Potassium bi-iodate standard solution,** 0.0021 mol/L. Dissolve 0.8125 g $KH(IO_3)_2$ in water in a 1 L volumetric flask and dilute to exactly 1 litre.

3.6 **Starch indicator.** Use an aqueous solution or a soluble starch powder.

3.7 **Potassium fluoride,** 40 g/100 mL solution of $KF.2H_2O$.

*Safety*

Toxic by inhalation, contact with skin and if swallowed. Wear gloves and safety glasses. If in contact with skin or eyes wash with large quantities of cold water. If swallowed, give about 250 mL water to dilute in stomach. Do not induce vomiting. Seek immediate medical advice.

**4.0 SAMPLES and SAMPLING**

4.1 **Sample bottles.** Use narrow neck, glass stoppered, glass bottles, 250-300 mL, prepared for sampling as follows:

(a) Wash bottle and stopper with detergent and tap water,
(b) Rinse thoroughly with tap water,
(c) Rinse with nitric acid (1+1),
(d) Rinse twice with distilled water,
(e) Drain and replace stopper.

4.2 **Sampling.** Collect samples very carefully. Do not let sample be agitated or remain in contact with air. Avoid entraining air or dissolving atmospheric oxygen.

Rinse the bottle by filling 2-3 times with sample. When sampling from a line under pressure attach a rubber tube and extend to the bottom of the bottle and allow the bottle to overflow 2-3 times its volume. Immediately replace stopper to exclude air.

Samples should be analysed immediately or stored for up to 4 hours after fixing by adding manganous sulphate solution, alkali iodide solution and $H_2SO_4$ as in 5.1 below.

**5.0 PROCEDURE**

NOTE: Add all reagents, except $H_2SO_4$, well below surface of sample from 10 mL graduated pipettes. Rinse pipettes before returning to reagent bottles.

5.1 Add 1 mL $MnSO_4$ solution (3.1) and 1 mL alkali-iodide-azide reagent (3.2) to the sample collected in the 250-300 mL bottle (4.2).

5.2 Stopper carefully to exclude air and mix by inverting several times.

5.3 After the precipitate has settled, leaving about 100 mm clear supernatant, remove stopper and add 1 mL of $H_2SO_4$ (3.3) down neck of bottle [If >100 mg/L ferric, ($Fe^{3+}$), iron is present add 1.0 mL KF solution (3.7) before acidifying].

5.4 Re-stopper and mix by inverting until precipitate is completely dissolved.

5.5 Immediately titrate 201 mL (1 mL is allowance for added reagents) with 0.025 mol/L $Na_2S_2O_3$ solution (3.4) to a pale straw colour. Add starch indicator (3.6) and continue titration to disappearance of blue colour. Record the volume of $Na_2S_2O_3$ used to 0.1 mL.

## 6.0 CALCULATION

$$\text{Dissolved oxygen, (mg/L)} = V \times \frac{c}{0.025}$$

where: V = mL, $Na_2S_2O_3$ used in titration (5.5)
c = conc. (moles/L) of $Na_2S_2O_3$ solution (3.4)

Record dissolved oxygen in sample to 1 mg/L.

## 7.0 PRECISION

The precision of the method has not been determined. As a guide the standard deviation for D.O. in waste waters is about 60 mg/L but can be much higher, due to bad sampling, suspended organic solids or heavy pollution.

## 8.0 REFERENCES

1. Standard Methods for the Examination of Water and Waste Water, 16th Edition, (1985); American Public Health Association.
2. Official Methods of Analysis, 14th Edition, (1984); Association of Official Analytical Chemists; U.S.A.

# METHOD 49
## SUCROSE SOLUBLE ALKALI — DETERMINATION IN LIME

**1.0 SCOPE AND FIELD OF APPLICATION**

This method has been adopted officially within the Australian Sugar Industry. The method is used to determine the alkali (expressed as CaO) in a sample of rock lime or slaked lime, which is available to react with sucrose to form a soluble calcium saccharate. It is a measure of the quality of lime purchased for use in juice clarification in mills or carbonatation in refineries.

**2.0 PRINCIPLE**

The sample is completely ground to less than 150 microns size. A small amount of ethanol is used to transfer the weighed sample into a flask, where it is mixed with a known volume of 10% sucrose solution. The flask is then shaken for 30 minutes and the suspended matter is allowed to settle out. The solution is coarse filtered, and an aliquot titrated with hydrochloric acid solution to a methyl orange end-point.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Mortar and pestle,** porcelain.

3.2 **Standard sieve,** 150 micron.

3.3 **Mechanical flask shaker,** to take 200 mL volumetric flask.

**4.0 REAGENTS**

Unless otherwise specified, use only analytical grade reagents and only distilled water which has been freed of carbon dioxide by boiling for 15 minutes and cooled to room temperature immediately before use.

4.1 **Sucrose solution, 100 g/L.** Dissolve 100 g sucrose in $CO_2$ free water and dilute to exactly 1 litre in a volumetric flask. Prepare the solution daily.

4.2 **Methyl Orange Indicator Solution, 500 mg/L.** Dissolve 0.5 g methyl orange in 1L of distilled water. Filter if necessary.

4.3 **Hydrochloric Acid Standard Solution 0.2 mol/L.** Prepare by diluting commercial concentrated volumetric solution with $CO_2$ free distilled water. Standardise weekly against sodium carbonate.

Dry about 2 g $Na_2CO_3$ at 260 °C for 30 minutes, and cool in a dessicator. Accurately weigh about 0.4 g dried $Na_2CO_3$ and transfer to a 250 mL conical flask. Record the weight of $Na_2CO_3$ to 0.1 mg. Add 3 drops of methyl orange, and titrate with the 0.2 mol/L hydrochloric acid. Record the titre to 0.1 mL.

Calculate the concentration, (c) in moles/L of the HCl

$$c = \frac{\text{g } Na_2CO_3 \text{ x } 1000}{\text{mL Acid x } 52.994}$$

4.4 **95% Ethyl alcohol.** Dilute 95.0 ± 0.1 mL ethyl alcohol in a 100 mL volumetric flask to 100 mL with water and mix thoroughly.

**5.0 PREPARATION OF SAMPLE**

The grinding operating should be carried out as quickly as possible to minimise reaction with atmospheric carbon dioxide.

5.1 For rock lime or slaked lime, grind approximately 20 g of well mixed sample using a mortar and pestle. Mix the sample thoroughly using a flat-bladed spatula. Cone and quarter, mix the two diagonally opposite portions, and discard the remaining sample.

5.2 Place the sample into a 150 micron sieve (with lid and base) and shake by hand until all material smaller than 150 micron has passed through.

5.3 Regrind sample larger than 150 micron and shake again. Repeat until all sample passes through mesh. Mix thoroughly and store in a clean, dry, well sealed container.

**6.0 PROCEDURE**

The analysis should be performed in duplicate using separately prepared lime samples. The titration of each sample should also be done in duplicate.

6.1 Weigh exactly 0.800 g of the finely ground lime and wash it into a clean, dry 200 mL volumetric flask with 4.00 ± 0.05 mL of 95% ethyl alcohol. To prevent lumping mix the sample and alcohol carefully, so that the sample is completely wet with alcohol before transferring to the flask.

6.2 Add 160 ± 1 mL of 10% sucrose solution (4.1), stopper and shake on a mechanical flask shaker for 30 minutes ± 30 secs. Let the flask stand for a few minutes to allow suspended matter to settle out. Dilute to the mark with 10% sucrose solution and mix thoroughly.

6.3 Filter through a coarse filter paper and reject the first 20 mLs of filtrate. Use the filtrate to rinse the filter flask.

6.4 Pipette a 50.0 mL aliquot of the filtrate into a 250 mL conical flask, without delay to avoid reaction with $CO_2$.

6.5 Add 4 drops of methyl orange indicator, and titrate with 0.2 mol/L HCl from a 50 mL burette until the clear yellow solution changes to a salmon colour. Over-titration leads to an orange colour.

Record the volume, (v) of 0.2 mol/L HCl used to 0.1 mL.

**7.0 CALCULATIONS**

1 mL of 1 mol/L HCl is equivalent to 0.02804 g of CaO.

$$\% \text{ sucrose soluble alkali, as CaO} = \frac{\text{v x c x } 0.02804 \text{ x } 200 \text{ x } 100}{50 \text{ x } 0.8000}$$

$$= \text{v x c x } 14.02$$

where v = volume of HCl used in the titration (6.5)

c = concentration of HCl, (moles/L) (4.3)

Record the % sucrose soluble alkali as CaO to 0.01% sucrose.

## 8.0 PRECISION

| Sample | Concentration Range (%) | 95% Repeatability r | 95% Reproducibility R |
|---|---|---|---|
| Quicklime | 70-97 | 0.33 | not determined |
| Slaked lime | 60-70 | 0.33 | not determined |

# METHOD 50

## SUCROSE — DETERMINATION IN EFFLUENTS BY THE PHENOL-SULPHURIC ACID METHOD

**1.0 SCOPE AND FIELD OF APPLICATION**

This method gives a semi-quantitative measure of sugar and sugar degradation products in factory effluents. The prime objective of the analysis is a measure of sucrose loss by leakage or entrainment into condensate streams and cooling waters in the factory.

A limitation imposed on the method is that it also effectively measures furfuraldehyde and its homologues. Furthermore, it has been established that $\alpha$- and $\beta$- keto acids and aliphatic aldehydes and ketones give a yellow colour with this reagent and they will interfere to a varying degree in the determination of carbohydrates.

**2.0 PRINCIPLE**

An aliquot of the test solution is mixed with the phenol reagent in a test tube and a measured volume of concentrated sulphuric acid added. The acid reacts with sucrose to give a furfural derivative which then combines with phenol to give an orange condensation product. The concentration of sucrose in the test solution is obtained by measuring the absorbance of light at 488 nm and then reading off the concentration from a previously prepared graph of absorbance versus sucrose concentration.

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware and

3.1 **Pyrex test tubes**, 20 mm x 150 mm

3.2 **Volumetric flasks**, Class A, 100 mL, 1L

3.3 **Bulb pipettes**, Class A, 0.5 mL, 1 mL, 2 mL, 3 mL, 4 mL, 6 mL, 8 mL

3.4 **Automatic dispenser**, to dispense 5.0 ± 0.03 mL sulphuric acid

3.5 **Vortex mixer**

3.6 **Spectrophotometer**, suitable for the measurement of absorbance at 488 nm, and 1 cm matched cuvettes.

**4.0 REAGENTS**

Unless otherwise specified, use only analytical grade reagents and only distilled water.

4.1 **Sulphuric Acid**, conc. ($\rho_{20}$ = 1.84 g/mL)

*Safety*

Safety glasses and gloves must be worn. Sulphuric acid is best dispensed behind a perspex safety shield.

4.2 **Phenol Reagent**, 50 ± 1 g/L solution in water*. (This should be stored in a dark bottle).

*Safety*

Avoid contact with the skin and breathing the vapour.

4.3 **Standard Sucrose**, 1000 mg/L solution.

Dissolve 1.000 ± 0.001 g sucrose in water and dilute to 1 litre with water.

4.4 **Working Standard Sucrose Solution**. Pipette 0.50, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 mL of the 1000 mg/L standard sucrose solution into separate 100 mL volumetric flasks and make to the mark with water. This will give standard dilutions of 5, 10, 20, 30, 40, 60 and 80 mg/L of sucrose.

**5.0 STANDARDISATION OF METHOD**

5.1 Pipette 1.00 ± 0.015 mL of water into a test tube. This will be the 0 mg/L standard or blank required for zeroing the spectrophotometer.

5.2 Pipette 1.00 ± 0.015 mL of each of the working standards respectively into seven test tubes. These will correspond to 5, 10, 20, 30, 40 and 80 mg/L of sucrose.

5.3 Add 1.00 ± 0.015 mL of the phenol reagent (4.2) to each tube and mix.

*Safety*

Avoid contact with the skin and breathing the vapour.

5.4 Add, with an automatic dispenser, 5.0 mL of concentrated sulphuric acid to each test tube. Add the acid in a continuous jet into the centre of the liquid, while holding the test tube in a vertical position. Do not allow the acid to run down the sides of the tube.

*Safety*

Safety glasses and gloves must be worn.

5.5 Using a vortex mixer, mix the contents of each test tube immediately, then stand the solutions for at least 30 minutes to allow the colour to develop.

After addition of the acid, the temperature rises spontaneously to approximately 110 °C and the heat so generated completes the development of the colour. The colour is stable for several hours.

5.6 Determine the absorbance of each standard solution in 1 cm cells in a spectrophotometer at 488 nm, with the 0 mg/L standard (blank) as reference.

5.7 Plot the absorbance for each standard against its sucrose content (mg/L) and draw a straight line of best fit. It should pass through the origin. If two or more points lie off the line of best fit by more than 5% of the line's absorbance value at that particular sugar concentration, the standardisation must be repeated.

**6.0 PROCEDURE**

In collecting samples, care must be taken to avoid contamination. Samples should be tested as soon as possible after being taken to avoid the risk of decomposition of sucrose in these dilute solutions. Determination in duplicate reduces errors and allows exclusion of gross anomalies resulting from accidental contamination.

6.1 Pipette 1.00 ± 0.015 mL of the test sample into a test tube.

6.2 Add 1.00 ± 0.015 mL of the phenol reagent (4.2) to the test tube and mix.

*Safety*

Avoid contact with skin and breathing the vapour.

* If solid phenol is not available, liquified phenol (commercial product, 30% phenol in water) can be diluted to 9.2% phenol (w/w), distilled as a two phase azeotrope (bp 99.5 °C), and diluted to 5%. This operation must be carried out in an efficient fume hood.

6.3 Add, with an automatic dispenser, 5.0 mL of concentrated sulphuric acid. Add the acid in a continuous jet into the centre of the liquid, while holding the test tube in a vertical position. Do not allow the acid to run down the sides of the test tube.

*Safety*

Safety glasses and gloves must be worn.

6.4 Using a vortex mixer, mix the contents of the test tube immediately and allow to stand for 30 minutes for colour to develop.

6.5 Determine the absorbance of the sample at 488 nm relative to the 0 mg/L standard.

A new 0 mg/L standard (blank) may have to be prepared if the old standard is more than a day old.

6.6 Estimate the sucrose content of the test sample from the plot of absorbance versus sucrose concentration. Record the result as mg/L sucrose in the test sample.

6.7 If the absorbance of the test solution exceeds that of the highest standard, dilute an aliquot of the test sample with water and repeat the test.

**7.0 CALCULATION**

Sucrose (mg/L) = estimated sucrose (mg/L) x dilution factor (see 6.6)

where:

$$\text{dilution factor} = \frac{\text{diluted volume}}{\text{test sample aliquot}} \quad \text{(see 6.7)}$$

**8.0 PRECISION**

Expected range of results: 0-100 mg/L
95% repeatability (r): 2.3 ppm

**9.0 REFERENCE**

M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. *Anal. Chem.,* Vol. 28, No. 3, 1956, p. 350.

# METHOD 51
## BIOCHEMICAL OXYGEN DEMAND ($BOD_5$) — DETERMINATION IN LIQUID EFFLUENTS

**1.0 SCOPE AND FIELD OF APPLICATION**

This method is a standard method for BOD analysis and is prescribed by the Water Quality Council of Queensland for the determination of the quality of effluents discharged to a water-course.

It is applied also to a broad-spectrum assessment of the level of organic matter in other water discharges e.g. discharge to an effluent disposal plant or from a cooling tower. However, the test will under-estimate to varying degrees the level of organic matter and the result in these instances is best interpreted in comparison with an analysis of Chemical Oxygen Demand (COD).

**2.0 PRINCIPLE OF METHOD**

The consumption of oxygen or oxygen-uptake by microorganisms metabolising organic matter follows roughly a sigmoidal curve with respect to time. The rate of growth of microorganisms is temperature-dependent. Therefore, by convention, a five-day time period and temperature of 20 °C have been accepted as standard. The five-day BOD is about 70% of the ultimate oxygen demand which takes 20 to 30 days to be exerted.

The test requires a determination of the dissolved oxygen content of a sample at the time of preparation and after five days incubation. The determination of dissolved oxygen is done by the azide modification method (Method No. 48). The saturation level of dissolved oxygen in water at 20 °C is only 9.2 mg/L. Therefore, samples contaminated with high levels of organic matter must be diluted sufficiently to obtain a titratable level of oxygen after five days. Also, as nitrifying microorganisms could oxidise the ammonium ion present in the dilution water, inhibition of nitrification is recommended for samples likely to contain large numbers of these organisms. Such wastewaters are secondary effluents, aerobic ponds, polluted streams and any of these samples can be when used as seed (see 5.2).

**3.0 APPARATUS**

Ordinary laboratory apparatus and glassware (Brown ground-glass-stoppered reagent bottles are preferable for incubated samples) and

3.1 **A refrigerator or water-bath,** controlled at 20 °C which permits incubation of samples in the dark.

(NOTE: Minute quantities of sugar or molasses or bio-degradable detergents will result in inflated BOD readings. BOD glassware should be separate from normal laboratory materials and not washed with detergent, only rinsed thoroughly with tap water followed by distilled water).

**4.0 REAGENTS**

Unless otherwise specified, use only analytical grade reagents and only distilled water.

4.1 **Phosphate buffer solution.** Dissolve 8.5 g potassium dihydrogen phosphate, 21.75 g dipotassium hydrogen phosphate, 33.4 g disodium hydrogen phosphate heptahydrate, and 1.7 g ammonium chloride in about 500 mL distilled water and dilute to 1L. The pH should be 7.2.

4.2 **Magnesium sulphate solution.** Dissolve 22.5 g magnesium sulphate heptahydrate in distilled water and dilute to 1L.

4.3 **Calcium chloride solution.** Dissolve 27.5 g anhydrous calcium chloride in distilled water and dilute to 1L.

4.4 **Ferric chloride solution.** Dissolve 0.25 g of ferric chloride hexahydrate in distilled water and dilute to 1L.

4.5 **Sulphuric acid,** 0.1 mol/L solution.

4.6 **Sodium hydroxide,** 0.1 mol/L solution.

4.7 **Sodium sulphite solution,** 0.013 mol/L. 1.575 g sodium sulphite per L distilled water. Prepare daily.

4.8 **Nitrification inhibitor.** Reagent grade 2-chloro-6-(trichloromethyl) pyridine, (e.g. N-Serve, Dow Chemical Co., or Nitrification inhibitor 25-33, Hach Chemical Co.)

4.9 **Glucose-Glutamic acid solution.** Dry at 105 °C for one hour reagent grade glucose and glutamic acid. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1L. Prepare fresh immediately before use when required as a check solution.

**5.0 PROCEDURE**

(a) $BOD_5$

5.1 **Dilution Water.** Store the distilled water in cotton wool-plugged bottles at 20 °C for sufficient time to allow the water to become saturated with dissolved oxygen. Alternatively, aerate overnight with filtered air at 20 °C. Add 1 mL of phosphate buffer (4.1), magnesium sulphate (4.2), calcium chloride (4.3) and ferric chloride (4.4) solutions per L.

5.2 **The Addition of Seed.** In many samples, e.g. wastewater from a treatment plant or pond or the overflow from a cooling tower, and in river water, there are sufficient numbers of microorganisms to initiate oxidation of organic matter. However, frozen samples or samples with extreme pH or that were very hot will require 'seeding'. Suitable sources of 'seed' are a river water taken from below a waste discharge point or an effluent plant overflow. A suitable seed concentration is 1 to 5 mL/L. The 'seed' can be added to the sample or to dilution water for a large number of samples but must be analysed for BOD and corrected for. The DO uptake of the seeded dilution water should be between 0.7 and 1.0 mg/L.

5.3 **Sample Pre-Treatment.** Samples containing caustic alkalinity or acidity should be adjusted to pH 7.0, without diluting the sample by more than 0.5%. Samples supersaturated with oxygen e.g. due to algal activity should be brought to 20 °C and agitated by vigorous shaking. If the sample has been chlorinated, seed the dilution water. If the sample contains residual

chlorine, dechlorinate or preferably sample ahead of the chlorination process. Chlorine may dissipate on standing the sample for 1 to 2 hours in the light. If not, destroy the chlorine residual by adding $Na_2SO_3$ solution to the neutralised sample. Determine the required volume of $Na_2SO_3$ solution on a 100 to 1000 mL portion of neutralised sample, by adding 10 mL of 1 + 1 acetic acid or 1 + 50 sulphuric acid, 10 mL potassium iodide solution (10 g/100 mL) and titrating with 0.025 mol/L thiosulphate titrant to the starch-iodine end-point. Add the required volume of $Na_2SO_3$ to the sample and check for residual chlorine after 10 to 20 minutes.

If the nitrification inhibitor is desired, add 10 mg 2-chloro-6 (trichloro methyl) pyridine per litre of dilution water at the same time as adding nutrient and buffer solutions. Samples that may require nitrification inhibition include, but are not limited to, biologically treated effluents, samples seeded with such effluents and river waters. Note the use of the nitrification inhibitor when reporting results.

5.4 **Sample Dilution.** A sample has to be diluted sufficiently to allow about 5 mg/L drop in DO in five days. Without prior knowledge of the BOD, a range of dilutions for a particular sample will be required. As a guide, unpolluted river water is not diluted, a treatment plant discharging near 20 mg/L $BOD_5$ would be diluted 1/4 or 1/5 and a cooling tower overflow 1/200 or 1/400. A useful rule of thumb is to divide the anticipated $BOD_5$ by five to obtain the dilution required.

5.5 **Procedure.** Add the desired quantity of sample to a 1L graduated cylinder or volumetric flask and dilute to 1L with standard dilution water. Mix well with a plunger-type mixing rod taking care to avoid excessive shaking or air entrainment. Siphon the sample into three 250 mL brown reagent bottles, fill to their brim and stopper. One sample is analysed for initial DO and the remaining two are stopped tightly, water-sealed and incubated for 5 days at 20°C.

Include seed controls, dilution water blanks (unseeded) and from time to time, glucose-glutamic acid solution checks. Their DO uptakes should be 0.6 to 1.0, $<0.1$ and 200 ± 37 mg/L respectively.

The dilution selected for BOD determination is one which gives closest to a 5 mg/L drop in dissolved oxygen. Grossly over-diluted samples should not be used for BOD calculation. Similarly, the 'seed' correction should be determined from a normal determination of BOD on the 'seed' sample and not one where the analysis is based on the 'seed' dilution used in the main sample. Grossly over-diluted samples are subject to erratic oxidation.

(b) Oxygen Determination

The initial sample, 5 day incubated samples, seed controls, dilution water blanks and glucose-glutamic acid solution checks are analysed for dissolved oxygen by Method No. 48 'Dissolved Oxygen (DO) — Determination in Water by the Azide Modification'.

## 6.0 CALCULATIONS

For 200 mL of original sample,

1 mL 0.0250 mol/L sodium thiosulphate = 1mg/L DO.

When seeding is not required

$$\text{mg/L BOD} = \frac{D_1 - D_2}{P}$$

where: $D_1$ = DO of diluted sample after 15 minutes
$D_2$ = DO of diluted sample after incubation
$P$ = decimal fraction of sample used

When seeding is required

$$\text{mg/L BOD} = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where: $B_1$ = DO of diluted seed control after 15 minutes
$B_2$ = DO of diluted seed control after incubation
$f$ = ratio of seed in sample to seed in control

## 7.0 PRECISION

The BOD test is a bioassay and not a precise chemical analysis. A check on the method can be obtained by analysis of a solution containing 150 mg/L of glucose and 150 mg/L of glutamic acid. The $BOD_5$ should be in the range 200 ± 37 mg/L.

## 8.0 REFERENCES

American Public Health Association, American Water Works Association and Water Pollution Council Federation (1980). In, *Standard Methods for the Examination of Water and Wastewater*, 15th Edition, APHA, Washington, DC, pp. 390-393, 483-489.