

THE PURIFICATION OF INDUSTRIAL WATER  
AND EFFLUENT REGULATIONS  
(under section 22 of the Act)

*Date of commencement: 21st April, 1967.*

*Citation.*

1. These regulations may be cited as the Purification of Industrial Water and Effluent Regulations, 1967.

*Purification of industrial water and effluents.*

2. A person using water for industrial purposes shall purify such water or effluent, waste or tailings produced by or resulting from the use of water so as to conform to the requirements set out in the First Schedule as determined by the methods of testing set out in the Second Schedule.

FIRST SCHEDULE

*Colour, Odour or Taste.*

1. Water used for industrial purposes, whether or not it contains waste or tailings produced by or resulting from such use, (hereinafter referred to as "waste water or effluent") shall contain no substance in concentrations capable of producing colour, odour or taste.

*pH.*

2. The pH of the waste water or effluent shall be between 5.5 and 9.5.

*Dissolved Oxygen.*

3. The waste water or effluent shall contain dissolved oxygen to the extent of at least 75 per cent saturation.

*Temperature.*

4. The temperature of the waste water or effluent shall not exceed 33°C.

*Chemical Oxygen Demand.*

5. The chemical oxygen demand of the waste water or effluent shall not exceed 75 milligrams per litre, after applying the chloride correction.

*Oxygen Absorbed.*

6. The oxygen absorbed by the waste water or effluent from N/80 potassium permanganate in four hours at 27°C shall not exceed 10 milligrams per litre.

*Total dissolved solids.*

7. (a) The total dissolved solids content of the waste water or effluent shall not have been increased by more than 500 milligrams per litre above that of the intake water.

(b) If the intake water is not public water, such waste water or effluent shall not contain total dissolved solids to an extent which, as a result of the disposal of the waste water or effluent, will increase the total dissolved solids content of any public or private water, including underground water, to such a degree that such water is rendered less fit for the purposes for which it is ordinarily used by other persons entitled to the use thereof, or for the propagation of fish or other aquatic life, or for recreational or other legitimate purposes.

*Suspended Solids.*

8. The waste water or effluent shall contain not more than 25 milligrams per litre of suspended solids.

*Sodium Content.*

9. (a) The total sodium content of the waste water or effluent shall not have been increased by more than 50 milligrams per litre above that of the intake water.

(b) If the intake water is not public water, such waste water or effluent shall not contain sodium to an extent which, as a result of the disposal of the waste water or effluent, will increase the sodium content of any public or private water, including underground water, to such a degree that such water is rendered less fit for the purpose for which it is ordinarily used by other persons entitled to the use thereof, or for the propagation of fish or other aquatic life, or for recreational or other legitimate purposes.

*Soap, oil or grease.*

10. The waste water or effluent shall contain not more than 5 milligrams per litre of soap, oil or grease.

*Other constituents.*

11. The waste water or effluent shall comply with the following requirements—

<i>Constituents</i>	<i>Maximum concentration in milligrams per litre</i>
Residual chlorine (as Cl)	0.5
Free and Saline Ammonia (as N)	10.0
Arsenic (as As)	0.5
Boron (as B)	1.0
Hexavalent chromium (as Cr)	0.05
Total chromium (as Cr)	0.5
Copper (as Cu)	1.0
Phenolic Compounds (as Phenol)	0.1
Lead (as Pb)	1.0
Cyanides (as CN)	0.5
Sulphides (as S)	1.0
Fluorine (as F)	1.0
Zinc (as Zn)	5.0

## SECOND SCHEDULE

## METHODS OF TESTING

## 1. DETERMINATION OF pH.

1.1. *Apparatus.*

- 1.1.1. Standard glass electrode.
- 1.1.2. Saturated calomel electrode.
- 1.1.3. pH electrometer with temperature compensator.

1.2. *Reagents.*

- 1.2.1. Distilled water for washing electrodes.
- 1.2.2. Standard buffer solutions of pH 4.0, pH 7.0 and pH 9.0. These are usually obtained with the instrument.

1.3. *Method.*

The method of operation of the electrometer is always given in full in the directions with the apparatus.

2. DETERMINATION OF DISSOLVED OXYGEN.

2.1. *Collection of Samples.*

- 2.1.1. *Sample bottles.* — Narrow mouth glass stoppered bottles with capacity of 250 to 300 ml should be used.
- 2.1.2. *Sampling.* — In general, the sampling arrangements should be such as to ensure a three-fold displacement of the liquid in the sampling bottle without entrainment of air bubbles. The temperature of the sampled water should be recorded to the nearest degree Centigrade, or more precisely as desired.
- 2.1.3. *Storage of Samples.* — When special dissolved oxygen samples cannot be analysed immediately the samples may be preserved for a 24-hour period, as follows:

Add 0.7 ml of concentrated sulphuric acid and 1 ml of a 2 per cent sodium azide solution. Stopper and store at the temperature of collection, or water-seal in a 20°C incubator, until analysis can be made. Then continue with the analytical procedure using 2 ml of manganous sulphate solution and 4 ml of alkaline-iodide solution and 3 ml of concentrated sulphuric acid for the final acidification.

2.2. *Reagents.*

- 2.2.1. *Manganous Sulphate Solution.* — Dissolve 480 g manganous sulphate ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ) or 400 g  $\text{MnSO}_4 \cdot 2\text{H}_2\text{O}$  or 364 g  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$  in distilled water and dilute to 1 litre. If doubt exists about the water of crystallisation make a solution with S.G. of 1.270 at 20°C.
- 2.2.2. *Alkaline-iodide/Sodium Azide Solution.* — Dissolve 500g of sodium hydroxide ( $\text{NaOH}$ ) or 100 g of potassium hydroxide ( $\text{KOH}$ ) and 133 g of sodium iodide ( $\text{NaI}$ ) or 150 g of potassium iodide ( $\text{KI}$ ) in distilled water and dilute to 1 litre. Dissolve 10 g sodium azide ( $\text{NaN}_3$ ) in 40 ml of distilled water, and add this solution with constant stirring to 950 ml of the alkaline-iodide solution.
- 2.2.3. *Sulphuric Acid.* — Concentrated
- 2.2.4. *Sodium Thiosulphate.* — Stock Solution, 0.25N. Dissolve 62.4 g sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ), in 1 litre of copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide to stabilise the solution. Allow to stand for several days before use.
- 2.2.5. *Working Solution, 0.025N.* — Dilute 100 ml of stock solution (2.2.4.) to 1 litre with copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide. This solution is reasonably stable but it should be standardised against potassium dichromate at frequent intervals. Store in an amber glass bottle with a rubber stopper and discard any solution remaining in the burette at the end of the day.

2.2.6. *Standard Potassium Dichromate Solution, 0.025N.* -- Dissolve 1.226 g previously dried  $K_2Cr_2O_7$  in distilled water and dilute to 1 litre.

2.2.7. *Starch.* -- Grind 1 g of soluble starch into a smooth paste with a little cold distilled water and pour it into 1 litre of boiling water with constant stirring. Boil for 1 minute, and allow to cool before use. The solution should be used freshly prepared.

2.3. *Procedure.*

2.3.1. *Standardisation of Sodium Thiosulphate Solution.* Dissolve approximately 2 g potassium iodide (KI) free from iodate in an Erlenmeyer flask with 100-150 ml distilled water, add 10 ml 1 : 1 sulphuric acid followed by exactly 20 ml standard dichromate solution. Place in the dark for 5 minutes, dilute to 400 ml and titrate with thiosulphate until a pale straw colour is reached, add starch and titrate until colourless. If the thiosulphate is not exactly 0.025N adjust it until it is.

2.3.2. *Method.*

To the sample as collected in a 250-300 ml bottle add 2 ml of manganous sulphate solution, followed by 2 ml alkaline iodide/sodium azide reagent well below the surface of the liquid. Stopper with care to exclude all air and mix well; let the precipitate settle and mix again. Let the precipitate settle well and then add 2 ml of concentrated sulphuric acid carefully, letting it run slowly down the neck of the bottle. Re-stopper and mix gently until solution is complete. Decant off an amount equal to 200 ml of original sample after correcting for increase in volume by the added reagents. Thus, if 4 ml of reagents as in this case have been added to a 300 ml bottle the volume taken for titration should be:--

$$200 \times \frac{300}{300 - 4} = 203 \text{ ml.}$$

Titrate with 0.025N sodium thio-sulphate to the starch end point.

2.4. *Expression of Results.*

If the equivalent of 200 ml original sample has been taken the dissolved oxygen will be numerically equal to the titration figure, and expressed thus:--

Dissolved Oxygen as  $O_2$  in mg/l.

2.5. *Calculations.*

2.5.1. *Percentage Saturation of Oxygen.*  $\frac{\text{Dissolved oxygen as } O_2 \text{ in mg/l} \times 100}{\text{Solubility of oxygen in water at the temperature, barometric pressure and salinity of the sample when taken, in mg/l.}}$

2.5.2. *Equation for Determining the Solubility at any Barometric Pressure.*

$$S_1 = \frac{SP}{760} \times \frac{SP_1}{29.92}$$

where  $S_1$  = solubility at P or  $P_1$ .  
 $S$  = solubility at 760 mm or 29.92 in.  
 (Refer to Table I)  
 $P$  = barometric pressure in millimetres.  
 $P_1$  = barometric pressure in inches.

TABLE 1

SOLUBILITY OF OXYGEN IN FRESH WATER AND IN SEA WATER OF STATED DEGREES OF SALINITY AT VARIOUS TEMPERATURES WHEN EXPOSED TO AN ATMOSPHERE CONTAINING 20.9 PER CENT OF OXYGEN UNDER A PRESSURE OF 760 MM.

(Calculated by G.C. Whipple and M.C. Whipple from measurements of C.J.J. Fox).

Chlorides in Sea Water, ppm.						Difference per 100 ppm. Chlorine, ppm.	Dissolved Oxygen in Chloride- free Water	
	0	5,000	10,000	15,000	20,000		°C.	ppm.
°C.	Dissolved Oxygen in ppm by Weight*							
0	14.02	13.79	12.97	12.14	11.32	0.0165	30	7.6
1	14.23	13.41	12.61	11.82	11.03	0.0160	31	7.5
2	13.84	13.05	12.28	11.52	10.76	0.0154	32	7.4
3	13.48	12.72	11.98	11.24	10.50	0.0149	33	7.3
4	13.13	12.41	11.69	10.97	10.25	0.0144	34	7.2
5	12.80	12.09	11.39	10.70	10.01	0.0140	35	7.1
6	12.48	11.79	11.12	10.45	9.78	0.0135	36	7.0
7	12.17	11.51	10.85	10.21	9.57	0.0130	37	6.9
8	11.87	11.24	10.61	9.98	9.36	0.0125	38	6.8
9	11.59	10.97	10.36	9.76	9.17	0.0121	39	6.7
10	11.33	10.73	10.13	9.55	8.98	0.0118	40	6.6
11	11.08	10.49	9.92	9.35	8.80	0.0114	41	6.5
12	10.83	10.28	9.72	9.17	8.62	0.0110	42	6.4
13	10.60	10.05	9.52	8.98	8.46	0.0107	43	6.3
14	10.37	9.85	9.32	8.80	8.30	0.0104	44	6.2
15	10.15	9.65	9.14	8.63	8.14	0.0100	45	6.1
16	9.95	9.46	8.96	8.47	7.99	0.0098	46	6.0
17	9.74	9.26	8.78	8.30	7.84	0.0095	47	5.9
18	9.54	9.07	8.62	8.15	7.70	0.0092	48	5.8
19	9.35	8.89	8.45	8.00	7.56	0.0089	49	5.7
20	9.17	8.73	8.30	7.86	7.42	0.0088	50	5.6
21	8.99	8.75	8.14	7.71	7.28	0.0086		
22	8.83	8.42	7.99	7.57	7.14	0.0084		
23	8.68	8.27	7.85	7.43	7.00	0.0083		
24	8.53	8.12	7.71	7.30	6.87	0.0083		
25	8.38	7.96	7.56	7.15	6.74	0.0082		
26	8.22	7.81	7.42	7.02	6.61	0.0080		
27	8.07	7.76	7.28	6.88	6.49	0.0079		
28	7.92	7.53	7.14	6.75	6.37	0.0078		
29	7.77	7.39	7.00	6.62	6.25	0.0076		
30	7.63	7.25	6.86	6.49	6.13	0.0075		

\*The second decimal place in the above table is not accurately known. The average difference from the mean of five different investigators represents 0.07 ppm. Until further data are obtained, however, the second decimal place has been retained in the table.

## 3. METHODS OF EXAMINATION FOR TYPICAL (FACIAL) COLI.

In establishing the presence and number of typical (facial) coli, the presence and number of presumptive coli forms is first determined and by sub-culturing the positive cultures and re-inoculating the presence and number of typical (facial) coli is then determined.

## 3.1. Apparatus. —

Contaminated bottles, jars and test tubes should be autoclaved at 121°C for 20 minutes, washed with soap powder, or other suitable washing compound and water, rinsed with distilled water until clear and finally dried in a hot air oven. All the apparatus shall be made sterile before use.

## 3.1.1. Sample bottles.

3.1.1.1. *Description.* The sample bottles shall be wide-mouthed and glass-stoppered or screw-capped with heat resistant screw caps. They shall preferably be of neutral glass, have a minimum capacity of 200 ml and shall be free from excessive alkali.

## 3.1.1.2.

*Sterilisation.* — When sterilising, place a small slip of paper between the edge of the bottle neck and the stopper, allowing about 7 to 10 mm to remain outside. Cover the stopper and neck with either brown paper or a heat-resistant cellophane paper and fasten with string or heat-resistant tape. Sterilise in an autoclave for 30 minutes at 121°C and drive off the external moisture by placing the bottles in an oven for 10 minutes at a temperature of 110°C for 5°C. Alternatively the bottles may be sterilised in a hot air oven for 1 hour at a temperature of 170°C for 5°C.

## 3.1.2. Test jars, Subculture Tubes and Durham Fermentation tubes.

3.1.2.1. *Description.*

(a) *Test jars.* — The test jars shall be of heat-resisting glass, 6.7 cm wide, 14-16 cm high and have a capacity of 350-350 ml. The mouths shall be 4.5-5 cm wide and covered with screw caps made of metal or other heat-resisting material. It is good practice to mark the jars at the 100 ml and 200 ml levels.

(b) *Subculture Tubes.* — Subculture tubes shall measure 6.8 cm long and 1.5-2 cm in diameter. Stopper these tubes with cotton wool plugs.

Instead of the subculture tubes described, metal screw-capped bottles of 25 ml capacity may be substituted.

(c) *Durham Fermentation Tubes (Large).* — Test tubes 12.2 cm long and 1.5-2 cm wide will serve as Durham fermentation tubes in the test jars.

(d) *Durham Fermentation Tubes (Small).* — Durham fermentation tubes used in subculture tubes shall measure 2.5-3.5 cm long and 0.7-1.0 cm in diameter.

## 3.1.2.2.

*Sterilisation.* — Within each test jar place one Durham fermentation tube (3.1.2.1(c)). In inverted position, screw the cap on and finally sterilise in a hot air oven for 1 hour at a temperature of 170°C for 5°C. Sterilising substance tubes similarly after a small Durham fermentation tube (3.1.2.1(d)) is placed in each.

3.1.3. *Incubators or Waterbaths.* — For incubation, use incubators or waterbaths capable of being maintained at temperatures of 37°C for 0.5°C or 44°C for 0.5°C.

3.1.4. *Thermometers.* — Thermometers used for reading the temperatures shall be of certified, Grade A quality.

3.2. *Media and Reagents.*

- 3.2.1. *Distilled Water.* -- All water used in the preparation of media shall be glass-distilled.
- 3.2.2. *0.04 per cent Bromo-Cresol Purple.* -- To 0.1 g of bromo-cresol purple add 18.5 ml 0.01N sodium hydroxide solution. Add enough distilled water to dissolve the indicator completely and to dilute to 250 ml.
- 3.2.3. *Preparation of MacConkey Broth.*
- 3.2.3.1. *Double Strength.* -- The double strength MacConkey broth consists of the following: 10 g sodium taurocholate (bile salt), 20 g lactose, 40 g peptone, 10 g sodium chloride and 1,000 ml distilled water.
- 3.2.3.1.1. *Alternative Broth.* -- Instead of making up MacConkey broth according to (3.2.3.1.), double quantities of a dehydrated MacConkey broth of bacteriological standard, dissolved in 1,000 ml of distilled water may be used.
- 3.2.3.1.2. *Preliminary Treatment of Double Strength MacConkey Broth.* -- Steam the solutions prepared (3.2.3.1. or 3.2.3.1.1.) for 2 hours at 98°-100°C, cool and transfer to an ice chest overnight. The following morning, while still cold, filter through Whatman No. 1 or similar grade filter paper. Adjust the pH of the filtrate to 7.4 and add 25 ml of a 0.04 per cent aqueous solution of bromo-cresol purple (3.2.2.).
- 3.2.3.1.3. *Distribution of Double Strength MacConkey Broth.* -- Using the jars described in (3.1.2.1.)*(a)* and *(c)*, add 100 ml of MacConkey broth to each test jar and sterilise for 15 minutes at 115°C in an autoclave. Alternatively, sterilise by steaming at 98°-100°C for 30 minutes on three consecutive days. The final pH shall be 7.3 ± or - 0.1. When required for test the jars shall be put in the 37°C incubator or waterbath in advance and taken out only for the addition of the water sample.
- 3.2.3.2. *Single Strength.* -- In addition to the double strength MacConkey broth described in (3.2.3.1.) prepare single strength MacConkey broth by taking half the weights of sodium taurocholate, lactose, peptone and sodium chloride given in (3.2.3.1.) and dissolve them in 1,000 ml of distilled water. Treat the broth further as in (3.2.3.1.2.). Alternatively prepare MacConkey broth from appropriate quantities of a dehydrated MacConkey broth of bacteriological standard dissolved in 1,000 ml of distilled water.
- Distribute this broth in 10 ml amounts in subculture tubes described in (3.1.2.1. *(b)* and 3.1.2.2.) and sterilise as described under (3.2.3.1.3.). When required for test the tube shall be put in the 37°C incubator or waterbath and taken out only for the addition of the water sample.
- 3.2.4. *Indole Medium.* -- Prepare a 1 per cent solution in distilled water of a bacteriological peptone containing tryptophan. Adjust pH to 7.0 ± or - 0.1, distribute in 5 ml quantities into subculture tubes, the Durham fermentation tubes being omitted. Stopper, and sterilise the tubes for 15 minutes at 121°C in an autoclave.
- 3.2.5. *Kovacs Indole Reagent.* -- Dissolve 5 grams of p-dimethylamino benzaldehyde in 75 ml of pure n-amyl alcohol and add 25 ml of 10N hydrochloric acid.

3.3. *Collection of Samples.*

- 3.3.1. *General.* -- Before taking a sample prepare a label giving the name of the sampler, the date and time of sampling and any special distinguishing mark. Immediately after sampling attach this label firmly to the sample.

- 3.3.2. *Sampling for Bacteriological Test.* — When handling the sterile sample bottle the inside portion of the stopper shall not touch the hand or come into contact with any object during the collection of the sample and on no account shall the stopper be laid down.
- 3.3.2.1. *Sampling from a Tap or Pump.* — Wash the mouth of the tap inside and outside with a clean cloth. With a spirit, or other type of burner, flame the mouth of the tap until well heated. Allow the water to run for 2 to 3 minutes, fill the bottle almost full, replace the stopper and the paper cover immediately, and return to the sample box. Fill the sample box with lumps of ice (about 1 to 2 in. in size).
- 3.3.2.2. *Sampling from a Stream, Reservoir, etc.* — Grasp the bottom part of the bottle in one hand, remove the stopper and immerse the bottle at once about 1 ft. below the surface, allowing to fill either up-stream in flowing water or by forward movement of the bottle in still water, to prevent collection of any water which has come into contact with the hand. After removal from the water, replace the stopper and the paper cover immediately and replace in sample box which has been filled with lumps of ice.
- 3.4. *Methods of Examination.*
- 3.4.1. *Presumptive Test.* Invert the sample bottle 25 times by a rapid rotary movement of the wrist in order to distribute any deposit uniformly throughout the water. After flaming the mouth of the bottle, transfer aseptically 100 ml of water to one of the prepared test jars containing 100 ml of double strength MacConkey broth. Replace the screw cap of the test jar and place the jar in a 37°C incubator or waterbath. After 20 to 24 hours' incubation examine the jars; the presence of acid and gas in the enclosed Durham fermentation tubes is regarded as a positive reaction but the absence of gas formation, whether or not growth or acid production is present, is not regarded as a negative reaction until the total period of incubation at 37°C is 48 hours. As soon as a jar shows acid and gas, it must be sub-inoculated at once into single strength MacConkey broth at 44°C as set out in (3.4.2).
- If any jar does not show the presence of acid and gas, incubate it until a total period (including the first period) of 48 hours has elapsed. The jars showing no acid and gas formation are then regarded as negative in reaction.
- 3.4.2. *Confirmatory Test.*
- 3.4.2.1. *Theory.* — The possibility of the presence of typical (faecal) coli in presumptive positive MacConkey jars at 37°C is ascertained by subculture from each positive jars into MacConkey medium at 44°C. If the temperature of incubation is kept strictly at 44°C (with a maximum tolerance of + or -0.5°C) the presence of gas within 48 hours is practically characteristic of *Escherichia coli*, Type 1, faecal.
- 3.4.2.2. *Procedure.* — From each positive presumptive jar at 37°C take a subculture into 10 ml single strength MacConkey broth by means of a sterile bacteriological loop or Pasteur pipette; transferring approximately 0.01 to 0.02 ml. Incubate these tubes in a waterbath at 44°C, care being taken that the water level of the bath is above the level of the liquid in the tubes and that the temperature is maintained throughout at 44°C ± 0.5°C.
- 3.4.2.3. *Interpretation.* The presence of gas in the enclosed Durham fermentation tubes is regarded as positive indication of the growth of typical (faecal) coli. No tube is regarded as negative until the total period of incubation of 48 hours has elapsed. Final confirmation depends on the production of indole at 44°C (3.4.2.4.)

- 3.4.2.4 *Test for Indole Production.* -- Transfer approximately 0.01 to 0.02 ml of the 44°C positive culture obtained in (3.4.2.2.) to 5 ml of indole medium (3.2.4.). Incubate these tubes as in (3.4.2.2.). After 24 hours add 0.3 ml of Kovacs reagent (3.2.5.) and shake well. A red colour developing within 5 minutes indicates a positive test for the presence of indole and confirms the presence of typical (faecal) *Escherichia coli*, Type 1.
- 3.4.3. *Method of Examination for the Most Probable Number of Typical (Faecal) Coli.* To establish the most probable number of typical *Escherichia Coli* Type 1 organisms present conduct the following tests simultaneously with those of Section (3.4.1.) and (3.4.2.)
- 3.4.3.1. *Apparatus.* -- In addition to the apparatus specified in (3.1) excepting (3.1.2.1.(iv)) Pipettes are required.
- 3.4.3.1.1. *Types.* Pipettes shall be graduated (10, 5 and 1 ml) and shall have straight sides with tapering tips. Used pipettes should be cleaned in 2 per cent sodium hydroxide solution, or in other suitable washing compounds, washed and rinsed in distilled water and dried in a hot air oven at 105°C  $\pm$  or  $\pm$  5°C.
- 3.4.3.1.2. *Sterilisation.* -- Fit a piece of cotton wool inside the upper end of pipettes, place in a pipette container (or wrap in kraft paper) and sterilise in a hot air oven for 1 hour at a temperature of 170°C  $\pm$  or  $\pm$  5°C.
- 3.4.3.2. *Media.* -- In addition to the media specified in (3.2.), the following is required: --
- Double Strength MacConkey Broth.* -- Prepare double strength MacConkey broth in accordance with (3.2.3.1.), except that instead of distributing the broth in 100 ml quantities by test jars distribute 10 ml quantities in sterile subculture tubes (3.1.2.1.(b)) containing Durham fermentation tubes (3.1.2.1.(d)) and then sterilise.
- 3.4.3.3. *Procedure.*
- 3.4.3.3.1. *Presumptive Test.* -- Invert the sample bottle 25 times by a rapid rotary movement of the wrist in order to distribute any deposits uniformly throughout the water. After flaming the mouth of the bottle, to each of five prepared, double strength MacConkey broth tubes (3.4.3.2.) add 10 ml of water sample. To each of five prepared, single strength MacConkey broth tubes (3.2.3.2.) add 1.0 ml of water sample. To each of another five prepared single strength MacConkey broth tubes add 1.0 ml of a diluted water sample. This diluted water is prepared by diluting 1.0 ml of water sample to 10.0 ml with sterile glass-distilled water. In the first series of tubes, each contains 10 ml of water sample, in the second series 1.0 ml and in the third series 0.1 ml. Immediately place these 15 tubes in the 37°C incubator or water-bath. After 20 to 24 hours' incubation examine the tubes; the presence of acid and gas in the enclosed Durham fermentation tubes is regarded as a positive reaction but the absence of gas formation, whether or not growth or acid production is present, is not regarded as a negative reaction until the total period of incubation at 37°C is 48 hours. As soon as any tube shows acid and gas, it must be sub-inoculated at once onto single strength MacConkey broth at 44°C as set out in (3.4.3.3.2.). If any tubes do not show the presence of acid and gas, incubate them until a total period (including the first period) of 48 hours has elapsed. The tubes showing no acid and gas formation are then regarded as negative in reaction.
- 3.4.3.3.2. *Confirmatory Test.* -- From each positive presumptive tube at 37°C take a subculture into single strength MacConkey broth by means of a sterile bacteriological loop or Pasteur pipette, capable of transferring 0.01 to 0.02

3.4.3.4. *Interpretation.* The presence of gas in the enclosed Durham fermentation tubes is regarded as positive indication of the growth of typical faecal coliforms per 100 ml. To arrive at the count of typical faecal coliforms per 100 ml the results obtained in the above tests are referred to a

3.4.3.5. *Estimation of Count.* To arrive at the count of typical faecal coliforms per 100 ml the results obtained in the above tests are referred to a

TABLE II  
MOST PROBABLE NUMBER TABLE

No. of Probable Tubes Count	giving positive Reaction (1) ml. (2)	No. of Probable Tubes Count	giving positive Reaction ml.	No. of Probable Tubes Count	giving positive Reaction ml.	No. of Probable Tubes Count	giving positive Reaction ml.
000	0	203	12	400	13	513	85
001	2	210	7	401	17	520	50
002	4	211	9	402	20	521	70
010	2	212	12	403	25	522	95
011	4	220	9	410	17	523	120
012	6	221	12	411	20	524	150
020	4	222	14	412	25	525	175
021	6	230	12	42*	20	530	80
030	6	231	1	421	25	531	110
100	2	240	14	422	30	532	140
101	4	300	8	430	25	533	175
102	6	301	11	431	30	534	200
103	8	302	14	432	40	535	250
110	4	310	11	440	25	540	130
111	6	311	14	441	40	541	170
112	8	312	17	450	40	542	250
120	6	313	20	451	50	543	300
121	8	320	14	500	25	544	350
122	10	321	17	501	30	545	450
130	8	322	20	502	40	550	250
140	10	330	17	503	60	551	350
141	11	331	20	504	75	552	600
200	5	340	20	510	35	553	900
201	7	341	25	511	45	554	1600
202	9	350	25	512	60	555	1800

Note: --  
1. A series of five tubes each containing 10 ml sample.

Note, continued. (Table II).

- b - series of five tubes each containing 1 ml sample.
  - c - series of five tubes each containing 0.1 ml sample.
2. The most probable numbers (shown as probable count per 100 ml in table) from 0 to 20 are correct to the nearest unit; from 20 to 200 are correct to the nearest 5; above 200 are correct to the nearest 50.

#### 4. DETERMINATION OF CHEMICAL OXYGEN DEMAND.

##### 4.1. Apparatus.

Reflux apparatus. - A round bottom boiling flask with ground glass neck, and a reflux condenser.

##### 4.2. Reagents.

- 4.2.1. *Sulphuric Acid.* - Concentrated.
- 4.2.2. *Ferroin Indicator Solution.* - Dissolve 1.485 g 1,10-phenanthroline (monohydrate), together with 0.695 g ferrous sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) in water and dilute to 100 ml.
- 4.2.3. *Standard Potassium Dichromate Solution, 0.25N.* Dissolve 12.2588 g potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) previously dried at  $103^\circ\text{C}$  for 2 hours, in distilled water and dilute to 1 litre.
- 4.2.4. *Standard Ferrous Ammonium Sulphate Solution, approximately 0.25N.* Dissolve 98 g ferrous ammonium sulphate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$ ) in distilled water. Add 20 ml concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ), cool and dilute to 1 litre. This solution must be standardised against the potassium dichromate daily.

##### 4.3. Procedure.

- 4.3.1. *Standardisation of Ferrous Ammonium Sulphate.* Dilute 25 ml standard dichromate solution to about 250 ml, add 20 ml concentrated sulphuric acid and allow to cool. Titrate against the ferrous ammonium sulphate, using 2 or 3 drops of the ferroin indicator.

$$\text{Normality} = \frac{\text{ml } \text{K}_2\text{Cr}_2\text{O}_7 \times 0.25}{\text{ml } \text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2}$$

##### 4.3.2. Method.

Place 50 ml sample in the round bottom flask, and add 25 ml standard dichromate solution. Carefully add 75 ml concentrated sulphuric acid, mixing after each addition.

(*Caution.* - The reflux mixture must be thoroughly mixed before heat is applied. If this is not done, local heating occurs in the bottom of the flask and the mixture may be blown out the side arm of the condenser).

Attach the flask to the condenser and reflux the mixture for 2 hours. Pumice granules or glass beads should be added to the reflux to prevent bumping. Cool and then wash down the condenser with about 25 ml distilled water.

Transfer the contents to a 500 ml Erlenmeyer flask, washing out the reflux flask 4 to 5 times with distilled water. Dilute the mixture to about 350 ml and titrate the excess dichromate with standard ferrous ammonium sulphate, using ferroin indicator. Generally 2 to 3 drops of the indicator are used. This, however, depends upon the individual analyst. The colour change is sharp, changing from a blue-green to a reddish-blue. The end-point, however, will not be as sharp as in the standardisation of the reagents because of the lower acid concentration. For this reason it is necessary that the sample be diluted to at least 350 ml before the titration is carried out.

A blank consisting of 50 ml distilled water instead of the sample, together with the reagents, is refluxed in the same manner.

4.4. *Calculation.*

$$\text{mg/l COD} = \frac{(a - b) \times N \times 8000}{\text{ml of sample}} - C$$

where COD = Chemical oxygen demand.

a = ml  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_6$  used for blank

b = ml  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_6$  used for sample.

C = Chloride correction =  $\text{mg/l Cl} \times 0.22$ . (See 4.5.)

N = Normality of  $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_6$ .

4.5. *Determination of Chloride.*

4.5.1. *Reagents.*

4.5.1.1. *Silver Nitrate Solution*, approximately 0.028N. --- Dissolve 4.8 g silver nitrate ( $\text{AgNO}_3$ ) in one litre of distilled water. Using the procedure described below (4.5.2.2), standardise against the standard sodium chloride (NaCl) solution. The factor for the  $\text{AgNO}_3$  solution is:

$$\frac{\text{ml of NaCl solution taken}}{\text{ml of AgNO}_3 \text{ solution used}}$$

4.5.1.2. *Standard Sodium Chloride Solution*, 0.028N. --- Dissolve 16.486 g sodium chloride (NaCl), dried by fusing at 900°C for ½ hour, in 500 ml distilled water. Dilute 50.0 ml to one litre. Each ml of this solution contains 1 mg of chloride.

4.5.1.3. *Potassium Chromate Indicator Solution*. --- Dissolve 50 g of potassium chromate ( $\text{K}_2\text{CrO}_4$ ) in a little distilled water. Add silver nitrate to produce a slight red precipitate. After it has stood at least over-night, filter and dilute to one litre with distilled water.

4.5.1.4. *Aluminium Hydroxide Suspension*. --- Dissolve 125 g potassium or ammonium alum in one litre of distilled water. Precipitate the aluminium by adding ammonium hydroxide slowly and with stirring. Wash the precipitate by successive decantation with numerous portions of distilled water until free from chloride.

4.5.1.5. *Phenolphthalein Indicator Solution*. --- Dissolve 5 g of phenolphthalein in 500 ml of ethanol and add 500 ml of distilled water. Then add 0.02N sodium hydroxide solution dropwise, until a faint pink colour appears.

4.5.1.6. *Sulphuric Acid*, 1 : 70, approximately 0.5N.

4.5.1.7. *Sodium Hydroxide*, approximately 0.5N. --- Dissolve 4 g of sodium hydroxide (NaOH) in 200 ml distilled water.

#### 4.5.2. Procedure.

##### 4.5.2.1. Pye-Treatment of Sample.

Use 100 ml sample or a suitable aliquot diluted to 100 ml.

If the sample is coloured, decolorise by adding 3 ml aluminium hydroxide suspension. Stir thoroughly and, after a few minutes, filter and wash with 10-15 ml distilled water.

4.5.2.2. *Titration*. --- Either a white porcelain dish, or an Erlenmeyer flask over a white surface should be used. Adjust the sample with either dilute sulphuric acid or sodium hydroxide so that it is just colourless to phenolphthalein. Add 1 ml potassium chromate solution. Titrate with silver nitrate solution until a colour change from pure yellow to pinkish-yellow is perceptible. The indicator blank should be determined by titrating distilled water in the same way. This blank, showing the end-point colour should be placed near the sample being titrated to aid in the detection of the colour change at the chosen end-point.

#### 4.5.3. Calculation.

$$\text{Chloride as Cl in mg/l} = \frac{(\text{ml AgNO}_3 \text{ for sample} - \text{ml AgNO}_3 \text{ for blank}) \times F \times 1,000}{\text{ml of sample}}$$

where F = AgNO<sub>3</sub> factor (determined in 4.5.1.1.)

### 5. DETERMINATION OF "OXYGEN ABSORBIBLE".

5.1. *Apparatus*. Incubator or waterbath capable of maintaining a temperature of 27 C ± 0.2 C.

#### 5.2. Reagents.

5.2.1. *Potassium Permanganate Solution*, approximately N/80. -- It is usually convenient to prepare 10 litres of this reagent at a time, and by a method which ensures stability over long periods. Even then, daily blank determinations should be made to check the strength. When the following method is carefully followed and the solution stored in amber bottles or in the dark, it is stable for several months.

Dissolve 4.0g potassium permanganate (KMnO<sub>4</sub>) in 1 litre of hot distilled water contained in a large beaker covered with a clock glass, preferably heating the solution to 90-95 C for 2-3 hours. Dilute to 10 litres with distilled water and set aside for several days in the dark to ensure complete oxidation of any organic matter and to allow any precipitated manganese dioxide to settle. Carefully decant or siphon off the supernatant liquid, avoiding disturbance of sediment. Alternatively, filter the solution through a sintered glass funnel, through glass wool or through asbestos fibre previously digested with nitric and hydrochloric acids and then thoroughly washed with water; *do not filter through paper*. Dust or organic matter must not be allowed to contaminate the solution.

- 5.2.2. **Sodium Thiosulphate, Stock Solution, N/8.** Dissolve 31.2 g of sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in 1 litre of copper-free, freshly boiled and cooled distilled water adding 1 ml chloroform or 10 mg mercuric iodide to stabilise the solution. Allow to stand for several days before use.
- 5.2.3. **Sodium Thiosulphate, Working Solution N/80.** Dilute 100 ml of stock solution to 1 litre with copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide. This solution is reasonably stable but it should be standardised against potassium dichromate at frequent intervals. Store in an amber glass bottle with a rubber stopper and discard any solution remaining in the burette at the end of the day.
- 5.2.4. **Standard Potassium Dichromate Solution N/80.** Dissolve 0.613 g previously dried potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) in distilled water and dilute to 1 litre.
- 5.2.5. **Dilute Sulphuric Acid, 1 : 9.** Add cautiously, small quantities at a time, 1 volume of concentrated sulphuric acid to 9 volumes of water. When heat is generated in the process and precipitations should be taken against splitting of acids and the cracking of glass vessels. After mixing, add sufficient N/80 permanganate solution to give a faint permanent pink tint to the mixture.
- 5.2.6. **Dilute Sulphuric Acid, 1 : 9.** Prepare as in (5.2.5), but add 1 volume of concentrated sulphuric acid to 9 volumes of distilled water.
- 5.2.7. **Potassium Iodide Solution.** Dissolve 10 g potassium iodide in 100 ml of water and store in an amber glass bottle.
- 5.2.8. **Starch.** Grind 1 g of soluble starch into a smooth paste with a little cold distilled water and pour it into 1 litre of boiling distilled water with constant stirring. Boil for 1 minute, and allow to cool before use.
- The solution should be used freshly prepared.
- 5.3. **Procedure.**
- 5.3.1. **Standardisation of Sodium Thiosulphate Solution.**  
 Dissolve approximately 2 g of potassium iodide (K.I) free from iodide in an Erlenmeyer flask with 100-150 ml distilled water, add 10 ml 1 : 9 sulphuric acid followed by exactly 20 ml standard dichromate solution. Place in the dark for 5 minutes, dilute to 100 - 400 ml and titrate with thiosulphate until a pale straw colour is reached, add starch and titrate until colourless. If the thiosulphate is not exactly N/80 adjust it until it is.
- 5.3.2. **Method.**  
 Into a clean 12 oz. glass-stoppered bottle place 10 ml dilute sulphuric acid and exactly 20 ml permanganate solution. Add 100 ml of the sample and mix immediately by gentle rotation of the bottle. Maintain at a temperature of 27°C for 4 hours by placing in a constant-temperature waterbath, removing the contents after one hour if the sample contains much suspended matter. After 4 hours add 5 ml of the iodide solution and after mixing titrate immediately with N/80 thiosulphate solution, adding 2 ml starch solution towards the end of the titration. Titrate until the blue colour just disappears and ignore any blueness which may return after standing. Make a blank determination using the same procedure without the sample but using 100 ml of distilled water instead.

54. *Calculation.*

"Oxygen absorbed" in mg/l = (a - b)

where a = ml thiosulphate used for blank.

b = ml thiosulphate used for sample.

6. DETERMINATION OF TOTAL DISSOLVED SOLIDS.

6.1. *Apparatus.*

Platinum, nickel or silica dish which has been heated at 180°C for 1 hour, cooled in a desiccator and weighed accurately.

6.2. *Method.*

Place the dish over aperture in a water-bath. Choose a volume of sample from 50-250 ml depending on the concentration of total solids, and run it into a glass-stoppered graduated flask. (The sample should first be filtered through Whatman No. 42 filter paper if it is turbid.)

Almost fill the evaporating dish with a portion of the sample and when this portion has evaporated to dryness add a further portion and so on until the whole sample has been transferred to the dish. Finally rinse out the flask with a few ml of distilled water and add this to the dish.

When completely dry, wipe the outside and place in an oven at 180°C for 2 hours, remove to a desiccator and weigh after an interval of between 5 and 10 minutes. The increase in weight represents the weight of solids in the sample taken.

6.3. *Calculation.*

$$\text{Total dissolved solids at 180°C in mg/l} = \frac{\text{weight of solids in mg} \times 1,000}{\text{ml of sample.}}$$

7. DETERMINATION OF SUSPENDED SOLIDS.

7.1. *Apparatus*

Sintered glass crucible, Pyrex Grade 2 (average pore diameter 40-60 microns). Wash it well with distilled water. Dry the crucible at 105°C for 2 hours or until the weight is constant, cool in a desiccator and weigh accurately.

7.2. *Method.*

Use 200 ml of the sample. With samples containing solid matter in very fine suspension where the sintered glass is liable to become "choked" it may be advisable to subject the sample to a preliminary centrifuging until the solids settle out. Using gentle suction, filter the supernatant portion of the sample and finally transfer all the sample to the filter, using distilled water for rinsing the sample beaker or the centrifuge tube, if used.

Dry the crucible at 105°C for 4 hours, transfer to a desiccator for 30 minutes and weigh. The increase in weight will be the weight of suspended solids.

7.3. *Calculation*

$$\text{Suspended solids in mg/l} = \frac{\text{weight of solids in mg} \times 1,000}{\text{ml of sample}}$$

8. DETERMINATION OF SODIUM.

8.1. *Apparatus.*

Flame photometer.

8.2. *Reagents.*

8.2.1. *Standard Sodium Solution.* - Dissolve 2,5413 g of dry sodium chloride (NaCl) (analytical reagent quality) in distilled water and make up to 1.0 litre. This solution contains 1.0 mg of sodium (Na) per millilitre. Keep for preference in a polythene bottle. Dilute as required for use.

8.2.2. *Sodium Radiation Buffer.* - Distilled water must be successively saturated and filtered with analytical reagent quality chlorides of calcium, potassium and magnesium.

8.3. *Method.*

Full instructions should be given by the makers of the instrument used, but the following are points that should be particularly noted.

The instrument is used for comparison against known standards from which a calibration curve is prepared over a range which may, for example, be 0 to 100 mg per litre. The sample has to be diluted as necessary for its content of sodium to fall within the required range. At least one standard should be observed every time the instrument is operated. The analyst should satisfy himself, by the use of a sufficient number of standards, that the *reproducibility and accuracy* of results given by the particular instrument is satisfactory for the purpose required.

Soft glass or other sources of possible contamination by traces of sodium should be avoided.

1.0 ml of the radiation buffer should be added to 25.0 ml of sample and standards to bring the interference from calcium, potassium and magnesium to a constant level.

8.4. *Expression of Results.* - The results should be expressed as follows:

Sodium as Na in mg/l.

9. DETERMINATION OF SOAP, OIL AND GREASE.

9.1. *Sampling.*

Care should be taken that the sample is representative. Samples should be taken in clean, glass-stoppered bottles, previously washed with solvent and air-dried before use. The bottle should not be completely filled, as a loss of floating oil may occur in stoppering. It is advisable to collect the desired quantity of sample in an oversized bottle that has previously been marked on the outside at the desired volume.

### 9.2. Storage.

Samples should be analysed as soon as possible after sampling, since many oils and hydrocarbons are utilised by bacteria.

If storage is unavoidable, use 5 ml 1 : 1 sulphuric acid per litre, to inhibit bacterial action.

### 9.3. Apparatus.

Separating funnel, of approximately two litres capacity, with no-lubrication stopcock or with all greasy lubricants removed from the ground glass surfaces.

### 9.4. Reagents.

9.4.1. Sulphuric Acid ( $H_2SO_4$ ), 1 : 1.

9.4.2. Petroleum Ether, boiling point 35° to 60° C.

Distil at least twice in an all-glass apparatus, discarding the last 10 per cent remaining in the flask at each distillation. The residue on evaporation should be less than 0.1 milligram per 100 millilitres.

### 9.5. Method.

Place the well-mixed sample, usually 1 litre, in a separating funnel, and acidify with 5 ml sulphuric acid per litre. Add 40 ml petroleum ether to the separating funnel shaking vigorously for 2 minutes. Allow the ether layer to separate, withdraw the aqueous portion of the sample into a clean container and transfer the solvent layer into a clean, weighed distilling flask capable of holding at least three volumes of solvent. If a clear ether layer cannot be obtained, filter the solvent layer into the weighed distilling flask through a funnel containing an ether-moistened Whatman No. 40 (or equivalent) filter paper. Use as small a funnel and filter as practical. After all the ether from the two extractions and the final rinsing is included, wash down the funnel and filter paper twice with fresh 5 ml increments of petroleum ether.

Return the sample to the separating funnel, rinsing the container with 15 ml ether. Add the ether washings and an additional 25 ml ether to the separating funnel, and agitate for another 2 minutes.

Allow the solvent layer to separate, and discard the aqueous phase. Add the ether extraction to the weighed distilling flask, and rinse the separating funnel with 20 ml ether. Add the ether washing to the weighed distilling flask.

Distil off all but approximately 10 ml of the ether extract, keeping the source of heat at about 70°C. Then disconnect the condenser, and boil off the remaining solvent at the same temperature. Dry the flask on a water-bath.

Cool in a desiccator and weigh.

### 9.6. Calculation.

The total gain in weight of the weighed flask less the calculated residue from the solvent, as determined by the distillation or evaporation of a measured quantity, indicates the amount of extracted soap, oil or grease in the water sample.

$$\text{Soap, grease, or oil in mg/l.} = \frac{(\text{mg weight gain} - \text{mg residue}) \times 1,000}{\text{ml sample}}$$

10. DETERMINATION OF RESIDUAL CHLORINE.

*Note:* --The determination of free chlorine should be carried out as soon as possible after the collection of the sample.

10.1. Reagents

- 10.1.1. *Potassium Iodide Solution.* -- Dissolve 50 g of potassium iodide (KI), free from iodine and iodate, in 1 litre of freshly boiled and cooled distilled water.
- 10.1.2. *Sodium Thiosulphate, Stock solution, 0.25N.* -- Dissolve 62.4 g sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in 1 litre of copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide to stabilise the solution. Allow to stand for several days before use.
- 10.1.3. *Sodium Thiosulphate, 0.025N.* -- Dilute 100 ml of stock solution to 1 litre with copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide. This solution is reasonably stable but it should be standardised against potassium dichromate at frequent intervals. Store in an amber glass bottle with a rubber stopper and discard any solution remaining in the burette at the end of the day.
- 10.1.4. *Standard Potassium Dichromate Solution, 0.025N.* Dissolve 1.226 g previously dried potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) in distilled water and dilute to 1 litre.  
*Standardisation of 0.025N Sodium Thiosulphate Solution.* -- Dissolve approximately 2 g potassium iodide (KI) free from iodate in an Erlenmeyer flask with 100 - 150 ml distilled water, add 10 ml 1:9 sulphuric acid followed by exactly 20 ml standard dichromate solution. Place in the dark for 5 minutes, dilute to 40 or 400 ml and titrate with thiosulphate until a pale straw colour is reached, add starch and titrate until colourless. If the thiosulphate is not exactly 0.025N adjust it until it is.
- 10.1.5. *Sodium Thiosulphate, 0.005N.* -- Pipette 20 ml of the standardised 0.025N sodium thiosulphate into a 100 ml volumetric flask and make up to the mark with distilled water. This solution should be prepared fresh daily. 1 ml = 0.178 mg of chlorine.
- 10.1.6. *Stock Iodine Solution, approximately 0.1N.* -- Place 40 g of potassium iodide and 13 g resublimed iodine together in a beaker and add small amounts of water, stirring until all the iodine has been dissolved. Transfer to a 1-litre flask and dilute to the mark.
- 10.1.7. *Iodine Working Solution, approximately 0.005N.* -- Pipette 25 ml of the iodine stock solution into a 500 ml flask and make up to the mark with distilled water. Standardise this solution against the 0.005N sodium thiosulphate in the normal way, using starch indicator, and calculate the factor F.

$$F = \frac{\text{ml sodium thiosulphate}}{\text{ml iodine}}$$

10.1.8. *Starch.* - Grind 1 g of soluble starch into a smooth paste with a little cold distilled water and pour it into 1 litre of boiling distilled water with constant stirring. Boil for 1 minute, and allow to cool before use.

The solution used should be freshly prepared.

10.1.9. *Acetate Buffer Solution, pH 4.0.* - Dissolve 146 g anhydrous sodium acetate in 400 ml distilled water, add 480 g glacial acetic acid and dilute to 1 litre with distilled water.

### 10.2. Method.

Place 5.0 ml of 0.005N sodium thiosulphate in a flask. Add 5 ml of potassium iodide solution and sufficient acetate buffer solution to reduce the pH to between 3.5 and 4.2 (approximately 10 ml). Pour in 1,000 ml of the sample and mix with a stirring rod. Just prior to titration with 0.005N iodine add 5 ml of starch solution. Titrate with iodine, from a micro-burette, to the first appearance of blue colour.

### 10.3. Calculation.

$$\text{Residual chlorine as Cl in mg/l} = \frac{(A - B) \times 0.178 \times 1,000}{\text{ml of sample}}$$

A = ml of sodium thiosulphate added to sample.

B = ml of iodine used in titration.

F = factor for iodine solution.

## 11. DETERMINATION OF FREE AND SALINE AMMONIA.

### 11.1. Reagents.

11.1.1. *Light Magnesium Oxide.*

11.1.2. *Sulphuric Acid, exactly 1.00N H<sub>2</sub>SO<sub>4</sub>.*

11.1.3. *Sulphuric Acid, 0.02N.* - Dilute 20.0 ml of 1.00N sulphuric acid to 1.0 litre. 1 ml = 0.28 mg nitrogen (N).

11.1.4. *Boric Acid Solution.* - Dissolve 40 g boric acid (H<sub>3</sub>BO<sub>3</sub>) in 1 litre ammonia-free distilled water.

11.1.5. *Indicator Solution.* - Dissolve 0.2 g of bromo-cresol green and 0.04 g of methyl red in 120 ml of 95 per cent alcohol.

### 11.2. Method.

Take 280 ml\* of sample diluted to 500 ml with ammonia-free distilled water. If the sample is acid or very alkaline neutralise it to a pH of approximately 7, and then add 0.4 g of magnesium oxide. The mixture is shaken and distilled as vigorously as possible from a 1 litre distillation flask fitted with a splash head and a vertical pyrex condenser into 50 ml of boric acid solution. The distillation must be continued until 250-300 ml has distilled over. An attachment to the condenser should dip below the surface of the boric acid.

The residue in the distillation flask should be retained for the determination of nitrogen as nitrate. (Section 12.)

To the distillate add 3 drops indicator solution and titrate the ammonia with 0.02N sulphuric acid, matching the end point with that of a blank containing the same amounts of boric acid and indicator diluted to the same volume with carbon dioxide-free distilled water. A blank should be run on the reagents used and the necessary corrections made.

11.3. Calculation.

$$\text{mg/l ammonia N} = \frac{\text{ml 0.02N acid} \times 0.28 \times 1,000}{\text{ml of sample}}$$

Therefore, if 250 ml of sample has been used the ammonia nitrogen in mg/l is numerically equal to the volume of acid used in the titration.

\*For low concentrations of ammonia it may be necessary to take a larger volume of sample (e.g. 360 ml).

12. THE DETERMINATION OF NITRATE.

After the determination of ammonia by the distillation method using magnesium oxide, nitrates and nitrites in the residue are reduced by boiling with Devarda's alloy and the ammonia so produced is distilled over and determined by the titration method.

12.1. Reagents.

- 12.1.1. All the reagents required for the determination of ammoniacal nitrogen (11.1.) plus;
- 12.1.2. *Devarda's Alloy*: -- Nitrogen-free and fine enough to pass through a 200 mesh sieve.

12.2. Method.

The ammonia is distilled off as described under "Determination of Free and Saline Ammonia" (11.2.) using 0.4 g of light magnesia and 280 ml of sample. The last portion of the distillate is tested with Nessler solution to make sure that all the ammonia has been distilled off, 1 g of Devarda's alloy is added and the mixture is distilled into 50 ml of boric acid solution. When bubbles of gas are visible as the distillation flask is being heated, the heat is turned down in order to avoid frothing and excessive spray formation on the commencement of boiling. The mixture is boiled gently for 5-10 minutes and then vigorously for another 20 minutes, provided this does not cause excessive frothing. Collect about 250-300 ml of distillate and titrate the ammonia with 0.02N sulphuric acid as in the previous method.

Periodically determine a blank on all reagents used in this method and make the necessary corrections.

12.3. Calculation.

$$\text{mg/l nitrate N} = \frac{\text{ml 0.02N acid} \times 0.28 \times 1,000}{\text{ml of sample}} \quad \text{--- nitrite N in mg/l}$$

Therefore if 280 ml of sample has been used the nitrate in mg/l N is numerically equal to the volume of acid used in the titration.

\*For low concentrations of nitrate it may be necessary to take a larger volume of sample (e.g. 500 ml).

12.4. Determination of Nitrite.

(To be used in conjunction with the determination of Nitrate).

12.4.1. Apparatus.

Spectrophotometer, for use at 520 nm providing a light path of 1 cm or longer.

12.4.2. Reagents.

All reagents must be prepared from chemicals which are white in colour.

12.4.2.1. Sulfanilic Acid Reagent. -- Completely dissolve 0.60 g sulfanilic acid in 70 ml hot distilled water, cool, add 30 ml concentrated hydrochloric acid, dilute to 100 ml with distilled water, and mix thoroughly.

12.4.2.2. Naphthylamine Hydrochloride Reagent. -- Dissolve 0.60 g 1-naphthylamine hydrochloride in distilled water to which 1.0 concentrated hydrochloric acid has been added. Dilute to 100 ml with distilled water, and mix thoroughly.

12.4.2.3. Sodium Acetate Buffer Solution. 2M. -- Dissolve 16.4 g sodium acetate ( $\text{NaC}_2\text{H}_3\text{O}_2$ ) or 27.2 g  $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$  in distilled water and dilute to 100 ml. Filter if necessary.

12.4.2.4. Sodium Nitrite Stock Solution. -- Dissolve 0.2463 g anhydrous sodium nitrite ( $\text{NaNO}_2$ ) in nitrite-free distilled water and dilute to 1,000 ml; 1,000 ml = 0.250 mg nitrite as N. Preserve by adding 1 ml chloroform.

12.4.2.5. Standard Sodium Nitrite Solution. -- Dilute 10.00 ml sodium nitrite stock solution to 1,000 ml with nitrite-free distilled water; 1.00 ml = 0.0005 mg nitrite as N. This solution may be preserved by adding 1 ml chloroform, and storing in a sealed bottle.

12.4.2.6. Aluminum Hydroxide. -- Dissolve 125 g potassium or ammonium aluminum sulfate,  $(\text{KAl}_3(\text{SO}_4)_3 \cdot 24 \text{H}_2\text{O}$  or  $(\text{NH}_4)_3\text{Al}_3(\text{SO}_4)_3 \cdot 24 \text{H}_2\text{O}$ ), in 1 litre distilled water. Warm to 60°C and add 55 ml concentrated ammonium hydroxide slowly, with stirring. After permitting the mixture to stand about 1 hour, transfer to a large bottle and wash the precipitate by successive additions (with thorough mixing) and decantations of distilled water, until free from ammonium, chloride, nitrite and nitrate.

12.4.3. Method.

If the sample contains suspended solids and colour, add 2 ml aluminium hydroxide suspension to 100 ml of sample, stir thoroughly, allow to stand for a few minutes, and filter, discarding the first portion of the filtrate.

To 50.0 ml of clear sample which has been neutralised to pH 7, or to an aliquot diluted to 50.0 ml, add 1.0 ml sulfanilic acid reagent. Mix thoroughly. At this point, the pH of the solution should be about 1.4. After it has been standing 3 to 10 minutes, add 1.0 ml naphthylamine hydrochloride reagent and 1.0 ml sodium acetate buffer solution; mix well. At this point, the pH of the solution should be 2.0 to 2.5. Measure the reddish-purple colour after 10 to 30 minutes.

Absorbance readings should be made at 520 *nm* against a reagent blank, and parallel checks should be run frequently against known nitrite standards, preferably in the nitrite range of the sample. Complete calibration curves should be determined following the preparation of new reagents.

An calibration curve should be constructed of absorbance against *mg* nitrite as N, by diluting quantities of standard nitrite solution to 50.0 ml and subjecting them to the same treatment as described for the sample.

12.4.4. *Calculation.*

$$\text{Nitrite as N in mg/l} = \frac{\text{mg nitrite N} \times 1,000}{\text{ml sample}}$$

13. DETERMINATION OF ARSENIC

13.1. *Apparatus.*

- 13.1.1. Arsenic generator (see Fig. 1) consisting of a 200 ml flask, a vertical guard column (scrubber) connected directly to the flask. The upper end of guard column is connected by means of a horizontal glass tube to a vertical absorption tube of approximately 4 inches in length and ¼ inch diameter.
- 13.1.2. Pyrex glass beads.
- 13.1.3. Spectrophotometer for use at 535 *nm*, with light path of 1 cm.

13.2. *Reagents.*

- 13.2.1. *Silver Diethyldithiocarbamate.* -- Dissolve 1 g of silver diethyldithiocarbamate (AgSCSN(C<sub>2</sub>H<sub>5</sub>)<sub>2</sub>) in 300 ml of pyridine. This solution is stable when stored in an amber bottle.
- 13.2.2. *Stannous Chloride Solution.* -- Dissolve 40 g of arsenic-free stannous chloride (SnCl<sub>2</sub> · 2H<sub>2</sub>O) in 100 ml of concentrated hydrochloric acid. This solution is stable.
- 13.2.3. *Potassium Iodide Solution.* -- Dissolve 15 g potassium iodide (KI) in 100 ml of water. This solution is stable when stored in an amber bottle.
- 13.2.4. *Lead Acetate Solution.* -- Dissolve 10 g of lead acetate (Pb(C<sub>2</sub>H<sub>3</sub>O<sub>2</sub>)<sub>2</sub> · 3H<sub>2</sub>O) in 100 ml water.
- 13.2.5. *Arsenic Trioxide Stock Solution (1 ml = 1.0 *mg* As).* -- Dissolve 1.32g of arsenious acid (As<sub>2</sub>O<sub>3</sub>), dried at least 1 hour at 116 C, in 100 ml of 40 per cent sodium hydroxide (NaOH) and dilute to 1 litre. This solution is stable.
- 13.2.6. *Arsenic Trioxide Working Solution (1 ml = 1 *µg* As).* -- Dilute 5 ml stock solution to 500 ml. Dilute 10 ml of resulting solution to 100 ml.
- 13.2.7. *Zinc.* Use granular zinc, about 20 mesh, with arsenic content not greater than 1 x 10<sup>-6</sup> per cent.
- 13.2.8. *Hydrochloric Acid, Concentrated.* Use analytical-grade acid with arsenic content not greater than 1 part per hundred million.

13.3. *Method.*

- 13.3.1. *Construction of calibration curve.* Pipette appropriate aliquots of the working standard, containing 0, 1, 2, 5, 10, 15, and 20 *µg* of As, into clean

numbered generating flasks and dilute to 35 ml. Clean apparatus with nitric acid before beginning the analysis.

To each flask add successively, with thorough mixing after each addition, 5 ml concentrated hydrochloric acid, 2 ml potassium iodide solution, and 8 drops of stannous chloride solution. Allow about 15 minutes for reduction of the arsenic to the trivalent state.

Place in each scrubber a plug of pyrex wool that has been impregnated with lead acetate solution and dried in a vacuum. Assemble the generator, scrubber, and absorber, making certain that all parts fit and are correctly adjusted. Add exactly 5 ml of silver diethylthiocarbamate-pyridine solution to each absorber. Add glass beads to the absorbers until the liquid just covers them.

Disconnect each generator, add 3 g of zinc, and reconnect immediately.

Allow 30 minutes for complete evolution of the arsine. Warm the generator flasks for a few minutes to make sure that all the arsine is released, and then pour the solution from the absorber directly into the spectrophotometer cells. Determine the absorbance of the standards against the blank (0  $\mu\text{g}$  As) without unnecessary delay, as the colour developed is not permanent.

Plot absorbances against concentrations of arsenic in the standards.

- 13.3.2. *Treatment of sample.* When analysing samples, follow the procedure used for the standards in preparation of the calibration curve. If concentrating the samples seems desirable, acidify a suitable sample volume, usually 100 or 200 ml, with hydrochloric acid and evaporate to 35 ml or less. From the graph determine the amount of arsenic in the sample taken.

#### 13.4. Calculation.

$$\text{Arsenic as As in } \mu\text{g/l} = \frac{\mu\text{g As from standard curve}}{\text{ml sample}}$$

### 14. DETERMINATION OF BORON.

#### 14.1. Apparatus.

Spectrophotometer, for use at 585  $m\mu$ , with minimum light path of 1 cm.

#### 14.2. Reagents.

- 14.2.1. *Standard Sodium Hydroxide Solution*, 1 N, Boron-free.  
 14.2.2. *Hydrochloric Acid*, Concentrated.  
 14.2.3. *Hydrochloric Acid*, 1 + 19.  
 14.2.4. *Sulphuric Acid*, Concentrated.  
 14.2.5. *Standard Boric Acid Solution*. -- Dissolve 0.5716 g dry boric acid ( $\text{H}_3\text{BO}_3$ ) in distilled water and dilute to 1 litre. (1 ml = 0.100 mg B).  
 14.2.6. *Carminic Acid Solution*. -- Dissolve 0.92 g carmine NF-40 or carminic acid in 1 litre concentrated sulphuric acid.

#### 14.3. Method.

- 14.3.1. *Preparation of standard curve.* -- Dilute portions of the standard boric acid solution to obtain standards over the range of 0 to 10  $\mu\text{g/l}$  boron

Treat 2.0 ml of each solution as described under 14.3.2.1.3 below and determine the photometer reading against a blank of distilled water carried through the same procedure as the standards. Set blank at 0 absorbance, through the same procedure as the standards. Because the carrying reagent deteriorates, the standard curve should be checked daily.

Plot the absorbance against the concentration.

#### 14.3.2 Treatment of Samples:

14.3.2.1 *Samples not Containing Organic Material:* — Pipette 2.0 ml sample into a small Erlenmeyer flask and add two drops concentrated hydrochloric acid. Add 10.0 ml concentrated sulphuric acid; mix well and cool. Add 10.0 ml carrying solution; mix well and allow to stand at least 45 minutes. Place the photometer reading at 585 nm against a blank of distilled water carried through the same procedure as the sample.

When the boron concentration is less than 1 mg/l, pipette a suitable aliquot into a platinum, silica, or porcelain dish; make alkaline with dilute NaOH and add a slight excess. Prepare a blank containing the same amount of alkali. Evaporate both sample and blank on steam bath to dryness. Place on steam bath to dryness. Treat 5.0 ml dilute hydrochloric acid and dilute with a certain percentage of water. If turbidity is present, pour the solution into a conical centrifuge tube and centrifuge until clear. Take 2.0 ml of the clear solution for analysis. For samples high in boron content dilute with distilled water and take a suitable aliquot for analysis.

14.3.2.2 *Waters Containing Organic Material:* — Make a suitable aliquot just as above with dilute sodium hydroxide (NaOH) and add a slight excess. Prepare a blank containing the same amount of alkali. Evaporate both samples and blank on a steam bath to dryness. Light at 500 °C, cool and add 5.0 ml dilute hydrochloric acid. Make sure that solution is acid. Treat rate with a rubber policeman. Pour the solution into a conical centrifuge tube and centrifuge until clear. Pipette 2.0 ml of the clear solution into an Erlenmeyer flask and proceed as above.

#### 14.4. Continuation

The concentration of boron tested is read from the calibration curve. If sodium hydroxide was used in preparing the sample, correct the result by subtracting the amount of B contributed by it as determined by the reagent blank. If the sample was concentrated or diluted prior to analysis, the result must be multiplied or divided by the appropriate factor to convert the concentration found to that present in the original sample. The results are expressed as follows:

Boron as B in mg/l

### 15. DETERMINATION OF CHROMIUM

#### 15.1. Total Chromium:

##### 15.1.1. Apparatus:

Spectrophotometer for use at 540 nm.

##### 15.1.2. Reagents:

- 15.1.2.1. *Distilled Water.* This should be specially distilled from tap water containing sulphuric acid and a few crystals of potassium permanganate. Atmospheric dust should be excluded during distillation and storage.  
This specially prepared water should be used for the reagents and throughout the procedure.
- 15.1.2.2. *Sulphuric Acid, S.G. 1.84.*
- 15.1.2.3. *Nitric Acid, S.G. 1.42.*
- 15.1.2.4. *Ammonium Oxalate Solution.* -- Saturated.
- 15.1.2.5. *Phosphoric Acid.* -- 60 per cent.
- 15.1.2.6. *Potassium Permanganate Solution.* -- 1 per cent W/V.
- 15.1.2.7. *Sodium Sulphite.* ---  $\text{Na}_2\text{SO}_3 \cdot 7\text{H}_2\text{O}$ .
- 15.1.2.8. *Sodium Hydroxide Solution.* -- 15 per cent W/V.
- 15.1.2.9. *Hydrogen Peroxide.* -- 3 per cent V/V (10 volumes).
- 15.1.2.10. *Diphenylcarbazide Solution.* -- Dissolve 0.25 g of diphenylcarbazide in 25 ml of alcohol and dilute to 100 ml with distilled water.
- 15.1.2.11. *Sulphuric Acid, Dilute.* -- 5 per cent W/V.
- 15.1.2.12. *Standard Chromium Solution.* -- Dissolve 0.3740 g of potassium chromate ( $\text{K}_2\text{CrO}_4$ ) in distilled water and dilute to 1 litre. Dilute 10 ml of this solution to 500 ml. This solution should be freshly prepared as required. (1 ml = 2  $\mu\text{g}$  of chromium).

15.1.3. *Method.*

15.1.3.1. *Construction of Calibration Curve.*

Measure appropriate amounts of standard chromium solution covering the range 0 to 20  $\mu\text{g}$  of chromium into a series of 25-ml calibrated flasks. Add to each 5 ml of dilute sulphuric acid and dilute the solution to about 20 ml. Add 2.5 ml of diphenylcarbazide solution and dilute each solution to the mark. Allow to stand for 5 minutes and then measure the absorbances. Construct a graph relating the absorbances to the number of micrograms of chromium.

15.1.3.2. *Treatment of Sample.*

Place 100 ml (or a suitable volume) of the effluent sample in a 250-ml Kjeldahl flask and dissolve in it 0.1 g of sodium sulphite. Add 2 ml of concentrated sulphuric acid and evaporate until white fumes of sulphur trioxide are evolved. If necessary add concentrated nitric acid drop by drop to oxidize any residual organic matter. Add 10 ml of saturated ammonium oxalate solution and evaporate once more to fumes. When cool, dilute with 10 ml of distilled water and transfer to a 25-ml volumetric flask, and dilute to the mark.

Mix well and place 5 ml or a larger aliquot if necessary, in a small beaker, add 5 drops of phosphoric acid and evaporate to fumes. Cool and add 1 ml of potassium permanganate solution, cover the beaker with a watch glass and heat on a water-bath for 20 minutes.

Neutralise the solution to litmus paper with sodium hydroxide solution and add 1 ml in excess. Add 2 ml of the hydrogen peroxide and allow the solution to simmer gently on a hot plate for 10 minutes. Cool the solution, dilute it to 20 ml in a volumetric flask and filter. Measure accurately a

volume of the filtrate (5 to 10 ml) into a 25 ml calibrated flask, add 5 ml of dilute sulphuric acid and dilute to about 20 ml. Add 2.5 ml of diphenyl carbazide solution and dilute the solution to the mark. Allow to stand for 5 minutes before the colour measurement.

Carry out a blank determination on all reagents used. Measure the absorbance in a spectrophotometer, using a wave-length of 540 mμ. Read the number of micrograms of chromium equivalent to the observed absorbance of the test and blank solutions from the calibration curve, and so obtain the net measure of chromium in the sample.

As the violet colour fades on standing the measurements should be done after 5 minutes.

15.1.4. *Calculation.*

$$\text{Total chromium as Cr in (mg/l)} = \frac{\mu\text{g total Cr}}{\text{ml of sample}}$$

15.2. *Hexavalent Chromium.*

15.2.1. *Reagents.*

Those marked with an asterisk (\*) in Section 15.1.2.

15.2.2. *Method.*

Place 25 ml of the effluent sample in a 50-ml cylinder and add 5 ml of diphenylcarbazide solution, then add 10 ml of dilute sulphuric acid, with mixing, and dilute to 50 ml. Allow to stand for 5 minutes and measure the absorbance as described in the method for "Total Chromium".

If the solution under test is turbid, clear it by centrifuging or filtering in an alkaline condition, using a filter aid.

15.2.3. *Calculation.*

$$\text{Hexavalent chromium - Cr in (mg/l)} = \frac{\mu\text{g total Cr}}{\text{ml of sample}}$$

16. DETERMINATION OF COPPER.

Copper ion has a tendency to be adsorbed on the surface of the sample container. Samples should, therefore, be analysed as soon as possible after collection.

16.1. *Apparatus.*

Spectrophotometer for use at 546 mμ.

16.2. *Reagents.*

16.2.1. *Tartaric Acid Solution*, 50 per cent aqueous. Prepare from analytical reagent grade tartaric acid.

16.2.2. *Hydroxylamine Hydrochloride*, 10 per cent aqueous. Prepare from analytical-reagent grade hydroxylamine hydrochloride, and store in a polythene bottle.

- 16.2.3. *Sodium Hydroxide, 5 N.* -- Prepare from analytical-reagent grade sodium hydroxide.
- 16.2.4. *2,2'-Diquinolyl Solution, 0.05 per cent, in amyl alcohol.*
- 16.2.5. *Sodium Sulphate, anhydrous.* -- Analytical-reagent grade.
- 16.2.6. *Amyl Alcohol.* -- Analytical-reagent grade.
- 16.2.7. *Strong Solution of Copper Sulphate.* -- Dissolve 0.1964 g crystalline copper sulphate in distilled water, add sufficient acid (hydrochloric or sulphuric) to make the final acidity about 0.1N in 500 ml volume. (1 ml = 0.1 mg Cu).
- 16.2.8. *Dilute Solution of Copper Sulphate.* -- Dilute 50.0 ml of the strong solution of copper sulphate to 250 ml with 0.1 N acid (HCl or H<sub>2</sub>SO<sub>4</sub>). A fresh solution must be made from time to time as copper is slowly adsorbed on the glass of the container. (1 ml = 0.02 mg Cu).
- Note:* It is necessary to remove traces of copper from the tartaric acid and hydroxylamine hydrochloride solutions by adding 5 ml of the 2,2'-diquinolyl solution and extracting with three 20 ml portions of amyl alcohol.

### 16.3. Method.

Place 400 ml of the sample in a 500-ml separating funnel, and add 2 ml each of tartaric acid and hydroxylamine hydrochloride solutions. Adjust the pH to between 5 and 6 by using 5 N sodium hydroxide and a narrow range indicator paper (this is critical as no coloured complex will form below pH 4 or above pH 7). Form the lilac-coloured complex by adding 10 ml of 2,2'-diquinolyl solution (an excess produces a milky cloud). Extract the complex in two 20 ml portions of amyl alcohol, dry the combined extracts by swirling with anhydrous sodium sulphate, and filter into a 50 ml calibrated flask. Dilute to the mark with amyl alcohol and measure this absorbance at 546 mμ in the spectrophotometer. Read off the amount of copper present in the aliquot, from a calibration graph. The calibration graph is constructed by submitting a series of copper standards to the same treatment as described for the sample. Plot milligrams of copper against absorbance.

### 16.4. Calculation.

$$\text{Copper as Cu in mg/l} = \frac{\text{mg Cu} \times 1,000}{\text{ml sample}}$$

## 17. DETERMINATION OF PHENOLIC COMPOUNDS.

*Note:* Phenolic compounds are subject to chemical change on standing due to bacterial oxidation. If the sample cannot be analysed within four hours of collection, it should be preserved by the addition of 1 g of copper sulphate per litre. The sample should then be analysed within 24 hours.

### 17.1. Preliminary Screening Procedure.

#### 17.1.1. Apparatus.

Distillation apparatus, all-glass, such as 1 litre Pyrex distilling flask with Graham condenser.

17.1.2. *Reagents.*

All reagents shall be prepared with distilled water free of phenols and chlorine.

17.1.2.1. *Copper Sulphate Solution.* -- Dissolve 100 g of copper sulphate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) in distilled water and dilute to 1 litre.

17.1.2.2. *Phosphoric Acid Solution.* -- Dilute 10 ml of 85 per cent phosphoric acid to 100 ml with distilled water.

17.1.3. *Method.*

To a 500-ml sample of water, add 5.0 ml of copper sulphate solution, unless this has previously been added as a preservative. Lower the pH of the mixture to below 4.0 with phosphoric acid solution; 0.7 ml is sufficient for most samples. Place the mixture in the all-glass distillation apparatus and distil over 450 ml. Stop the distillation and, when boiling ceases, add 50 ml of distilled water to the distilling flask. Continue the distillation until a total of 500 ml has been collected.

17.2. *4-Aminocantipyrine Method.*

17.2.1. *Apparatus.*

Spectrophotometer for use at 460 m $\mu$ , having a light path of 1-10 cm.

17.2.2. *Reagents.*

17.2.2.1. *4-Aminocantipyrine Solution.* -- Dissolve 2.0 g of 4-aminocantipyrine in distilled water and dilute to 100 ml. This solution should be prepared fresh weekly.

17.2.2.2. *Potassium Ferricyanide Solution.* -- Dissolve 8 g of potassium ferricyanide ( $\text{K}_3\text{Fe}(\text{CN})_6$ ) in distilled water and dilute to 100 ml. Filter if necessary. This solution is not stable and should be made up fresh when decomposition occurs, as evidenced by darkening of the solution, usually within a week.

17.2.2.3. *Ammonium Chloride Solution.* -- Dissolve 50 g of ammonium chloride in distilled water and dilute to 1 litre.

17.2.2.4. *Potassium Bromate-Bromide Solution,* approximately 0.1 N. -- Dissolve 2.784 g of potassium bromate in distilled water, add 10 g of potassium bromide, dissolve, and dilute to 1 litre with distilled water.

17.2.2.5. *Potassium Iodide.* -- Crystals.

17.2.2.6. *Ammonium Hydroxide.* -- Concentrated.

17.2.2.7. *Chloroform.*

17.2.2.8. *Starch.* -- Grind 1 g of soluble starch into a smooth paste with a little cold distilled water and pour it into 1 litre of boiling distilled water with constant stirring. Boil for 1 minute, and allow to cool before use.

This solution should be used freshly prepared.

17.2.2.9. *Sodium Thiosulphate Stock Solution,* 0.25 N. -- Dissolve 62 g sodium thiosulphate, ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in 1 litre of copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide to stabilise the solution. Allow to stand for several days before use.

17.2.2.10. *Sodium Thiosulphate Working Solution,* 0.025 N. -- Dilute 100 ml stock solution to 1 litre with copper-free freshly boiled and cooled distilled water,

adding 1 ml chloroform or 10 mg mercuric iodide. This solution is reasonably stable but it should be standardised against potassium dichromate at frequent intervals. Store in an amber glass bottle with a rubber stopper and discard any solution remaining in the burette at the end of the day.

- 17.2.2.11. *Standard Potassium Dichromate Solution, 0.025 N.* -- Dissolve 1.226 g of previously dried ( $K_2Cr_2O_7$ ) in distilled water and dilute to 1 litre.

*Standardisation of Sodium Thiosulphate Solution.* --

Dissolve approximately 2 g potassium iodide (KI), free from iodate, in an Erlenmeyer flask with 100-150 ml distilled water, add 10 ml 1:9 sulphuric acid followed by exactly 20 ml standard dichromate solution. Place in the dark for 5 minutes, dilute to 1 or 2 400 ml and titrate with thiosulphate until a pale straw colour is reached, add starch and titrate until colourless. If the thiosulphate is not exactly 0.025 N adjust it until it is.

- 17.2.2.12. *Phenol Solutions.* --

- (a) *Stock Solution.* -- Dissolve 1 g of phenol in distilled water and dilute to 1 litre. Standardise as directed below.
- (b) *Intermediate Solution.* -- Dilute 10 ml of stock solution to 1 litre with distilled water. One ml of this solution contains 0.01 mg (10 µg) of phenol.
- (c) *Standard Solution.* -- Dilute 5 ml of intermediate solution to 500 ml with distilled water. One ml of this solution contains 0.1 µg of phenol.

*Standardisation of Phenol Solutions.* -- Place approximately 100 ml of distilled water in a 500-ml glass-stoppered Erlenmeyer flask and add 50 ml of phenol stock solution. To this add exactly 10.0 ml of potassium bromate-bromide solution followed by approximately 5 ml of concentrated hydrochloric acid. Swirl gently with flask stoppered. If brown colour of free bromine does not persist, add bromate-bromide solution in exact 10-ml portions until bromine colour does persist. Stopper and allow to stand for 10 minutes, and then add approximately 1 g of potassium iodide. Four 10-ml portions of the bromate-bromide solution are required if the stock solution contains 1,000 mg/l phenol.

Prepare a blank in exactly the same manner, using distilled water and 10 ml of potassium bromate-bromide solution. Titrate both blank and sample with 0.025 N sodium thiosulphate solution, using starch solution as indicator.

Calculate the strength of the phenol solution by the following formula:

$$\text{Phenol in mg/l} = \left\{ \frac{A \times B}{10} - C \right\} \times 7.835$$

where --

A -- ml of 0.025 N sodium thiosulphate solution used for blank.

B -- ml of potassium bromate-bromide solution used for sample.

C -- ml of 0.025 N sodium thiosulphate solution used for sample.

17.2.3. *Method.*

After completion of the preliminary screening, determine by a preliminary check the proper aliquot of the distillate to use for the final determination. This may be done by carrying out the reaction in 50-ml Nessler tubes and comparing against *stable standards*. In this case no chloroform extraction is necessary.

Dilute the indicated aliquot to 500 ml with distilled water and place in a 1-litre beaker. Similarly prepare a blank and a series of 500-ml phenol standards containing 5, 10, 20, 30, 40 and 50 µg of phenol.

Treat the sample, blank and standards as follows: Add 10 ml of ammonium chloride solution and adjust with ammonium hydroxide to pH of 10.0 ± 0.2. This usually requires from 3.5 to 5.0 ml of ammonium hydroxide. Transfer to separating funnels, then add 3.00 ml aminoantipyrine solution. Mix immediately, add 3.00 ml potassium ferricyanide solution, and again mix immediately. Allow to stand 3 minutes and extract immediately with chloroform, using 25-ml for a 5-cm cell and 50-ml for a 10-cm cell. Filter each of the chloroform extracts through fritted-glass funnels containing a 5-g layer of anhydrous sodium sulphate, collecting the dried extracts in dry beakers. Do not add more chloroform. Read the absorbance of the sample and standards against the blank in the spectrophotometer, at a wavelength of 460 mµ, using a 5-cm cell and 10-cm cell with the 25-ml and 50-ml extracts respectively. Plot absorbance values against phenol concentrations (in micrograms) of the standards, estimate the phenol content of the sample.

17.2.4. *Calculation.*

$$\text{Phenolic compounds as phenol in mg/l} = \frac{\text{µg phenol in the aliquot taken}}{\text{ml of aliquot taken.}}$$

## 18. DETERMINATION OF LEAD.

*Note:* The analysis should be carried out in diffused light as bright sunlight tends to destroy dithizone and dithizonates.

Great care should be taken that the reagents and apparatus are free from lead and other metals.

18.1. *Apparatus.*

Spectrophotometer for use at 510 mµ.

18.2. *Reagents.*

18.2.1. *Lead-free Double-Distilled Water.* For preparation of reagents and dilution water.

18.2.2. *Ammonium Hydroxide.* - - 28-29 per cent, lead-free.

18.2.3. *Ammonium Cyanide Solution.* - - Dissolve 40 g potassium cyanide (KCN) in 80 ml water. Extract this solution repeatedly with 10 ml portions of 0.005 per cent dithizone until the last portion remains green. Then wash solution with chloroform until the extract remains clear. Add 1,160 ml ammonium hydroxide to the potassium cyanide solution and make up the entire mixture to 2 litres with water. Store in a glass-stoppered pyrex bottle.

- 18.2.1. *Standard Lead Solution*. -- Dissolve 50 mg diphenylthio-carbazone in 1 litre chloroform. This solution is stable for several weeks if kept at 40 F (4 C) in the dark.
- 18.2.6. *Standard Dithizone Solution*, 0.001 per cent. -- Dissolve 100 ml stock dithizone solution to 500 ml with chloroform. Standardise with spectrophotometer using air blank at 510 m $\mu$ . As this dilute solution will show progressive loss in strength, its strength should be checked before use. Keep solution at 40 F in the dark. This solution is stable for several days.
- 18.2.7. *Hydroxylamine Hydrochloride Solution*. -- Dissolve 20 g hydroxylamine hydrochloride (FH11011, EIC) in distilled water and make up to 100 ml.
- 18.2.8. *Hydrochloric Acid*. -- 1:1.
- 18.2.9. *Nitric Acid*. -- 1:99.
- 18.2.10. *Standard Lead Solution*. -- Dry 1.598 g lead nitrate, Pb(NO<sub>3</sub>)<sub>2</sub> at 110 C. Dissolve and dilute to 500 ml with 1:99 nitric acid. One ml of this solution is equivalent to 0.2 mg lead as Pb. From this prepare a 1 in 20 dilution. This dilute solution will be equivalent to 0.01 mg Pb per ml.
- 18.2.11. *Sodium Citrate Solution*. -- Dissolve 50 g sodium citrate (F442, 1125, 2332) in 90 ml water. Extract with 10 ml portions of 0.005 per cent dithizone until the last portion remains green. Wash with chloroform to remove excess dithizone.
- 18.3.1. *Preparation of Calibration Curve*. -- To 100 ml portions of double-distilled water, add 0 (blank), 0.01, 0.02, 0.03, 0.04, 0.05 mg lead and 1 ml of 1:1 hydrochloric acid. Evaporate each portion to approximately 40 ml, add 10 ml sodium citrate solution and 2 ml ammonium hydroxide. Mix and transfer to a separating funnel. Extract by shaking vigorously for 10 seconds with 5 ml portions of 0.005 per cent dithizone until the colour in the last portion remains unchanged. To the combined extracts add 25 ml of 1:99 nitric acid and shake 1 minute. Discard the chloroform. To the acid extract add 5 ml hydroxylamine hydrochloride solution, 5 ml ammoniacal cyanide solution and exactly 20 ml 0.001 per cent dithizone.
- The order of the addition is important.* Shake vigorously for 1 minute and allow the layers to separate. Discard the first 2 ml chloroform extract and transfer the rest into a dry absorption cell of about 2 cm light path. Set the blank at 100 per cent transmittance and determine the absorbance of the standard solutions at 510 m $\mu$ . Plot an absorbance-concentration calibration curve which should be linear.
- 18.3.2. *Procedure for Water Sample*. -- Take a suitable volume of water containing 0.110 - 0.050 mg lead as Pb, add 1 ml of 1:1 hydrochloric acid and evaporate to about 40 ml. Prepare a comparison blank using double-distilled water and treat in the same way as the unknown. Unless the calibration curve is being determined at the same time, it is advisable also to prepare one or two standard solutions and run them in conjunction with the unknown. Proceed as for the standards above. Read mg Pb from calibration curve.
- 18.4. *Calculation*.
- $$\text{mg Pb in mg/l} = \frac{\text{mg Pb} \times 1,000}{\text{ml sample}}$$
- 31

## 19. DETERMINATION OF PHOSPHATE.

### Apparatus.

- 19.1.1. *Acid-washed Glassware.* -- This may be of great importance, particularly when determining low concentrations of phosphate. Phosphate contamination is common owing to the formation of thin films or adsorption of iron oxide films on glassware. *Commercial detergents containing phosphate should be avoided.* Glassware should be cleaned with hot dilute hydrochloric acid and rinsed well with distilled water.
- 19.1.2. *Spectrophotometer.* -- For use at 690 m $\mu$  providing a light path of at least 1 cm.

### 19.2. Reagents.

- 19.2.1. *Phenolphthalein Indicator Solution.* -- Dissolve 5 g phenolphthalein in 500 ml 95 per cent alcohol and add 500 ml of distilled water. Then add 0.02 N sodium hydroxide until a faint pink colour appears.
- 19.2.2. *Strong Acid Solution.* -- Slowly add 300 ml concentrated sulphuric acid to about 600 ml distilled water. When cool add 4.0 ml concentrated nitric acid and dilute to 1 litre.
- 19.2.3. *Ammonium Molybdate Strong Acid Solution.* -- Dissolve 31.4 g ammonium molybdate ( $(NH_4)_2MoO_4 \cdot 11H_2O$ ) in about 200 ml distilled water. Cautiously add 252 ml concentrated sulphuric acid to 400 ml distilled water. Cool, add 3.4 ml concentrated nitric acid, add the molybdate solution, and dilute to 1 litre.
- 19.2.4. *Amino Naphthol Sulphonic Acid Solution.* -- Weigh out separately 0.75 1-amino-2-naphthol-4-sulphonic acid (use only a powder which is pale pink in colour); 42 g anhydrous sodium sulphite ( $Na_2SO_3$ ); and 70 g sodium metabisulphite (also called sodium pyrosulphite) ( $Na_2S_2O_5$ ). Thoroughly grind the sulphonic acid with a small portion of the  $Na_2SO_3$  powder in a clean dry mortar. Dissolve the remaining salts in about 500 ml distilled water; dissolve the finely ground sulphonic acid in this mixture and dilute to 1 litre. Store in a brown glass-stoppered bottle at a temperature not exceeding 30 C. This solution will become slightly discoloured with time; however, if not contaminated, it will give satisfactory results for most work for 4 months or more. For the most precise work, discard the solution when tests made with standards show a deviation from calibration of 2 per cent of concentration.
- 19.2.5. *Stock Phosphate Solution.* -- Dissolve in distilled water 0.7165 g potassium dihydrogen phosphate, ( $KH_2PO_4$ ) which has been dried in an oven at 105°C. Dilute the solution to 1,000 ml; 1.00 ml -- 0.500 mg  $P_2O_5$ .
- 19.2.6. *Working Phosphate Solution.* -- Dilute 100.0 ml stock phosphate solution to 1,000 ml with distilled water. This solution contains 0.050 mg  $P_2O_5$  per 1.00 ml.

### 19.3. Method.

If precipitation has occurred during the transporting of the sample, mix thoroughly and filter a portion of the sample through an acid-washed, hard-finish, fine filter paper. If the pH of the sample is less than 4, dilute 50.0 ml to 100 ml in a volumetric flask with distilled water, and mix thoroughly. Use this diluted sample in the following steps. If the pH is greater than 10,

add 1 drop of phenolphthalein indicator to 50.0 ml of sample and discharge the red colour with strong-acid solution before diluting to 100 ml. When dilutions are made, the correct interpretation of "ml sample" in the final calculation must be made. For example, 50.0 ml of original sample has been diluted to 100 ml for pH adjustment, the "ml sample" in the calculation is 25 ml, although 50.0 ml of diluted sample is still used in the following steps.

Pipette 50.0 ml of filtered or clear sample into a clean, dry 125-ml Erlenmeyer flask. Add 2.0 ml molybdate-acid solution and mix by swirling. Add 2.0 ml ascorbic acid reagent and mix again. Since the rate and intensity of colour development are dependent on temperature, the reagents, standards, and samples should be at the same temperature (20 — 30 °C).

After exactly 5 minutes, measure the colour photometrically, adjusting the instrument to zero absorbance with a proper blank. Interference from colour, turbidity not removed by filtration and chlorine is greatly reduced or eliminated by preparing the blank from the sample in exactly the same manner except that strong-acid solution is used in place of the molybdate solution. Distilled water treated in the same manner with the strong-acid and ascorbic acid solutions can be used where such interferences are absent. One distilled water blank can be used for a number of interence-free samples. Obtain the weight of ortho-phosphate in the sample taken by referring the reading to the standard curve. This curve is obtained by plotting the absorbance readings from a suitable number of phosphate standards, treated as described above. At least one standard should be tested with each set of samples or once each day that tests are made.

19.4. Calculation

$$\text{Phosphate as } PO_4 \text{ in } \mu\text{g/l} = \frac{\text{mg } PO_4 \times 1,000}{\text{ml sample}} \times 0.127 \times \text{phosphate as } PO_4 \text{ in } \mu\text{g/l}$$

20. DETERMINATION OF IRON

20.1. Apparatus

- 20.1.1. *Reduced Glassware*. — All glassware must be washed with concentrated hydrochloric acid and rinsed with distilled water prior to use, in order to remove the thin film of adsorbed iron oxide which is frequently present as a result of employing glassware for other purposes.
- 20.1.2. *Spectrophotometer*. — For use at 510 nm providing a light path of 1 cm or longer.

20.2. Reagents

All reagents must be low in iron. Iron-free distilled water is required. Glass-stoppered bottles are recommended for storage. The hydrochloric acid, ammonium acetate solution and stock iron solutions are stable and - finally if tightly stoppered. The hydroxyferric and perchloric solutions are stable for several months. The working iron solutions are not stable and must be prepared freshly as needed by diluting the stock solution.

- 20.2.1. *Hydrochloric Acid.* -- Concentrated.
- 20.2.2. *Hydroxylamine Reagent.* -- Dissolve 10 g hydroxylamine hydrochloride ( $\text{NH}_2\text{OH}\cdot\text{HCl}$ ) in 100 ml distilled water.
- 20.2.3. *Ammonium Acetate Buffer Solution.* -- Dissolve 250 g ammonium acetate ( $\text{CH}_3\text{COONH}_4$ ) in 150 ml distilled water. Add 700 ml glacial acetic acid and dilute to 1 litre. (Since even good grade ammonium acetate contains a significant amount of iron, new reference standards should be prepared with each buffer preparation).
- 20.2.4. *Phenanthroline Solution.* -- Dissolve 0.1 g of 1,10-phenanthroline monohydrate ( $\text{C}_{12}\text{H}_8\text{N}_2\cdot\text{H}_2\text{O}$ ) in 100 ml distilled water by stirring and heating to  $80^\circ\text{C}$ ; do not boil. Discard the solution if it darkens. Heating is not necessary if 2 drops of concentrated hydrochloric acid are added to the distilled water. (Note that 1 ml of this reagent is sufficient for no more than 0.1 mg Fe.)
- 20.2.5. *Iron Stock Solution.* -- Slowly add 20 ml concentrated sulphuric acid to 20 ml of distilled water and dissolve in it 0.7022 g of ferrous ammonium sulphate ( $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2\cdot 6\text{H}_2\text{O}$ ). Add dropwise 0.1 N potassium permanganate solution until a faint pink colour persists. Dilute with iron-free distilled water to 1,000 ml and mix. This stock solution contains 0.10 mg of Fe per 1.00 ml.
- 20.2.6. *Iron Working Solutions.* -- These should be prepared the day they are to be used.
- Pipette 100.00 ml stock solution into a 1 litre volumetric flask and dilute to the mark with iron-free distilled water. This solution contains 0.010 mg Fe per 1.00 ml.
  - Pipette 10.00 ml of stock solution into a 1 litre volumetric flask and dilute to the mark with iron-free distilled water. This solution contains 0.001 mg Fe per 1.00 ml.

### 20.3. Method.

Mix the sample thoroughly and measure 50.0 ml into a 125-ml Erlenmeyer flask. Add 2 ml concentrated hydrochloric acid and 1 ml hydroxylamine reagent. Add a few glass beads and heat to boiling. To insure solution of all the iron, boiling may be continued until the volume is reduced to 15-20 ml. Cool to room temperature and transfer to a 100-ml volumetric flask. Add 10 ml acetate buffer solution and 2 ml phenanthroline solution, and dilute to the mark with distilled water. Mix thoroughly and allow at least 10-15 minutes for maximum colour development.

### 20.4. Colour Measurement.

Prepare a series of standards by accurately pipetting the calculated volumes of working iron solutions (the weaker solution should be used to measure the 0.001 - 0.010 mg portions) into 125-ml Erlenmeyer flasks, diluting to 50 ml, and carrying out the procedure as set out above. A light path of 1 cm should be used. The standards should be read against distilled water set at zero absorbance and a calibration curve plotted of absorbance against mg of Fe. If the samples are at all coloured or turbid a second set of identical aliquots of the samples may be carried through all steps of the procedure except that no phenanthroline is to be added. Then instead of distilled water, the prepared blanks are used to set the photometer to zero absorbance, and each developed sample, with

phenanthroline is read against the corresponding blank, without phenanthroline. Observed photometer readings are translated into iron values by means of the calibration curve. If colour and turbidity are absent, it is quicker and just as satisfactory to read the developed samples, as well as the standards, against distilled water.

#### 20.5. Calculation.

$$\text{Iron as Fe in mg/l} = \frac{\text{mg Fe} \times 1,000}{\text{ml sample}}$$

### 21. DETERMINATION OF MANGANESE.

#### 21.1. Sampling and Storage.

Manganese should be determined very soon after collection. If delay is unavoidable, the sample should be acidified at the time of collection.

#### 21.2. Apparatus.

Spectrophotometer for use at 525 m $\mu$ , providing a light path of at least 1 cm.

#### 21.3. Reagents.

21.3.1. "Special Solution". -- Dissolve 75 g mercuric sulphate in 400 ml of concentrated nitric acid and 200 ml distilled water. Add 200 ml 85 per cent phosphoric acid and 0.035 g silver nitrate, and dilute the cooled solution to 1 litre.

21.3.2. Ammonium Persulphate. -- Solid.

21.3.3. Manganous Sulphate Standard Solution. -- Prepare a 0.1 N potassium permanganate solution in the usual manner by dissolving 3.2 g potassium permanganate in distilled water and making up to 1 litre. This solution must be aged for several weeks in sunlight or heated for several hours near the boiling point, then filtered, through a fritted-glass filter and carefully standardised against sodium oxalate. Calculate the volume of this solution necessary to prepare 1 litre of solution of such strength that 1.00 ml = 0.050 mg of manganese as Mn, as follows:

$$\text{ml permanganate} = \frac{4.55}{\text{normality of permanganate solution}}$$

To this volume add 2 to 3 ml of concentrated sulphuric acid and then calcium bisulphite solution (10 g NaHSO<sub>3</sub> plus 100 ml distilled water) dropwise with stirring, until the permanganate colour disappears.

Boil to remove excess sulphur dioxide, cool, and dilute to 1 litre with distilled water. This solution may be diluted further in order to measure small amounts of manganese.

21.3.4. Hydrogen Peroxide Solution. -- 30 per cent.

#### 21.4. Method.

21.4.1. Treatment of Sample. -- To a suitable aliquot of the sample, add 5 ml of the "Special solution". Concentrate to 90 ml by boiling or dilute to

90 ml. Add 1 g ammonium persulphate and bring to boiling in about 2 minutes over a flame. Do not heat on a waterbath. Remove from the flame for 1 minute, then cool under the tap. (Too long boiling time or too slow cooling results in decomposition of excess persulphate and subsequent loss of permanganate colour). Dilute 100 ml with distilled water, free from reducing substances, and mix. Make photometric measurements against a distilled water blank using a wave-length of 525 nm. Standards containing 0, 0.005, 0.010, etc., to 1.5 mg Mn should be treated in the same way and a calibration graph constructed.

21.4.2. *Correction for Turbidity or Interfering Colour.* - Filtration is not recommended because of the possibility that some of the permanganate may be retained on the filter paper. The following "Bleaching" method should be used: As soon as the photometer reading has been made, add 0.05 ml hydrogen peroxide solution directly to the sample in the optical cell. Mix the solution and, as soon as the permanganate colour has completely faded and no bubbles are left, take the absorbance reading again. The absorbance reading can be subtracted from the original reading, or it can be read from the calibration curve as "interference as manganese" and then subtracted from the "apparent manganese" to give the true manganese figure.

21.5. *Calculation.*

$$\text{Manganese as Mn in mg/l} = \frac{\mu\text{g Mn} \times 1,000}{\text{ml sample}}$$

22. DETERMINATION OF CYANIDES AND RELATED COMPOUNDS.

22.1. *Preservation of Samples.*

Since most cyanides are very reactive and unstable, analysis should be made as soon as possible after sampling. If the sample cannot be analysed immediately, add sodium hydroxide, to raise the pH to 11.0 or above and store in a cool place.

22.2. *Preliminary Screening Procedure.*

*Caution.*

Due care should be exercised in the manipulation of cyanide samples because of the toxicity involved.

22.2.1. *Interference.*

22.2.1.1. *Sulphide interference.* - Is removed by treating the alkaline sample at pH 11.0 with small increments of powdered lead carbonate. Black lead sulphide precipitates in samples containing sulphide. Repeat this operation until no more lead sulphide forms. Filter and rinse the precipitate, add the rinse water to the filtrate and use an aliquot for analysis. Avoid a large excess of lead carbonate and a long period of contact in order to minimise complexing or occlusion of the cyanide with the precipitated material.

22.2.1.2. *Oxidizing agents* - if present will give a positive test with starch-iodide paper and can be removed by titrating with sodium sulphite (12.6 g anhydrous Na<sub>2</sub>SO<sub>3</sub> per litre of solution) until a negative test is obtained.

Other interfering substances can be removed by the distillation procedure outlined below:

## 22.2.2. Apparatus.

The following apparatus (see Fig. 2) is required:—

- 22.2.2.1. *Modified Claissen flask*, 500 ml, having ground glass joints, with side-arm delivery tube removed and the opening sealed.
- 22.2.2.2. *Air inlet*, consisting of a thistle tube with a ground glass joint, having a glass tube (3 mm internal diameter) fused to the joint and extended to 1 inch from the bottom of the modified Claissen flask.
- 22.2.2.3. *Condenser*. — with ground glass cone and socket, the bottom cone to fit the modified Claissen flask.
- 22.2.2.4. *Gas washer*. — spiral flow type, liquid capacity 100-250 ml, (such as the Fisher-Milligan spiral gas washer), fitted if possible with a rubber drain tube with a pinch clamp at the bottom.
- 22.2.2.5. *Suction flask*, 500 ml capacity.
- 22.2.2.6. *Water aspirator*.
- 22.2.2.7. *Heating element*. — for the modified Claissen flask.
- 22.2.2.8. *Connecting tubing*. — rubber or glass as indicated.

## 22.2.3. Reagents.

- 22.2.3.1. *Sodium Hydroxide Solution, 1N*. — Dissolve 4 g sodium hydroxide (NaOH) in 100 ml distilled water.
- 22.2.3.2. *Mercuric Chloride Solution*. — Dissolve 34 g mercuric chloride ( $\text{HgCl}_2$ ) in 500 ml distilled water. (CAUTION: Toxic; take care to avoid ingestion).
- 22.2.3.3. *Magnesium Chloride Solution*. — Dissolve 51 g magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) in 100 ml distilled water.
- 22.2.3.4. *Sulphuric acid*. — Concentrated.

## 22.2.4. Procedure.

Add 250 ml of the sample or an aliquot diluted to 250 ml, to the Claissen flask. Add exactly 50 ml of sodium hydroxide solution to the gas washer and dilute if necessary with distilled water to obtain an adequate depth of liquid in the absorber. Connect the train, consisting of boiling flask air inlet, flask, condenser, gas washer, suction flask trap, and aspirator. Adjust the suction so that approximately one bubble of air per second enters the boiling flask through the air inlet. This air rate will effectively act as a carrier for the HCN gas from flask to absorber and will usually prevent a reverse flow of HCN through the air inlet. If the air rate does not prevent the sample from backing up in the delivery tube, the air flow rate may be increased up to two bubbles a second without loss in efficiency. If the air rate becomes too high, the gas washer will not trap all the HCN.

Add 20 ml mercuric chloride solution and 10 ml magnesium chloride solution through the air inlet tube. Rinse tube with distilled water and allow the air flow to mix the flask contents for three minutes. Slowly add concentrated sulphuric acid in an amount equal to 5 ml for each 100 ml of solution in the distilling flask, and rinse the air inlet once more.

Heat at a rate which will provide rapid boiling but will not flood the condenser inlet or permit vapours to rise more than half-way into the condenser. Reflux for 1 hour. Turn off the heat but continue the air flow. After 15 minutes of cooling, drain the gas washer contents into a

250 ml volumetric flask. Rinse the connecting tube from the condenser to the gas washer with distilled water, add the rinse water to the drained liquid, and dilute to 250 ml.

Refill the gas washer with a fresh charge of sodium hydroxide solution and repeat the reflux procedure.

Determine the cyanide content of both portions of absorption liquid by the colorimetric method outlined below, (22.3.).

If the sample contains only readily hydrolysed cyanides the absorber liquid from the first reflux will contain all of the cyanide; if stable complex cyanides are present, a significant yield will appear in the absorber liquid from the second reflux. The total of the two concentrations represents the total concentration of cyanide in the sample.

### 22.3. Colorimetric Determination.

#### 22.3.1. Apparatus.

- 22.3.1.1. *Spectrophotometer*, for use at 620 m $\mu$ , providing a light path of 1 cm.
- 22.3.1.2. *Reaction tubes* consisting of test tubes, approximately 1 x 8 inches, fitted with rubber stoppers.

#### 22.3.2. Reagents.

- 22.3.2.1. *Sodium Hydroxide Solution, 0.2N.* - Dissolve 8 g sodium hydroxide (NaOH) in 1 litre distilled water.
- 22.3.2.2. *Acetic Acid (glacial), 1+4.*
- 22.3.2.3. *Stock Cyanide Solution.* - Dissolve 2.51 g potassium cyanide (KCN) in 1 litre water. Standardize against silver nitrate as outlined below. The solution loses strength gradually and must be rechecked every week. Approximate strength, 1 ml = 1  $\mu$ g CN. (CAUTION: Toxic; take care to avoid ingestion).
- 22.3.2.4. *Standard Cyanide Solution.* Dilute 10 ml stock cyanide solution to 1 litre with distilled water, *mb.*, and make a second dilution of 10 ml of this solution to 100 ml with sodium hydroxide (0.2N; 1.00 ml = 1.0  $\mu$ g CN. (Actual strength is determined by standardization of stock solution). This solution must be prepared daily. (CAUTION: Toxic; take care to avoid ingestion).
- 22.3.2.5. *Chloramine-T Solution.* Dissolve 1 g in 100 ml water. Prepare daily.
- 22.3.2.6. *1-phenyl-3-methyl-5-pyrazolone solution.* Prepare a saturated aqueous solution (approximately 0.5 g/100 ml) by adding the pyrazolone to water at approximately 75 C. Agitate occasionally as the solution cools to room temperature. If necessary, the pyrazolone (melting point 127 C-128 C) can be purified by recrystallization from ethyl alcohol. Usually this is not required.
- 22.3.2.7. *Pyridine.*
- 22.3.2.8. *Bis-pyrazolone.* - Dissolve 17.4 g of 1-phenyl-3-methyl-5-pyrazolone in 100 ml ethyl alcohol. Add 25 g phenyl hydrazine, freshly distilled under reduced pressure. Reflux in an all-glass apparatus. Several hours of refluxing is necessary to produce the bis-pyrazolone, which is indicated by formation of crystals in the reflux mixture. A reflux of 6-8 hours, followed by standing at room temperature overnight, and 1-2 hour reflux the following day are generally adequate for a good yield. Filter while hot, wash with hot 95 per cent ethyl alcohol, and air dry. The product (melting point greater than 320 C) is stable indefinitely in a dry form.

22.3.2.9. *Mixed pyridine-pyrazolone reagent.* — Mix 125 ml of the filtered, saturated aqueous solution of pyrazolone with a filtered solution containing 0.025 g bis-pyrazolone dissolved in 25 ml pyridine. Several minutes of mixing is usually necessary to dissolve the bis-pyrazolone in pyridine. The mixed reagent develops a pink colour on standing but, if used within 24 hours, this does not affect the colour production with cyanide. Prepare daily.

### 22.3. Standardization of Stock Cyanide Solution.

#### 22.3.3.1. Reagents.

- (a) *Indicator solution.* — Dissolve 0.02 g pyridimethylamino-benzal-rhodanine in 100 ml acetone.
- (b) *Standard silver nitrate solution, 0.0192N.* — Dissolve 3.27 g silver nitrate ( $\text{AgNO}_3$ ) in 1 litre distilled water. Standardize against standard sodium chloride solution using the Mohr method with potassium chromate ( $\text{K}_2\text{CrO}_4$ ) indicator as indicated in Section 4.4. 1.00 ml of this solution is equivalent to 1.00 mg CN.

#### 22.3.3.2. Procedure.

Take an aliquot (10 ml) of the stock cyanide solution and adjust the pH to 11.0 or above with sodium hydroxide solution. Dilute the aliquot to 250 ml. Add 0.5 ml indicator solution.

Titrate with standard silver nitrate solution to the first change from a canary yellow to a salmon hue. Titrate a blank containing the same amount of alkali and water.

Strength of cyanide stock solution in mg CN/ml

$$\frac{\text{ml standard silver nitrate for aliquot} - \text{blank}}{\text{ml of aliquot taken}}$$

#### 22.3.4. Procedure for Colorimetric Determination of Cyanide.

Prepare one or more aliquots of the absorption liquid obtained from the distillation procedure in reaction tubes which, together with their stoppers, have been carefully rinsed with distilled water. Dilute each to 15 ml with 0.2N sodium hydroxide, and neutralize with acetic acid to pH 6-7.

Prepare a blank by adding 15 ml 0.2N sodium hydroxide to a carefully rinsed reaction tube. Also prepare a series of standards containing 0.5 to 6.0  $\mu\text{g}$  CN, all made up to 15 ml with 0.2N sodium hydroxide. Neutralize blank and standards to pH 6-7 with acetic acid. The amount of the acid for all diluted aliquots, the blank, and the standards should be approximately the same.

Add 0.2 ml chloramine-T solution, stopper, and mix by inversion two or three times. Allow 1-2 minutes for the reaction.

Add 5.0 ml of mixed pyridine-pyrazolone reagent, stopper and mix by inversion. Allow 20 minutes for colour development.

Dilute all reaction tubes accurately to a definite volume -- usually 25 ml -- mix, and read absorbance at 620 m $\mu$ , on all samples and standards. If the colour of the distillate aliquot is too intense repeat the test using a smaller aliquot.

Construct a calibration graph of absorbance versus  $\mu\text{g}$  CN.

22.3.3. *Calculation.*

Cyanides and related compounds as CN in mg l<sup>-1</sup> =

$$\frac{\text{ug CN in test aliquot}}{\text{ml test aliquot}} \times \frac{250}{\text{ml original sample}}$$

23. DETERMINATION OF SULPHIDE.

23.1. *Treatment of Sample.*

In the determination of sulphide care must be taken to ensure that errors do not arise from any of the following causes:—

- (a) Loss of free hydrogen sulphide from the sample to the atmosphere.
- (b) Entry of oxygen into the sample with resultant loss of sulphide by oxidation.
- (c) Formation of hydrogen sulphide due to storing the sample under anaerobic conditions.

The best results will be obtained if the sample is analysed immediately and if loss of free hydrogen sulphide is prevented by addition of a small volume of zinc acetate solution to the sample when it is taken.

23.2. *Reagents.*

23.2.1. *Ammonio-sulphuric Acid Solution.*—

- (a) *Stock Solution.* — Distill para-amino-dimethyl-aniline in an all-glass apparatus from which air has been displaced by an inert gas. Mix very cautiously 50 ml of concentrated sulphuric acid with 30 ml water and cool. Add to this 20 g of the purified amine, stirring until solution is complete. Make up to 100 ml with water.

<sup>a</sup>If any of the amine gets on the skin wash it off immediately with dilute hydrochloric acid.

Alternatively, mix 46 ml of concentrated sulphuric acid with 30 ml water and cool. Add to this 27.2 g para-amino-dimethylaniline sulphate, stirring until solution is complete. Make up to 100 ml with water.

*Note:* This assumes that the amine sulphate is available in a much purer state than the amine itself.

- (b) *Working Solution.* — Dilute 25 ml of the stock solution to 1 litre with 1:1 sulphuric acid.

23.2.2. *Dilute Sulphuric Acid (1:1).* — Mix very cautiously equal volumes of concentrated sulphuric acid and distilled water.

23.2.3. *Ferric Chloride Solution.* — Dissolve 100 g of ferric chloride (FeCl<sub>3</sub>·6H<sub>2</sub>O) in water and make up to 100 ml.

23.2.4. *Ammonium Phosphate Solution.* — Dissolve 400 g of ammonium phosphate (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> in water and make up to 1 litre.

23.2.5. *Methylene Blue Solution.* —

- (a) *Strong Solution.* - Dissolve 1.00 g of methylene blue in water and make up to 1 litre. Standardise the solution as described below, (23.4.)
- (b) *Dilute Solution.* - Dilute 10 ml of the strong solution to 100 ml with distilled water.
- 23.2.6. *Aluminium Sulphate Solution.* - Dissolve 24 g of aluminium sulphate ( $Al_2(SO_4)_3 \cdot 18H_2O$ ) in water and dilute to 100 ml.
- 23.2.7. *Sodium Hydroxide Solution.* - Dissolve 9 g of sodium hydroxide in distilled water. Cool and dilute to 100 ml.
- 23.2.8. *Sodium Thiosulphate.* -
- 23.2.8.1. *Preparation.*
- (a) *Stock Solution, 0.25N.* - Dissolve 67.4 g sodium thiosulphate, ( $Na_2S_2O_3 \cdot 5H_2O$ ) in 1 litre of copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide to stabilise the solution. Allow to stand for several days before use.
- (b) *Working Solution, 0.025N.* - Dilute 100 ml of stock solution to 1 litre with copper-free, freshly boiled and cooled distilled water, adding 1 ml chloroform or 10 mg mercuric iodide. This solution is reasonably stable but it should be standardised against potassium dichromate at frequent intervals. Store in an amber glass bottle with a rubber stopper and discard any solution remaining in the burette at the end of the day.
- 23.2.8.2. *Standardisation of Sodium Thiosulphate Solution.* -
- Dissolve approximately 2 g potassium iodide (KI) free from iodate in an Erlenmeyer flask with 100-150 ml distilled water, add 10 ml 1:9 sulphuric acid followed by exactly 20 ml standard dichromate solution. Place in the dark for 5 minutes, dilute to ~400 ml and titrate with thiosulphate until a pale straw colour is reached, add starch and titrate until colourless. If the thiosulphate is not exactly 0.025N adjust it until it is.
- 23.2.9. *Standard Potassium Dichromate Solution, 0.25N.* - Dissolve 1.226 g previously dried potassium dichromate ( $K_2Cr_2O_7$ ) in distilled water and dilute to 1 litre.
- 23.2.10. *Iodine Solution, 0.025N.* - Add 3.175 g iodine to about 25 g potassium iodide and moisten with water, slowly add more water with shaking until dissolved; dilute to 1 litre and standardise against 0.025N sodium thiosulphate.
- 23.2.11. *Starch Solution.* - 0.5 g to 200 ml freshly prepared

### 23.3. Method.

Pipette 7.5 ml portions of sample into each of two test tubes which are accurately matched as to diameter. To the first tube add 0.5 ml amine-sulphuric-acid working solution.

To the second tube add 0.5 ml dilute sulphuric acid. Add 2 drops ferric chloride solution to each tube, close them with the thumbs and slowly invert them once or twice to mix the contents. When sulphide is present a blue colour will appear in the first tube; development of colour is complete after approximately 1 minute. After 2 to 5 minutes add 1.6 ml of ammonium

phosphate solution to each tube and mix. (This diminishes the colour due to the ferric chloride and reduces the acidity of the solution enabling a more intense blue colour to form).

After 5 minutes add methylene blue solution a drop at a time to the second of the two tubes, mixing between each addition, until the colour matches that of the first tube. The solution should be added either from standard dropping pipettes which deliver 20 drops per ml or from 1 ml burettes. If the methylene blue is of the correct strength 1 drop of the strong solution (0.05 ml) is equivalent to 1 mg/l sulphide and 1 drop of the dilute solution is equivalent to 0.1 mg/l sulphide. For highest accuracy an equal volume of water should be added to the first tube before the colours are matched thus eliminating any error due to the changing volume in the second tube.

#### 23.4. *Standardisation of the Methylene Blue.*

Completely fill a one-gallon bottle with acidified distilled water, add a washed crystal of sodium sulphide (about 100 to 200 mg), stopper the bottle, and then thoroughly mix.

Pipette 20 ml of 0.025N iodine solution into a 500 ml volumetric flask. Siphon in sulphide solution from the bottom of the gallon bottle to fill the flask to the mark. Transfer the contents to a suitable beaker, add 1 ml concentrated sulphuric acid and titrate the excess iodine with 0.025N thio-sulphate solution using starch's indicator. Multiply the net volume of 0.025N iodine solution used by 0.831 to obtain the concentration of sulphide in mg/l.

Test the sulphide solution by the methylene blue procedure given above and adjust the strength of the methylene blue solutions to give the same value for the concentration of sulphide as was obtained by the titration method.

#### 23.5. *Expression of Results.*

Sulphide as S in mg/l.

### 24. DETERMINATION OF FLUORIDE.

#### 24.1. *Distillation Procedure.*

The distillation procedure is recommended as giving greater reliability with unknown samples. It eliminates the necessity for complete analysis and has the advantage of eliminating suspected as well as known interferences. In general, distillation is resorted to when the total correction due to interfering substances will exceed  $\pm$  or  $\pm$  0.1 mg/l.

##### 24.1.1. *Apparatus.*

A Claisen flask with a thermometer and steam inlet extending to 1/16th inch of the bottom of the flask. The other outlet is connected to a vertical Liebig condenser. The steam inlet is connected to a steam generating flask over a separate source of heat. However, a separating funnel containing either distilled water or the sample may be used in place of the steam inlet. When a steam generator is used, the steam inlet should terminate in a fan-shaped outlet facing downward of about 45°. When a separating funnel is used, the inlet to the flask should terminate with a capillary tip. Bumping is minimised by placing glass beads in the steam generator and Claisen flask.

**24.1.2. Reagents.**

- 24.1.2.1. *Silver Sulphate*. — Solid.
- 24.1.2.2. *Sodium Hydroxide Solution*. — Dissolve 10 g sodium hydroxide (NaOH) in 100 ml distilled water.
- 24.1.2.3. *Sulphuric Acid*. — Concentrated.
- 24.1.2.4. *Phenolphthalein indicator solution*.

**24.1.3. Procedure.**

- 24.1.3.1. When the sample contains less than 0.6 mg/l of fluoride as F<sup>-</sup> concentrate the sample in the following way:—

Take 150 ml sample alkaline with sodium hydroxide (NaOH) using phenolphthalein indicator, and add a few drops (NaOH) in excess. Concentrate to 15-20 ml. When cool transfer quantitatively to the distillation flask, and carefully add 13 ml of concentrated sulphuric acid. If the amount of chloride in the aliquot exceeds 1 mg add about 5 mg solid silver sulphate for each mg of chloride. Connect the condenser, thermometer, and steam inlet assembly with the steam exit valve open. Heat the mixture to boiling.

As the distillate is collected, the water in the steam generator is brought to the boil and the steam permitted to escape. As soon as the temperature reaches 110°C, the steam is introduced into the distillation flask. Heat from the two burners is regulated to maintain a temperature of 140-150°C (never go over 150°C), and a distillation rate not less than 3 ml per minute.

The distillation is continued until 150 ml are collected.

- 24.1.3.2. When the sample contains 0.6 mg/l of fluoride as F<sup>-</sup> or more, a 50 ml portion is transferred to the distillation flask, treated as described above for the sample concentrate, and the distillate collected without prior evaporation.

A total of 150 ml of distillate is collected, the excess over the original volume coming from the steam.

Treat the distillate as in the following method to determine the fluoride.

**24.2. Colorimetric Method.****24.2.1. Interference.**

The important known interferences and their effects are listed in the table.

This information may be used to correct the apparent fluoride concentration, if the total correction is not greater than 0.1 mg/l. It may also be used to estimate whether distillation is necessary. Unless otherwise noted, the interference is independent of the fluoride concentration, and is assumed to be algebraically additive. If the interference from any one substance is 0.1 mg/l, the sample should be distilled even though a compensating interference may be present.

TABLE III

## THE EFFECT OF INTERFERING SUBSTANCES ON FLUORIDE DETERMINATION.

Interfering Substance	Concentration (mg/l)	Effect on F Reading.	
		Increase or Decrease.	Amount in mg/l.
Alkalinity (as CaCO <sub>3</sub> )	325	Decrease.	0.1
Al <sup>3+</sup> F <sup>-</sup> F <sup>-</sup>	0.2	Decrease	0.1
Cl <sup>-</sup>	1,800	Decrease	0.1
Fe <sup>3+</sup> F <sup>-</sup> F <sup>-</sup>	5	Increase	0.1
Cl <sub>2</sub> (must be completely removed.)		Increase	
PO <sub>4</sub> <sup>3-</sup>	1.0	Increase	0.1
(NaPO <sub>3</sub> ) <sub>2</sub>	1.1	Increase	0.1
SO <sub>4</sub> <sup>2-</sup>	400	Increase	0.1
Ca <sup>2+</sup> F <sup>-</sup> F <sup>-</sup>	400	No effect	0.0
Mg <sup>2+</sup> F <sup>-</sup> F <sup>-</sup>	200	No effect	0.0

- Notes:- (a) The value given applies only to fluoride concentrations above 0.5 mg/l. Below 0.5 mg/l F<sup>-</sup>, the error varies from 0.1-0 mg/l as the fluoride concentration is reduced to 0.
- (b) When fluoride is absent, phosphate exerts a much greater effect than when fluoride is present.

## 24.2.2. Apparatus.

*Spectrophotometer.* -- For use at 525 mμ, providing a light path of 1 cm.

## 24.2.3. Reagents.

- 24.2.3.1. *Standard Sodium Fluoride Solution.* -- Dissolve 0.2210 g sodium fluoride (NaF) in distilled water and dilute to 1 litre. Dilute 100 ml of this stock solution to 1 litre with distilled water. 1.00 ml is equivalent to 0.0100 mg fluorine as F.
- 24.2.3.2. *Sodium Arsenite Solution,* approximately 0.028N. -- Dissolve 1.83 g sodium arsenite (Na<sub>2</sub>AsO<sub>3</sub>) in 1 litre distilled water. Prepare fresh every 6 months.
- 24.2.3.3. *Alizarin Red Solution.* -- Dissolve 0.375 g Alizarin Red S in distilled water and dilute to 500 ml. If insoluble material is present, filter through Whatman No. 40 filter paper. *Protect from direct sunlight.*
- 24.2.3.4. *Zirconyl-acid Solution.* -- Dissolve 0.184 g zirconium oxychloride (ZrOCl<sub>2</sub>·8H<sub>2</sub>O) in 50 ml water and make up to 500 ml with Scott's Mixed Acid (24.2.3.5.).
- 24.2.3.5. *Scott's Mixed Acid.* -- Add 68 ml concentrated hydrochloric acid to 182 ml water and add 22 ml concentrated sulphuric acid to 228 ml water and then mix the two acid solutions together.

## 24.24. Method.

24.24.1. *Preparation of Standard Curve*. - Prepare fluoride standards in the range 0.00 - 3.00 mg/l by diluting appropriate quantities of the standard fluoride solution to 100 ml with distilled water. Adjust the temperature of the standards to the temperature at which subsequent analyses are to be done (23-27°C). Add 5.00 ml of alizarin red solution and 5.00 ml of the zinc-lead solution to each standard, mix well and allow the reaction to proceed for 60 - or - 2 minutes.

Set the photometer to zero absorbance (100 per cent transmittance) with distilled water. (If the instrument drifts the zero standard cannot be used to check the original reference point at a later stage due to progressive colour development in the standard).

At the end of 60 - or - 2 minutes take the absorbance readings of the standards at 575 nm. A new standard curve must be plotted whenever a new batch of either of the reagents is made up. The standard curve must be checked at several points whenever analyses are made.

24.24.2. *Treatment of Sample* (If it has not been distilled). - If sample contains free chlorine, remove it by adding 2 drops 0.1 ml ascorbic solution for each 0.1 mg Cl<sub>2</sub> and mix. Add 2 drops in excess. If sample is turbid filter it.

24.24.3. *Analysis of Sample*. - Use 100 ml of sample or aliquot diluted to 100 ml. Adjust the temperature of the sample to that of the standards. Add 5.00 ml alizarin red solution and 5.00 ml zinc-lead solution and mix well. Read the absorbance after 60 - or - 2 minutes, first setting the reference point of the photometer in the same way as was done for the standard curve.

## 24.25. Calculation.

Read mg fluoride as F<sup>-</sup> from the standard curve. -

$$\frac{\text{mg F} \times 1,000}{\text{ml sample}} = \text{mg/l}$$

(Correct the fluoride value if necessary from Table III.)

## 25. DETERMINATION OF ZINC

## 25.1. Interference.

Ferric iron, chlorine and other oxidizing agents decompose dithizone to form a usually yellow brown product. Carelessness should be very carefully cleaned with dilute nitric acid, then distilled water and finally with a mixture of sodium citrate and dithizone solution.

## 25.2. Collection of Samples and Storage.

Samples should preferably be analysed within 6 hours after collection. "Packing" with hydrochloric acid will preserve the metallic ion content, but requires that -

(a) the acid is zinc-free;

(b) the sample bottles are rinsed with acid before use;

- (c) The samples are evaporated to dryness in silica dishes before they are analysed to remove excess hydrochloric acid.

25.3. Apparatus.

- 25.3.1. *Spectrophotometer.* For use at 535 nm, providing a light path of 2 cm.  
 25.3.2. *Separating Funnel.*  
 25.3.3. *pH Meter.*

25.4. Reagents.

- 25.4.1. *Zinc-free Double-distilled Water.*  
 25.4.2. *Dithizone Solutions.*  
     (a) Dissolve 0.10 g diphenylthiocarbazone in 1 litre of carbon tetrachloride. Store in a brown bottle in a refrigerator. If the solution is of doubtful quality or has been stored for a long time, the following test for deterioration can be applied: Shake 10 ml with 10 ml 1 + 99 ammonium hydroxide. If the lower, carbon tetrachloride, layer is only slightly yellow, the reagent is in good condition.  
     (b) Dilute one volume of dithizone solution (a) with nine volumes of carbon tetrachloride. If stored in a brown glass-stoppered bottle in the refrigerator, this solution is satisfactory for several weeks.  
 25.4.3. *Sodium Citrate Solution for Cleaning Glassware.*  
     Dissolve 10 g sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) in 90 ml water. Extract with 10-ml portions of dithizone solution (a) until the last extract remains green, then extract with carbon tetrachloride to remove excess dithizone.  
 25.4.4. *Hydrochloric Acid Solution* approximately 0.02N. Dilute 1.0 ml concentrated hydrochloric acid to 100 ml with re-distilled water.  
 25.4.5. *Sodium Acetate Solution*, approximately 2N. Dissolve 68 g sodium acetate ( $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ ) and make up to 250 ml with re-distilled water.  
 25.4.6. *Acetate Buffer Solution.* Mix equal volumes sodium acetate solution and acetic acid solution. Purify by dithizone extraction as described for sodium citrate solution.  
 25.4.7. *Acetic Acid Solution*, approximately 2*M*. Mix 1 volume glacial acetic acid ( $\text{C}_2\text{H}_4\text{O}_2$ ) with 7 volumes re-distilled water.  
 25.4.8. *Sodium Thiosulphate Solution.* Dissolve 25 g sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in 100 ml re-distilled water. Purify by dithizone extraction as described for sodium citrate solution.  
 25.4.9. *Standard Zinc Stock Solution.* Dissolve 0.100 g 30-mesh zinc metal in a slight excess of 1 + 1 hydrochloric acid; about 1 ml is required. Then dilute to 1 litre with re-distilled water.  
 25.4.10. *Standard Zinc Working Solution.* Dilute 10.0 ml standard zinc stock solution (25.4.9.) to 1 litre with redistilled water. 1 ml = 0.001 mg of Zn.  
 25.4.11. *Carbon Tetrachloride.*

## 5. Method.

- 25.5.1. *Preparation of Colorimetric Standards.* -- To a series of 125 ml Squibb separating funnels, thoroughly cleansed as described, add 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ml standard zinc working solution (25.4.10.) equivalent to 0.000, 0.001, 0.002, 0.003, 0.004 and 0.005 mg Zn. Bring each volume up to 10.0 ml by adding re-distilled water. To each funnel add 5.0 ml acetate buffer and 1.0 ml sodium thiosulphate solution and mix. The pH should be between 4 and 5.5 at this point. To each funnel add 10.0 ml dithizone solution (b). Stopper and shake vigorously for 4 min. Allow the layers to separate. Dry the stem of the funnel with strips of filter paper and run the lower, carbon tetrachloride layer into a clean, dry colorimeter cell.
- 25.5.2. *Colorimetric Measurement.* -- Set the spectrophotometer at zero absorbance with the blank at 535 m $\mu$ . Plot calibration curve. A new curve should be plotted with each set of samples.
- 25.5.3. *Procedure for Water Samples.* -- If the zinc content is not within the working range, dilute the sample with re-distilled water or concentrate it in a silica dish. Using a pH meter, and accounting for any dilution, adjust the sample to pH 7 to 8 with 0.02 N hydrochloric acid. Transfer 10.0 ml to a separating funnel. Complete the analysis as described in (25.5.1.) "preparation of standards", beginning with the words "to each funnel add 5.0 ml acetate buffer . . . . ."

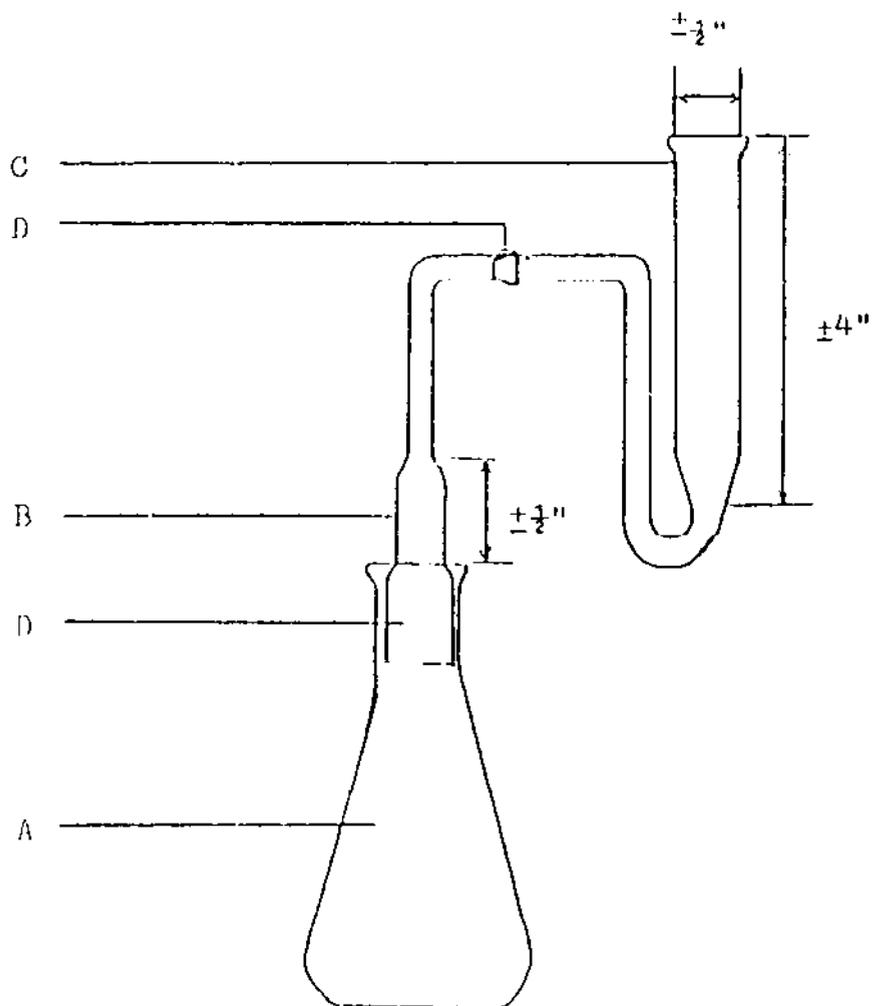
Read the mg Zn on the calibration curve.

## 25.6. Calculation.

$$\text{Zinc as Zn in mg/l} = \frac{\text{mg Zn} \times 1,000}{\text{ml sample}}$$

FIGURE 1.

ARSINE GENERATOR

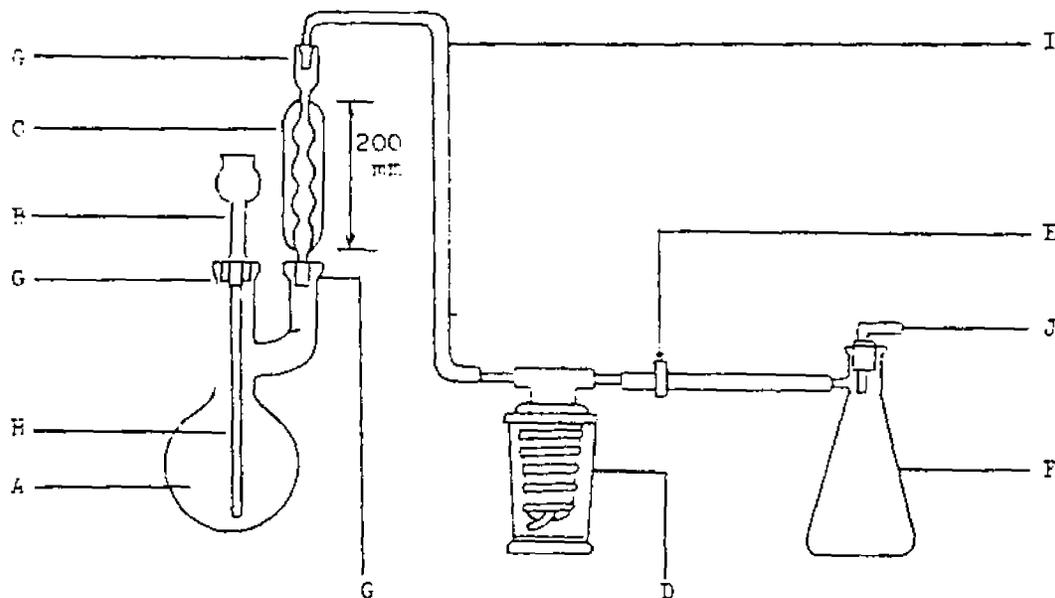


- A - Generator Flask
- B - Scrubber
- C - Absorber
- D - Ground-Glass Joints.

FIGURE 2.

CYANIDE DISTILLATION APPARATUS

- A = 500 ml Modified Claissner Flask
- B = Thistle Tube Air Inlet
- C = Water-cooled Condenser
- D = Fisher-Miligan Spiral Gas Washer
- E = Screw Clamp
- F = 500 ml Suction Flask
- G = Ground Glass Joints
- H = Tube 3 mm Internal Diameter
- I = Tube 9 mm External Diameter
- J = To Suction.



The Government Printer, Madras.

(1)