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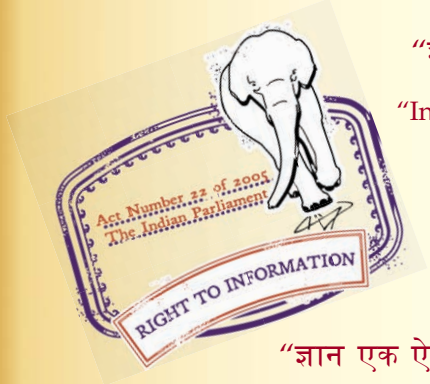

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Indian Standard

METHODS OF TEST FOR DAIRY INDUSTRY

PART II CHEMICAL ANALYSIS OF MILK

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MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

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Indian Standard

METHODS OF TEST FOR DAIRY INDUSTRY

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Indian Standard

METHODS OF TEST FOR DAIRY INDUSTRY

PART II CHEMICAL ANALYSIS OF MILK

0. FOREWORD

0.1 This Indian Standard (Part II) was adopted by the Indian Standards Institution on 21 September 1961, after the draft finalized by the Dairy Industry Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 Part II of this standard covers methods of chemical analysis which are generally required for detailed analysis of milk. The physical, chemical and bacteriological methods of analysis, which are used for assessing *rapidly* the quality of raw milk supplies for processing and manufacture, have already been covered in Part I of this standard. Parts III, IV and V of this standard, which are under preparation, would cover in detail (a) Methods of Bacteriological Analysis of Milk, (b) Method of Determination of Freezing Point of Milk, and (c) Methods of Dairy Plant Control respectively.

0.3 It is expected that the adoption of this standard in India will help in achieving uniformity in the methods of analysis of milk, thereby facilitating the interpretation and comparison of results.

0.4 This standard (Part II) is one of a series of Indian Standards on methods of testing milk and equipment used for these tests. Other standards published so far in the series are:

*IS: 1183-1957 Specification for Density Hydrometers for Use in Milk

IS: 1223-1958 Specification for Apparatus for the Determination of Fat in Whole Milk, Evaporated (Unsweetened) Milk, Separated Milk, Skim Milk, Buttermilk and Cream by the Gerber Method

IS: 1224-1958 Determination of Fat in Whole Milk, Evaporated (Unsweetened) Milk, Separated Milk, Skim Milk, Buttermilk and Cream by the Gerber Method

IS: 1479 (Part I)-1960 Methods of Test for Dairy Industry Part I Rapid Examination of Milk

*Since revised.

0.5 In the formulation of Part II of this standard, considerable assistance has been derived from the following publications:

B.S. 1741: 1951 METHODS FOR THE CHEMICAL ANALYSIS OF LIQUID MILK. British Standards Institution.

STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS. American Public Health Association, 11th ed. 1960.

LABORATORY MANUAL. Milk Industry Foundation, Washington.

OFFICIAL METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 9th ed. Washington, 1960.

Full use has also been made of the valuable information received from the National Dairy Research Institute, Karnal.

0.6 Metric system has been adopted in India and all quantities and dimensions in this standard have been given in this system.

0.7 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS: 2-1960 Rules for Rounding Off Numerical Values (*Revised*).

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1. SCOPE

1.1 This standard (Part II) specifies chemical methods commonly used for the detailed analysis of milk. The specific method(s) to be employed would depend upon the object of analysis.

1.1.1 This part does not include rapid methods of analysis already covered in Part I of this standard. It also does not include the cryoscopic method for the determination of freezing point.

2. QUALITY OF REAGENTS

2.1 Unless otherwise specified, pure chemicals and distilled water shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the experimental results.

3. PREPARATION OF SAMPLES

3.1 Before withdrawing portions for analytical determinations, bring the sample to a temperature of 26 to 28°C and mix thoroughly by pouring gently into a clean dry receptacle and back, until a homogeneous mixture is assured.

3.1.1 If lumps of cream do not completely disappear, warm the sample to about 40°C, mix thoroughly, then cool to 26 to 28°C. In case a

measured volume is required in a determination, bring the temperature of the sample to about 27°C before pipetting.

3.1.2 All samples shall be allowed to stand for three to four minutes after mixing to allow air bubbles to rise; the sample bottle shall then be inverted three or four times immediately prior to taking the milk for analysis.

NOTE — If a sample is curdled, excessively churned or decomposed, the sample shall not be taken up for analysis.

4. DETERMINATION OF TOTAL SOLIDS (GRAVIMETRIC METHOD)

4.1 Apparatus

4.1.1 *Shallow Flat-Bottomed Dishes* — of aluminium alloy, nickel, stainless steel, porcelain or silica, 7 to 8 cm diameter, about 1·5 cm in height and provided with easily removable but closely fitting lids.

4.2 Procedure — Weigh accurately the clean, dry empty dish with the lid. Pipette into the dish about 5 ml of the prepared sample of milk and weigh quickly, with the lid on the dish. Place the dish, uncovered, on a boiling water-bath. Keep the base of the dish horizontal to promote uniform drying and protect it from direct contact with the metal of the water-bath. After at least 30 minutes, remove the dish, wipe the bottom and transfer to a well-ventilated oven at 98 to 100°C, placing the lid by the dish. The bulb of the oven control thermometer shall be immediately above the shelf carrying the dish. The dish shall not be placed near the walls of the oven, and shall be insulated from the shelf, for example, by a silica or glass triangle. The shelf used shall be near the middle of the oven. After three hours, cover the dish and immediately transfer it to a desiccator. Allow to cool for about 30 minutes and weigh. Return the dish, uncovered, and the lid to the oven and heat for one hour. Remove to the desiccator, cool and weigh, as before. Repeat, if necessary, until the loss of weight between successive weighings does not exceed 0·5 mg. Note the lowest weight.

4.3 Calculation

$$\text{Total solids, percent by weight} = \frac{100\,w}{W}$$

where

w = weight in g of the residue after drying, and

W = weight in g of the prepared sample taken for the test.

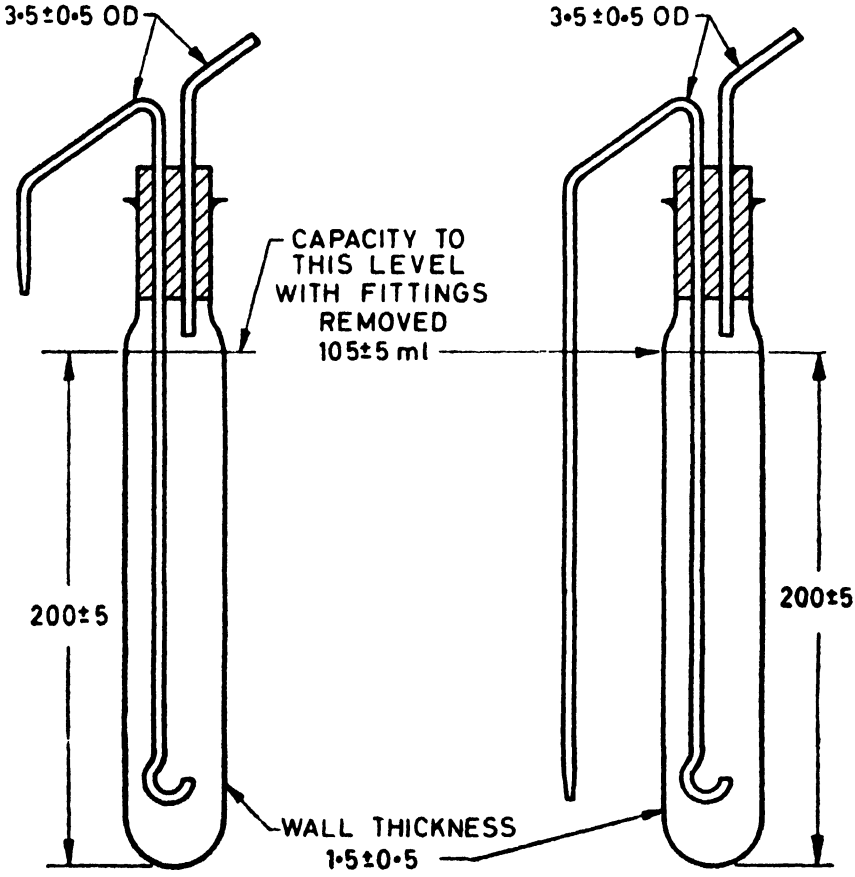
5. DETERMINATION OF FAT (RÖSE-GOTTLIEB METHOD)

5.1 Apparatus

5.1.1 *Fat-Extraction Apparatus* — Either of the following apparatus may be used:

a) A fat-extraction tube conforming to the dimensions and capacities given in Fig. 1, 2 or 3, fitted, as shown, with either a wash-bottle

top or a siphon, carrying the two tubes in a two-holed bark cork, and provided also with a ground-glass stopper or with a solid bark cork. The solid bark cork used to close the tube shall be sound, free from pores and channels which would allow leakage of solvent, and previously extracted with ether. The narrow tube with the

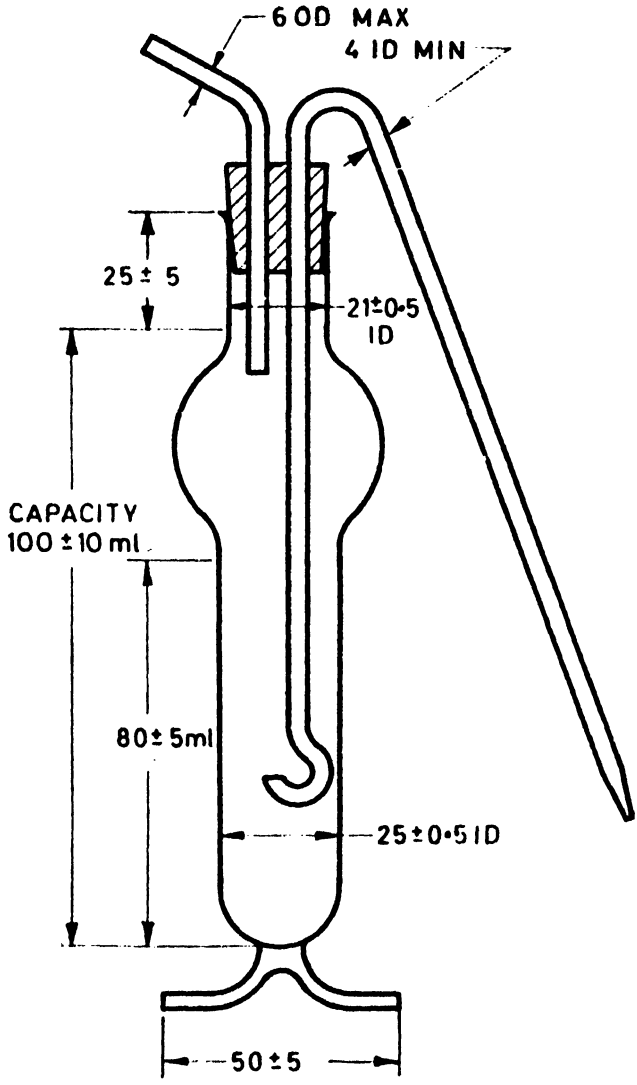


All dimensions in millimetres unless otherwise stated.

FIG. 1 FAT-EXTRACTION TUBE WITH WASH-BOTTLE FITTINGS

All dimensions in millimetres unless otherwise stated.

FIG. 2 FAT-EXTRACTION TUBE WITH SIPHON



All dimensions in millimetres unless otherwise stated.

FIG. 3 ALTERNATIVE FAT-EXTRACTION TUBE

hook-shaped lower end (*see* Fig. 1, 2 and 3) is a sliding fit in the cork and of such length that the opening at its lower end may be placed, if necessary, at a distance of 25 mm from the bottom of the tube.

NOTE — Other modifications of the Röhrig tube of the same capacity may also be used.

b) A Mojonnier fat-extraction tube of the dimensions and capacity given in Fig. 4, closed with a solid bark or ground-glass stopper.

5.1.2 *A Well-Ventilated Electrically Heated Oven* — set to operate at 98 to 100°C.

5.2 Reagents

5.2.1 *Concentrated Ammonia Solution* — sp-gr 0·88.

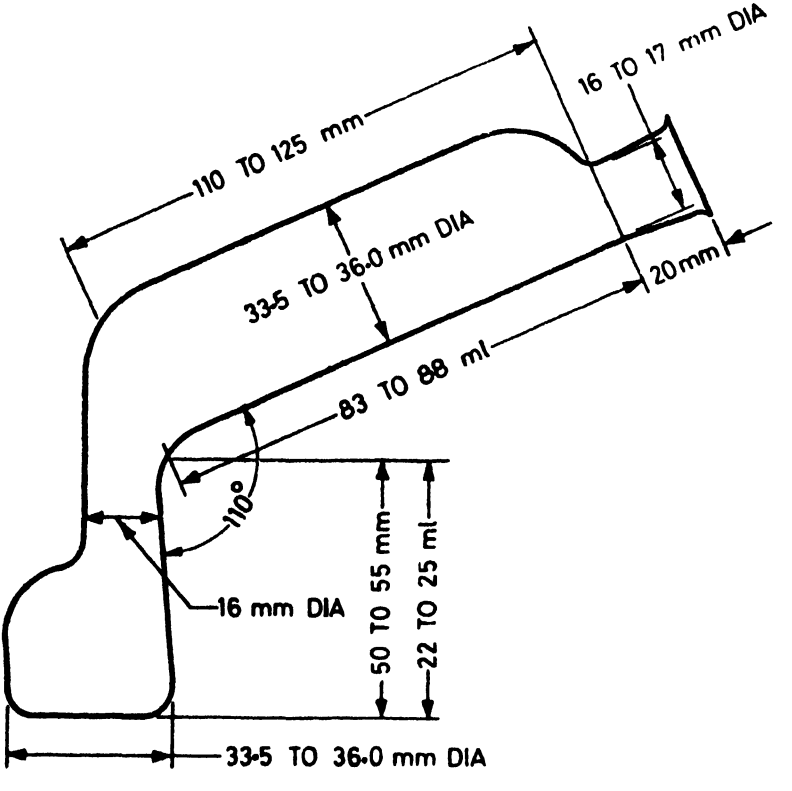


FIG. 4 MOJONNIER FAT-EXTRACTION TUBE

5.2.2 *Ethyl Alcohol* — 95 to 96 percent (*v/v*).

5.2.3 *Diethyl Ether* — sp gr 0·720, peroxide-free. It may be maintained free from peroxide by adding wet zinc foil (approx 80 cm per litre, cut in strips long enough to reach at least half-way up the container) that has been completely immersed in dilute acidified copper sulphate for 1 minute and subsequently washed with water.

5.2.4 *Light Petroleum* — boiling range 40 to 60°C.

5.2.5 *Mixed Solvent* — prepared by mixing equal volumes of the ether and light petroleum.

5.3 Procedure

5.3.1 *Using the Fat-Extraction Tube* (*Fig.* 1, 2 *or* 3) — Weigh accurately 10 to 11 g of the prepared sample into the extraction tube, add one millilitre of concentrated ammonia solution and mix well. Add 10 ml of alcohol and again mix well. Complete extraction of the fat is dependent on satisfactory mixing at each stage.

Add 25 ml of ether, close the tube with the cork (or stopper), which is wetted with water before insertion, and shake vigorously for one minute. Remove the cork and, with 25 ml of light petroleum, wash the cork, and neck of the tube so that the washings run into the tube. Replace the cork, again wetted with water, and shake vigorously for 30 seconds.

[It is essential that the cork (or stopper) be wetted with water before each insertion and washed with solvent during each removal. Also, before each removal, to avoid spurting of the solvent, a slightly reduced pressure should be induced in the tube by cooling. Rubber stoppers shall not be used.]

Allow the tube to stand until the ethereal layer is clear and completely separated from the aqueous layer, usually for not less than 30 minutes. (For extraction tubes shown in Fig. 1 and 2, separation of layers may be achieved by the use of a centrifuge.) Remove the cork and insert the siphon (or wash-bottle) fitting so adjusted for length that the inlet is 2 to 3 mm above the interface between the ethereal and aqueous layers, and transfer the ethereal layer to a suitable flask. Add 5 ml of mixed solvent to the extraction tube, using it to wash the siphon or wash-bottle fitting which is raised sufficiently to permit this but not removed, and the inside of the tube. Lower the fittings and transfer the solvent, without shaking, to the flask. Repeat this operation with a further 5 ml of mixed solvent. Wash the tip of the siphon fitting into the flask with mixed solvent.

Remove the siphon fitting, and repeat the extraction of the milk residue, using 15 ml of ether and 15 ml of light petroleum, and repeat the subsequent operations, as before. Use the ether to wash the inner limb of the siphon (or wash-bottle) fitting during its removal from the tube. Finally, repeat the extraction once more with 15 ml each of ether and petroleum.

Distil carefully the solvents from the flask and dry the residual fat in the oven at 98 to 100°C for one hour taking precautions to remove all traces of volatile solvent and cooling the flask to room temperature in a desiccator charged with an efficient desiccant. Repeat this procedure for periods of half an hour until successive weighings do not show a loss in weight by more than one milligram.

Extract completely the fat from the flask by repeated washing with light petroleum, allowing any sediment to settle before each decantation, dry the flask in the oven, cool and weigh as before. The difference in weights before and after the petroleum extractions, subject, if necessary, to a correction for the blank described below, is the weight of fat contained in the weight of milk taken.

Make a blank determination using the specified quantities of reagents throughout, and water in place of the milk, and deduct the value found, if any, from the apparent weight of fat. A flask, similar to that used to contain the fat, shall receive the same heating and cooling treatments and shall be used as a counterweight.

5.3.2 *Using the Mojonnier Fat-Extraction Tube* (Fig. 4) — Weigh accurately 10 or 11 g of the prepared sample into the tube. Add one millilitre of concentrated ammonia solution and mix well in the lower bulb. Add 10 ml of the alcohol and mix by allowing the liquid to flow backwards and forwards between the two bulbs. (Avoid bringing the liquid too near the neck of the tube.) Allow the tube to cool in cold, running water or by immersing in chilled water.

Add 25 ml of ether, close with a bark cork or glass stopper which is wetted with water before insertion, and shake vigorously for one minute.

[It is essential that the cork (or stopper) be wetted with water before each insertion and washed with solvent during each removal. Also, before each removal to avoid spurting of the solvent a slightly reduced pressure should be induced in the tube by cooling. Rubber stoppers shall not be used.]

Open the tube and add 25 ml of light petroleum, close the tube, and shake vigorously for one minute. Allow the tube to stand on the flat bottom of the lower bulb until the ethereal layer is clear and completely separated from the aqueous layer, usually for not less than 30 minutes, or centrifuge until clear. Examine the tube to see if the junction of the liquid is at the lower end of the narrow neck of the tube. If it is below this, it should be raised by the addition of a little distilled water run down the side of the tube.

Carefully decant as much as possible of the supernatant layer into a suitable flask by gradually bringing the cylindrical bulb of the tube into a horizontal position. When as much as possible has been poured off, wash the outside of the neck of the tube and the cork or stopper with mixed solvent, collecting the rinsings in the flask. With the Mojonnier tube

in a vertical position, wash the inside of the neck with 4 to 5 ml of mixed solvent, and decant.

Repeat the extraction of the milk residue and the subsequent operations but using 15 ml of ether and 15 ml of petroleum.

Finally repeat the extraction and subsequent operations once more with 15 ml each of ether and petroleum.

Distil carefully the solvents from the flask and dry the residual fat in the oven at 98 to 100°C for one hour taking precautions to remove all traces of volatile solvent and cool the flask to room temperature in a desiccator charged with an efficient desiccant. Repeat this procedure for periods of half an hour until successive weighings do not show a loss in weight by more than one milligram.

Extract completely the fat from the flask by repeated washing with light petroleum, allowing any sediment to settle before each decantation, dry the flask in the oven, cool and weigh as before. The difference in weights before and after the petroleum extractions, subject, if necessary, to a correction for the blank described below, is the weight of fat contained in the weight of milk taken.

Make a blank determination using the specified quantities of reagents throughout, and water in place of milk, and deduct the value found, if any, from the apparent weight of fat. A flask similar to that used to contain the fat shall receive the same heating and cooling treatments and shall be used as a counterweight.

6. DETERMINATION OF *p*H

6.1 Methods — The *p*H of milk may be determined by either the *colorimetric (comparator) method* or the *potentiometric method.*

6.2 Colorimetric (Comparator) Method — The method is based on the fact that certain indicators show definite changes of colour, depending on the *p*H of the sample tested. Colorimetric *p*H determination shall be used only as an aid for the detection of abnormal milk and not as conclusive evidence of such abnormality. Accuracy higher than 0·2 *p*H unit may not be expected from this method.

Various models are available on the market for the colorimetric determination of *p*H consisting basically of a comparator, standard disc to be used with each indicator, standard indicator solution, 1-ml graduated pipette and cells or test-tubes in duplicate. The detailed instructions for making the test are furnished with each set which shall be followed. Usually 10 ml of milk are used and 0·2 ml of a standard indicator solution is added. Another cell containing milk without indicator serves as a control for matching the colour. The blank tube is placed on the left, and milk containing indicator on the right in the slots provided in the comparator. The standard disc is rotated to get an even match.

The determination is carried out in the daylight or with good daylight lamp.

The indicators most suitable for determining the *p*H of milk colorimetrically are bromothymol blue (*p*H 6·0 to 7·6) and bromocresol purple (*p*H 5·2 to 6·8).

6.3 Potentiometric Method

6.3.0 This is the most accurate and reliable method for measuring the *p*H of milk. Several types of apparatus are available for the electrometric measurement either working with the help of dry cells, battery (bench types) or directly from the mains, where steady DC supply is available. Instruments adopted for use with AC supply are also available. With each instrument, the instructions of the maker shall be followed.

Two types of electrodes are used, glass and quinhydrone. The former is usable almost throughout the *p*H range but is fragile and great care is necessary in handling. The quinhydrone electrode (platinum wire or disc dipped in a saturated solution of quinhydrone) has a limited range but is quite satisfactory for the usual milk control work and gives reliable results, provided it is not used in solutions having *p*H over 8·0.

6.3.1 *Apparatus*

a) *Milk half cell* — Place in a 25-ml beaker or wide mouth test-tube enough milk to cover the electrode. Add about 0·1 to 0·2 g quinhydrone and mix well. Place a clean platinum or gold electrode, rinsed with glass-distilled water and suitably supported on a stand, into the sample. The platinum electrode should preferably be immersed in boiling water, or flamed to dull red heat in a spirit lamp, just before use.

b) *The standard saturated calomel half cell* — The calomel electrode consists of a layer of purified mercury, covered with a mixture of mercury and calomel, above which is a saturated solution of potassium chloride saturated with calomel. The mixture of mercury and calomel is prepared by grinding them into a paste with a pestle and mortar. The mixture is washed with saturated potassium chloride solution by decantation several times. It is then shaken with the remainder of the potassium chloride solution. When saturated with calomel, the solution is decanted and kept in a stoppered bottle for further use.

There are many forms of electrode vessels available to set up the half cell. Connection between the mercury and the potentiometer is made by a platinum wire. The two half cells are conveniently connected by an agar-agar bridge (thin glass tube, bent into a U-shape and filled with agar solution containing 5 g of agar-agar per 100 ml of saturated potassium chloride solution). The bridge shall be kept in saturated potassium chloride solution when not used.

6.3.2 *Procedure* — Standardize the instrument against a buffer solution of known *p*H and check against another buffer of different *p*H. Then connect the saturated calomel and milk half cells to the potentiometer. Balance the potentiometer to make the reading. Equilibrium occurs almost instantaneously and no drift is experienced. The *p*H is indicated directly on the instrument.

NOTE — When a batch of samples is to be examined, remove the agar-agar bridge after each testing sample, wash the end dipping into the milk carefully with glass-distilled water and replace in the next sample.

6.4 Indication — In normal milk, the *p*H is well below 6·9. On an average, cow milk gives a *p*H of 6·6, and buffalo milk 6·8. Milk of *p*H over 6·9 should be regarded with suspicion as indication of some diseases of the udder, or late lactation milk.

7. DETERMINATION OF TOTAL NITROGEN

7.1 Reagents

7.1.1 *Concentrated Sulphuric Acid* — approximately 98 percent by weight and nitrogen-free.

7.1.2 *Copper Sulphate*

7.1.3 *Potassium Sulphate, or Anhydrous Sodium Sulphate* — nitrogen-free.

7.1.4 *Sodium Hydroxide Solution* — 50 percent by weight.

7.1.5 *Standard Sulphuric Acid Solution* — 0·1 N.

7.1.6 *Standard Sodium Hydroxide Solution* — carbonate-free, 0·1 N.

7.1.7 *Indicator Solution* — Mix equal volumes of a saturated solution of methyl red in ethanol (95 percent by volume) and a 0·1 percent solution of methylene blue in ethanol (95 percent by volume).

7.1.8 *Sucrose* — anhydrous pure.

7.2 Apparatus

7.2.1 A recommended apparatus, as assembled, is shown in Fig. 5.

7.2.1.1 *Description* — The apparatus consists of a round-bottomed flask *A* of 1 000 ml capacity fitted with a rubber stopper through which passes one end of the connecting bulb tube *B*. The other end of the bulb tube *B* is connected to the condenser *C* which is attached by means of a rubber tube to a dip tube *D* which dips into the liquid contained in a beaker *E* of 250 ml capacity.

7.3 Procedure

7.3.1 Transfer approximately 10 g of the prepared sample of milk, accurately weighed, to a 800-ml Kjeldahl flask. Add 25 ml of concentrated sulphuric acid, pouring this down the neck of the flask in such a way as to wash any milk into the body of the flask, and add 0·2 g of the copper

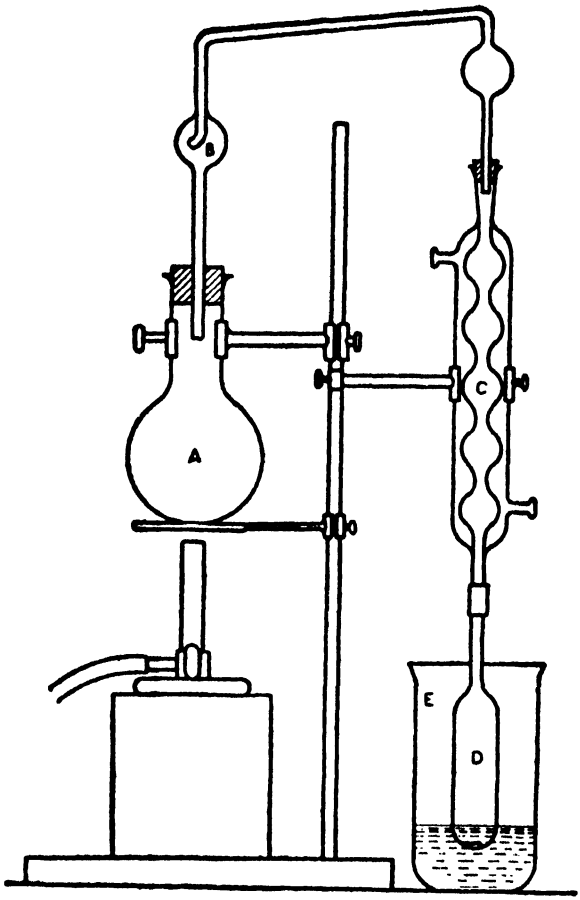


FIG. 5 APPARATUS FOR DETERMINATION OF NITROGEN

sulphate. Gently rotate the flask so that the whole of the contents are well mixed.

Place the flask on a frame so that the neck is inclined at an angle of 45° to the horizontal and the bulb rests in the hole of an asbestos sheet so that the flame does not touch the flask above the level of the liquid. Heat to gentle boiling, and when frothing has ceased, add 10 g of potassium or anhydrous sodium sulphate. Boil the contents of the flask briskly until clear and free from yellowish colour, and for a further period of one hour.

Allow the liquid to cool and wash down the sides with a fine jet of distilled water. Continue heating the contents of the flask for a further period of one hour. Allow the liquid to cool, dilute with approximately 200 ml of distilled water, transfer to a 1 000-ml flask, rinsing thoroughly, and add a few pieces of granulated zinc or a small amount of powdered pumice to prevent bumping. Add a suitable excess (usually 75 to 80 ml) of sodium hydroxide solution carefully down the neck of the flask to form a layer under the acid liquor.

Fit the flask with a splash head and connect it to a condenser. Pipette a suitable excess of the standard sulphuric acid (usually 50 ml) into a 250-ml beaker. Assemble the apparatus as shown in Fig. 5 taking care that the tip of the condenser extends below the surface of the standard sulphuric acid in the beaker. Mix the contents of the flask by shaking and distil until all ammonia has passed over into the standard sulphuric acid. Detach the flask from the condenser and shut off the burner. Rinse the condenser thoroughly with water into the beaker. Wash the dip tube carefully so that all traces of condensate are transferred to the beaker. Titrate the excess acid in the beaker with the standard sodium hydroxide solution using the indicator solution.

Carry out a blank determination by taking 0·5 g of sucrose in place of milk, and by using the same quantities of reagents and the same conditions of test.

7.4 Calculation

$$\text{Total nitrogen, percent by weight} = \frac{1{\cdot}4(A-B)N}{W}$$

where

A = volume in ml of the standard sodium hydroxide required for the blank determination,

B = volume in ml of the standard sodium hydroxide required for the test,

N = normality of the standard sodium hydroxide, and

W = weight in g of the prepared sample taken for the test.

8. DETERMINATION OF CRUDE PROTEIN

8.1 Crude protein percentage is obtained by multiplying by 6·38 the percentage of total nitrogen as determined in 7.

9. DETERMINATION OF TRUE PROTEIN

9.1 Reagent

9.1.1 *Trichloroacetic Acid Solution* — 15 percent by volume.

9.2 Procedure — Pipette 10 ml of the prepared sample into a 50-ml tared graduated flask and weigh accurately. Dilute to the mark with trichloroacetic acid solution and mix immediately. When the precipitate has settled, leaving a clear supernatant liquid, filter on a dry pleated 9-cm filter paper (Whatman No. 40 or its equivalent) into a dry flask. Wash the precipitate on the filter paper twice with the trichloroacetic acid solution

Determine the nitrogen in the washed precipitate and paper as prescribed in **7**. Obtain the percentage of true protein by multiplying the percentage of protein nitrogen by the factor 6·38. Preserve the filtrate for the determination of non-protein nitrogen (*see* **12**).

10. DETERMINATION OF CASEIN

10.1 Two methods, namely, *method I* (*see* **10.2**) and *method II* (*see* **10·3**) may be used for the determination of casein.

NOTE — Casein should be determined in fresh milk or nearly fresh milk. If it is not possible to make this determination within 8 hours, add 2·5 parts of formaldehyde to 2 500 parts of milk and keep the sample in a cool place.

10.2 Method I

10.2.1 *Reagents*

a) *Acetic acid* — 10 percent by volume.

b) *Sodium acetate solution* — 1 N.

10.2.2 *Procedure* — Pipette, and accurately weigh, 5 ml of the prepared sample into a 100-ml beaker. Add 50 ml of water at about 40°C and 0·5 ml of acetic acid and mix the contents of the beaker. After 10 minutes, add 0·5 ml of sodium acetate solution and mix again. Allow the contents of the beaker to cool to about 20°C (30 min) and decant into a pleated 9-cm filter paper. Wash the precipitate with water three times, and by decantation, transfer it to the paper and wash it twice on the paper. [Preserve the filtrate for the determination of albumin (*see* **11**).]

Determine the nitrogen in the washed precipitate and paper by the method described for total nitrogen (*see* **7**), using 25 ml of the 0·1 N sulphuric acid solution, and a paper in addition to 5 ml of water for the blank determination. Calculate the percentage of nitrogen and multiply by 6·38 to obtain the percentage of casein.

10.3 Method II

10.3.1 *Apparatus*

10.3.1.1 *Burette with soda-lime guard tube*

10.3.2 *Reagents*

a) *Phenolphthalein indicator solution* — Dissolve 0·5 g of phenolphthalein in 100 ml of ethyl alcohol (95 percent by volume).

b) *Potassium oxalate solution* — saturated and neutral to phenolphthalein.

c) *Standard sodium hydroxide solution* — 0·1 N.

d) *Formaldehyde* — 40 percent.

10.3.3 *Procedure* — Place 10 g of the prepared sample into each of two flasks. Add one millilitre of phenolphthalein indicator solution, followed by 0·4 ml of the potassium oxalate solution. Set aside for 2 minutes. Neutralize the contents of one of the flasks with the standard sodium hydroxide solution using the other flask as a blank. Add 2 ml of neutralized formaldehyde and again titrate with the standard sodium hydroxide solution to the same pink shade.

10.3.4 *Calculation* — The first titration value is not required, but the volume in millilitres of the standard sodium hydroxide used in the second titration shall be noted. Multiply this value by 1·38 to obtain the percentage of casein.

11. DETERMINATION OF ALBUMIN

11.1 Reagents

11.1.1 *Sodium Hydroxide Solution* — 10 percent *w/v*.

11.1.2 *Dilute Acetic Acid* — 1 : 9 by volume.

11.2 Procedure — Exactly neutralize the filtrate obtained in **10.2.2** with sodium hydroxide solution. Add 0·3 ml of dilute acetic acid, and heat on a steam-bath until the albumin is completely precipitated. Collect the precipitate on an acid-washed filter and wash with cold water. Determine the nitrogen in the precipitate by the method described in **7**.

11.3 Calculation — Multiply the percentage of nitrogen by 6·38 to obtain the percentage of albumin.

12. DETERMINATION OF NON-PROTEIN NITROGEN

12.1 Procedure — Determine the nitrogen in 20 ml of the filtrate (*see* **9.2**) by the method described in **7**.

12.2 Calculation

$$\text{Non-protein nitrogen, percent by weight} = \frac{3{\cdot}5(A-B)N}{W}$$

where

A, B and N have the same connotation as in **7.4**, and

W = weight in g of the prepared sample taken for the test (*see* **9**).

13. DETERMINATION OF LACTOSE

13.1 Methods — Four methods, namely the *polarimetric method* (*see* **13.2**), the *Munson and Walker* gravimetric method (*see* **13.3**), the *Munson and Walker volumetric method* (*see* **13.4**), and the *Lane-Eynon method* (*see* **13.5**), may be used for the determination of lactose.

13.2 Polarimetric Mtehod

13.2.1 *Apparatus* — polarimeter, with 400-mm and 200-mm tubes and sodium lamp.

13.2.2 *Reagents*

a) *Acid mercuric nitrate solution* — Dissolve purified mercury in twice its weight of nitric acid (sp gr 1·42) and dilute with 5 volumes of water.

b) *Mercuric iodide solution* — Dissolve 33·2 g of potassium iodide and 13·5 g mercuric chloride in 200 ml of glacial acetic acid and 640 ml of water.

c) *Phosphotungstic acid solution* — 5 percent (*w/v*).

13.2.3 *Procedure* — Take two graduated flasks, one of 100 ml and the other of 200 ml capacity. Weigh accurately 65·8 g (2 normal weights) of the prepared sample into each flask. Add to each flask 20 ml of acid mercuric nitrate solution or 30 ml of mercuric iodide solution. To the 100-ml flask, add phosphotungstic acid solution to the mark, and to the 200-ml flask, add 15 ml of phosphotungstic acid solution and dilute to the mark with water. Shake both the flasks frequently during 15 minutes, filter through dry filter paper, and polarize. (It is preferable to read solution from 200-ml flask in 400-mm tube to reduce error of reading. Solution from the 100-ml flask may be read in 200-mm tube.)

13.2.4 *Calculation* — Calculate percentage of lactose in the sample as follows:

a) Subtract reading of solution from the 200-ml flask (using 400-mm tube) from reading of solution from the 100-ml flask (using 200-mm tube);

b) Multiply difference by 2;

c) Subtract result from reading of solution from the 100-ml flask; and

d) Divide result by 2.

13.3 Munson and Walker Gravimetric Method

13.3.1 *Reagents and Materials*

a) *Fehling solution* — Prepared by mixing immediately before use, equal volumes of solutions (*A*) and (*B*).

Solution (*A*) — Dissolve 34·639 g of copper sulphate ($CuSO_4$, $5H_2O$) in water. Dilute to 500 ml and filter through prepared asbestos.

Solution (*B*) — Dissolve 173 g of Rochelle salt ($KNaC_4H_4O_6$, $4H_2O$) and 50 g of sodium hydroxide in water. Dilute to 500 ml, allow to stand for two days, and filter through prepared asbestos.

b) *Standard sodium hydroxide solution* — 0·5 N.

c) *Asbestos* — Digest the asbestos, which should be of the amphibole variety, with hydrochloric acid (1 : 3) for 2 to 3 days. Wash free from acid, digest for similar period with 10 percent (*w/v*) sodium hydroxide solution, and then treat for a few hours with hot alkaline tartrate solution [old solution (*B*) that has stood for some time may be used for this purpose] of the strength used in sugar determinations [*see* **13.3.1**(a)]. Wash the asbestos free from alkali, digest for several hours with dilute nitric acid (1 : 3), and, after washing free from acid, shake with water into a fine pulp. In preparing a Gooch crucible, make a film of asbestos 6 mm thick and wash thoroughly with water to remove fine particles of asbestos.

d) *Ethyl alcohol* — 95 percent by volume.

e) *Ethyl ether*

13.3.2 *Procedure* — Dilute 25 g of the prepared sample, accurately weighed, with 400 ml of water in a 500-ml graduated flask. Add 10 ml of Fehling solution (*A*), and 8·8 ml of the standard sodium hydroxide solution. After the addition of the alkali solution, the mixture shall still have an acid reaction and contain copper in solution. Fill the flask to the mark with water, mix, filter through a dry filter paper. The filtrate should have a faint blue colour indicating a slight excess of copper.

Prepare a tared Gooch crucible by making a film of asbestos 6 mm thick. Wash the crucible with 10 ml of ethyl alcohol, then with 10 ml of ethyl ether. Dry for 30 minutes at 98 to 100°C, cool in a desiccator and weigh. Transfer 25 ml of each of the Fehling solutions (*A*) and (*B*) to a 400-ml beaker of alkali resistant glass and add 50 ml of the filtrate. If a smaller volume of sugar solution is used, add water to make final volume of 100 ml. Heat the beaker on asbestos gauze over a Bunsen burner, regulate the flame so that boiling begins in 4 minutes, and continue boiling for exactly 2 minutes. (It is important that these directions be strictly observed. To regulate burner for this purpose, it is advisable to make preliminary tests, using 50 ml of the reagent and 50 ml of water before proceeding with the actual determination.) Keep the beaker covered with a watch-glass during heating. Filter the hot solution at once through asbestos mat in a porcelain Gooch crucible, using suction (*see* Note). Wash the precipitate of cuprous oxide thoroughly with water

at about 60°C and either weigh directly as cuprous oxide, after drying, or determine the quantity of reduced copper volumetrically (*see* **13.4**). Conduct a blank determination, using 50 ml of the reagent and 50 ml of water, and if the weight of cuprous oxide obtained exceeds 0·5 mg, correct the result of reducing sugar determination accordingly. The alkaline tartrate solution deteriorates on standing and the quantity of cuprous oxide obtained in the blank increases.

NOTE — If sucrose is to be determined, the filtrate should be collected in a 250 ml graduated flask and the precipitated cuprous oxide washed well with about 80 ml of hot water (for the determination of sucrose refer **14**).

If cuprous oxide is to be determined by weighing, after washing the precipitate thoroughly with hot water, wash it respectively with 10 ml each of ethyl alcohol and ethyl ether. Dry the precipitate for 30 minutes in an oven at the temperature of boiling water, cool and weigh.

13.3.3 *Calculation* — Obtain, from Munson and Walker's table for lactose as given in Table I, the lactose equivalent of cuprous oxide and calculate the percentage of lactose in the sample as follows:

$$\text{Lactose, percent by weight} = \frac{M}{25}$$

where

M = lactose equivalent, in mg, of the cuprous oxide precipitate.

13.4 Munson and Walker Volumetric Method

13.4.1 *Reagents* — In addition to the reagents mentioned in **13.3.1** the following reagents are required:

a) *Ferric sulphate solution in 20 percent sulphuric acid* — saturated.

b) *Standard potassium permanganate solution* — 0·10 N, standardized against sodium oxalate made acidic with sulphuric acid.

13.4.2 *Procedure* — After washing cuprous oxide as described in **13.3.2**, transfer the asbestos film to a 250-ml beaker, add about 30 ml of hot water, and heat to precipitate the asbestos thoroughly. Rinse the crucible with 50 ml of hot saturated solution of ferric sulphate, receiving the rinsings in the beaker containing the precipitate. After the cuprous oxide is dissolved, wash the solution into a large Erlenmeyer flask and immediately titrate with the standard solution of potassium permanganate.

13.4.3 *Calculation* — Find out the weight of cuprous oxide in milligrams in 50 ml of the filtrate used in the precipitation on the basis that one millilitre of 0·10 N permanganate is equivalent to 0·006 35 g of copper. From Table I, find the lactose equivalent of cuprous oxide and calculate the percentage of lactose in the sample from the formula given in **13.3.3**.

TABLE I MUNSON AND WALKER'S TABLE FOR CALCULATING LACTOSE (EXPRESSED IN MILLIGRAMS)

(*Clauses* 13.3.3 and 13.4.3)

Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)	Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)
10	6·3	4·5	90	59·7	40·4
12	7·5	5·4	92	61·1	41·4
14	8·8	6·3	94	62·5	42·3
16	10·0	7·2	96	63·8	43·2
18	11·3	8·1	98	65·2	44·1
20	12·5	8·9	100	66·6	45·0
22	13·8	9·8	102	68·0	46·0
24	15·0	10·7	104	69·3	46·9
26	16·3	11·6	106	70·7	47·8
28	17·6	12·5	108	72·1	48·7
30	18·8	13·4	110	73·5	49·6
32	20·1	14·3	112	74·8	50·6
34	21·4	15·2	114	76·2	51·5
36	22·8	16·1	116	77·6	52·4
38	24·2	16·9	118	79·0	53·3
40	25·5	17·8	120	80·3	54·3
42	26·9	18·7	122	81·7	55·2
44	28·3	19·6	124	83·1	56·1
46	29·6	20·5	126	84·5	57·0
48	31·0	21·4	128	85·8	58·0
50	32·3	22·3	130	87·2	58·9
52	33·7	23·2	132	88·6	59·8
54	35·1	24·1	134	90·0	60·8
56	36·4	25·0	136	91·3	61·7
58	37·8	25·9	138	92·7	62·6
60	39·2	26·8	140	94·1	63·6
62	40·5	27·7	142	95·5	64·5
64	41·9	28·6	144	96·8	65·4
66	43·3	29·5	146	98·2	66·4
68	44·7	30·4	148	99·6	67·3
70	46·0	31·3	150	101·0	68·3
72	47·4	32·3	152	102·3	69·2
74	48·8	33·2	154	103·7	70·1
76	50·1	34·1	156	105·1	71·1
78	51·5	35·0	158	106·5	72·0
80	52·9	35·9	160	107·9	73·0
82	54·2	36·8	162	109·2	73·9
84	55·6	37·7	164	110·6	74·9
86	57·0	38·6	166	112·0	75·8
88	58·4	39·5	168	113·4	76·8

TABLE I MUNSON AND WALKER'S TABLE FOR CALCULATING LACTOSE (EXPRESSED IN MILLIGRAMS) — *Contd*

Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)	Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)
170	114·8	77·7	250	170·1	116·4
172	116·1	78·7	252	171·5	117·4
174	117·5	79·6	254	172·5	118·4
176	118·9	80·6	256	174·2	119·4
178	120·3	81·5	258	175·6	120·4
180	121·6	82·5	260	177·0	121·4
182	123·1	83·4	262	178·4	122·4
184	124·3	84·4	264	179·8	123·4
186	125·8	85·3	266	181·2	124·4
188	127·2	86·3	268	182·6	125·4
190	128·5	87·2	270	184·0	126·4
192	129·9	88·2	272	185·3	127·4
194	131·3	89·2	274	186·7	128·4
196	132·7	90·1	276	188·1	129·4
198	134·1	91·1	278	189·5	130·4
200	135·4	92·0	280	190·9	131·4
202	136·8	93·0	282	192·3	132·4
204	138·2	94·0	284	193·7	133·4
206	139·6	94·9	286	195·1	134·4
208	141·0	95·9	288	196·5	135·4
210	142·3	96·9	290	197·8	136·4
212	143·7	97·8	292	199·2	137·4
214	145·1	98·8	294	200·0	138·4
216	146·5	99·8	296	202·0	139·4
218	147·9	100·8	298	203·4	140·5
220	149·3	101·7	300	204·8	141·5
222	150·7	102·7	302	206·2	142·5
224	152·0	103·7	304	207·6	143·5
226	153·4	104·6	306	209·0	144·5
228	154·8	105·6	308	210·4	145·5
230	156·2	106·6	310	211·8	146·6
232	157·6	107·6	312	213·2	147·6
234	159·0	108·6	314	214·6	148·6
236	160·3	109·5	316	216·0	149·6
238	161·7	110·5	318	217·3	150·7
240	163·1	111·5	320	218·7	151·7
242	164·5	112·5	322	220·1	152·7
244	165·9	113·5	324	221·5	153·7
246	167·3	114·5	326	222·9	154·8
248	168·7	115·5	328	224·3	155·8

TABLE I MUNSON AND WALKER'S TABLE FOR CALCULATING LACTOSE (EXPRESSED IN MILLIGRAMS) — *Contd*

Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)	Cuprous Oxide Cu_2O (1)	Lactose ($C_{12}H_{22}O_{11}.H_2O$) (2)	Invert Sugar (3)
330	225·7	156·8	410	281·7	199·1
332	227·1	157·9	412	283·2	200·2
334	228·5	158·9	414	284·6	201·3
336	229·9	159·9	416	286·0	202·4
338	231·3	161·0	418	287·4	203·5
340	232·7	162·0	420	288·8	204·6
342	234·1	163·1	422	290·2	205·7
344	235·5	164·1	424	291·6	206·7
346	236·9	165·1	426	293·0	207·8
348	238·3	166·2	428	294·4	208·9
350	239·7	167·2	430	295·8	210·0
352	241·1	168·3	432	297·2	211·1
354	242·5	169·3	434	298·6	212·2
356	243·9	170·4	436	300·0	213·3
358	245·3	171·4	438	301·4	214·4
360	246·7	172·5	440	302·8	215·5
362	248·1	173·5	442	304·2	216·6
364	249·5	174·6	444	305·6	217·8
366	250·9	175·6	446	307·0	218·9
368	252·3	176·7	448	308·4	220·0
370	253·7	177·7	450	309·9	221·1
372	255·1	178·8	452	311·3	222·2
374	256·5	179·8	454	312·7	223·3
376	257·9	180·9	456	314·1	224·4
378	259·3	182·0	458	315·5	225·5
380	260·7	183·0	460	316·9	226·7
382	262·1	184·1	462	318·3	227·8
384	263·5	185·2	464	319·7	228·9
386	264·9	186·2	466	321·1	230·0
388	266·5	187·3	468	322·5	231·2
390	267·7	188·4	470	323·9	232·3
392	269·1	189·4	472	325·3	233·4
394	270·5	190·5	474	326·8	234·5
396	271·9	191·6	476	328·2	235·7
398	273·3	192·7	478	329·6	236·8
400	274·7	193·7	480	331·0	237·9
402	276·1	194·8	482	332·4	239·1
404	277·5	195·9	484	333·8	240·2
406	278·9	197·0	486	335·2	241·4
408	280·3	198·1	488	336·6	242·5
			490	338·0	243·6

13.5 Lane-Eynon Method

13.5.1 *Reagents*

a) *Solution* (*A*) — [*See* **13.3.1**(a)].
b) *Solution* (*B*) — [*See* **13.3.1**(a)].
c) *Methylene blue* — one percent (*w*/*v*) solution in water.
d) *Pure lactose*

13.5.2 *Procedure*

13.5.2.1 *Standardization of Fehling solution* — Pipette accurately 10 ml or 25 ml of the Fehling solution [*see* **13.3.1**(a)] or pipette 5 ml or 12·5 ml of each of the solutions (*A*) and (*B*) into a flask of 300 to 400 ml capacity. The quantity of copper taken will differ slightly between the two methods of pipetting, and the method used shall be followed consistently during standardization and determination. Prepare a standard solution of pure lactose of such concentration that more than 15 ml and less than 50 ml will be required to reduce all the copper. The titre may be calculated as follows:

$$\frac{\text{Factor}}{\text{mg lactose in 1 ml}}$$

Add almost the whole of the lactose solution required to effect reduction of all the copper, so that not more than 0·5 to 1·0 ml is required later to complete titration. Heat the cold mixture to boiling on wire gauze and maintain in moderate ebullition for two minutes, lowering the flame sufficiently to avoid bumping. Without removing flame, add 2 to 5 drops of one percent aqueous methylene blue solution and complete the titration within a total boiling time of about three minutes, by small additions of sugar solution, to the point of decolourization of the indicator.

Multiply the titre by the number of milligrams in one millilitre of the standard solution to obtain the factor. Compare with the tabulated factor (*see* Table II) to determine correction, if any, to be applied to the table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within one percent) are required, the standardization may be omitted provided specifications of the analysis are rigidly observed.

13.5.2.2 *Determination* — If approximate concentration of lactose in sample is unknown, proceed by the incremental method of titration. Add to 10 ml or 25 ml of the Fehling solution 15 ml of the test solution and heat to boiling over wire gauze. Boil for about 15 seconds and add rapidly further quantities of the solution until only faintest perceptible blue colour remains. Then add 2 to 5 drops of methylene blue and complete the titration by adding the test solution dropwise. (The error resulting from this titration will not generally exceed one percent.)

For higher precision, repeat the titration, adding almost the whole of the test solution required to reduce all the copper and proceed as directed above. From the Table II, find the factor corresponding to titre and apply correction previously determined.

TABLE II FACTORS FOR FEHLING SOLUTION TO BE USED IN CONNECTION WITH THE LANE-EYNON GENERAL VOLUMETRIC METHOD

(*Clauses* 13.5.2.1 *and* 13.5.2.2)

Titre	10 ml Fehling Solution		25 ml Fehling Solution	
	Anhydrous Lactose $C_{12}H_{22}O_{11}$	Hydrated Lactose $C_{12}H_{22}O_{11}$, H_2O	Anhydrous Lactose $C_{12}H_{22}O_{11}$	Hydrated Lactose $C_{12}H_{22}O_{11}.H_2O$
(1)	(2)	(3)	(4)	(5)
15	64·9	68·3	163·9	172·5
16	64·8	68·2	163·5	172·1
17	64·8	68·2	163·1	171·7
18	64·7	68·1	162·8	171·4
19	64·7	68·1	162·5	171·1
20	64·6	68·0	162·3	170·9
21	64·6	68·0	162·0	170·6
22	64·6	68·0	161·8	170·4
23	64·5	67·9	161·6	170·2
24	64·5	67·9	161·5	170·0
25	64·5	67·9	161·4	169·9
26	64·5	67·9	161·2	169·7
27	64·4	67·8	161·0	169·5
28	64·4	67·8	160·8	169·3
29	64·4	67·8	160·7	169·2
30	64·4	67·8	160·6	169·0
31	64·4	67·8	160·5	168·9
32	64·4	67·8	160·4	168·8
33	64·4	67·8	160·2	168·6
34	64·4	67·9	160·1	168·5
35	64·5	67·9	160·0	168·4
36	64·5	67·9	159·8	168·2
37	64·5	67·9	159·7	168·1
38	64·5	67·9	159·6	168·0
39	64·5	67·9	159·5	167·9
40	64·5	67·9	159·4	167·8
41	64·6	68·0	159·3	167·7
42	64·6	68·0	159·2	167·6
43	64·6	68·0	159·2	167·6
44	64·6	68·0	159·1	167·5
45	64·7	68·1	159·0	167·4
46	64·7	68·1	159·0	167·4
47	64·8	68·2	158·9	167·3
48	64·8	68·2	158·8	167·2
49	64·8	68·2	158·8	167·2
50	64·9	68·3	158·7	167·1

13.5.3 *Calculation* — Calculate the percentage of lactose in the sample as follows:

$$\text{Lactose, percent} = \frac{\text{Factor}}{\text{Titre} \times 100}$$

14. DETERMINATION OF SUCROSE

14.1 Reagents

14.1.1 *Hydrochloric Acid* — 1 : 1 by volume.

14.1.2 *Sodium Hydroxide Solution* — 50 percent (*w/v*).

14.1.3 *Ethyl Alcohol* — 95 percent by volume.

14.2 Procedure — Place the filtrate from lactose determination (*see* **13.3.2**) in a 250-ml flask, add 34 ml of hydrochloric acid and place the flask immediately in a vigorously boiling water-bath. (The contents shall be at such a temperature as will show a temperature of 21° ± 2°C after the addition of acid.) Leave in the bath for exactly 5 minutes, remove and cool rapidly to room temperature. Neutralize with sodium hydroxide solution, taking care not to have either a local or a general excess of alkali in the warm solution lest some of the sugar be destroyed. Cool to room temperature, make up to the mark, shake well and determine the reducing sugar by the Munson and Walker gravimetric method using a 50-ml aliquot (*see* **13.3**). Filter through a tared Gooch filter, wash four or five times with hot water and once with alcohol and dry for 30 minutes at 98 to 100°C. Weigh the dry precipitate of cuprous oxide.

14.3 Calculation — From Table I find out the invert sugar equivalent of the cuprous oxide precipitate and multiply the result by 0·95 to obtain its sucrose value in milligrams. Calculate the percentage of sucrose in the sample as follows.

$$\text{Sucrose, percent by weight} = \frac{M}{5}$$

where

M = number of mg of sucrose equivalent to cuprous oxide precipitate.

15. DETERMINATION OF CHLORIDE

15.1 Reagents

15.1.1 *Silver Nitrate Solution* — approximately 0·05 N.

15.1.2 *Concentrated Nitric Acid* — sp gr 1·42.

15.1.3 *Standard Potassium Thiocyanate Solution* — 0·05 N, standardized against standard potassium chloride or sodium chloride.

15.1.4 *Saturated Iron Alum Solution* — in 10 percent nitric acid, prepared by boiling excess of iron alum, cooling and filtering.

15.2 Procedure — Place 15 g of the prepared sample, accurately weighed, in a 250-ml Erlenmeyer flask. Mix with it 10 ml of silver nitrate solution. Add 10 ml of concentrated nitric acid and digest the whole until reddish brown fumes are evolved. Add one millilitre of saturated iron alum solution. Determine the excess of silver nitrate by titrating with the standard potassium thiocyanate solution, until the first appearance of an orange tint that persists for 15 seconds.

In the same manner, determine the volume of the standard thiocyanate solution equivalent to 10 ml of silver nitrate using the same volumes of reagents and water.

15.3 Calculation

$$\text{Chlorine, percent by weight} = 0{\cdot}017\,73\ (B-A)$$

$$\text{Chloride as sodium chloride, percent by weight} = 0{\cdot}029\,23\ (B-A)$$

where

B = volume in ml of the standard potassium thiocyanate solution required by the blank, and

A = volume in ml of the standard potassium thiocyanate solution required by the sample.

16. DETERMINATION OF ASH

16.1 Reagent

16.1.1 *Dilute Hydrochloric Acid* — 1 : 4 by volume.

16.2 Procedure — Weigh accurately about 10 g of the prepared sample in a platinum, silica or other suitable dish which has been ignited, cooled in a desiccator charged with an efficient desiccant and weighed. Evaporate to dryness and ignite in a muffle furnace at a temperature not more than 550°C until the ash is free from carbon. Cool in a desiccator and weigh.

NOTE — For further analysis of the ash (*see* **17** to **19**), dissolve it in dilute hydrochloric acid and transfer it to a 100-ml graduated flask, make up to the mark and preserve.

16.3 Calculation

$$\text{Ash, percent by weight} = \frac{100\,w}{W}$$

where

w = weight in g of the ash, and

W = weight in g of the sample taken for ashing.

17. DETERMINATION OF CALCIUM

17.1 Reagents

17.1.1 *Ammonium Hydroxide* — 1 : 1 by volume.

17.1.2 *Ammonium Oxalate Solution* — saturated.

17.1.3 *Hydrochloric Acid* — 1 : 9 by volume.

17.1.4 *Standard Potassium Permanganate Solution* — 0·1 N. Prepare a stock solution by dissolving 33 g of potassium permanganate in a litre of water by heating to 70 to 80°C. Allow to stand in a stoppered amber glass bottle for several days. Then filter through an asbestos mat (*see* Note) on a small Buchner funnel direct in another glass-stoppered amber coloured bottle without aid of suction. Place 110 ml of the stock solution in a litre graduated flask and make up to the mark with recently boiled and cooled distilled water. Pipette 25 ml of 0·1 N sodium oxalate or oxalic acid solution into a 250-ml conical flask, add 25 ml of water and 15 ml of sulphuric acid (1 : 4). Heat to boiling and titrate the potassium permanganate solution to the first appearance of a faint but permanent pink colour. Make the necessary dilution to make it 0·1 N.

NOTE — Digest asbestos, prepared as given in **13.3.1**(c), for one hour with about 0·1 N potassium permanganate solution that has been acidified with sulphuric acid (1 : 3) and wash with water.

17.1.5 *Dilute Sulphuric Acid* — 1 : 4 by volume.

17.2 Procedure — Transfer 20 ml of the solution, prepared as given in the Note to **16.2**, to a beaker and dilute to about 50 ml. Make slightly alkaline with ammonium hydroxide. While still hot, add ammonium oxalate solution dropwise as long as any precipitate forms, and then an excess sufficient to convert magnesium salts also to oxalate. Heat to boiling. Allow to stand for 3 hours or longer. Decant the clear solution through a filter paper (Whatman No. 42 or its equivalent). Pour 15 to 20 ml of hot water on precipitate and again decant the clear solution through the filter. Dissolve any precipitate remaining on the filter by washing with hot hydrochloric acid into the original beaker and wash 6 times with hot water. Then reprecipitate by adding ammonium hydroxide and a little ammonium oxalate solution. Allow to stand as before, filter through the same filter paper, and wash with hot water until chloride-free. Preserve the filtrates and washings from both precipitations for the determination of magnesium (*see* **18**).

Perforate the apex of the filter cone. Wash the calcium oxalate precipitate into the beaker used for precipitation. Then wash the filter paper with hot dilute sulphuric acid and titrate at 85 to 90°C with the standard potassium permanganate solution.

17.3 Calculation

$$\text{Calcium, percent by weight} = \frac{1{\cdot}002\,V}{W}$$

where

V = volume in ml of the standard potassium permanganate required for titration, and

W = weight in g of the prepared sample taken for the determination of ash (*see* **16.2**).

18. DETERMINATION OF MAGNESIUM

18.1 Reagents

18.1.1 *Citric Acid* — molar solution.

18.1.2 *Ammonium Hydroxide* — sp gr 0·88.

18.1.3 *Ethyl Alcohol* — 95 percent by volume.

18.1.4 *Phosphate Solution* — Dissolve 100 g ammonium hydrogen phosphate in hot water. Dilute to one litre, and add 5 ml of chloroform.

18.1.5 *Ammonium Hydroxide* — 1 : 9 by volume.

18.1.6 *Dilute Nitric Acid* — 1 : 4 by volume.

18.1.7 *Dilute Ammonium Nitrate* — Saturated ammoniacal solution.

18.1.8 *Ammonium Hydroxide* — 1 : 10 by volume.

18.2 Procedure — To the combined filtrates and washings (*see* **17.2**) add 2 ml of citric acid solution, 100 ml of ammonium hydroxide (sp-gr 0·88) and 50 ml of alcohol. Then add with constant stirring 25 ml of phosphate solution and let stand for 12 to 24 hours. Filter, wash twice with ammonium hydroxide (1 : 9) and dissolve the precipitate in dilute nitric acid, washing the solution into the original beaker to make a volume of 100 to 150 ml. Add ammonium hydroxide (about $\frac{1}{10}$ volume of the quantity of solution in the beaker) and 2 drops of phosphate solution. Stir vigorously and allow to stand for 3 hours or longer. Filter through Gooch filter and wash with ammonium hydroxide (1 : 10). Moisten the filter with dilute ammonium nitrate solution. Ignite, and weigh as magnesium pyrophosphate.

18.3 Calculation

$$\text{Magnesium, percent by weight} = \frac{109{\cdot}24\,w}{W}$$

where

w = weight in g of magnesium pyrophosphate (*see* **18.2**), and

W = weight in g of the prepared sample taken for the determination of ash (*see* **15.2**).

19. DETERMINATION OF PHOSPHORUS

19.1 Reagents

19.1.1 *Nitric Acid* — sp gr 1·42.

19.1.2 *Ammonium Hydroxide* — sp-gr 0·88.

19.1.3 *Nitric Acid* — 1 : 3 by volume.

19.1.4 *Molybdate Solution* — Dissolve 100 g of molybdenum oxide in a mixture of 144 ml of ammonium hydroxide and 271 ml of water. Cool, and pour the solution slowly and with constant stirring into a cool mixture of 489 ml of nitric acid and 1148 ml of water. Keep the final mixture in a warm place for several days or until a portion heated to 40°C deposits no yellow precipitate of ammonium phosphomolybdate. Decant the solution from any sediment and preserve in glass-stoppered vessels. To 100 ml of the molybdate solution, add 5 ml of nitric acid. Filter this solution immediately before using.

19.1.5 *Standard Sodium or Potassium Hydroxide Solution* — Dilute 324·03 ml of normal strength alkali, free from carbonates, to one litre. 100 ml of this solution should neutralize 32·40 ml of normal acid.

19.1.6 *Standard Acid Solution* — Prepare a solution of hydrochloric acid or of nitric acid corresponding to the strength of alkali (*see* **19.1.5**) and standardize by titration against that solution using phenolphthalein indicator.

19.1.7 *Phenolphthalein Indicator Solution* — one percent in rectified spirit.

19.2 Procedure — Use 20 ml of the solution, prepared as given in the **Note** to **16.2**. Add 10 ml of nitric acid (sp gr 1·42). Add ammonium hydroxide (sp gr 0·88) until the precipitate that forms dissolves but slowly on stirring vigorously. Dilute to 75 to 100 ml and adjust to a temperature of 25 to 30°C. If the sample does not give a precipitate with ammonium hydroxide as test of neutralization, make the solution slightly alkaline to litmus paper with ammonium hydroxide and then slightly acidic with nitric acid (1 : 3). Add 20 to 25 ml of freshly filtered molybdate solution. Place the solution in a shaking or stirring apparatus and shake or stir for 30 minutes at room temperature. Decant at once through a filter paper (Whatman No. 42 or its equivalent) and wash the precipitate twice by decantation with 25 to 30 ml portions of water containing 2 percent of sodium nitrate, agitating thoroughly and allowing to settle. Transfer the precipitate to the filter and wash with cold water containing 2 percent of sodium nitrate until the filtrate from the fillings of the filter yields a pink colour upon the addition of phenolphthalein indicator and one drop of standard alkali. Transfer the precipitate and the filter paper to a beaker. Dissolve the precipitate in a small excess of the standard alkali, add a few drops of phenolphthalein indicator and titrate with standard acid solution. One millilitre of the standard sodium or potassium hydroxide solution = 0·436 4 mg of phosphorus.

19.3 Calculation

$$\text{Phosphorus, percent by weight} = \frac{0{\cdot}436\,4\,(V_1 - V_2)}{2W_1}$$

where

V_1 = volume in ml of the standard alkali used to dissolve the precipitate,

V_2 = volume in ml of the standard acid solution required for neutralization of excess alkali, and

W_1 = weight in g of the prepared sample taken for the determination of ash (*see* **16.2**).

20. DETERMINATION OF COPPER

20.1 Reagents

20.1.1 *Dilute Hydrochloric Acid* — 1 : 4 by volume.

20.1.2 *Sulphuric Acid* — sp gr 1·84.

20.1.3 *Sodium Thiosulphate Solution* — saturated.

20.1.4 *Ammonium Sulphate Solution* — one percent (*w/v*).

20.1.5 *Dilute Nitric Acid* — 2 : 5 by volume.

20.1.6 *Ammonium Hydroxide* — sp gr 0·88.

20.1.7 *Dilute Acetic Acid* — 1 : 1 by volume.

20.1.8 *Potassium Iodide* — crystals.

20.1.9 *Standard Copper Solution* — Dissolve 318 mg of pure electrolytic copper in nitric acid (sp gr 1·42) and evaporate to dryness on a steam-bath. Add sufficient water and a few drops of acetic acid to dissolve the copper nitrate and again evaporate to dryness on steam-bath. Redissolve the copper nitrate in water, using a few drops of acetic acid and dilute to one litre.

20.1.10 *Sodium Thiosulphate Solution* — 0·01 N. Dissolve 24·82 g of sodium thiosulphate ($Na_2S_2O_3$, $5H_2O$) in one litre of carbon dioxide-free water to make about 0·1 N solution. Allow to stand preferably for about 2 weeks. Prepare about 0·01 N solution by dilution of this reagent with carbon dioxide-free water. Standardize daily against the standard copper solution in the following manner:

Place 20 ml of the standard copper solution in a 250-ml Erlenmeyer flask and add an excess of ammonium hydroxide. Boil gently to drive off excess ammonia. Make acid to litmus paper with dilute acetic acid, add 10 ml in excess, boil the solution for one minute, and cool to room temperature. Add 2 g of the potassium iodide dissolved in enough water, and dilute to make the final volume about 50 ml and titrate the free iodine immediately with the standard sodium thiosulphate solution until the end point is nearly reached. Add 2 ml of starch indicator solution and continue the titration

dropwise to the disappearance of blue colour. Find the copper equivalent of one millilitre of the standard sodium thiosulphate solution.

20.1.11 *Starch Indicator Solution* — Make one gram of soluble starch into a paste with a little cold water and add with constant stirring to 100 ml of boiling water. Cool and decant the clear solution. Add 2 ml of chloroform as a preservative.

20.2 Procedure — Weigh accurately about 10 g of the prepared sample and ash it as described in **16.2**. Dissolve the ash in 100 ml dilute hydrochloric acid. Add 5 ml of sulphuric acid. Dilute the solution to 200 ml and boil for one minute. Add cautiously 10 ml of hot saturated sodium thiosulphate solution and continue boiling for 5 minutes. With larger quantities of copper, the precipitate coagulates and the liquid becomes practically clear. Four millilitres of ammonium sulphate solution may be added to hasten coagulation. Filter the precipitate through Whatman No. 42 or its equivalent and wash six times with hot water. Fold the filter paper with precipitate within, place in a small crucible and ignite in an electric muffle furnace at about 500°C. Treat the residue with one millilitre of dilute nitric acid and dry on a steam-bath. Add 5 ml of water and again dry on the steam-bath. Add 20 ml of water. Add an excess of ammonium hydroxide and heat on the steam-bath until copper salts are dissolved. Transfer to a 250-ml Erlenmeyer flask. Boil gently to drive off excess ammonia. Make acid to litmus paper with dilute acetic acid, add 10 ml in excess, boil the solution for one minute, and cool to room temperature. Add 2 g of potassium iodide dissolved in enough water and make the final solution to 50 ml. Titrate the free iodine immediately with the standard sodium thiosulphate solution (according to quantity of copper present, as shown by degree of blue colour in ammoniacal solution) until the end point is nearly reached. Add 2 ml of starch indicator solution and continue the titration dropwise to the disappearance of blue colour.

20.3 Calculation — Calculate the percentage of copper from the volume in millilitres of the standard sodium thiosulphate solution used for titration by the following formula:

$$\text{Copper, percent by weight} = \frac{VM}{10W}$$

where

V = volume in ml of the standard sodium thiosulphate solution used (*see* **20.2**),

M = weight in mg of copper equivalent to one millilitre of the standard sodium thiosulphate solution as determined (*see* **20.1.10**), and

W = weight in g of prepared sample taken for the test.

21. DETERMINATION OF CITRIC ACID

21.1 Reagents

21.1.1 *Tartaric Acid*

21.1.2 *Dilute Sulphuric Acid* — normal.

21.1.3 *Phosphotungstic Acid Solution* — 20 percent (*w*/*v*).

21.1.4 *Ethyl Alcohol* — 95 percent by volume.

21.1.5 *Potassium Bromide* — pure.

21.1.6 *Concentrated Sulphuric Acid* — sp gr 1·84.

21.1.7 *Potassium Permanganate Solution* — Dissolve 5 g of potassium permanganate in water and dilute to 100 ml.

21.1.8 *Ferrous Sulphate Solution* — Dissolve 40 g of ferrous sulphate ($FeSO_4$, $7H_2O$) in 100 ml of water containing one millilitre of concentrated sulphuric acid.

21.1.9 *Sodium Sulphate* — anhydrous.

21.1.10 *Ethyl Ether*

21.2 Procedure

21.2.1 *Preparation of Sample* — Weigh accurately about 50 g of the prepared sample in a 150-ml beaker. Add about 100 mg of tartaric acid and 6 ml of dilute sulphuric acid and heat on a steam-bath for 15 minutes. Immediately add 3 ml of phosphotungstic acid solution, mix well, and return to the steam-bath for 5 minutes. Transfer to a 250-ml graduated flask with ethyl alcohol, cool, dilute to the mark with alcohol, mix and filter through a folded filter paper.

21.2.2 Evaporate 200 ml of the prepared solution to about 20 ml, rinse into a 250 to 300-ml tared glass-stoppered Erlenmeyer flask, and adjust with water to a net weight of about 40 g. Add 2 g of potassium bromide and 5 ml of concentrated sulphuric acid, and, if necessary, heat to about 50°C and let stand for 5 minutes. Add 20 ml of potassium permanganate solution from a pipette or burette slowly (in 1 or 2-ml portions), swirling the flask for a few seconds after each addition. Let it stand undisturbed for 5 minutes and cool to about 15°C. Add ferrous sulphate solution slowly with constant agitation until the mixture starts to clear. Shake for one minute, continue addition of ferrous sulphate solution until manganese dioxide is dissolved, and add a few millilitres in excess. Add 20 g of anhydrous sodium sulphate with swirling to assure solution (if sodium sulphate remains substantially undissolved, repeat the determination). Cool to about 15°C and shake vigorously for 5 minutes.

Immediately, while still cold, collect the pentabromacetone on asbestos in a Gooch crucible and wash the residual precipitate from the flask with a portion of the filtrate. Finally, wash the crucible with 50 ml of

cold water and let the crucible remain under suction for a few minutes. Dry the crucible overnight in a sulphuric acid desiccator and weigh, or place the crucible in a drying train and aerate until loss in weight does not exceed one tenth of a milligram, making the first weighing after 20 minutes.

Remove the pentabromacetone from the crucible with alcohol followed by ethyl ether, filling the crucible 3 times with each solvent. Dry the crucible for 10 minutes at 98 to 100°C, cool in the desiccator, and weigh. The difference between the two weighings gives the weight of pentabromacetone.

21.3 Calculation

21.3.1 Weight of citric acid in aliquot (x) = $0{\cdot}424\ P$

where

P = weight in g of pentabromacetone.

21.3.2 Anhydrous citric acid, percent by weight = $\frac{125\ x}{W}$

where

x = weight in g of citric acid in aliquot (*see* **21.3.1**), and

W = weight in g of the prepared sample taken for the test.

22. DETECTION OF NEUTRALIZERS

22.0 General — Neutralizers in the form of lime water, or sodium bicarbonate, may be added to neutralize developed acidity before milk is processed. Such a practice is not permissible. The presence of these is detected by the following methods.

22.1 Rosalic Acid Test for Presence of Carbonate

22.1.1 *Reagents*

a) *Ethyl alcohol* — 95 percent by volume.

b) *Rosalic acid solution* — one percent (*w/v*) in ethyl alcohol.

22.1.2 *Procedure* — To about 5 ml of milk in a test-tube, add 5 ml of ethyl alcohol, a few drops of rosalic acid solution and mix. If carbonate is present, a rose red colour appears whereas pure milk shows only a brownish colouration.

22.2 Alkalinity of Ash

22.2.0 *General* — Neutralization of milk whether with lime, soda, or caustic soda, invariably increases the ash content and the total alkalinity of the ash from a fixed quantity of milk.

22.2.1 *Reagent*

Standard hydrochloric acid — 0·1 N.

22.2.2 *Procedure* — Measure 20 ml of milk in a porcelain dish and ash it well. Disperse the ash in 10 ml of water. Titrate with the standard hydrochloric acid.

22.2.3 *Interpretation* — The amount of the standard hydrochloric acid required in the absence of neutralizers will not exceed 1·2 ml.

22.3 Determination of *p*H — A determination of the *p*H of milk, suspected of being neutralized, will enable the detection of only an overneutralized product. A hydrogen-concentration of *p*H 7·0 or over indicates over-neutralization. The *p*H may be determined either colorimetrically or by means of the potentiometer as described in **6**.

23. DETECTION AND DETERMINATION OF PRESERVATIVES

23.1 Detection and Determination of Boric Acid and Borates

23.1.1 *Turmeric Paper Test*

23.1.1.1 *Reagents*

a) *Turmeric paper* — dried.

b) *Concentrated hydrochloric acid* — sp gr 1·16.

c) *Ammonium hydroxide* — sp gr 0·88.

d) *Lime water or caustic soda*

23.1.1.2 *Procedure* — Either of the methods given at (a) or (b) below shall be followed. The latter method is, however, more sensitive:

a) Immerse a strip of turmeric paper in a sample of milk previously acidified with concentrated hydrochloric acid in the proportion of 7 ml of concentrated hydrochloric acid to each 100 ml of milk. Allow the paper to dry spontaneously. If boric acid or borax is present, the paper will acquire a characteristic red colour. The addition of the ammonium hydroxide will change the colour of the paper to a dark green, but the red colour may be restored by hydrochloric acid.

b) Make about 25 ml of the sample strongly alkaline with lime water or caustic soda and evaporate to dryness on a water-bath. Ignite the residue at a low red heat to destroy organic matter. Cool, digest with about 15 ml of water, add concentrated hydrochloric acid drop by drop until the ignited residue is dissolved. Then add one millilitre in excess. Saturate a piece of turmeric paper with this solution, and allow the paper to dry without the aid of heat. The colour change will be the same as described in (a).

23.1.2 *Glycerol Test*

23.1.2.1 *Reagents*

a) *Phenolphthalein indicator solution* — one percent (*w*/*v*) in ethyl alcohol (95 percent by volume).

b) *Sodium hydroxide solution* — 0·1 N.

c) *Glycerine solution* — 50 percent (*w/v*) in water, neutral.

23.1.2.2 *Procedure* — To 10 ml of sample in a test-tube, add a few drops of phenolphthalein indicator solution. Then add sodium hydroxide solution drop by drop, till a faint pink colour is obtained. Divide the neutralized sample between two test-tubes. Add to one an equal volume of water. To the other test-tube add an equal volume of glycerine solution. Compare the colours. If the milk to which the glycerine has been added is lighter in colour than the other, it is an indication of the presence of boric acid. The milk will turn completely white, if the amount of boric acid is considerable. Presence of about 0·1 percent boric acid is detectable by this test.

23.1.3 *Quantitative Method*

23.1.3.1 *Reagents*

a) *Sodium hydroxide solution* — approximately 10 percent.

b) *Concentrated hydrochloric acid* — sp gr 1·16.

c) *Lime water*

d) *Dilute hydrochloric acid* — 1 : 3 by volume.

e) *Calcium chloride* — anhydrous.

f) *Dilute sulphuric acid* — 1 N.

g) *Methyl orange indicator solution* — 0·1 percent (*w/v*) in water.

h) *Standard sodium hydroxide solution* — 0·20 N.

j) *Mannitol* — neutral.

k) *Phenolphthalein indicator solution* — one percent (*w/v*) in ethyl alcohol (95 percent by volume).

23.1.3.2 *Procedure* — Make 50 to 100 g of sample, accurately weighed, distinctly alkaline with sodium hydroxide solution and evaporate to dryness in a platinum dish. Ignite the residue until the organic matter is thoroughly charred, avoiding intense red heat; cool, digest with about 20 ml of hot water and add concentrated hydrochloric acid dropwise until the reaction is distinctly acid. Filter into a 100-ml graduated flask and wash with a little hot water. (Volume of the filtrate should not exceed 60 ml.) Return the filter containing any unoxidized carbon to the platinum dish, make alkaline by wetting thoroughly with lime water, dry on a steam-bath, and ignite to white ash. Dissolve the ash in a few millilitres of hydrochloric acid (1 : 3) and add to the liquid in the 100-ml flask, rinsing the dish with a few millilitres of water. To the combined solutions, add 0·5 g of calcium chloride and a few drops of phenolphthalein indicator solution, then sodium hydroxide solution until a permanent light pink colour is produced. Finally, dilute to the mark with lime water, mix, and filter through a dry filter paper. To 50 ml of the filtrate, add dilute sulphuric acid, until the pink colour disappears, then add methyl

orange indicator and continue addition of the acid until the yellow colour is changed to pink. Boil for about one minute to expel carbon dioxide. Cool, and carefully add the standard sodium hydroxide until the liquid assumes a yellow tinge, avoiding excess of alkali. (All boric acid is now in the free state with no uncombined sulphuric acid present.) Add 1 to 2 g of neutral mannitol and a few drops of phenolphthalein indicator solution, note the burette reading, and again titrate the solution with the standard sodium hydroxide solution until pink colour develops. Add a little more of mannitol, and if the pink colour disappears, continue addition of the standard sodium hydroxide solution until pink colour reappears. Repeat alternate addition of mannitol and standard alkali until the permanent end point is reached. A volume of glycerol (neutral to phenolphthalein) equal to the volume of the solution to be titrated may be substituted for mannitol.

23.1.3.3 *Calculation*

$$\text{Boric acid, percent by weight} = \frac{2{\cdot}48\,V}{W}$$

where

V = volume in ml of the standard sodium hydroxide solution required for the titration, and

W = weight in g of sample taken for the test.

23.2 Detection and Determination of Formaldehyde

23.2.1 *Leach Test*

23.2.1.1 *Reagents*

a) *Concentrated hydrochloric acid* — sp gr 1·16.

b) *Ferric chloride solution* — 10 percent (*w/v*).

23.2.1.2 *Procedure* — Mix in a casserole about 10 ml of milk with an equal volume of concentrated hydrochloric acid containing one millilitre of ferric chloride solution to each 500 ml of acid. Heat slowly but directly over a gas flame for about 5 minutes to 80 to 90°C. Rotate the casserole to break up the curd. A violet colour indicates the presence of formaldehyde.

23.2.2 *Hehner Test*

23.2.2.1 *Reagent*

Concentrated sulphuric acid — commercial, sp gr 1·84.

23.2.2.2 *Procedure* — To about 10 ml of milk in a wide mouth testtube, add about half the volume of concentrated sulphuric acid pouring the acid carefully down the side of the tube so that it forms a layer at the bottom without mixing with the milk. A violet, or blue colour, at the junction of the two liquids indicates the presence of formaldehyde.

NOTE — The test is given only in the presence of a trace of ferric chloride or other oxidizing agents. This test may be combined with the determination

of fat by the Gerber method, noting whether a violet colour forms on addition of milk to the sulphuric acid in the butyrometer.

23.2.3 *Chromotropic Acid Test*

23.2.3.1 *Reagent* — Saturated solution of 1,8-dihydroxynaphthalene-3,6 disulphonic acid in about 72 percent sulphuric acid (about 500 mg/100 ml). Light straw-coloured solution should result.

23.2.3.2 *Preparation of the sample* — Dilute 100 ml of sample with 100 ml of water. Make it acidic to litmus with phosphoric acid, and then add one millilitre in excess. Connect with condenser through trap, and slowly distil 50 ml.

23.2.3.3 *Procedure* — Place 5 ml of the reagent in a test-tube and add with mixing one millilitre of distillate (*see* **23.2.3.2**). Place in a boiling water-bath for 15 minutes, and observe during the heating period. The presence of formaldehyde is indicated by the appearance of a light to deep purple colour (depth of colour depending on the quality of formaldehyde present).

23.2.4 *Phenylhydrazine Hydrochloride and Ferric Chloride Test*

23.2.4.1 *Reagents*

a) *Phenylhydrazine hydrochloride solution* — one percent (*w/v*).

b) *Ferric chloride solution* — one percent (*w/v*).

c) *Concentrated hydrochloric acid* — sp gr 1·16.

23.2.4.2 *Procedure* — Treat about 15 ml of milk with one millilitre of phenylhydrazine hydrochloride solution, then add a few drops of ferric chloride solution, and finally 2 ml of concentrated hydrochloric acid. The presence of formaldehyde is indicated by the formation of a red colour, which changes after some time to orange-yellow.

This method gives indication of the presence of one part of formaldehyde in about 100 000 parts of milk.

23.2.5 *Quantitative Method*

23.2.5.1 *Reagents*

a) *Standard sodium hydroxide solution* — 1·0 N standardized against standard sulphuric acid solution, using bromothymol blue indicator (one gram in 500 ml of 50 percent alcohol), or litmus (solution of purified litmus of such concentration that three drops will impart a distinct blue colour in 50 ml of water).

b) *Hydrogen peroxide solution* — Containing about three percent of H_2O_2. If acid, neutralize with standard sodium hydroxide solution, using bromothymol blue or litmus indicator.

c) *Standard sulphuric acid solution* — 0·1 N.

23.2.5.2 *Preparation of the sample* — Dilute 100 ml of milk with 100 ml of water in a 800-ml Kjeldahl flask. Acidify with phosphoric acid and

add one millilitre in excess. Connect with the condenser through a splash head with trap, and slowly distil 50 ml.

23.2.5.3 *Procedure* — Measure 50 ml of the standard sodium hydroxide solution into a 500-ml conical flask and add 50 ml of hydrogen peroxide solution. Add 25 ml of the prepared distillate (*see* **23.2.2.2**), place a funnel in the neck of flask and heat on a steam-bath for 5 minutes, shaking occasionally. Remove from the steam-bath, wash the funnel with water, cool the flask to room temperature and titrate the excess of sodium hydroxide solution with the standard sulphuric acid, using bromothymol blue or litmus indicator.

NOTE — It is necessary to cool the flask before titration to obtain a sharp end point with litmus.

23.2.5.4 *Calculation*

$$\text{Formaldehyde, g per 100 ml} = 0{\cdot}060\,06\ V$$

where

V = volume in ml of the standard sodium hydroxide solution used for neutralization.

NOTE — If the distillate of the milk sample (**23.2.5.2**) contains appreciable quantity of free acid, titrate separately a portion and calculate the acidity as percent formaldehyde (HCHO). Make correction for this acidity in calculating percent formaldehyde (HCHO).

23.3 Detection and Determination of Benzoic Acid

23.3.1 *Ferric. Chloride Test*

23.3.1.1 *Reagents*

a) *Dilute hydrochloric acid* — 1 : 3 by volume.

b) *Ethyl ether*

c) *Ammonium hydroxide* — sp gr 0·88.

d) *Ferric chloride solution* — 0·5 percent (*v*/*v*), neutral.

e) *Sodium hydroxide solution* — 10 percent (*w*/*v*).

f) *Potassium nitrate* — crystals.

g) *Concentrated sulphuric acid* — sp gr 1·84.

h) *Ammonium sulphide* — freshly prepared, colourless.

23.3.1.2 *Procedure* — Acidify 100 ml of milk with 5 ml of dilute hydrochloric acid. Shake until curdled. Filter and extract the filtrate with 50 to 100 ml of ethyl ether. Wash the ether extract with two 5-ml portions of water. Evaporate the greater portion of the ether in a porcelain dish on a water-bath and allow the remainder to evaporate spontaneously. If benzoic acid is present in considerable quantity, it will crystallize from the ether in shining leaflets which give a characteristic colour on heating.

Dissolve the residue in hot water, divide into two portions and test as follows:

a) Make one portion alkaline with a few drops of ammonium hydroxide, expel the excess of ammonia by evaporation, dissolve the residue in a few millilitres of hot water and filter, if necessary. Then add a few drops of ferric chloride solution. A salmon coloured precipitate of ferric benzoate indicates the presence of benzoic acid.

b) To the other portion, add one or two drops of sodium hydroxide solution and evaporate to dryness. To the residue, add from five to ten drops of concentrated sulphuric acid and a small crystal of potassium nitrate. Heat for 10 minutes in a glycerol bath at 120 to 130°C, or for 20 minutes in a boiling water-bath. The temperature shall not exceed 130°C. After cooling, add one millilitre of water, make distinctly ammoniacal, and boil the solution to decompose any ammonium nitrate that may have been formed. Cool, pour into a test-tube, and add a drop of ammonium sulphide, without allowing the layers to mix.

A red-brown ring indicates the presence of benzoic acid. On mixing, the colour diffuses through the whole liquid, and on heating finally, changes to greenish yellow. This differentiates benzoic acid, from salicylic acid. The latter forms coloured compounds, which are not destroyed by heating.

23.3.2 *Quantitative Method*

23.3.2.1 *Reagents*

a) *Sodium chloride* — pulverized.

b) *Sodium hydroxide solution* — 10 percent (*w*/*v*).

c) *Milk of lime* — One part powdered, recently slaked calcium hydroxide suspended in 3 parts of water.

d) *Dilute hydrochloric acid* — 1 : 3 by volume.

e) *Chloroform*

f) *Ethyl alcohol* — 95 percent by volume, neutral.

g) *Phenolphthalein indicator solution* — one percent (*w*/*v*) in ethyl alcohol (95 percent by volume).

h) *Standard sodium hydroxide solution* — 0·05 N.

23.3.2.2 *Preparation of the sample* — Mix the sample thoroughly and transfer 150 g to a 500-ml graduated flask, add enough of sodium chloride to saturate the water in the sample, make alkaline to litmus paper with sodium hydroxide solution or with milk of lime and dilute to the mark with saturated sodium chloride solution. Shake thoroughly, allow to stand for at least 2 hours, with frequent shaking, and filter. If the sample contains large quantities of fat, portions of which may

contaminate the filtrate, add a few millilitres of sodium hydroxide solution to the filtrate and extract with ether before proceeding as given in **23.3.2.3**.

23.3.2.3 *Procedure* — Pipette a convenient portion (100 to 200 ml) of the filtrate (*see* **23.3.2.2**) into a separating funnel. Neutralize to litmus paper with dilute hydrochloric acid and add an excess of 5 ml. Extract carefully with chloroform, using successively portions of 70, 50, 40 and 30 ml. To avoid formation of emulsion, shake cautiously each time, using rotary motion. Layer of chloroform usually separates readily after standing for a few minutes. If an emulsion forms, break it by stirring the chloroform layer with a glass rod, by drawing it off into a second separator and giving one or two sharp shakes from one end of the separator to the other, or by centrifuging for a few minutes. As this is a progressive extraction, draw off carefully as much of the clear chloroform solution as possible after each extraction, but do not draw off any of the emulsion with chloroform layer. If this precaution is taken, the chloroform extract need not be washed.

Transfer the combined chloroform extracts to a porcelain evaporating dish, rinse the container several times with a few millilitres of chloroform, and evaporate to dryness at room temperature in a current of dry air.

The extract may also be transferred from the separating funnel to a 300-ml Erlenmeyer flask, rinsing the separating funnel 3 times with 5 to 10-ml portions of chloroform. Distil very slowly at low temperature to about one-fourth of the original volume. Transfer the residue to a porcelain evaporating dish, rinsing the flask 3 times with 5 to 10-ml portions of chloroform, and evaporate to dryness at room temperature in a current of dry air.

Dry the residue overnight in a desiccator containing concentrated sulphuric acid. Dissolve the residue of benzoic acid in 30 to 50 ml of ethyl alcohol and titrate with the standard sodium hydroxide solution.

23.3.2.4 *Calculation*

$$\text{Benzoic acid (as sodium benzoate) percent by weight} = \frac{0{\cdot}72\, V_1 V_2}{V_3 W}$$

where

V_1 = volume in ml of the standard sodium hydroxide used for titration,

V_2 = volume in ml to which the filtrate was made up (*see* **23.3.2.2**),

V_3 = volume in ml of the aliquot used for the test (*see* **23.3.2.3**), and

W = weight in g of the sample taken for the test.

23.4 Detection and Determination of Salicylic Acid

23.4.1 *Ferric Chloride Test*

23.4.1.1 *Reagents*

a) *Dilute hydrochloric acid* — 1 : 3 by volume.

b) *Ethyl ether*

c) *Ferric chloride solution* — 0·5 percent (*w*/*v*), neutral.

23.4.1.2 *Procedure* — Acidify 100 ml of the sample with 5 ml of dilute hydrochloric acid. Shake until curdled and filter. Extract with 50 to 100 ml of ethyl ether. Wash the ether layer with two 5-ml portions of water. Evaporate the greater portion of the ether in a porcelain dish on a steam-bath, allow the remainder to evaporate spontaneously. Add one drop of the ferric chloride solution. A violet colour indicates the presence of salicylic acid.

23.4.2 *Jorissen Test*

23.4.2.1 *Reagents*

a) *Dilute hydrochloric acid* — 1 : 3 by volume.

b) *Ethyl ether*

c) *Potassium nitrite solution* — 10 percent (*w*/*v*).

d) *Acetic acid solution* — 50 percent (*w*/*v*).

e) *Copper sulphate solution* — one percent (*w*/*v*).

23.4.2.2 *Procedure* — Acidify, filter, and extract the sample as described in **23.4.1.2**. Dissolve the residue from the ether extract in about 10 ml of hot water. Cool and add four or five drops of potassium nitrite solution, four or five drops of the acetic acid solution and one drop of copper sulphate solution. Mix thoroughly. Boil for 30 seconds and allow to stand for one or two minutes. In the presence of salicylic acid, a blood red colour will develop.

23.4.3 *Quantitative Method*

23.4.3.1 *Reagents*

a) *Dilute hydrochloric acid* — 1 : 3 by volume.

b) *Ethyl ether*

c) *Petroleum ether* — boiling below 60°C.

d) *Methyl orange indicator solution*

e) *Sodium hydroxide solution* — 0·1 N.

f) *Carbon disulphide or petroleum ether boiling below* 60°C.

g) *Ferric alum solution* — 2 percent (*w*/*v*).

Or

Ferric chloride solution — 0·5 percent.

h) *Dilute ammonium hydroxide* — one percent by volume.

23.4.3.2 *Extraction* — Transfer to a separating funnel 100 ml of the sample, neutralize to litmus with dilute hydrochloric acid and add an excess of hydrochloric acid at the rate of 2 ml of acid for each 100 ml of solution. Extract with 4 separate portions of ethyl ether using for each extraction a volume of ether equal to half the volume of aqueous layer. If an emulsion forms on shaking, this may usually be broken by adding a little (one-fifth volume of ether layer) petroleum ether and shaking again, or by centrifuging. If a small quantity of emulsion still persists, allow it to remain with the aqueous layer, where frequently it is broken during the next extraction. If an emulsion remains after the fourth extraction, separate it from the clear ether and the clear aqueous layer and extract it separately with 2 or 3 small portions of ether. Combine the ether extracts, wash with a volume of water equal to one-tenth of the total volume of ether extracts, allow the layers to separate, and reject the aqueous layer. Wash in this way until the aqueous layer, after separation yields yellow colour upon addition of methyl orange solution and 2 drops of sodium hydroxide solution. Distil slowly the greater part of ether, transfer the remainder to a porcelain dish, and allow it to evaporate spontaneously. If no interfering substances are present, proceed as described in **23.4.3.3**; if interfering substances are present, purify the residue by one of the following methods:

a) Thoroughly dry the residue in vacuo over concentrated sulphuric acid. Extract it 10 times with 10 to 15-ml portions of carbon disulphide or petroleum ether rubbing the contents of the dish with a glass rod, and filter successive portions of the solvent through a dry filter paper into the porcelain dish. Test the extracted residue with a drop of ferric alum solution, and if it gives the reaction for salicylic acid, dissolve it in water; acidify the solution with dilute hydrochloric acid, extract with ether, evaporate, extract the dry residue thus obtained with carbon disulphide or petroleum ether, and add to the extract first obtained. Distil the greater portion of the carbon disulphide or petroleum ether and allow the remainder to evaporate spontaneously. Proceed as described in **23.4.3.3**.

b) Dissolve the residue in 40 to 50 ml of ether. Transfer the ether solution to a separating funnel and extract with 3 successive 15-ml portions of dilute ammonium hydroxide (if fat is known to be present in the original ether extract, extract the latter directly with 4 portions of dilute ammonium hydroxide instead of 3). Combine the alkaline aqueous extracts, acidify, again extract with ether, and wash the combined ether extracts as before. Slowly distil the greater portion of ether, allow the remainder to evaporate spontaneously, and proceed as described in **23.4.3.3**.

23.4.3.3 *Determination* — Dissolve the residue (*see* **23.4.3.2**) in a small quantity of hot water and after cooling, dilute to a definite volume (usually

50 or 100 ml). If the solution is not clear, filter through dry filter paper. Dilute aliquots of the solution and treat with a few drops of ferric chloride solution or ferric alum solution until maximum colour is developed.

(Ferric alum solution should be boiled until a precipitate appears, then allowed to settle, and filtered. Acidity of the solution is slightly increased in this manner, but the solution remains clear for a considerable time, and the turbidity caused by its dilution with water is much less and does not appear so soon as when an unboiled solution is used. This turbidity interferes with the exact matching of colour.)

Compare the colour developed with the colour obtained when a standard salicylic acid solution (containing one milligram of salicylic acid in 50 ml) is similarly treated, using Nessler tubes or a colorimeter. In either case, and especially with ferric chloride, avoid an excess of the reagent, although an excess of 0·5 ml of ferric alum solution may be added to 50 ml of the comparison solution of salicylic acid without vitiating the results.

23.5 Detection of Hydrogen Peroxide

23.5.1 *Reagent*

Para phenylenediamine solution — 2 percent (*w*/*v*).

23.5.2 *Procedure* — Add to about 5 ml of milk in a test-tube, an equal volume of the sample followed by five drops of para phenylenediamine solution. A blue colour is developed in the presence of hydrogen peroxide.

NOTE — Hydrogen peroxide is destroyed when milk is heated, or stored for a long interval.

23.6 Detection of Hypochlorites and Chloramines

23.6.1 *Detection of Hypochlorites*

23.6.1.1 *Apparatus*

a) *Centrifuge*

b) *Tubes for centrifuge* — of 12·5-ml capacity.

c) *Mercury vapour lamp* — fitted with a Wood's filter.

23.6.1.2 *Reagents*

Stannous chloride solution — 0·025 percent (*w*/*v*) in 73·5 percent sulphuric acid [prepared by mixing three volumes of concentrated sulphuric acid (sp gr 1·84) and one volume of water].

23.6.1.3 *Procedure* — Cool about 3 ml of milk taken in a test-tube in a freezing mixture of ice and salt to 2 to 5°C. In another test-tube, take an equal volume of stannous chloride solution and similarly cool and add to the milk. Shake the tube whilst in the freezing mixture and hold for 3 minutes. Place the mixture in a 12·5-ml centrifuge tube and centrifuge for 3 minutes at 2 500 rpm. A yellow-green colour is produced in the presence of hypochlorite. Alternatively, after centrifuging, examine

the tube in ultra-violet light from a mercury vapour lamp fitted with a Wood's filter, for the presence of any yellow fluorescene.

23.6.2 *Detection of Hypochlorites and Chloramines*

23.6.2.1 *Reagents*

a) *Potassium iodide solution* — Prepare fresh by dissolving 7 g of potassium iodide in 100 ml of water.

b) *Dilute hydrochloric acid* — To 100 ml of concentrated hydrochloric acid (sp gr 1·16), add 200 ml of water.

c) *Starch solution* — Boil one gram of starch in 100 ml of water. Cool before using.

23.6.2.2 *Tests*

a) To 5 ml of sample in a medium size test-tube, add 1·5 ml of potassium iodide solution, mix thoroughly by shaking, and observe the colour of milk.

b) If unaltered, add 4 ml of dilute hydrochloric acid, mix thoroughly by means of a glass rod flattened at one end, and note colour of the curd.

c) Next, place tube in large water-bath, previously heated to 85 ± 1°C, allow to remain for 10 minutes (during this interval the curd will rise to the surface), then cool rapidly by placing in cold water. Note the colour of the curd and the liquid.

d) Then add 0·5 to 1 ml of starch solution to the liquid below curd and note the colour.

The proportion of available chlorine may be ascertained from Table III.

NOTE — The method is not reliable in the presence of more than 2·5 ppm of copper.

23.7 Quaternary Ammonium Compounds (QAC)

23.7.1 *Reagents*

a) *Tetrachloroethane, technical* — (Not all technical grades are satisfactory. If pink colour is not obtained at one and two parts per million levels, redistil the solvent under reduced pressure, rejecting both the first and the last 10 percent of the distillate. Some old batches are unsatisfactory even after redistillation. Absence of pink colour is sometimes due to residual acids, detergents, etc, on glassware.)

b) *Lactic acid solution* — 50 percent (*w/v*), aqueous.

c) *Eosin* (*yellowish dye*) *CI* 768 — 0·05 percent (*w/v*), aqueous solution (0·5 mg/ml).

d) *Standard sodium hydroxide solution* — 4 N.

TABLE III REACTIONS WITH VARIOUS TESTS

(*Clause* 23.6.2.2)

TEST	CONCENTRATION OF AVAILABLE CHLORINE					
	1 : 1 000	1 : 2 000	1 : 5 000	1 : 10 000	1 : 25 000	1 : 50 000
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Test (a)	Yellowish brown	Deep yellow	Pale yellow, Fades	—	—	—
Test (b)	Yellowish brown	Deep yellow	Light yellow	—	—	—
Test (c)	Yellowish brown	Deep yellow	Yellow	Yellow	Pale yellow	Yellowish
Test (d)	Blue purple	Blue purple	Blue purple	Dark red-purple	Red purple	Pale red-purple

e) *Citric acid* — monohydrate buffer. (Adjust 25 percent aqueous citric acid to *p*H 4·5 with 50 percent sodium hydroxide solution.)

f) *Dioctyl sodium sulfosuccinate solution* — 0·01 percent (*w*/*v*), aqueous.

23.7.2 *Procedure*

23.7.2.1 Place 5 ml of tetrachloroethane in a test-tube suitable for centrifuging, with cork (or glass) stopper. Add 2 ml of lactic acid solution and 5 ml of the milk sample. Shake the contents vigorously for one minute. Add 2 ml of standard sodium hydroxide solution and invert the tube 6 times. Centrifuge in a 25-cm diameter machine at 3 200 rpm (or other type giving equivalent force) for 5 minutes. Three layers are formed. Decant or aspirate off the top layer. The second layer consists chiefly of white precipitated protein. The bottom layer should be clear tetrachloroethane containing any QAC originally present in milk. Loosen the curd layer and pour or pipette as much as possible the bottom layer to another tube.

23.7.2.2 Transfer a 2-ml aliquot of the bottom layer to a clean test-tube containing 0·5 ml of the citric acid buffer and 0·2 ml of eosin solution. Close the tube and shake vigorously. A pink or red colour in the tetrachloroethane indicates the presence of QAC. Add dioctyl sodium sulfosuccinate solution dropwise, shaking the contents vigorously after each addition, and titrate to a colourless end point. One millilitre of 0·01 percent of the dioctyl sodium sulfosuccinate solution is equivalent to about 50 ppm of QAC.

23.7.2.3 The exact equivalent strength of solution depends upon relation between the molecular weights of dioctyl sodium sulfosuccinate and

particular QAC present. This may be determined by titration of a standard solution of the QAC, using the procedure outlined in **23.7.2.1** and **23.7.2.2**. The standard solution may be prepared from weighed portion of the dry QAC.

24. DETECTION OF NITRATES

24.1 Reagents

24.1.1 *Mercuric Chloride Solution* — 2·5 percent (*w*/*v*) in one percent hydrochloric acid.

24.1.2 *Diphenylbenzidine or Diphenylamine Sulphate Reagents* — Add 50 ml of water to 0·085 g of diphenylamine sulphate or diphenylbenzidine contained in a large flask, and gradually add 450 ml of concentrated sulphuric acid (sp gr 1·84).

24.2 Procedure — Mix 25 ml of milk with 25 ml of mercuric chloride solution. After thorough mixing, filter through a 11-cm filter paper. Place one millilitre of the filtrate in a test-tube and add 4 ml of the reagent (*see* **24.1.2**). On mixing, a blue colour is formed in the presence of nitrates.

NOTE — Use of diphenylbenzidine increases the sensitivity of the test ten times.

25. QUANTITATIVE ESTIMATION OF SEDIMENT IN MILK

25.1 Apparatus

25.1.1 *Trommsdorf Centrifuge Tubes* — with capacity of graduated capillary portion 0·02 ml and 0·05 ml (*see* Fig. 6).

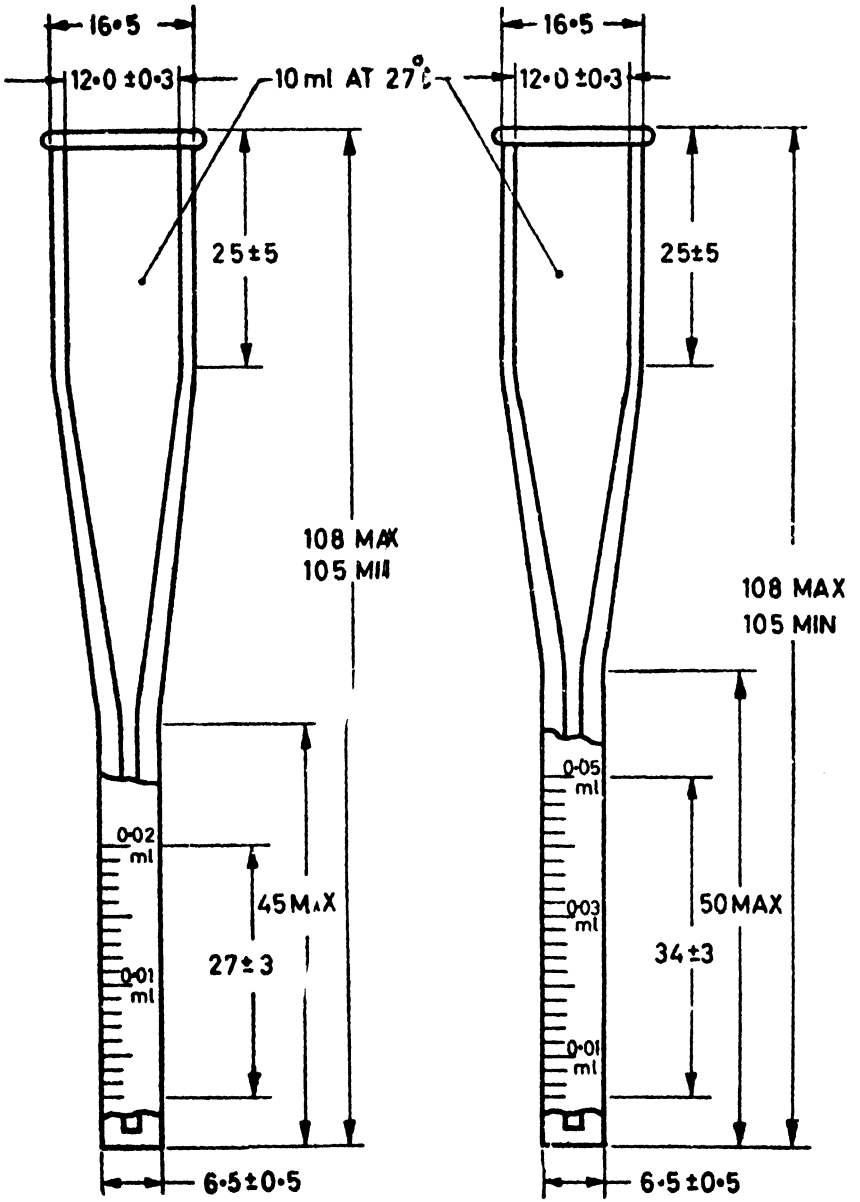
25.1.2 *Centrifuge* — of a suitable type, for use with centrifuge tubes (*see* **25.1.1**).

25.2 Procedure — Place 10 ml of the well-mixed sample in the graduated Trommsdorf tube and centrifuge for 5 minutes. Pour off the supernatant liquid. Read the volume of the deposit.

25.3 Interpretation — Normally with clean milk the amount of deposit should not exceed 0·002 percent. The deposit consists not only of visible dirt but also of cells. A large deposit may be due either to the presence of visible dirt, or mastitis or both.

26. PHOSPHATASE TEST

26.0 General — The test is used to judge the efficiency of pasteurization of milk. According to the accepted practice in this country, milk for liquid trade is pasteurized by the holding method (63 to 66°C for 30 minutes), or by the High Temperature-Short Time (HTST) method



6a 6b

All dimension in millimetres.

FIG. 6 TROMMSDOR CENTRIFUGE TUBES

(holding at 72·2 to 72·8°C for 15 seconds). To test whether the heat-treatment by either of these methods was properly carried out, the treated milk is subjected to the phosphatase test which helps to indicate the presence or absence of phosphatase enzyme. Phosphatase present in milk is destroyed by just about the same heat-treatment necessary for the destruction of *Mycobacterium tuberculosis*, the most heat resistant pathogen likely to be present in milk. When heat-treatment is less than that specified above, some of the phosphatase remains active and will liberate phenol from disodium phenylphosphate in a buffered solution at a *p*H of approximately 9·5. The presence of free phenol is determined by a colorimetric test.

The sample of pasteurized milk shall be tested within 48 hours after pasteurization. The sample shall be stored at a temperature not exceeding 5°C.

Several modifications of phosphatase test are in use. The method described here is based on the Aschaffenburg and Mullen technique. The test yields valuable information about the pasteurization of milk after incubation for only 30 minutes, and results comparable with those of the Kay and Graham phosphatase test are obtained after the incubation period of 2 hours instead of 24 hours. Faulty heat-treatment may thus be quickly detected and remedied. Moreover, the new test is simple to carry out, requires the minimum of equipment and, being highly specific, is unlikely to be adversely affected by interfering substances.

In the test, milk is added to a suitably buffered solution of disodium *p*-nitrophenyl phosphate which is readily hydrolyzed by the enzyme phosphatase to form *p*-nitrophenol which is yellow in alkaline solution.

26.1 Apparatus

26.1.1 *Lovibond All-Purpose Comparator with Stand*

26.1.2 *Standard Discs* — giving 0, 6, 10, 18, 42 or 0, 6, 10, 14, 18, 25, 42 readings.

26.1.3 *Fused Glass cells* — 25 mm.

26.1.4 *Test-Tubes* — 15 × 1·9 cm, with ring at 10 ml fitted with rubber stoppers.

26.2 Reagents

26.2.1 *Buffer Solution* — 3·5 g of sodium carbonate analytical reagent grade (*see* IS: 296-1951*), and 1·5 g of sodium bicarbonate analytical reagent grade (*see* IS: 491-1954†) dissolved in one litre of water.

26.2.2 *Substrate* — disodium *p*-nitrophenyl phosphate not less than 95 percent pure.

26.2.3 *Buffer Substrate* — Transfer 0·15 g of the substrate into a 100-ml measuring cylinder or stoppered graduated flask and make up to the mark with the buffer solution. The solution should not be stored for long

*Since revised.

†Since superseded by IS : 2124-1962 Specification for sodium bicarbonate. (Since revised).

periods but may normally be kept in a refrigerator for up to one week. The solution is practically colourless; when viewed through a 25-mm cell in the all-purpose comparator, it should give a reading of less than 10 on the disc.

26.3 Procedure — Fill 10 ml (5 ml may be used) quantities of the buffer substrate solution into test-tubes marked at 10 ml and bring to 37 to 38°C in a water-bath. Add 2 ml (one millilitre if 5 ml of buffer substrate are used) of the milk to be tested, close the tubes with rubber stoppers and invert to mix. Prepare in the same way a blank from a boiled milk of the same type as that under test. Incubate all the tubes at 37 to 38°C. Read the yellow colour after 30 minutes, return to the bath, and take a second reading after incubation for a further 90 minutes. The yellow colour is read in a Lovibond all-purpose comparator on a resazurin stand, fitted with the disc calibrated in microgram *p*-nitrophenol. The blank is placed on the left of the stand and the sample on the right. Readings are taken by looking down on to the two apertures with the comparator facing a good source of north daylight; the disc is revolved until the sample is matched; readings falling between two standards are recorded to the nearest reading.

26.4 Interpretation of Results

Disc Reading After 30 Minutes Incubation	*Interpretation*
0 or trace	Properly pasteurized
6	Doubtful
10 or over	Underpasteurized
Disc Reading After 2 Hours Incubation	
0 to 10	Properly pasteurized
Over 10	Under pasteurized

The 30-minute test will reveal any serious fault in pasteurization, but to enable minor errors to be detected, readings shall be taken after further incubation for 90 minutes.

27. TEST FOR STERILIZED MILK

27.0 General — When milk is heated to sterilizing temperatures (104 to 110°C for 20 to 40 minutes), the albumin completely gets precipitated. The test is made by adding sufficient ammonium sulphate to precipitate other substances such as casein, filtering the mixture of milk and ammonium sulphate, and then heating the filtrate. If milk has not been heated to 100°C for at least a short time, the albumin remaining in solution will be revealed by turbidity in the heated filtrate.

27.1 Reagents

27.1.1 *Ammonium Sulphate*

27.1.2 *Whatman Filter Paper No.* 12 *or Equivalent* — 12·5-cm dia.

27.2 Procedure — Weigh accurately 4·0 g of ammonium sulphate into a 50-ml conical flask. Measure out 20·0 ± 0·5 ml of sample, and pour into the conical flask containing ammonium sulphate. Mix properly to ensure that ammonium sulphate is dissolved. Leave for not less than 5 minutes and then filter through Whatman No. 12 filter paper or equivalent into a test-tube. When not less than 5 ml of a clear filtrate have collected, place the tube in a beaker of water, which is kept boiling. Keep it there for 5 minutes. Transfer the tube to a beaker of cold water. When the tube is cool, examine the contents for turbidity by observing in north light, or under daylight electric lamp.

27.3 Interpretation — Milk which shows no sign of turbidity has been satisfactorily sterilized.

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