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TEST METHODS FOR TOBACCO IN TOBACCO PRODUCTS

Part 1 - Loss on Heating, Freedom From Mould and Weevil Attack, Total Alkaloids, Total Nitrogen, Total Ash, Acid Insoluble Ash, Total Chlorine, Total and Reducing Sugars

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BUREAU OF CEYLON STANDARDS

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S. L. S. 309 : 1974

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BUREAU OF CEYLON STANDARDS
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Colombo 3.

Sri Lanka Standards are subject to periodical revision in order to accommodate the progress made by industry. Suggestions for improvement will be recorded and brought to the notice of the Committees to which the revisions are entrusted.

This Standard does not purport to include all the necessary provisions of a contract.

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In the preparation of this standard considerable assistance derived from the publications of the Indian standard Institution is acknowledged.

SRI LANKA STANDARD TEST METHODS FOR TOBACCO IN TOBACCO PRODUCTS

PART - 1

Loss on Heating, Freedom from Mould and Weevil Attack, Total Alkaloids, Total Nitrogen, Total Ash, Acid Insoluble Ash, Total Chlorine, Total And Reducing Sugars.

FOREWORD

This Sri Lanka Standard Test Method has been prepared by the Drafting sub committee on Test methods for Tobacco. It was approved by the Agricultural and Chemicals Divisional Committee of the Bureau of Ceylon Standards and was authorised for adoption and publication by the Council of the Bureau on 31st October 1974.

This Standard prescribes a simplified scheme of tests which could be adopted with minimum costs and will lower the cost of quality control appreciably. This standard prescribes an additional method for determining chlorine and it covers two methods for determining total alkaloids; it includes technical details for sample preparation and tobacco examination for freedom from mould and weevil attack. It is expected that the adoption of this standard will help in achieving uniformity in the methods of analysis, thereby facilitating the interprotation and comparison of results.

Any further tests will published at a later stage as Part 2 of this standard.

In reporting the result of a test or analysis made according with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with CS. 102.*

In the preparation of this standard considerable assistance derived from the publications of the Indian standard Institution is acknowledged.

1. SCOPE

1·1 This standard prescribes the test methods commonly used for testing of tobacco in tobacco products.

* CS 102 – Presentation of numerical values.

2. QUALITY AND REAGENTS

2·1 Unless specified otherwise 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. SAMPLE PREPARATION AND ITS SIZE

3·1 Take 300 g of the test sample. Keep aside 50 g of the sample for the tests given under clauses 4 and 5 Grind the remaining sample to a particular size so that all shall pass through 500μm C8 sieve* Preserve the sample in a clean and dry containner for carrying out the tests given under clauses 6 to 11.

4. DETERMINATION OF LOSS ON HEATING

4·1 Principle – The method is based on dehydration of the sample in a hot air oven at a temperature of 100·0 ± 0·5°C for 15 hours.

4·2 Apparatus

4·2·1 Dish – made of aluminium, stainless steel, silica or porcelain and provided with a perforated cover. The diameter of the dish shall be at least 50 mm and the depth not more than 40 mm.

4·2·2 Oven – fitted with a ventilator and means for forced internal circulation of air and maintained at a temperature 100·0 ± 0·5°C.

4·3 Procedure – Place about 10 g of the material (see clause 3·1) in a tared dish, close it with the perforated cover, weigh, and place it in the oven which shall previously have been brought to a temperature of 100 0 ± 0 5°C. Maintain this temperature in the oven for the drying period of 15 hours. Remove the dish and allow it to cool in a desiccator. Weigh the dish with cover and the contents and note the mass of the material.

4 4 Calculation

$$\text{Loss on heating, per cent by mass} = \frac{m_0 - m_1}{m_0} \times 100$$

Where

m_0 = mass in g, of the material taken for the test, and
m_1 = mass, in g, of the material after drying

* CS 124 – Test Sieves

5. EXAMINATION FOR FREEDOM FROM MOULD AND WEEVIL ATTACK

5·1 Procedure – Take about 10 g of the material (see clause 3·1) on a large, clean sheet of paper. Loosen the lumps of the material and examine for the presence or absence of mould and weevil (dead and alive) by naked eye (corrected for abnormal vision). A hand lens (magnification x 10) may also be used. In case a larger magnification is used, this shall be stated in the test report.

6. DETERMINATION OF TOTAL ALKALOIDS

Determination of total alkaloids may be carried out either by silicotungstic acid method (see clause 6·1) or by spectrophotometric method (see clause 6·2). In case of dispute the spectrophotometric method shall be used.

6·1 Silico – Tungstic Method

6·1·1 **Principle** – The method consists of steam distilling the nicotine from alkaline medium, converting it into a salt by collecting the distillate in an acid medium, precipitating with sillicotungstic acid and finally weighing the residue (oxides of silica and tungsten – $WO_3 \cdot SiO_2$) obtained after ignition.

6·1·2 **Apparatus**

6·1·2·1 Kjeldahl flask – 500 ml capacity.

6·1·2·2 Pipette – 25 ml capacity.

6·1·2·3 Volumetric flask – 100 and 250 ml capacity.

6·1·2·4 Crucible – silica or platinum.

6·1·2·5 Distillation assembly – The assembly consists of 500 ml kjeldahl flask or a round - bottom flask fitted with a rubber stopper through which passes a stem of a trap bulb and the inlet tube for steam. The free end of the trap bulb is connected to a well – cooled condenser, the lower end of which dips below the surface of dilute hydrochloric acid contained in a receiving flask.

6·1·3 **Reagents**

6·1·3·1 Liquid paraffin

6·1·3·2 Sodium hydroxide solution – aqueous, 30 per cent (m/v).

6·1·3 3 Sodium chloride

6·1·3·4 Phenolphathalein indicator solution – 0·2 per cent (m/v) Dissolve 0 2 g of phenolpathalein in 60 ml of rectified spirit (95%) and dilute to 100 ml with water.

6·1·3·5 Dilute hydrochloric acid–1 : 4 and 1 : 1 000 (v/v).

6 1·3·6 Silico – tungstic acid solution – Dissolve 120 g of silico – tungstic acid ($4H_2O \cdot SiO_2 \cdot 12 WO_3 \cdot 22H_2O$) in water and dilute to one litre The solution should be free from cloudiness and green colour.

Note: The silico – tungstic acids, should be white or pale yellow crystals free from green colour. Of the several silico – tungstic acids, $4H_2O \cdot SiO_2 \cdot 3H_2O$ and $4 H_2O \cdot SiO_2 \cdot 12 WO_3 \cdot 20 H_2O$ do not give crystalline precipitates with nicotine and should not be used.

6 1·3 7 Methyl orange indicator solution – 0 04 per cent (m/v) in aqueous ethyl alcohol (20 per cent by volume).

6·1·4 **Procedure**

6·1·4·1 Weigh accurately a quantity of the material that shall contain preferably 0 1 to 1·0 g of nicotine in a suitable tared vessel Quantitatively transfer the weighed material to a 500 ml Kjeldahl flask or a round bottom flask using water to wash the last traces of the material in to the flask If necessary, add to the contents of the Kjeldahl flask a little of liquid paraffin to prevent frothing during distillation and a few small pieces of pumice to prevent bumping. Add

10 g of sodium chloride and make the contents of the flask alkaline by adding 10 ml of sodium hydroxide solution, using phenolphthalein as indicator. Fit the mouth of the Kjeldahl flask with a two – holed rubber stopper through which pass the stem of a trap bulb and an inlet tube for steam. Connect the free end of the trap bulb to a well – cooled condenser, the lower end of which dips below the surface of 10 ml of dilute hydrochloric acid (1 : 4) contained in a suitable receiving flask. Connect the inlet tube to the source of steam and distil rapidly with the current steam. When the distillation is well under way, heat the Kjeldahl flask using a bunsen burner to reduce the volume of the contents of the flask as far as practicable without causing bumping or undue separation of insoluble matter. Continue the distillation until a small quantity of the distillate shows no cloudiness or opalescence when treated with a drop of silico-tungstic acid and a drop of dilute hydrochloric acid (1 : 4). Confirm the alkalinity of the residue in the Kjeldahl flask with phenolphthalein indicator solution. Reduce the volume of the distillate by concentrating it on a steam-bath (see Note) and make up the volume of the concentrated distillate to a convenient volume in a graduated flask with water at room temperature. Thoroughly mix the contents of the graduated flask and filter through a dry filter paper, if it is not clear. Clloect the filtrate in a convenient flask. Test a portion of the filtrate with methyl orange indicator solution to confirm its acidity.

Note: By heating on a steam-bath, the nicotine content of the distillate is not affected.

6·1·4·2 Pipette an aliquot (see Note) of the filtrate, containing about 0·1 g of nicotine into a beaker and add at the rate of 3 ml of dilute hydrochloric acid (1 : 4) for each 100 ml of the aliquot and one millilitre of silico-tungstic acid solution for every 0·01 g of nicotine supposed to be present in the aliquot. Stir the ontents of the beaker thoroughly and let stand overnight at room temperature. Before filtering, stir the contents of the beaker to see that the precipitate settles down quickly and is in a crystalline form. Filter the contents of the beaker through an ashless filter paper. Wash all the residue remaining in the beaker on to the filter using dilute hydrochloric acid (1 : 1 000) at room temperature. Wash the filter with dilute hydrochloric acid (1 : 1 000) until a few millilitres of the filtrate do not produce a precipitate or opalescence when tested with a few drops of the distillate containing nicotine. Transfer the filter paper containing the precipitate to a tared platinum crucible, dry carefully and ignite the filter paper untill all carbon is oxidised. Finally heat the platinum crucible over a Meker burner for not more than 10 min. Cool the crucible in a desiccater and weigh.

Note: If the nicotine content of the material is very low, an aliquot containing at least 0·01 g of nicotine should be used.

6·1·5 **Calculation**

Total alkaloid as nicotine (on dry basis), per cent by mass

$$= \frac{1\ 141\ m_0 V_0}{m_1 V_1\ (100 - M)}$$

where

m_0 = mass, in g, of the residue;

V_0 = total volume, in ml, of the concentrated distillate after making up at room temperature;

m_1 = mass, in g, of the material taken for steam distillation.

V_1 = Volume, in ml, of the aliquot of the filtrate taken for the precipitation; and

M = loss on heating, per cent by mass (see clause 4.4)

6·2 Spectrophotometric Method

6·2·1 **Principle** – The sample is submitted to steam distillation under strongly alkaline conditions, the total alkaloid content of the distillate measured spectrophotometricallay and calculated as percentage of nicotine.

6·2·2 **Apparatus**

6·2·2·1 Analytical balance–minimum accuracy ± 1·0 mg.

6·2·2·2 Oven – ventilated natural convection type.

6·2·2·3 Volumetric flasks – 100 and 250 ml capacity narrow-neck type with ground stopper.

6·2·2·4 Pipettes – 25 ml capacity.

6·2·2·5 Funnels of glass, about 55 mm diameter.

6·2·2·6 Distillation assembly – See clause 6·1·2·5

6·2·2·7 Spectrophotometer – wavelength range 230 to 290 nm.

6·2·2·8 Quartz cells – optical path length 1 cm.
Note: Absorbance of the cells must be equal before and after each measurement.

6·2·2·9 Filter paper, fast filtering grade.

6·2·3 **Reagents**

6·2·3·1 Sodium hydroxide solution – 8 N.

6·2·3·2 Sulphuric acid – 0·05 N and 2 N.

6·2·3·3 Sodium chloride.

6·2·4 Procedure – Weigh 0·2 to 2·0 g (depending on the expected alkaloid content) of the well mixed test sample to the nearest milligramme. Transfer the test sample to the distillation flask and wash down with 5 to 25 ml of distilled water. Add 20 to 40 g of sodium chloride and 5 ml of sodium hydroxide solution to the liquid in the distillation flask.

Note: The amount of sodium chloride shall be sufficient to leave some undissolved salt at the end of the distillation.

Steam distil the mixture into a 250 ml volumetric flask containing 15 ml of 2 N sulphuric acid. Collect 220 to 250 ml of the distillate and fill up to the mark with distilled water (volme = V_1). Remove cloudiness in the distillate, if any, by filtration.

Note: The rate of disstillation shall be at least 10 to 12ml of distillate/min The volume of the liquid in this distillation flask shall not be allowed to change appreciably during distillation- Auxiliary heating shall be employed, if necessary.

Pipette an aliquot (Volume V_2, normally 25 ml), of the filtered distillate into a second volumetric flask (capacity V_3 normally 100 ml) and diluted to the mark with 0·05 N sulphuric acid.

Prepare a blank solution by diluting 10 ml of 2 N sulphuric acid to 250 ml with distilled water, in a volumetric flask and diluting further by the procedure described, for the test solution.

Measure the absorbance of the test solution with the spectrophotometer using the blank solution as reference at the wavelengths of 236 nm 259 nm, and 282 nm.

If the absorbance at 259 nm exceeds 0·7 repeat the measurement using a smaller volume V_2 in the dilution stage.

If the absorbance at 236 nm is greater than twice the absorbance at 282 nm the determination should be repeated because the background is too high and the distillation has been ineffective.

6·2·5 Calculation

6·2·5·1 Calculate the corrected absorbance A (extinction) from the observed values of absorbance by mean of the following formula;

$$A = 1.059 \left[A_{259} - \frac{A_{236} + A_{282}}{2} \right]$$

Where,

A_{236}, A_{259} and A_{282} are the observed absorbance at 236, 259 and 282 nm respectively.

6·2·5·2 Total alkaloids as nicotine (on dry basis) per cent by mass =

$$\frac{100 \times A \times V_1 \times V_3}{a \times V_2 \times l \times m \frac{(100 - b)}{100}}$$

Note: Other frequently used formulae are;

(i) Nicotine content c of the diluted distillate, mg nicotine/ml solution

$$c = \frac{A}{a \times l}$$

(ii) Absorptivity:

$$a = \frac{A}{c \times l} \; ; \; {}^{a}259 = 34.3$$

(iii) Total amount of tobacco alkaloids, expressed as mg nicotine, in the test sample:

$$C = \frac{A \times V_1 \times V_3}{a \times V_2 \times l}$$

a = absorptivity (decadic extinction coefficient) of nicotine in 0·05 N sulphuric acid. (ie, 34·3 at the absorption maximum of 259 nm);

A = Corrected absorbance (extinction);

b = residual water content per cent by mass, of the test sample,

c = nicotine concentration of the diluted distillate, in g/1 000 ml;

C = total amount of tobacco alkaloids in mg.

l = optical path length in cm;

m = mass in g, of sample used for distillation

V_1 = volume, in ml, of distillate;

V_2 = aliquot, in ml, of distillate taken for further dilution;

V_3 = capacity, in ml, of dilution flask.

6·2·6 **Repeatability** – The difference between two determinations carried out simultaneously in rapid succession by the same analyst shall not exceed 0·05% of nicotine, expressed in per cent by mass of the test sample If not, further duplicate determinations should be made until this requirement is fulfilled.

7. DETERMINATION OF TOTAL NITROGEN

7·1 **Principle** – The sample is heated with sulphuric acid in the presence of a catalyst to oxidize the sample and convert the nitregenous matter into ammonium sulphate; excess of sodium hydroxide solution is added and the liberated ammonia is distilled into standard sulphuric acid solution, followed by titration of the latter with standard sodium hydroxide solution.

7·2 **Apparatus**

7·2·1 **Kjeldahl Flask** – 500 ml capacity.

7·2·2 **Distillation Assembly** – the assembly consists of a round-bottom flask of 1 000 ml capacity connected to the condenser by means of a glass tube having ground glass taper joints (see Fig. 1). The other end of the condenser is extended so as to dip into a known quantity of standard sulphuric acid contained in a conical flask of 500 ml capacity to which 3 to 4 drops of methyl red indicator solution have been added.

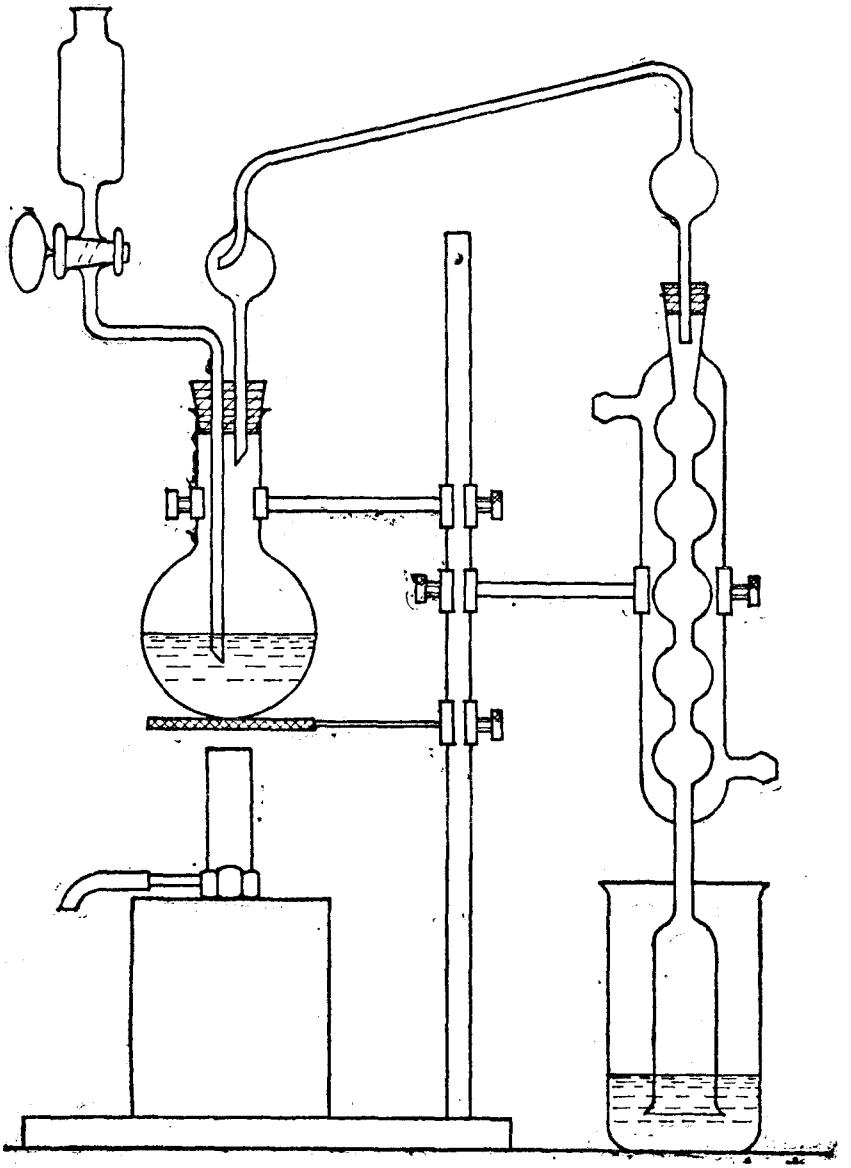

Fig. 1 — DISTILLATION ASSEMBLY

7·3 Reagents

7·3·1 Standard Sulphuric acid – 0·1 N.

7·3·2 Standard Sodium Hydroxide Solution – 0·1 N.

7·3·3 Sodium Hydroxide Solution – Dissolve 500 g of commercial sodium hydroxide free from nitrates and 40 g of sodium thiosulphate in 1 000 ml of water.

7·3·4 Concentrated Sulphuric acid – relative density 1·84.

7·3·5 Sodium Thiosulphate.

7·3·6 Potassium Sulphate.

7·3·7 Sodium Sulphate – anhydrous.

7·3·8 Mercuric Oxide.

7·3·9 Phenolphthalein Indicator.

7·3·10 Methyl Red Indicator – Dissolve 0·4 g of methyl red in 200ml of ethyl alcohol.

7·3·11 Salicylic acid.

7·4 Procedure

7·4·1 Place 0·7 to 3·5 g of sample (see clause 3·1), accurately weighed, in a digestion flask and add 30 ml of sulphuric acid containing one gramme of commercial salicylic acid. Shake until thoroughly mixed and allow to stand shaking frequently for at least 30 min. Add 5 g of sodium thiosulphate and heat the solution for 5 min. Cool, add 10 g of potassium sulphate or anhydrous sodium sulphate, and 0·7g of mercuric oxide. Heat very gently keeping the flask in an inclined position until foaming ceases. Increase the heat till acid boils briskly and digest for a time after the mixture becomes colourless or nearly so, until oxidation is complete (approximately 2 hours).

7·4·2 Distillation – After cooling, dilute with approximately 200 ml of water, transfer the contents to a round – bottom flask and add a few pieces of granulated zinc or pumice-stone to prevent bumping. Shake and add a few drops of

phenolphthalein and add 50 ml of sodium hydroxide solution to make the contents strongly alkaline. (The phenolphthalein loses colour in excess alkali). Pour the sodium hydroxide solution down the sides of flasks so that it does not mix at once with the acid solution. Connect the flask to the condenser by means of Kjeldahl connecting bulb taking care that the top of the condenser extends below the surface of the standard sulphuric acid in the receiver. Mix the contents by shaking and distill until ammonia has passed over into measured quantity of standard sulphuric acid (first 150 ml of the distillate contains generally all the ammonia) Titrate this with standard sodium hydroxide solution using methyl red indicator.

7·4·3 Carry out a blank determination using all reagents in the same quantities but without the material to be tested.

7·5 Calculation

Total nitrogen (on dry basis),

$$\text{per cent by mass} = \frac{140\,(v_0 - v_1)\,N}{m\,(100-M)}$$

Where

v_o = volume in ml, of standard sodium hydroxide solution used to neutralize the acid in blank determination.

v_1 = volume in ml, of the standard sodim hydroxide solu ion used to neutralize the excess acid in the test with the material.

N = normality of the standard sodium hydroxide solution.

m = mass, in g, of the material taken for the test, and

M = loss on heating per cent by mass (see clause 4·4).

8. DETERMINATION OF TOTAL ASH

8·1 Procedure

8·1·1 Accurately weigh about 10 g of the material (see clause 3 1) into a tared 90 mm diameter platinum, porcelain or silica dish Carefully dry the material on a bunsen flame and char it completely until all organic matter is destroyed. Ignite the charred material by placing the dish in a muffle furnace maintained at a temperature of $550 \pm 25^{\circ}C$ for 2 hours Cool the dish and weigh. Note the mass of the ash contained in the dish.

8·1·2 Preserve the ash for the determination of acid insoluble ash.

8·2 Calculation

Total ash content of the material (on dry basis), per cent by mass

$$= \frac{10\,000\, m_0}{m_1\,(100-M)}$$

Where

m_0 = mass, in g, of the ash,

m_1 = mass, in g. of the material taken for the test and

M = loss on heating, per cent by mass (see clause 4·4).

9. DETERMINATION OF ACID INSOLUBLE ASH

9·1 Reagents

9·1·1 Dilute Hydrochloric Acid – 1:1 (v/v).

9·1·2 Concentrated Nitric acid - relative density 1·42.

9·2 Procedure

9·2·1 Moisten the ash contained in the dish (see clause 8·1·2) with water. Cover the dish and carefully add 20 ml of dilute hydrochloric acid avoiding loss due to effervescence. Place the covered dish on a water-bath and digest for 20 to 30min. Remove and rinse the cover, add one millilitre of concentrated nitric acid to oxidize any ferrous salts and evaporate the contents to dryness. Heat for about 30 min on the water-bath to dehydrate silica. If necessary, heat for one hour in an oven at 110°C to complete the dehydration. Moisten the dry salt with 10 ml of dilute hydrochloric acid and 50 ml of water. Heat on the water-bath until all soluble salts are in solution. Filter through a filter paper (Whatman No. 44 or equivalent) and collect the filtrate in a 500 ml volumetric flask. Transfer the residue to the filter paper and wash several times with hot dilute hydrochloric acid.

9·2·2 Transfer the filter paper along with the residue to a platinum dish or silica crucible and ignite. Finish at bright red heat. Cool and weigh the material.

9·3 Calculation

Acid insoluble ash (on dry basis), per cent by mass

$$= \frac{10\,000\,(m_2 - m_0)}{m_1\,(100 - M)}$$

Where

m_0 = mass, in g, of empty dish,

m_1 = mass, in g, of the material taken for the test,

m_2 = mass, in g, of dish with acid insoluble ash, and

M = loss on heating, per cent by mass (see clause 4·4)

10. DETERMINATION OF TOTAL CHLORINE

10·1 Principle – Total chlorine is determined by potentiometric titration using silver nitrate solution.

10·2 Apparatus

10·2·1 pH Meter - Equipped with silver and glass electrodes.

10·2·2 Burette – 10 ml capacity, graduated in 0·2 to 0·05 ml units.

10 3 Reagents

10 3·1 Standard Silver Nitrate Solution – 0·1 N. Standardize against potassium chloride as in clause 10·4.

10·3·2 Dilute Nitric Acid – 1 : 9 (v/v).

10·4 Procedure – Weigh accurately about 2 g of tobacco (see clause 3·1) into a 250 ml electrolytic beaker. Add 100 ml of water, a small amount in the first instance to wet the tobacco thoroughly and then the remainder. Let it stand at least for 5 min at room temperature stirring intermittently. Pipette 5 ml of dilute nitric acid into the mixture and insert the clean electrodes. Start the magnetic stirrer and continue stirring throughout titration at a rate sufficient to produce vigorous agitation without spattering. Titrate with standard silver nitrate solution to the potential previously established as equivalent point. Determine equivalence point graphically by making several titrations on one or more tobacco samples Recheck occasionally and determine when either electrode is replaced. Record volume of titrant.

10·5 Calculation

Total chlorine (on dry basis), per cent by mass

$$= \frac{V \times N \times 354 \cdot 53}{m\,(100 - M)}$$

Where

V = volume, in ml, of silver nitrate solution required for the test,

N = normality of silver nitrate solution,

m = mass, in g, of the sample taken for the test. and

M = loss on heating per cent by mass (see clause 4·4).

11. DETERMINATION OF TOTAL AND REDUCING SUGARS (USING SOMOGYI'S PHOSPHATE COPPER REAGENT)*

11·1 Reagents –

11·1·1 Phosphate - Copper reagnet - 500 ml –
14 g of anhydrous disodium hydrogen phosphate (Na_2HPO_4) and 20 g of Rochelle salt are dissolved in 350 ml. of distilled water 50 ml of normal Sodium hydroxide solution and 40 ml of 10% Copper Sulphate solution are slowly added to it while stirring Fine ly 90 g of anhydrous sodium sulphate and 12 5 ml of normal potassium iodate (3·567 g of KIO_3/ 100 ml) solution are added. The entire solution is then made up to 500 ml and let stand for two days during which time i purities separate out. The clear top part of the solution is decanted and the remainder filtered through good grade of filter paper. If kept in a stoppered Pyrex bottle and protected from strong light, the solution will remain unchanged for months.

11·1 2 Potassium iodide solution - 2·5 per cent alkalinised with Sodium carbonate. By making a new solution each week and keeping it in a dark coloured bottle deterioration may be avoided.

11·1·3 Approximately 2 N sulphuric acid solution - made by diluting Concentrated sulphuric acid (56·8 ml / 1.)

11·1·4 Thiosulphate solution 0·005 N – This is prepared by diluting 50 ml of 0·1 N Thiosulphate solution to a litre, incorporating 2 ml of 10% Sodium hydroxide solution in it for protection against atmospheric carbon dioxide and for increasing the stability of the solution.

* Somogyi, Michael – A new reagent for dererminatiun of Sugars, Journal of Biochemistry Vol. 160, 1945, P 61 – 68.

11·2 Extraction – 2·5 g sample of tobacco powder is placed in a 250 ml· Pyrex beaker. 0·5 g of Calcium Carbonate and 100 ml of distilled water are added to it. The contents of the beaker are boiled gently for 15 min. on a hot plate. The beaker is cooled to room temperature and the contents are quantitatively transferred to a 250 ml volumetric flask and 5 ml of saturated neutral lead acetate solution is added and the contents of the flask are thoroughly mixed and allowed to a stand for 15 min. Volume is made upto the mark with distilled water and the contents are shaken well. 30 ml to 50 ml portion is decanted off. Sufficient anhydrous disodium phosphate (Na_2HPO_4) is added to the solution in the flask to precipitate all the lead. The contents are shaken well once again and filtered through dry Whatman No. 42 fluted filter paper. A few drops of the filterate are tested with disodium phosphate ($Na_2H\,PO_4$) to make sure that all lead is precipitated. Filtrate is refiltered if necessary using a fresh dry filter paper. The filtrate thus obtained is called "Clarified extract" and is stored in stoppered flask in a refrigerator and is later used for determination of total and reducing sugars.

11·3 Determination of reducing sugars – 10 ml of clarified extract is placed in 50 ml volumetric flask, diluted to volume and mixed. 5 ml of this solution is used for determination of reducing sugars using Somogyi's phosphate – copper reagent on the same day as per the iodometric procedure given in clause 11·5.

11·4 Determination of total sugars – 10 ml of clarified extract is placed in 50 ml volumetric flask. 5 ml of (1 + 4) HCl is added. The contents are mixed well and allowed to stand for 24 hours at room temparature to invert.

The solution is neutralised with normal NaOH solution using a piece of red litmus paper as indicator and keeping the flask in ice-cold water bath while sodium hydroxide solution is added slowly. Solution is made upto volume and mixed. 5 ml of this solution is used for determination of total sugars using Somogyi's phosphate-copper reagent on the same day as per the iodometric procedure given in clause 11·5.

11·5 Iodometric procedure – 5 ml of Phosphate-copper reagent and 5 ml of sugar solution are mixed in a 25 mm × 200 mm Pyrex test-tube, covered with cotton plug and heated by immersion in a vigorously boiling water bath for exactly 15 min. They are firmly held in metal racks during the heating to avoid undue agitation. At the end of heating period the tubes are removed to a container with running tap water and cooled for 3 min. to about 30°C. After cooling, 2 ml of 2·5% Potassium iodide solution is added by stirring or agitation. Following this, about 1·5 ml of sulphuric acid solution is rapidly dropped, rather than permitted to flow into the test tube with simultaneous agitation, so that the entire contents of the tube are mixed and acidified at once. A graduated pipette with a cracked - off tip serves the purpose. The cotton plug is replaced and the solution is allowed to stand for 5 min. and then titrated with thiosulphate solution using starch solution (1 ml of 1%) as indicator at the end stage.

A pair of blanks (5 ml of water with 5 ml of phosphate copper reagent) is run with each set of ten determinations.

11·6 Calculation

Per cent reducing sugars in tobacco (as glucose)
or per cent total sugars in tobacco (as glucose)

$$= \frac{338 \times (V_0 - V_1)}{m \times (100 - M)}$$

where

V_0 = Titre value, in ml, for blank,

V_1 = Titre value, in ml, for tobacco sample,

m = mass in g, of tobacco and,

M = loss on heating, per cent by mass (see clause 4·4)

The above calculation is based on the assumption that 1·0 mg of pure glucose solution gives a $(V_0 - V_1)$ value of 7·40 ml of 0·005 N thiosulphate solution.

Note:

1. With these reagents, it is possible to determine reducing sugar equivalent to 0·01 to 3·0 mg of glucose in 5 ml of sugar solution.

2. The reagent is quite suitable when the $(V_0 - V_1)$ value falls between 15 to 20 ml of 0·005 N thiosulphate solution.

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