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GOVERNMENT NOTICE.

DEPARTMENT OF WATER AFFAIRS.

No. R. 969.] [22 June 1962.

REGIONAL STANDARDS FOR INDUSTRIAL EFFLUENTS.

METHODS OF TESTING.

It is hereby notified in terms of paragraphs 1.14 and 2.14 of Government Notice R. 553 of 5 April, 1962, that the Minister of Water Affairs has prescribed the following methods of testing waste water or effluents.

1. DETERMINATION OF pH.

Apparatus.

1. Standard glass electrode.
2. Saturated calomel electrode.
3. pH electrometer with temperature compensator.

Reagents.

1. *Distilled water for washing electrodes.*
2. *Standard buffer solutions* of pH 4.0, pH 7.0 and pH 9.0. These are usually obtained with the instrument.

Method.

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

2. DETERMINATION OF DISSOLVED OXYGEN.

Collection of Samples.—Narrow mouth glass stoppered bottles with capacity of 250 to 300 ml should be used.

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.

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.

Reagents.

1. *Manganous Sulphate Solution.*—Dissolve 480 g manganous sulphate ($MnSO_4.4H_2O$) or (400 g $MnSO_4.2H_2O$ or 364 g $MnSO_4.H_2O$) 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.

GOEWERMENTSKENNISGEWING.

DEPARTEMENT VAN WATERWESE.

No. R. 969.] [22 Junie 1962.

STREEKSTANDAARDE VIR NYWERHEIDSAFVALWATER.

TOETSMETODES.

Hierby word bekendgemaak ingevolge paragrawe 1.14 en 2.14 van Goewermentskennisgewing R. 553 van 5 April 1962, dat die Minister van Waterwese die volgende metodes voorskryf om afvalwater of uitvloeisels te toets.

1. BEPALING VAN pH.

Apparatuur.

1. Standaardglaselektrode.
2. Versadigde kalomel-elektrode.
3. pH-elektrometer met temperatuurkompenseerder.

Reagense.

1. *Gedistilleerde water* vir was van elektrodes.
2. *Standaardbufferoplossings* met pH 4.0, pH 7.0 en pH 9.0. Hierdie word gewoonlik saam met die instrument gelewer.

Metode.

Volledige gebruiksaanwysings word altyd saam met die apparaat gelewer.

2. BEPALING VAN OPGELOSTE SUURSTOF.

Versameling van monsters.—Stopflesse met 'n glasprop en 'n inhoudsmaat van 250 tot 300 ml behoort gebruik te word.

Oor die algemeen behoort die monsterneming so gereël te word dat die vloeistof in die monsterfles driemaal vervang word sonder dat daar lugborrels ontstaan. Die temperatuur van die water waaruit die monster geneem is, behoort tot die naaste graad Celsius, of nog noukeuriger indien gewens, aangeteken te word.

Wanneer spesiale monsters opgeloste suurstof nie onmiddellik ontleed kan word nie, kan hulle op onderstaande wyse 'n etmaal lank goed gehou word:—

Voeg 0·7 ml sterk swawelsuur en 1 ml van 'n 2-persentige natriumasiedoplossing by die monster. Prop toe en bewaar by die temperatuur waarby die monster geneem is, of sit met 'n waterafsluiting in die 20° C-inkubator totdat die ontleding gedoen kan word. Gaan dan aan met die analise met gebruikmaking van 1 ml mangaan-II-sulfaat en 4 ml alkaliese jodiedoplossing, en 3 ml sterk swawelsuur vir die eindversuring.

Reagense.

1. *Manganosulfaatoplossing.*—Los 480 g manganosulfaat ($MnSO_4.4H_2O$) (of 400 g $MnSO_4.2H_2O$ of 364 g $MnSO_4.H_2O$) in gedistilleerde water op en verdun tot 1 liter. Indien daar twyfel omtrent die kristalwater bestaan, maak dan 'n oplossing met S.G. van 1·270 by 20° C.

2. *Alkaline-iodide Sodium Azide Solution.*—Dissolve 500 g of sodium hydroxide (NaOH) or 700 g of potassium hydroxide (KOH) and 135 g of sodium iodide (NaI) or 150 g of potassium iodide (KI) in distilled water and dilute to 1 litre. Dissolve 10 g sodium azide (NaN_3) in 40 ml of distilled water, and add this solution with constant stirring to 950 ml of the alkaline-iodide solution.

3. *Sulphuric Acid.*—Concentrated.

4. *Sodium Thiosulphate.*—Stock Solution, O.25N. Dissolve 63 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$), 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.

5. *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

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.

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 distilled water with constant stirring. Boil for 1 minute, and allow to cool before use.

The solution should be used freshly prepared.

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 0·025 N adjust it until it is.

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 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 0·025 N sodium thiosulphate to the starch end point. If the equivalent of 200 ml original sample have been taken the dissolved oxygen will be numerically equal to the titration figure, and expressed thus:—

Dissolved oxygen as O_2 in mg/l.

Calculation of Percentage Saturation of Oxygen.

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

Equation for Determining the Solubility at any Barometric Pressure.

$$S^1 = \frac{SP}{760} = \frac{SP^1}{29{\cdot}92}$$

where S^1 = solubility at P or P^1.
S = solubility at 760 mm or 29·92 in.
P = barometric pressure in millimetres.
P^1 = barometric pressure in inches.

2. *Oplossing van alkaliese jodied natriumasied.*—Los 500 g natriumhidroksied (NaOH) of 700 g kaliumhidroksied (KOH) en 135 g natriumjodied (NaI) of 150 g kaliumjodied (KI) in gedistilleerde water op en verdun tot 1 liter. Los 10 g natriumasied (NaN_8) in 40 ml gedistilleerde water op, en voeg hierdie oplossing, al roerende, by 950 ml van die alkaliese jodiedoplossing.

3. *Swawelsuur.*—Sterk.

4. *Natriumtiosulfaat.*—Voorraadoplossing, 0·25N. Los 63 g natriumtiosulfaat ($Na_2S_2O_3.5H_2O$) in 1 liter kopervrye, pasgekookte en afgekoelde gedistilleerde water op en voeg 1 ml chloroform of 10 mg kwik-II-jodied by om die oplossing te stabiliseer. Laat voor gebruik 'n paar dae staan.

5. *Werkoplossing,* 0·025N.—Verdun 100 ml voorraadoplossing tot 1 liter met kopervrye, pasgekookte en afgekoelde gedistilleerde water, en voeg 1 ml chloroform of 10 mg kwik-II-jodied by. Hierdie oplossing is taamlik bestendig, maar dit moet met kort tussenpose teen kaliumdichromaat gestandaardiseer word. Bewaar in 'n amberkleurige glasbottel met 'n rubberprop en gooi enige oplossing wat aan die einde van die dag in die buret agterbly, weg.

6. *Standaardkaliumdichromaatoplossing,* 0·025N.—Los 1·226 g K_2Cr_2O7 nadat dit eers gedroog is, in gedistilleerde water op, en verdun tot 1 liter.

7. *Stysel.*—Vryf 1 g oplosbare stysel met 'n bietjie koue gedistilleerde water tot 'n gladde pasta fyn. Giet, al roerende, in 1 liter kokende gedistilleerde water. Laat 1 minuut kook en voor gebruik afkoel.

Die oplossing moet telkens voor gebruik vars aangemaak word.

Standaardisering van natriumtiosulfaatoplossing.

Los ongeveer 2 g kaliumjodied (KI), vry van jodaat, in 'n Erlenmeyerfles met 100–150 ml gedistilleerde water op, voeg 10 ml swawelsuur (1: 9) daarby, gevolg deur presies 20 ml. standdarddichromaatoplossing. Laat 5 minute in die donker staan, verdun tot ± 400 ml en titreer met tiosulfaat tot 'n ligte strooikleur te voorskyn tree; voeg stysel by en titreer tot die vloeistof kleurloos is. Indien die tiosulfaat nie presies 0·025N is nie, moet dit gereël word totdat dit wel presies daardie normaliteit het.

Metode.

Voeg by die monster wat in 'n fles van 250–300 ml versamel is, 2 ml manganosulfaatoplossing, gevolg deur 2 ml alkaliese jodiedreagens goed onder die oppervlak van die vloeistof, prop sorgvuldig toe om alle lug uit te sluit, en meng goed; laat die neerslag besink en meng weer. Laat die neerslag weer besink en voeg dan 2 ml sterk swawelsuur daarby terwyl dit versigtig by die fles se nek afgegiet word. Sit weer die prop op en skud versigtig tot alles opgelos is. Giet 'n hoeveelheid gelyk aan 200 ml van die oorspronklike monster af na korrigering vir die volumetoename deur die bygevoegde reagense veroorsaak. Indien dus, soos in hierdie geval, 4 ml reagense by 'n fles van 300 ml gevoeg is, behoort die volume vir die titrering geneem, as volg te wees:—

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

Titreer met 0·025N natriumtiosulfaat tot die omslagpunt van die stysel. Indien die ekwivalent van 200 ml oorspronklike monster geneem is, sal die opgeloste suurstof numeries gelyk aan die titrasiesyfer wees en as volg aangegee word:—

Opgeloste suurstof as O_2 in mg/l.

Berekening van persentasie suurstofversadiging.

$$\text{Persentasie suurstofversadiging} = \frac{\text{Opgeloste suurstof as } O_2 \text{ in mg/l} \times 100}{\text{Oplosbaarheid van suurstof in water by die temperatuur, lugdruk en southeid van die monster wanneer geneem, in mg/l.}}$$

Vergelyking vir die bepaling van oplosbaarheid by enige lugdruk.

$$S^1 = \frac{SP}{760} = \frac{SP^1}{29{\cdot}92}$$

waarin S^1 = oplosbaarheid by P of P^1.
S = oplosbaarheid by 760 mm of 29·92 dm.
P = lugdruk in millimeters.
P^1 = lugdruk in duime.

The solubility of oxygen at 760 mm pressure at various temperatures and stated degrees of salinity can be obtained from Table I.

TABLE I.

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 760MM.

(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.	Dissolved Oxygen in Chloride-free Water.	
	0.	5,000.	10,000.	15,000.	20,000.			
°C.	Dissolved Oxygen in ppm by Weight.					ppm.	°C.	ppm.
0	14·62	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	8·57	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 (FAECAL) COLI.

In establishing the presence and number of typical (faecal) coli, the presence and number of presumptive coliforms is first determined and by sub-culturing the positive cultures and re-incubating, the presence and number of typical (faecal) 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.*—(*a*) 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.

Die oplosbaarheid van suurstof in vars wate· en in seewater van 'n gegewe southeidsgraad by 'n druk van 760 mm en by verskillende temperature kan uit Tabel I gesien word.

TABEL I.

OPLOSBAARHEID VAN SUURSTOF IN VARS WATER EN IN SEEWATER MET 'N GEGEWE SUURHEIDSGRAAD BY VERSKILLENDE TEMPERATURE WANNEER BLOOTGESTEL AAN 'N ATMOSFEER WAT 20·9 PERSENT SUURSTOF ONDER DRUK VAN 760 MM BEVAT.

(Bereken deur G. C. Whipple en M. C. Whipple uit metings deur C. J. J. Fox gedoen.)

	Chloriede in seewater, dpm.*					Verskil per 100 dpm chloor.	Opgeloste suurstof in chloried-water.	
	0.	5,000.	10,000.	15,000.	20,000.			
°C.	Opgeloste suurstof in dpm volgens gewig.					dpm.	°C.	dpm.
0	14·62	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	8·57	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		

* Die tweede desimaal in bostaande tabel is nie noukeurig bekend nie. Die gemiddelde verskil van die gemiddelde van vyf verskillende ondersoekers verteenwoordig 0·07 dpm. Tot tyd en wyl nadere gegewens egter bekend is, word die tweede desimaal in die tabel behou.

3. METODES VIR DIE ONDERSOEK VAN TIPIESE (FEKALE) COLI.

Om die aanwesigheid van tipiese (fekale) coli, en hul aantal te bepaal, word eers die aanwesigheid van vermoedelike colivorms, en hul aantal vasgestel, en deur die bereiding van subkulture van die positiewe kulture en herinkubering word dan die aanwesigheid van die tipiese (fekale) coli, en hul aantal bepaal.

3.1 *Apparatuur.*—Besoedelde bottels, flesse en proefbuisies moet vir 20 minute by 121° C in 'n outoklaaf gesit word, daarna met seeppoeier of ander geskikte wasmiddel en water gewas en met gedistilleerde water uitgespoel word tot hulle helder is, en vervolgens in 'n warmelugoond gedroog word. Al die apparate moet voor gebruik gesteriliseer word.

3.1.1 *Monsterflesse.*—(*a*) Die monsterflesse moet 'n wye bek hê en met 'n glasprop of skroefdeksel wat teen hitte bestand is, toegemaak word. Hulle moet by voorkeur van neutrale glas gemaak wees, 'n minimum inhoudsmaat van 200 ml hê en geen oormaat alkali bevat nie.

(*b*) *Sterilisation.*—When sterilising, place a small slip of paper between the edge of the bottle neck and the stopper, allowing about ½ in. to remain outside. Cover the stopper and neck with either brown paper or a heat-resistant cellophane paper and fasten with string or heat-resisting 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° ± 5° C. Alternatively the bottles may be sterilised in a hot air oven for 1 hour at a temperature of 170° ± 5° C.

3.1.2 *Test Jars, Subculture Tubes and Durham Fermentation Tubes.*—(*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 330–350 ml. The mouths shall be 4½–5 cm wide and covered with screw caps made of metal or other heat-resistant 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 1½–2 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 6–8 cm long and 1½–2 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.

(*e*) *Sterilisation.*—Within each test jar place one Durham fermentation tube [3.1.2 (*c*)], in inverted position, screw the cap on and finally sterilise in a hot air oven for 1 hour at a temperature of 170 ± 5° C. Sterilise subculture tubes similarly after a small Durham fermentation tube [3.1.2. (*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 ± 0·5° C or 44 ± 0·5°C.

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

3.2. *Media.*

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 0·01 N 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. 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.

3.2.4 *Alternative Broth.*—Instead of making up MacConkey broth according to (3.2.3), double quantities of a dehydrated MacConkey broth of bacteriological standard, dissolved in 1,000 ml of distilled water may be used.

3.2.5 *Preliminary Treatment of Double Strength MacConkey Broth.*—Steam the solutions prepared (3.2.3) 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).

3.2.6 *Distribution of Double Strength MacConkey Broth.*—Using the jars described in (3.1.2) (*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 ± 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.

3.2.7 *Single Strength MacConkey Broth.*—In addition to the double strength MacConkey broth described in (3.2.3) prepare single strength MacConkey broth by taking half the weights of sodium taurocholate, lactose, peptone

(*b*) *Sterilisering.*—Plaas by sterilisering 'n klein strokie papier so tussen die fles se rand en die prop dat ½ dm daarvan na buite uitsteek. Bedek die prop en die nek met bruin papier of met sellofaanpapier wat teen hitte bestand is, en bind met 'n lyntjie of 'n hittebestande bandjie vas. Steriliseer 30 minute in 'n outoklaaf by 121° C en verdryf die buitenste vog deur die fles vir 10 minute in 'n oond te plaas by 'n temperatuur van 110° ± 5° C. Die flesse kan ook 1 uur lank in 'n warmelugoond gesteriliseer word by 'n temperatuur van 170° ± 5° C.

3.1.2 *Toetsflesse, subkultuurbuise en Durham-fermenteerbuisies.*—(*a*) *Toetsflesse.*—Die toetsflesse moet van glas wat teen hitte bestand is, gemaak, 6–7 cm wyd en 14–16 cm hoog wees, en 'n inhoudsmaat van 330–350 ml hê. Die bek moet 4½–5 cm wyd wees en met 'n skroefdeksel van metaal of 'n ander hittebestande materiaal toegemaak word. Dit is aanbevelenswaardig om die flesse op hoogtes van 100 ml en 200 ml te merk.

(*b*) *Subkultuurbuise.*—Subkultuurbuise moet 6–8 cm lank en 1½–2 cm in deursnee wees. Prop hulle met watte toe.

In plaas van hierdie buise kan skroefdekselflesse met 'n inhoudsmaat van 25 ml gebruik word.

(*c*) *Durham-fermenteerbuisies* (*groot*).—Proefbuisies 6–8 cm lank en 1½–2 cm wyd dien as Durham-fermenteerbuisies in die toetsflesse.

(*d*) *Durham-fermenteerbuisies* (*klein*).—Durham-fermenteerbuisies wat in subkultuurbuise gebruik word, moet 2·5–3·5 cm lank en 0·7–1·0 cm in deursnee wees.

(*e*) *Sterilisering.*—Plaas een Durham-buisie [3.1.2(c)], onderstebo in elke toetsfles; skroef die deksel op en steriliseer 1 uur lank in 'n warmelugoond by 'n temperatuur van 170 ± 5° C. Steriliseer subkultuurbuise op dieselfde manier nadat klein Durham-buisies [3.1.2(d)] daarin gesit is.

3.1.3 *Inkubators of waterbaddens.*—Gebruik vir inkubering 'n inkubator of 'n waterbad waarvan die temperatuur respektiewelik op 37° C ± 0·5° C of 44 ± 0·5° C gehou kan word.

3.1.4 *Termometers.*—Termometers vir temperatuuraflesing moet van kwaliteit A gesertifiseer wees.

3.2 *Voedingsbodems.*

3.2.1 *Gedistilleerde water.*—Die water vir die bereiding van voedingsbodems gebruik moet in glas gedistilleer wees.

3.2.2 *Broomkresolpers, 0·04-persentig.*—Voeg by 0·1 g broomkresolpers eers 18·5 ml 0·01N natriumhidroksiedoplossing en dan voldoende water om die indiktator heeltemal op te los en tot 250 ml te verdun.

3.2.3 *Bereiding van MacConkey-boeljon.*

Dubbel sterkte.—Die MacConkey-boeljon van dubbel sterkte bestaan uit die volgende: 10 g natriumtourocholaat (galsout), 20 g laktose, 40 g peptoon, 10 g natriumchloried en 1,000 ml gedistilleerde water.

3.2.4 *Alternatiewe boeljon.*—In plaas van MacConkey-boeljon volgens 3.2.3 aan te maak, kan dubbel hoeveelhede van 'n ontwaterde MacConkey-boeljon van bakteriologiese standaard in 1,000 ml gedistilleerde water gebruik word.

3.2.5 *Voorafgaande behandeling van MacConkey-boeljon van dubbel sterkte.*—Stoom die bereide oplossings (3.2.3) 2 uur by 98°–100° C, laat afkoel en sit 'n nag in die yskas. Filtreer die volgende oggend terwyl dit nog koud is, deur filtreerpapier Whatman No. 1 of van 'n dergelike graad. Reël die pH van die filtraat tot 7·4 en voeg 25 ml van 'n 0·04-persentige waterige oplossing van broomkresolpers (3.2.2) daarby.

3.2.6 *Verdeling van MacConkey-boeljon van dubbel sterkte.*—Giet 100 ml MacConkey-boeljon in elk van de flesse in 3.1.2(*a*) en (*c*) beskryf en steriliseer 15 minute by 115° C in 'n outoklaaf. Die boeljon kan ook gesteriliseer word deur dit op drie agtereenvolgende dae telkens 30 minute by 98–100° C te stoom. Die finale pH moet 7·3 ± 0·1 wees. Wanneer die flesse vir die toetse benodig word, moet hulle vantevore in die 37°-inkubator of die waterbad geplaas word en slegs daaruit verwyder word vir byvoeging van die monster water.

3.2.7 *MacConkey-boeljon van enkel sterkte.*—Berei benewens die MacConkey-boeljon van dubbel sterkte (3.2.3), MacConkey-boeljon van enkel sterkte deur die helfte van die gewigte aan natriumtourocholaat, laktose,

and sodium chloride given in (3.2.3) and dissolve them in 1,000 ml of distilled water. Treat the broth further as in (3.2.5). Alternately 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 (*b*)] and (*e*) and sterilise as described under (3.2.6). 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.

3.2.8 *Indole Medium.*—Prepare a 1 per cent solution in distilled water of a bacteriological peptone containing tryptophan. Adjust pH to 7·0 ± 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.

3.2.9 *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.

3.3.3 *Sampling from a Tap or Pump.*—Wash the mouth of the tap inside and outside with clean material. 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).

3.3.4 *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 sampling box which has been filled with lumps of ice.

3.4 *Method of Examination.*—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, even though 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 (3.5).

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.

3.5 *Examination for Typical (Faecal) Coli.*

3.5.1 *Method–Theory.*—The possibility of the presence of typical (faecal) coli in presumptive positive MacConkey jars at 37° C is ascertained by subculture from such positive jars into MacConkey medium at 44° C. If the temperature of incubation is kept strictly at 44° C (with a maximum

peptoon en natriumchloried in 3.2.3 aangegee, in 1,000 ml gedistilleerde water op te los. Behandel die boeljon verder volgens 3.2.5. Berei as alternatief MacConkey-boeljon deur geskikte hoeveelhede van 'n ontwaterde MacConkey-boeljon van bakteriologiese standaard in 1,000 ml gedistilleerde water op te los.

Plaas hoeveelhede van 10 ml van hierdie boeljon in die subkultuurbuise in 3.1.2 (*b*) en (*e*) beskryf, en steriliseer volgens 3.2.6. Wanneer die buise vir die toetse benodig word, moet hulle in die 37° C-inkubator of die waterbad gesit en slegs vir byvoeging van die monster water daaruit verwyder word.

3.2.8 *Indoolvoedingsbodem.*—Berei 'n 1-persentige oplossing in gedistilleerde water van 'n bakteriologiese peptoon wat triptofaan bevat. Reël die pH tot 7·0 ± 0·1, sit 'n hoeveelheid van 5 ml in elke subkultuurbuis sonder Durham-buisie. Prop die buise toe en steriliseer hulle 15 minute by 121° C in 'n outoklaaf.

3.2.9 *Kovacs-indoolreagens.*—Los 5 g p-dimetielamino-bensaldehied in 75 ml suiwere n-amielalkohol op en voeg 25 ml 10N soutsuur daarby.

3.3 *Versameling van monsters.*

3.3.1 *Algemeen.*—Skryf voor 'n monster geneem word, 'n etiket uit met vermelding van die naam van die monsternemer, die datum en tyd van monsterneming, en eventuele spesiale onderskeidingsmerke. Heg hierdie etiket onmiddellik na monsterneming stewig aan die monster vas.

3.3.2 *Monsterneming vir bakteriologiese toets.*—By hantering van die steriele monsterfles moet gesorg word dat die binnedeel van die prop nie aan die hand raak of met enigiets in aanraking kom gedurende die versameling van die monster nie, en in geen geval mag die prop neergelê word nie.

3.3.3 *Monsterneming uit 'n kraan of pomp.*—Was die bek van die kraan van binne en van buite met skoon materiaal af. Brand die bek van die kraan met 'n spiritus- of ander soort brander totdat hy goed warm is. Laat die water van 2 tot 3 minute loop, maak die fles amper vol, sit direk die prop op en bind die papier om; sit terug in die monsterkissie. Vul die kissie met stukke ys (ongeveer 1 tot 2 dm groot).

3.3.4 *Monsterneming uit 'n stroom, reservoir, ens.*—Hou die fles met een hand aan sy boom vas, haal die prop af en dompel die fles onmiddellik omtrent 1 voet onder die watervlak; laat die fles vol loop hetsy deur hom met sy bek stroom-op te hou of, in stilstaande water, deur hom vorentoe te stoot, om te voorkom dat water wat met die hand in aanraking was in die fles kom. Haal uit, sit onmiddellik die prop en papierbedekking op en plaas terug in die monsterkissie wat met ysblokke gevul is.

3.4 *Metode van ondersoek.*—Keer die monsterfles 25 maal onderstebo deur 'n vinnige draaibeweging van die pols om eventuele besinksel gelykmatig oor die water te verdeel. Brand die bek van die fles met 'n vlam, giet asepties 100 ml van die water in een van die voorbereide toetsflesse met 100 ml MacConkey-boeljon van dubbel sterkte. Skroef die deksel weer op die fles en plaas in die 37°-inkubator of die waterbad. Ondersoek die flesse na 20–24 uur se inkubering; die aanwesigheid van suur en gas in die ingeslote Durham-buisies word as 'n positiewe reaksie beskou, dog die afwesigheid van gasvorming, selfs al is daar tekens van groei of aanwesigheid van suur, word nie as 'n negatiewe reaksie beskou nie tensy die totale inkuberingstydperk by 37° C 48 uur geduur het. Sodra 'n fles die aanwesigheid van suur en gas vertoon, moet 'n subokulering op MacConkey-boeljon van enkel sterkte by 44° C uitgevoer word volgens 3.5. As daar 'n fles is wat geen tekens van suur of gas vertoon nie, moet hy geïnkubeer word totdat 'n totale tydperk (met inbegrip van die eerste tydperk) van 48 uur verloop het. Die flesse wat geen suur- en gasvorming vertoon nie, word dan as negatief beskou wat reaksie betref.

3.5 *Ondersoek vir tipiese (fekale) coli.*

3.5.1 *Metode—Teorie.*—Die moontlikheid van die aanwesigheid van tipiese (fekale) coli in vermoedelik positiewe MacConkey-flesse by 37° C word vasgestel deur subkulture van sulke positiewe flesse op 'n MacConkeyvoedingsbodem te maak by 44° C. As die inkuberingstemperatuur streng op 44° C gehou word (met 'n maksimum toleransie

tolerance of ± 0·5° C) the presence of gas within 48 hours is practically characteristic of *Escherichia coli.* Type I, faecal.

3.5.2 *Method of Examination.*—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 ± 0·5° C.

3.5.3 *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 (3.5.4).

3.5.4 *Test for Indole Production.*—Transfer approximately 0·01–0·02 ml of the 44° C positive culture obtained in 3.5.2 to 5 ml of indole medium (3.2.8). Incubate these tubes as in 3.5.2. After 24 hours add 0·3 ml of Kovacs reagent (3.2.9) 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 I.

3.6 *Method of Examination for the most probable number of Typical (Faecal) Coli.*—To establsih the most probable number of typical *Escherichia coli* type I organisms present conduct the following tests simultaneously with those of Section 3.4 and 3.5.

3.6.1 *Apparatus.*—In addition to the apparatus specified in 3.1 excepting [3.1.2 (*a*)], the following is required:—

3.6.1.1 *Pipettes.*—(*a*) *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° ± 5° C.

(*b*) *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 ± 5° C.

3.6.2 *Media.*—In addition to the media specified in 3.2 the following is required:—

3.6.2.1 *Double Strength MacConkey Broth.*—Prepare double strength MacConkey broth in accordance with 3.2.3, 3.2.4, 3.2.5 and 3.2.6, except that instead of distributing the broth in 100 ml quantities in test jars distribute 10 ml quantities in sterile subculture tubes [3.1.2 (*b*)] containing Durham fermentation tubes [3.1.2 (*d*)] and then sterilise.

3.6.3 *Method of Examination.*—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 (3.6.2.1) add 10 ml of water sample. To each of five, prepared, single strength MacConkey broth tubes (3.2.7) 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. The first series of tubes each contains 10 ml of water sample, the second series 1·0 ml and the third series 0·1 ml. Immediately place these 15 tubes in the 37° C incubator or waterbath. 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, even though 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 into single strength MacConkey broth at 44° C as set out

van ± 0·5° C), is die aanwesigheid van gas binne 48 uur amper seker 'n bewys dat daar fekale *Escherichia coli,* tipe I, aanwesig is.

3.5.2 *Metode van ondersoek.*—Bring uit elke vermoedelik positiewe fles waarvan die temperatuur 37° C is, met behulp van 'n steriele bakteriologiese draadogie of 'n Pasteur-pipet ongeveer 0·01–0·02 ml oor na 10 ml MacConkey-boeljon van enkel sterkte in inkubeerbuise, om 'n subkultuur te vorm. Inkubeer hierdie buise in 'n waterbad by 44° C en sorg dat die watervlak in die bad bo die vloeistofvlak in die buise is en dat die temperatuur dwarsdeur 44 ± 0·5° C bly.

3.5.3 *Vertolking.*—Die aanwesigheid van gas in die ingeslote Durham-buisie word beskou as 'n positiewe aanduiding van die groei van tipiese (fekale) coli. Geen buisie word as negatief beskou nie alvorens die totale inkuberingstydperk van 48 uur verby is. Finale bevestiging hang van die produksie van indool by 44° C (3.5.4) af.

3.5.4 *Toets vir indoolproduksie.*—Bring ongeveer 0·01–0·02 ml van die positiewe kultuur (44° C) volgens 3.5.2 verkry na 5 ml indoolvoedingsbodem (3.2.8) oor. Inkubeer hierdie buise volgens 3.5.2. Voeg na 24 uur 0·3 ml Kovacs-reagens (3.2.9) daarby en skud goed. As daar binne 5 minute 'n rooi kleur tevoorskyn tree, is dit 'n positiewe bewys van die aanwesigheid van indool en bevestig dit die aanwesigheid van tipiese (fekale) *Escherichia coli,* tipe I.

3.6 *Metode vir die bepaling van die mees waarskynlike aantal tipiese (fekale) coli.*—Om die mees waarskynlike aantal tipiese organismes van *Escherichia coli,* tipe I, wat aanwesig is, te bepaal, moet onderstaande toetse gelyktydig met dié van afdeling 3.4 en 3.5 gedoen word.

3.6.1 *Apparatuur.*—Benewens die apparate in 3.1 voorgeskryf, met uitsondering van 3.1.2(*a*), is die volgende nodig:—

3.6.1.1 *Pipette.*—(*a*) *Tipes.*—Pipette moet van graadverdelings voorsien wees (10, 5 en 1 ml), reguit kante hê en spits toeloop.

Gebruikte pipette moet in 'n 2-persentige natriumhidroksiedoplossing of 'n ander geskikte wasmiddel skoongemaak, word, daarna in gedistilleerde water gewas en afgespoel en in 'n warmelugoond by 105° ± 5° C gedroog word.

(*b*) *Sterilisering.*—Prop 'n stuk watte in die bo-ent van die pipette en plaas hulle dan in 'n houer of draai hulle in Kraft-papier toe en steriliseer hulle 'n uur lank by 'n temperatuur van 170 ± 5° C in 'n warmelugoond.

3.6.2 *Voedingsbodems.*—Benewens die voedingsbodems in 3.2 voorgeskryf, is die volgende nodig:—

3.6.2.1 *MacConkey-boeljon van dubbel sterkte.*—Berei MacConkey-boeljon van dubbel sterkte volgens 3.2.3, 3.2.4, 3.2.5 en 3.2.6, en sit i.p.v. hoeveelhede van 100 ml daarvan in toetsflesse, hoeveelhede van 10 ml in steriele subkultuurbuise [3.1.2(*b*)] wat Durham-buisies bevat [3.1.2(*c*)], en steriliseer daarna.

3.6.3 *Metode van ondersoek.*—Keer die monsterfles 25 maal onderstebo deur 'n vinnige draaibeweging van die pols om eventuele besinksel gelykmatig oor die water te verdeel. Brand die bek van die fles met 'n vlam en giet 10 ml van die monster water in elk van vyf buisies met MacConkey-boeljon van dubbel sterkte (3.6.2.1). Giet 1·0 ml van die monster water in elk van vyf buisies met MacConkey-boeljon enkel sterkte. Giet dan nog 1·0 ml van 'n verdunde monster water in elk van vyf buise met MacConkey-boeljon van enkel sterkte. Hierdie verdunde water word berei deur 1·0 ml. van die monster water met steriele, in glas gedistilleerde water tot 10·0 ml te verdun. Die eerste reeks buise bevat elk 10 ml van die monster water, die tweede reeks 1·0 ml en die derde reeks 0·1 ml. Plaas hierdie 15 buise onmiddellik in die 37°-inkubator of -waterbad. Ondersoek die buise na 20 tot 24 uur se inkubering; as daar suur en gas in die ingeslote Durham-buise aanwesig is, word die reaksie as positief beskou, dog as daar geen gasvorming plaasgevind het nie, selfs al is daar tekens van groei of aanwesigheid van suur, word die reaksie nie as negatief beskou nie alvorens die totale inkuberingstydperk by 37° C 48 geduur het. Sodra 'n buis suur en gas vertoon, moet dit onmiddellik in 'n MacConkey-boeljon van enkel sterkte by 44° C oorgeënt word,

in 3.6.4. 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.

3.6.4 *Examination for Typical (Faecal) Coli.*—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 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. It should be noted from which tube each sub-inoculation is performed in order to be able to use Table II to arrive at the count of typical (faecal) coli.

3.6.5 *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. Conduct the test described in Section 3.5.4 to confirm the presence of typical (faecal) coli.

3.6.6 *Estimation of Count.*—To arrive at the count of typical (faecal) coli organisms per 100 ml the results obtained in the above tests are referred to in Table II.

TABLE II.

MOST PROBABLE NUMBER TABLE.

No. of Tubes giving Positive Reaction. A B C	Probable Count per 100 ml.	No. of Tubes giving Positive Reaction. A B C	Probable Count per 100 ml.	No. of Tubes giving Positive Reaction. A B C	Probable Count per 100 ml.	No. of Tubes giving Positive Reaction. A B C	Probable Count per 100 ml.
0 0 0	0	2 0 3	12	4 0 0	13	5 1 3	85
0 0 1	2	2 1 0	7	4 0 1	17	5 2 0	50
0 0 2	4	2 1 1	9	4 0 2	20	5 2 1	70
0 1 0	2	2 1 2	12	4 0 3	25	5 2 2	95
0 1 1	4	2 2 0	9	4 1 0	17	5 2 3	120
0 1 2	6	2 2 1	12	4 1 1	20	5 2 4	150
0 2 0	4	2 2 2	14	4 1 2	25	5 2 5	175
0 2 1	6	2 3 0	12	4 2 0	20	5 3 0	80
0 3 0	6	2 3 1	14	4 2 1	25	5 3 1	110
1 0 0	2	2 4 0	14	4 2 2	30	5 3 2	140
1 0 1	4	3 0 0	8	4 3 0	25	5 3 3	175
1 0 2	6	3 0 1	11	4 3 1	30	5 3 4	200
1 0 3	8	3 0 2	14	4 3 2	40	5 3 5	250
1 1 0	4	3 1 0	11	4 4 0	35	5 4 0	130
1 1 1	6	3 1 1	14	4 4 1	40	5 4 1	170
1 1 2	8	3 1 2	17	4 5 0	40	5 4 2	250
1 2 0	6	3 1 3	20	4 5 1	50	5 4 3	300
1 2 1	8	3 2 0	14	5 0 0	25	5 4 4	350
1 2 2	10	3 2 1	17	5 0 1	30	5 4 5	450
1 3 0	8	3 2 2	20	5 0 2	40	5 5 0	250
1 3 1	10	3 3 0	17	5 0 3	60	5 5 1	350
1 4 0	11	3 3 1	20	5 0 4	75	5 5 2	600
2 0 0	5	3 4 0	20	5 1 0	35	5 5 3	900
2 0 1	7	3 4 1	25	5 1 1	45	5 5 4	1600
2 0 2	9	3 5 0	25	5 1 2	60	5 5 5	1800

NOTE.—

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

Apparatus.

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

Reagents.

1. *Sulphuric Acid.*—Concentrated.

2. *Ferroin Indicator Solution.*—Dissolve 1·485 g 1, 10-phenanthroline (monohydrate), together with 0·695 g ferrous sulphate ($FeSO_4 \cdot 7H_2O$) in water and dilute to 100 ml.

soos in 3.6.4 beskryf. As daar buisies is wat geen suur en gas vertoon nie, moet hulle geïnkubeer word tot 'n totale tydperk (met inbegrip van die eerste tydperk) van 48 uur verstryk het. Die reaksie van die buisies wat dan nog geen suur- en gasvorming toon nie, word as negatief beskou.

3.6.4 *Ondersoek vir tipiese (fekale) coli.*—Maak uit elke vermoedelik positiewe buis by 37° C, 'n subkultuur in MacConkey-boeljon van enkel sterkte deur met behulp van 'n steriele bakteriologiese draadogie of Pasteurpipet 0·01 tot 0·02 ml daarvan oor te bring. Inkubeer hierdie buise in 'n waterbad by 44° C en sorg dat die watervlak van die bad bo die vloeistofvlak in die buise is en dat die temperatuur dwarsdeur op 44° C ± 0·5° C bly. Aantekening moet gehou word van uit watter buisie elke sub-okulering gedoen is, sodat tabel II gebruik kan word om die totale telling van tipiese (fekale) coli te bepaal.

3.6.5 *Vertolking.*—Die aanwesigheid van gas in die ingeslote Durham-buisies word as 'n positiewe aanduiding van die groei van tipiese (fekale) coli beskou. Geen buisie word as negatief beskou nie alvorens die totale inkuberingstydperk van 48 uur verby is. Finale bevestiging hang van die produksie van indool by 44° C af. Voer die toets in 3.5.4 beskryf uit om die aanwesigheid van tipiese (fekale) coli te bevestig.

3.6.6 *Telling.*—Om tot die telling van tipiese (fekale) coli-organismes per 100 ml te geraak, word die resultate met bovermelde toetse verkry, na dié in tabel II verwys.

TABEL II.

MEES WAARSKYNLIKE AANTAL.

Aantal buise wat 'n positiewe reaksie toon. A B C	Waarskynlike telling per 100 ml.	Aantal buise wat 'n positiewe reaksie toon. A B C	Waarskynlike telling per 100 ml.	Aantal buise wat 'n positiewe reaksie toon. A B C	Waarskynlike telling per 100 ml.	Aantal buise wat 'n positiewe reaksie toon. A B C	Waarskynlike telling per 100 ml.
0 0 0	0	2 0 3	12	4 0 0	13	5 1 3	85
0 0 1	2	2 1 0	7	4 0 1	17	5 2 0	50
0 0 2	4	2 1 1	9	4 0 2	20	5 2 1	70
0 1 0	2	2 1 2	12	4 0 3	25	5 2 2	95
0 1 1	4	2 2 0	9	4 1 0	17	5 2 3	120
0 1 2	6	2 2 1	12	4 1 1	20	5 2 4	150
0 2 0	4	2 2 2	14	4 1 2	25	5 2 5	175
0 2 1	6	2 3 0	12	4 2 0	20	5 3 0	80
0 3 0	6	2 3 1	14	4 2 1	25	5 3 1	110
1 0 0	2	2 4 0	14	4 2 2	30	5 3 2	140
1 0 1	4	3 0 0	8	4 3 0	25	5 3 3	175
1 0 2	6	3 0 1	11	4 3 1	30	5 3 4	200
1 0 3	8	3 0 2	14	4 3 2	40	5 3 5	250
1 1 0	4	3 1 0	11	4 4 0	35	5 4 0	130
1 1 1	6	3 1 1	14	4 4 1	40	5 4 1	170
1 1 2	8	3 1 2	17	4 5 0	40	5 4 2	250
1 2 0	6	3 1 3	20	4 5 1	50	5 4 3	300
1 2 1	8	3 2 0	14	5 0 0	25	5 4 4	350
1 2 2	10	3 2 1	17	5 0 1	30	5 4 5	450
1 3 0	8	3 2 2	20	5 0 2	40	5 5 0	250
1 3 1	10	3 3 0	17	5 0 3	60	5 5 1	350
1 4 0	11	3 3 1	20	5 0 4	75	5 5 2	600
2 0 0	5	3 4 0	20	5 1 0	35	5 5 3	900
2 0 1	7	3 4 1	25	5 1 1	45	5 5 4	1600
2 0 2	9	3 5 0	25	5 1 2	60	5 5 5	1800+

OPMERKING.—

1. A — Reeks van vyf buise wat elk 10 ml monster bevat.
   B — Reeks van vyf buise wat elk 1 ml monster bevat.
   C — Reeks van vyf buise wat elk 0·1 ml monster bevat.
2. Die mees waarskynlike aantalle (in die tabel aangegee as waarskynlike telling per 100 ml) van 0 tot 20 is korrek tot die naaste eenheid; van 20 tot 200 korrek tot die naaste 5; bo 200 korrek tot die naaste 50.

4. BEPALING VAN CHEMIESE SUURSTOFVEREISTE.

Apparatuur.

'n Terugvloei-apparaat: 'n Rondeboomkookfles met 'n geslypte nek en 'n terugvloeikoeler.

Reagense.

1. *Swawelsuur.*—Sterk.

2. *Ferroïenindikatoroplossing.*—Los 1·485 g 1, 10-fenantrolien (monohidraat) saam met 0·695 g ferrosulfaat ($FeSO_4 \cdot 7H_2O$) in water op en verdun tot 100 ml.

3. *Standard Potassium Dichromate Solution*, 0·25N.—Dissolve 12·2588 g potassium dichromate ($K_2Cr_2O_7$) previously dried at 103° C for 2 hours, in distilled water and dilute to 1 litre.

4. *Standard Ferrous Ammonium Sulphate Solution*, approximately 0·25N.—Dissolve 98 g ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$) in distilled water. Add 20 ml concentrated sulphuric acid (H_2SO_4), cool and dilute to 1 litre. This solution must be standardised against the potassium dichromate daily.

Standardisation Procedure.—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.

$$\text{Normality} = \frac{\text{ml } K_2Cr_2O_7 \times 0{\cdot}25}{\text{ml } FeSO_4(NH_4)_2SO_4}$$

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 mixture 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.

Calculation—

$$\text{mg/l COD} = \frac{(a-b) \times \text{Normality of } FeSO_4(NH_4)_2SO_4 \times 8{,}000}{\text{ml sample}} - \text{chloride correction.}$$

COD = Chemical oxygen demand.

a = ml $FeSO_4(NH_4)_2SO_4$ used for blank.

b = ml $FeSO_4(NH_4)_2SO_4$ used for sample.

Chloride correction = mg/l Cl × 0·23.

4A. Determination of Chloride.

(To be used in conjunction with the determination of Chemical Oxygen Demand.)

Reagents.

1. *Standard Silver Nitrate Solution*, 0·0282N.—Dissolve 4·791 g silver nitrate ($AgNO_3$) in one litre of distilled water. Each ml is equivalent to 1 mg of chloride. Using the procedure described below, standardise against the standard sodium chloride (NaCl) solution. The factor for the $AgNO_3$ solution is—

$$F = \frac{\text{ml of NaCl solution taken}}{\text{ml of } AgNO_3 \text{ solution used}}$$

2. *Standard Sodium Chloride Solution*, 0·0282N.—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.

3. *Potassium Chromate Indicator Solution.*—Dissolve 50 g potassium chromate (K_2CrO_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.

3. *Standaardkaliumdichromaatoplossing*, 0·25N.—Los 12·2588 g kaliumdichromaat ($K_2Cr_2O_7$) wat eers 2 uur lank by 103° C gedroog is, in gedistilleerde water op en verdun tot 1 liter.

4. *Standaardferro-ammoniumsulfaatoplossing.*—Ongeveer 0·25N: Los 98 g ferro-ammoniumsulfaat [$(Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O)$] gedistilleerde water op. Voeg 20 ml sterk swawelsuur (H_2SO_4) daarby, laat afkoel en verdun tot 1 liter. Hierdie oplossing moet daagliks teen die kaliumdichromaat gestandaardiseer word.

Standaardisering.—Verdun 25 ml standaardichromaatoplossing tot ongeveer 250 ml. Voeg 20 ml sterk swawelsuur by en laat afkoel. Titreer teen die ferro-ammoniumsulfaat en gebruik 2 of 3 druppels van die ferroïen-indikator—

$$\text{Normaliteit:} \frac{\text{ml } K_2Cr_2O_7 \times 0{\cdot}25}{\text{ml } FeSO_4(NH_4)_2SO_4}$$

Metode.

Plaas 50 ml monster in die rondeboomfles, en voeg 25 ml standaard-dichromaatoplossing daarby. Voeg versigtig 75 ml sterk swawelsuur daarby en meng na elke byvoeging.

(Waarskuwing.—Die terugvloeimengsel moet goed gemeng word voordat hitte aangewend word. As dit nie gedoen word nie, vind plaaslike verhitting op die boom van die fles plaas en bestaan die kans dat die mengsel uit die sy-arm van die verkoeler uitgeblaas word).

Verbind die fles met die verkoeler en laat die mengsel 2 uur lank terugvloei. Puimsteenkorrels of glaskrale behoort in die terugvloeimengsel gesit te word om onegalige kook te verhoed. Laat afkoel en spoel dan die verkoeler met ongeveer 25 ml gedistilleerde water deur.

Bring die inhoud na 'n Erlenmeyerfles van 500 ml oor, spoel die terugvloeifles 4 tot 5 maal met gedistilleerde water uit. Verdun die mengsel tot ongeveer 350 ml en titreer die oormaal dichromaat met standaardferro-ammoniumsulfaat; gebruik ferroïen as indikator. Gewoonlik word 2 tot 3 druppels indikator gebruik. Dit hang egter van die individuele ontleder af. Die kleuromslag is skerp van blou-groen tot rooierige blou. Die omslagpunt sal egter nie so skerp wees as by die standaardisering van die reagense nie vanweë die laer suurkonsentrasie. Om hierdie rede is dit nodig om die monster tot minstens 350 ml te verdun voordat met die titrering begin word.

'n Kontrolehoeveelheid van 50 ml gedistilleerde water i.p.v. die monster, saam met die reagense, word op dieselfde manier behandel.

Berekening.

$$\text{mg/l CSV} = \frac{(a-b) \times \text{normaliteit van } FeSO_4(NH_4)_2SO_4 \times 8{,}000}{\text{ml monster}} - \text{chloried-korreksie}$$

waarin—

CSV = chemiese suurstofvereiste.

a = ml $FeSO_4(NH_4)_2SO_4$ vir kontrole gebruik.

b = ml $FeSO_4(NH_4)SO_4$ vir monster gebruik.

Chloriedkorreksie = mg/l Cl × 0·23.

4A. Bepaling van Chloried.

(Moet tesame met die bepaling van die chemiese suurstofvereiste gedoen word.)

Reagense.

1. *Standaardsilvernitraatoplossing*, 0·0282N—. Los 4·791 g silvernitraat ($AgNO_3$) in een liter gedistilleerde water op. Elke ml is gelyk aan 1 mg chloried. Gaan soos hieronder beskryf te werk, standaardiseer teen die standaardnatriumchloried (NaCl)-oplossing. Die faktor vir die $AgNO_3$-oplossing is—

$$F = \frac{\text{ml NaCl-oplossing geneem}}{\text{ml } AgNO_3\text{-oplossing gebruik}}$$

2. *Standaardnatriumchloriedoplossing*, 0·0282N—. Los 16·486 g natriumchloried (NaCl), ½ uur gedroog deur by 900° C te smelt, in 500 ml gedistilleerde water op. Verdun 50·0 ml tot een liter. Elke ml van hierdie oplossing bevat 1 mg chloried.

3. *Kaliumchromaatindikatoroplossing.*—Los 50 g kaliumchromaat (K_2CrO_4) in 'n bietjie gedistilleerde water op. Voeg silvernitraat by tot 'n geringe mate van rooi neerslag vorm. Filtreer nadat dit minstens 'n nag lank gestaan het, en verdun met gedistilleerde water tot een liter.

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.

5. *Phenolpthalein Indicator Solution.*

6. *Sulphuric Acid* 1 +70, approximately O·5N.

7. *Sodium Hydroxide*, approximately 0·5N.—Dissolve 4 g sodium hydroxide (NaOH) in 200 ml distilled water.

Method.

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

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

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 phenolpthalein. 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.

Calculation.

$$\text{Chloride as Cl in mg/l} = \frac{(\text{ml } AgNO_3 \text{ for sample} - \text{ml } AgNO_3 \text{ for blank}) \times F \times 1{,}000}{\text{ml of sample}}$$

F = $AgNO_3$ factor (determined above).

5. Determination of "Oxygen Absorbed".

Reagents.

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 4·0 g potassium permanganate ($KMnO_4$) in 1 L. 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.

2. *Sodium Thiosulphate*—Stock Solution (0·25N).—Dissolve 63 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) 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.

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.

3. *Standard Potassium Dichromate Solution*—0·025N.—Dissolve 1·226 g previously dried potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1 litre.

4. *Aluminiumhidroksiedsuspensie.*—Los 125 g kalium- of ammonium-aluin in een liter gedistilleerde water op. Laat die aluminium neerslaan deur al roerende stadig ammoniumhidroksied by te voeg. Was die neerslag deur dit meermale met 'n hoeveelheid gedistilleerde water af te giet totdat al die chloried uitgewas is.

5. *Fenolftaleïenindikatoroplossing.*

6. *Swawelsuur.*—1 + 70, naastenby 0·5 N.

7. *Natriumhidroksied.*—Naastenby 0·5 N. Los 4 g natriumhidroksied (NaOH) in 200 ml gedistilleerde water op.

Metode.

Gebruik 100 ml monster of 'n geskikte deelvolume tot 100 ml verdun.

Indien die monster gekleurd is, moet dit ontkleur word deur 3 ml aluminiumhidroksiedsuspensie by te voeg. Roer flink, filtreer na 'n paar minute, en was met 10–15 ml gedistilleerde water.

Titrering.—Gebruik òf 'n wit porseleinbakkie òf 'n Erlenmeyerfles op 'n wit oppervlak. Voeg òf verdunde swawelsuur òf natriumhidroksied by die monster totdat dit net kleurloos is teenoor fenolftaleïen. Voeg vervolgens een ml kaliumchromaatoplossing daarby. Titreer met silvernitraatoplossing totdat die kleur omslaan van suiwer geel tot pienkeriggeel. Die indikatorkontrole behoort bepaal te word deur gedistilleerde water op dieselfde manier te titreer. Wanneer hierdie kontrolehoeveelheid die omslagpunkleur het, behoort dit naby die monster wat getitreer word, geplaas te word om te help om die kleurverandering by die gekose omslagpunt te gewaar.

Berekening.

$$\text{Chloried as Cl in mg/l} = \frac{(\text{ml } AgNO_3 \text{ vir monster} - \text{ml } AgNO_3 \text{ vir kontrole}) \times F \times 1{,}000}{\text{ml monster}}$$

waarby—

F = $AgNO_3$-faktor (soos hierbo bepaal).

5. Bepaling van "geabsorbeerde suurstof".

Reagense.

1. *Kaliumpermanganaatoplossing—naastenby N/80.*—Gewoonlik is dit gerieflik om telkens 10 liter van hierdie reagens aan te maak volgens 'n metode wat bestendigheid oor lang tydperke verseker. Selfs dan behoort daaglikse kontrolebepalings gedoen te word om die sterkte na te gaan. Wanneer onderstaande metode trou gevolg word en die oplossing in amberkleurige bottels of in die donker bewaar word, bly dit verskeie maande bestendig.

Los 4·0 g kaliumpermanganaat ($KMnO_4$) op in 1 liter warm gedistilleerde water in 'n groot beker wat met 'n horlosieglas toegemaak is; die oplossing behoort verkieslik 2–3 uur lank deur verhitting op 90–95° C gehou te word. Verdun met gedistilleerde water tot 10 liter en laat 'n paar dae in die donker staan om volledige oksidering van eventuele organiese stowwe te verseker en om eventuele mangaandioksiedneerslag te laat besink. Giet die boonste laag vloeistof versigtig af of hewel dit oor; sorg dat die neerslag nie in beroering gebring word nie. As alternatief kan die oplossing deur 'n sinterglastregter, deur glaswol of deur asbesvesel wat eers met salpeter- en soutsuur gedigereer is, gefiltreer en dan goed met water gewas word; *moenie deur papier filtreer nie.* Daar moet verhoed word dat die oplossing deur stof of organiese stowwe besoedel word.

2. *Natriumtiosulfaat.*—Voorraadoplossing (0·25 N). Los 63 g natriumtiosulfaat ($Na_2S_2O_3 \cdot 5H_2O$) in 1 liter koppervrye, pasgekookte en afgekoelde gedistilleerde water op en voeg 1 ml chloroform of 10 mg kwik-II-jodied by om die oplossing te stabiliseer. Laat voor gebruik 'n paar dae staan.

Werkoplossing (0·025 N).—Verdun 100 ml voorraadoplossing met kopervrye, pasgekookte en afgekoelde gedistilleerde water tot 1 liter en voeg 1 ml chloroform of 10 mg kwik-II-jodied by. Hierdie oplossing is redelik bestendig, maar dit behoort met kort tussenpose teen kaliumdichromaat gestandaardiseer te word. Bewaar in 'n amberkleurige glasbottel met 'n rubberprop en gooi die oplossing wat aan die einde van die dag in die buret agterbly weg.

3. *Standaardkaliumdichromaatoplossing*, 0·025 N.—Los 1·226 g eers gedroogde kaliumdichromaat ($K_2Cr_2O_7$) in gedistilleerde water op en verdun tot 1 liter.

4. *Diluted Sulphuric Acid.*—Add cautiously, small quantities at a time, 1 volume of concentrated sulphuric acid to 3 volumes of water. Much heat is generated in the process and precautions should be taken against spitting of acid 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. *Potassium Iodide Solution.*—Dissolve 10 g potassium iodide in 100 ml of water and store in an amber glass bottle.

6. *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.

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.

Method.

Into a clean 12 oz. glass-stoppered bottle place 10 ml dilute sulphuric acid and 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 water-bath, remixing the contents after one hour if the sample contains much suspended matter. After 4 hours add 5 ml of the iodide solution or about 0·5 g of potassium iodide and after mixing titrate immediately with 0·025N 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.

Calculation.

$$\text{"Oxygen absorbed" mg/l} = \frac{(a - b) \times 20}{a}$$

a = ml thiosulphate used for blank.
b = ml thiosulphate used for sample.

6. Determination of Total Dissolved Solids.

Apparatus.

Platinum, nickel or silica dish which has been heated at 105° C for 1 hour, cooled in a desiccator and weighed accurately.

Method.

Place the dish over an 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.

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 105° 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.

Calculation.

$$\text{Total dissolved solids at 105° C in mg/l} = \frac{\text{weight of solids in mg} \times 1{,}000}{\text{ml of sample}}$$

Note:—For the determination of dissolved solids, the sample, if turbid, must be filtered through Whatman No. 42 filter paper and the filtered sample used for the determination.

4. *Verdunde Swawelsuur.*—Voeg versigtig met klein hoeveelhede op 'n slag 1 volume sterk swawelsuur by 3 volumes water. Baie hitte word in die proses vrygestel en voorsorg moet getref word om te voorkom dat die suur spat en die glashouer kraak. Voeg na menging soveel N/80 permanganaat-oplossing daarby dat die mengsel 'n ligte blywende pienk kleur kry.

5. *Kaliumjodiedoplossing.*—Los 10 g kaliumjodied in 100 ml water op en bewaar in 'n amberkleurige glasbottel.

6. *Stysel.*—Vryf 1 g oplosbare stysel met 'n bietjie koue gedistilleerde water fyn tot 'n gladde pasta en giet dit al roerende in 1 liter kokende gedistilleerde water. Laat 1 minuut kook en voor gebruik afkoel.

Daar behoort elke keer 'n vars oplossing gebruik te word.

Standaardisering van natriumtiosulfaatoplossing.

Los ongeveer 2 g kaliumjodied (KI), vry van jodaat, in 'n Erlenmeyerfles met 100–150 ml gedistilleerde water op, voeg 10 ml swawelsuur (1 + 9) by, gevolg deur presies 20 ml standaarddichromaatoplossing. Plaas 5 minute in die donker, verdun tot ± 400 ml en titreer met tiosulfaat totdat 'n ligte strooikleur te voorskyn tree, voeg stysel by en titreer tot die oplossing kleurloos is. Indien die tiosulfaat nie presies 0·025 N is nie, moet dit gereël word totdat dit wel presies daardie normaliteit het.

Metode.

Plaas 10 ml verdunde swawelsuur en 20 ml permanganaatoplossing in 'n skoon fles van 12 ons met 'n glasprop. Voeg 100 ml van die monster daarby en meng onmiddellik deur die fles versigtig te draai. Hou 4 uur lank op 'n temperatuur van 27° C deur dit in 'n waterbad met konstante temperatuur te sit, terwyl die inhoud na 1 uur weer geskud word indien die monster baie gesuspendeerde stowwe bevat. Voeg na 4 uur 5 ml van die jodied-oplossing of omtrent 0·5 g kaliumjodied daarby, en titreer onmiddellik nadat dit gemeng is, met 0·025 N tiosulfaat-oplossing, terwyl teen die einde van die titrering 2 ml styseloplossing toegevoeg word. Titreer totdat die blou kleur net verdwyn en neem geen notisie daarvan as die blou kleur nadat die oplossing gestaan het, weer te voorskyn tree nie. Doen 'n kontrolebepaling waarby dieselfde werkwyse sonder die monster toegepas word maar met gebruikmaking van 100 ml gedistilleerde water in plaas daarvan.

Berekening.

$$\text{Geabsorbeerde suurstof, mg/l} = \frac{(a - b) \times 200}{a}$$

waarby—

a = ml tiosulfaat vir die kontrolebepaling gebruik.
b = ml tiosulfaat vir die monstergebruik.

6. Bepaling van totale opgeloste vaste stowwe.

Apparatuur.

'n Platinum-, nikkel- of silikabakkie wat 1 uur lank by 105° C verhit, daarna in 'n desikkator afgekoel en noukeurig geweeg is.

Metode.

Plaas die bakkie oor 'n opening in 'n waterbad. Giet omtrent 50–250 ml van die monster, al na gelang van die konsentrasie van die totale vaste stowwe, in 'n maatfles met 'n glasprop.

Maak die verdampingsbakkie amper vol met 'n gedeelte van die monster, en voeg wanneer hierdie gedeelte tot droog verdamp is, nog 'n gedeelte daarby, en so voorts tot die hele monster na die bakkie oorgebring is.

Vee die buitekant van die bakkie wanneer dit heeltemal droog is, af en plaas dit dan vir 2 uur by 105° C in 'n oond; sit dit vervolgens in 'n desikkator en weeg na omtrent 5 tot 10 minute. Die toename in gewig gee die gewig van die vaste stowwe in die monster weer.

Berekening.

$$\text{Totale opgeloste vaste stowwe by 105° C, in mg/l} = \frac{\text{gewig van vaste stowwe in mg} \times 1{,}000}{\text{ml monster}}$$

Opmerking.—As die monster troebel is, moet dit voor die bepaling van die opgeloste vaste stowwe, eers deur Whatmanfiltreerpapier No. 42 gefiltreer word.

7. Determination of Suspended Solids.

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.

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.

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.

Apparatus.

Flame photometer.

Reagents.

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 millimetre. Keep for preference in a polythene bottle. Dilute as required for use.

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

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.

The results should be expressed as follows:—

Sodium as Na in mg/l.

9. Determination of Soap, Oil and Grease.

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.

Storage.

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

7. Bepaling van gesuspendeerde vaste stowwe.

Apparatuur.

'n Sinterglaskroesie, Pyrex graad 2 (gemiddelde porie-diameter 40–60 mikron). Spoel goed uit met gedistilleerde water. Droog daarna 2 uur lank by 105° C of weeg totdat die gewig konstant bly; laat in 'n desikkator afkoel en weeg noukeurig.

Metode.

Gebruik 200 ml van die monster. Wanneer die vaste stowwe in die monster baie fyn gesuspendeer is, is dit miskien raadsaam om die monster eerste te sentrifugeer totdat die vaste stowwe afgeskei is, aangesien die sinterglas anders maklik "verstop" raak. Filtreer eers die boonste laag van die monster onder ligte suiging, en bring daarna die hele monster na die filter oor; spoel die monsterbeker of die sentrifugeerbuis, indien gebruik, met gedistilleerde water uit.

Laat die kroesie 4 uur lank by 105° C droog, sit daarna vir 30 minute in 'n desikkator en weeg. Die toename in gewig is die gewig van die gesuspendeerde vaste stowwe.

Berekening.

$$\text{Gesuspendeerde vaste stowwe, in mg/l} = \frac{\text{gewig van vaste stowwe in mg} \times 1{,}000}{\text{ml monster}}$$

8. Bepaling van natrium.

Apparatuur.

Vlamfotometer.

Reagense.

1. *Standaardnatriumoplossing.*—Los 2·5413 g droë natriumchloried (NaCl) (kwaliteit: analitiese reagens) in gedistilleerde water op en vul by tot 1·0 liter. Hierdie oplossing bevat 1·0 mg natrium (Na) per milliliter. Bewaar by voorkeur in 'n politeenbottel. Verdun net voor gebruik.

2. *Natriumuitstralingsbuffer.*—Versadig en filtreer gedistilleerde water agtereenvolgens met kalsium-, kalium- en magnesiumchloried van die kwaliteit van analitiese reagense.

Metode.

Die vervaardigers van die instrument behoort 'n volledige gebruiksaanwysing te verskaf, dog besondere aandag behoort aan onderstaande punte gegee te word.

Die instrument word gebruik vir vergelyking met bekende standaarde, waarvoor 'n ykingskromme gemaak word oor 'n gebied van, bv. 0 tot 100 mg per liter. Die monster moet, wanneer nodig, verdun word sodat sy natriumgehalte binne die vereiste gebied val. Telkens wanneer die instrument gebruik word, behoort minstens een standaard waargeneem te word. Die analis moet homself oortuig deur die gebruik van 'n voldoende aantal standaarde, dat die reproduseerbaarheid en die noukeurigheid van die resultate deur die bepaalde instrument aangegee, geskik vir die vereiste doel is.

Die gebruik van sagte glas of ander moontlike bronne van besoedeling deur spore van natrium moet vermy word.

1·0 ml van die uitstralingsbuffer behoort by 25·0 ml van die monster en van die standaard gevoeg te word om die interferensie deur kalsium, kalium en magnesium tot 'n konstante peil te bring.

Die resultate behoort soos volg aangegee te word:—

Natrium as Na in mg/l.

9. Bepaling van seep, olie en ghries.

Monsterneming.

Sorg behoort gedra te word dat die monster verteenwoordigend is. Monsters behoort in skoon stopflesse geneem te word wat voor gebruik met 'n oplosmiddel uitgespoel en in die lug gedroog is. Die fles moet nie heeltemal vol gemaak word nie, aangesien daar met die toeprop van die fles van die bodrywende olie verlore sou kan gaan. Dit is raadsaam om die verlangde hoeveelheid monster in 'n te groot fles te versamel waarop vantevore aan die buitekant 'n merk aangebring is by die verlangde volume.

Bewaring.

Monsters behoort so spoedig moontlik nadat hulle geneem is, ontleed te word, aangesien baie olies en koolwaterstofverbindings deur bakterieë aangetas word.

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

Apparatus.

1. Round-bottomed, ground glass neck flask, of 2 litres capacity.
2. Oil trap, with a total graduation of 2 millilitres and each division equalling not more than 0·05 millilitres. All joints to be of ground glass.
3. Cold water condensor, with ground glass joints.
4. Separating funnel, of approximately two litres capacity, and with no-lubrication stopcock or with all greasy lubricants removed from the ground glass surfaces.

Reagents.

1. *Sulphuric Acid* (H_2SO_4) 1 + 1.
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 milligrams per 100 millilitres.

Method.

(*a*) *Volatile Oils.*—Place the well-mixed sample, usually 1 litre, in the round-bottomed flask, add boiling beads and place the flask in a suitable heating mantle. Fit the oil trap to the neck of the flask, and connect the condensor to the top of the oil trap.

Switch on the heat to the mantle, and when the sample commences refluxing record the volume of volatile oils in the trap after 5 minutes, 15 minutes and every 15 minutes following from the beginning of refluxing. Turn off the heat when the volume of oil in the trap increases by not more than 0·05 millimetres in any 15 minute period.

(*b*) *Extracted Soap, Grease and Oil.*—Allow the above sample to cool to room temperature. Transfer to the separating funnel, and acidify with 5 ml sulphuric acid per litre. Rinse the flask carefully with 15 ml of petroleum ether and add the ether washings to the separating funnel. Add an additional 25 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 condensor, and boil off the remaining solvent at the same temperature. Dry the flask on a water-bath.

Cool in a desiccator and weigh.

Calculation.

(*a*) *Volatile Oils.*—The average S.G. of volatile oils is taken as 0·7.

$$\text{Volatile oil in mg/l} = \frac{\text{ml oil found} \times 0{\cdot}7 \times 1{,}000}{\text{ml sample}}$$

(*b*) *Extracted Soap, Grease and Oil.*—The total gain in weight of the weighed flask less the calculated residue from the solvent, as determined by the distillation or

Indien dit nie onmiddellik gedoen kan word nie, moet 5 ml swawelsuur (1 + 1) per liter gebruik word om bakterie-aantasting te voorkom.

Apparatuur.

1. 'n Rondeboomfles met 'n geslypte nek en 'n inhoudsmaat van 2 liter.
2. 'n Olie-opvanger met 'n totale graadverdeling van 2 milliliters; elke verdeling mag nie meer as 0·05 milliliters wees nie. Alle verbindingstukke moet van slypglas wees.
3. Kouwaterkoeler met slypglasverbindings.
4. 'n Skeitregter, met 'n inhoudsmaat van ongeveer twee liter, en 'n afsluitkraan wat sonder smeermiddel werk, of met alle vetterige smeermiddels van die geslypte vlakke verwyder.

Reagense.

1. *Swawelsuur* (H_2SO_4) 1 + 1.
2. *Petroleumeter*, kookpunt 35° tot 60° C.—Distilleer minstens twee maal in 'n apparaat wat heeltemal van glas is; gooi die laaste 10 persent wat by elke distillering in die fles agterbly, weg. Die residu by verdamping behoort minder as 0·1 milligram per 100 milliliters te wees.

Metode.

(*a*) *Vlugtige olies.*—Plaas die goedgemengde monster, gewoonlik 1 liter, in die rondeboomfles, saam met 'n paar krale, en omring die fles met 'n geskikte verwarmingsmantel. Bevestig die olie-opvanger aan die fles se nek, en verbind die koeler met die bopunt van die opvanger.

Slaan die verwarmingstroom na die mantel aan, en teken die volume vlugtige olies in die opvanger aan wanneer die monster begin terugvloei en weer na 5 minute en daarna elke 15 minute. Slaan die verwarmingstroom af wanneer die olie in die opvanger hoogstens 0·05 milliliters in volume toeneem gedurende enigeen van die tydperke van 15 minute.

(*b*) *Geëkstraheerde seep, ghries en olie.*—Laat bogenoemde monster tot kamertemperatuur afkoel. Bring na die skeitregter oor en suur aan met 5 ml swawelsuur per liter. Spoel die fles versigtig met 15 ml petroleumeter uit en giet die eter wat daarvoor gebruik is, in die skeitregter oor. Giet nog 25 ml petroleumeter in die skeitregter en skud flink 2 minute lank. Laat die eterlaag afskei, onttrek die waterige gedeelte van die monster in 'n skoon houer en bring die laag oplosmiddel oor na 'n skoon, geweegde distilleerkolf wat minstens drie volumes van die oplosmiddel kan bevat. Indien geen helder laag eter verkry kan word nie, filtreer dan die laag oplosmiddel in die geweegde distilleerkolf deur 'n tregter met 'n stuk Whatman-filtreerpapier nr. 40 (of 'n dergelike papier) wat met eter vogtig gemaak is. Die tregter en filter moet so klein moontlik wees. Nadat al die eter van die twee ekstraksies en die laaste spoelsel in die kolf beland het, moet die tregter en die filtreerpapier twee maal met vars 5 ml-hoeveelhede petroleumeter afgespoel word.

Sit die monster terug in die skeitregter en spoel die houer met 15 ml eter uit. Giet die spoelsel en nog 25 ml eter in die skeitregter, en skud weer 2 minute lank.

Laat die laag oplosmiddel afskei en gooi die waterige fase weg. Giet die geëkstraheerde eter in die geweegde distilleerkolf en spoel die skeitregter met 20 ml eter uit. Giet die eterspoelsel in die geweegde distilleerkolf.

Distilleer alles af op ongeveer 10 ml van die eterekstrak na en hou die warmtebron op ongeveer 70° C. Maak dan die koeler los en laat die oorblywende oplosmiddel by dieselfde temperatuur kook. Maak die fles op 'n waterbad droog.

Laat in 'n desikkator afkoel en weeg.

Berekening.

(*a*) *Vlugtige olies.*—Daar word aangeneem dat die gemiddelde s.g. van vlugtige olies 0·7 is.

$$\text{Vlugtige olie, in mg/l} = \frac{\text{ml olie gevind} \times 0{\cdot}7 \times 1{,}000}{\text{ml monster}}$$

(*b*) *Geëkstraheerde seep, ghries en olie.*—Die totale gewigstoename van die geweegde fles min die berekende residu van die oplosmiddel, soos deur diltillasie of verdamping van 'n afgemete hoeveelheid bepaal, gee die

evaporation of a measured quantity, indicates the amount of extracted soap, oil or grease in the water sample.

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

The values for (*a*) and (*b*) are added to give the total concentration of soap, grease and oil in the effluent.

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.

Reagents.

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.

2. *Sodium Thiosulphate.*—Stock solution, 0·25 N. Dissolve 63 g sodium thiosulphate ($Na_2S_2O_3,5H_2O$) 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.

3. *Sodium Thiosulphate*, 0·025 N.—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.

4. *Standard Potassium Dichromate Solution*, 0·025 N.—Dissolve 1·226 g previously dried potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1 litre.

Standardisation of 0·025 *N 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 0·025 N adjust it until it is.

5. *Sodium Thiosulphate*, 0·005 N.—Pipette 20 ml of the standardised 0·025 N sodium thiosulphate into a 100 ml volumetric flask and make up to the mark with distilled water: This olution should be prepared fresh daily. 1 ml = 0·178 mg of chlorine.

6. *Stock Iodine Solution* ± 0·1 *N.*—Place 40 g of potassium iodide and 13 g resublimed iodine together in a beaker and add small amounts of water, with stirring until all the iodine has been dissolved. Transfer to a 1-litre flask and dilute to the mark.

7. *Iodine Working Solution* ± 0·005 *N.*—Pipette 25 ml of the iodine stock solution into a 500-ml flask and make to the mark with distilled water. Standardise this solution against the 0·005 N sodium thiosulphate in the normal way, using starch indicator, and calculate the factor F.

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

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.

9. *Acetate Buffer Solution*, pH 4·0.—Dissolve 146 g anhydrous sodium acetate in 400 ml distilled water, add 480 g glocial acetic acid and dilute to 1 litre with distilled water.

Method.

Place 5·0 ml of 0·005 N 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

hoeveelheid geëkstraheerde seep, olie of ghries in die monster water weer.

$$\text{Geëkstraheerde seep, ghries en olie, in mg/l} = \frac{\text{mg gew: toename} - \text{mg residu} \times 1{,}000}{\text{ml monster}}$$

Die waardes vir (*a*) en (*b*) word daarby opgetel om die totale konsentrasie seep, ghries en olie in die afvalwater te verkry.

10. BEPALING VAN OORBLYWENDE CHLOOR.

Opmerking.

Die bepaling van ongebonde chloor behoort so spoedig moontlik na monsterneming te geskied.

Reagense.

1. *Kaliumjodiedoplossing.*—Los 50 g kaliumjodied (KI) wat geen jodium of jodaat bevat nie, in 1 liter pasgekookte en afgekoelde gedistilleerde water op.

2. *Natriumtiosulfaat.*—Voorraadoplossing, 0·25 N. Los 63 g natriumtiosulfaat ($Na_2S_2O_3 \cdot H_2O$) in 1 liter kopervrye, pasgekookte en afgekoelde gedistilleerde water op en voeg 1 ml chloroform of 10 mg kwik-II-jodied daarby om die oplossing bestendig te maak. Laat voor gebruik 'n paar dae staan.

3. *Natriumtiosulfaat*, 0·025 N.—Verdun 100 ml voorraadoplossing tot 1 liter met kopervrye, pasgekookte en afgekoelde gedistilleerde water, en voeg 1 ml chloroform of 10 mg kwik-II-jodied daarby. Hierdie oplossing is redelik bestendig maar dit moet met kort tussenpose teen kaliumdichromaat gestandaardiseer word. Bewaar in 'n amberkleurige bottel met 'n rubberprop en gooi die oplossing wat aan die einde van die dag in die buret agterbly, weg.

4. *Standaardkaliumdichromaatoplossing*, 0·025 N.—Los 1·226 g vantevore gedroogde kaliumdichromaat ($K_2Cr_2O_7$) in gedistilleerde water op en verdun tot 1 liter.

Standaardisering van 0·025 N *natriumtiosulfaatoplossing.*

Los ongeveer 2 g kaliumjodied (KI) wat geen jodaat bevat nie, in 'n Erlenmeyerfles met 100–150 ml gedistilleerde water op; voeg dan eers 10 ml swawelsuur (1 + 9- en daarna presies 20 ml standaarddichromaatoplossing daarby. Plaas vir 5 minute in die donker, verdun tot ± 400 ml en titreer met tiosulfaat totdat 'n ligte strooikleur te voorskyn tree; voeg dan stysel by en titreer tot dit kleurloos is. As die tiosulfaat nie presies 0·025 N is nie, moet dit so gereël word dat dit wel hierdie normaliteit het.

5. *Natriumtiosulfaat*, 0·005 N.—Pipetteer 20 ml van die gestandaardiseerde 0·025 N natriumtiosulfaat in 'n maatfles van 100 ml en vul met gedistilleerde water op tot by die merk. Hierdie oplossing moet elke dag vars aangemaak word. 1 ml = 0·178 mg chloor.

6. *Jodiumvoorraadoplossing*, ± 0·1 N.—Plaas 40 g kaliumjodied en 13 g geresublimeerde jodium tesame in 'n beker en voeg klein hoeveelhede water daarby; roer tot al die jodium opgelos is. Bring na 'n literfles oor en verdun tot by die merk.

7. *Jodiumwerkoplossing*, ± 0·005 N.—Pipetteer 25 ml van die jodiumvoorraadoplossing in 'n fles van 500 ml en vul met gedistilleerde water op tot by die merk. Standaardiseer hierdie oplossing op die gewone manier teen die 0·005 N natriumtiosulfaat en gebruik stysel as indikator en bereken die faktor F.

$$F = \frac{\text{ml natriumtiosulfaat}}{\text{ml jodium}}$$

8. *Stysel.*—Vryf 1 g oplosbare stysel met 'n bietjie koue gedistilleerde water fyn tot 'n gladde pasta en giet, al roerende, in 1 liter kokende gedistilleerde water oor. Laat 1 minuut kook en afkoel voor gebruik.

Hierdie oplossing moet telkens voor gebruik vars aangemaak word.

9. *Asetaatbufferoplossing*, pH 4·0.—Los 146 g anhidriese natriumasetaat in 400 ml gedistilleerde water op, voeg 480 g ysasynsuur daarby en verdun met gedistilleerde water tot 1 liter.

Metode.

Plaas 5·0 ml natriumtiosulfaat (0·005 N) in 'n fles. Voeg 5 ml kaliumjodiedoplossing en voldoende asetaatbufferoplossing daarby om die pH te verlaag tot tussen 3·5 en 4·2 (ongeveer 10 ml). Giet 1,000 ml van die monster

sample and mix with a stirring rod. Just prior to titration with 0·005 N iodine add 5 ml of starch solution. Titrate with iodine, from a micro-burette, to the first appearance of blue colour.

Calculation.

$$\text{Residual chlorine as } Cl_2 \text{ in mg/l} = \frac{(A - BF) \times 0{\cdot}178 \times 1{,}000}{\text{ml of sample}}$$

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.

Reagents.

1. *Light Magnesium Oxide.*

2. *Sulphuric Acid* exactly 1·000 N H_2SO_4.

3. *Sulphuric Acid* 0·02 N.—Dilute 20·0 ml of 1·000 N sulphuric acid to 1·0 litre. 1 ml = 0·28 mg nitrogen as N.

4. *Boric Acid Solution.*—Dissolve 40 g boric acid (H_3BO_3) in 1 litre ammonia-free distilled water.

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.

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 (see subsequent method).

To the distillate add 3 drops indicator solution and titrate the ammonia with 0·02 N 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.

Calculation.

$$\text{mg/l ammonia N} = \frac{\text{ml 0·02 N acid} \times 0{\cdot}28 \times 1{,}000}{\text{ml of sample}}$$

∴ If 280 ml of sample has been used the ammonia 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. 560 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.

Reagents.

1. All the reagents required for the determination of ammoniacal nitrogen plus:

2. *Devarda Alloy.*—Nitrogen-free and fine enough to pass through a 200 mesh sieve.

Method.

The ammonia is distilled off as described under "Determination of Free and Saline Ammonia" 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 0·02 N sulphuric acid as in the previous method.

in die fles en roer met 'n stafie. Voeg net voor titrering met 0·005 N jodium 1 ml styseloplossing by. Titreer met jodium met behulp van 'n mikroburet totdat 'n blou kleur te voorskyn tree.

Berekening.

$$\text{Oorblywende chloor as } Cl_2, \text{ in mg/l} = \frac{(A - BF) \times 0{\cdot}178 \times 1{\cdot}000}{\text{ml monster}}$$

waarby—

A = ml natriumtiosulfaat by monster gevoeg.
B = ml jodium by titrering gebruik.
C = faktor vir jodiumoplossing.

11. BEPALING VAN ONGEBONDE AMMONIAK EN AMMONIUMSOUTE.

Reagense.

1. *Ligte magnesiumoksied.*

2. *Swawelsuur*, presies 1·000 N H_2SO_4.

3. *Swawelsuur*, 0·02 N.—Verdun 20·0 ml 1·000 N swawelsuur tot 1·0 liter. 1 ml = 0·28 mg stikstof as N.

4. *Boorsuuroplossing.*—Los 40 g boorsuur (H_3BO_3) in 1 liter gedistilleerde water wat geen ammoniak bevat nie, op.

5. *Indikatoroplossing.*—Los 0·2 g broomkresolblou en 0·04 g metielrooi in 120 ml 95-persentige alkohol op.

Metode.

Neem 280 ml* monster met ammoniakvrye gedistilleerde water verdun. As die monster suur of baie alkalies is, neutraliseer dit dan tot 'n pH van ongeveer 7, en voeg daarna 0·4 g magnesiumoksied daarby. Die mengsel moet so flink moontlik geskud en gedistilleer word uit 'n distilleerkolf van 1 liter met 'n spatkop en 'n vertikale pyrex-koeler in 50 ml boorsuuroplossing. Die distillering moet voortgesit word tot 250–300 ml oorgedistilleer is. 'n Hulpstuk aan die koeler behoort tot onder die oppervlak van die boorsuur te reik.

Die residu in die distilleerkolf behoort bewaar te word vir die bepaling van stikstof as nitraat (sien volgende metode).

Voeg 3 druppels indikatoroplossing by die distillaat en titreer die ammoniak met 0·02 N swawelsuur, tot die omslagpuntkleur dieselfde is as dié van 'n kontroletoets waarby ewe groot hoeveelhede boorsuur en indikator gebruik is wat tot dieselfde volume verdun is met gedistilleerde water wat geen koolsuurgas bevat nie. 'n Kontrolebepaling behoort op die gebruikte reagense gedoen en die nodige korreksies gemaak te word.

Berekening.

$$\text{mg/l N-ammoniak} = \frac{\text{ml 0·02 N suur} \times 0{\cdot}28 \times 1{,}000}{\text{ml monster}}$$

∴ As 280 ml monster gebruik is, is die ammoniak in mg/l numeries gelyk aan die volume suur by die titrasie gebruik.

*By lae konsentrasies ammoniak sal dit miskien nodig wees om 'n groter volume monster te neem (bv. 560 ml).

12. BEPALING VAN NITRAAT.

Na bepaling van ammoniak d.m.v. die distilleermetode waarby magnesiumoksied gebruik word, word nitrate en nitriete in die residu gereduseer deur met Devarda se legering te kook; die aldus geproduseerde ammoniak word oorgedistilleer en d.m.v. titrasie bepaal.

Reagense.

1. Dieselfde reagense as vir die bepaling van stikstof gebruik word, plus:

2. *Devarda se legering.*—Wat geen stikstof bevat nie en fyn genoeg is om deur 'n sif met maas 200 te gaan.

Metode.

Die ammoniak word afgedistilleer soos onder „ Bepaling van ongebonde ammoniak en ammoniumsoute " beskryf, met gebruik van 0·4 g ligte magnesia en 280 ml* monster. Die laaste deel van die distillaat word met Nessler se oplossing getoets om seker te maak dat al die ammoniak afgedistilleer is, 1 g Devarda se legering word toegevoeg en die mengsel word in 50 ml boorsuuroplossing oorgedistilleer. Wanneer gasborrels ontstaan as die distilleerkolf verhit word, word die hittebron laer gedraai om skuimvorming en 'n oormaat sproeisel te voorkom wanneer die mengsel begin om te kook. Die mengsel word eers 5–10 minute saggies en daarna nog 20 minute flink aan die kook gehou, mits nie al te veel skuim vorm nie. Versamel ongeveer 250–300 ml distillaat en titreer die ammoniak met 0·02 N swawelsuur netsoos by die voorgaande metode.

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

Calculation.

$$\text{mg/l nitrate N} = \frac{\text{ml } 0{\cdot}02 \text{ N acid} \times 0{\cdot}28 \times 1{,}000}{\text{ml of sample}} - \text{nitrite in mg/l}$$

∴ 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. 560 ml).

12A. DETERMINATION OF NITRITE.

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

Apparatus.

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

Reagents.

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

1. *Sulphanilic Acid Reagent.*—Completely dissolve 0·60 g sulphanilic acid in 70 ml hot distilled water, cool, add 20 ml concentrated hydrochloric acid, dilute to 100 ml with distilled water, and mix thoroughly.

2. *Naphthylamine Hydrochloride Reagent.*—Dissolve 0·60 g 1-naphthylamine hydrochloride in distilled water to which 1·0 ml concentrated hydrochloric acid has been added. Dilute to 100 ml with distilled water, and mix thoroughly. The reagent becomes discoloured and a precipitate may form after 1 week, but it is still usable. It should be discarded when the sensitivity or reproducibility is affected. Storage in a refrigerator extends the useful life of the reagent. Filter before using.

3. *Sodium Acetate Buffer Solution*, 2M.—Dissolve 16·4 g sodium acetate ($NaC_2H_3O_2$) or 27·2 g $NaC_2H_3O_2.3H_2O$ in distilled water and dilute to 100 ml. Filter if necessary.

4. *Sodium Nitrate Stock Solution.*—Dissolve 0·2463 g anhydrous sodium nitrite ($NaNO_2$) in nitrite-free distilled water and dilute to 1,000 ml; 1·00 ml = 0·050 mg nitrite as N. Preserve by adding 1 ml chloroform.

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 sterilised bottle.

6. *Aluminium Hydroxide.*—Dissolve 125 g potassium or ammonium alum [$K_2Al_2(SO_4)_4.24H_2O$ or $(NH_4)_2Al_2(SO_4)_4.24H_2O$], 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 ammonia, chloride, nitrite and nitrate.

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 pH7, or to an aliquot diluted to 50·0 ml, add 1·0 ml sulphanilic 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 mμ 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.

A 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.

Calculation.

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

Doen van tyd tot 'n kontrolebepaling op alle reagense by hierdie metode gebruik en maak die nodige korreksies.

Berekening.

$$\text{mg/l N-nitraat} = \frac{\text{ml } 0{\cdot}02 \text{ N suur} \times 0{\cdot}28 \times 1{\,}000}{\text{ml monster}} - \text{nitriet in mg/l}$$

∴ Indien 280 ml monster gebruik is, is die nitraat in mg/l N numeries gelyk aan die volume suur by die titrasie gebruik.

*Vir lae nitraatkonsentrasies sal dit miskien nodig wees om 'n groter volume monster te neem (bv. 560 ml)

12A. BEPALING VAN NITRIET.

(Moet tesame met die nitraatbepaling gedoen word).

Apparatuur.

'n Spektrofotometer.—Vir gebruik by 520 mμ, wat 'n optiese pad van 1 cm of langer lewer.

Reagense.

Alle reagense moet van chemikalieë wat wit van kleur is, berei word.

1. *Sulfanielsuurreagens.*—Los 0·60 g sulfanielsuur heeltemal op in 70 ml warm gedistilleerde water, laat afkoel, voeg 20 ml sterk soutsuur by, verdun met gedistilleerde water tot 100 ml, en meng goed.

2. *Naftielamienhidrochloriedreagens.*—Los 0·60 g 1-naftielamienhidrochloried in gedistilleerde water op waarby 1·0 ml sterk soutsuur gevoeg is. Verdun met gedistilleerde water tot 100 ml, en meng goed. Die reagens verkleur en miskien sal daar na 'n week 'n neerslag vorm, maar dit bly dan tog nog bruikbaar. Dit moet weggegooi word wanneer die gevoeligheid of reproduseerbaarheid aangetas word. Bewaring in 'n elektriese yskas verleng die gebruiksduur van die reagens. Filtreer voor gebruik.

3. *Natriumasetaatbufferoplossing*, 2 M.—Los 16·4 g natriumasetaat ($NaC_2H_3O_2$) of 27·2 g $NaC_2H_3O_2.3H_2O$ in gedistilleerde water op en verdun tot 100 ml. Filtreer indien nodig.

4. *Natriumnitrietvoorraadoplossing.*—Los 0·2463 g anhidriese natriumnitriet ($NaNO_2$) in nitrietvrye gedistilleerde water op en verdun tot 1,000 ml; 1·00 ml = 0·050 mg nitriet as N. Preserveer deur 1 ml chloroform by te voeg.

5. *Standaardnatriumnitraatoplossing.*—Verdun 10·00 ml natriumnitrietvoorraadoplossing tot 1,000 ml met nitrietvrye gedistilleerde water; 1·00 ml = 0·0005 mg nitriet as N. Hierdie oplossing kan goed gehou word deur toevoeging van 1 ml chloroform en bewaring in 'n gesteriliseerde bottel.

Metode.

Indien die monster gesuspendeerde vaste en kleurstowwe bevat voeg dan 2 ml aluminiumhidroksiedsuspensie by 100 ml monster, roer flink, laat 'n paar minute staan, en filtreer; gooi die eerste deel van die filtraat weg.

Voeg by 50·0 ml helder monster tot pH7 geneutraliseer, of by 'n deelvolume tot 50·0 ml verdun, 1·0 ml sulfanielsuurreagens. Meng goed. Op hierdie tydstip behoort die pH van die oplossing omtrent 1·4 te wees. Voeg nadat dit van 3 tot 10 minute gestaan het, 1·0 ml naftielamienhidrochloriedreagens en 1·0 ml natriumasetaatbufferoplossing by; meng goed. Op hierdie tydstip behoort die pH van die oplossing 2·0 tot 2·5 te wees. Meet die rooi-pers kleur na 10 tot 30 minute. Die optiese digtheid (absorpsie)-aflesings behoort by 520 mμ gedoen te word teen 'n kontrolereagens en parallelle kontroles behoort dikwels uitgevoer te word teen bekende nitrietstandaarde, verkieslik in die nitrietgebied van die monster. Volledige ykkrommes behoort gemaak te word na bereiding van nuwe reagense.

'n Ykkromme behoort geteken te word van optiese digtheid teen mg nitriet as N, deur hoeveelhede standaardnitrietoplossing tot 50·0 ml te verdun en hulle netso te behandel as die monster.

Berekening.

$$\text{Nitriet as N, in mg/l} = \frac{\text{mg nitriet N} \times 1{,}000}{\text{ml monster}}$$

13. DETERMINATION OF ARSENIC.

Apparatus.

1. Gutzeit generator consisting of a 200-ml flask with a single hole rubber stopper through which passes a glass column of about ½ inch diameter with a constricted lower end. (This is henceforth referred to as the guard column.)

The upper end of the guard column is connected by means of suitable rubber stoppers and glass tubing to a vertically placed absorbtion tube of approximately five inches length and ¾ inch diameter.

2. Pyrex glass beads, 2–3 mm diameter.

3. Spectrophotometer, for use at 820 mμ with light path of 1–2 cm.

Reagents.

1. *Saturated Bromine Water.*—Add 2 ml liquid bromine to 200 ml water in a 250 ml glass-stoppered bottle; shake well; and allow to stand.

2. *Sodium Hypobromite Solution.*—To 30 ml saturated bromine water add 30 ml distilled water and then exactly 20 ml 0·500 N sodium hydroxide. Prepare fresh daily.

3. *Ammonium Molybdate Solution.*—Dissolve 25 g ammonium molybdate ($(NH_4)_6Mo_7O_{24}.4H_2O$) in 300 ml distilled water. Add 75 ml concentrated sulphuric acid to approximately 100 ml distilled water, cool, and dilute to 200 ml. Add this solution to the molybdate solution. Store in pyrex or plastic bottle.

4. *Hydrazine Sulphate Solution.*—Dilute 50 ml saturated hydrazine sulphate solution with 50 ml distilled water.

5. *Standard Sulphuric Acid Solution*, 2·0 N.—This reagent must be standardised since too strong an acid inhibits colour development.

6. *Sulphuric Acid Solution*, approx. 24 N.—Add two volumes concentrated sulphuric acid carefully to one volume distilled water.

7. *Standard Arsenic Solution.*—Dissolve 0·3949 g arsenious oxide (As_2O_3) in 25 ml 10 per cent sodium hydroxide. Acidify with 6 N sulphuric acid and dilute to 1 litre with distilled water. 1 ml = 0·30 mg As.

Prepare fresh dilute solutions from stock as needed.

8. *Roll Cotton.*—Cut a roll of dentist's cotton into 1 in. lengths.

9. *Lead Acetate Solution.*—Dissolve 10 g lead acetate [$Pb(CH_3COO)_2.3H_2O$)] in 100 ml distilled water.

10. *Stannous Chloride Solution.*—Dissolve 40 g arsenic-free stannous chloride ($SnCl_2.2H_2O$) in 25 ml concentrated hydrochloric acid and make up to 100 ml with distilled water.

11. *Potassium Iodide Solution.*—Dissolve 15 g potassium iodide (KI) in water and dilute to 100 ml. Prepare fresh.

12. *Zinc.*—20 to 30 mesh, arsenic-free.

Procedure.

1. *Concentration of Sample (and Oxidation of any Organic matter).*—To a suitable aliquot of sample containing 0·002–0·040 mg As, add 5 ml of 24 N sulphuric acid and 5 ml concentrated nitric acid and evaporate to sulphur trioxide fumes. Cool, add about 25 ml distilled water, again evaporate to sulphur trioxide fumes to discharge oxides of nitrogen. Maintain an excess of nitric acid during evaporation, do not allow the solution to darken as arsenic may be lost. Dilute to 25 ml.

2. *Preparation of Guard Column and Absorption Tube.*—Dip the cotton roll into the lead acetate solution and put into the guard column. To the absorption tube add 2–3 inches of glass beads and 3 ml sodium hypobromite solution.

3. *Treatment of Concentrated Sample.*—Place the 25 ml of concentrate in the Gutzeit generator. Add 5 ml 24 N sulphuric acid, cool, and add 5 ml potassium iodide solution and 4 drops stannous chloride solution. Place 2–3 g zinc in the generator and immediately connect with the absorption tube. Place the generator in a water-bath at 20–25° C for 1–1½ hours. After complete evolution of arsine, wash the contents of the absorption tube into a calibrated test tube or measuring cylinder, with 6, 2 ml portions of distilled water. Add with mixing after each addition, 5·0 ml 2·0 N sulphuric acid, 1·0 ml molybdate solution, and 1·0 ml hydrazine sulphate solution. Dilute to 25 ml with distilled water, let stand for an hour and read the absorbance at 820 mμ and read off the graph.

13. BEPALING VAN ARSEEN.

Apparatuur.

1. 'n Gutzeit-generator wat bestaan uit 'n fles van 200 ml met 'n rubberprop met 'n gat waardeur 'n glaspyp, omtrent ½ dm in deursnee, en aan die onderent vernou, steek. (Dit word verder hierin die veiligheidspyp genoem). Die bo-ent van die veiligheidspyp is d.m.v. geskikte rubberproppe en glasbuise verbind met 'n vertikale absorpsiebuis omtrent 5 duim lank en ¾ dm in deursnee.

2. Pyrex-glaskrale, 2–3 mm in deursnee.

3. 'n Spektrofotometer vir gebruik by 820 mμ met 'n optiese pad van 1–2 cm.

Reagense.

1. *Versadigde broomwater.*—Voeg 2 ml vloeibare broom by 200 ml water in 'n stopfles van 250 ml; skud goed en laat staan.

2. *Natriumhipobromietoplossing.*—Voeg by 30 ml versadigde broomwater 30 ml gedistilleerde water en vervolgens presies 20 ml 0·50 N natriumhidroksied. Maak elke dag vars aan.

3. *Ammoniummolibdaatoplossing.*—Los 25 g ammoniummolibdaat [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] in 300 ml gedistilleerde water op. Voeg 75 ml sterk swawelsuur by ongeveer 100 ml gedistilleerde water, laat afkoel, en verdun tot 200 ml. Voeg hierdie oplossing by die molibdaatoplossing. Bewaar in 'n pyrex- of plastiekbottel.

4. *Hidrasiensulfaatoplossing.*—Verdun 50 ml versadigde hidrasiensulfaatoplossing met 50 ml gedistilleerde water.

5. *Standaardswawelsuuroplossing*, 2·0 N—. Hierdie reagens moet gestandaardiseer word aangesien 'n suur wat te sterk is, kleurontwikkeling stuit.

6. *Swawelsuuroplossing*, ongeveer 24 N—. Voeg twee volumes sterk swawelsuur versigtig by een volume gedistilleerde water.

7. *Standaardarseenoplossing.*—Los 0·3949 g arseenoksied (As_2O_3) in 25 ml 10-persentige natriumoksied op. Suur aan met 6 N swawelsuur en verdun met gedistilleerde water tot 1 liter. 1 ml = 0·30 mg As. Berei vars verdunde oplossings van die voorraad namate dit nodig word.

8. *'n Rol katoen.*—Sny 'n rol tandheelkundige katoen in stukkies van 1 dm lengte.

9. *Loodasetaatoplossing.*—Los 10 g loodasetaat ($Pb(CH_3COO)_2\cdot 3H_2O$) in 100 ml gedistilleerde water op.

10. *Tin-II-chloriedoplossing.*—Los 40 g arseenvrye tin-II-chloried ($SnCl_2\cdot 2H_2O$) in 25 ml sterk soutsuur op en vul met gedistilleerde water tot 100 ml by.

11. *Kaliumjodiedoplossing.*—Los 15 g kaliumjodied (KI) in water op en verdun tot 100 ml. Maak vars aan.

12. *Sink.*—20 tot 30 maas, arseenvry.

Werkwyse.

1. *Konsentrasie van monster (en oksidasie van organiese stowwe, as daar is).*—Voeg by 'n geskikte deelvolume monster wat 0·002—0·040 mg. As bevat, 5 ml 24 N swawelsuur en 5 ml sterk salpetersuur en laat verdamp tot swaweltrioksieddampe afgegee word. Laat afkoel, voeg ongeveer 25 ml gedistilleerde water daarby, laat weer verdamp tot swaweltrioksieddampe afgegee word om die swaweloksiede te verdryf. Sorg dat daar gedurende verdamping altyd 'n oormaat salpetersuur is, en moenie die oplossing donker laat word nie aangesien arseen sodoende verlore kan gaan. Verdun tot 25 ml.

2. *Bereiding van veiligheidspyp en absorpsiebuis.*—Doop die katoenrol in die loodasetaatoplossing en sit dit in die veiligheidspyp. Sit glaskrale in die absorpsiebuis tot 'n hoogte van 2—3 dm en 3 ml natriumhipobromietoplossing.

3. *Behandeling van gekonsentreerde monster.*—Plaas die 25 ml konsentraat in die Gutzeit-generator. Voeg 5 ml 24 N swawelsuur daarby, laat afkoel, en voeg 5 ml kaliumjodiedoplossing en 4 druppels tin-II-chloriedoplossing toe. Plaas 2–3 g sink in die generator en verbind onverwyld met die absorpsiebuis. Plaas die generator vir 1–1½ uur in 'n waterbad met 'n temperatuur van 20–25° C. Spoel na volledige evolusie van arsien die inhoud van die absorpsiebuis in 'n gekalibreerde proefbuisie of 'n maatsilinder, met 6 hoeveelhede gedistilleerde water van 2 ml elk. Voeg by 5·0 ml 2·0 N swawelsuur, 1·0 ml molibdaatoplossing en 1·0 ml hidrasiensulfaatoplossing met menging na elke byvoeging. Verdun met gedistilleerde water tot 25 ml, laat een uur lank staan en lees die optiese digtheid (absorpsie) by 820 mμ af en doen ook 'n aflesing op die kromme.

4. *Preparation of Standards.*—Carry at least one standard through the whole procedure to check the recovery. Measure, using the diluted stock arsenic solution, standards containing from 0·000–0·040 mg of arsenic (As) into calibrated tubes. Add 3 ml sodium hypobromite solution, dilute to about 15 ml with distilled water. Add with mixing after each addition, 5·0 ml 2 N sulphuric acid, 1·0 ml molybdate solution and 1·0 ml hydrazine sulphate solution. Make up to 25 ml, mix and let stand for 1 hour. Read the absorbances at 820 mμ with zero standard set at 100 per cent transmission and plot a calibration curve of mg arsenic (As) against absorbance.

Calculation.

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

14. Determination of Boron.

Apparatus.

Spectrophotometer, for use at 585 mμ, with minimum light path of 1 cm.

Reagents.

1. *Standard Sodium Hydroxide Solution,* 1 N.—Boron-free.

2. *Hydrochloric Acid.*—Concentrated.

3. *Hydrochloric Acid.*—1 + 19.

4. *Sulphuric Acid.*—Concentrated.

5. *Standard Boric Acid Solution.*—Dissolve 0·5716 g dry boric acid (H_3BO_3) in distilled water and dilute to 1 litre (1 ml = 0·100 mg B).

6. *Carmine Solution.*—Dissolve 0·92 g carmine NF40 or carminic acid in 1 litre concentrated sulphuric acid.

Method.

Preparation of standard curve. Dilute portions of the standard boric acid solution to obtain standards over the range of 0 to 10 mg/l boron. Treat 2·0 ml of each solution as described under "samples not containing organic matter", 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, at a wavelength of 585 mμ. Because the carmine reagent deteriorates, the standard curve should be checked daily.

Plot the absorbance against the concentration.

Samples not containing Organic Matter.—Pipette 2·0 ml. sample into 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 carmine solution, *mix well* and allow to stand at least 45 minutes. Determine the photometer reading at 585 mμ 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; cool; add 5·0 ml dilute hydrochloric acid and triturate with a rubber policeman. 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.

Waters containing Organic Material.—Make a suitable aliquot just alkaline with dilute sodium hydroxide (NaOH) and add a slight excess. Prepare a blank containing the same amount of alkali. Evaporate both sample and blank on a steam bath to dryness. Ignite at 500°–550° C. Cool and add 5·0 ml dilute hydrochloric acid. Make sure that solution is acid. Triturate 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.

4. *Bereiding van standaarde.*—Onderwerp minstens een standaard aan die hele prosedure om die herstel na te gaan. Meet met behulp van die verdunde arseenvoorraadoplossing, standaarde wat van 0·000–0·040 mg arseen (As) bevat, in gekalibreerde buisies af. Voeg 3 ml natriumhipobromietoplossing, by, verdun met gedistilleerde water tot omtrent 15 ml. Voeg by 5·0 ml 2 N swawelsuur, 1·0 ml molibdaatoplossing en 1·0 ml hidrasiensulfaatoplossing met menging na elke byvoeging. Vul by tot 25 ml, meng en laat 1 uur staan. Lees die optiese digtheid (absorpsie) by 820 mμ af met die nulstandaard op 100 persent transmissie gestel en teken 'n ykkromme van mg arseen (As) teen optiese digtheid (absorpsie) uitgesit.

Berekening.

$$\text{Arseen as As, in mg/l} = \frac{\text{mg As} \times 1{,}000}{\text{ml monster}}$$

14. Bepaling van boor.

Apparatuur.

'n Spektrofotometer vir gebruik by 585 mμ, met 'n minimum optiese pad van 1 cm.

Reagense.

1. *Standaardnatriumhidroksiedoplossing* 1 N, vry van boor.
2. *Soutsuur,* sterk.
3. *Soutsuur,* 1 + 19.
4. *Swalwelsuur,* sterk.
5. *Standaardboorsuuroplossing.*—Los 0·5716 g droë boorsuur (H_3BO_3) in gedistilleerde water op en verdun tot 1 liter. (1 ml = 0·100 mg B).
6. *Karmynoplossing.*—Los 0·92 g karmyn NF4O of karmynsuur in 1 liter sterk swawelsuur op.

Metode.

Teken van standaardkromme.—Verdun hoeveelhede van die standaardboorsuuroplossing om standaarde te verkry oor die reeks van 0 tot 10 mg/l boor. Behandel 2·0 ml van elke oplossing volgens die metode onder „ monsters wat geen organiese stowwe bevat nie " hieronder aangegee, en lees die fotometer af teen 'n kontrolehoeveelheid gedistilleerde water wat net soos die standaarde behandel is. Stel die kontrolehoeveelheid op 0 optiese digtheid (absorpsie) by 'n golflengte van 585 mμ. Omdat die karmynreagens versleg, moet die standaardkromme daagliks gekontroleer word. Sit die optiese digtheid (absorpsie) uit teen die konsentrasie.

Monsters wat geen organiese stowwe bevat nie.—Pipetteer 2·0 ml monster in 'n klein Erlenmeyerfles en voeg twee druppels sterk soutsuur daarby, gevolg deur 10·0 ml sterk swawelsuur; *skud goed* en laat afkoel. Voeg 10·0 ml karmynoplossing toe, *meng goed* en laat minstens 45 minute staan. Lees die fotometer af by 585 mμ teen 'n kontrolehoeveelheid gedistilleerde water wat net soos die monster behandel is.

Indien die boorkonsentrasie minder as 1 mg/l, pipetteer dan 'n geskikte deelvolume in 'n platinum-, silika- of porseleinbakkie; maak alkalies met verdunde NaOH en voeg 'n klein oormaat by. Berei 'n kontrolehoeveelheid wat net so veel alkali bevat. Laat sowel die monster as die kontrolehoeveelheid op 'n stoombad tot droog verdamp; laat afkoel; voeg 5·0 ml verdunde soutsuur by en verpoeier met 'n rubberpuntstafie. Indien die oplossing troebel is, giet dit dan in 'n koniese sentrifugeerbuisie en sentrifugeer tot dit helder is. Neem 2·0 ml van die helder oplossing vir ontleding. Monsters met 'n hoë boorgehalte moet met gedistilleerde water verdun word; neem in dié geval 'n geskikte deelvolume vir ontleding.

Water wat organiese stowwe bevat.—Maak 'n geskikte deelvolume net alkalies met verdunde natriumhidroksied (NaOH) en voeg 'n klein oormaat by. Berei 'n kontrolehoeveelheid met net so veel alkali. Laat sowel die monster as die kontrolehoeveelheid op 'n stoombad tot droog verdamp. Gloei uit by 500°–550° C. Laat afkoel en voeg 5·0 ml verdunde soutsuur daarby. Maak seker dat die oplossing suur is. Verpoeier met 'n rubberpuntstafie. Giet die oplossing in 'n koniese sentrifugeerbuis en sentrifugeer totdat dit helder is. Pipetteer 2·0 ml van die helder oplossing in 'n Erlenmeyerfles en gaan soos hierbo aangegee te werk.

Calculation.

The concentration of boron tested is read from the calibration curve. If sodium hydroxide was used in pretreating 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.

(A) Total Chromium.

Apparatus.

Spectrophotometer for use at 540 mμ.

Reagents.

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.

2. *Sulphuric Acid.*—S.G. 1·84.
3. *Nitric Acid.*—S.G. 1·42.
4. *Ammonium Oxalate Solution.*—Saturated.
5. *Sodium Sulphite.*—$Na_2SO_3.7H_2O$.
6. *Phosphoric Acid.*—60 per cent.
7. *Potassium Permanganate Solution.*—1 per centW/V
8. *Sodium Hydroxide Solution.*—15 per cent W/V
9. *Hydrogen Peroxide.*—3 per cent V/V (10 volumes).

*10. *Diphenylcarbazide Solution.*—Dissolve 0·25 g of diphenylcarbazide in 25 ml of alcohol and dilute to 100 ml with distilled water.

*11. *Sulphuric Acid, Dilute.*—5 per cent V/V

*12. *Standard Chromium Solution.*—Dissolve 0·3740 g of potassium chromate (K_2CrO_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 = 2μg of chromium.)

Method.

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 permangenate 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 diphenylcarbazide 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 wavelenth 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.

Berekening.

Die konsentrasie getoetste boor word van die ykkuwre afgelees. Indien natriumhidroksied by die voorbehandeling van die monster gebruik is, moet die resultaat gekorrigeer word deur die hoeveelheid B wat dit volgens die reagenskontrolebepaling bygedra het, af te trek. Indien die monster voor ontleding gekonsentreer of verdun was, moet die resultaat met die toepaslike faktor vermenigvuldig of daardeur gedeel word, om die konsentrasie wat bepaal is, te herlei tot die konsentrasie in die oorspronklike monster. Die resultate word soos volg aangegee:—

Boor as B, in mg/l.

15. BEPALING VAN CHROOM.

A. Totale Chroom.

Apparatuur.

'n Spektrofotometer vir gebruik by 540 mμ.

Reagense.

1. *Gedistilleerde water.*—Dit moet spesiaal van kraanwater wat swawelsuur en 'n paar kristalle kaliumpermanganaat bevat, gedistilleer word. Gedurende distillering en bewaring mag dit nie aan stof uit die lug blootgestel wees nie. Hierdie spesiaal bereide water behoort vir die reagense en by al die werk gebruik te word.
2. *Swawelsuur.*—S.g. 1·84.
3. *Salpetersuur.*—S.g. 1·42.
4. *Ammoniumoksalaatoplossing.*—Versadig.
5. *Natriumsulfiet.*—$Na_2SO_3 \cdot 7H_2O$.
6. *Fosforsuur.*—60-persentig.
7. *Kaliumpermanganaatoplossing.*—1 persent G/V.
8. *Natriumhidroksiedoplossing.*—15 Persent G/V.
9. *Waterstofperoksiedoplossing.*—3 Volume persent (10 volumes).

*10. *Difenielkarbasiedoplossing.*—Los 0·25 g difenielkarbasied in 25 ml alkohol op en verdun met gedistilleerde water tot 100 ml.

*11. *Swawelsuur, verdunde.*—5 Volumepersent.

*12. *Standaardchroomoplossing.*—Los 0·3740 g kaliumchromaat (K_2CrO_4) in gedistilleerde water op en verdun tot 1 liter. Verdun 10 ml van hierdie oplossing to 500 ml. Hierdie oplossing moet telkens vars aangemaak word (1 ml = 2μg chroom).

Metode.

Plaas 100 ml (of 'n ander geskikte volume) van hierdie afvalwater in 'n Kjeldahl-fles van 250 ml en los 0·1 g natriumsulfiet daarin op. Voeg 2 ml sterk swawelsuur daarby en verdamp tot wit swaweltrioksieddampe afgegee word. Voeg, indien nodig, druppelsgewyse sterk salpetersuur toe om organiese reste te oksideer. Voeg 10 ml versadigde ammoniumoksilaatoplossing by en laat weer verdamp tot dampe afgegee word. Verdun wanneer afgekoel met 10 ml gedistilleerde water en giet oor in 'n maatfles van 25 ml; verdun tot by die merk.

Meng goed en plaas 5 ml of 'n groter deelvolume, indien nodig, in 'n klein bekertjie, voeg 5 druppels fosforsuur daarby en verdamp tot dampe afgegee word. Laat afkoel en voeg 1 ml kaliumpermanganaatoplossing daarby, maak die beker met 'n horlosieglas toe en verhit 20 minute op 'n waterbad.

Neutraliseer met natriumhidroksiedoplossing onder gebruikmaking van lakmoespapier, en voeg 'n oormaat van 1 ml toe. Voeg 2 ml waterstofperoksied by en laat die oplossing 10 minute saggies op 'n verwarmingsplaat kook. Laat die oplossing afkoel, verdun in 'n maatfles tot 20 ml en filtreer. Meet noukeurig 'n volume filtraat (5 tot 10 ml) in 'n maatfles van 25 ml af, voeg 5 ml verdunde swawelsuur daarby en verdun tot omtrent 20 ml. Voeg 2·5 ml difenielkarbasiedoplossing toe en verdun tot by die merk. Laat 5 minute staan alvorens die kleurmeting gedoen word.

Doen 'n kontrolebepaling op al die reagense wat gebruik word. Meet die optiese digtheid (absorpsie) met behulp van 'n spektrofotometer by golflengte 540 mμ. Lees die aantal mikrogramme chroom wat ekwivalent is aan die waargenome optiese digtheid (absorpsie) van die toets- en die kontroleoplossings van 'n ykkromme af, en verkry aldus die netto gehalte aan chroom in die monster.

Aangesien die violetkleur ligter word as die oplossing bly staan, behoort die metings na 5 minute gedoen te word.

Construction of Calibration Curve.

Measure appropriate amounts of standard chromium solution covering the range 0 to 20 μg 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.

B. Hexavalent Chromium.

Reagents.

Those marked with an asterisk (*) in the method for "Total Chromium".

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, 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 it in an alkaline condition, using a filter aid.

Calculation.

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

B. Hexavalent chromium as Cr in mg/l = $\frac{\mu g \text{ hexavalent Cr}}{\text{ml of sample}}$

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.

Apparatus.

Spectrophotometer for use at 546 mμ.

Reagents.

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

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

3. *Sodium Hydroxide*, 5 N.—Prepare from analytical-reagent grade sodium hydroxide.

4. 2:2′-*Diquinolyl Solution*, 0·05 per cent, in amyl alcohol.

5. *Sodium Sulphate*, anhydrous.—Analytical-reagent grade.

6. *Amyl Alchohol.*—Analytical-reagent grade.

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 0·1 N in 500 ml volume (1 ml = 0·1 mg Cu).

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_2SO_4). 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.

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

Konstruksie van ykkromme.

Meet toepaslike hoeveelhede standaardchroomoplossing af oor die gebied 0 tot 20 μg chroom in 'n reeks maatflesse van 25 ml. Voeg by elkeen van hulle 5 ml verdunde swawelsuur en verdun die oplossing tot omtrent 20 ml. Voeg 2·5 ml difenielkarbasiedoplossing daarby en verdun elke oplossing tot by die merk. Laat 5 minute staan en meet dan die optiese digthede (absorpsies). Konstrueer 'n kurwe deur die optiese digthede teen die aantal mikrogramme chroom uit te sit.

B. Seswaardigde Chroom.

Reagense.

Dié wat met 'n sterretjie* gemerk is onder die reagense vir die bepaling van "totale chroom".

Metode.

Plaas 25 ml van die monster afvalwater in 'n silinder van 50 ml en voeg 5 ml difenielkarbasiedoplossing daarby; voeg vervolgens 10 ml verdunde swawelsuur daarby, meng en verdun tot 50 ml. Laat 5 minute staan en meet die optiese digtheid (absorpsie) soos in die metode vir "totale chroom" beskryf.

Indien die toetsoplossing troebel is, moet dit eers helder gemaak word, hetsy deur sentrifugering, hetsy deur filtrering in 'n alkaliese toestand met gebruik van 'n filterhulpmiddel.

Berekening.

A. Totale chroom as Cr, in mg/l = $\frac{\mu g \text{ totale Cr}}{\text{ml monster}}$

B. Seswaardige chroom as Cr, in mg/l = $\frac{\mu g \text{ seswaardige Cr}}{\text{ml monster}}$

16. BEPALING VAN KOPER.

Koperione is geneig om teen die oppervlak van die monsterhouer geabsorbeer te word. Monsters behoort dus so spoedig moontlik na versameling ontleed te word.

Apparatuur

'n Spektrofotometer vir gebruik by 546 mμ.

Reagense.

1. *Tartaarsuuroplossing.*—50 Persent in water—Berei van tartaarsuur van die analitiese reagensgraad.

2. *Hidroksielamien-hidrochloried.*—10 Persent in water. Berei van hidroksielamien-hidrochloried van die analitiese reagensgraad, en bewaar in 'n politeenbottel.

3. *Natriumhidroksied*, 5 N.—Berei van natriumhidroksied van die analitiese reagensgraad.

4. 2:2 *Dikinolieloplossing.*—0·05 Persent in amielalkohol.

5. *Natriumsulfaat*, anhidries.—Analitiese reagensgraad.

6. *Amielalkohol.*—Analitiese reagensgraad.

7. *'n Sterk oplossing van kopersulfaat.*—Los 0·1964 g kopersulfaat kristalle in gedistilleerde water op, voeg soveel suur (sout- of swawel-) by dat die finale asiditeit ongeveer 0·1 N is in 500 ml volume (1 ml = 0·1 mg Cu).

8. *'n Verdunde oplossing van kopersulfaat.*—Verdun 50·0 ml van die sterk kopersulfaatoplossing tot 250 ml met 0·1 N suur (HCL of H_2SO_4). Van tyd tot tyd moet 'n vars oplossing gemaak word aangesien koper stadig op die glas van die houer geabsorbeer word (1 ml = 0·02 mg Cu).

Opmerking—Dit is noodsaaklik om spore van koper uit die tartaarsuur- en die hidroksielamienoplossing te verwyder deur toevoeging van 5 ml van die 2:2′-dikinolieloplossing en ekstrahering met drie hoeveelhede amielalkohol van 20 ml elk.

Metode.

Plaas 400 ml van die monster in 'n 500 ml-skeitregter en voeg 2 ml elk van die tartaarsuur- en die hidroksielamienhidrochloriedoplossing daarby. Reël die pH tot tussen 5 en 6 deur 5 N natriumhidroksied en 'n indikatorpapier wat 'n klein gebied dek, te gebruik (dit is uiters belangrik want onder pH4 of bo pH7 ontstaan daar geen kleurkompleks nie). Vorm die lilakleurige kompleks deur 10 ml van die 2:2′-dikinolieloplossing te gebruik ('n oormaat veroorsaak 'n melkerige wolk). Ekstraheer die kompleks in twee hoeveelhede amielalkohol van 20 ml elk, droog die saamgevoegde ekstrakte deur met anhidriese natriumsulfaat te draaiskud, en filtreer in 'n maatfles van 50 ml. Verdun met amielalkohol tot by die merk en meet die optiese

with amyl alcohol and measure the 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.

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.

A. Preliminary Screening Procedure.

Apparatus.

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

Reagents.

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

1. *Copper Sulphate Solution.*—Dissolve 100 g of copper sulphate ($CuSO_4$, $5H_2O$) in distilled water and dilute to 1 litre.

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

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.

B. 4-Aminoantipyrine Method.

Apparatus.

Spectrophotometer for use at 460 mμ, having a light path of 1–10 cm.

Reagents.

1. *4-Aminoantipyrine Solution.*—Dissolve 2·0 g of 4-aminoantipyrine in distilled water and dilute to 100 ml. This solution should be prepared fresh weekly.

2. *Potassium Ferricyanide Solution.*—Dissolve 8 g of potassium ferricyanide ($K_3Fe(CN)_6$) 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.

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

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.

5. *Potassium Iodide.*—Crystals.

6. *Ammonium Hydroxide.*—Concentrated.

7. *Chloroform.*

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

The solution should be used freshly prepared.

9. *Sodium Thiosulphate.* Stock Solution, 0·25 N.—Dissolve 63 g sodium thiosulphate, $Na_2S_2O_3.5H_2O$ 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.

digtheid (absorpsie) by 546 mμ met die spektrofotometer. Lees die hoeveelheid koper in die deelvolume aanwesig van die ykkromme af. Die ykkromme moet gekonstrueer word deur 'n reeks koperstandaarde aan dieselfde behandeling te onderwerp as vir die monster beskryf. Sit milligramme koper teen optiese digtheid (absorpsie) uit.

Berekening.

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

17. BEPALING VAN FENOLVERBINDINGS.

Opmerking.—Wanneer fenolverbindings staan, ondergaan hulle maklik chemiese verandering as gevolg van oksidasie deur bakterieë veroorsaak. As die monster nie binne vier uur nadat dit geneem is, ontleed word nie, behoort daar 1 g kopersulfaat per liter bygevoeg te word as preserveermiddel. In dié geval behoort die monster binne 24 uur ontleed te word.

A. Preliminêre sifmetode.

Apparatuur.

Distilleertoestel, heeltemal van glas, soos bv. 'n Pyrex-distilleerkolf van 1 liter met 'n Graham-koeler.

Reagense.

Alle reagense moet met gedistilleerde water wat geen fenole en chloor bevat nie, berei word.

1. *Kopersulfaatoplossing.*—Los 100 g kopersulfaat ($CuSO_4 \cdot 5H_2O$) in gedistilleerde water op en verdun tot 1 liter.

2. *Fosforsuuroplossing.*—Verdun 10 ml 85-persentige fosforsuur met gedistilleerde water tot 100 ml.

Metode.

Voeg by 'n monster water van 100 ml 5·0 ml kopersulfaatoplossing, tensy dit reeds as preserveermiddel bygevoeg is. Reël die pH van die mengsel tot onder 4·0 met fosforsuuroplossing; 0·7 ml is vir die meeste monsters genoeg. Plaas die mengsel in die glasdistilleertoestel en distilleer 450 ml oor. Sit die distillasie stop en voeg wanneer die vloeistof ophou kook, 50 ml gedistilleerde water by die distilleerkolf. Gaan aan met distilleer totdat 500 ml in die geheel versamel is.

B. 4-Amino-antipirienmetode.

Apparatuur.

'n Spektrofotometer vir gebruik by 460 mμ, met 'n optiese pad van 1 tot 10 cm.

Reagense.

1. *4-Amino-antipirienoplossing.*—Los 2·0 g 4-amino-antipirien in gedistilleerde water op en verdun tot 100 ml. Hierdie oplossing moet weekliks vars aangemaak word.

2. *Kaliumferrisianiedoplossing.*—Los 8 g kaliumferrisianied ($K_3Fe(CN)_6$) in gedistilleerde water op en verdun tot 100 ml. Filtreer indien nodig. Hierdie oplossing is nie bestendig nie en behoort hernu te word wanneer ontbinding intree, wat blyk uit donkerwording van die oplossing, gewoonlik binne 'n week.

3 *Ammoniumchloriedoplossing.*—Los 50 g ammoniumchloried in gedistilleerde water op en verdun tot 1 liter.

4. *Kaliumbromaat-bromiedoplossing*, ongeveer 0·1 N.—Los 2·784 g kaliumbromaat in gedistilleerde water op, voeg 10 g kaliumbromied daarby, los op en verdun met gedistilleerde water tot 1 liter.

5. *Kaliumjodiedkristalle.*

6. *Ammoniumhidroksied.*—Gekonsentreer.

7. *Chloroform.*

8. *Stysel*—Vryf 1 g oplosbare stysel met 'n bietjie koue gedistilleerde water fyn tot 'n gladde pasta en giet, al roerende, in 1 liter kokende gedistilleerde water. Laat 1 minuut kook, en weer afkoel voor gebruik.

Die oplossing behoort vars aangemaak gebruik te word.

9. *Natriumtiosulfaat.*—Voorraadoplossing, 0·25 N. Los 63 g natriumtiosulfaat $Na_2S_2O_3 \cdot 5H_2O$, in 1 liter kopervrye, pasgekookte en afgekoelde gedistilleerde water op, en voeg 1 ml chloroform of 10 mg kwik-II-jodied daarby om die oplossing bestendig te maak. Laat voor gebruik verskeie dae staan.

(*a*) *Working Solution*, 0·025 N.—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. *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 ± 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.

11. *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 (10μg) 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:—

$$\text{Phenol (mg/l)} = \left(\frac{A \times B}{10} - C\right) \times 7{\cdot}835$$

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.

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 suitable 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.

(*a*) *Werkoplossing*, 0·025 N.—Verdun 100 ml voorraadoplossing tot 1 liter met kopervrye pasgekookte en afgekoelde gedistilleerde water, terwyl 1 ml chloroform of 10 mg kwik-II-jodied toegevoeg word. Hierdie oplossing is redelik bestendig, dog dit behoort met kort tussenpose teen kaliumdichromaat gestandaardiseer te word. Bewaar in 'n amberkleurige glasbottel met 'n rubberprop en as daar aan die einde van die dag van die oplossing in die buret agterbly, moet dit weggegooi word.

10. *Standaardkaliumdichromaatoplossing*, 0·025 N.—Los 1·226 g $K_2Cr_2O_7$ nadat dit eers gedroog is, in gedistilleerde water op en verdun tot 1 liter.

Standaardisering van natriumtiosulfaatoplossing.—Los ongeveer 2 g kaliumjodied (KI), vry van jodaat, met 100 tot 150 ml gedistilleerde water in 'n Erlenmeyerfles op, voeg 10 ml swawelsuur (1 + 9) daarby en vervolgens presies 20 ml standaarddichromaatoplossing. Plaas 5 minute in die donker, verdun tot ± 400 ml en titreer met tiosulfaat tot 'n ligte strooikleur te voorskyn tree, voeg dan stysel by en titreer tot dit kleurloos is. Indien die tiosulfaat nie presies 0·025 N is, moet dit gereël word totdat dit wel daardie normaliteit het.

11. *Fenoloplossings.*

(*a*) *Voorraadoplossing.*—Los 1 g fenol in gedistilleerde water op en verdun tot 1 liter. Standaardiseer soos hieronder aangegee.

(*b*) *Tussenoplossing.*—Verdun 10 ml voorraadoplossing met gedistilleerde water tot 1 liter. Een ml van hierdie oplossing bevat 0·01 mg (10 μg) fenol.

(*c*) *Standaardoplossing.*—Verdun 5 ml tussenoplossing met gedistilleerde water tot 500 ml. Een ml van hierdie oplossing bevat 0·1 μg fenol.

Standaardisering van fenoloplossings.—Plaas ongeveer 100 ml gedistilleerde water in 'n Erlenmeyerfles van 500 ml met 'n glasprop, en voeg 50 ml fenolvoorraadoplossing daarby, gevolg deur presies 10·0 ml kaliumbromaat-bromiedoplossing en ongeveer 5 ml sterk soutsuur. Draaiskud versigtig in die toegepropte fles. Indien die bruin kleur van die ongebonde broom nie bly voortbestaan nie, moet bromaat-bromiedoplossing in hoeveelhede van presies 10 ml bygevoeg word totdat die bruin kleur bly. Prop toe en laat 10 minute staan; voeg daarna ongeveer 1 g kaliumjodied daarby. Vier hoeveelhede bromaat-bromiedoplossing van 10 ml elk is nodig as dié voorraadoplossing 1,000 mg/l fenol bevat. Maak op dieselfde manier 'n kontrolehoeveelheid aan van gedistilleerde water en 10 ml kaliumbromaat-bromiedoplossing. Titreer sowel die kontrole hoeveelheid as die monster met 0·025 N natriumsulfaatoplossing en gebruik styseloplossing as indikator.

Bereken die sterkte van die fenoloplossing met behulp van die volgende formule:—

$$\text{Fenol (mg/l} = \left(\frac{A \times B}{10} - C\right) \times 7{\cdot}835$$

A = ml 0·025 N natriumtiosulfaatoplossing vir kontrolebepaling gebruik.
B = ml kaliumbromaat-bromiedoplossing vir monster gebruik.
C = ml 0·025 N natriumtiosulfaatoplossing vir monster gebruik.

Metode.

Bepaal na voltooiing van die preliminêre sifting d.m.v. 'n voorlopige kontrolering die geskikte deelvolume van die distillaat wat vir die finale bepaling gebruik moet word. Dit kan gedoen word deur Nesslerbuisies van 50 ml vir die reaksie te gebruik en met geskikte standaarde te vergelyk. In hierdie geval is geen chloroformekstraksie nodig nie.

Verdun die geskikte deelvolume met gedistilleerde water tot 500 ml en plaas in 'n literbeker. Berei ook 'n kontrolehoeveelheid en 'n reeks fenolstandaarde van 500 ml wat 5, 10, 20, 30, 40 en 50 μg fenol bevat.

Behandel die monster, die kontrolehoeveelheid en die standaarde soos volg: Voeg 10 ml ammoniumchloried-oplossing by en reël die pH met ammoniumhidroksied tot 10·0 + 0·2. Dit vereis gewoonlik van 3·5 tot 5·0 ml ammoniumhidroksied. Giet oor in skeitregters, voeg dan 3·00 ml amino-antipirienoplossing by. Meng onmiddellik, voeg 3·00 ml kaliumystersianiedoplossing by en meng weer onmiddellik. Laat 3 minute staan en ekstraheer onverwyld met chloroform; gebruik hierby 25 ml vir 'n 5-cm-sel en 50 ml vir 'n 10-cm-sel. Filtreer elk van die chloroform-ekstrakte deur 'n sinterglastregter wat 'n 5-g-laag anhidriese natriumsulfaat bevat, en versamel die gedroogde ekstrakte in droë bekers. Moenie chloroform byvoeg nie.

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 a 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.

Calculation.

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

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.*

Apparatus.

Spectrophotometer for use at 510 mμ.

Reagents

1. *Lead-free Double-distilled water.*—For preparation of reagents and dilution water.

2. *Ammonium Hydroxide.*—28–29 per cent, lead-free.

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.

4. *Chloroform.*

5. *Stock Dithizone Solution*, 0·005 per cent.—Dissolve 50 mg diphenylthiocarbazone in 1 litre chloroform. This solution is stable for several weeks if kept at 40° F (4° C) in the dark.

6. *Standard Dithizone Solution*, 0·001 per cent.—Dilute 100 ml stock dithizone solution to 500 ml with chloroform. Standardise with spectrophotometer using air blank at 510 mμ. 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.

7. *Hydroxylamine Hydrochloride Solution.*—Dissolve 20 g hydroxylamine hydrochloride ($NH_2OH.HCl$) in distilled water and make up to 100 ml.

8. *Hydrochloric Acid.*—1 + 1.

9. *Nitric Acid.*—1 + 99.

10. *Standard Lead Solution.*—Dry 0·1598 g lead nitrate, $Pb(NO_3)_2$ 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.

11. *Sodium Citrate Solution.*—Dissolve 10 g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) 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.

Method.

Preparation of Calibration Curve.—To 100 ml portions of double-distilled water, add 0 (blank), 0·010, 0·020, 0·030, 0·040, 0·050 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 30 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

Lees die optiese digtheid (absorpsie) van die monster en die standaarde af teen die kontrolehoeveelheid in die spektrofotometer by 'n golflengte van 460 mμ; gebruik hierby 'n 5-cm-sel en 'n 10-cm-sel onderskeidelik vir die 25 ml en 50 ml ekstrak. Sit optiese digtheidsabsorpsiewaardes uit teen die fenolkonsentrasies (in mikrogramme) van die standaarde. Bereken die hoeveelheid fenol in die monster.

Berekening.

$$\text{Fenolverbindings as fenol in mg/l} = \frac{\mu\text{g fenol in deelvolume geneem}}{\text{ml in deelvolume geneem}}$$

18. BEPALING VAN LOOD.

Opmerking.—Die ontleding moet in diffuse lig gedoen word, aangesien helder sonlig geneig is om ditisoon en ditisonate te vernietig. *Daar behoort veral gesorg te word dat die reagense en apparate geen lood of ander metale bevat nie.*

Apparatuur.

'n Spektrofotometer vir gebruik by 510 mμ.

Reagense.

1. *Loodvrye, dubbelgedistilleerde water.*—Vir die bereiding van reagense en verdunningswater.

2. *Ammoniumhidroksied.*—28–29 Persent, loodvry.

3. *Ammoniumsianiedoplossing.*—Los 40 g kaliumsianied (KCN) in 80 ml water op. Ekstraheer hierdie oplossing herhaaldelik met 10 ml-hoeveelhede 0·005-persentige ditisoon totdat die laaste hoeveelheid groen bly. Was die oplossing vervolgens met chloroform totdat die oplossing helder bly. Voeg 1,160 ml ammoniumhidroksied by die kaliumsianiedoplossing en vul die mengsel met water by tot 2 liter. Bewaar in 'n pyrex-bottel met 'n glasprop.

4. *Chloroform.*

5. *Voorraadditisoonoplossing*, 0·005-persentig.—Los 50 mg difenieltiokarbasoon in 1 liter chloroform op. Hierdie oplossing bly verskeie weke bestendig wanneer in die donker by 40° F (4° C) bewaar.

6. *Standaardditisoonoplossing*, 0·001-persentig.—Verdun 100 ml voorraadditisoonoplossing met chloroform tot 500 ml. Standaardiseer met die spektrofotometer met niks (lug) in die optiese pad by 510 mμ. Aangesien hierdie oplossing se sterkte progressief sal verminder, behoort sy sterkte voor gebruik nagegaan te word. Bewaar die oplossing in die donker by 40° F. Hierdie oplossing bly verskeie dae bestendig.

7. *Hidroksielamien-hidrochloriedoplossing.*—Los 20 g hidroksielamien-hidrochloried ($NH_2OH \cdot HCl$) in gedistilleerde water op en vul tot 100 ml by.

8. *Soutsuur*, 1 + 1.

9. *Salpetersuur*, 1 + 99.

10. *Standaardloodoplossing.*—Droog 0·1598 g loodnitraat, $Pb(NO_3)_2$ by 110° C. Los op en verdun tot 500 ml met salpetersuur (1 + 99). Een ml van hierdie oplossing is ekwivalent aan 0·2 mg lood as Pb. Berei hiervan 'n verdunning van 1 op 20. Hierdie verdunde oplossing sal ekwivalent wees aan 0·01 mg Pb per ml.

11. *Natriumsitraatoplossing.*—Los 10 g natriumsitraat ($Na_3C_6H_5O_7 \cdot 2H_2O$) in 90 ml water op. Ekstraheer met hoeveelhede 0·005-persentige ditisoon van 10 ml elk, totdat die laaste hoeveelheid groen bly. Was met chloroform om die oormaat ditisoon te verwyder.

Metode.

Teken van ykkurwe.—Voeg by hoeveelhede dubbelgedistilleerde water van 100 ml elk 0(kontrole), 0·010, 0·020, 0·030, 0·040, 0·050 mg lood en 1 ml soutsuur (1 + 1). Laat elke hoeveelheid tot ongeveer 40 ml verdamp, voeg 10 ml natriumsitraatoplossing en 2 ml ammoniumhidroksied daarby. Meng en bring na 'n skeitregter oor. Ekstraheer deur 30 sek. lank flink te skud met hoeveelhede 0·005-persentige ditisoon van 5 ml elk, totdat die kleur in die laaste hoeveelheid onveranderd bly. Voeg by die saamgevoegde ekstrakte 25 ml salpetersuur (1 + 99) en skud 1 minuut lank. Gooi die chloroform weg. Voeg by die suur ekstrak 5 ml hidroksielamien-hidrochloriedoplossing, 5 ml ammoniakaliese sianiedoplossing en presies 20 ml 0·001-persentige ditisoon. *Die volgorde van byvoeging is belangrik.* Skud 1 minuut lank flink en laat die lae van mekaar skei. Gooi die eerste 2 ml chloroformekstrak weg en giet die res oor in 'n droë absorpsie-sel

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 mμ. Plot an absorbance-concentration calibration curve which should be linear.

Procedure for Water Sample.—Take a suitable volume of water containing 0·010–0·050 mg lead as Pb, add 1 ml 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 unknowns. 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.

Calculation.

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

19. DETERMINATION OF PHOSPHATE.

Apparatus.

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 on 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.

2. *Spectrophotometer.*—For use at 690 mμ providing a light path of at least 1 cm.

Reagents.

1. *Phenolphthalein Indicator Solution.*—Dissolve 5 g phenophthalein 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.

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.

3. *Ammonium Molybdate-strong-acid Solution.*—Dissolve 31·4 g ammonium molybdate ($(NH_4)_6M0_7O_{24}.4H_2O$) 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.

4. *Amino Naphthol Sulphonic Acid Solution.*—Weigh out separately 0·75 g 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 anhydrous sodium metabisulphite (also called sodium pyrosulphite) ($Na_2S_2O_5$). Thoroughly grind the sulphonic acid with a small portion of the $Na_2S_2O_5$ powder in a clean, dry mortar. Dissolve the remaining salts in about 900 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.

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 PO_4.

6. *Working Phosphate Solution.*—Dilute 100·0 ml stock phosphate solution to 1,000 ml with distilled water. This solution contains 0·050 mg PO_4 per 1·00 ml.

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 met 'n optiese pad van omtrent 2 cm. Stel die kontrolehoeveelheid op 100 persent transmittansie en bepaal die optiese digtheid (absorpsie) van die standaardoplossings by 510 mμ. Teken 'n ykkurwe deur die optiese digtheid teen die konsentrasie uit te sit; die kurwe behoort lineêr te wees.

Werkwyse t.o.v. monster water.—Neem 'n geskikte volume water wat 0·010–0·050 mg lood as Pb bevat, voeg 1 ml soutsuur (1 + 1) daarby en laat tot ongeveer 40 ml verdamp. Berei 'n kontrolehoeveelheid met gebruikmaking van dubbelgedistilleerde water en gaan op dieselfde manier tewerk as by die onbekendes. Tensy die ykkurwe tegelykertyd geteken word, is dit raadsaam om een of twee standaardoplossings te berei en gelyk met die onbekende te gebruik. Gaan net so te werk as in die geval van die standaarde hierbo genoem. Lees mg Pb van die ykkurwe af.

Berekening.

$$\text{Lood as Pb. in mg/l} = \frac{\text{mg Pb} \times 1{,}000}{\text{ml monster}}$$

19. BEPALING VAN FOSFAAT.

Apparatuur.

1. *Glaswerk met suur skoongemaak.*—Dit kan belangrik wees veral wanneer lae konsentrasies fosfaat bepaal word. Fosfaatbesoedeling kom baie voor as gevolg van die vorming van dun lagies daarvan direk op die glaswerk of van absorpsie daarvan op ysteroksiedlagies op die glaswerk. *Die gebruik van kommersiële detergense wat fosfaat bevat, behoort vermy te word.* Glaswerk behoort met warm, verdunde soutsuur skoongemaak en goed met gedistilleerde water afgespoel te word.

2. *'n Spektrofotometer*, vir gebruik by 690 mμ wat 'n optiese pad van minstens 1 cm lewer.

Reagense.

1. *Fenolftaleïenindikatoroplossing.*—Los 5 g fenolftaleïen in 500 ml 95-persentige alkohol op en voeg 500 ml gedistilleerde water daarby. Voeg daarna 0·02 N natriumhidroksied daarby tot 'n ligpienk kleur te voorskyn tree.

2. *'n Oplossing van sterk sure.*—Voeg stadig 300 ml sterk swawelsuur by ongeveer 600 ml gedistilleerde water. Voeg, wanneer hierdie mengsel afgekoel het, 4·0 ml sterk salpetersuur daarby en verdun tot 1 liter.

3. *'n Oplossing van ammoniummolibdaat en sterk sure.*—Los 31·4 g ammoniummolibdaat ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) in ongeveer 200 ml gedistilleerde water op. Voeg versigtig 252 ml gekonsentreerde swawelsuur by 400 ml gedistilleerde water. Laat afkoel, voeg 3·4 ml sterk salpetersuur daarby, gevolg deur die molibdaatoplossing en verdun tot 1 liter.

4. *Aminonaftolsulfoonsuuroplossing.*—Weeg afsonderlik 0·75 g 1-amino-2-naftol-4-sulfoonsuur (gebruik slegs 'n poeier wat ligpienk van kleur is), 42 g anhidriese natriumsulfiet (Na_2SO_3) en 70 g anhidriese natriummetabisulfiet (ook natriumperosulfiet genoem ($Na_2S_2O_5$) af. Vryf die sulfoonsuur met 'n klein hoeveelheid van die $Na_2S_2O_5$-poeier in 'n skoon, droë vysel goed fyn. Los die oorblywende soute in ongeveer 900 ml gedistilleerde water op; los die fyngevryfde sulfoonsuur in hierdie mengsel op en verdun tot 1 liter. Bewaar in 'n bruin bottel met 'n glasprop by 'n temperatuur van hoogstens 30° C. Hierdie oplossing sal met verloop van tyd effens verkleur; indien onbesoedel, sal dit egter vir die meeste werk oor 'n tydperk van 4 maande of meer bevredigende resultate verseker. In die geval van baie noukeurige werk moet die oplossing weggegooi word wanneer toetse met standaarde gedoen 'n ykafwyking van 2 persent van die konsentrasie toon.

5. *Voorraadfosfaatoplossing.*—Los 0·7165 g kaliumdiwaterstoffosfaat, KH_2PO_4, wat in 'n oond by 105° C gedroog is, in gedistilleerde water op. Verdun die oplossing tot 1,000 ml; 1·00 ml = 0·500 mg PO_4.

6. *Werkoplossing van fosfaat.*—Verdun 100·0 ml voorraadfosfaatoplossing met gedistilleerde water tot 1,000 ml. Hierdie oplossing bevat 0·050 mg PO_4 per 1·00 ml.

Metode.

Die monster moet indien 'n neerslag tydens vervoer daarin gevorm het, goed gemeng word en 'n gedeelte daarvan moet deur 'n stuk fyn filtreerpapier met 'n harde afwerking en in suur gewas, gefiltreer word. Indien die pH van die monster minder as 4 is, verdun dan 50·0 ml met gedistil-

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, when 50·0 ml of original sample has been diluted to 100 ml for pH adjustment, the " ml sample " in the calculation is 25 not 50, 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 sulphonic 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 chromate 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 sulphonic acid solutions can be used where such interferences are absent. One distilled water blank can be used for a number of interference-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.

Calculation.

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

Phosphate as P = 0·327 × phosphate as PO_4 in mg/l.

20. Determination of Iron.

Apparatus.

1. *Acid-washed 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 absorbed iron oxide which is frequently present as a result of employing glassware for other purposes.

2. *Spectrophotometer.*—For use at 510 mμ providing a light path of 1 cm or longer.

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 indefinitely if tightly stoppered. The hydroxylamine and phenanthroline 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.

1. *Hydrochloric Acid.*—Concentrated.

2. *Hydroxylamine Reagent.*—Dissolve 10 g hydroxylamine hydrochloride ($NH_2OH.HCl$) in 100 ml distilled water.

3. *Ammonium Acetate Buffer Solution.*—Dissolve 250 g ammonium acetate (CH_3COONH_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.)

4. *Phenanthroline Solution.*—Dissolve 0·1 g of 1, 10-phenanthroline monohydrate ($C_{12}H_8N_2.H_2O$), in 100 ml distilled water by stirring and heating to 80° C; do not boil. Discard the solution if it darkens. Heating is not

leerde water in 'n maatfles tot 100 ml en meng goed. Volg by die gebruik van hierdie verdunde monster die volgende stappe: Voeg indien die pH hoër as 10 is, 1 druppel fenolftaleïenindikator by 50·0 ml monster en verdryf die rooi kleur met die oplossing van sterk sure, en verdun dan tot 100 ml. (Wanneer verdunnings gemaak word, moet die uitdrukking „ ml monster " in die finale berekening reg vertolk word. Bv. wanneer 50·0 ml van die oorspronklike monster tot 100 ml verdun is vir reëling van die pH, beteken „ ml monster " in die berekening 25 en nie 50 nie, hoewel 50·0 ml van die verdunde monster nog by die volgende stappe gebruik word).

Pipetteer 50·0 ml van die gefiltreerde of helder monster in 'n skoon, droë Erlenmeyerfles van 125 ml. Voeg 2·0 ml molibdaat-suuroplossing daarby en meng deur te draaiskud. Voeg 2·0 ml sulfoonsuurreagens by en meng weer. Aangesien die tempo en die tyd van die kleurontwikkeling van die temperatuur afhang, behoort die reagense, standaarde en monsters dieselfde temperatuur te hê (20–30° C).

Meet die kleur fotometries na presies 5 minute en stel die instrument daarby op nulabsorpsie met 'n geskikte kontrolestof. Die mate van steuring deur kleur, troebelheid wat nie deur filtrasie verwyder is nie, en chromaat word aansienlik verminder of verdryf deur die kontrolestof op presies dieselfde manier van die monster te maak, behalwe dat die oplossing van sterk sure i.p.v. die molibdaatoplossing gebruik word. Gedistilleerde water wat op dieselfde manier met die oplossing van sterk sure en sulfoonsuur behandel is, kan gebruik word wanneer daar nie sulke steurings is nie. Een kontrolehoeveelheid van gedistilleerde water kan vir 'n aantal monsters waarin geen steurende elemente aanwesig is nie, gebruik word.

Bepaal die gewig aan ortofosfaat in die monster deur die aflesing met die standaardkromme te vergelyk. Hierdie kromme word verkry deur die optiese densiteit(absorpsie) aflesings van 'n geskikte aantal fosfaatstandaarde wat soos hierbo behandel is, grafies voor te stel. Tenminste een standaard behoort saam met elke stel monsters of eenmaal op elke dag wat proefnemings plaasvind, getoets te word.

Berekening.

$$\text{Fosfaat as } PO_4 \text{ in mg/l} = \frac{\text{mg } PO_4 \times 1{,}000}{\text{ml monster}}$$

Fosfaat as P = 0·327 × fosfaat as PO_4 n mg/l.

20. Bepaling van Yster.

Apparatuur.

1. *Glaswerk met suur skoongemaak.*—Alle glaswerk moet voor gebruik in sterk soutsuur gewas en met gedistilleerde water afgespoel word ten einde die dun lagie geabsorbeerde ysteroksied wat dikwels aanwesig is as gevolg van gebruik van die glaswerk vir ander doeleindes, te verwyder.

2. *'n Spektrofotometer* vir gebruik by 510 mμ, wat 'n optiese pad van 1 cm of meer lewer.

Reagense.

Alle reagense moet min yster bevat. Gedistilleerde water vry van yster is nodig. Dit is raadsaam om bottels met glasproppe vir bewaring te gebruik. Die soutsuur-ammoniumasetaat- en voorraadysteroplossings bly onbepaalde tyd goed as hulle styf toegeprop gehou word. Die hidroksielamien- en fenantrolienoplossings bly verskeie maande goed. Die werkoplossings om yster is onbestendig en moet vars aangemaak word namate hulle benodig word, deur verdunning van die voorraadoplossing.

1. *Soutsuur.*—Sterk.

2. *Hidroksielamienreagens.*—Los 10 g hidroksielamienhidrochloried ($NH_2OH \cdot HCl$) in 100 ml gedistilleerde water op.

3. *Ammoniumasetaatbufferoplossing.*—Los 250 g ammoniumasetaat (CH_3COONH_4) in 150 ml gedistilleerde water op. Voeg 700 ml ysasynsuur daarby en verdun tot 1 liter. (Aangesien selfs 'n goeie graad ammoniumasetaat 'n aansienlike hoeveelheid yster bevat, behoort nuwe vergelykingsstandaarde vir elke bufferbereiding gemaak te word.)

4. *Fenantrolienoplossing.*—Los 0·1 g 1, 10-fenantroliënmonohidraat ($C_{12}H_8N_2 \cdot H_2O$) in 100 ml gedistilleerde water op deur te roer en tot 80° C te verhit; moenie kook

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.)

5. *Iron Stock Solution.*—Slowly add 20 ml concentrated sulphuric acid to 50 ml of distilled water and dissolve in it 0·7022 g of ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2.6H_2O$). 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.

6. *Iron Working Solutions.*—These should be prepared the day they are to be used.

(*a*) 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.

(*b*) 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.

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.

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 of the 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.

Calculation.

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

21. DETERMINATION OF MANGANESE.

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.

Apparatus.

Spectrophotometer for use at 525 mμ, providing a light path of at least 1 cm.

Reagents.

1. "*Special Solution*".—Dissolve 75 g mercuric sulphate in 400 ml of cencentrated 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.

2. *Ammonium Persulphate.*—Solid.

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 nie. Gooi die oplossing weg as dit donker word. Verhitting is onnodig as 2 druppels sterk soutsuur by die gedistilleerde water gevoeg word. (Let op dat 1 ml van hierdie reagens vir hoogstens 0·1 mg Fe voldoende is).

5. *Voorraadysteroplossing.*—Voeg stadig 20 ml sterk swawelsuur by 50 ml gedistilleerde water en los 0·7022 g ferro-ammoniumsulfaat $(Fe)(NH_4)_2(SO_4)_2 \cdot 6H_2O$ daarin op. Voeg druppelsgewyse 0·1 N kaliumpermanganaatoplossing toe tot die kleur ligpienk bly. Verdun met ystervrye gedistilleerde water tot 1,000 ml en meng. Hierdie voorraadoplossing bevat 0·10 mg Fe per 1·00 ml.

6. *Werkoplossings van yster.*—Hierdie oplossings moet berei word op die dag waarop hulle gebruik gaan word.

(*a*) Pipetteer 100·00 ml voorraadoplossing in 'n literfles en verdun tot by die merk met ystervrye gedistilleerde water. Hierdie oplossing bevat 0·010 mg Fe per 1·00 ml.

(*b*) Pipetteer 10·00 ml voorraadoplossing in 'n literfles en verdun tot by die merk met ystervrye gedistilleerde water. Hierdie oplossing bevat 0·001 mg Fe per 1·00 ml.

Metode.

Meng die monster goed en meet 50·0 ml in 'n Erlenmeyerfles van 125 ml af. Voeg 2 ml sterk soutsuur en 1 ml hidroksielamienreagens daarby. Sit 'n paar glaskrale in die oplossing en bring aan die kook. Om te verseker dat al die yster oplos, kan dit aan die kook gehou word totdat die volume tot 15–20 ml verminder het. Laat tot kamertemperatuur afkoel en bring na 'n maatfles van 100 ml oor. Voeg 10 ml asetaatbufferoplossing en 2 ml fenantrolienoplossing daarby en verdun met gedistilleerde water tot by die merk. Meng goed en laat minstens 10–15 minute staan sodat die kleur op sy sterkste te voorskyn kan tree.

Kleurmeting.—Berei 'n reeks standaarde deur noukeurig die berekende werkoplossings (die swakker oplossing behoort gebruik te word om hoeveelhede van 0·00–0·010 mg te meet) in Erlenmeyerflesse van 125 ml te pipetteer; verdun tot 50 ml en gaan soos hierbo aangegee te werk. 'n Optiese pad van 1 cm behoort gebruik te word. Die standaarde behoort teen gedistilleerde water op nulabsorpsie gestel afgelees te word, en 'n ykkromme moet geteken word deur die optiese digtheid (absorpsie) teen mg Fe uit te sit. As die monsters ook maar effens gekleurd of troebel is, mag 'n tweede stel identiese deelvolumes van die monsters op dieselfde manier getoets word maar net sonder byvoeging van fenantrolien. Dan word i.p.v. gedistilleerde water die bereide kontrolehoeveelhede gebruik om die fotometer op nulabsorpsie te stel, en elke ontwikkelde monster, met fenantrolien, word teen die ooreenstemmende kontrolehoeveelheid, sonder fenantrolien afgelees. Waargenome fotometeraanwysings word tot ysterwaardes herlei met behulp van die ykkromme. As daar geen kleur of troebelheid is nie, gaan dit gouer en net so doeltreffend om die ontwikkelde monsters sowel as die standaarde teen gedistilleerde water af te lees.

Berekening.

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

21. BEPALING VAN MANGAAN.

Monsterneming en bewaring.—Mangaan behoort baie kort na monsterneming bepaal te word. Indien vertraging onvermybaar is, behoort die monster sodra dit geneem is, aangesuur te word.

Apparatuur.

'n Spektrofotometer vir gebruik by 525 mμ, wat 'n optiese pad van minstens 1 cm lewer.

Reagense.

1. „*Spesiale oplossing*".—Los 75 g kwiksulfaat in 400 ml sterk salpetersuur en 200 ml gedistilleerde water op. Voeg 200 ml 85-persentige fosforsuur en 0·035 g silwernitraat daarby en verdun die afgekoelde oplossing tot 1 liter.

2. *Ammoniumpersulfaat* in vaste vorm.

3. *Mangaansulfaat-standaardoplossing.*—Berei op die gewone manier 'n 0·1 N kaliumpermanganaatoplossing deur 3·2 g kaliumpermanganaat in gedistilleerde water op te los en tot 1 liter by te vul. Hierdie oplossing moet toegelaat word om 'n paar weke in sonlig te verouder, of dit moet 'n paar uur tot naby die kookpunt verhit word, en

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:—

$$\text{ml permanganate} = \frac{4{\cdot}55}{\text{normality of permanganate solution.}}$$

To this volume add 2 to 3 ml of concentrated sulphuric acid and then sodium bisulphate solution (10 g $NaHSO_3$ 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.

4. *Hydrogen Peroxide Solution.*—30 per cent.

Method.

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 water-bath. 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 to 100 ml with distilled water, free from reducing substances, and mix. Make photometric measurements against a distilled water blank using a wavelength of 525 mμ. 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.

Correction for Turbidity or Interfering Colour.—Filtration is not recommended because of the possiblity 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.

Calculation.

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

22. Determination of Cyanides and Related Compounds.

Note: Determination of cyanide should be made as soon as possible after collection of sample, since most cyanides are relatively unstable. If delay in analyses is unavoidable, the pH of the sample should be raised to 11 or above by the addition of sodium hydroxide, and the sample subsequently stored in a cool place.

Apparatus.

Spectrophotometer for use at 520 mμ.

Reagents.

1. *Bromine Water.*—Saturated.

2. *Arsenious Acid Solution.*—Dissolve 2 g of arsenious oxide, As_2O_3, in 100 ml of distilled water.

3. *Benzidine Reagent.*—Dissolve 5 g of benzidine hydrochloride in 100 ml of distilled water containing 2 ml of hydrochloric acid, sp. gr. 1·18. Prepare this solution freshly as required.

4. *Pyridine Solution.*—An approximately 60 per cent v/v solution in distilled water (the mixture of constant boiling-point).

5. *Acetic Acid.*—Approximately 2 N.

6. *Standard Cyanide Solution.*—Dissolve 1·25 g of potassium cyanide in 500 ml of distilled water. Standardise this solution frequently with 0·01 N silver nitrate solution (1 ml = 0·52 mg of CN′), using rhodanine indicator solution. Adjust the solution so that 1 ml contains 1 mg of CN′.

daarna deur 'n sinterglasfilter gefiltreer en noukeurig teenoor natriumoksalaat gestandaardiseer word. Bereken die volume van hierdie oplossing wat nodig is om 1 liter oplossing te berei wat so sterk is dat 1·00 ml = 0·050 mg mangaan as Mn, soos volg:—

$$\text{ml permanganaat} = \frac{4{\cdot}55}{\text{normaliteit van permanganaatoplossing}}$$

Voeg by hierdie volume 2 tot 3 ml sterk swawelsuur en dan druppelsgewyse en al roerende natriumbisulfietoplossing (10 g $NaHSO_3$ plus 100 ml gedistilleerde water) totdat die permanganaatkleur verdwyn. Kook om die oormaat swaweldioksied te verwyder; laat afkoel en verdun met gedistilleerde water tot 1 liter. Hierdie oplossing kan verder verdun word om klein hoeveelhede mangaan te meet.

4. *Waterstofperoksiedoplossing.*—30-persentig.

Metode.

Behandeling van monster: Voeg by 'n geskikte deelvolume van die monster 5 ml van die „ spesiale oplossing ". Konsentreer tot 90 ml deur dit te kook, of verdun tot 90 ml. Voeg 1 g ammoniumpersulfaat daarby en bring in ongeveer 2 minute oor 'n vlam aan die kook. Moenie op 'n waterbad verhit nie. Haal vir 1 minuut van die vlam af; laat dan onder die kraan afkoel. (As te lank gekook of te stadig afgekoel word, ontbind die oormaat persulfaat, gevolg deur verdwyning van die permanganaatkleur). Verdun met gedistilleerde water tot 100 ml, vry van reduserende stowwe, en meng. Doen fotometriese metings teen 'n kontrolehoeveelheid gedistilleerde water met gebruikmaking van 'n golflengte van 525 mμ. Standaarde wat 0, 0·005, 0·010, ens., tot 1·5 mg Mn bevat, behoort op dieselfde manier behandel te word; 'n ykkromme moet dan geteken word.

Korreksie vir troebelheid of steurende kleur.—Filtrering word nie aanbeveel nie vanweë die moontlikheid dat daar van die permanganaat op die filtreerpapier kan agterbly. Die volgende „ bleekmetode " behoort gebruik te word:— Voeg sodra die fotometer afgelees is, 0·05 ml waterstofperoksiedoplossing regstreeks by die monster in die optiese sel. Meng die oplossing en lees sodra die permanganaatkleur volkome verdwyn het en daar geen borrels meer is nie, die optiese digtheid (absorpsie) af. Hierdie aflesing kan van die oorspronklike aflesing afgetrek word, of dit kan van die ykkromme afgelees word as „ steuring in die vorm van mangaan " en dan van die „ skynbare mangaan " afgetrek word om die regte mangaansyfer te verkry.

Berekening.

$$\text{Mangaan a Mn, in mg/l} = \frac{\text{mg Mn} \times 1{,}000}{\text{ml monster}}$$

22. Bepaling van sianiede en verwante verbindings.

Opmerking.—Sianied behoort so spoedig moontlik na neming van die monster bepaal te word, aangesien die meeste sianiede betreklik onbestendig is. Indien vertraging onvermydelik is, behoort die pH van die monster tot 11 of meer verhoog te word deur toevoeging van natriumhidroksied, en die monster op 'n koel plek gebêre te word.

Apparatuur.

'n Spektrofotometer vir gebruik by 520 mμ.

Reagense.

1. *Broomwater.*—Versadig.

2. *Arsenigsuuroplossing.*—Los 2 g arseen-III-oksied, As_2O_3, in 100 ml gedistilleerde water op.

3. *Bensidienreagens.*—Los 5 g bensidienhidrochloried op in 100 ml gedistilleerde water wat 2 ml soutsuur, s.g. 1·18 bevat. Berei hierdie oplossing vars wanneer benodig.

4. *Piridienoplossing.*—'n Ongeveer 60-persentige volume-oplossing in gedistilleerde water (die mengsel moet 'n konstante kookpunt hê).

5. *Asynsuur.*—Ongeveer 2 N.

6. *Standaardsianiedoplossing.*—Los 1·25 g kaliumsianied in 500 ml gedistilleerde water op. Standaariseer hierdie oplossing dikwels met 0·01 N silwernitraatoplossing (1 ml = 0·52 mg CN′); gebruik rodanienoplossing as indikator. Reël die oplossing sodat 1 ml 1 mg CN′ bevat.

From this stock solution prepare a dilute solution freshly as required by diluting 10·0 ml to 1 litre with distilled water, and diluting this a further 10 times.

1 ml = 1 μg of cyanide (CN').

7. *Rhodanine Indicator Solution.*—A 0·02 per cent w/v solution of p-dimethylaminobenzylidine rhodanine in acetone.

8. *Silver Nitrate Solution.*—0·01 N.

9. *Sodium Hydroxide Solution.*—Approximately 2·5 N.

Standardisation of Cyanide Solution.

Add 10 ml of sodium hydroxide solution to a conical flask, add 20·0 ml of the cyanide solution and 2 drops of rhodanine indicator. Titrate with 0·01 N silver nitrate until the end-point which is indicated by the appearance of a red colour, is reached.

1 ml of 0·01 N silver nitrate = 0·52 mg of cyanide ion.

Method.

Into a glass-stoppered tube (calibrated at 10 ml) measure 2 ml of the effluent sample, adjusted by dilution if necessary to contain not more than 2μg of cyanide ion. Acidify with acetic acid; then add 0·2 ml of bromine water and mix thoroughly. Add 0·2 ml of arsenious acid to remove excess of bromine; remove any bromine vapour by blowing across the mouth of the tube. Mix 3 ml of pyridine solution with 0·6 ml of benzidine reagent, add this mixture to the contents of the tube, dilute to the mark with distilled water and mix thoroughly. Stopper the tube and allow the mixture to stand in the dark for 25 to 30 minutes at a temperature between 15° and 20° C.

Carry out a blank procedure on all the reagents used.

Express the result in terms of cyanide (as CN') as milligrams per litre of sample.

Measure the optical densities of the test and blank solutions in a spectrophotometer, using 1-cm cells, and using a wavelength of 520 mμ. Use distilled water in the comparison cell. Read the number of micrograms of cyanide ion equivalent to the observed optical densities of the test and blank solutions from a previously prepared calibration graph, and so obtain the net measure of cyanide ion in the sample.

Establish the calibration graph as follows:—

Into a series of stoppered tubes measure appropriate amounts of standard cyanide solution, covering the range 0 to 2 μg of cyanide ion, and proceed as for the test solution. Measure the optical densities and construct a graph relating the optical densities to the number of micrograms of cyanide ion.

Calculation.

$$\text{Cyanide and related compounds as CN in mg/l} = \frac{\mu\text{g Cn in aliquot taken}}{\text{ml of aliquot taken}}$$

23. DETERMINATION OF SULPHIDE.

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

1. Loss of free hydrogen sulphide from the sample to the atmosphere.

2. Entry of oxygen into the sample with resultant loss of sulphide by oxidation.

3. 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.

Reagents.

1. *Amine-sulphuric Acid Solution.*—(*a*) *Stock Solution.*—Distil para-amino-di-methyl-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.

*If any of the amine gets on the skin wash it off immediately with dilute hydrochloric acid.

Berei van hierdie voorraadoplossing 'n vars verdunde oplossing namate dit nodig word, deur 10·0 ml met gedistilleerde water tot 1 liter te verdun, en hierdie verdunning nog 10 maal te verdun.

1 ml = 1 μg sianied (CN').

7. *Rodanienindikatoroplossing.*—'n 0·02-persentige oplossing, gewig per volume, van p-dimetielaminobensilidienrodanien in asetoon.

8. *Silwernitraatoplossing*, 0·01 N.

9. *Natriumhidroksiedoplossing.*—Ongeveer 2·5 N.

Standaardisering van sianiedoplossing.—Sit 10 ml natriumhidroksiedoplossing in 'n koniese fles, voeg 20·0 ml van die sianiedoplossing en 2 druppels rodanienindikator daarby. Titreer met 0·01 N silwernitraat tot die omslagpunt bereik word wat blyk uit die tevoorskyntreding van 'n rooi kleur.

1 ml 0·01 N silwernitraat = 0·52 mg sianiedioon.

Metode.

Meet in 'n buis met 'n glasprop (by 10 ml gemerk) 2 ml van die afvalwatermonster af, indien nodig deur verdunning so gereël dat dit hoogstens 2 μg sianiedioon bevat. Suur met asynsuur aan; voeg dan 0·2 ml broomwater daarby en meng goed. Voeg 0·2 ml arsenigsuur by om die oormaat broom te verwyder; as daar broomdampe bo die bek van die buis verskyn, moet hulle weggeblaas word. Meng 3 ml piridienoplossing met 0·6 ml bensidienreagens, voeg hierdie mengsel by die inhoud van die buis, verdun met gedistilleerde water tot by die merk en meng goed. Prop die buis toe en laat die mengsel van 25 tot 30 minute by 'n temperatuur van tussen 15° en 20° C in die donker staan.

Voer 'n kontrolebepaling op al die gebruikte reagense uit.

Gee die resultaat aan in terme van sianied (as CN') as milligramme per liter monster.

Meet die optiese dightheid van die toets- en die kontroleoplossing met 'n spektrofotometer en gebruik daarby 1 cm-selle en 'n golflengte van 520 mμ. Gebruik gedistilleerde water in die vergelyking-sel. Lees die aantal mikrogramme sianiedioon gelyk aan die waargenome optiese digtheid van die toets- en die kontrole-oplossing van 'n vantevore getekende ykkromme af, en verkry sodoende die netto meting van sianiedioon in die monster.

Teken die ykkromme as volg:—

Meet in 'n reeks toegepropte buise toepaslike hoeveelhede standaardsianiedoplossing oor 'n gebied van 0 tot 2 μg sianiedioon, en gaan net so te werk as by die toetsoplossing. Meet die optiese digthede en teken 'n kromme waarop die optiese digthede teen die aantal mikrogramme sianiedioon uitgesit word.

Berekening.

$$\text{Sianied en verwante verbindings as CN, in mg/l} = \frac{\mu\text{g CN in deelvolume geneem}}{\text{ml deelvolume geneem}}$$

23. BEPALING VAN SULFIED.

By die bepaling van sulfied moet gesorg word dat geen foute deur die volgende oorsake ontstaan nie:—

1. Ontsnapping van ongebonde waterstofsulfied uit die monster in die atmosfeer.

2. Opname van suurstof in die monster met gevolglike verlies van sulfied deur oksidasie.

3. Vorming van waterstofsulfied deur die bewaring van die monster in anaërobiese toestande.

Die beste resultate sal verkry word as die monster onmiddelik ontleed word en as verlies van ongebonde waterstofsulfied verhoed word deur byvoeging van 'n klein volume sinkasetaatoplossing by die monster sodra dit geneem is.

Reagense.

1. *Amienswawelsuuroplossing.*—(*a*) *Voorraadoplossing.*—Distilleer para-amino-dimetielanilien* in 'n glasapparaat waarin die lug deur 'n inerte gas vervang is. Meng baie versigtig 50 ml sterk swawelsuur met 30 ml water en laat afkoel. Voeg 20 g gesuiwerde amien by en roer tot alles opgelos is. Vul met water tot 100 ml by. Of meng anders 46 ml sterk swawelsuur met 30 ml water en laat afkoel. Voeg 27·2 g para-amino-dimetielaniliensulfaat daarby en roer tot alles opgelos is. Vul met water tot 100 ml by.

*As daar van die amien op die vel kom, moet dit onmiddellik met verdunde soutsuur afgewas word.

Alternatively, mix 46 ml of concentrated sulphuric acid with 30 ml water and cool. Add to this 27·2 g para-amino-di-methyl-aniline 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.

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

3. *Ferric Chloride Solution.*—Dissolve 100 g of ferric chloride ($FeCl_3.6H_2O$) in water and make up to 100 ml.

4. *Ammonium Phosphate Solution.*—Dissolve 400 g of ammonium phosphate ($(NH_4)_2HPO_4$) in water and make up to 1 litre.

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.

(*b*) *Dilute Solution.*—Dilute 10 ml of the strong solution to 100 ml with distilled water.

6. *Aluminium Sulphate Solution.*—Dissolve 24 g aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) in water and dilute to 100 ml.

7. *Sodium Hydroxide Solution.*—Dissolve 9 g of sodium hydroxide in distilled water. Cool and dilute to 100 ml.

8. *Sodium Thiosulphate.*—(*a*) *Stock Solution*, 0·25 N.—Dissolve 63 g sodium thiosulphate, ($Na_2S_2O_3.5H_2O$) 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*, 0·25 N.—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.

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 0·025 N adjust it until it is.

9. *Standard Potassium Dichromate Solution*, 0·025 N.—Dissolve 1·226 g previously dried potassium dichromate ($K_2Cr_2O_7$) in distilled water and dilute to 1 litre.

10. *Iodine Solution*, 0·025 N.—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 0·025 N sodium thiosulphate.

11. *Starch Solution.*—0·5 g to 200 ml, freshly prepared.

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

N.B.—Hierby word aangeneem dat die amiensulfaat in 'n baie suiwerder staat beskikbaar is as die amien self.

(*b*) *Werkoplossing.*—Verdun 25 ml voorraadoplossing met swawelsuur (1: 1) tot 1 liter.

2. *Verdunde swawelsuur* (1: 1).—Meng baie versigtig gelyke volumes sterk swawelsuur en gedistilleerde water.

3. *Yster-III-Chloried.*—Los 100 g yster-II-chloried ($FeCl_3\cdot 6H_2O$) in water op en vul tot 100 ml by.

4. *Ammoniumfosfaatoplossing.*—Los 400 g ammoniumfosfaat ($(NH_4)_2HPO_4$) in water op en vul tot 1 liter by.

5. *Metileenblou-oplossing.*—(*a*) *Sterk oplossing.*—Los 1·00 g metileenblou in water op en vul tot 1 liter by. Standaardiseer die oplossing soos hieronder beskryf.

(*b*) *Verdunde oplossing.*—Verdun 10 ml van die sterk oplossing met gedistilleerde water tot 10 ml.

6. *Aluminiumsulfaatoplossing.*—Los 24 g aluminiumsulfaat ($Al_2(SO_4)_3\cdot 18H_2O$) in water op en verdun tot 100 ml.

7. *Natriumhidroksiedoplossing.*—Los 9 g natriumhidroksied in gedistilleerde water op. Laat afkoel en verdun tot 100 ml.

8. *Natriumtiosulfaat.*—(*a*) *Voorraadoplossing*, 0·025 N. Los 63 g natriumtiosulfaat ($Na_2S_2O_3\cdot 5H_2O$) in 1 liter kopervrye, pasgekookte en afgekoelde gedistilleerde water op en voeg 1 ml chloroform of 10 mg kwik-II-jodied daarby om die oplossing bestendig te maak. Laat voor gebruik 'n paar dae staan.

(*b*) *Werkoplossing*, 0·025 N.—Verdun 100 ml voorraadoplossing tot 1 liter met kopervrye, pasgekookte en afgekoelde gedistilleerde water, en voeg 1 ml chloroform of 10 mg kwik-II-jodied daarby. Hierdie oplossing is redelik bestendig, maar behoort met kort tussenpose teen kaliumdichromaat gestandaardiseer te word. Bewaar in 'n amberkleurige glasbottel met 'n rubberprop, en as daar aan die einde van die dag van die oplossing in die buret agterbly, moet dit weggegooi word.

Standaardisering van natriumtiosulfaatoplossing.—Los ongeveer 2 g kaliumjodied (KI), wat geen jodaat bevat nie, met 100 tot 150 ml gedistilleerde water in 'n Erlenmeyerfles op, voeg 10 ml swawelsuur (1 + 9) daarby, gevolg deur presies 20 ml standaarddichromaatoplossing. Plaas 5 minute in die donker, verdun tot ± 400 ml en titreer met tiosulfaat totdat 'n ligte strooikleur te voorskyn tree, voeg stysel by en titreer tot dit kleurloos is. Indien die tiosulfaat nie presies 0·025 N is nie, moet dit tot daardie normaliteit gebring word.

9. *Standaardkaliumdichromaatoplossing*, 0·025 N.—Los 1·226 g vantevore gedroogde kaliumdichromaat ($K_2Cr_2O_7$) in gedistilleerde water op en verdun tot 1 liter.

10. *Jodiumoplossing*, 0·025 N.—Voeg 3·175 g jodium by ongeveer 25 g kaliumjodied en maak klam met water; voeg stadig meer water by en skud voortdurend tot alles opgelos is; verdun tot 1 liter en standaardiseer teen 0·025 N natriumtiosulfaat.

11. *Styseloplossing*, 0·5 g by 200 ml, vars aangemaak.

Metode.

Pipetteer hoeveelhede van 7·5 ml van die monster in elk van twee proefbuisies met presies dieselfde deursnee. Voeg by die eerste buisie 0·5 ml van die werkoplossing amienswawelsuur en by die tweede buisie 0·5 ml verdunde swawelsuur. Voeg dan 2 druppels ystertrichloried by elke buisie, hou hulle met die duim toe en keer hulle stadig een of twee maal onderstebo om die inhoud te meng. Wanneer die sulfied aanwesig is, sal 'n blou kleur in die eerste buisie te voorskyn tree; na omtrent 1 minuut is die kleur volkome ontwikkel. Voeg na 2 tot 5 minute 1·6 ml ammoniumfosfaatoplossing by elke buisie en meng. (Dit verminder die kleur deur die ystertrichloried veroorsaak en laat die asiditeit van die oplossing afneem sodat 'n meer intense blou kleur kan ontstaan).

Voeg na 5 minute druppelsgewyse metileenblou-oplossing by die tweede buisie; meng na elke byvoeging, totdat die kleur dieselfde is as dié van die eerste buisie. Die oplossing behoort òf met 'n standaarddruppipet wat 20 druppels per ml drup, òf met 1 ml-burette bygevoeg te word. Indien die metileenblou van die regte sterkte is, is 1 druppel van die sterk oplossing (0·05 ml) gelyk aan 1 mg/l sulfied, en 1 druppel van die verdunde oplossing gelyk aan 0·1 mg/l

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.

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 0·025 N 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 0·025 N thiosulphate solution using starch as indicator. Multiply the net volume of 0·025 N iodine solution used by 0·835 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.

Expression of Results.

Sulphide as S in mg/l.

24. DETERMINATION OF FLUORIDE.

A. 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 ± 0·1 mg/l.

Apparatus.

A Claissen flask with a thermometer and steam inlet extending to $\frac{1}{16}$ 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 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 Claissen flask.

Reagents.

1. *Silver Sulphate.*—Solid.

2. *Sodium Hydroxide Solution.*—Dissolve 10 g sodium hydroxide (NaOH) in 100 ml distilled water.

3. *Sulphuric Acid.*—Concentrated.

4. *Phenolpthalein indicator solution.*

Procedure.

1. When the sample contains less than 0·6 mg/l of fluoride as F concentrate the sample in the following way:—

Make 150 ml sample alkaline with sodium hydroxide (NaOH) using phenolpthalein indicator, add a few drops NaOH in excess. Concentrate to 15–20 ml. When cool transfer quantitatively to the distillation flask, and carefully add 15 ml of concentrated sulphuric acid. If the amount of chloride in the aliquot exceeds 5 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 140° 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.

sulfied. Vir die grootste noukeurigheid behoort 'n ewe groot volume water by die eerste buisie gevoeg te word alvorens die kleure vergelyk word; dit verhoed foute te wyte aan die volumeverandering in die tweede buisie.

Standaardisering van die metileenblou.

Maak 'n gellingbottel vol met aangesuurde gedistilleerde water, voeg 'n gewaste natriumsulfiedkristal (omtrent 100 tot 200 mg) by, prop die bottel toe, en meng dan goed.

Pipetteer 20 ml 0·025 N jodiumoplossing in 'n maatfles van 500 ml. Hewel sulfiedoplossing van die boom van die gellingbottel oor in die fles tot by die merk. Giet die inhoud oor in 'n geskikte beker, voeg 1 ml sterk swawelsuur toe en titreer die oormaat jodium met 0·025 N tiosulfaatoplossing en gebruik stysel as indikator. Vermenigvuldig die netto volume 0·025 N jodiumoplossing wat gebruik is, met 0·835 om die konsentrasie sulfied in mg/l te verkry.

Toets die sulfiedoplossing d.m.v. bobeskrewe metileenblouметode, en reël die sterkte van die metileenblouoplossings sodat dieselfde waarde vir die konsentrasie sulfied verkry word as dié volgens die titreermetode bepaal.

Weergawe van resultate.

Sulfied as S, in mg/l

24. BEPALING VAN FLUORIED.

A. Distilleermetode.

Die distilleermetode word aanbeveel omdat dit betroubaarder resultate met onbekende monsters oplewer. Dit skakel die noodsaaklikheid vir volledige ontleding uit en bied dié voordeel dat sowel vermoede as bekende interferensies uitgeskakel word. Oor die algemeen word toevlug tot distillasie geneem wanneer die totale korreksie weens interferensiestowwe meer as ± 0·1 mg/l sal wees.

Apparatuur.

'n Claissen-kolf met 'n termometer en 'n stoominlaat wat tot $\frac{1}{16}$ dm van die boom van die kolf reik. Die ander uitlaat word met 'n vertikale Liebigkoeler verbind. Die stoominlaat word met 'n stoomontwikkelfles bo 'n afsonderlike hittebron verbind. 'n Skeitregter wat òf gedistilleerde water òf die monster bevat, kan egter i.p.v. die stoominlaat gebruik word. Wanneer 'n stoomgenerator gebruik word, behoort die stoominlaat 'n waaiervormige uitlaat te hê wat onder 'n hoek van ongeveer 45° ondertoe gerig is. Wanneer 'n skeitregter gebruik word, behoort die inlaat na die fles 'n kapillêre punt te hê. Onegalige kook word tot 'n minimum terug gebring deur glaskrale in die stoomgenerator en die Claissen-kolf te sit.

Reagense.

1. *Silwersulfaat.*—In vaste vorm.

2. *Natriumhidroksiedoplossing.*—Los 10 g natriumhidroksied (NaOH) in 100 ml gedistilleerde water op.

3. *Swawelsuur.*—Sterk.

4. *Fenolftaleïenindikatoroplossing.*

Werkwyse.

1. Wanneer die monster minder as 0·6 mg/l fluoried as F bevat, moet dit soos volg gekonsentreer word:—

Maak 150 ml monster alkalies met natriumhidroksied (NaOH) met gebruikmaking van fenolftaleïen as indikator; voeg 'n oormaat van 'n paar druppels NaOH daarby. Konsentreer tot 15 tot 20 ml. Bring, wanneer afgekoel, kwantitatief na die distilleerkolf oor, en voeg versigtig 15 ml sterk swawelsuur by. Indien daar meer as 5 mg chloried in die deelvolume is, voeg dan ongeveer 5 mg vaste silwersulfaat vir elke mg chloried daarby. Verbind die koeler, termometer en stoominlaat met mekaar met die stoomuitlaatklep oop. Bring die mengsel aan die kook. Namate die distillaat opgevang word, word die water in die stoomgenerator aan die kook gebring en die stoom toegelaat om te ontsnap. Sodra die temperatuur 140° C bereik, word die stoom in die distilleerkolf gelei. Die hitte van die twee branders word so gereël dat 'n temperatuur van 140° tot 150° gehandhaaf bly (laat dit nooit 150° C oorskry nie) en die distilleertempo mag nie minder as 3 ml per minuut wees nie. Die distillasie word voortgesit tot 150 ml opgevang is.

2. When the sample contains 0·6 mg/l of fluoride as F 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.

B. Colorimetric Method.

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 $CaCO_3$)	325	Decrease	0·1
Al^{+++}	0·2	Decrease	0·1 (Note *a*
Cl^-	1,800	Decrease	0·1
Fe^{+++}	5	Decrease	0·1
Cl_2 (must be completely removed)	—	Increase	—
PO_4^{---}	1·0	Increase	0·1 (Note *b*)
$(NaPO_3)_6$	1·1	Increase	0·1
SO_4^-	400	Increase	0·1
Ca^{++}	400	No effect	0·0
Mg^{++}	200	No effect	0·0

(*a*) The value given applies only to fluoride concentrations above 0·5 mg/l. Below 0·5 mg/l F, 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.

Apparatus.

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

Reagents.

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.

2. *Sodium Arsenite Solution*, approximately 0·028 N.—Dissolve 1·83 g sodium arsenite ($NaAsO_2$) in 1 litre distilled water. Prepare fresh every 6 months.

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.*

4. *Zirconyl-acid Solution.*—Dissolve 0·184 g zirconium oxychloride ($ZrOCl_2.8H_2O$) in 50 ml water and make up to 500 ml with Scott's Mixed Acid.*

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.

Method.

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

2. Wanneer die monster 0·6 mg/l of meer fluoried as F bevat, word 'n hoeveelheid van 50 ml daarvan in 'n distilleerkolf gesit, soos hierbo vir die monsterkonsentraat beskryf behandel en die distillaat word opgevang sonder voorafgaande verdamping. In die geheel word 150 ml distillaat opgevang; die verskil in vergelyking met die oorspronklike volume is aan die stoom toe te skrywe.

Behandel die distillaat soos volg om die fluoried te bepaal.

B. Kolorimetriese metode.

Interferensie.

Die belangrike bekende interferensies en hul uitwerking word in die tabel aangegee. Hierdie inligting kan gebruik word om die skynbare fluoriedkonsentrasie te korrigeer, indien die totale korreksie nie meer as 0·1 mg/l is nie. Dit kan ook gebruik word om te skat of distillasie nodig is. Tensy anders waargeneem, is die interferensie onafhanklik van die fluoriedkonsentrasie en word aangeneem dat dit algebraïes additief is. Indien die interferensie van 'n stof 0·1 mg/l is, dan behoort die monster gedistilleer te word, selfs al is daar 'n kompenserende interferensie aanwesig.

TABEL III.

DIE UITWERKING VAN INTERFERENSIESTOWWE OP DIE FLUORIEDBEPALING.

Interferensiestof.	Konsentrasie mg/l.	Uitwerking of F-aanwysing.	
		Toename of afname.	Hoeveelheid in mg/l.
Alkaliniteit ($AsCaCo_3$)	325	Afname	0·1
Al^{+++}	0·2	Afname	0·1 (Opm. a)
Cl^-	1,800	Afname	0·1
Fe^{+++}	5	Afname	0·1
Cl_2 (moet heeltemal verwyder word)	—	Toename	—
PO_4^{---}	1·0	Toename	0·1 (Opm. b)
$(NaPO_3)_6$	1·1	Toename	0·1
SO_4^-	400	Toename	0·1
Ca^{++}	400	Geen uitwerking nie	0·0
Mg^{++}	200	Geen uitwerking nie	0·0

(*a*) Die aangegewe waarde geld alleen vir fluoriedkonsentrasies bo 0·5 mg/l. Onder 0·5 mg/l F varieer die fout van 0·1 tot 0 mg/l namate die fluoriedkonsentrasie tot 0 afneem.

(*b*) Wanneer geen fluoried aanwesig is nie, het fosfaat 'n baie sterker uitwerking as wanneer daar wel fluoried aanwesig is.

Apparatuur.

'n Spektrofotometer vir gebruik by 525 mμ, wat 'n optiese pad van 1 cm lewer.

Reagense.

1. *Standaardnatriumfluoriedoplossing.*—Los 0·2210 g natriumfluoried (NaF) in gedistilleerde water op en verdun tot 1 liter. Verdun 100 ml van hierdie voorraadoplossing met gedistilleerde water tot 1 liter. 1·00 ml is gelyk aan 0·0100 mg fluoor as F.

2. *Natriumarsenietoplossing*, ongeveer 0·028 N.—Los 1·83 g natriumarseniet ($NaAsO_2$) in 1 liter gedistilleerde water op. Berei elke 6 maande 'n vars oplossing.

3. *Alisarienrooi-oplossing.*—Los 0·375 g alisarienrooi op in gedistilleerde water en verdun tot 500 ml. Indien daar onoplosbare stowwe aanwesig is, filtreer dan deur Whatman-filtreerpapier nr. 40. *Beskut teen direkte sonlig.*

4. *Sirkoniel-suuroplossing.*—Los 0·184 g sirkoniumoksidichloried ($ZrOCl_2 \cdot 8H_2O$) in 50 ml water op en vul met Scott se Gemengde Sure* tot 500 ml by.

5. **Scott se gemengde sure.*—Voeg 68 ml soutsuur by 182 ml water en voeg 22 ml sterk swawelsuur by 228 ml water en meng dan die twee suuroplossings.

Metode.

Teken van die standaardkromme.—Berei fluoriedstandaarde in die reeks 0·00 tot 3·00 mg/l deur toepaslike hoeveelhede van die standaardfluoriedoplossing met gedistilleerde water tot 100 ml te verdun. Reël die temperatuur

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 zirconyl-acid solution to each standard, mix well and allow the reaction to proceed for 60 ± 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 ± 2 minutes take the absorbance readings of the standards at 525 mμ. 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.

Pre-treatment of Sample (if it has not been distilled).—If sample contains free chlorine, remove it by adding 2 drops (0·1 ml) arsenite solution for each 0·1 mg Cl_2 and mix. Add 2 drops in excess. If sample is turbid filter it.

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 zirconyl-acid solution and mix well. Read the absorbance after 60 ± 2 minutes, first setting the reference point of the photometer in the same way as was done for the standard curve.

Calculation.

Read mg fluoride as F from the standard curve:—

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

Correct the fluoride value if necessary from the Table III.

25. Determination of Zinc.

Interference.

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

Collection of Samples and Storage.

Samples should preferably be analysed within 6 hours after collection. "Pickling" 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.

Apparatus.

1. *Spectrophotometer.*—For use at 535 mμ, providing a light path of 2 cm.

2. *Separating Funnels.*

3. *pH Meter.*

Reagents.

1. *Zinc-free Double-distilled Water.*

2. *Dithizone Solution 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.

3. *Dithizone Solution 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.

van die standaarde tot die temperatuur waarby later ontledings gedoen moet word (23 tot 27° C). Voeg 5·00 ml alisarienrooi-oplossing en 5·00 ml van die sirkoniel-suuroplossing by elke standaard, meng goed en laat die reaksie 60 ± 2 minute voortduur.

Stel die fotometer met gedistilleerde water op nulabsorpsie (100 persent transmittansie). (As die instrument nie konstant bly nie, kan die nulstandaard nie gebruik word om die oorspronklike vergelykingspunt op 'n later tydstip te kontroleer nie weens voortskrydende kleurontwikkeling in die standaard.)

Lees na 60 ± 2 minute die optiese digtheid (absorpsie) van die standaarde by 525 mμ af. Telkens wanneer 'n nuwe hoeveelheid van die reagense aangemaak word, moet 'n nuwe standaardkromme geteken word. Die standaardkromme moet op verskeie punte gekontroleer word wanneer ontledings gedoen word.

Voorbehandeling van monster.

(Indien dit nie gedistilleer is nie.) As die monster ongebonde chloor bevat, moet dit verwyder word deur byvoeging van 2 druppels (0·1 ml) arsenietoplossing vir elke 0·1 mg Cl_2, en dan gemeng word. Voeg nog 2 druppels ekstra by. As die monster troebel is, moet dit gefiltreer word.

Ontleding van monster.

Gebruik 100 ml monster of 'n deelvolume tot 100 ml verdun. Bring die monster op dieselfde temperatuur as die standaarde. Voeg 5·00 ml alisarienrooi-oplossing en 5·00 ml sirkoniel-suuroplossing daarby en meng goed. Lees die optiese digtheid (absorpsie) na 60 ± 2 minute af, terwyl die vergelykingspunt op die fotometer op dieselfde manier gestel word as vir die standaardkromme.

Berekening.

Lees mg fluoried as F van die standaardkromme af:

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

Korrigeer die fluoriedwaarde, indien nodig, d.m.v. tabel III.

25. Bepaling van sink.

Interferensie.

Ferri-yster, chloor en ander oksideermiddels laat ditisoon ontbind in intens geelbruin produkte. Glaswerk behoort sorgvuldig eers met verdunde salpetersuur, dan met gedistilleerde water en ten slotte met 'n mengsel van natriumsitraat- en ditisoonoplossings skoongemaak te word.

Versameling van monsters en bewaring.

Monsters behoort by voorkeur binne 6 uur na versameling ontleed te word. Byvoeging van soutsuur sal die metaalione-inhoud teen ontbinding vrywaar mits—

(*a*) die suur geen sink bevat nie;

(*b*) die monsterbottels voor gebruik met suur uitgespoel word;

(*c*) die monsters in silikabakkies tot droog verdamp voor hulle ontleed word om die oormaat soutsuur te verwyder.

Apparatuur.

1. *'n Spektrofotometer.*—Vir gebruik by 535 mμ wat 'n optiese pad van 2 cm lewer.

2. *Skeitregters.*

3. *'n pH-meter.*

Reagense.

1. *Sinkvrye, dubbelgedistilleerde water.*

2. *Ditisoonoplossing a.*—Los 0·10 g difenieltiokarbasoon in een liter koolstoftetrachloried op. Bewaar in 'n bruin bottel in 'n elektriese yskas. Indien die oplossing van twyfelagtige kwaliteit is of 'n lang tyd gestaan het, kan die volgende toets gedoen word, om verslegting te bepaal: Skud 10 ml met 10 ml Ammoniumhidroksied (1 + 99). Indien die onderste laag, d.w.s. die koolstoftetrachloried slegs effens gelerig is, is die reagens in goeie toestand.

3. *Ditisoonoplossing b.*—Verdun een volume ditisoonoplossing *a* met nege volumes koolstoftetrachloried. Indien hierdie oplossing in 'n bruin glasbottel in 'n yskas bewaar word, bly hy verskeie weke lank bruikbaar.

4. *Sodium Citrate Solution for Cleaning Glassware.*—Dissolve 10 g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in 90 ml water. Extract with 10-ml portions of dithizone solution until the last extract remains green, then extract with carbon tetrachloride to remove excess dithizone.

5. *Hydrochloric Acid Solution.* approximately 0·02 N.—Dilute 1·0 ml concentrated hydrochloric acid to 600 ml with re-distilled water.

6. *Sodium Acetate Solution*, approximately 2 N.—Dissolve 68 g sodium acetate ($NaC_2H_3O_2.3H_2O$) and make to 250 ml with re-distilled water.

7. *Acetic Acid Solution*, approximately 2 N.—Mix 1·0 volume glacial acetic acid (CH_3COOH) with 7 volumes re-distilled water.

8. *Acetate Buffer Solution.*—Mix equal volumes sodium acetate solution and acetic acid solution. Purify by dithizone extraction as described for sodium citrate solution.

9. *Sodium Thiosulphate Solution.*—Dissolve 25 g sodium thiosulphate ($Na_2S_2O_3.5H_2O$) in 100 ml re-distilled water. Purify by dithizone extraction as described for sodium citrate solution.

10. *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.

11. *Standard Zinc Working Solution.*—Dilute 10·0 ml standard zinc stock solution to 1 litre with re-distilled water; 1 ml = 0·001 mg Zn.

12. *Carbon Tetrachloride.*

Method.

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 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 4·0 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.

Colorimetric Measurement.—Set the spectrophotometer at 0·0 absorbance with the blank at 535 mμ. Plot a calibration curve. A new curve should be plotted with each set of samples.

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 2 to 3 with 0·02 N hydrochloric acid. Transfer 10·0 ml to a separating funnel. Complete the analysis as described in " preparation of standards ", beginning with the word " to each funnel add 5·0 ml acetate buffer . . . ".

Read the mg Zn on the calibration curve.

Calculation.

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

4. *Natriumsitraatoplossing vir die skoonmaak van glaswerk.*—Los 10 g natriumsitraat ($Na_3C_6H_5O_7 \cdot 2H_2O$) in 90 ml water op. Ekstraheer met hoeveelhede ditisoonoplossing van 10 ml elk totdat die laaste ekstrak groen bly; ekstraheer daarna met koolstoftetrachloried om die oormaat ditisoon te verwyder.

5. *Soutsuuroplossing*, ongeveer 0·02 N.—Verdun 1·0 ml sterk soutsuur met herhaaldelik gedistilleerde water tot 600 ml.

6. *Natriumasetaatoplossing*, ongeveer 2 N.—Los 68 g natriumasetaat ($Na\ C_2H_3O_2 \cdot 3H_2O$) op en vul met herhaaldelik gedistilleerde water by tot 250 ml.

7. *Asynsuuroplossing*, ongeveer 2 N.—Meng 1·0 volume ysasynsuur (CH_3COOH) met 7 volumes herhaaldelik gedistilleerde water.

8. *Asetaatbufferoplossing.*—Meng gelyke volumes natriumasetaatoplossing en asynsuuroplossing. Suiwer d.m.v. ditisoonekstraksie soos onder natriumsitraatoplossing beskryf.

9. *Natriumtiosulfaatoplossing.*—Los 25 g natriumtiosulfaat ($Na_2S_2O_3 \cdot 5H_2O$) in 100 ml herhaaldelik gedistilleerde water op. Suiwer d.m.v. ditisoonekstraksie soos onder natriumsitraatoplossing beskryf.

10. *Standaardsinkvoorraadoplossing.*—Los 0·100 g 30-maas-sinkmetaal in 'n klein oormaat soutsuur (1 + 1) op; omtrent 1 ml is daarvoor nodig. Verdun dan met herhaaldelik gedistilleerde water tot 1 liter.

11. *Standaardsinkwerkoplossing.*—Verdun 10·0 ml standaardsinkvoorraadoplossing met herhaaldelik gedistilleerde water tot 1 liter; 1 ml = 0·001 mg Zn.

12. *Koolstoftetrachloried.*

Metode.

Bereiding van kolorimetriese standaarde.—Voeg by 'n reeks Squibb-skeitregters van 125 ml, wat volgens die voorskrifte skoongemaak is, 0·0, 1·0, 2·0, 3·0, 4·0 en 5·0 ml standaardsinkwerkoplossing gelyk aan 0·000, 0·001, 0·002, 0·003, 0·004 en 0·005 mg Zn. Vul elke volume met herhaaldelik gedistilleerde water by tot 10·0 ml. Voeg by elke tregter 5·0 ml asetaatbuffer en 1·0 ml natriumtiosulfaat oplossing en meng. Die pH behoort by hierdie punt tussen 4 en 5·5 te lê. Voeg by elke tregter 10·0 ml ditisoonoplossing *b*; prop toe en skud flink 4·0 minute lank. Laat die lae skei. Maak die steel van die tregter met strokies filtreerpapier droog en laat die onderste, d.w.s. die koolstoftetrachloriedlaag, in 'n skoon *droë* kolorimeter-sel afloop.

Kolorimetriese meting.—Stel die spektrofotometer op 0·0 absorpsie met die kontrolehoeveelheid by 535 mμ. Teken 'n ykkromme. 'n Nuwe kromme moet vir elke stel monsters geteken word.

Werkwyse in die geval van monsters water.—Indien die gehalte aan sink nie binne die werkgebied val nie, moet die monster met herhaaldelik gedistilleerde water verdun of in 'n silikabakkie gekonsentreer word. Reël die monster se pH met 0·02 N soutsuur tot 2 of 3 met behulp van 'n pH-meter en neem enige verdunning in aanmerking. Giet 10·0 ml in 'n skeitregter oor. Voltooi die ontleding soos onder „ bereiding van standaarde " aangegee, vanaf die woorde „ Voeg by elke tregter 5·0 ml asetaatbuffer . . . ".

Lees die mg Zn op die ykkromme af.

Berekening.

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

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