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SRI LANKA STANDARD 394 : 1976

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METHODS FOR THE ANALYSIS OF WATER-SOLUBLE COAL-TAR DYES PERMITTED FOR USE IN FOODS

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BUREAU OF CEYLON STANDARDS

METHODS FOR THE ANALYSIS OF WATER-SOLUBLE COAL-TAR DYES PERMITTED FOR USE IN FOODS

S. L. S. 394 : 1976

Gr. 10

BUREAU OF CEYLON STANDARDS
53, Dharmapala Mawatha,
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.

BUREAU OF CEYLON STANDARDS
53, DHARMAPALA MAWATHA,
COLOMBO 3.

Telephone : 26055
26054
26051

Telegrams : " PRAMIKA "

SRI LANKA STANDARD METHODS FOR THE ANALYSIS OF WATER-SOLUBLE COAL-TAR DYES PERMITTED FOR USE IN FOODS

FOREWORD

This Sri Lanka Standard specification has been prepared by the Drafting Committee of the Bureau on Food Additives. 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 1976-08-04.

This standard prescribes the general methods of analysis that are applicable to the water-soluble coal-tar dyes. Reference is made to these methods in the Sri Lanka Standards covering the individual colouring matters.

Values given in this standard are in SI units.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with CS 102 : 1971*. The number of significant figures to be retained in the rounded off value shall be the same as that of the specified value in this standard.

The assistance obtained from the publication of the British Standards Institution is gratefully acknowledged.

1. SCOPE

This standard specifies methods for the analysis of water-soluble coal-tar dyes permitted for use in foods.

In some of these methods, different reagents or factors are used according to the particular dye being analysed. Details of such variations are given in the Sri Lanka standard specifications for the dyes concerned.

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*CS 102 : 1971 - Presentation of Numerical Values.

2. METHODS

2.1 Method for the Determination of Matter Volatile at 135°C Weigh to the nearest mg 2 — 3 g of the dye sample in a tared weighing bottle fitted with a ground lid. A weighing bottle of squat form about 50 mm in diameter and 30 mm high is suitable. Heat at 135 ± 5°C until a constant mass is obtained. Express the loss in mass as a percentage of the mass of sample taken.

2.2 Method for the Determination of Matter Insoluble in Water Weigh to the nearest mg 4·5 — 5·5 g of the dye sample into a 250 ml beaker. Add about 200 ml of hot distilled water (80 — 90°C), stir to dissolve the dye and allow the solution to cool to room temperature. Filter the solution through a tared Grade 4 sintered glass filter and wash with cold water until the washings are colourless. Dry the filter and residue at 135°C until a constant mass is obtained. Express the mass of the residue as a percentage of the mass of sample taken.

2.3 Method of Extraction with Di-Isopropyl Ether

2.3.1 Apparatus — Upward-displacement type liquid/liquid extractor with sinter distributor, working capacity 200 ml. A piece of bright copper wire is suspended through the condenser and a small coil of copper wire (0·5 g) is placed in the distillation flask.

2.3.2 Reagents — The reagents shall be of a recognized analytical reagent quality. Water used shall be distilled water.

(*a*) **Di-isopropyl Ether** — Immediately before use the freshly distilled ether should be passed through a 300 mm column of chromatography-grade aluminium oxide in order to remove peroxides and inhibitors. Test to ensure the absence of peroxides, as follows:

Prepare a colourless solution of ferrous thiocyanate by mixing equal volumes of 0·1N solutions of ferrous sulphate and ammonium thiocyanate and carefully discharging any red coloration, due to ferric ions, with titanous chloride. To 50 ml of this solution add 10 ml of the ether and shake the mixture vigorously for 2 — 3 min. No red colour should develop.

(*b*) Sodium hydroxide, 2·5N solution.

(*c*) Sodium hydroxide, 0·1N solution.

(*d*) Hydrochloric acid, 3N solution.

(*e*) Hydrochloric acid, 0·1N solution.

2.3.3 Procedure

(*a*) Neutral ether extract — Weigh to the nearest mg about 5 g of the dye sample, dissolve in 150 ml of water and transfer the solution to the extractor, diluting to approximately 200 ml with water in the process. In a suitable apparatus the level of the liquid will be about 650 mm below the vapour arm. Add 200 ml of di-isopropyl ether into the distillation flask and extract for 2 hours with a reflux rate of about 15 ml/min. Reserve the dye solution. Transfer the ether extract to a separating funnel and wash with two 25 ml portions of water. Distil the ether in portions from a tared 150 ml flasks containing a clean copper coil, reducing the volume to about 5 ml.

(*b*) Alkaline ether extract — To the dye solution reserved from (*a*) and 2 ml of 2·5N sodium hydroxide solution and mix by raising and lowering the sinter distributor. Extract with a further quantity of the ether as in (*a*) and reserve the dye solution. Wash the ether extract with two 25 ml portions of 0·1N sodium hydroxide solution and then with water. Transfer in portions to the flask containing the evaporated neutral extract and distil as before.

(*c*) Acid ether extract — To the dye solution reserved from (*b*) add 5 ml of 3N hydrochloric acid, mix and extract with a further quantity of the ether as in (*a*). Wash the ether extract with two 25 ml portions of 0·1N hydrochloric acid and then with water. Transfer in portions to the flask containing the evaporated neutral and alkaline extracts and carefully remove all the ether.

Complete the drying in an oven at 85°C for 20 min. then allow the flask to cool in a desiccator for 30 min. and weigh. Repeat the drying and cooling until constant mass is obtained.

Note : It is recommended that the tared flask be counterpoised by a similar flask and that both be subject to the drying and cooling operations.

The increase in mass of the tared flask, expressed as a percentage of the mass of sample taken, is the 'ether extract'.

2.4 Method for the Determination of Subsidiary Dyes

2.4.1 Principle — The subsidiary dyes are separated from the main dye by ascending paper chromatography and are extracted separately from the paper. The optical densities of the extracts are measured at their wavelengths of maximum absorption in the visible spectrum and are used to calculate the contents of subsidiary dyes as a percentage by mass of the sample.

2.4.2 Apparatus

(*a*) Chromotography tank and ancillary equipment. Suitable apparatus is shown in Figs. 1 and 2 and comprises :

(i) A glass tank (A) and cover (B)

(ii) A supporting frame (C) for the chromatography paper sheets

(iii) A tray (D) for developing solvent

(iv) A secondary frame (E) supporting 'drapes' of filter paper

(v) Sheets of chromatography grade paper*, not less than 200 mm × 200 mm.

(*b*) A micro-syringe, capable of delivering 0·1 ml with a tolerance of ± 0·002 ml.

(*c*) A Spectrophotometer.

* Whatman No. 1 Chromatography grade paper is suitable.

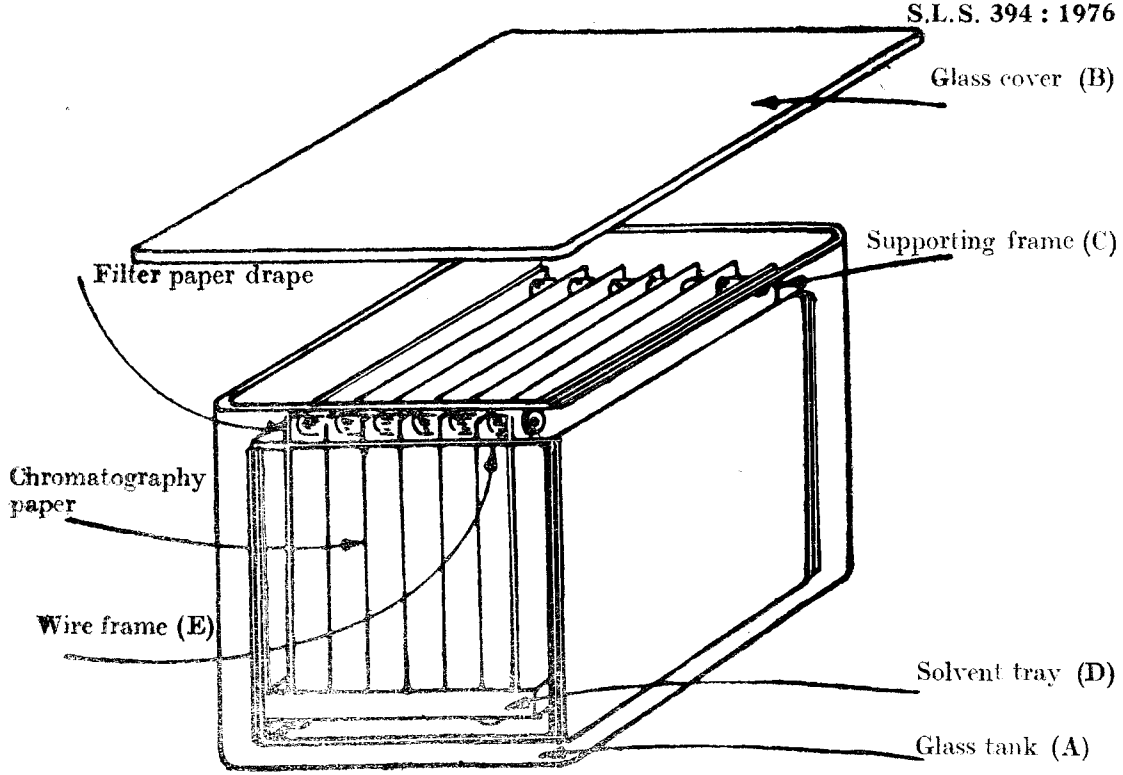

Fig. 1—Assembly of Chromatography Apparatus

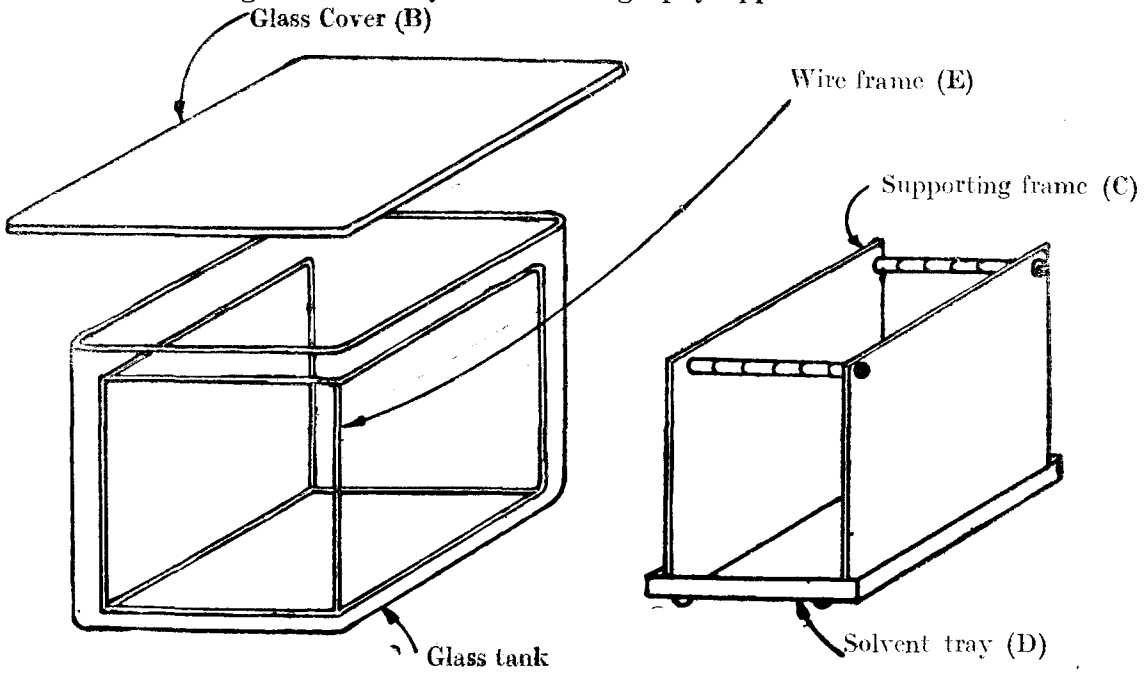

Fig. 2—Components of Chromatography Apparatus

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2.4.3 Reagents — All reagents shall be of a recognized analytical reagent quality and freshly prepared. Water used shall be distilled water.

(*a*) Atmosphere-saturating solvent
(*b*) Developing solvent
(*c*) Extracting solvent

} specified in the appropriate Sri Lanka Standard for each dye.

(*d*) Sodium hydrogen carbonate, 0·05N solution.

2.4.4 Procedure

(*a*) Not less than 2 h before carrying out the determinations, arrange the filter-paper drapes in the glass tank and pour over the drapes and into the bottom of the tank sufficient of the atmosphere-saturating solvent to cover the bottom of the tank to a depth of approximately 10 mm. Place the solvent tray (D) in position and fit the cover to the tank.

(*b*) Mark out a sheet of chromatography paper as shown in Fig. 3. Apply 0·10 ml of a 1·0 per cent aqueous solution of the dye as uniformly as possible within the confines of the 180 mm × 7 mm rectangle, holding the nozzle of the micro-syringe steadily in contact with the paper. Allow the paper to dry at room temperature for 1 — 2 hours, or at 50°C for 5 min followed by 15 min at room temperature. Mount the sheet together with a plain sheet to act as a blank, in frame C. Pour sufficient of the developing solvent into the tray D to bring the surface of the solvent about 10 mm below the base line of the chromatogram sheet. The volume necessary will depend on the dimensions of the apparatus and should be predetermined. Put frame C into position and replace the cover. Allow the solvent front to ascend the specified distance above the base line, then remove frame C and transfer it to a drying cabinet at 50 — 60°C for 10 — 15 min. Remove the sheets from frame C.

Note : If required, several chromatograms may be developed simultaneously.

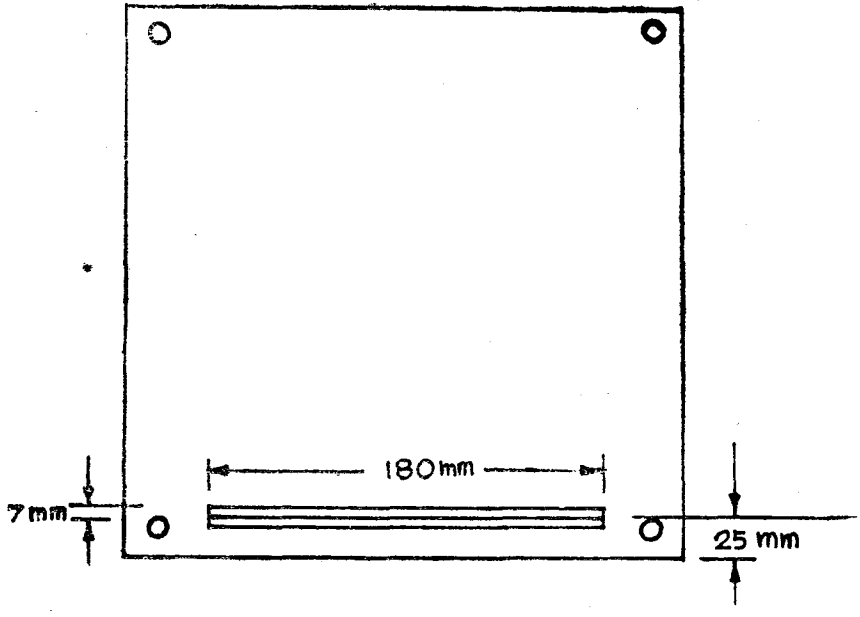

Fig. 3—Method of marking out Chromatography Paper

(*c*) Cut each subsidiary band from the sheet as a strip, and cut an equivalent strip from the corresponding position of the plain sheet. Place each strip, subdivided into a suitable number of approximately equal portions, in a separate test tube. Add 5·0 ml of extracting solvent to each test tube, swirl for 2 — 3 min, add 15·0 ml of the sodium hydrogen carbonate solution and shake the tube to ensure mixing. Filter the coloured extracts and blanks through a 9 mm filter paper of open texture and determine the optical densities of the coloured extracts at their wavelengths of maximum absorption, using 40 mm closed cells, against a filtered mixture of 5·0 ml of extracting solvent and 15·0 ml of the sodium hydrogen carbonate solution. Measure the optical densities of the extracts of the blank strips at the wavelengths at which those of the corresponding coloured extracts were measured.

The content of the subsidiary dye, expressed as a percentage (S) of the sample, is given by

$$S = F\,(D_1 + D_2 + \ldots.) - (b_1 + b_2 + \ldots..)$$

where F is a conversion factor specified in the appropriate Sri Lanka Standard for each dye,

D_1, D_2 etc. are the optical densities of the subsidiary dye extracts,

b_1, b_2 etc. are the optical densities of the extracts of the corresponding blanks.

The conversion factor F in the above expression is derived from the extinction coefficient of the main colour, not that of the subsidiaries, and from the other constants of the determination.

2.5 Method for the Determination of Chloride

2.5.1 Apparatus — Potentiometric titration apparatus, with silver indicator electrode, calomel reference electrode and saturated potassium sulphate bridge.

2.5.2 Reagents —The reagents shall be of a recognized analytical reagent quality. Water used shall be distilled water.

(*a*) Silver nitrate, 0·1N solution, accurately standardized.

(*b*) Nitric acid, 1·5N solution.

2.5.3 Procedure — Accurately weigh 0·5 — 1·0 g of the dye sample, dissolve in 100 ml of water, and acidify with 5 ml of the nitric acid solution. Place the silver electrode in the dye solution and connect the calomel electrode to the solution by means of the saturated potassium sulphate bridge.

Determine the chloride content of the solution by titration against the 0·1N silver nitrate solution, and calculate the result as sodium chloride.

1 ml of 0·1N $AgNO_3$ solution $\equiv$ 0·00585 g of NaCl.

Express the result as a percentage of the mass of sample taken.

2.6 Method for the Determination of Sulphate

2.6.1 Reagents — The reagents shall be of a recognized analytical reagent quality. Water used shall be distilled water.

(*a*) Sodium chloride, sulphate-free.

(*b*) Sodium chloride, saturated solution in water.

(*c*) Hydrochloric acid, relative density 1·16 — 1·18.

(*d*) Barium chloride, 0·25N solution.

2.6.2 Procedure — Weigh to the nearest mg about 5 g of the dye sample, transfer it to a 250 ml conical flask and dissolve in about 100 ml of water by heating on a water bath. Add 35 g of the sodium chloride, stopper the flask and swirl at frequent intervals during one hour. Cool, transfer with saturated sodium chloride solution to a 250 ml one-mark graduated flask, and dilute to the mark at 20°C. Shake the flask, and filter the solution through a dry filter paper. Pipette 100 ml of the filtrate into a 500 ml beaker, dilute to 300 ml with water and acidify with concentrated hydrochloric acid, adding 1 ml in excess. Heat the solution to boiling, and add an excess of barium chloride solution drop by drop, with stirring. Allow the mixture to stand on a hot plate for 4 hours or leave it overnight at room temperature and then bring it to about 80°C, and allow the precipitate to settle. Filter off the precipitated barium sulphate, wash with hot water and ignite at a dull red heat in a tared crucible until a constant mass is obtained.

Carry out a blank determination, apply any necessary correction to the mass of barium sulphate found in the test and calculate the result as sodium sulphate.

Mass of Na_2SO_4 in sample = 2·5 × corrected mass of $BaSO_4$ × 0·6086

Express the result as a percentage of the mass of sample taken.

2.7 Method for the Determination of Dye Content

2.7.1 Apparatus

(*a*) Carbon dioxide generator, eg., a Kipp apparatus.

(*b*) Titration apparatus, as shown in Fig. 4*, comprising an aspirator and a burette fitted with a double-oblique tap and side arm. The aspirator is closed at the top by a rubber stopper with two holes, through one of which passes a glass tube connected to the carbon dioxide generator. Through the second hole passes a glass tube connected to the top of the burette. The side arm of the burette is connected to the bottom of the aspirator.

(*c*) Carbon dioxide flasks or titanous reduction flasks (see Fig. 5*) of 500 ml capacity.

2.7.2 Reagents — All reagents shall be of a recognized analytical reagent quality. Water used shall be distilled water.

(*a*) Ammonium ferrous sulphate, $(NH_4)_2SO_4.FeSO_4.6H_2O$.

(*b*) Potassium dichromate, 0·1N solution, accurately standardized.

(*c*) Ammonium thiocyanate, 20 per cent (m/v) solution in water.

* The dimensions in Figs. 4 and 5 are included for guidance only and are not mandatory.

(*d*) **Sulphuric acid, 10N solution.**

(*e*) **Buffer salt,** Trisodium citrate dihydrate or sodium hydrogen tartrate, as specified in the appropriate Sri Lanka Standard for each dye.

(*f*) Titanous chloride, 0·2N solution. Measure into a large round flask a volume of 15 per cent titanous chloride solution containing 31 — 33 g of titanous chloride for each litre of solution required. Add 100 ml of concentrated hydrochloric acid (relative density 1·16 — 1·18) for each litre of solution required. Heat the mixture to boiling, and boil it for 1 — 2 min and then pour it into cold water. Make up the required volume in the aspirator, mix well and preserve the solution in an atmosphere of carbon dioxide. As a further precaution against oxidation, cover the solution with a layer of medicinal paraffin (about 6 mm deep).

2.7.3 Procedure

(*a*) Standardization of titanous chloride solution — Weigh 3g of ammonium ferrous sulphate into a carbon dioxide flask or titanous reduction flask, and pass a stream of carbon dioxide through the flask continuously until the end of the determination. Add 50 ml of water and 25 ml of the sulphuric acid solution, then 50·0 ml of 0·1N potassium dichromate solution. Titrate with the titanous chloride solution until the calculated end point is nearly reached. Add 5 ml of the ammonium thiocyanate solution and continue the titration until the red colour is discharged and the solution remains green. Carry out a blank determination on 3g of ammonium ferrous sulphate, using the same quantities of water, acid and ammonium thiocyanate solution and passing a continuous current of carbon dioxide through the flask as before.

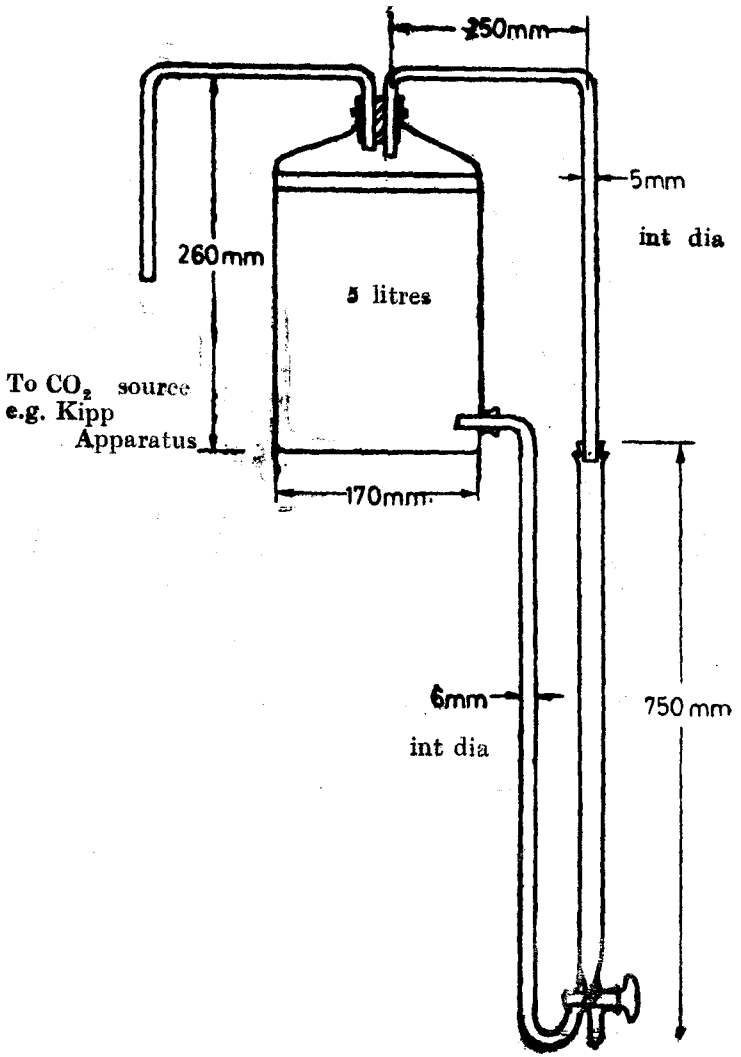

Fig. 4—Apparatus for storing titanous chloride solution.

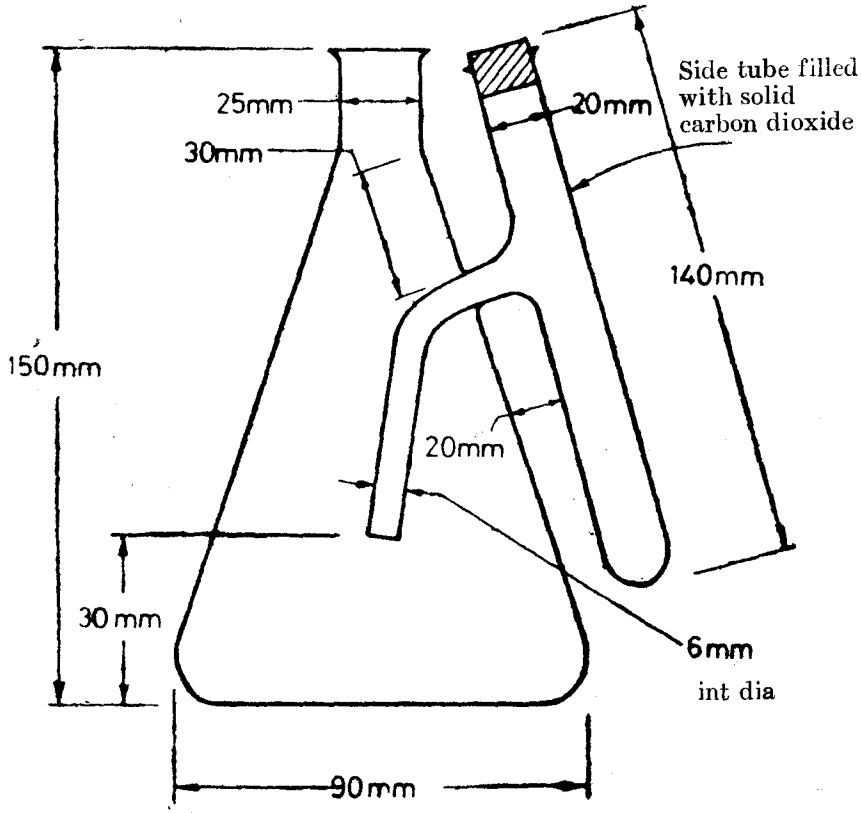

Fig. 5—Titanous reduction flask.

F = Factor for 0·2N titanous chloride solution.

$$= \frac{25}{\text{ml (corrected) of titanous chloride solution required}}$$

(*b*) Determination of dye content of sample — Accurately weigh the quantity of the dye sample specified in the appropriate Sri Lanka Standard into a carbon dioxide flask or titanous reduction flask, and add 10 g of trisodium citrate dihydrate or 15 g of sodium hydrogen

tartrate, as similarly specified, and 150 ml of water. Pass a stream of carbon dioxide through the flask, heat the solution to boiling and titrate with the stan-

Let A = ml of 0·2 N $TiCl_3$ solution required (corrected),

D = mass of dye equivalent to 1·00 ml of 0·2N $TiCl_3$ solution (specified in the appropriate Sri Lanka Standard for each dye)

$$\text{Then percentage of dye in sample} = \frac{A \times D \times 100 \times F}{\text{mass of sample}}$$

2.8 Method for the Determination of Copper, Arsenic and Lead

2.8.1 Principle — The method described below enables the elements copper, arsenic and lead to be determined in the solution obtained from wet oxidation of a single 2·5 g sample. The metals are separated entirely by solvent extraction methods and then determined photometrically. The method has been devised primarily for organic compounds with a very low specification limit for metals. It only applies in general when the amounts of other metals present do not exceed 500 mg/kg.

2.8.2 Apparatus

(*a*) Kjeldahl flasks, of silica or borosilicate glass (nominal capacity 100 ml) fitted with an extension to the neck by means of a B24 ground joint as shown in Fig. 6. The extension serves to condense the fumes and carries a tapfunnel through which the reagents are introduced.

(*b*) 'Cold finger' condensers, consisting of test tubes 70 — 75 mm long × 13 mm diameter with flanged

mouths to fit loosely 50 ml conical flasks, reaching to within 10—15 mm of the bottom of the flask (see Fig. 7*).

(*c*) Separating funnels. Graduated cylindrical funnels of 25 ml, 50 ml and 100 ml capacity, with the stems cut short to within 6 — 13 mm of the stopcock barrel.

(*d*) Spectrophotometer or photoelectric absorptiometer.

2.8.3 Reagents

(*a*) Nitric acid, relative density 1·42 redistilled from an all-glass apparatus. This acid is used at full strength or diluted with water as required.

(*b*) Nitric acid, dilute. Dilute 30 ml of the concentrated acid to 100 ml with water.

(*c*) Perchloric acid, 60 per cent (m/m) solution, metal-free. If the material gives too high a blank, particularly in lead, redistil from a perfectly clean all-glass apparatus under reduced pressure (about 2700 Pa).

Note : It is important that no grease should be used at the joints.

(*d*) Sulphuric acid, 96 — 98 per cent H_2SO_4, pure, metal-free. Redistil if necessary.

(*e*) Sulphuric acid, dilute. Mix equal volumes of the concentrated acid with water, and cool.

(*f*) Sulphuric acid, N solution, metal-free.

(*g*) Hydrochloric acid, relative density 1·16 — 1·18, metal-free.

*The dimensions of the flask in Fig. 7 are not mandatory.

(*h*) Hydrochloric acid, 5N solution, metal-free.

(*i*) Hydrochloric acid, 0·1N solution, metal-free.

(*j*) Ammonia solution, 10N.

(*k*) Dithizone 0·008 per cent (m/v) solution in chloroform. Prepare from purified solid diphenylthiocarbazone (dithizone) by dissolving the required weighed amount in the appropriate volume of redistilled chloroform. If high quality diphenylthiocarbazone is not available, purify the commercial product as follows :

Dissolve 1 g of the commercial product in 75 ml of redistilled chloroform, filter, and shake the filtered solution, contained in a 250 ml separating funnel, with four successive 100 ml portions of 10 per cent (m/v) ammonia solution. Combine the orange-coloured aqueous solutions, filter, and precipitate the dithizone from the filtrate by rendering it slightly acid with hydrochloric acid (1 : 1 by volume) or sulphuric acid (1 : 3 by volume). After settling, collect the precipitate on a clean sintered glass crucible, using suction, and wash with distilled water until free from acid. Dry the well drained precipitate over concentrated sulphuric acid in vacuo for 3 or 4 days, protecting from light. The purified solid, so prepared, when stored in the dark is stable for at least six months.

(*l*) Diethylammonium diethyldithiocarbamate, 1 per cent solution (m/v) in chloroform. Dissolve 1 g of the pure crystalline reagent in 100 ml of redistilled chloroform. Preserve in an amber-coloured bottle and discard after one week. The solution is hereafter referred to as ' carbamate reagent '.

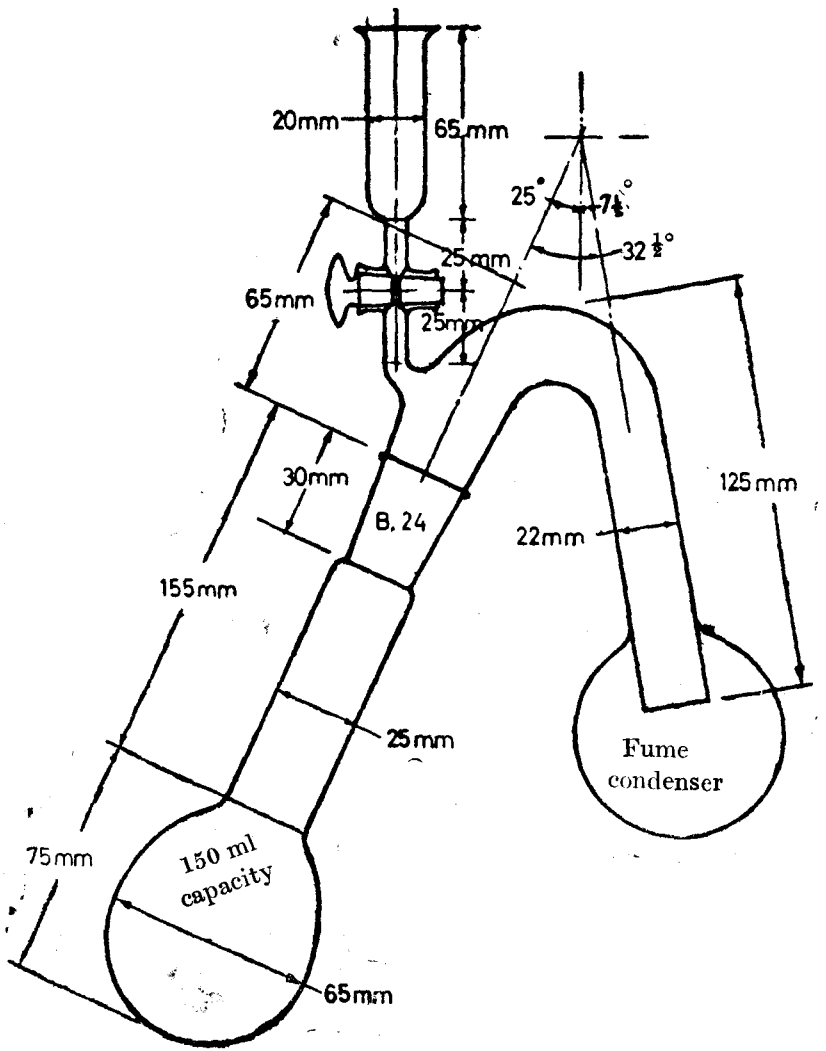

Fig. 6—Modified Kjeldahl flask (open type)

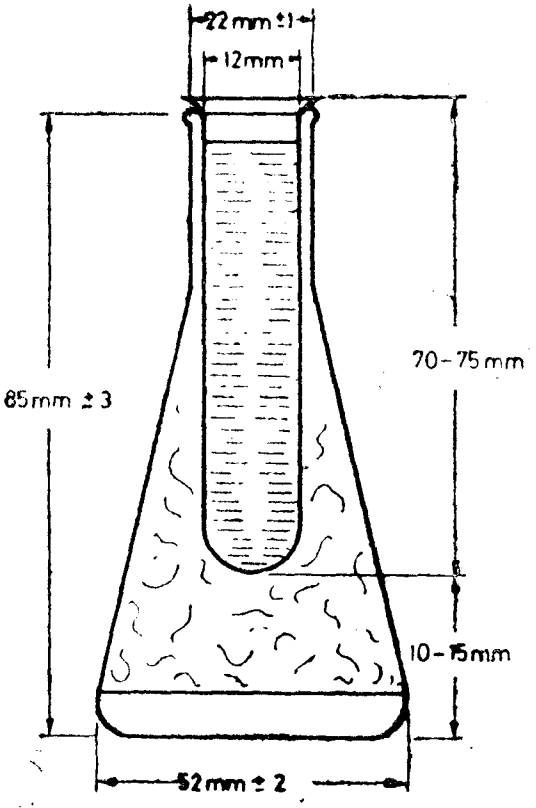

Fig. 7—"Cold-Finger" Condenser.

(m) Sodium citrate, approx. 25 per cent (m/v) solution. Dissolve 150 g of trisodium citrate dihydrate in water, and dilute to 500 ml. Transfer to a 1 litre separating funnel, add 0·5 ml of concentrated ammonia solution (relative density 0·88 — 0·89) and shake thoroughly with 50 ml of a 0·02 per cent (m/v) solution of dithizone in chloroform. Separate, and repeat the extraction with 50 ml portions of diluted dithizone solution (10 ml of the 0·02 per cent solution and 40 ml chloroform) until the last extract remains green. Finally, add 5 ml of 20 per cent (m/v) citric acid solution, and extract with 50 ml portions of chloroform until the final extract is colourless. Store the reagent in a polythene bottle.

(*n*) Potassium cyanide, 10 per cent (m/v) solution. Dissolve 50 g of potassium cyanide in water and dilute to 100 ml. Repeatedly extract this solution with the 0·02 per cent dithizone solution used in (m) until the last portion retains its original green colour. Then extract the excess dithizone from the aqueous phase with 10 ml portions of chloroform. Use as small an excess of dithizone as possible, since a large excess is difficult to extract. Dilute the extracted cyanide solution to 500 ml with water, and keep it in a polythene bottle.

Warning.—Do not measure solutions containing cyanide by means of a pipette filled by suction with the mouth ; use a burette or other dispensing device.

(*o*) Ammoniacal cyanide solution. Dilute 1 ml of the potassium cyanide solution and 2 ml of 10N ammonia solution to 100 ml with water.

(*p*) Sodium iodide, 20 per cent (m/v) solution. Dissolve 20 g of pure sodium iodide in 100 ml of water, add 0·2 ml of ammonia solution (relative density 0·88 — 0·89) and extract with 10 ml of carbamate reagent, shaking for 30 s. Reject the chloroform layer and shake the aqueous layer with 10 ml of chloroform, rejecting the chloroform wash.

(*q*) Ammonium molybdate solution. Dissolve 17·5 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 250 ml of water and mix with exactly 250 ml of 10N sulphuric acid solution (accurately standardized). Filter into a one-mark graduated flask, wash the filter with water, add exactly 250 ml of 4N perchloric acid solution (accurately standardized) and dilute to 1 l with water. Store the reagent in a polythene bottle.

(*r*) Sodium metabisulphite. 5 per cent (m/v) solution in water. Prepare fresh once a week, and filter.

(*s*) Hydrazine sulphate, 0·03 per cent (m/v) solution in water.

(*t*) Standard arsenic solution—Dissolve 1·320 g of arsenous oxide, As_2O_3 by warming at a temperature not exceeding 60°C with 14 ml of 5N sodium hydroxide solution in a 100 ml beaker. Cool, add 0·2 ml of phenolphthalein indicator and neutralize with 6N sulphuric acid. Transfer the solution to a 1 l one-mark graduated flask containing 10 g of sodium hydrogen carbonate dissolved in water, washing out the beaker with water. Dilute to the mark with water at 20°C and mix. Dilute 10 ml of this solution to 1 l at 20°C with water, in a one-mark graduated flask as required. 1 ml ≡ 0·00001 g As (10 µg As).

Warning—Do not measure solutions containing arsenic by means of a pipette filled by suction with the mouth; use a burette or other dispensing device.

(*u*) Standard copper solution—Dissolve 0·3928 g of pure copper sulphate, $CuSO_4.5H_2O$ in water, dilute to 1 l at 20°C with water in a one-mark graduated flask. Dilute 10·0 ml of this solution to 100 ml at 20°C with water in a one-mark graduated flask as required. 1 ml ≡ 0·00001 g Cu (10 µg Cu).

(*v*) Standard lead solution—Dissolve 1·60 g of lead nitrate, $Pb(NO_3)_2$, in nitric acid (10 ml of concentrated nitric acid diluted with 20 ml water, boiled to remove nitrous fumes, and cooled) and dilute to 1 l with water in a one-mark graduated flask. Dilute 5·0 ml of this solution to 500 ml at 20°C with water in a one-mark graduated flask as required. 1 ml ≡ 0·00001 g Pb (10 µg Pb).

(*w*) Sodium sulphate, anhydrous, granular.

(*x*) Chloroform, redistilled—Use chloroform of the highest possible purity.
(It should be noted that B.P. quality chloroform often contains zinc, so that redistillation is essential.)

(*y*) Distilled water, metal-free—Redistil from an all-glass apparatus if necessary. Test by adding 1 drop of 10N ammonia solution to 100 ml of water, followed by 5 ml of chloroform and one or two drops of dithizone solution. Shake for 30s and allow the layers to separate. The chloroform (lower) layer should not be

more than a faint pink in colour, indicating traces of lead, zinc or both. Add 1 ml of 10 per cent (m/v) potassium cyanide solution, shake again and allow to separate. The chloroform layer should be virtually colourless, indicating the absence of lead.

2.8.4 Preparation of Test Solutions

(*a*) General precautions—Owing to the minute amounts of metal involved, take special care to reduce the reagent blanks to as low a value as possible, and avoid contamination during the test. Thoroughly clean all apparatus with a mixture of hot dilute acids (1 part hydrochloric acid, 1 part of concentrated nitric acid and 3 parts of water), followed by thorough washing with water, immediately before use. Follow exactly the method of preparation and purification described. Carry out all extractions with vigorous shaking (about 100 to 200 shakes per minute) and for the full time specified. (Instead of shaking by hand, a mechanical flask shaker may be used).

Warning— Do not measure solutions containing arsenic or cyanide with a pipette filled by suction with the mouth ; use a burette or other dispensing device.

(*b*) Wet decomposition— Accurately weigh about 5 g of the dye sample into a 100 — 150 ml Kjeldhal flask and add 10 ml of the dilute nitric acid. As soon as any initial reaction subsides, heat gently until further vigorous reactions cease and then cool. Add gradually 8 ml of concentrated sulphuric acid at such a rate as not to cause excessive frothing on heating (5 — 10 min. are usually required) and then heat until the liquid darkens appreciably in colour, i.e. begins to char.

Add concentrated nitric acid slowly in small portions, heating between additions until darkening again takes place. Do not heat so strongly that charring is excessive, or loss of arsenic may occur ; a small but not excessive amount of free nitric acid should be present throughout. Continue this treatment until the solution is only pale yellow in colour and fails to darken in colour on prolonged heating. Now run in 0·5 ml of the perchloric acid solution and a little concentrated

nitric acid and heat for about 15 min, then add a further 0·5 ml of the perchloric acid solution and heat for a few minutes longer. Note the total amount of concentrated nitric acid used. Allow to cool somewhat and dilute with 10 ml of water. The solution should be quite colourless (if much iron is present it may be faintly yellow). Boil down gently, taking care to avoid bumping, until white fumes appear. Allow to cool, add a further 5 ml of water and again boil down gently to fuming. Finally, cool and dilute with 15 ml of water.

Prepare a reagent blank by adding 8·0 ml of concentrated sulphuric acid, the same amount of concentrated nitric acid as was used in the test (usually 20 — 25 ml) and 1 ml of the perchloric acid solution to a Kjeldahl flask and boiling down to fuming. Allow to fume for about 20 min, then evaporate twice with 5 ml portions of water, and finally dilute with 15 ml of water.

Cool the solutions and transfer to 50 ml one-mark graduated flasks, washing out the Kjeldahl flasks with 2 ml portions of water. Add 10·0 ml of 5N hydrochloric acid solution to each Kjeldahl flask, heat to incipient boiling, swirl vigorously to wash the sides of the flasks, cool and drain the acid into the graduated flasks. Finally, wash out the Kjeldahl flasks with portions of water, add the washings to the graduated flasks and dilute to the mark with water. If, after the wet decomposition, the test solution contains an insoluble deposit or suspended matter, however small in amount, filter it through a suitable 70 mm filter paper into the 50 ml graduated flask, the subsequent washings, including particularly the hot hydrochloric acid wash, being also passed in turn through the filter. If much residue is present retain it as far as possible in the Kjeldahl flask, and boil gently for about 1 min with the hydrochloric acid before the acid is drained through the filter. If much calcium is present, calcium sulphate will crystallize out from the contents of the 50 ml graduated flask on cooling. In this case, cool the contents of the flask to below 10°C in ice and water, re-filter through a 70 mm filter paper into a second 50 ml graduated flask, and wash the original

flask with three 2 ml portions of ice-cold water, which in turn, are passed through the filter. Dilute to 50 ml at 20°C with water. Call this Solution A.

(*c*) Extraction of copper and arsenic. Measure 25·0 ml of Solution A into a 100 ml conical flask, add 10 ml of concentrated hydrochloric acid and 2·0 ml of the sodium iodide solution and warm to 35 — 40°C. Add 0·5 ml of the sodium metabisulphite solution, cool to 20 — 25°C, and transfer the solution to a 50 ml graduated cylindrical separating funnel. Wash out the conical flask with 1 ml portions of water. It is necessary that the volume of the solution in the separating funnel shall not exceed 40 ml in order that the hydrochloric acid concentration shall be not less than 3N.

Introduce 5·0 ml of the carbamate reagent and shake the solution vigorously for 40 s, then carefully release the pressure. Allow the mixture to separate, and run the lower layer into a 25 ml graduated separating funnel, taking care not to allow any of the aqueous layer to enter the stem of the first funnel. Wash the aqueous layer with 0·5 ml of chloroform, without mixing, and run the chloroform washing into the second funnel containing the main chloroform extract. Extract the aqueous layer with a further 2·0 ml of carbamate reagent, shaking for 30 s, allow to separate, run the lower chloroform layer into the second funnel, and wash the aqueous layer with 0·5 ml of chloroform as before, adding the chloroform washing to the contents of the second funnel (Solution B). Return the acid layer to the original conical flask, and wash out the separating funnel with a little water, adding the washings to the flask. Reserve this acid solution (Solution C), which contains the lead, for the determination of this element (see Clause 2.8.11). Extract the copper and arsenic from the reagent blank in exactly the same way as from the test solution.

Determination of Copper

2.8.5 **Preparation of Solution for Test** — Add 10 ml of N sulphuric acid solution to the combined chloroform extracts (Solution B) contained in the 25 ml funnel, and shake for 5 s. (The washing with sulphuric acid is introduced to prevent

any possible interference by entrainment of phosphate, possibly present in the material under test. Even a trace of entrained phosphate would give fictitiously high results for arsenic.) Allow the mixture to separate, make sure that the stem of the funnel is dry, run the chloroform layer into a dry 10 ml measuring cylinder, wash the acid layer with 0·5 ml of chloroform, add the chloroform washing to the main chloroform extract, and reject the acid layer. Adjust the volume of the chloroform extract to 9·0 ml with chloroform and drain into a dry 25 or 50 ml flask. Wash the cylinder with 1·0 ml of chloroform, and add the washing to the total extract, which has now a total volume of 10·0 ml. Add 0·5 — 1·0 g of anhydrous sodium sulphate, swirl gently until the supernatant liquid clears, close the mouth of the flask with a cork, and allow to stand for 30 min. (This is necessary because an extraneous yellow colour not due to copper may appear in the chloroform layer during extraction, but this colour slowly fades out on standing.) Treat the chloroform extract from the reagent blank in an exactly similar way to the test solution. Reserve the chloroform extracts for the determination of copper (Clause 2.8.7).

2.8.6 Preparation of Calibration Graph — Into each of seven 100 ml conical flasks measure 6·0 ml of dilute sulphuric acid, 20·0 ml of 5N hydrochloric acid solution and 0, 1·0, 2·0, 3·0, 4·0, 5·0 and 7·0 ml of the standard copper solution. To each flask add 2·0 ml of the sodium iodide solution, warm to 35—40°C, add 0·5 ml of the sodium metabisulphite solution, cool to 20 — 25°C and transfer each solution in turn to a 50 ml separating funnel. Adjust the volume to 35 — 40 ml and extract with carbamate reagent as described in Clause 2.8.4 (c) finally adjusting the volume of the extract to 10·0 ml with chloroform. Clarify each extract with anhydrous sodium sulphate, and allow to stand for 30 min. as described in Clause 2.8.5.

Measure the optical density of each extract in turn in a 10 mm cell at the wavelength of maximum absorption (about 435 nm), against the extract containing no added copper. Plot a graph relating optical density to microgrammes of Cu.

2.8.7 Determination of Copper in Test Solution — Measure the optical density of the test solution and the reagent blank as in Clause 2.8.6 against a solution blank consisting

of 7 ml of carbamate reagent and 8 ml of chloroform clarified with anhydrous sodium sulphate. Deduct the value for the reagent blank from that for the test solution and read from the calibration graph the number of micrograms of Cu corresponding to this corrected value. Then

$$\text{mg/kg Cu} = \frac{\text{micrograms Cu} \times 2}{\text{mass in g of dye sample taken}}$$

After determining the optical density, return each solution without loss to its appropriate flask, and wash out the cell with the minimum amount of chloroform from a wash bottle with a fine jet, adding the washings to the flask. Decant the chloroform solution in the flask into another 50 ml conical flask, and wash the residue of sodium sulphate by decantation with three small washings of chloroform, adding the washings to the main solution. Take care not to wash any particles of sodium sulphate into the decanted solution. Reject the residue and reserve the decanted solution for the determination of arsenic.

Note : If the amount of copper in the test solution exceeds 50 micrograms, dilute the whole of the solution further with chloroform, e.g. to 25 ml, diluting the reagent blank similarly. Measure the optical density as specified above. Then

$$\text{mg/kg Cu} = \frac{\text{micrograms Cu} \times \text{dilution of test solution} \times 2}{\text{mass in g taken} \times \text{dilution of graphed solution}}$$

Determination of Arsenic

2.8.8 Preparation of Solution for Test — Add to the chloroform solution of the arsenic, reserved from the determination of copper (see Clause 2.8.7), $2{\cdot}0 \pm 0{\cdot}02$ ml of the ammonium molybdate solution, close the mouth of the flask with a glass bulb, and cautiously evaporate the chloroform on a water bath. When the chloroform is removed, transfer the flask to a hotplate and evaporate till fumes of perchloric acid appear, accompanied by sudden reaction between perchloric acid and the organic matter. Allow this reaction to continue for about one minute (not more) to ensure complete destruction of the organic matter, remove

the flask from the hotplate, allow to cool and remove the glass bulb without washing, since the acid should not be diluted. However, for very accurate work wash the bulb with a few drops of water and gently evaporate the water from the open flask on the hotplate until fumes of perchloric acid just appear. Place a ' cold-finger ' condenser into the flask and fill the tube with cold water. The outside of the condenser should be quite clean and dry. Place the flask on the hotplate and heat for 10 min at such a temperature that a ' blanket ' of fumes about half fills the flask, and the temperature of the water in the condenser rises to about 90 $\pm$ 5°C at the end of the 10 min heating period. Allow the solution to cool.

Note : This is the most critical part of the arsenic determination and it is essential to follow the above instructions exactly. It is necessary to destroy all organic matter, leaving a colourless residue, with a minimum loss of acid fumes, otherwise the acidity of the final solution will not be sufficient to prevent interference by silica in the arsenic determination. A hot plate with a surface temperature of 250°C has been found most suitable for the procedure. With hotter plates the flask should be placed on an asbestos sheet on the plate.

Treat the reagent blank in exactly the same way as the test solution.

2.8.9 **Preparation of Calibration Graph** — Into each of six 50 ml conical flasks measure 0, 0·2, 0·5, 1·0, 1·5 and 2·0 ml of standard arsenic solution, 2·0 ml of the ammonium molybdate solution and close the mouth of the flask with a glass bulb, evaporate to fuming, remove the bulb, wash with a few drops of water and evaporate the contents of the open flask just to fuming. Insert a ' cold-finger ' condenser and continue as described for the test solution.

Measure the optical density of each solution in turn in a 20 mm cell at the wavelength of maximum absorption (about 840 nm) against the first solution containing no added arsenic. Plot the graph relating optical density to microgrammes of As.

2.8.10 Determination of Arsenic in Test Solution — After washing down the sides of the condenser with 5·0 ml of N sulphuric acid solution from a pipette, followed by about 2·0 ml of water, boil for 2 — 3 min to remove traces of chlorine and cool to room temperature. Add 1·0 ml of the hydrazine sulphate solution and drain into a 10 ml stoppered measuring cylinder. Dilute to 10·0 ml at 20°C with water. Mix thoroughly and drain back into the conical flask. Close the mouth of the flask with a glass bulb and heat on the water bath for 15 min. Remove from the bath, stand for 30 min and cool to room temperature. Treat the reagent blank similarly. Measure the optical density of the test solution against the reagent blank from Clause 2.8.9. Deduct the value of the reagent blank from that for the test solution, and read from the calibration graph the number of microgrammes of As corresponding to this corrected value.

$$\text{Then mg/kg As} = \frac{\text{microgrammes of As} \times 2}{\text{mass in g of dye sample taken}}$$

Determination of Lead

2.8.11 Preparation of Solution for Test — Place the flask containing the acid solution retained after extraction of copper and arsenic (Solution C) on the hotplate, and evaporate until no more iodine is evolved and strong fuming commences.

(This is conveniently done while the copper and arsenic determinations are in progress). Allow to cool, dilute with 10 ml of water, add 5 ml of concentrated hydrochloric acid and 0·5 ml of the sodium metabisulphite solution, heat to boiling, and cool. Add 2 ml of the sodium citrate solution and 0·2 ml of thymol blue indicator solution, and neutralize the solution by adding 10 N ammonia solution to give the blue-green colour indicating pH 9·0 — 9·5. Cool the solution to room temperature.

Transfer the solution to a 50 ml separating funnel. Add 5 ml of chloroform and 1 ml of the dithizone solution and shake well for 30 s. The dithizone colour should change from green to a bright pink. If this does not occur, add a further 0·1 ml of 10 N ammonia solution and shake again. Continue by adding more dithizone solution, 1 ml or other

suitable increment, at a time, until with the last addition, after shaking, the colour changes from bright pink to a duller shade owing to the presence of a slight excess of unchanged dithizone. In the absence of cadmium, nickel and cobalt, the amount of dithizone used gives an approximate measure of the lead plus zinc content of the sample.

Shake for 15 s, allow the two layers to separate completely, and run the chloroform layer into a clean 50 ml conical flask. Wash the aqueous layer with a few drops of chloroform, without mixing, and add the washing to the main extract. Add to the aqueous layer 2 ml of chloroform and 1 ml of the dithizone solution, shake for 15 s and allow to separate. The chloroform layer should remain green or purplish-green. If it becomes pink, extraction is incomplete and more dithizone solution should be added with shaking until a definite excess of at least 1 ml is present. Add the chloroform layer to the main chloroform extract, which contain the lead, zinc, etc., and reject the aqueous layer.

Transfer the combined chloroform extracts to the separating funnel, washing out the flask with a little chloroform. Add 10 ml of 0·1N hydrochloric acid solution, shake vigorously for 30 s, and allow to separate. Transfer the chloroform layer to another separating funnel or flask, wash the acid with a little chloroform solution. Transfer the acid extract to the conical flask, washing the separating funnel with a little water. Re-extract the chloroform solution with a further 10 ml of 0·1N hydrochloric acid solution, as before, reject the chloroform layer, and add the acid extract to the first acid extract. To the combined acid extracts, which now contain the whole of the lead and zinc, add 0·5 ml of the sodium citrate solution and 0·5 ml of 10 N ammonia solution, followed by 0·5 ml of the potassium cyanide solution.

Transfer the solution to a 50 ml separating funnel, washing out the flask with a little water, add 2 ml of chloroform and 0·5 ml of the dithizone solution, and shake well. If the chloroform layer becomes bright pink, continue the addition of dithizone solution, 0·5 ml at a time, until the chloroform layer develops a slightly duller purplish tint and the aqueous layer a yellow tint, due to the presence of a slight excess of free dithizone. Run the lower chloroform layer into the original 50 ml conical flask, washing the

aqueous layer with two 1 ml portions of chloroform, which are added to the main extract. Shake the aqueous layer for 15 s with 2 ml of chloroform.

If all the lead has been extracted the chloroform layer will become pale green or purplish green. If the colour becomes pink, continue the addition of dithizone solution, 0·5 ml at a time, until a slight excess is present, and add the chloroform layer to the main extract, washing with a few drops of chloroform. The combined chloroform extracts should have a volume of less than 10 ml, since the amount of dithizone solution required to extract all the lead is almost invariably less than 5 ml and usually less than 3 ml. Reject the aqueous layer.

Transfer the combined chloroform extracts to a 25 ml separating funnel, wash out the flask with a few drops of chloroform and add the washing to the funnel. Add 10 ml of ammoniacal cyanide solution, shake until no further change in colour occurs (about 10 s) and allow to separate. If the ammoniacal layer is coloured only pale yellow, the one washing will suffice. If it is deeply coloured, indicating that a rather large excess of free dithizone is present, transfer the chloroform layer to another separating funnel, washing the aqueous layer with a little chloroform, which is added to the main chloroform layer, and give it a second washing with 10 ml of the ammoniacal cyanide solution.

After this washing, dry the stem of the separating funnel with filter paper, and run the chloroform layer into a dry 10 ml measuring cylinder, separating as completely as possible. Wash the aqueous layer with a little chloroform, adding the washing to the main solution, adjust the volume to 10·0 ml with chloroform and mix well. Allow to stand for a few minutes in the dark or subdued light, and if the solution is not then optically clear, filter it through a dry 90 mm filter paper into a dry vial.

Treat the reagent blank in exactly the same way as the test solution.

2.8.12 Preparation of Calibration Graph — Into each of six 50 ml conical flasks, measure 20 ml of 0·1N hydrochloric acid solution and 0, 1·0, 2·0, 3·0, 4·0 and 5·0 ml of

standard lead solution, and continue as described in Clause 2.8.11, for the test solution, beginning at paragraph 4. 'Add 0·5 ml of the sodium citrate solution . . . .'

Measure the optical density of each chloroform solution in turn in a 10 mm cell at the wavelength of maximum absorption (about 515 nm) against the first solution containing no added lead. Plot the graph relating optical density to micrograms of Pb.

2.8.13 Determination of Lead in Test Solution — Measure the optical density of the test solution and reagent blank as in Clause 2.8.12 against pure chloroform. Deduct the value for the reagent blank from that for the test solution and read from the calibration graph the micrograms of Pb corresponding to this corrected value. Then

$$\text{mg/kg Pb} = \frac{\text{micrograms of Pb} \times 2}{\text{mass in g of dye sample taken}}$$

2.9 Limit Test for Heavy Metals (as Sulphides)

2.9.1 Apparatus — Hydrogen sulphide generator, e.g. a Kipp apparatus.

2.9.2 Reagents — The reagents shall be of a recognized analytical reagent quality. Distilled water shall be used.

(*a*) Hydrochloric acid, dilute, Mix 1 part of concentrated hydrochloric acid (relative density 1·16 — 1·18) and 39 parts of water (by volume).

(*b*) Ascorbic acid.

(*c*) Cobaltous chloride solution. Dissolve about 65 g of cobaltous chloride, $CoCl_2.6H_2O$ in the dilute hydrochloric acid, and dilute to 1 l with the acid. Determine the strength of the solution by the following procedure :

Place exactly 5 ml of the solution in a 250 ml glass-stoppered flask, add 5 ml of hydrogen peroxide solution (containing 2·5 — 3·5 per cent (m/v) of H_2O_2) and 15 ml of 5N sodium hydroxide solution, boil for

10 min, cool and add 2 g of potassium iodide and 20 ml of sulphuric acid (25 per cent v/v). When the precipitate has dissolved, titrate the liberated iodine with 0·1N sodium thiosulphate solution. Each ml of 0·1 N sodium thiosulphate solution is equivalent to 23·80 mg of $CoCl_2.6H_2O$.

Adjust the final volume of the main bulk of the solution, by adding enough of the dilute hydrochloric acid, so that 1 ml contains 59·5 mg of $CoCl_2.6H_2O$.

(*d*) Copper sulphate solution. Dissolve about 65 g of copper sulphate, $CuSO_4.5H_2O$, in the dilute hydrochloric acid, and dilute to 1 l with the acid. Determine the strength of this solution by the following procedure :

Dilute exactly 10 ml of the solution to 50 ml with water, add 4 ml of glacial acetic acid and 3 g of potassium iodide, and titrate the liberated iodine with 0·1N sodium thiosulphate solution, using starch solution as indicator. Each ml of 0·1N sodium thiosulphate solution is equivalent to 24·97 mg of $CuSO_4.5H_2O$.

Adjust the final volume of the main bulk of the solution, by adding enough of the dilute hydrochloric acid, so that 1 ml contains 62·4 mg of $CuSO_4.5H_2O$.

(*e*) **Ferric Chloride Solution** — Dissolve about 55 g of ferric chloride, $FeCl_3.6H_2O$, in the dilute hydrochloric acid and dilute to 1 l with the acid. Determine the strength of this solution by the following procedure :

Measure exactly 10 ml of the solution into a glass-stoppered flask, add 15 ml of water, 5 ml of concentrated hydrochloric acid (relative density 1·16 — 1·18) and 4 g of potassium iodide, stopper the flask and allow it to stand for 15 min in the dark. Dilute with 100 ml of water and titrate the liberated iodine with 0·1N sodium thiosulphate solution, using starch solution as the indicator. Perform a blank determination in same manner with the same quantities of the reagents, and make any necessary

correction. Each ml of 0·1N sodium thiosulphate solution is equivalent to 27·03 mg of $FeCl_3$. $6H_2O$.

Adjust the final volume of the main bulk of the solution, by adding enough of the dilute hydrochloric acid, so that 1 ml contains 45 mg of $FeCl_3$. $6H_2O$.

(*f*) **Inorganic Reference Colour Standard** — Mix together 10·0 ml of the cobaltous chloride solution, 16·0 ml of the copper sulphate solution, 7·0 ml of ferric chloride solution and 67·0 ml of the dilute hydrochloric acid.

Place 20 ml of this mixture in a 150 mm × 25 mm boiling tube and seal.

2.9.3 Procedure — Measure 10·0 ml of Solution A (from the determination of copper, arsenic and lead — see Clause 2.8.4 b of that method) into a 50 ml beaker and evaporate on a hotplate or silica radiant heater until a white solid comes out of solution, or until there is only a trace of sulphuric acid left. Cool, add 8 ml of the dilute hydrochloric acid and swirl to dissolve any solid. Transfer to a 150 mm × 25 mm boiling tube, using 12 ml of water to effect the transfer, and add 0·1 g of ascorbic acid.

Pass a stream of hydrogen sulphide, washed with water, through the solution for 10 min. Compare the colour of the test solution with that of the inorganic reference colour standard.

Report as lighter than, similar to, or darker than the inorganic reference colour standard.

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