THE CHEMICAL ANALYSIS OF FRESH WATER

By
M. P. STAINTON
M. J. CAPEL
F. A. J. ARMSTRONG

Environment Canada
Fisheries and Marine Service

Environnement Canada
Service des pêches et des sciences de la mer

Miscellaneous Special Publication No. 25

Department of the Environment
Fisheries and Marine Service
Research and Development Directorate

MISCELLANEOUS SPECIAL PUBLICATION No. 25

Ministère de l'Environnement
Service des Pêches et des Sciences de la mer
Direction de la Recherche et Développement

PUBLICATION DIVERSE SPÉCIALE No. 25

The Chemical Analysis of Fresh Water

by

M. P. STAINTON, M. J. CAPEL and F. A. J. ARMSTRONG

Research and Development Directorate
Freshwater Institute
Winnipeg, Manitoba

Recherche et Développement
Institut des eaux douces
Winnipeg, Manitoba

1974

STAINTON, M.P., M.J. CAPEL, and F.A.J. ARMSTRONG

The Chemical Analysis of Fresh Water,

Fish. Res. Board Can. Misc. Spec. Publ. 25:125 p.

This publication describes analytical methods used in the Freshwater Institute for the chemical analysis of fresh water. Emphasis is on the determination of carbon, nitrogen, phosphorus and silicon in dilute waters. Recommendations are made concerning sampling techniques, sample manipulation and storage. Estimates of analytical precision are included.

Le présent ouvrage décrit les méthodes analytiques employées à l'Institut des eaux douces pour l'analyse chimique de l'eau douce. On spécialise dans la détermination du carbone, de l'azote, du phosphore et du silicium, en solutions diluées (en faibles concentrations). On inclut des recommendations relativement aux techniques d'échantillonage, à la transformation des échantillons et à leur entrepasage. On estime la précision analytique.

TABLE OF CONTENTS

ACKNOWLEDGMENTS

We are indepted to G. Porth, J. Boughen and S. Edwards for rendering the manuscript intelligible. Diagrams were constructed by C. Royal and L. Davies. The manuscript was critically read by B. Graham, B. Hauser, J. Prokopowich, R. Schmidt, and D. Schindler. Members of the Freshwater Institute staff, too numerous to mention, have assisted in the development of these methods by giving of their ideas, time and encouragement.

INTRODUCTION

These methods have been in use in this Institute for some time, for analysis of fresh waters, mostly of low solute content, and often highly coloured. Care should be taken in applying them to alkaline, hard, or saline waters, although many are adaptations of methods for sea water.

They have been used mainly in studies of aquatic productivity and are therefore mostly for determination of micronutrients rather than for pollutants or assessment of water quality. They are not intended in any way as standard methods, and can be modified as experience demands, if they can be made to yield results closer to what is actually present.

None of them should require any great analytical skill, and we have tried to lay out the instructions as simply as possible, and to keep theory and explanatorymatter to the minimum. It is, of course, to the advantage of the analyst, and especially to the supervisor, to get all the background knowledge that he can, and we recommend reference to the sources mentioned with the methods, and to the texts listed below. Our own indebtedness to these sources should be obvious. In choosing among methods we have gone for simplicity and speed, partly for greater productivity in a busy laboratory, but also because the longer an analysis takes, and the more manipulation there is, the greater the chances of contamination.

We have evaluated errors of analysis at one or more levels for most of these methods, and so have an indication of the precision in

replication from a single sample. Sampling error, that is, the variability of separate samples taken successively from the same spot, is another matter. It is known, for example, that the suspended matter in a lake, or the phyto- or zooplankton, may be patchily distributed so that analyses of successive samples, even if quickly taken, may show a relative standard deviation of ±20% or more. In comparing analyses related to particulate material, it will be wise to test for significance.

We have generally used research type instruments for our own analyses, because these are what we use in developing methods and we have them and are familiar with them. Much can be done with simpler instruments, and good absorptiometric work does not necessarily demand instruments with monochromators, double beam systems, or strip-chart or digital read-out. The laboratory with limited means should be able to work with a filter absorptiometer for most of these determinations if care is taken to choose the correct filter each time. Sophisticated instrumentation will not compensate for errors of timing, contamination or incorrect storage of samples.

At summer temperatures changes in nutrient concentrations can be rapid, as bacteria or phytoplankters multiply, and storage for 24 hours can make analyses meaningless. Generally, changes can be arrested by freezing and keeping samples frozen until analyzed, though this can upset silicate analyses, and probably those for calcium and magnesium as well. Methods of storage are indicated for each determination.

Usually analyses are done on filtered samples. If the suspended matter is also analyzed, some nutrients can be assigned to "dissolved"

or "suspended" fractions. The distinction is, of course, an artificial one and has to depend on the porosity of the filter used. To have some physical meaning, the dividing line, in particle size, between particulate and dissolved matter could reasonably be put between 0.1 and 1.0 μm, where the smaller particles might be kept from settling by Brownian movement. Recently there has been a tendency to specify membrane filters of A.P.D. 0.45 μm. However, most cellulose ester membrane filters of this porosity are slow and apt to clog. We prefer Whatman GF/C glass fiber filters, which are faster, and being inorganic, may be used for carbon and nitrogen determination. The pore size is greater, and not so well defined, being around 2-3 μm, insofar as it can be specified for this type of filter material. However, there is very little difference in the quantity of suspended matter retained by these filters, compared with membrane filters of 0.45 μm A.P.D.

We will greatly appreciate correspondence from users of these methods, indicating errors or difficulties with any of them, and suggesting improvements. It is our intention to update the manual annually. Those wishing to receive these supplements should fill out the information form provided inside the back cover.

Bibliography

Riley, J.P. (1968). Analytical chemistry of sea water. Chapter 21, pp 295-424 in Chemical Oceanography, Vol. 2, J.P. Riley and G. Skirrow, Academic Press, London and New York.

Strickland, J.D.H. and T.R. Parsons (1968). A practical handbook of sea water analysis. Bull. Fish. Res. Bd Canada, No. 167.

Golterman, H.L. (1969). Methods for chemical analysis of fresh waters. I.B.P. Handbook No. 8. International Biological Program, London and Blackwell. Oxford and Edinburgh. 166 p.

American Public Health Association (1965). Standard methods for the examination of water and wastewater. 12th ed. APHA, New York, N.Y. 769 p.

SAMPLE COLLECTION

Because of the dilute nature of much of Canadian fresh water, gross changes in sample composition can arise through contamination from sampling equipment and personnel and from poor sampling technique. Below are listed recommendations concerning samplers, sample containers and special sampling techniques and precautions.

Using the methods we have described, about 1 liter of water is required for complete analysis (500 ml if automated techniques are used).

Samplers

Two types of sampler have been found useful for work with lakes and slow moving rivers. The 2-liter van Dorn type sampler constructed of clear plastic is an excellent general purpose instrument; easy to load and trigger under all weather conditions and light enough for work in remote areas. Being transparent this sampler permits viewing samples prior to transfer to sample bottles which is essential when sampling near the sediment-water interface.

For shallow lakes and situations where good resolution of stratification is required, a 12 volt D.C. peristaltic pump has some advantage. Little perturbance of the source occurs with this sampler as only small diameter weighted tube is lowered to the desired depth. However, this equipment is heavy and cannot be used for winter sampling.

Sample Bottles

We have found the 500 ml translucent polyethylene screw-cap bottle to be the most useful sample container for field use. Polypropylene appears to be too brittle, particularly at low temperatures, to be serviceable. Opaque sample bottles should be avoided, it being quite difficult to tell when they are clean. Before use bottles should be washed with a phosphate-free detergent followed by rinses with dilute hydrochloric acid and distilled and deionized water. When attempting to measure trace nutrients and metals the effect of sample container walls must be investigated.

When sampling, bottles should be rinsed twice before filling. Samples should not be stored in polyethylene sample bottles for more than a few days as bacterial films form on bottle walls which are difficult to remove and which may contaminate subsequent samples.

Special Techniques and Precautions

Dissolved oxygen

It is essential to minimize the contact of sample with the atmosphere. The sampler used must be equipped with an 18" flexible tube to dispense sample. Samples must be collected in specially designed glass "B.O.D." bottles having conical stoppers to prevent bubble entrapment. When transferring sample the sampler dispensing tube must be placed at the bottom of the sample bottle and held below the liquid surface while

filling. At no time should sample be allowed to cascade into the bottle. Liquid should be allowed to overflow the bottle by at least one bottle volume. The bottle should then be quickly stoppered taking care not to trap any air bubbles.

Nutrient elements

When sampling for nutrient elements the usually low levels involved make contamination a significant problem. While the need for clean samplers and sample bottles is obvious there are several other contamination sources which must be avoided. Small amounts of tobacco ash, dandruff and perspiration contributed by field personnel or plant pollen and other atmospheric particulates all can introduce significant errors into nutrient element analysis. Field personnel must be made aware of these and other possible sources of contamination.

Silicon

Samples collected for silicon analysis must not be stored in glass containers, particularly if they are alkaline. Samples should never be allowed to freeze as the freeze concentration which occurs in the sample bottle, causes precipitation of silicon in a form which does not quantitatively redissolve on thawing.

Deoxygenated waters

When sampling anaerobic waters for dissolved iron, manganese and phosphate, it is important to avoid aeration of sample which will precipitate metal oxides and coprecipitate phosphorus. Samples should be treated as for dissolved oxygen, filtered and acidified as soon as possible after sampling.

SAMPLE HANDLING

We have found it useful, if not essential, to filter samples as soon as possible (within hours) after collection. This permits analytical distinction between dissolved and particulate phases and minimizes changes in sample composition arising from biological uptake and excretion. Since parameters differ in their storage condition and time limits it is useful to sub-divide a sample into different containers, each receiving appropriate storage treatment. The sample handling scheme found useful in our laboratories appears in Figure A.

Three particulate samples are obtained, one each for: chlorophyll, particulate solids and/or particulate phosphorus and particulate carbon and nitrogen. Filtrate is divided into two portions. One is acidified and used for cation analysis. The other is used for nutrient elements, major anions and dissolved solids. A portion of sample remains unfiltered for analysis of ammonia, carbon dioxide, conductivity, pH and soluble reactive silicon.

Dissolved and particulate phases are arbitrarily defined by what passes through or is retained on a pre-ignited (16 hours @ 550 C) Whatman 45 mm GF/C glass fiber filter of undefined but consistent pore size.

SAMPLE HANDLING SCHEME FOR UNFILTERED WATER

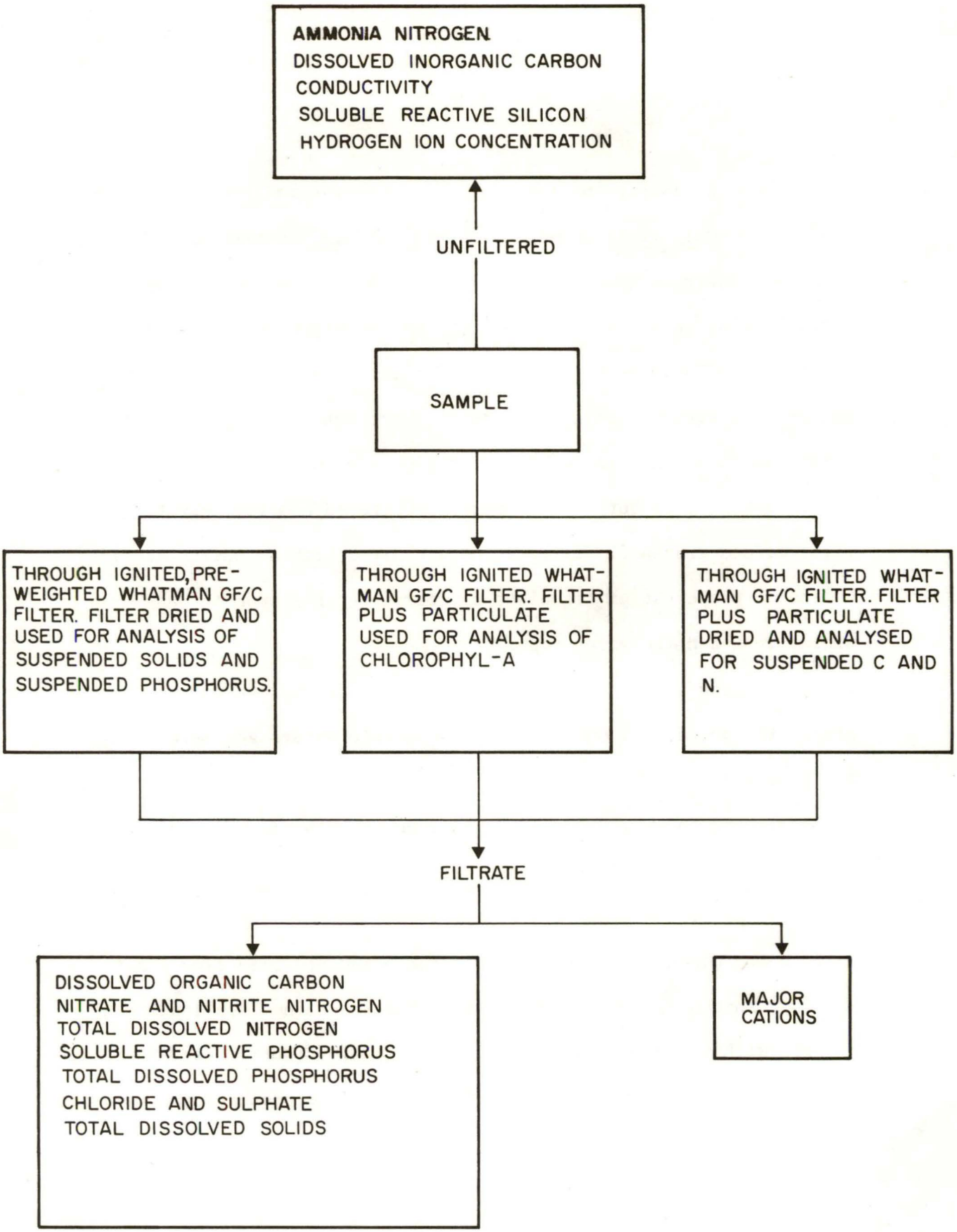


Figure A

SAMPLE STORAGE

Table I lists guidelines for the treatment and storage of samples and is a summary of recommendations made within the text of individual methods. While several preservation methods have been reported (Hellwig 1963, Brezonik and Lee 1966) and widely used, none are satisfactory for preserving samples for all analyses. For determinations of nutrient elements we have found no substitute for rapid analysis. Where sample storage is necessary samples should, at the very least, be refrigerated (5 C) but more preferably filtered and refrigerated, filtered and frozen or filtered and preserved with mercuric chloride. Choice of preservation technique of course depends upon the analysis of interest and the water type.

Freezing samples inevitably produces a freeze concentration causing precipitation of silicon in neutral to acidic waters and metal salts in alkaline waters. These precipitates often do not quantitatively redissolve on thawing. Addition of mercuric chloride to samples interferes with both nitrate and chloride analysis.

The permissable storage times listed in Table I are guidelines only, being a compromise between desirable and possible time limits.

It should be obvious that sample deterioration is a continuous process with the rate dependent on the parameter (nutrients faster than major cations and anions), the level (trace levels proportionatly faster than major levels) and the type of source.

BIBLIOGRAPHY

1. Brezonik, P.L. (1966). Preservation of water samples for inorganic nitrogen analysis with mercuric chloride. Air & Wat. Pollut. Int. J. 10:549-53.

2. Hellwig, D.H.R. (1964). Preservation of water samples. Air & Wat. Pollut. Int. J. 8:215-28.

Table 1. Guidelines for treatment and storage of samples.

Analysis	Storage conditions and duration prior to first manipulation	Initial manipulation	Storage conditions and allowable duration prior to analysis
Dissolved inorganic carbon	5 C 24 hours	Analysis	None
Dissolved organic carbon	5 C 48 hours	Filtration-chemical oxidation in ampoule	Room temp. Indefinitely
Particulate carbon and nitrogen	5 C 48 hours	Particulate collected on glass fiber filter	Filter @ -10 C. Indefinitely
Ammonia nitrogen	5 C 24 hours	Analysis	None
Nitrite nitrogen	5 C 24 hours	Filtration - analysis	None
Nitrate nitrogen	5 C 48 hours	Filtration - analysis	None
Total dissolved nitrogen	5 C 48 hours	Filtration - U.V. photocombustion	5 C 5 days after U.V.
Soluble reactive phosphorus	5 C 24 hours	Filtration analysis	None
Total dissolved phosphorus	5 C 48 hours	Filtration - U.V. photocombustion	5 C 5 days after U.V.
Particulate phosphorus	5 C 48 hours	Particulate collected on glass fiber	Room temp. Indefinitely
Soluble reactive silicon	5 C 7 days	Analysis	None
Chloride	5 C 7 days	Filtration	5 C 30 days
Sulphate	5 C 7 days	Filtration	5 C 30 days
Ca, Fe, Mg, Mn, K, Na	5 C 7 days	Filtration - acidification	Room temp. - 30 days
Specific conductance	5 C 7 days	Analysis	None
Chlorophyll	5 C 48 hours	Particulate collected on glass fiber	-10 C in dark Indefinitely
Dissolved oxygen	5 C 1 hour	Analysis	None
Dissolved solids	5 C 7 days	Filtration	5 C 30 days
pH	5 C 24 hours	Analysis	None
Particulate solids	5 C 48 hours	Particulate collected on glass fiber	Dry room temp. Indefinitely

METHOD PRECISION

We have obtained estimates of the precision attainable using the methods described in this manual, both for synthetic standards and natural water. Replicate samples (n=6) of synthetic standards and natural (Canadian Sheild) water (six subsamples from a homogenized 10-liter sample) were analysed in our routine laboratory. Standard devaition values (n=6) for natural water are given with each method and are compiled along with those for synthetic standards in Table III.

Errors for synthetic standards indicate the precision limits of method chemistry and instrumentation and have been termed Method Error (Table III). Errors for natural water include the additional errors arising from filtration, voluming and other manipulative errors and are termed Laboratory Error.

We have not included estimates of error involved in sampling a point source as these errors vary too greatly with source to permit a generalization. Since sampling errors are often quite large (10-30% for particulate material) we cannot urge too strongly that persons involved in sampling obtain estimates of these errors before interpreting chemical data.

Table III. Some estimates of method precision for synthetic standards and Canadian Shield water. (Errors expressed as relative standard deviation n = 6)

ANALYSIS	SYNTHETIC STANDARDS		CANADIAN SHIELD LAKE WATER	
	Level	Method Error	Level	Lab Error
Dissolved inorganic carbon	1000 µM	<1%	400 µM	<1%
Dissolved organic carbon	1000 µM	2%	700 µM	2%
Particulate carbon	200 µg/l	4%	1000 µg/l	3%
Ammonia - nitrogen	600 µg/l	2%	370 µg/l	1%
Nitrite - nitrogen	300 µg/l	<1%	5 µg/l	10%
Nitrate - nitrogen	300 µg/l	<1%	120 µg/l	3%
Total dissolved nitrogen	-	-	900 µg/l	1%
Particulate - nitrogen	25 µg/l	5%	140 µg/l	4%
Soluble reactive phosphorus	250 µ	<1%	-	-
Total dissolved phosphorus	-	-	20 µg/l	13%
Particulate phosphorus	150 µg/l	<1%	10 µg/l	4%
Silicon	10 mg/l	<1%	2.10 mg/l	<1%
Chloride	25 mg/l	3%	0.4 mg/l	21%
Sulphate	25 mg/l	5%	5.0 mg/l	3%
Calcium	1.0mg/l	<1%	2.5 mg/l	<1%
Iron	1.0mg/l	3%	0.52 mg/l	6%
Magnesium	1.0mg/l	1%	0.80 mg/l	2%
Manganese	1.0mg/l	1%	-	-
Potassium	1.0mg/l	2%	0.30 mg/l	4%
Sodium	1.0mg/l	2%	4.80 mg/l	<1%
Specific conductance	140 µS/cm	<1%	50 µS/cm	<1%
Chlorophyll	-	-	18 µg/l	7%
Dissolved oxygen	-	-	10.0 mg/l	2%
Dissolved solids	-	-	46 mg/l	9%
pH	8.50	<1%	-	-
Particulate solids	-	-	5 mg/l	10%

DISSOLVED INORGANIC CARBON [TOTAL CARBON DIOXIDE]

PRECISION

Level	- 300 µM CO_2/liter
Standard deviation	- ± 10 µM CO_2/liter

RANGE - 10-2000 µM/liter total inorganic C

SAMPLE CONDITION
- Sample collected in a 50 ml polypropylene syringe or
- in a bottle which is filled completely, tightly sealed and stored at 5 C.
- Less than 24 hours old.
- Do not acidify, filter or freeze.
- Free from suspended carbonates.

METHOD PRINCIPLE - A sample containing H_2CO_3[1], HCO_3^- and $CO_3^=$ is acidified [pH-4] converting carbonate and bicarbonate to H_2CO_3. Agitation of the sample with helium partitions gaseous CO_2 out of solution into the gas phase. The helium-CO_2 mixture (containing also N_2 and O_2) is then injected into a gas chromatograph for separation and detection of CO_2 by thermal conductance.

REAGENTS

A. CO_2-free distilled water.

Pass distilled water over a mixed bed resin to remove CO_2.
Collect effluent without bubbling.
Prepare fresh as needed.

B. Sulphuric acid - 1 N

---

1. H_2CO_3 is used to represent all dissolved carbon dioxide (i.e. CO_2 +H_2CO_3) following the convention of Garrels & Christ (Garrels, R.M. and Christ, C.L. 1965. Solution, Minerals and Equilibria. Harper & Row, N.Y.).

C. Carbon dioxide standard

Dissolve 0.8401 g of dry $NaHCO_3$ in CO_2-free water. Make to 1 liter with CO_2-free water without aeration.

1.00 ml = 10 μmoles CO_2
Prepare fresh daily.

D. Carbon dioxide standard (alternate method)

Dilute B.D.H. N/50 sodium carbonate concentrated volumetric solution to volume specified by manufacturer.

1.00 ml = 10 μmoles CO_2
Prepare fresh daily.

APPARATUS

A. Fisher Hamilton gas partitioner Model 29 (or equivalent equipment) set up as follows:

1. One 4-foot by 1/8" Poropak Q (80-100 mesh) chromatographic column (Bypass loop in position of second column).

2. Sample loop valve - 2 ml volume.

3. Helium carrier gas flow 20-30 ml/min.

4. Detector current - 240 mA.

5. Detector signal to 10", 1 mV full-scale recorder - 0.2 inch per minute.

B. "Cornwall" type dispensing syringe - 1 ml volume with double female luer coupling.

C. Disposable polypropylene syringes - 50 ml with caps.

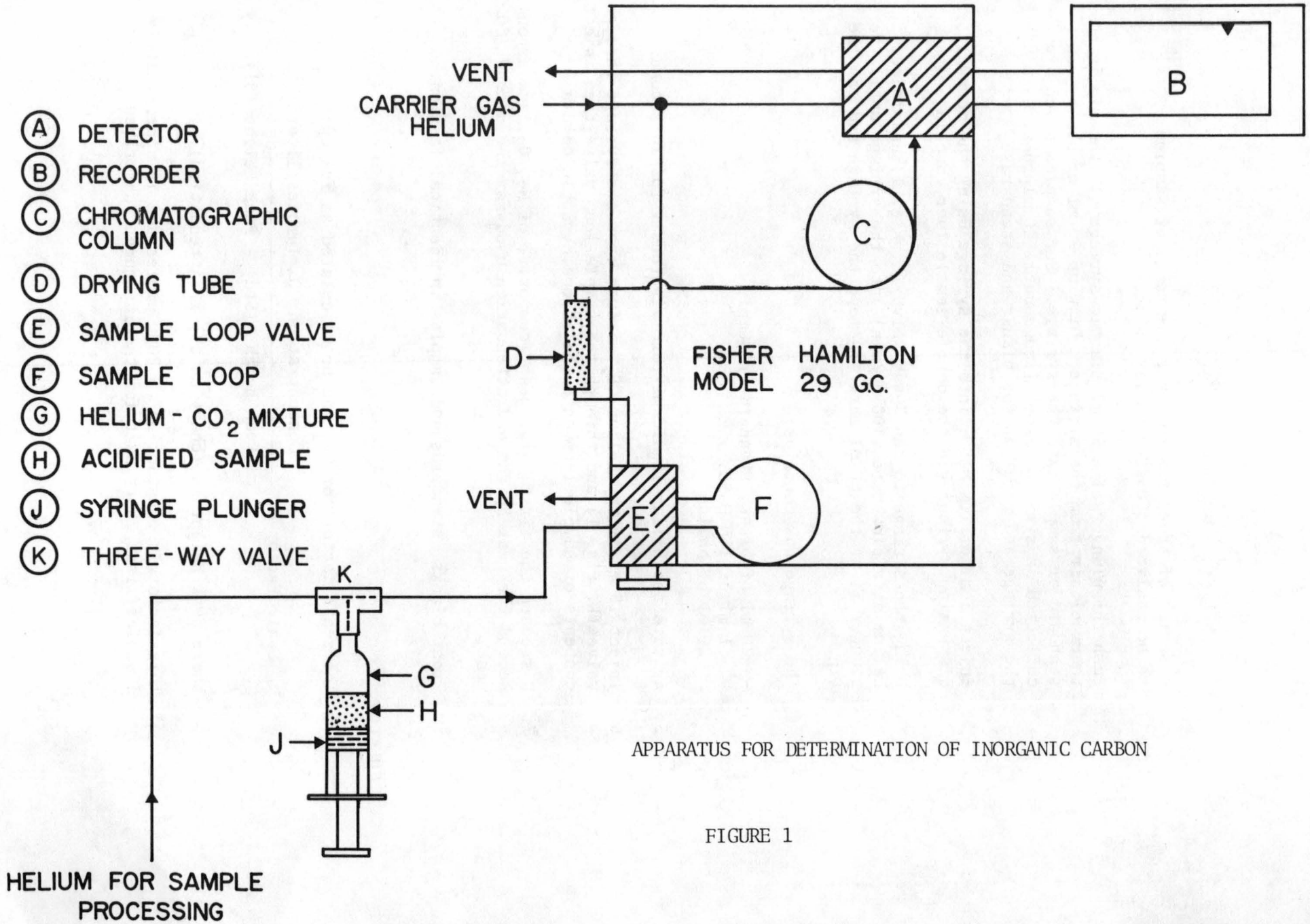
D. Plastic 3-way valves (6) with male and female couplings (Ace Glass #5851-10).

E. 2 cylinders of helium with regulators or 1 cylinder of helium with a stream splitting valve.

Assemble apparatus as in Figure 1.

PROCEDURE

1. Using CO_2-free water to dilute the stock CO_2 standard, prepare



APPARATUS FOR DETERMINATION OF INORGANIC CARBON

FIGURE 1

a series of standards to cover the range of samples to be analyzed (50-2000 µMolar CO_2).

2. Push the plunger of a 50 ml plastic syringe all the way in to expel air from the syringe. Draw up 20 ml of blank, standard or sample into the syringe. For samples which are collected in syringes expel liquid until 20 ml remains in the syringe and treat as for blanks and standards.

3. Inject 1 ml of 1 N H_2SO_4 into the syringe using the Cornwall dispensing syringe via the double female luer coupling.

4. Bring the syringe to the 3-way valve (K, Fig. 1) and inject helium at a slow rate (100 ml/min) into the syringe until the plunger is at the 50 ml mark. Remove the syringe and cap the tip.

   The syringe now contains:

   - 20 ml of blank, standard or sample
   - 1 ml of 1 N H_2SO_4
   - 29 ml of helium

5. Agitate the helium-liquid mixture vigorously for 10 seconds.

6. Inject 15 ml of the helium-CO_2 mixture from the syringe through valve (K, Fig. 1) and through the sample loop valve; then inject contents of sample loop onto the chromatographic column.

7. Disregard the first large peak (composite of N_2, O_2, and argon). Record the peak height and attenuation used for the second (CO_2) peak.

8. Treat blanks, standards and samples in identical fashion.

CALCULATIONS

1. The scale factor for CO_2 can be calculated as follows:

$$\text{Scale Factor } CO_2 = \frac{\text{Concentration } CO_2 \text{ (µmoles/liter)}}{\text{Peak height (Std) - Peak height (Bl)}}$$

   Where peak height is (chart units) x (attenuation).

   This factor is constant up to 2000 µmoles and is approximately 3 µmoles/liter/chart unit for the instrument described.

2. Sample concentration is found by multiplying the observed peak height for the sample by the CO_2 scale factor.

3. The calculations above give a value for the total CO_2 content of the water sample. If pH and temperature values for the sample at the time of collection are available then (H_2CO_3), (HCO_3^-), and $(CO_3^=)$ can be computed.

   See Method on Dissolved Carbonate System.

REFERENCE

Stainton, M.P. (1973). A syringe gas-stripping procedure for gas chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. J. Fish. Res. Board Can. 30:1441-1445.

DISSOLVED ORGANIC CARBON

PRECISION

Level - 700 µmoles C/liter

Standard deviation - ± 16 µmoles C/liter

OPERATING RANGE - 50-2000 µmoles dissolved organic carbon per liter.

SAMPLE CONDITION - Filtered through ignited Whatman GF/C and stored at 5 C.
- Less than 48 hours old.

METHOD PRINCIPLE - If a water sample is acidified (below pH 4) and bubbled vigorously with helium, the inorganic carbon can be removed. Remaining carbon is considered to be dissolved organic carbon (DOC). If the acidified sample is then treated with potassium persulphate at elevated temperature and pressure, organic carbon compounds are fragmented and oxidized to CO_2. The reaction is carried out in sealed ampoules which are autoclaved at 121 C for 1 hour.

The CO_2 produced is measured as for inorganic carbon and is a measure of the organic carbon content of the sample.

REAGENTS

A. Low-carbon water - Effluent from Millipore "Super Q" system.

B. Potassium persulphate - Analytical reagent grade $K_2S_2O_8$.

C. Perchloric acid - Sp. Gr. - 1.70.

D. Glucose - organic carbon standard

Dissolve 0.3003 g of glucose in low-carbon water and make to 1 liter. Store at 5 C. Discard if a precipitate develops.

1.00 ml = 10 μmoles glucose-C

APPARATUS

A. Inorganic carbon stripping manifold as in Figure 2, assembled from 20 gauge, 6 inch, stainless steel needles, tygon tubing and ganged valves.

B. Helium and regulator.

C. Glass ampoules - pre-scored necks to contain 25 ml.

D. Oxy-propane torch to seal ampoules.

E. Metal racks for ampoules.

F. Muffle furnace to pre-ignite ampoules.

G. Autoclave with slow exhaust cycle.

PROCEDURE

1. Pre-ignite (550 C for 8 hours) enough ampoules for the samples to be analyzed.

2. Place 0.1 g (approximately) of potassium persulphate in each ampoule.

3. Prepare a series of glucose standards to cover the range of the samples to be analyzed (50-2000 μmolar C).

4. Place 25 ml of blank, standard, or filtered sample into an ampoule containing potassium persulphate.

5. Add 0.1 ml of perchloric acid to the ampoule.

6. Insert one of the stainless steel needles of the CO_2 stripping manifold into the ampoule and turn on helium.

7. Bubble sample for 20 minutes to drive off inorganic carbon.

8. Quickly seal the ampoule with the oxy-propane torch taking

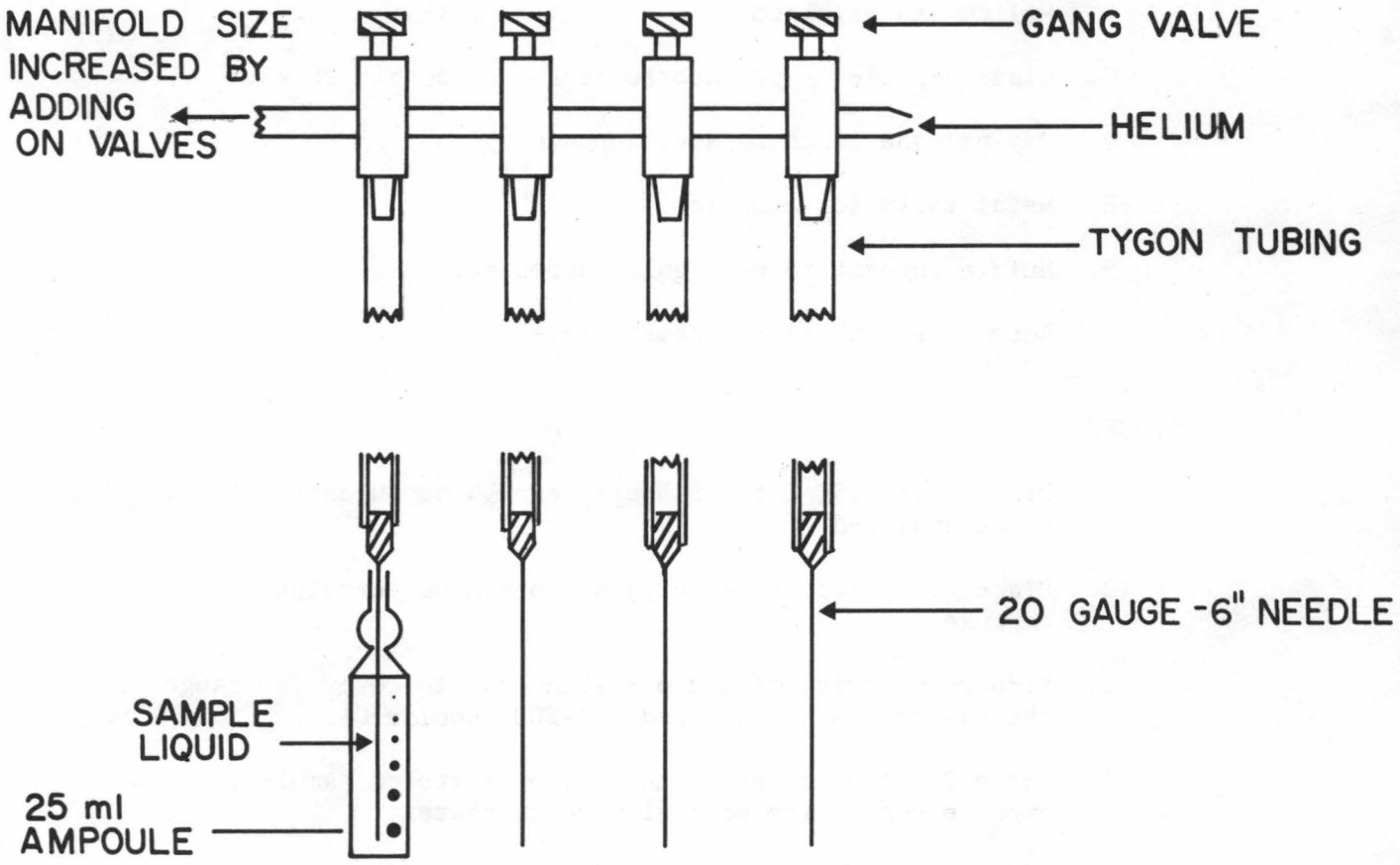

MANIFOLD SIZE
INCREASED BY
ADDING
ON VALVES
GANG VALVE
HELIUM
TYGON TUBING
20 GAUGE -6" NEEDLE
SAMPLE
LIQUID
25 ml
AMPOULE

FIGURE 2

care not to entrap combustion gases from the flame in the ampoule. A rounded bubble seal is desirable for strength.

9. Autoclave samples at 121 C for 1 hour.

   Ampoules may be stored indefinitely after this is done.

10. An autoclaved sample is cooled to room temperature and opened. 20 ml of the ampoule contents are transferred to a 50 ml plastic syringe through a wide-bore cannula. It is important to carry out this transfer quickly but without degassing the sample which results if the syringe plunger is pulled out too fast.

11. The sample in the 50 ml syringe is analyzed as for inorganic carbon (page).

12. Obtain peak height (chart units x attenuation) values for low-carbon water, glucose standards, and samples.

CALCULATIONS

1. A scale factor for the gas chromatograph can be calculated as follows:

$$\text{Scale Factor (Organic C)} = \frac{\text{Glucose-C concentration (µmoles/liter)}}{\text{Peak height (Std) - Peak height (Bl)}}$$

   where peak height = (Chart Units) x (Attenuation).

2. Sample concentration is found by multiplying observed peak height for the sample by the scale factor. Reagent blank is usually negligible.

3. Dilution calculations -

   Organic carbon concentration in samples requiring dilution with low carbon water can be calculated using the equation below. Organic carbon content of dilution water must be determined.

$$C_1 = (C_2 \times D) - (C_3 \times (D-1))$$

   where C_1 = conc. of organic carbon in sample.
   C_2 = conc. of organic carbon in diluted sample.
   C_3 = conc. of organic carbon in dilution water
   D = dilution factor

REFERENCES

1. Menzel, D.W., and Vaccaro, R.F. (1964). The measurement of dissolved organic and particulate carbon in sea water. Limnol. Oceanogr. 138-142.

2. Stainton, M.P. (1973). A syringe gas stripping procedure for gas chromatographic determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. J. Fish. Res. Board Can. 30:1441-1445.

DISSOLVED CARBONATE SYSTEM

The equilibrium levels of the various components of the carbonate system depend on temperature, pH, ionic strength and dissolved inorganic carbon levels. Measurement of these permits computation of the levels of H_2CO_3, $CO_3^{=}$, and HCO_3^{-} in the original sample (if pH and temperature measurements are taken upon sampling).

The carbonate equilibrium system involves the following chemical reactions:

1. $CO_2 + H_2O \rightleftharpoons H_2CO_3$

2. $H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$

3. $HCO_3^- \rightleftharpoons H^+ + CO_3^=$

The equilibrium constants for these reactions are:

1. $Kc = \dfrac{[H_2CO_3]}{P_{CO_2}} = 10^{-(1.131 \times T)}$

where P_{CO_2} = the partial pressure of CO_2 in the atmosphere $\cong 10^{-3.5}$ atm.

2. $Ka = \dfrac{[H^+]\,[HCO_3^-]}{[H_2CO_3]} = 10^{-(6.57 - (0.00926 \times T))}$

3. $Kb = \dfrac{[H^+]\,[CO_3^=]}{[HCO_3^-]} = 10^{-(10.614 - (0.0117 \times T))}$

In all these equations T is the temperature (centigrade) of the water sample at the time of collection. It should also be noted that the values given for the constants only apply to waters with a low ionic strength.

To calculate the concentrations of the various components of the system the following equations are used:

$$A.\quad [H_2CO_3] = \frac{[H^+]^2 \times \Sigma CO_2}{[H^+]^2 + ([H^+] \times Ka) + KaKb}$$

where $\Sigma CO_2 = [H_2CO_3] + [HCO_3^-] + [CO_3^-]$ = dissolved inorganic carbon (moles/liter)

$$B.\quad [H\,CO_3^-] = \frac{[H^+] \times Ka \times \Sigma CO_2}{[H^+]^2 + [H^+]\,Ka + KaKb}$$

$$C.\quad [CO_3^=] = \frac{KaKb \times \Sigma CO_2}{[H^+]^2 + [H^+]\,Ka + KaKb}$$

H^+ is calculated from the pH of the sample measured at the time of collection. $H^+ = 10^{-pH}$.

REFERENCE

Garrels, R.M. and Christ, C.L. 1965. Solutions, Minerals and Equilibria. Harper & Row, N.Y.

PARTICULATE CARBON AND NITROGEN

PRECISION

Nitrogen	Level	- 140 µg N/liter
	Standard deviation	- ± 6 µg N/liter
Carbon	Level	- 1000 µg C/liter
	Standard deviation	- ± 30 µg C/liter

OPERATING RANGE - 0.1-100 µg N or 1-500 µg C per filter paper. Operating range in terms of water samples depends on the volume available or possible to filter.

SAMPLE CONDITION
- Water sample stored at 5 C.
- Less than 48 hours old.
- Particulate collected on a pre-ignited (16 hours at 550 C) Whatman 4.25 cm GF/C glass fiber filter paper.
- Filter paper with particulate, vacuum dèsiccated in the dark to dryness.
- Dry filter papers stored in individual plastic petri dishes at -10 C for an indefinite period.

METHOD PRINCIPLE - Particulate material on a glass fiber filter paper is combusted in an oxygen helium atmosphere at 700 C. The combustion products are then swept by helium carrier gas over a heated copper oxide-silver vanadate bed where they are oxidized to CO_2, H_2O, and oxides of nitrogen. The gas stream then passes over hot copper where oxides of nitrogen are reduced to N_2 and excess O_2 is removed. The remaining CO_2, H_2O, and N_2 in the helium carrier is then treated differently depending on the instrument used.

In the Carlo Erba Model 1102, a dynamic, continuous flow, CHN analyzer, the three gases are separated chromatographically and analyzed with a single thermal conductance detector. (See Hauser 1973 for details of operation.)

In the Perkin Elmer Model 240, a stop-flow type CHN analyzer, the gas mixture is introduced into a reservoir where CO_2, H_2O, N_2, and He are mixed. The gas mixture is then diverted through a series of columns which sequentially remove H_2O and CO_2. Each of these columns is bracketed by a pair of thermal conductance detectors, the difference between their signal outputs being proportional to the amount of H_2O or CO_2 removed in the column (see Figure 3).

The final gas stream containing N_2 and helium goes through a detector whose signal output when referred to pure helium, is proportional to N concentration.

REAGENTS

Acetanilide - Calibration standard for both CHN analyzers (e.g. NBS standard #141B)

Carbon	71.09%
Nitrogen	10.36%
Hydrogen	6.71%

The following are required to construct combustion and reduction tubes, traps for CO_2 and H_2O, and for instrument operation. See instrument manuals for specifics of column construction.

PE 240
- Copper oxide
- Copper metal (60-100 mesh)
- Silver vanadate
- Magnesium perchlorate
- Colorcarb
- Helium 99.995 mole %
- Oxygen 99.995 mole %

Carlo Erba 1102

- Copper oxide
- Copper metal (60-100 mesh)
- Silver vanadate
- Magnesium perchlorate
- Helium - low purity
- Oxygen - 99.5%
- Compressed air
- MnO_2 catalyst-ignited 16 hours @ 550 C

APPARATUS

1. Perkin Elmer Model 240 Elemental Analyzer as in Figure 3.

   - A slight modification to manufacturers ladles was made to facilitate handling of filter papers.
   - Cycle time is about 14 minutes to obtain both C and N values.

or

2. Carlo Erba Model 1102 Elemental Analyzer.

   - Considerable modification (Hauser 1973) has been made to the arrangement of combustion tubes and gas flows.
   - Cycle time is about 7 minutes to obtain both C and N values.

3. Cahn Gram Electrobalance - Model G. or equivalent, to weigh to nearest µg.

PROCEDURE

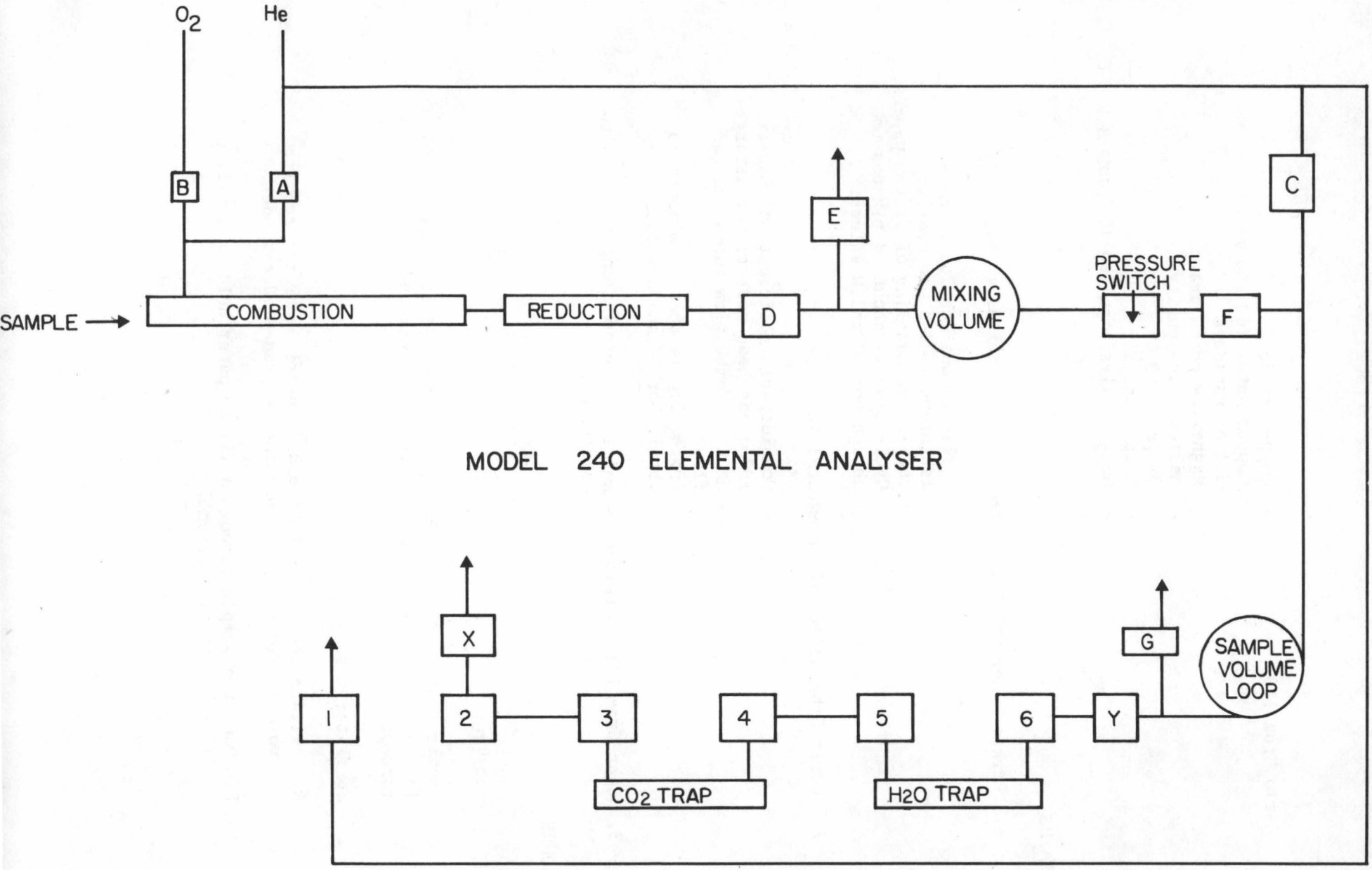
SAMPLE PREPARATION

1. Ignite Whatman 4.25 cm GF/C glass fiber filter papers at 550 C for 16 hours. Store in dust-free container and handle only with forceps.

2. To filter a sample:

   A. Agitate the sample bottle and quickly pour the required volume V (usually 100 ml) into a graduated cylinder.

   B. Pour the sample through a filter paper held in a Millipore 47 mm filtration assembly.



A - G SOLENOID CONTROL VALVES

X - Y SAFETY SENSORS

1 - 2 NITROGEN DETECTORS

3 - 4 CARBON DECTECTORS

5 - 6 HYDROGEN DETECTORS

Figure 3

C. Filter at 15 inches Hg vacuum just to dryness then break vacuum.

3. Place the damp paper in a vacuum desiccator in the dark over night.

4. Place the dry sample, face up, in a labelled plastic, covered petri dish (50 mm diam.) and place in a freezer at -10 C.

INSTRUMENTAL ANALYSIS

The following is a general outline of the procedure followed. The procedure below should be followed only when the analyst is thoroughly familiar with the complexities of the instruments described.

1. Samples of acetanilide standard from 0.1-0.4 mg are weighed in platinum boats to the nearest µg.

2. With the instrument ready to operate (see manual for machine settings) an acetanilide standard, ignited filter paper blank or ignited filter paper with sample is placed in a ladle.

3. The ladle is then placed in the cool zone of the instrument's combustion tube and atmosphereic contaminants purged for 2-3 minutes with helium.

4. The ladle is then injected into the hot zone of the combustion tube where ladle contents are combusted.

5. Chart units and attenuation for C and N signals are recorded.

CALCULATIONS

1. Scale factors for nitrogen and carbon are calculated as follows:

$$\text{Scale Factor (C or N)} = \frac{\text{µg C or N per ladle}}{\text{Peak height (C or N)}}$$

where Peak height = (chart units) x (attenuation)

2. Peak heights for samples are corrected for blank paper values and multiplied by the C and N scale factors above to give µg C and N per sample (on the filter).

3. The µg C and N per filter paper values obtained above are multiplied by the volume factor to give the suspended N and C concentration in the original water sample.

   i.e. µg C or N/paper $x(\frac{1000}{V})$ = µg/liter C or N in the water sample

   where V is the volume filtered in mls.

REFERENCES

1. Hauser, B.W. (1973). Modifications to Carlo Erba elemental analyzer for rapid determination of carbon and nitrogen in suspended matter of natural water. FRB Technical Report No. 412.

2. Stockner, J.G., and Armstrong, F.A.J. (1971). Periphyton of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 28:215-229.

AMMONIA-NITROGEN (MANUAL METHOD)

PRECISION

Level	- 400 μgNH_3-N/liter
Standard deviation	- ± 5 μgNH_3-N/liter

OPERATING RANGE

-0-1000 µg NH_3-N/liter

SAMPLE CONDITION

- Unfiltered stored at 5 C.
- Less than 24 hours old
- Free from H_2S (If present acidify sample to pH3 and bubble with helium until free of sulphide odour).

METHOD PRINCIPLE

- Ammonia reacts with phenol and hypochlorite under alkaline condition to form indophenol blue; the colour intensity being proportional to ammonia concentration. Nitroprusside is used as a catalyst to facilitate colour development at room temperature. While the exact nature of the reaction is in some doubt, the order of reagent addition appears to be important. The order of reagent addition outlined below is that recommended by Solorzano (1969) and has been found to perform quite reliably.

REAGENTS

A. Distilled and deionized water

Pass distilled water over a strong acid cation exchange resin in hydrogen form. Prepare fresh as needed.

B. Hypochlorite stock - Commercially available household bleach (5.5% available chlorine). This reagent deteriorates with time and should be checked periodically. Normality should be at least 1.5.

C. Alkaline stock solution

Sodium citrate	100 g
Sodium hydroxide	5 g

Dissolve and make to 500 ml with distilled and deionized water.

D. Oxidizing reagent

Prepare fresh daily from 4 parts of C plus 1 part of B.
Keep in stoppered bottle until used.

E. Sodium nitroprusside reagent

Dissolve 1.0 g sodium nitroprusside in distilled and deionized water, and make to 200 ml.

F. Phenol reagent

Dissolve 100 g phenol in 95% ethanol and make to 1 liter.

G. Ammonium standard

Dissolve 3.818 g of dry ammonium chloride in distilled and deionized water and make to 1 liter.

1.00 ml = 1000 µg NH_3-N

APPARATUS

A. 50 ml stoppered graduated cylinders.

PROCEDURE

1. Prepare duplicate 100 ml aliquots of ammonia standard having a concentration approximating that of samples to be analyzed.

2. Place 50.0 ml of distilled and deionized water, ammonia standards or sample(s) in 50 ml stoppered graduates.

3. Add 2.0 ml of phenol reagent F. Mix.

4. Add 2.0 ml of nitroprusside reagent E. Mix.

5. Add 5.0 ml of oxidizing reagent D and mix by inverting the stoppered cylinders.

6. Between 1 and 24 hours later measure:

E_{Bl} = Absorbance of distilled water plus reagents.

E_1 = Absorbance of standards and samples plus reagents

Absorbance is measured at 640 nm using an appropriate path length for the level of ammonia present.

i.e. 1 cm path length 0-1000 N μgNH_3-N/liter
5 cm path length 0-200 μgNH_3-N/liter
10 cm path length 0-20 μgNH_3-N/liter

CALCULATIONS

1. Unit extinction factor F for ammonia-N is calculated as follows:

$$F = \frac{\text{Concentration of std. } (\mu gNH_3\text{-N/liter})}{E_1 \text{ (standard)} - E_{B1}}$$

This should be around 1000 μgNH_3-N/liter for a 1.00 cm path length.

2. The concentration of ammonia - N in samples can be calculated by multiplying the extinction due to ammonia in the sample:

i.e. E_1 (sample) - E_{B1}

by the unit extinction factor F obtained above.

REFERENCES

1. Bolleter, C.J., Bushman, D.J., and Tidwell, P.W. (1961). Spectrophotometric determination of ammonia as indophenol. Anal. Chem. 33:592-594.

2. Harwood, J.E. and Huyser, D.J. (1970). Some aspects of the phenol-hypochlorite reaction as applied to ammonia analysis. Water Research. 4:501-515.

3. Solorzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. 14:799-801.

AMMONIA NITROGEN (AUTOMATED METHOD)

PRECISION

Level	- 600 μgNH_3-N/liter
Standard deviation	- $\pm$ 12 μgNH_3-N/liter

OPERATING RANGE - 0-1000 μg NH_3-N/liter

SAMPLE CONDITION
- Same as for manual method.
- Samples containing much suspended matter require filtering.

METHOD PRINCIPLE - Same as for manual method.

REAGENTS

A. Distilled and deionized water. See manual method for details.

B. Buffer

Potassium hydrogen phosphate	200 g
EDTA (disodium dihydrate)	5 g
Sodium citrate	1 g
Sodium hydroxide (1 N)	130 ml

Dissolve dry reagents in order given in 800 ml of distilled and deionized water. Add 1 N sodium hydroxide and make to 1 liter with distilled and deionized water.

C. Phenol stock

Dissolve 625 g phenol in methanol and make to 1 liter.

D. Sodium hydroxide stock

Dissolve 270 g of sodium hydroxide pellets in distilled and deionized water. When cool, make to 1 liter with distilled and deionized water.

E. Phenate reagent

Dilute 200 ml of phenol stock plus 450 ml of sodium hydroxide stock to 1 liter with distilled and deionized water.

F. Nitroprusside reagent

Dissolve 1.0 g sodium nitroprusside in distilled and deionized water and make to 1 liter. Store in low actinic glass bottle.

G. Hypochlorite

Dilute commerical bleach with 6 parts distilled and deionized water to 1 part bleach. Prepare daily.

APPARATUS

1. Technicon Autoanalyser^{R} or comparable equipment (sampler, proportioning pump, colorimeter and recorder). Flow colorimeter should be equipped to measure absorbance at 640 nm.

2. Manifold assembly as in Figure 4.

PROCEDURE

1. Prepare duplicate 100 ml aliquots of ammonia standard having a concentration approximating that of the samples to be analyzed.

2. Using the manifold arrangement of Figure 4:

   A. Set the sampler rate to 20-40 samples per hour with a sample to wash ratio of 1:2.

   B. With the sample tube drawing distilled deionized water allow the system to equilibrate for 30 minutes.

   C. With the system sampling distilled deionized water, balance and zero the colorimeter. This automatically subtracts E_{B1} from all subsequent readings. (See Note 1)

3. Place samples and standards on the sampler turntable and start sampler. Each group of 20 samples should be preceded and followed by pairs of standards.

4. Obtain absorbance values (E_1) for samples and standards.

Note 1. E_{B1} should be zero initially but may show positive drift as reagents absorb NH_3 from room air.

AMMONIA MANIFOLD

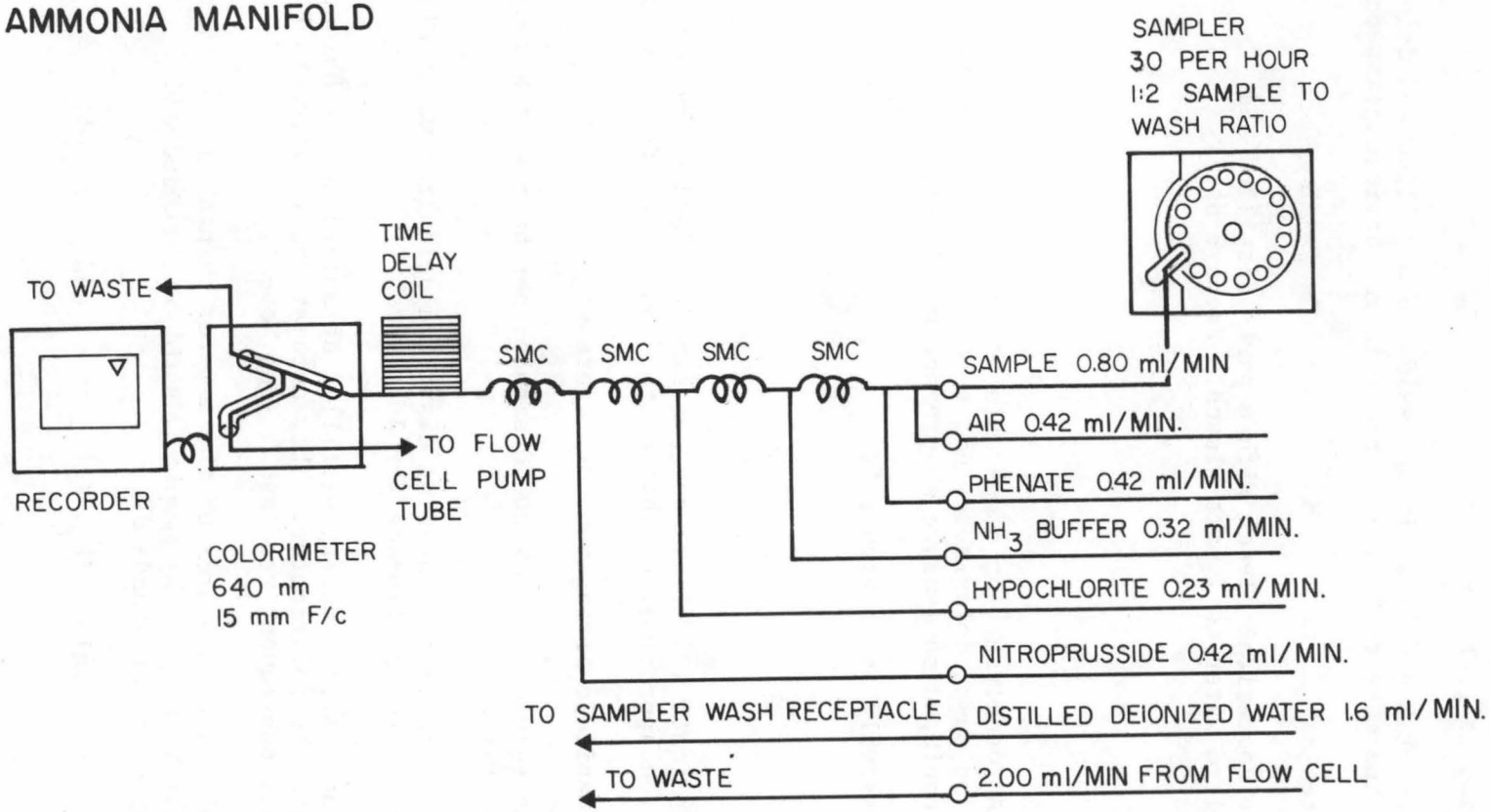


FIGURE 4

CALCULATIONS

1. The unit extinction factor for ammonia -N is calculated as follows:

$$F = \frac{\text{Standard concentration } (\mu gNH_3\text{-N/liter})}{E_1 \text{ (standard)} - E_{B1}}$$

This factor should be around 2000 μgNH_3-N/liter.

2. Concentrations of ammonia -N in samples can be calculated by multiplying the extinction due to NH_3 in the sample;

i.e. E_1 (sample) - E_{B1}

by the unit extinction factor F from above.

REFERENCES

1. Harwood, J.E. and Hayer, D.J. (1970). Automated analysis of ammonia in water. Water Research 5:695-704.

2. See also manual ammonia method references.

NITRITE AND NITRATE NITROGEN (MANUAL METHOD)

PRECISION

Nitrate Level	-	300 μgNO_3-N/liter
Standard deviation	-	± 4 μgNO_3-N/liter
Nitrite Level	-	5.0 μgNO_2-N/liter
Standard deviation	-	± 0.5 μgNO_2-N/liter

OPERATING RANGE

0-500 µg NO_3-N or NO_2-N per liter.

SAMPLE CONDITION

- Filtered through ignited Whatman GF/C.
- Stored at 5 C.
- Less than 48 hours old.
- Acid buffering capacity of sample must not exceed the alkaline buffering capacity of Reagent "A" below.

METHOD PRINCIPLE

- Acidification (0.02 N HCl) of a solution of nitrite yields nitrous acid. By addition of an aromatic amine (sulphanilamide) a diazonium salt forms which quantitatively couples with a second aromatic amine (N-1 Naphthylethylene diamine dihydrochloride). This coupling yields a pink azo dye with an absorbance maximum at 543 nm. Beer's law is obeyed.

  Nitrate in alkaline-buffered solution (pH-8) can be quantitatively reduced to nitrite by a cadmium-copper couple. The resulting nitrite can then be determined by forming the pink azo dye mentioned above.

REAGENTS

A. Buffer solution

Ammonium chloride	100 g
Sodium tetraborate	20 g
EDTA (disodium dihydrate)	1 g

Dissolve in distilled water and make to 1 liter.

B. Sulphanilamide

Dissolve 5 g of suphanilamide in a mixture of 100 ml of concentrated HCl and 300 ml of distilled water. Make to 500 ml.

C. N-(1-naphthyl) ethylene diamine dihydrochloride (NNED).

Dissolve 0.5 g of NNED in 500 ml of distilled water. Store in a low actinic glass bottle (dark brown or deep red). Renew if reagent develops a dark brown coloration.

D. Nitrate standard

Dissolve 0.722 g of potassium nitrate in distilled water and make to 1 liter. Stable 2 months.

1.00 ml = 100 µg NO_3-N

E. Nitrite standard

Dissolve 0.4926 g of sodium nitrite in distilled water. Make to 1 liter. Stable 2 months.

1.00 ml = 100 µg NO_3-N

F. Cupric sulphate

Dissolve 20 g of cupric sulphate penta-hydrate in 1000 ml of distilled water.

G. 2 N Hydrochloric acid

Dilute 85 ml of concentrated HCl to 500 ml with distilled water.

H. Cadmium filings - about 0.5 mm diameter.

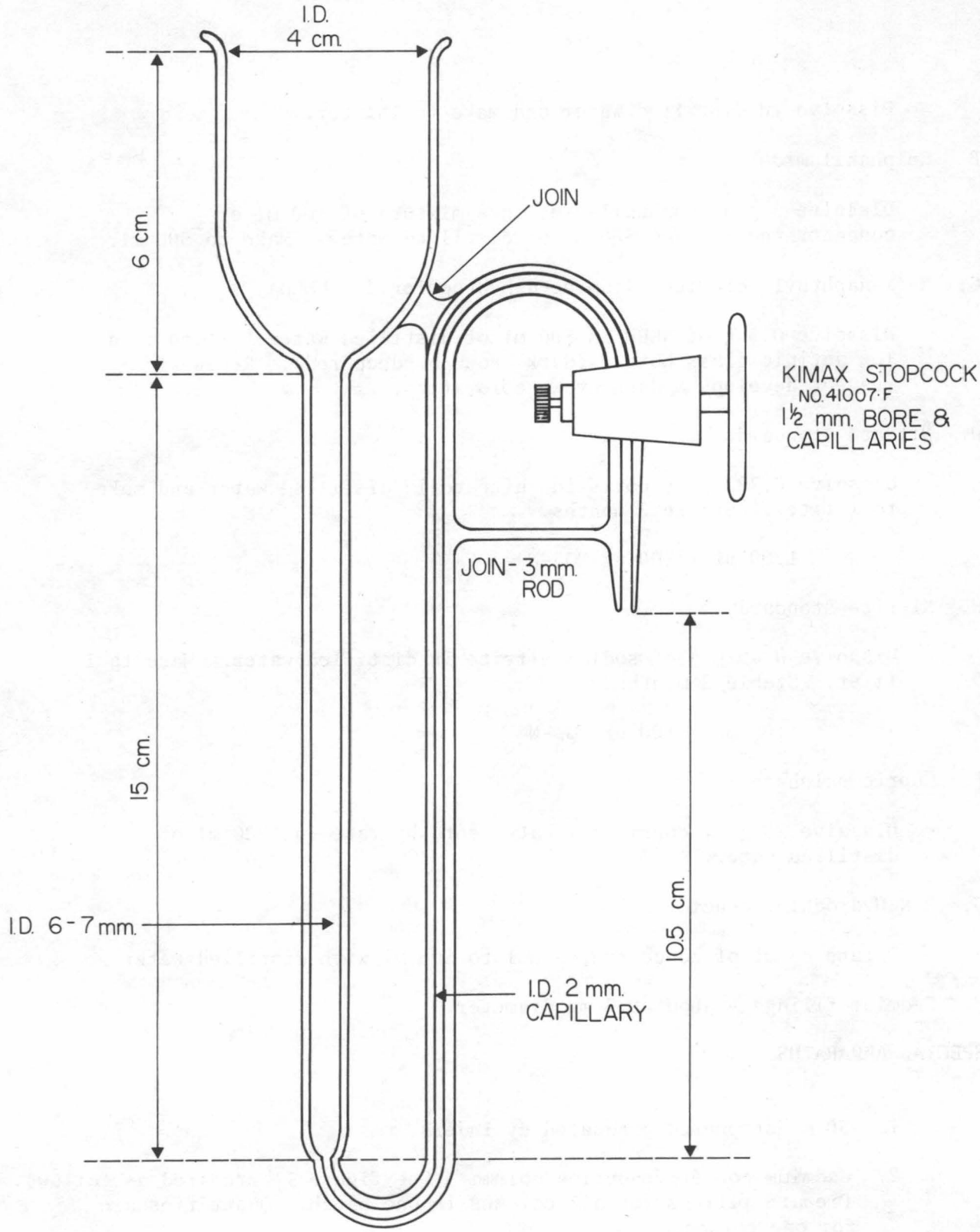
SPECIAL APPARATUS

1. 50 ml stoppered graduated cylinders.

2. Cadmium-copper reduction column (See figure 5) prepared as follows. Prepare filings for all columns in one batch. Quantities are for one column.

   A. Place 5 g of cadmium filings in a beaker, wash with 25 ml of 2 N HCl and rinse with distilled water.

   B. Add 10 ml of cupric sulphate solution and swirl until all copper is deposited (blue colour disappears).



NITRATE REDUCTION COLUMN - BOROSILICATE GLASS

Figure 5

C. Plug bottom of column(s) with a small piece of glass wool, fill columns with water and add treated cadmium to a point level with the outlet. There should be no entrapped air bubbles.

D. Flush column twice with a solution of 50 ml of distilled water plus 5 ml of buffer solution A.

E. Adjust metering valve(s) so that the delivery rate for 25 ml is 240 seconds ± 10 seconds.

PROCEDURE

NITRITE NITROGEN

1. Prepare, duplicate 100 ml aliquots of nitrite standard having a concentration approximating that of the samples to be analyzed.

2. Place 50 ml of: distilled water, nitrite standards, or sample(s), into stoppered graduated cylinders. Add 5.0 ml of buffer solution (A) and mix by inverting the stoppered cylinders. (See Note 1)

3. Add 1.0 ml of suphanilamide solution (B) and mix.

4. After 5 minutes add 1.0 ml of NNED solution (C) and mix.

5. Between 10 minutes and 2 hours later measure:

   E_{Bl} = Absorbance of distilled water + reagents

   E_o = Absorbance of sample without NNED reagent (i.e. the natural color of the sample). (See Note 2)

   E_1 = Absorbance of standards and samples with reagents.

Absorbance is measured at 543 nm using a path length appropriate for the level of nitrite present.

i.e.	1 cm path length	60-300 μgNO_2-N/liter
	5 cm path length	30-60 μgNO_2-N/liter
	10 cm path length	0-30 μgNO_2-N/liter

Note 1. Allows use of nitrate reagents and simplifies correction for NO_2 levels when computing NO_3.

2. E_o must be measured where water samples show visible brownish coloration.

CALCULATIONS

1. Unit extinction factor F for nitrite - N is calculated as follows:

$$F = \frac{\text{Standard concentration } (\mu g NO_2\text{-N/liter})}{E \text{ (standard)} - E_{B1}}$$

This should be around 330 for a 1.00 cm path length.

2. The concentration of nitrite - N in samples can be calculated by multiplying the extinction due to nitrite - N in the sample;

i.e. E_1 sample - $(E_o + E_{B1})$

by the unit extinction factor calculated above.

NITRATE NITROGEN

1. Prepare in duplicate enough nitrate standard to provide 50 ml for each cadmium-copper reduction column (Figure 5) to be used. Concentration should approximate that of the samples to be analyzed.

2. Place 50 ml of distilled water, nitrate standard or sample(s) into stoppered graduated cylinders. Add 5 ml of buffer solution (A) and mix by inverting the stoppered cylinders.

3. Place 10 ml of buffered sample on the column, allow it to run through and discard the effluent.

4. Add remainder of buffered solution to the column, collect 2 ml of effluent in the same cylinder; rinse and discard. Collect 25 ml of column effluent.

5. Carry a distilled water blank and a standard through each column used.

6. Add 0.5 ml of sulphanilamide (B) to the 25 ml of column effluent and mix.

7. After 5 minutes add 0.5 ml of NNED (C) solution and mix.

8. Between 10 minutes and 2 hours later measure

E_{B1} = Absorbance of distilled water plus reagent

E_o = Absorbance of sample without NNED reagent (natural color). (See Note 1)

Note 1. E_o must be measured where water samples show visible brownish coloration.

E_1 = Absorbance of standards and samples plus reagents.

Absorbance is measured at 543 nm using a path length appropriate for the level of nitrate present.

i.e.	1 cm path length	60-300 µg NO_3-N/liter
	5 cm path length	30-60 µg NO_3-N/liter
	10 cm path length	0-30 µgNO_3-N/liter

CALCULATIONS

1. Unit extinction Factor for NO_3-N is calculated as follows:

$$F = \frac{\text{Standard concentration (µg}NO_3\text{-N/liter)}}{E_1 \text{ (standard)} - E_{B1}}$$

This should be around 330 for a 1.00 cm path length.

Unit extinction factors should be obtained for standards passed through each of the columns in use. Values should agree within 3%. If so, a mean is taken.

2. The concentration of nitrate -N in samples can be calculated by multiplying the extinction due to NO_3-N in the sample

i.e. E_1 samples - $(E_o + E_{B1})$

by the unit extinction factor calculated above.

3. Reduction efficiency of cadmium-copper column can be determined by measuring the extinction of a 100 µg/l NO_3 standard, and a 100 µg/l NO_2 standard. Efficiency should be above 95%. If not, re-pack the column(s).

4. If NO_2-N is present in the sample it will be measured as NO_3 and results will be NO_3 + NO_2 nitrogen. If NO_2-N is present in significant amounts relative to NO_3-N it must be determined separately and nitrate values corrected as follows:

Given 1) concentration NO_3-N + NO_2-N = x

2) concentration NO_2-N = y

3) reduction column efficiency % = Z

the NO_3-N = $x - [\frac{100}{z}]y$

If reduction columns are close to 100% efficient

NO_3-N = x - y

REFERENCES

1. Morris, A.W. and Riley, J.P. (1963). The determination of nitrate in sea water. Analytica Chim Acta. 29:272-79.

2. Strickland, J.D.H. and Parsons, T.R. (1967). A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Canada, No. 167.

3. Woods, E.D., Armstrong, F.A.J. and Richards, F.A. (1967). Determination of nitrate in sea water by cadmium-copper reduction to nitrite. J. Mar. Biol. Ass., U.K. 47:23-31.

4. Bendschneider, K., and Robinson, R.J. (1952). A new spectrophotometric determination of nitrite in seawater. J. Mar. Res. 11:87-96.

NITRITE AND NITRATE NITROGEN (AUTOMATED METHOD)

PRECISION

Nitrite Level - 300 μgNO_2-N/liter

Standard deviation - $\pm$ 1 μgNO_2-N/liter

Nitrate Level

Standard deviation - $\pm$ 1 μgNO_3-N/liter

OPERATING RANGE

- 0-500 $\mu g(NO_2$-N or NO_3-N)/liter

SAMPLE CONDITION

- Same as for manual method.
- Sample must be free of sulphide which poisons the cadmium reduction column.

METHOD PRINCIPLE

- Same as manual method.

REAGENTS

- Same as manual method.

APPARATUS

1. Technicon Autoanalyser^R or comparable equipment (sampler, proportioning pump, colorimeter and recorder). Flow colorimeter should be equipped to measure absorbance at 540 nm.

2. Manifold assembled as in Figure 6.

3. In-line cadmium-copper reduction coil prepared as follows:

   A. Thread 1 metre of 1 mm diameter cadmium wire inside 1/32" I.D. Teflon^R tubing and wind tubing into a coil. Place coil in line as shown in Figure 6.

   B. With all reagent tubes pumping distilled water, draw 10 ml of 2 N HCl through the sample tube followed by a distilled water rinse.

C. Draw cupric sulphate solution through the sample tube until deposition of copper ceases. (Blue color is not removed by coil).

D. Rinse with distilled water and resume pumping of reagents.

PROCEDURES

NITRITE

1. Prepare in duplicate 100 ml of nitrite standard having a concentration approximating that of the samples to be analyzed. Samples should be in the range 0-500 µg/liter NO_2-N. If concentration exceeds this, dilute with distilled water.

2. Using the manifold arrangement of Figure 6.

   A. Set the 3-way valves to bypass the cadmium-copper reduction column.

   B. Set sampler rate to 20-40 samples per hour with a sample to wash ratio of 1:2.

   C. With the sample tube drawing distilled water allow the system to equilibrate for 30 minutes.

   D. With the system sampling distilled water, balance and zero the colorimeter. This automatically subtracts the E_{B1} value from all subsequent readings. (See note 1)

3. Add samples and standards to the sampler and start sampler. Each group of 20 samples should be preceeded and followed by pairs of standards.

4. Obtain absorbance values (E_1) for samples and standards.

5. If samples are visibly brownish in colour their natural colour (E_o) must be measured. Replace NNED reagent with distilled water. Allow the system to equilibrate for 10 minutes and reprocess samples to obtain E_o.

CALCULATIONS

1. The unit extinction factor for NO_2-N is calculated as follows:

$$F = \frac{\text{Standard concentration } (\mu g NO_2\text{-N/liter})}{E_1 \text{ (standard)} - E_{B1}}$$

This factor is around 500 µgNO_2-N/liter for a 15 mm flow cell.

Note 1. E_{B1} should initially be set to zero but may drift during the course of a sample series.

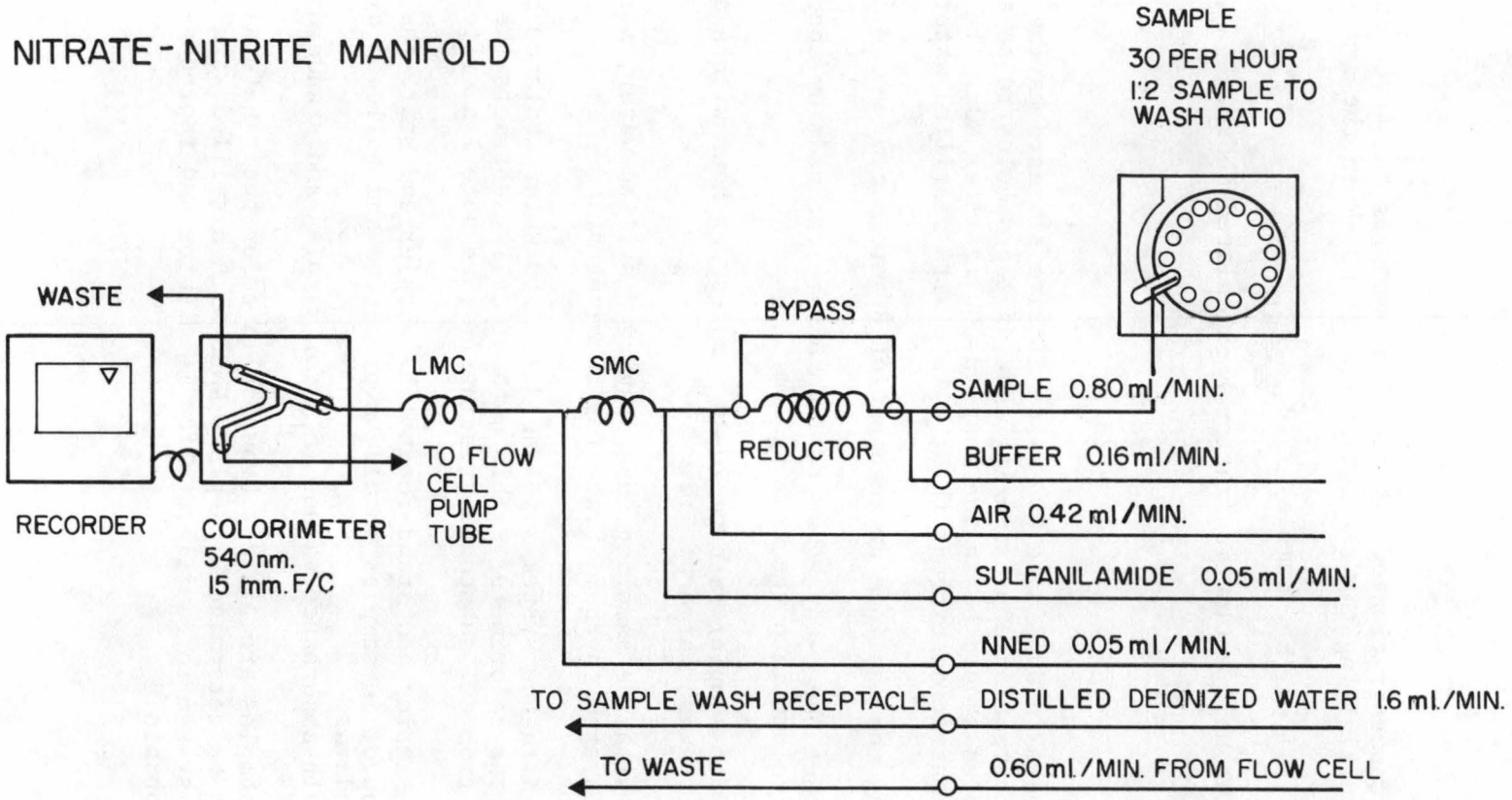

NITRATE - NITRITE MANIFOLD
SAMPLE
30 PER HOUR
1:2 SAMPLE TO
WASH RATIO
WASTE
BYPASS
LMC
SMC
SAMPLE 0.80 ml /MIN.
REDUCTOR
BUFFER 0.16 ml/MIN.
TO FLOW
CELL
PUMP
TUBE
AIR 0.42 ml/MIN.
RECORDER
COLORIMETER
540 nm.
15 mm. F/C
SULFANILAMIDE 0.05 ml/MIN.
NNED 0.05 ml/MIN.
TO SAMPLE WASH RECEPTACLE
DISTILLED DEIONIZED WATER 1.6 ml./MIN.
TO WASTE
0.60 ml./MIN. FROM FLOW CELL

FIGURE 6

2. The concentration of nitrite -N in samples can be calculated by multiplying the extinction due to NO_2-N in the sample

   i.e. E_1 (sample) - $(E_o + E_{B1})$

   by the unit extinction factor F calculated above.

NITRATE

1. Prepare in duplicate 100 ml of nitrate standard having a concentration approximating that of the samples to be analyzed. Samples should be in the range 0-500 µg/liter NO_3. If sample concentration exceeds this, dilute with distilled water.

2. Using the manifold arrangement of Figure 6.:

   A. Set the 3-way valves to incorporate the cadmium-copper reduction column into the flow system.

   B. Set sampler rate to 20-40 samples per hour with a sample to wash ratio of 1:2.

   C. With the sampling tube drawing distilled water, allow the system to equilibrate for 30 minutes.

   D. With the system sampling distilled water, balance and zero the colorimeter. This automatically subtracts the E_{B1} value from all subsequent readings. (See note 1 nitrite method).

3. Add samples and standards to the sampler and start the sampler. Each group of 20 samples should be preceeded and followed by pairs of standards.
4. Obtain absorbance values (E_1) for samples and standards.

5. If samples are visibly brownish in color their natural color (E_o) must be determined. Replace NNED with distilled water. Allow the system to equilibrate for 10 minutes and reprocess samples to obtain E_o.

CALCULATIONS

1. The unit extinction factor for NO_3-N is calculated as follows:

$$F = \frac{\text{Standard concentration } (\mu g NO_3\text{-N/liter})}{E_1 \text{ (standard)} - E_{B1}}$$

The factor is around 500 μgNO_3-N/liter for a 15 mm path length.

2. The concentration of nitrate -N in samples can be calculated by multiplying the extinction due to NO_3-N in the sample

   i.e. E_1 (sample) - (E_o + E_{B1})

   by the unit extinction factor F calculated above.

3. Reduction efficiency of the cadmium-copper column can be determined by measuring the absorbance of a 100 μgNO_3-N/liter standard and a 100 μgNO_2-N/liter standard with the reduction column in line.

   Efficiency may be anywhere from 60-100% depending upon age of column and sampling rate.

4. If NO_2-N is present in the sample it will be measured as NO_3-N and results will be NO_3-N + NO_2-N. If NO_2-N is present in significant amounts relative to nitrate it must be determined separately and nitrate values corrected as follows:

   Given 1) concentration of NO_3-N + NO_2 = x $\mu g/l$

   2) concentration of NO_2-N = y $\mu g/l$

   3) reduction column efficiency = z%

   then $NO_3\text{-}N = x - [\frac{100}{z}]y$

   If reduction column is 100% efficient

   NO_3-N = x - y

REFERENCES

1. Brewer, P.G. and Riley, J.P. (1965). The automatic determination of nitrate in sea water. Deep Sea Res. 12:765-72.

2. See also manual nitrate method references.

TOTAL DISSOLVED NITROGEN

PRECISION

Level	- 500 µg Nitrogen/liter
Standard deviation	- ± 25 µg Nitrogen/liter

OPERATING RANGE - 0-1000µg total dissolved N per liter.

SAMPLE CONDITION
- Filtered through Whatman GF/C, stored at 5 C.
- Less than 48 hours old
- Less than 10 mg/liter dissolved organic carbon.

METHOD PRINCIPLE - Organic nitrogen compounds and ammonia, under appropriate conditions, (pH 9.0 and adequate oxygen supply) can be decomposed and oxidized with short U.V. radiation. Products of this photo-oxidation are a mixture of nitrate, nitrite, and ammonia, the relative proportions of which depend on oxygen level, pH and duration of exposure to the radiation source.

After 12 hours of irradiation, essentially all organic nitrogen is photo-oxidized to nitrite with a small variable fraction remaining as nitrate. Exceptions are samples containing high organic nitrogen, ammonia, and organic carbon, where considerable ammonia can remain unoxidized.

Products of photo-combustion are measured as the sum of nitrate, nitrite and ammonia. (See Nitrate, Nitrite, and Ammonia Methods).

REAGENTS

A. Low nitrogen distilled water

This is used where dilution of sample is required. The following methods do not remove all nitrogenous compounds. When used, a dilution water blank must also be carried through the photocombustion process.

1. Pass distilled water over a mixed bed ion exchange resin. Effluent is typically 30-50 µgN/liter. Use immediately.

2. Re-distill water from acid permanganate. Reject first 10%, collect next 70% and reject remainder. Use immediately.

3. Collect effluent from Millipore "Super Q" ultra pure water preparation system, and use immediately.

B. Sodium bicarbonate 0.1 molar

Dissolve 8.40 g sodium hydrogen carbonate in low nitrogen distilled water and make to 1 liter.

C. Hydrogen peroxide

Reagent grade 30% w/w. This reagent deteriorates if left open at room temperature. Store at 5 C. Caution: This is a dangerous oxidant. Avoid contact with skin. Never pipette by mouth. Wash hands after handling.

APPARATUS

A. Short-U.V. irradiator

The apparatus used for this method consists of the following components:

1. 550 watt medium pressure mercury vapour lamp.

2. Cooling fan to prevent boiling of samples.

3. Quartz jacket tube to shield mercury lamp from the cooling effect of the fan.

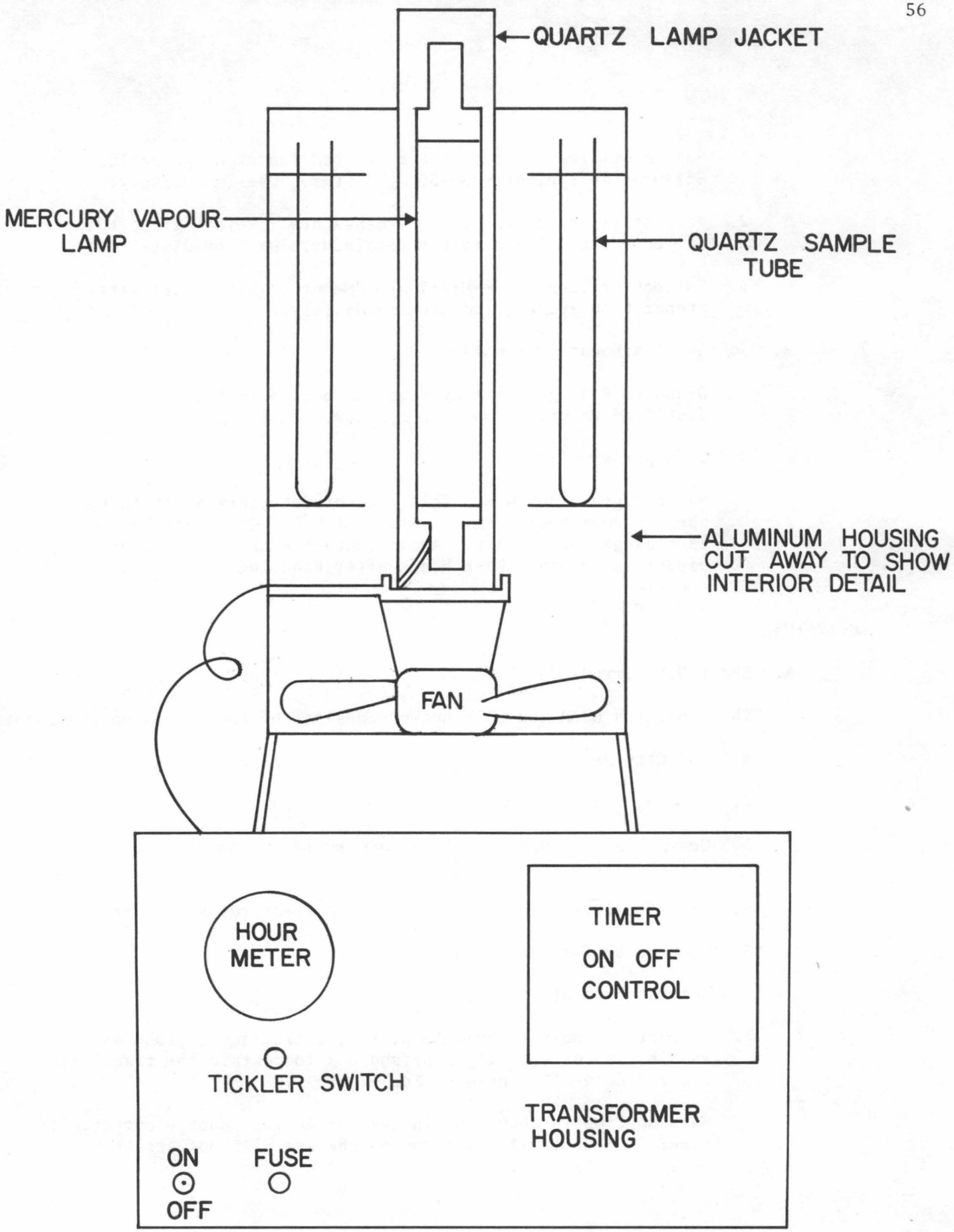
4. Transformer to supply power at the correct voltage to the lamp.

5. Hour meter for lamp life monitoring.

6. Automatic on-off timer.

7. Support assembly to hold lamp, fan, quartz jacket tube and samples in correct alignment,and box to contain the transformer, timer and on-off control. See Figure 7.

Warm-up time is about 10 minutes for maximum energy output. If power is momentarily interrupted the arc will not strike until lamp is cool.



U.V. PHOTO OXIDIZER

FIGURE 7

PRECAUTIONS:

1. Do not look at arc with the naked eye or expose skin to arc for prolonged periods.

2. Do not handle quartz surface of lamp or quartz jacket tube with the fingers. Salt deposits ($NaHCO_3$ in particular) will permanently etch quartz surfaces, reduce their transmittance and hence the photo-combustion efficiency.

3. Quartz surfaces should be wiped regularly with soft tissue, moistened first with distilled water, then ethanol.

B. Sample Tubes

1. Size required depends on the quantity of sample available, the number of samples to be analyzed and the volume of liquid required for analysis. A convenient size is 30 ml which allows 16 tubes in a 4.5 inch radius

2. These quartz tubes should be treated with the care outlined above.

3. Some samples during the course of irradiation form a precipitate. This should be removed from tubes (with 1 N HCl) before re-using.

4. No ink marking should be placed on the tubes. A diamond tip pen may be used to write identification on the tubes.

PROCEDURE

1. Rinse the quartz sample tube with sample and add the required volume using a graduated cylinder or pipette.

2. Add 1 ml of 0.1 M bicarbonate per 100 ml of sample.

3. Add 2 drops of 30% peroxide and swirl to mix.

4. Wipe outer surface of tube dry with soft tissue.

5. Stopper tubes with a glass marble.

6. Place sample tube(s) in U.V. irradiator and set automatic timer to shut off after 12 hours.

7. After completion of photo-oxidation allow samples to cool. Make up to volume with distilled water if necessary.

8. Analyze samples for nitrate, nitrite, and ammonia nitrogen.

9. If total N exceeds 1000 µgN/liter, dilute samples with low N distilled water before photo-combustion. Determine the blank of the dilution water also carried through the photo-combustion stage.

CALCULATIONS

Total dissolved nitrogen is equal to the sum of nitrate, nitrite, and ammonia nitrogen in the irradiated sample. An estimate of organic nitrogen can be obtained by determining the sum of nitrate, nitrite, and ammonia in the un-irradiated sample and subtracting this from T.D.N. The difference is organic nitrogen.

Dilution Calculations:

$$C_1 = (C_2 \times D) - (C_3 \times (D-1))$$

Where C_1 = conc. of nitrogen in sample.

C_2 = conc. of nitrogen in diluted sample.

C_3 - conc. of nitrogen in dilution water.

D = dilution factor.

REFERENCES

1. Armstrong, F.A.J., and Tibbitts, S. (1968). Photochemical combustion of organic matter in seawater for nitrogen, phosphorus and carbon determination. J. Mar. Biol. Ass. U.K. 48:143-52.

2. Henriksen, A. (1970). Determination of total nitrogen, phosphorus and iron in freshwater by photo-oxidation with ultra-violet radiation. Analyst. 95:601-608.

SOLUBLE REACTIVE PHOSPHORUS

PRECISION

Level	- 100 µg PO_4-P/liter
Standard deviation	- ±1 µg PO_4-P/liter

OPERATING RANGE - 5-250 µg PO_4-P/liter

SAMPLE CONDITION
- Filtered through Whatman GF/C, stored at 5 C.
- Unacidified.
- Less than 24 hours old.
- Less than 5 µg AsO_4^{-3}.

METHOD PRINCIPLE - Phosphate, silicate, arsenate and germanate ions react under acidic conditions with molybdate to form heteropoly acids which are yellow in color and can be used to determine these oxy-anions in the mg/liter range. These heteropoly molybdic acids can be converted by suitable reducing agents to blue compounds of uncertain composition.

The formation of the blue complex is highly dependent on acid strength, molybdate concentration and the reductant used, with conditions being different for group IV b elements (Si and Ge) than for group V b (P and As), permitting analytical distinction between these groups.

Using appropriate acid and molybdate strength, ascorbic acid as reductant and antimony as a color enhancing species an intensely blue colored complex is formed with PO_4^{-3} and AsO_4^{-3} having an absorbance maximum at 885 nm.

It should be noted that some observers feel this method, while specific for PO_4-P, is capable of hydrolysing labile organic phosphorus compounds giving an overestimate of PO_4-P and hence biologically available phosphorus.

REAGENTS

A. Acid molybdate-antimony

Distilled water	500 ml
Ammonium paramolybdate $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	7.5 g
Antimony potassium tartrate	0.14 g
Sulphuric acid (S.G. = 1.84)	88 ml

Mix in order given, cool and make to 1000 ml. Keep in dark glass bottle.

B. Ascorbic acid

Dissolve 2.5 g of L-ascorbic acid in 100 ml of distilled water. This reagent is stable for a few days if kept refrigerated.

C. Mixed molybdate for natural colour determination.

Mix 4 parts of reagent A. with 1 part of distilled water.

D. Mixed molybdate for orthophosphate determination.

Mix 4 parts of Reagent A. with 1 part of Reagent B. Stable 1 day.

E. Phosphate standard

Dissolve 0.2197 g potassium dihydrogen phosphate (KH_2PO_4) in 100 ml of water and make to 1000 ml with water saturated with chloroform.

$$1.00\ ml = 50.0\ \mu g\ PO_4\text{-}P$$

PROCEDURE

1. Prepare in duplicate 100 ml of orthophosphate standards having a concentration approximating that of the samples to be analyzed.

2. Place 25 ml of standards and distilled water into 125 ml Erlenmeyer flasks. Add 5.0 ml of mixed molybdate reagent D.

3. Place 25 ml of sample into a 125 ml Erlenmeyer flask. Add 5.0 ml of mixed molybdate reagent C.

4. Place 25 ml of sample into a second 125 ml Erlenmeyer flask. Add 5.0 ml of mixed molybdate reagent D.

5. After 5 minutes and within 3 hours measure

E_{B1} = Absorbance of distilled water + reagents.

E_o = Absorbance of sample without reductant.

E_1 = Absorbance of standards + sample with reductant.

Absorbance is measured at 885 nm using a 10.00 cm path length.

CALCULATIONS

1. Unit extinction factor for PO_4-P is calculated as follows:

$$F = \frac{\text{Standard concentration } (\mu g \text{ P/liter})}{E_1 \text{ (standard)} - E_{B1}}$$

This should be around 170 $\mu g PO_4$-P/liter for a 10 cm path length. Concentration of orthophosphate in sample can be calculated by multiplying the extinction due to PO_4-P in the sample

i.e. E_1 sample - $(E_o + E_{B1})$

by the unit extinction factor F calculated above.

REFERENCE

1. Murphy, J. and Riley, J.P. (1962).
A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27:31-36.

SOLUBLE REACTIVE PHOSPHORUS (AUTOMATED METHOD)

PRECISION

Level	-	250 µgPO_4-P/liter
Standard deviation	-	±1 µgPO_4-P/liter

OPERATING RANGE - 1-500 µgPO_4-P/liter

SAMPLE CONDITION - See manual method.

METHOD PRINCIPLE - See manual method.

REAGENTS

A. Ammonium molybdate

Dissolve 7.5 g of ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$) in 500 ml of distilled water. Add 0.14 g of antimony potassium tartrate ($KSbO\cdot C_4H_4O_6$), 88 ml conc. sulphuric acid, and 1 ml of phosphate standard (see Note 1). When cool dilute to 1 liter and store in dark glass bottle.

B. Ascorbic acid

Dissolve 2.5 g of ascorbic acid in 100 ml of distilled water. This reagent is stable for a few days if kept refrigerated.

C. Phosphate standard (See manual method).

1 ml = 50 µg PO_4-P

APPARATUS

Technicon Autoanalyzer ^{R} II system with manifold assembled as in Figure 8. System comprised of sampler, proportioning pump, manifold, colorimeter, recorder, and digital printer.

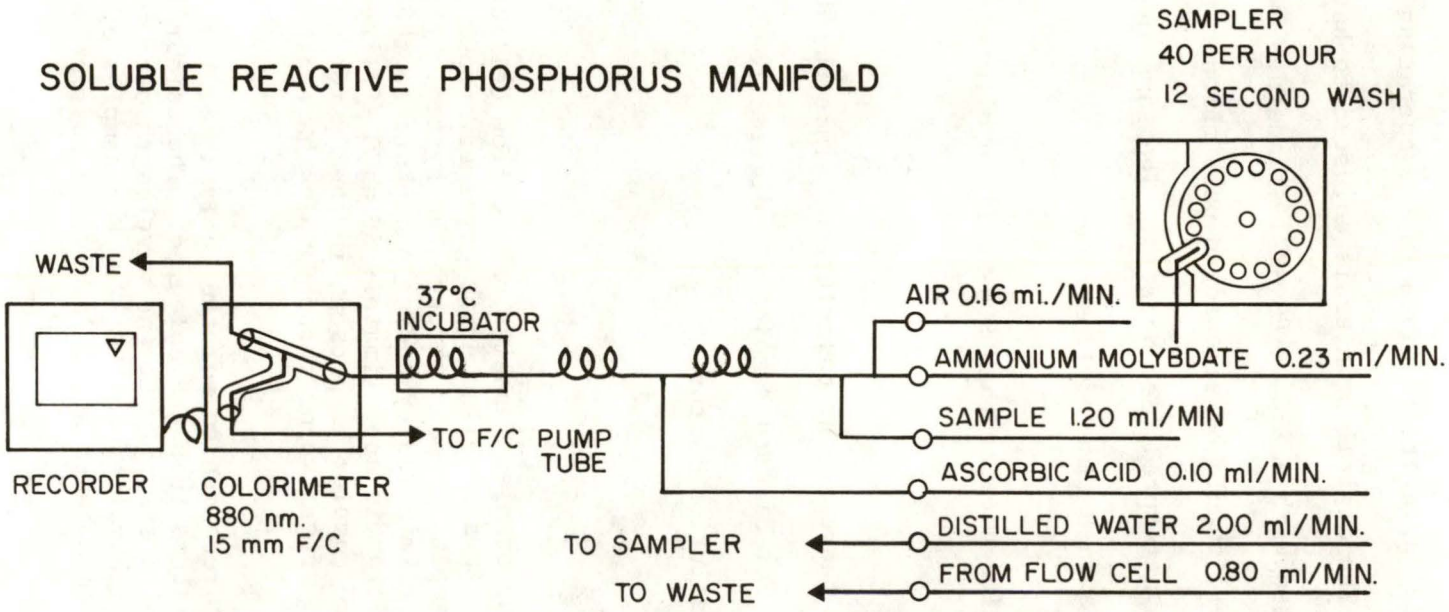

SOLUBLE REACTIVE PHOSPHORUS MANIFOLD
SAMPLER
40 PER HOUR
12 SECOND WASH
WASTE
37°C
INCUBATOR
AIR 0.16 ml./MIN.
AMMONIUM MOLYBDATE 0.23 ml/MIN.
SAMPLE 1.20 ml/MIN
TO F/C PUMP
TUBE
ASCORBIC ACID 0.10 ml/MIN.
RECORDER
COLORIMETER
880 nm.
15 mm F/C
TO SAMPLER
DISTILLED WATER 2.00 ml/MIN.
TO WASTE
FROM FLOW CELL 0.80 ml/MIN.

FIGURE 8

PROCEDURE

Detailed instructions for the adjustment and calibration of the colorimeter and digital printer are given in the operating manual supplied with the equipment. The digital printer is adjusted to have a range of 0-500 and is calibrated with a 250 µg PO_4-P/1 standard. Two or three samples of the standard should be allowed to pass through the system before calibration is attempted (See Note 1). The sampling rate is set at 40 samples/hour with a wash time of 12 seconds.

Samples of concentration greater than 500 µg/1 are diluted prior to analysis.

CALCULATIONS

For undiluted samples there are no calculations since the digital readout is in concentration. For diluted samples the digital readout value must be multiplied by the dilution factor.

NOTES

1. The molybdenum blue complex deposits on the flow cell causing the base-line to increase. This baseline drift gives erroneous results for the first 15-20 samples to pass through the system. The problem is almost completely eliminated by adding phosphate to the molybdate so that the concentration is approximately 50 µgPO_4-P/liter in the reagent. The molybdenum blue complex is present in the system at all times and the basline stabilizes after two or three samples have passed through the system.

2. This method is not recommended for the analysis of low levels of PO_4-P in natural waters that are coloured. The colour in the water usually absorbs quite strongly at the wavelength used for the PO_4-P analysis, giving high values for PO_4-P. For samples of this type the manual method is recommended as it involves a correction for natural colour.

REFERENCE

See manual method.

TOTAL DISSOLVED PHOSPHORUS

PRECISION

Level	-	200 µgP/liter
Standard deviation	-	±21 µgP/liter

OPERATING RANGE - 5-250 µg P/liter

SAMPLE CONDITION
- Filtered through Whatman GF/C, stored at 5 C.
- Less than 48 hours old.

METHOD PRINCIPLE - Organic phosphorus compounds under acidic conditions and with adequate oxygen supply, can be photo-oxidized to phosphate with short U.V. radiation. Breakdown is fairly quick (2-4 hours compared with 12 for dissolved nitrogen). This method does not, however, appear to hydrolyze polyphosphate. The resulting phosphate is measured by the soluble reactive phosphorus method.

REAGENTS

A. Sulphuric acid-8N

Add 220 ml of concentrated sulphuric acid (S.G. 1.84) to about 700 ml of distilled water. Cool and make to 1 liter.

B. Hydrogen peroxide

Reagent grade 30% w/w. This reagent deteriorates if left open at room temperature. Store at 5 C.

CAUTION: This is a dangerous oxidant. Avoid contact with skin. Never pipette by mouth. Wash hands after handling.

APPARATUS

The apparatus required is the same as used for Total Dissolved Nitrogen. Consult this method before using this equipment.

PROCEDURE

1. Rinse the quartz sample tube with sample and add the required volume using a graduated cylinder or pipette.

2. Add 1 ml of 8N H_2SO_4 per 100 ml of sample.

3. Add 2 drops of 30% peroxide and swirl to mix.

4. Wipe outer surface of tube dry with soft tissue.

5. Stopper tubes with a glass marble and place in U.V. reactor with automatic timer set to shut off after 4 hours.

6. Upon completion of photo-oxidation allow to cool and make up to volume if necessary with distilled water.

7. Analyze samples as for soluble reactive phosphate.

CALCULATIONS

Measured phosphate level is the total dissolved phosphorus in the sample minus polyphosphate. If significant amounts of polyphosphates are suspected to be present a separate analysis should be made to add their P content to the T.D.P. value.

REFERENCES

1. Armstrong, F.A.J., Tibbitts, S. (1968). Photochemical combustion of organic matter in sea water for nitrogen, phosphorus, and carbon determination. J. Mar. Biol. Ass. U.K. 48:143-152.

2. Henriksen, A. (1970) Determination of total nitrogen, phosphorus and iron in freshwater by photo-oxidation with ultraviolet radiation. Analyst. 95:601-605.

PARTICULATE PHOSPHORUS

PRECISION

Level	-	25 µg P/liter
Standard deviation	-	±1.5 µg P/liter

OPERATING RANGE - 0.05-20 µg P per filter paper. Operating range in terms of water samples depends on the volume available or possible to filter.

SAMPLE CONDITION
- Water sample stored at 5 C with no acidification.
- Less than 48 hours old.
- Particulate collected on a preignited (16 hours at 550 C) Whatman 4.25 cm GF/C glass fiber filter paper.
- Damp filter placed in a glass screw cap vial (70 mm x 20 mm -- 20 ml volume). Vial stored at room temperature.

METHOD PRINCIPLE - Particulate matter collected on a glass fiber filter is ignited at low temperature to destroy organic matter. The ignited filter is heated with dilute HCl which extracts the phosphorus and converts it to ortho-phosphate. Phosphorus is determined by a scaled down version of the soluble reactive phosphorus method.

REAGENTS

A. 1 N HCl

B. Acid molybdate - antimony solution.

In 500 ml of distilled water dissolve in order listed:

Ammonium paramolybdate	7.5 g
Antimony potassium tartrate	0.14 g
Sulphuric acid	88 ml

Cool and make to 1000 ml. Store in a dark glass bottle.

C. Ascorbic acid

Dissolve 2.5 g of l-ascorbic acid in 100 ml of distilled water. This reagent is stable for a few days if kept in a refrigerator.

D. Mixed molybdate reagent

Mix 400 ml of reagent B with 100 ml of reagent C. Stable approximately 6 hours.

E. Phosphate standard - stock

Dissolve 0.2197 g potassium dihydrogen phosphate in about 100 ml of distilled water and make to 1000 ml with water saturated with chloroform.

$$1.00 \text{ ml} = 50 \text{ µg } PO_4\text{-P}$$

APPARATUS

1. Spectrophotometer or colorimeter to read absorbance at 885 nm. A 1 cm flow-through cuvette is desirable.
2. Muffle furnace set at 550 C.
3. Oven set at 104 C.
4. Cornwall type dispensing syringes to deliver 10 ml and 2.5 ml.
5. Pyrex screw-cap vials, 20 ml capacity - 70 mm x 20 mm with screw caps and cap inserts.
6. Metal rack to hold the above vials and capable of standing 550 C.
7. Millipore type filtration apparatus to hold 47 mm filter papers.
8. Whatman GF/C glass fiber filter papers - 4.25 cm in diameter. Pore size is 2-3 microns.
9. Microburette - 0.2 ml total volume to dispense phosphate standard.

PROCEDURE

1. Filter an appropriate volume (Vml) of sample through a 4.25 cm GF/C filter paper. Apply no more than 15 inches Hg vacuum and do not allow vacuum to remain on after samples have been sucked to dryness. Samples may be stored wet at room temperature for an indefinite period. It is convenient to store the filter paper in a pyrex screw-cap vial with identification on the lid. This pyrex storage container can then be used as the reaction vessel for the subsequent analysis for phosphorus.

2. Place sample vials, minus plastic lids, in a muffle furnance at 550 C for 1 hour to ignite organic matter. When processing samples include 4 blank papers in 4 separate vials to serve as blanks and standards.

3. After cooling, add 2 ml of 1 N HCl and 10 ml of distilled water to the ignited samples. Add 2.5 µg of PO_4-P to two of the blank papers included in step 2. Replace screw caps tightly and place in an oven at 104 C for 2 hours.

4. Remove samples from the oven and after cooling remove screw caps. Add 2.5 ml of mixed molybdate reagent to each vial. A rapid injection rate ensures mixing of reagent with sample.

5. Allow 30 minutes for color development (blue) and for glass fiber particles to settle.

6. Using a cuvette of appropriate path length (1-5 cm) measure absorbance of blanks, standards and samples at 885 nm.

CALCULATIONS

Having obtained

E_{Bl} = Absorbance of blank

E_1 = Absorbance of standard

E_o = Absorbance of sample

substract E_{Bl} from standard and sample.

Using the standard absorbance corrected for blank, calculate a unit extinction factor in terms of µg of PO_4-P per sample vial:

$$\text{i.e. } F = \frac{2.50 \text{ µg} PO_4\text{-P (per vial)}}{E_1 - E_{Bl}}$$

Calculate μg PO_4-P per sample vial by multiplying the absorbance due to PO_4-P in samples by F

i.e. PO_4-P/sample vial = $F (E_o - E_{Bl})$

To obtain the concentration of suspended P in the original water sample, multiply PO_4-P content of the vial by 1000/V where V is the volume, in milliliters, of water filtered through the glass fiber filter.

SOLUBLE REACTIVE SILICON (MANUAL METHOD)

PRECISION

Level	-	4000µg (diluted for analysis)/liter
Standard deviation	-	± 64 µg Si/liter

OPERATING RANGE - 5-1500 µg Si/liter

SAMPLE CONDITION
- Unfiltered, stored in polyethylene at 5 C.
- DO NOT FREEZE. DO NOT STORE IN GLASS CONTAINERS.
- Less than 48 hours old.

METHOD PRINCIPLE - Orthosilicate and possibly the dimer ($Si_2O_7^{-2}$) react with molybdate in acid solution to form heteropoly molybdates which can be reduced to silicomolybdenum blues. The absorption spectrum, intensity and stability of the color depend greatly on the acidity and other experimental conditions (Strickland 1951). In this method stannous chloride is used as reductant. Phosphate interference is prevented by the addition of tartrate which complexes excess molybdate, preventing its reduction. The absorption maximum is in the near infra-red (800-820 nm).

REAGENTS

A. Silica-free distilled water

A still pot with a silver plated or aluminum condenser will produce silica-free distilled water. Collect in polyethylene.

B. Ammonium molybdate - 5% w/v

Dissolve 50 g of ammonium paramolybdate $(NH_4)_6\ Mo_7\ O_{24} \cdot 4H_2O$ in 1 liter of silica-free distilled water.

C. Hydrochloric acid - Approximately 1 N

Dilute 83 ml of concentrated HCl to 1 liter with silica-free distilled water.

D. Mixed molybdate

Mix 2 volumes of molybdate reagent B. with 3 volumes of hydrochloric acid C. Prepare fresh daily.

E. Tartaric acid - 10% w/v

Dissolve 100 g of tartaric acid in 1 liter of silica-free distilled water.

F. Stannous chloride - stock - 3.5 N

Dissolve 40 g $SnCl_2 \cdot 2H_2O$ in 100 ml of 50% v/v HCl.

G. Stannous chloride - 0.05 N

Prepare by dilution of stock stannous chloride. The normality of the final reducing solution should be between 0.04 and 0.06 and can be checked periodically by titration with standard iodine. Prepare fresh daily.

H. Silica standard

Grind a sample of quartz "Vitreosil", "Spectrosil", etc., to a fine powder. Heat powder to redness in a platinum crucible. Cool in a desiccator and weigh 0.107 g silica plus 0.6 g Na_2CO_3 into a platinum crucible. Heat to 800-1000 C in gas flame to melt flux and dissolve quartz powder completely (cessation of bubbles of CO_2 evolving from the transparent liquid flux). Cool and dissolve in 100.0 ml of silica-free distilled water.

1.00 ml = 500 µg Si

APPARATUS

Spectrophotometer, or absorptiometer to measure extinction at 820 nm.

PROCEDURE

1. Prepare in duplicate 100 ml aliquots of silicate standard having a concentration approximating that of samples to be analyzed.

2. Place 3 ml of mixed molybdate reagent D in a 125 ml Erlenmeyer flask and add 50 ml of distilled water, silicate standard or sample and mix.

Note: Do not add sample to flask without first adding the acidic mixed molybdate. If the sample is alkaline a significant quantity of silicate can be solubilized from the flask.

3. Wait 15 minutes. (See Note 1)

4. Add 2 ml of tartaric acid E and mix.

5. Wait 2 minutes. (See Note 1).

6. Add 1ml of stannous chloride reductant G and mix.

7. Between 5 minutes and 1 hour later measure:

   E_{B1} = Absorbance of distilled water plus reagents.

   E_1 = Absorbance of standards and samples plus reagents.

   Absorbance is measured at 820 nm using an appropriate path length:

1 cm path length	100-1000 µg Si/l
5 cm path length	25-250 µg Si/l
10 cm path length	0-100 µg Si/l

CALCULATIONS

1. Unit extinction factor for silicate is calculated as follows:

$$F = \frac{\text{Standard concentration (µg Si/liter)}}{E_1 \text{ (standard)} - E_{B1}}$$

   This should be around 1400 µg Si/liter for a 1 cm path length.

2. Concentration of silicate in samples can be calculated by multiplying the extinction due to silicate in the sample.

   i.e. $(E_1 \text{ Sample} - E_{B1})$

   by the unit extinction factor F calculated above.

   Note 1: These times should be consistent for all samples and standards.

REFERENCE

1. Armstrong, F.A.J. (1951). The determination of silicate in sea water. J. Mar. Biol. Ass. U.K. 30:149-60.

2. Armstrong, F.A.J. and Butler, E.I. (1962). Chemical changes in sea water off Plymouth during 1960. J. Mar. Biol. Ass. U.K. 42:253-58.

3. Strickland, J.D.H., (1952). The preparation and properties of silico-molybdic acid. (In three parts). J. Am. Chem. Soc. 74:862-76.

SOLUBLE REACTIVE SILICON (AUTOMATED METHOD)

PRECISION

Level	- 1000 µg Si/liter
Standard deviation	- ±4 µg Si/liter
Level	- 10,000 µg Si/liter (diluted for analysis)
Standard deviation	- ± 44 µg Si/liter

OPERATING RANGE - -5-1000 µg-Si/liter for undiluted samples

- 1000-15,000 µg-Si/liter for diluted samples

SAMPLE CONDITION - See manual method.

METHOD PRINCIPLE - Same as manual method, except that ascorbic acid is used as a reductant.

REAGENTS

A. Ammonium molybdate

Dissolve 10 g of ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 1 liter of 0.1 N hydrochloric acid (8.3 ml concentrated HCl/1). Store in amber bottle.

B. Tartaric acid

Dissolve 100 g of tartaric acid in distilled water and dilute to 1 liter.

C. Ascorbic acid

Dissolve 17.6 g ascorbic acid in distilled water. Add 50 ml of acetone and dilute to one liter. Add 0.5 ml of Levor IV.

D. Silica standard

See manual method.

APPARATUS

Technicon Autoanalyzer^{R} II system with manifold assembled as in Figure 9. System comprised of sampler, proportioning pump, manifold, colorimeter, recorder, and digital printer.

Hook and Tucker Auto Diluter Mk II or equivalent.

PROCEDURE

Detailed instructions for the adjustment and calibration of the colorimeter and digital printer are given in the operating manual supplied with the equipment. The digital printer is adjusted to have a range of 0-1000 and calibrated with a 500 µg Si/liter standard. The sampling rate is set at 40 samples/hour with a wash time of 12 seconds.

Samples of concentration greater than 1000 µg Si/liter are diluted with a Hook and Tucker Auto-Diluter Mk II. The dilution factor is obtained by diluting a 10,000 µg Si/liter standard and running it in duplicate, with each batch of diluted samples.

CALCULATIONS

1. For undiluted samples there are no calculations since the digital readout is in concentration.
2. For diluted samples multiply the digital readout value by the dilution factor.

REFERENCE

See manual method.

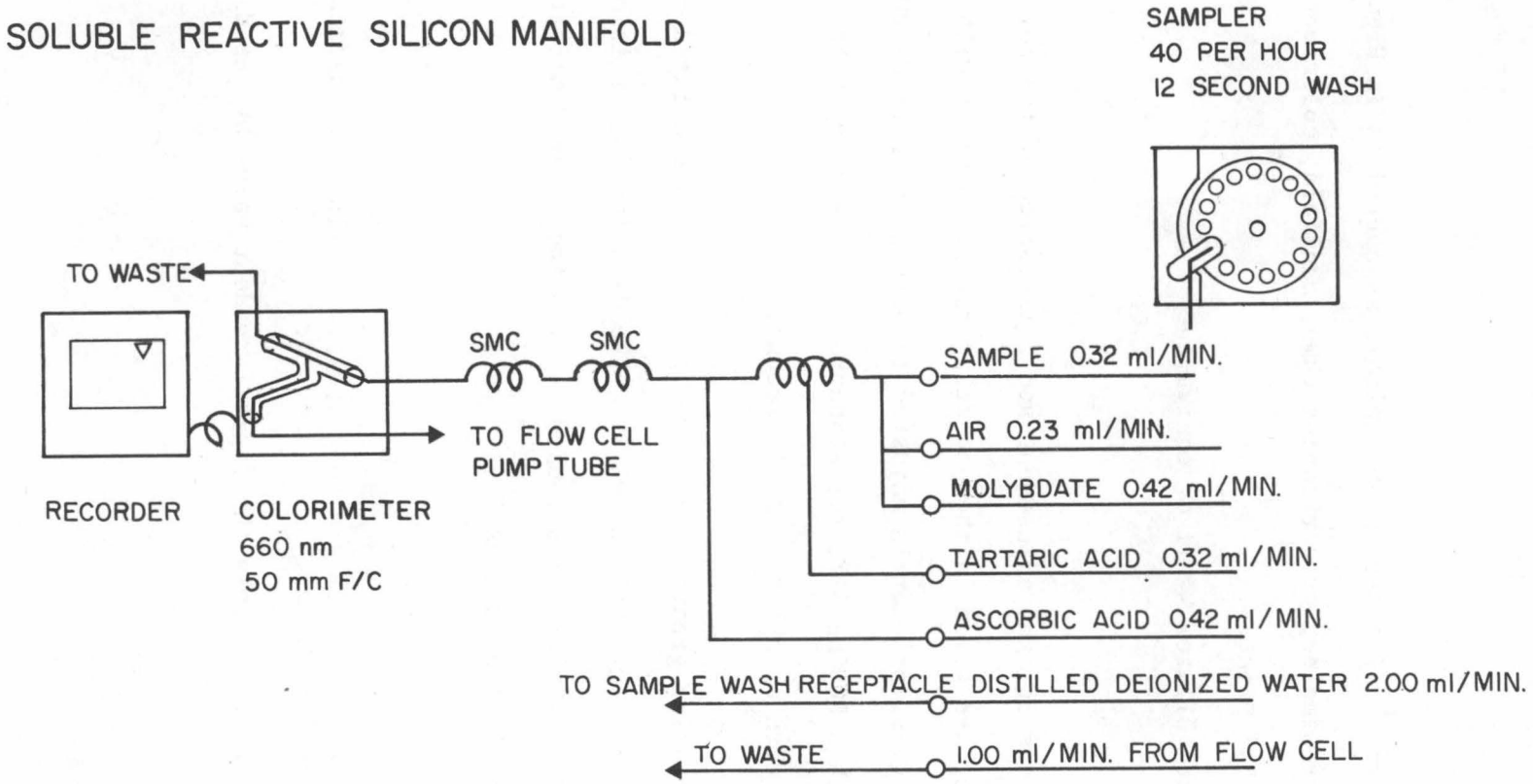

SOLUBLE REACTIVE SILICON MANIFOLD
SAMPLER
40 PER HOUR
12 SECOND WASH
TO WASTE
SMC
SMC
SAMPLE 0.32 ml/MIN.
AIR 0.23 ml/MIN.
MOLYBDATE 0.42 ml/MIN.
TARTARIC ACID 0.32 ml/MIN.
ASCORBIC ACID 0.42 ml/MIN.
TO FLOW CELL
PUMP TUBE
RECORDER
COLORIMETER
660 nm
50 mm F/C
TO SAMPLE WASH RECEPTACLE DISTILLED DEIONIZED WATER 2.00 ml/MIN.
TO WASTE
1.00 ml/MIN. FROM FLOW CELL

FIGURE 9

CHLORIDE (TITRIMETRIC)

PRECISION

Level	- 30 mgCI /liter
Standard deviation	- ±1 mgCI /liter

OPERATING RANGE - 1.0 mg/liter upwards.
1.00 ml of titrant = 0.50 mg chloride.

SAMPLE PREPARATION
- Filtered through Whatman GF/C, stored at 5 C.
- Less than 30 days old.

METHOD PRINCIPLE - Mercuric ion is added to the sample, forming soluble, but undissociated mercuric chloride with any chloride ion present. When an amount of mercuric ion equivalent to chloride ion has been added, free mercuric ion reacts with diphenylcarbazone indicator forming a purple complex in the pH range 2.3-2.8. Br^- and I^- respond in the same manner and hence interfere with this method. End point is pH dependent therefore sample pH must be adjusted before analysis.

REAGENTS

A. Chloride standard 0.0141 N

Dissolve 0.8241 g NaCl in distilled water and make to 1 liter.

1.00 ml = 0.500 mg chloride.

B. Nitric acid 0.1 N

C. Sodium hydroxide 0.1 N

D. Indicator-acidifier reagent

s-Diphenylcarbazone	0.250 g
Concentrated nitric acid	4.0 ml
Xylene cyanol FF	0.030 g

Dissolve in order given in 95% ethanol. Make to 100 ml. Store in low actinic glass bottle at 5 C. Stable several months.

E. Standard mercuric nitrate titrant

Dissolve 2.3 g $Hg(NO_3)_2$ or 2.5 g $Hg(NO_3)_2 \cdot H_2O$ in 100 ml of distilled water containing 0.25 ml of concentrated nitric acid. Dilute to 1 liter.

Solution is approximately 0.014 N in mercury.
1.00 ml approximately equals 0.500 mg chloride.

APPARATUS

1. Burette to contain 25 ml and graduated to nearest 0.1 ml.

2. Magnetic stirrer with lighted base for ease of end point detection.

PROCEDURE

1. Place 10.0 ml of standard sodium chloride solution in each of three 125 ml Erlenmeyer flasks.

   Add 90 ml of distilled water.
   These flasks contain 5.0 mg of chloride.

2. Place 100 ml of distilled water blank in each of three 125 ml Erlenmeyer flasks.

3. Place 100 ml of sample(s) in 125 ml Erlenmeyer flasks. If samples are strongly alkaline or acid adjust pH to approximately 2.5 with 0.1 nitric acid or 0.1 N sodium hydroxide.

4. Add 1.0 ml of indicator-acidifier reagent D to each standard, blank, and sample. Color should be green-blue. If green, the pH is too low. If blue, the pH is too high.

5. Titrate blanks, standards, and samples with mercuric nitrate to a definite purple colored end point. Record volume to the nearest 0.05 ml

V_{Bl} = Volume in ml of titrant to blank end point

V_{Std} = Volume in ml of titrant to standard end point

V_{sample} = Volume in ml of titrant to sample end point

CALCULATIONS

1. An equivalence factor for the titrant can be calculated as follows:

$$F = \frac{5.0 \text{ mg Chloride}}{V_{Std} - V_{Bl}}$$

The factor should be around 0.50 mg chloride per ml of titrant.

2. Quantity of chloride in the sample flasks is calculated as follows:

$$\text{Quantity of chloride} = (F)\ (V_{Sample} - V_{Bl})$$

3. Multiplication of the above value by 10 gives the concentration in mg chloride/liter in the original sample.

REFERENCE

1. American Public Health Association. (1965). Standard methods for examination of water and waste water. 12th ed. APHA, New York, N.Y. 769 pp.

CHLORIDE AND SULPHATE AUTOMATED METHOD

PRECISION

Chloride Level	- 10 mg Cl/liter
Standard deviation	- ± 0.2 mg Cl/liter
Sulphate Level	- 10 mg SO_4/liter
Standard deviation	- ± 0.2 mg SO_4/liter

OPERATING RANGE

- Chloride - 0.2-20 mg Cl/liter
- Sulphate - 0.2-30 mg SO_4/liter

SAMPLE PREPARATION

- Filtered through Whatman GF/C.
- Stored at 5 C.
- Less than 30 days old.

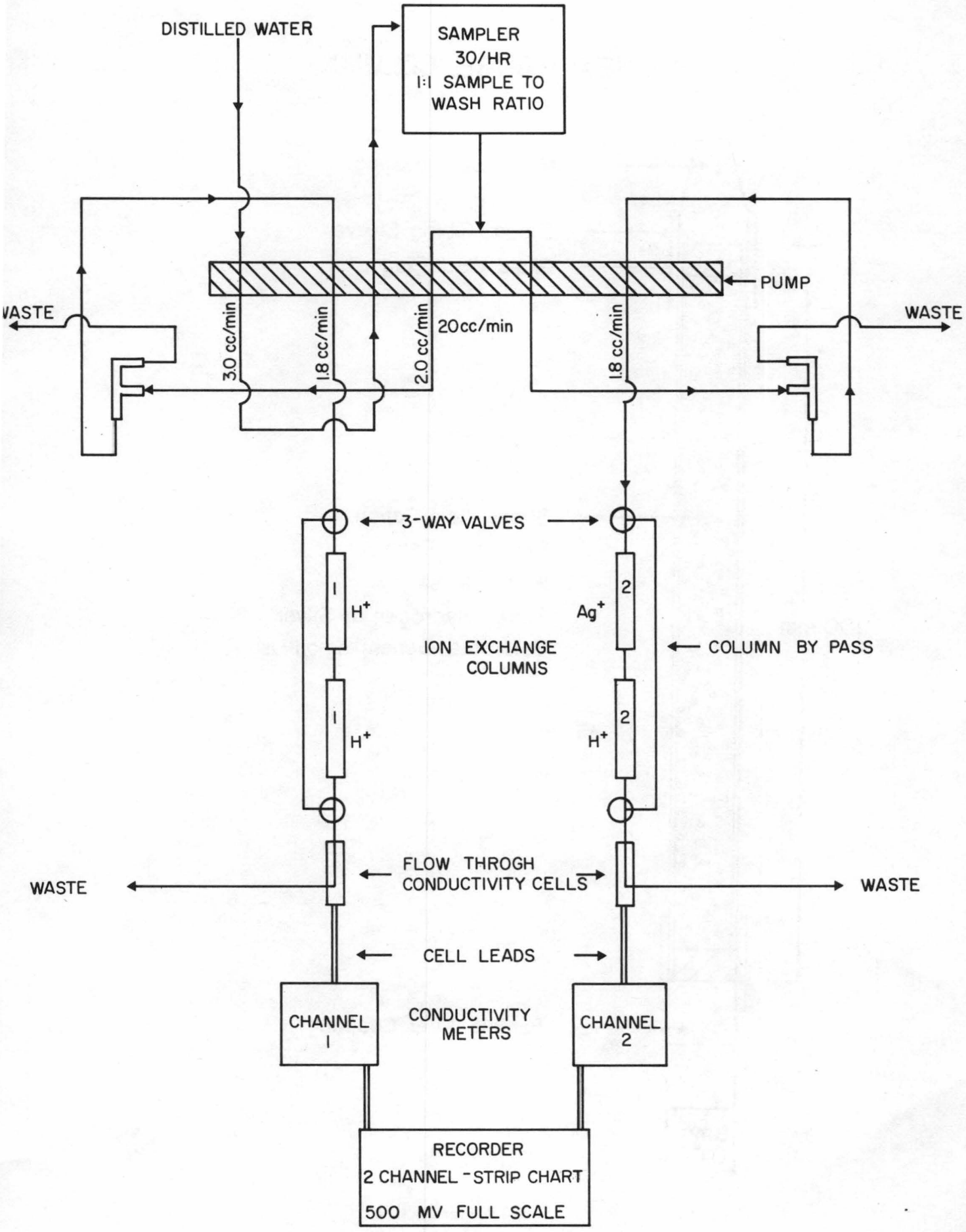
METHOD PRINCIPLE

Sample is divided into two portions, each being passed through an ion exchange column. (See Figure 10) One half of the sample goes to column 1 (Figure 10) where a strong acid cation exchange resin exchanges sample cations for hydrogen ions. Effluent from column 1 is acidic and contains HCl, H_2SO_4, HNO_3, H_3PO_4, H_4SiO_4, HF, and H_2CO_3. At low pH, only HCl, H_2SO_4, and HNO_3 are completely dissociated. Usually only HCl and H_2SO_4 are present at significant levels. Hydrogen ion concentration in the effluent from column 1 is therefore equivalent to chloride plus sulphate.

The other half of the sample goes to column 2 which contains a 2-stage resin bed. The first stage contains silver saturated resin. Silver exchanges for sample cations and precipitates chlorides as silver chloride. The second stage contains hydrogen saturated resin which exchanges silver for hydrogen ion. The resulting effluent is identical to that of column 1 except that HCl has been quantitatively removed. Hydrogen ion concentration in the effluent from column 2 is equivalent to sulphate.

The difference in hydrogen ion concentration in the effluents of columns 1 and 2 is equivalent to chloride.

Hydrogen ion concentration in column effluents is measured using



CHLORIDE SULPHATE MANIFOLD

FIGURE 10

ION EXCHANGE COLUMN

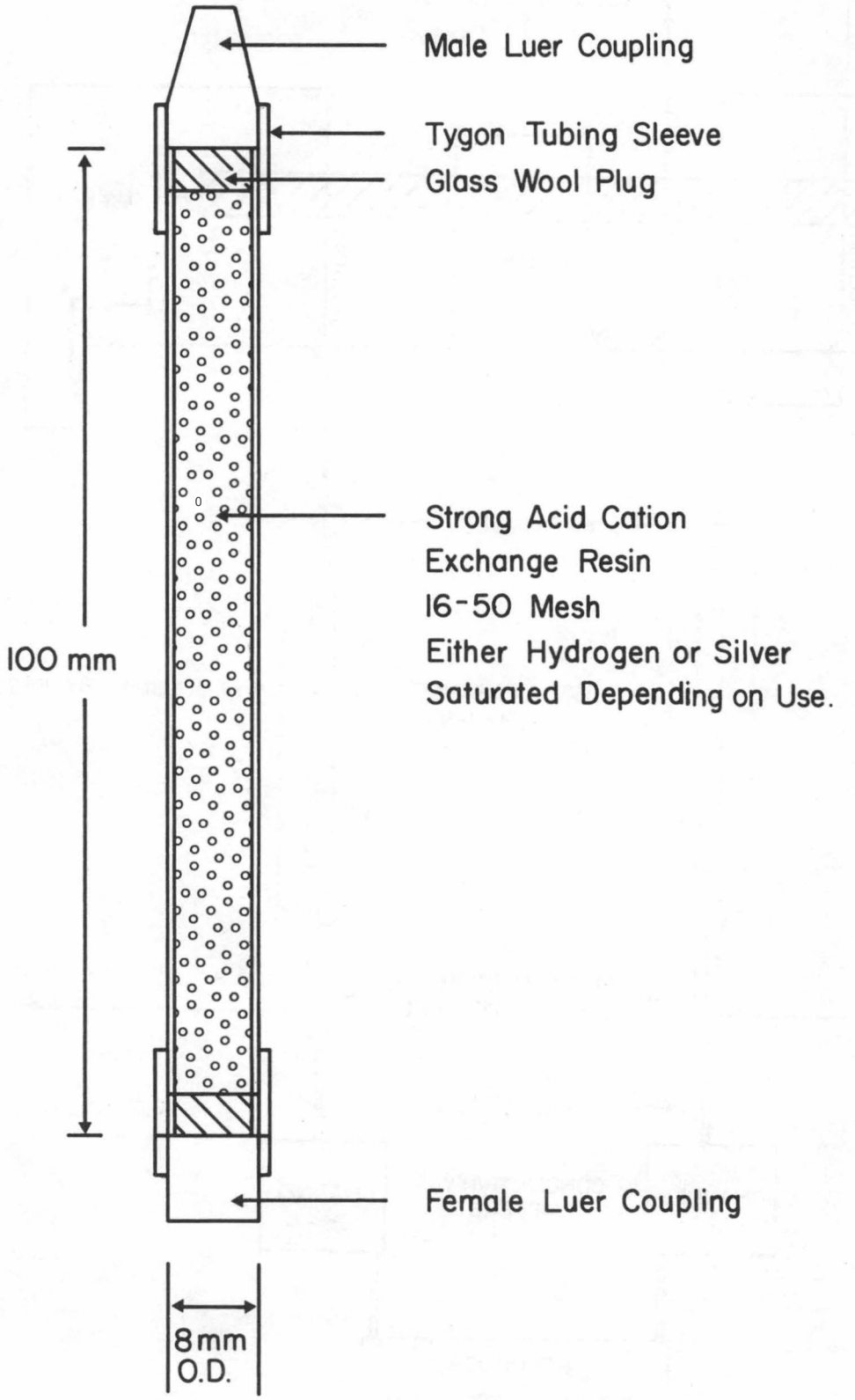


FIGURE 11

a conductivity meter and a flow through conductance cell with signal output to a strip chart recorder.

Since Cl and SO_4 solutions of equivalent strength give equivalent response, only a sulphate standard is required to calibrate the response of both channels 1 and 2.

INTERFERENCES: In some waters nitrate, orthophosphate, and fluoride may be present at significant levels and their sulphate interference must be corrected for. See Table 1 for correction factors.

REAGENTS

A. 0.1 N HCl and 0.1 N $AgNO_3$

B. Strong acid cation exchange resin

"Rexyn 101" or equivalent having a wet volume exchange capacity of 2.0×10^{-3} equivalents per ml. Mesh size 16-50.

C. Sulphate standard

Dissolve 7.102 g of reagent grade Na_2SO_4 in distilled water and make to 1 liter.

1.00 ml = 100 μ equivalent SO_4 or Cl

APPARATUS

1. Manifold assembled as in Figure 10 consisting of sampler, proportioning pump, 2 flow-through conductivity cells (cell constant 0.316/cm), 2 conductivity meters (500μ Siemens full-scale), and a 2-channel strip chart recorder.

2. Ion exchange columns constructed as in Figure 11 and prepared as follows:

   A. Columns packed with strong acid resin were saturated with hydrogen ion by pumping 0.1 N HCl through the column at 2 ml per minute for 20 minutes. Columns were then rinsed with distilled water pumped at 2 ml per minute for 20 minutes. Three such columns are required for the manifold of Figure 10.

   B. Silver columns were prepared as above using 0.1 N $AgNO_3$.

   C. Life of columns depends on sample size, exchange capacity of resin used and ionic strength of samples analyzed, but should be at least 2000 samples.

D. Column 1 can be regenerated simply by treatment with 0.1 N HCl. The first stage of column 2 cannot be easily regenerated and is discarded. The second stage of column 2 contains much silver and should be used to prepare a silver saturated column.

PROCEDURE

1. Prepare duplicate sulphate standards containing 500μ eq SO_4/liter.

2. Using the manifold arrangement of Figure 10:

   A. Set sampler at 30 per hour with a 1:1 sample to wash ratio.

   B. While pumping distilled water through the system, zero conductivity meter and recorders.

   C. Add samples and standards to sampler and start sampler.

   D. Obtain peak heights for standards and samples for channels 1 and 2.

CALCULATIONS

1. Compute a scale factor for channels 1 and 2.

$$\text{i.e. } F = \frac{\text{Standard concentration (}\mu\text{ eq/liter)}}{\text{Peak height (chart units)}}$$

   Factor should be approximately 7 for the system described.

2. Multiply sample peak height for channels 1 and 2 by their respective scale factor to obtain μ eq/liter values for channels 1 and 2. Values for channel 1 are μ eq/liter of chloride plus sulphate. Values for channel 2 are μ eq sulphate/liter. The difference between channels 1 and 2 is μ eq chloride/liter.

3. Chloride levels in μ eq/liter are multiplied by 0.0355 to give mg/liter chloride.

4. Sulphate levels in μ eq/liter are multiplied by 0.048 to give mg SO_4/liter.

   The principle of this method can be used for the manual determination of Cl and SO_4. See Mackereth (1955).

REFERENCES

1. Mackereth, F.J.H. (1955). (a) Ion exchange procedures for the estimation of (I) total ionic concentration; (II) chlorides; and (III) sulphate in natural waters. Mitt. int. Ver. Limnol. 4:1-16.

2. Mackereth, F.J.H. (1955). (b) Rapid microestimation of the major ions of freshwater. Proc. Soc. Wat. Trtmnt. Exmn. 4:27-42.

3. Mackereth, F.J.H. (1963). Some methods of water analysis for limnologists. Freshw. Biol. Ass. Sci. Pub. No. 21, Kendal, England. 70 pp.

4. Stainton, M.P. (1973). An automated method for determination of chloride and sulphate in freshwater using cation exchange and measurement of electrical conductance. In press.

ANION 1 mg/liter	SULPHATE INTERFERENCE mg/liter
NO_3-N	3.42
F	2.53
PO_4-P	1.29
HCO_3-C	0.06
SiO_4-Si	no interference

Table 1. Sulphate interference caused by various anions.

SULPHATE GRAVIMETRIC METHOD

PRECISION

Level	- 50 mg SO_4/liter
Standard deviation	- $\pm$ 2 mg SO_4/liter

OPERATING RANGE - 1-200 mg SO_4/liter

SAMPLE CONDITION
- Filtered through Whatman GF/C.
- Stored at 5 C.
- Less than 30 days old.

METHOD PRINCIPLE - Hot barium chloride solution is added to boiling sample to precipitate barium sulphate. The precipitate is digested, collected on a filter paper, washed with boiling water and ignited. The resulting barium sulphate is weighed and sulphate concentration in the original sample calculated.

REAGENTS

A. Methyl red indicator

Dissolve 100 mg methyl red (sodium salt) in 100 ml of distilled water.

B. Hydrochloric acid - 6 N

Dilute concentrated HCl 1:1 with distilled water

C. Barium chloride

Dissolve 100 g of $BaCl_2.2\ H_2O$ in 1 liter of distilled water. Filter before use.

1 ml precipitates approximately 40 mg SO_4.

APPARATUS

1. Muffle furnace set at 800 C.
2. Hot plate with control to maintain beakers of water at 80-90 C.
3. Porcelain crucibles to contain 10 ml.
4. Ashless filter papers. Whatman No. 42 - 8 cm diameter.
5. Filter funnels and rack.

PROCEDURE

1. Measure 100 ml of filtered sample into a 250 ml beaker and heat to boiling.
2. Add one drop of indicator.
   Add acid (B) until indicator turns red.
   Add 2 ml of acid (B) in excess.
3. Add slowly 5 ml of hot barium chloride solution.
4. Cover beaker with a watch glass and allow to digest at 80-90 C overnight.
5. Quantitatively transfer the barium sulphate precipitate to an ashless filter paper and wash the precipitate with hot distilled water.
6. Transfer the filter paper with precipitate to a pre-ignited (1 hour at 800 C) pre-weighed (nearest 0.1 mg) crucible.
7. Ignite crucible, paper and precipitate for 1 hour at 800 C.
8. Re-weigh the cool ignited crucible to the nearest 0.1 mg.

CALCULATIONS

1. Multiply the weight of barium sulphate precipitate (in mg) by 0.4115 to obtain the weight (mg) of sulphate in the 100 ml of sample used. Multiply this by 10 to obtain the sulphate concentration in mg SO_4/liter.

REFERENCE

1. American Public Health Association (1965). Standard methods for the examination of water and waste water, 12th ed. APHA, New York, N.Y. 769 p.

METALS BY ATOMIC ABSORPTION AND EMISSION SPECTROSCOPY

OPERATING RANGE - See individual metals.

SAMPLE CONDITION
- Stored at 5 C for less than 48 hours before filtering.
- Filtered through Whatman GF/C.
- Acidified with 1 ml 1 N HCl per 100 ml of sample.
- Do not freeze samples.

METHOD PRINCIPLE

An aqueous solution containing the metal of interest is aspirated into a laminar flow, air acetylene flame. At high temperature, metal ions are either excited, emitting a line spectra, or remain unionized, unbound and capable of absorbing radiant energy. Whether metals emit or absorb radiation depends upon their ionization potential, flame temperature and sample matrix.

For elements that ionize readily the flame is used as a point emission source with emitted light being chopped and passed through a slit and monochromator to a photo tube. Emitted light is proportional to concentration of emitting element.

For elements that absorb radiant energy the flame is analogous to the glass windowed cuvette of conventional U.V.-visible spectroscopy and is placed between a light source and the slit, monochromator and phototube assembly.

Sensitivity and specificity is obtained by using a source lamp whose cathode contains the elements of interest. Source lamp emission is a line spectrum of which a principal line is coincident with the principal absorption line of the element of interest. Radiant energy absorbed is proportional to element concentration, there usually being a linear relationship.

Significant interferences are of three types: ionization, chemical and non-specific absorption.

Ionization interference occurs when the degree to which the element of interest is ionized depends on other ionizable metals in the sample. The resulting signal whether it be emission or absorbance is then dependent both on the level of the element of interest and the level of other ionizable metals present. Potassium for example

when present in large excess to sodium, causes a decrease in the ionization of sodium which increases the emission signal and increases the absorbance signal. This form of interference can be overcome by matching the level of interfering metal in standards and samples.

Chemical interference occurs when the element of interest combines with other elements in the sample or flame. The signal is either enhanced or depressed depending on whether the compound formed is more labile or refractory than the uncombined element. The net effect is an increase or decrease in the ground state atoms available for absorption. Methods for eliminating this interference depend largely on the element of interest and the interfering species. In the case of calcium and magnesium,phosphate interference is eliminated by adding excess lanthanum which preferentially forms a refractory compound with phosphate. Often a hotter flame temperature (nitrous oxide-acetylene) will dissociate refractory compounds.

Non-specific absorption occurs when the flame contains particles (salts or unevaporated solvent) which scatter light or molecules capable of absorbing over a broad spectral range. A false positive absorption error results. This can be corrected by measuring absorbance at a line near the analytical line where the element of interest does not absorb. The non-specific absorbance is then subtracted from that measured at the analytical line. This correction is analogous to natural color correction required in some colorimetric methods.

APPARATUS

1. Methods outlined require a Perkin Elmer Model 403 atomic absorption spectrophotometer or equivalent instrument having:

   A. Double beam optics
   B. Premix burner
   C. Optical response 180 to 900 nm
   D. Digitized readout in absorbance
   E. Time integrating, signal averaging circuitry
   F. Emission and absorbance operation modes
   G. Scaling amplifier to give readout in concentration units
   H. Curvature correction circuitry
   I. Analog and digital recorder output.

2. Hollow cathode lamps, single or multi-element type for each element of interest.

REAGENTS

See element of interest.

PROCEDURE

With instrument settings as listed under individual metals:

1. Prepare blanks and standards to cover the concentration range of samples to be analyzed.

2. Aspirate blank solution for 10 seconds and set zero.

3. Aspirate lowest standard concentration and adjust "Concentration" potentiometer to obtain desired digital display.

4. Aspirate successively higher standards until deviation from linearity is observed. Use "Onset" to bring first deviant point into line.

5. Aspirate remaining standards and use "Magnitude" potentiometer to correct deviation.

6. Repeat aspiration of standards to check linearity of response.

7. Aspirate samples and record readings.

8. Repeat standards after samples to check machine stability.

CALCULATIONS

None required unless samples are diluted. With equipment described readout is in concentration.

REFERENCE

1. Christian, G.D. and Feldman, F.J. (1970). Atomic absorption spectroscopy: applications in agriculture, biology and medicine. John Wiley & Sons, Inc., New York, N.Y. 490 p.

CALCIUM BY ATOMIC ABSORPTION

PRECISION

Level	- 20.0 mg Ca/liter
Standard deviation	- ± 0.3 mg Ca/liter

OPERATING RANGE

- Light path through length of flame 0.01-12 mg Ca/l.
- Light path through width of flame 0.1-200 mg Ca/l.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 423 nm
U.V. or Visible	- Visible
Slit	- 4
Emission chopper	- Off
Filter	- Out
Readout mode	- "Concentration" "Repeat"
Signal conditioning	- 10 average
Recorder response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8.0 units

INTERFERENCE

Calcium absorption is subject to chemical interference from a number of sources. In freshwater the most significant of these are silicate, phosphate and carbonate which in the flame form refractory compounds with calcium, yielding low estimates. Most suppressing interference can be overcome by adding about 1% lanthanum chloride.

REAGENTS

A. Lanthanum chloride buffer

Lanthanum oxide	58.65 g
Hydrochloric acid	250 ml

Wet lanthanum oxide with 100 ml of distilled water. Add hydrochloric acid very slowly while stirring. Cool and make to 1 liter with distilled water.

B. Calcium standard

Calcium carbonate 2.497 g

Dissolve calcium carbonate in a minimum of 1 N HCl and make to 1 liter with distilled water.

1.00 ml = 1.00 mg Ca

PROCEDURE

1. Prepare calcium standards to cover the range of sample concentrations.
2. Add lanthanum chloride solution to blanks, standards, and samples in the ratio 1 part lanthanum solution plus 4 parts sample. The absolute ratio is not critical; it is, however, very important that the ratio used be the same for standards and samples.
3. Aspirate lanthanum blank and zero instrument.
4. Aspirate standards to calibrate instrument and adjust curvature.
5. Aspirate samples and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual.

IRON BY ATOMIC ABSORPTION

PRECISION

Level	- 1.00 mg Fe/liter
Standard deviation	- ± 0.03 mg Fe/l

OPERATING RANGE

- Light path through length of flame 0.01-10 mg Fe/liter.
- Light path through width of flame 0.1-100 mg Fe/liter.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 249 nm
U.V. or Visible	- U.V.
Slit	- 4
Emission Chopper	- Off
Filter	- Out
Readout Mode	- "Concentration" "Repeat"
Signal Conditioning	- 10 Average
Recorder response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8 units

REAGENTS

A. Iron standard

A commercially prepared standard is used, having a concentration of 1 g Fe/liter.

1.00 ml = 1 mg Fe

PROCEDURE

1. Prepare iron standards to cover the range of sample concentrations.
2. Aspirate a distilled water blank and zero instrument.
3. Aspirate standards to calibrate instrument and adjust curvature (see general AAS outline).
4. Aspirate sample and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual.

MAGNESIUM BY ATOMIC ABSORPTION

PRECISION

Level	- 1.00 mg Mg/liter
Standard deviation	- ± 0.01 mg Mg/liter

OPERATING RANGE

- Light path through length of flame 0.005-5 mg Mg/1.
- Light path through width of flame 0.5-50 mg Mg/1.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 285 nm
U.V. or Visible	- U.V.
Slit	- 5
Emission Chopper	- Off
Filter	- Out
Readout Mode	- "Concentration" "Repeat"
Signal conditioning	- 10 Average
Recorder Response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8 units

INTERFERENCES

Magnesium absorption is subject to several chemical interferences. In freshwater the most significant of these are phosphate and silicate which in the flame form refractory compounds with magnesium. Addition of 1% lanthanum chloride to samples overcomes these interferences.

REAGENTS

A. Lanthanum chloride buffer

Lanthanum oxide	58.65 g
Hydrochloric acid	250 ml

Wet lanthanum oxide with 100 ml of distilled water. Add hydrochloric acid very slowly with stirring. Cool and make to 1 liter with distilled water.

B. Magnesium carbonate

Magnesium carbonate 3.469 g

Dissolve magnesium carbonate in a minimum of 1 N HCl and make to 1 liter with distilled water.

1.00 ml = 1.00 mg Mg

PROCEDURE

1. Prepare magnesium standards to cover the range of sample concentrations.

2. Add lanthanum chloride solution to blanks, standards, and samples in the ratio 1 part lanthanum solution plus 4 parts sample. While the absolute ratio is not critical it is very important that the ratio be the same for standards and samples.

3. Aspirate blank and zero instrument.

4. Aspirate standards to calibrate instrument and adjust curvature (see general AAS outline).

5. Aspirate samples and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual.

MANGANESE BY ATOMIC ABSORPTION

PRECISION

Level	- 1.00 mg Mn/liter
Standard deviation	- ± 0.01 mg Mn/liter

OPERATING RANGE

- Light path through length of flame 0.01-10 mg Mn/1.
- Light path through width of flame 0.1-100 mg Mn/1.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 279 nm
U.V. or Visible	- U.V.
Slit	- 4
Emission Chopper	- Off
Filter	- Out
Readout Mode	- "Concentration" "Repeat"
Signal Conditioning	- 10 Average
Recorder Response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8 units

REAGENTS

A. Manganese standard

Manganous sulphate monohydrate 3.076 g

Dissolve manganous sulphate in distilled water and make up to 1 liter.

1.00 ml = 1.00 mg Mn

PROCEDURE

1. Prepare manganese standards to cover the range of sample concentrations.
2. Aspirate distilled water blank and zero instrument.
3. Aspirate standards to calibrate instrument and adjust curvature (see general AAS outline).
4. Aspirate sample and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual

POTASSIUM BY ATOMIC EMISSION

PRECISION

Level	- 5.0 mg K/liter
Standard deviation	- ± 0.16 mg K/liter

OPERATING RANGE

- Light path through length of flame 0.05-10 mg K/1.
- Light path through width of flame 0.5-50 mg K/1.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 766.5 nm
U.V. or Visible	- Visible
Slit	- 4
Emission Chopper	- On
Filter	- In
Readout mode	- "Intensity" "Repeat"
Signal conditioning	- 10 Average
Recorder response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8 units

INTERFERENCES

Ionization interference from high concentrations of sodium is possible in some samples. This interference suppresses observed potassium levels but can be eliminated by preparing potassium standards containing sodium at the same level as samples.

REAGENTS

A. Potassium standard

Potassium chloride 1.907 g

Dissolve potassium chloride in distilled water and make to 1 liter.

1.00 ml = 1.00 mg K

PROCEDURE

1. With instrument settings as listed use a potassium hollow cathode lamp to adjust the monochromator to the principle emission line at 766.5 nm (383 on monochromator dial). Remove lamp.

2. Aspirate distilled water blank and zero instrument.

3. Aspirate standards and adjust concentration dial to give required readout. In "Intensity" mode the "onset" and "magnitude" potentiometers have no effect. If the calibration curve is non-linear this can be remedied by careful adjustment of ratio of flame gases and burner height.

4. Aspirate samples and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual.

SODIUM BY ATOMIC ABSORPTION

PRECISION

Level	- 20.0 mg Na/liter
Standard deviation	- ± 0.2 mg Na/liter

OPERATING RANGE

- Light path through length of flame 0.01-5 mg Na/l.
- Light path through width of flame 0.05-50 mg Na/l.

INSTRUMENT SETTINGS

- For Perkin Elmer Model 403.

Wavelength	- 590 nm
U.V. or Visible	- Visible
Slit	- 3
Emission Chopper	- Off
Filter	- Out
Readout mode	- "Concentration" "Repeat"
Signal conditioning	- 10 Average
Recorder response	- 1
Fuel - Acetylene	- 28 units
Oxidant - Air	- 37 units
Burner Height	- 8 units

INTERFERENCES

There appear to be no significant interferences for sodium determination in freshwater.

REAGENTS

A. Sodium standard

Sodium chloride 2.542 g

Dissolve sodium chloride in distilled water and make to 1 liter.

1.00 ml - 1.00 mg Na

PROCEDURE

1. Prepare sodium standards to cover the range of sample concentration.

2. Aspirate distilled water blank and zero instrument.

3. Aspirate standards to calibrate instrument and adjust curvature (see general AAS outline).

4. Aspirate samples and record digital display when stable.

REFERENCE

Perkin Elmer Methods Manual.

SPECIFIC CONDUCTANCE

PRECISION

Level	- 150 µS/cm
Standard deviation	- ± 0.01 µS/cm

OPERATING RANGE - This is dependent on instrument used and cell constant of electrode.

Cell constant = 0.1 cm^{-1} (0.1 µS-150 mS)
Cell constant = 1.0 cm^{-1} (1 µS-1500 mS)

SAMPLE CONDITION
- Unfiltered.
- Stored at 5 C less than 30 days.

METHOD PRINCIPLE

Solutions of dissociated ions can conduct electrical current and like metals obey ohms law. The resistance (R) of electrolyte solutions is a function of electrode surface area (A) distance between electrodes (ℓ) solution temperature, and the concentration and nature of ion in solution. For given solution and temperature:

$$R(\text{ohms}) \propto \frac{\ell \text{ cm}}{(A_{cm}2)} \qquad (1)$$

Resistance is measured with platinum electrodes (coated with platinum black) using a bridge circuit with an alternating current supply at 60 -3000 Hz to minimize polarization effects.

A more commonly used measurement than resistance is conductance (L) measured in Siemens (S) where:

$$L_{(\text{Siemens})} = \frac{1}{R(\text{ohms})} \qquad (2)$$

From equations (1) and (2) then

$$L \propto \frac{(A)}{\ell} \qquad (3)$$

or $$L = K\left(\frac{A}{\ell}\right) \qquad (4)$$

where K is a proportionality constant termed the specific conductance and is the observed conductance of a solution measured with a cell of unspecified geometry (A and ℓ) normalized to the conductance one would obtain with a cell having A = 1 cm^2 and ℓ = 1 cm. K is usually measured at 25 C.

From (4) $$K_{(S \cdot cm^{-1})} = \frac{\ell\ (cm)}{A\ (cm^2)} \cdot L\ (Siemens) \qquad (5)$$

$\frac{\ell}{A}$ is termed the cell constant which varies with the geometry of the cell used (area of electrode plates (A) and their separation (ℓ)). Commercially prepared cells are labelled with an approximate cell constant but must be calibrated before use by measuring the conductance (L) of a KCl solution of known specific conductance (K) at 25 C and calculating $\left(\frac{\ell}{A}\right)$ from equation (5). Specific conductance of samples is obtained by measuring (L) for samples at 25 C and calculating using equation (5) and the calibrated cell constant.

REAGENTS

A. Distilled and deionized water - Specific conductance (25 C) not to exceed 10 µS cm^{-1}.

This can be prepared by passing distilled water through a mixed bed ion exchange resin.

B. Potassium chloride - 0.2 N

Dissolve 14.912 g of dry KCl in distilled and deionized water and make to 1 liter.

C. Potassium chloride - 0.01 N

Dilute 50.0 ml of 0.2 N KCl to 1 liter with distilled water.

Specific conductance (25 C) = 1411 µS cm^{-1} (see Kortum et al.).

SPECIFIC CONDUCTANCE

PRECISION

Level	- 150 µS/cm
Standard deviation	- ± 0.01 µS/cm

OPERATING RANGE - This is dependent on instrument used and cell constant of electrode.

Cell constant = 0.1 cm^{-1} (0.1 µS-150 mS)
Cell constant = 1.0 cm^{-1} (1 µS-1500 mS)

SAMPLE CONDITION
- Unfiltered.
- Stored at 5 C less than 30 days.

METHOD PRINCIPLE

Solutions of dissociated ions can conduct electrical current and like metals obey ohms law. The resistance (R) of electrolyte solutions is a function of electrode surface area (A) distance between electrodes (ℓ) solution temperature, and the concentration and nature of ion in solution. For given solution and temperature:

$$R(\text{ohms}) \propto \frac{\ell \text{ cm}}{(A_{cm}2)} \qquad (1)$$

Resistance is measured with platinum electrodes (coated with platinum black) using a bridge circuit with an alternating current supply at 60 -3000 Hz to minimize polarization effects.

A more commonly used measurement than resistance is conductance (L) measured in Siemens (S) where:

$$L_{(\text{Siemens})} = \frac{1}{R(\text{ohms})} \qquad (2)$$

From equations (1) and (2) then

CHLOROPHYLL-a FLUOROMETRIC METHOD

PRECISION

Level	- 1.2 µg Chlorophyll-a/liter
Standard deviation	- ± 0.12 µg Chlorophyll-a/liter

OPERATING RANGE

- Depends on volume of liquid available or capable of being filtered. Minimum detectable quantity of chlorophyll-a in 10 ml of 90% acetone ranges from 0.01-0.05 µg depending on the individual instrument used.

SAMPLE CONDITION

- Stored at 5 C less than 48 hours.
- Do not acidify or freeze water samples.
- Particulate collected on Whatman GF/C. 4.25 cm glass fiber papers. Filters can be frozen and stored in dark indefinitely.

METHOD PRINCIPLE

- Particulate matter collected on a glass fiber paper is mechanically ground in an acetone medium. Plant pigments appear to be quantitatively extracted into the acetone.

  Solutions of photosynthetic pigments when excited by light in the blue region, emit strongly in the red region. For chlorophyll-a maximum excitation is produced by radiation between 430-450 nm with an emission maximum between 650 and 675 nm. In the method described a fluorometer, equipped to provide excitation energy around 440 nm, and detect fluorescent emission at around 660 nm is used. The signal output of the instrument is in arbitrary units and it must be calibrated using a chlorophyll-a solution of known concentration.

Since standard chlorophyll-a solutions are not easily obtained one must prepare a stock chlorophyll solution and calibrate it using a spectrophotometer and the following calibration equation of Strickland and Parsons (1965):

$$C = (11.6)\ E_{665} - (1.31)\ E_{645} - (0.41)\ E_{630}$$

where E_{665}, E_{645}, and E_{630} are absorbance values for a 1.0 cm path length, corrected for absorbance at 750 nm and blank absorbances at 665, 645 and 630 nm. Blank absorbances are also corrected for any absorbance at 750 nm.

C = mg Chlorophyll/liter 90% acetone

REAGENTS

A. Acetone 90% v/v.

Mix 900 ml of acetone (acid free) with 100 ml of distilled water.

B. Chlorophyll-a stock solution.

Using 90% acetone, extract green lettuce leaf to obtain 25 ml of chlorophyll solution which is deep green in colour. Store in the dark at 5 C.

APPARATUS

1. Tissue grinder - 15 ml volume

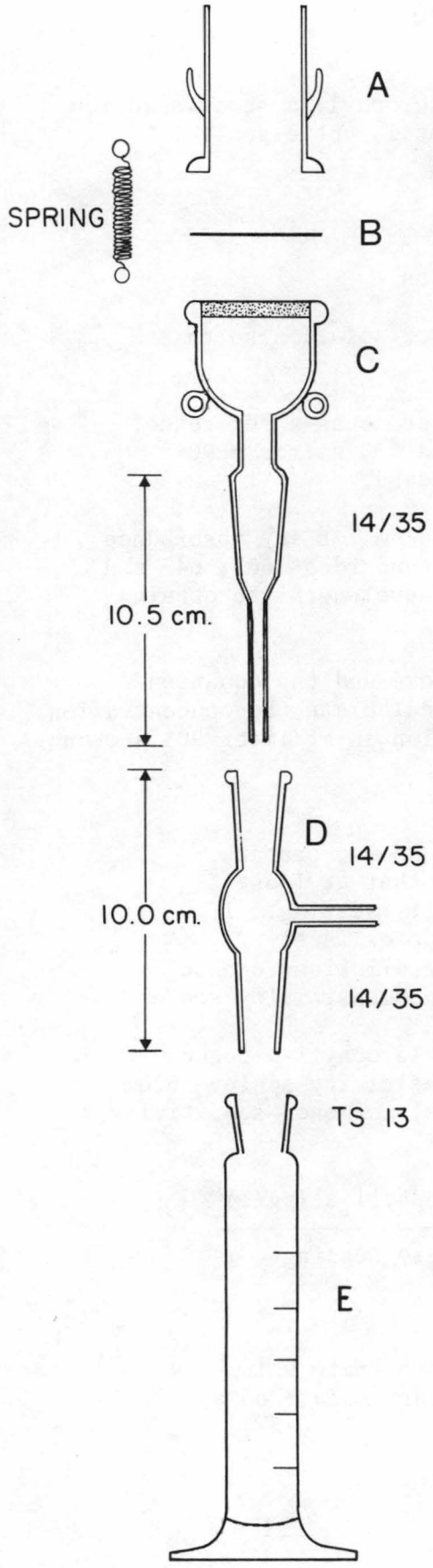
Scientific Glass Apparatus Co. Inc #JT-4110 or equivalent. These tissue grinders are designed for manual use but can be modified so that the pestle can be driven with a 1/4" electric drill. The glass handle on the pestle is cut off and a stainless steel shaft inserted down into the hollow pestle and fixed in place with epoxy cement.

2. Electric drill - 1/4"-variable speed.

3. Filtration assembly - All glass, small surface area (Figure 12).

4. Turner Model III Fluorometer equipped with:

ALL GLASS FILTRATION ASSEMBLY



(A) GLASS FUNNEL - 50 ml.

(B) GLASS FIBER FILTER

(C) SINTERED GLASS FILTER SUPPORT WITH GROUND GLASS FILTER

(D) GLASS ADAPTER WITH SIDE ARM TO VACUUM AND GROUND GLASS FITTINGS

(E) 10 ml. GRADUATED CYLINDER

Figure 12

A. Primary filter - Kodak Wratten No. 76
B. Secondary filter - Kodak Wratten No. 26
C. Blue Source Lamp - Turner No. 110-853
D. Low sensitivity door

5. Spectrophotometer to calibrate the chlorophyll-a stock solution. A scanning model is to be preferred but is not essential.

PROCEDURE

Calibration of standard Chlorophyll solution

1. Prepare a suitable dilution of the stock solution so that E_{665} is about 0.6 for a 1.0 cm path length.

2. Using a spectrophotometer, with 90% acetone as a reference obtain absorbances at 750, 665, 645 and 630 nm for a 90% acetone blank and the chlorophyll standard.

3. Correct absorbances at 665, 645 and 630 nm for any absorbance at 750 nm and correct absorbances of standard at 665, 645 and 630 nm for blank absorbances at these wavelengths to obtain E_{665}, E_{645} and E_{630}.

4. From the extinction values obtained above and the equation given in the method principle section calculate the concentration of chlorophyll-a in the standard solution in mg/liter 90% acetone.

Calibration of Fluorometer

1. Using the standard solution calibrated above, prepare accurate dilutions with 90% acetone so that at least 3 readings are obtained on each sensitivity setting. If possible the readings should come at approximately 25, 50, and 75 divisions. Several of these dilutions can be used to obtain readings on more than one sensitivity scale.

2. Plot graphs of scale reading against chlorophyll-a concentration (µg/l) in acetone, for each sensitivity scale. From the graphs obtain a scale factor (slope) for each sensitivity scale

$$\text{i.e. } F_{(1x,3x,10x,30x)} = \frac{\text{Chlorophyll-a (µg/l)}}{\text{Scale Reading}}$$

Analysis of Samples

1. Agitate water sample and measure an appropriate volume (V_1) into a graduated cylinder and collect particulate on a

glass fiber filter. Vacuum not to exceed 15 inches of Hg.

2. If filters are not to be processed immediately, store in the dark at -10 C.

3. Place filter paper in tissue grinder with 2-3 ml of 90% acetone.

4. Grind for 1 minute taking care not to lose any of the solvent through splashing.

5. Transfer the paper and acetone slurry to an all-glass filtration assembly using 2-3 ml of 90% acetone to rinse out the tissue grinder. Collect acetone filtrate in a 10 ml graduated cylinder.

6. Wash the filter assembly with 2-3 ml of acetone.

7. Make the acetone extract up to 10 ml or appropriate volume (V_2).

8. Measure fluorescence to nearest 0.2 units.

Note: It is essential that the time from extraction to measurement of fluorescence be short with minimum exposure to light. We have found extracted pigments to be quite stable at room temperature if maintained in total darkness.

CALCULATIONS

1. Chlorophyll-a concentrations (µg/l) in the original water sample can be obtained from the following equation:

$$\text{Chlorophyll-a } (\mu g/\text{liter}) = F \times (\text{fluorometer reading}) \times \left(\frac{1000}{V_1}\right) \times \left(\frac{V_2}{1000}\right)$$

where V_1 (ml) is the volume of water filtered and V_2 (ml) is the volume of 90% acetone into which chlorophyll-a is extracted.

REFERENCES

1. Strickland, J.D.H. and Parsons, T.R. (1965). A manual of sea water analysis. Fish. Res. Bd. Canada. 125:203 pp.

2. Yentsch, C.S. and Menzel, D.W. (1963). A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep Sea Res. 10:221-31.

OXYGEN DISSOLVED

PRECISION

Level - 10.0 mg 0_2/liter

Standard deviation - $\pm$ 0.2 mg 0_2/liter

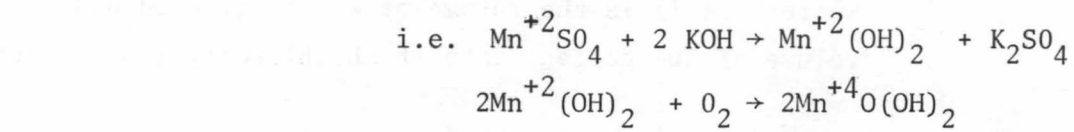
OPERATING RANGE - 0-20 mg 0_2 per liter

SAMPLE CONDITION

- Sampling technique is critical for this method - see section on sampling methods.
- Sample is collected in specially designed glass bottles having a capacity of 300 ml and ground glass stoppers with conical ends to prevent trapping air bubbles.
- Sample must be collected without exposure to atmosphere.
- Stored in sealed bottle at temperature sampled.
- Less than 1 hour old.
- Free of chemical reductants.

METHOD PRINCIPLE

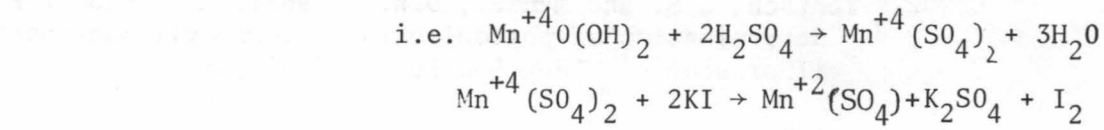
- Manganous sulphate reacts with potassium or sodium hydroxide to give a white precipitate of manganous hydroxide. In the presence of oxygen, brown manganic basic oxide is formed.



i.e. $Mn^{+2}SO_4 + 2\ KOH \rightarrow Mn^{+2}(OH)_2 + K_2SO_4$

$2Mn^{+2}(OH)_2 + O_2 \rightarrow 2Mn^{+4}O(OH)_2$

Addition of sulphuric acid dissolves the brown manganic oxide yielding manganic sulphate which reacts instantly with iodide to yield iodine.



i.e. $Mn^{+4}O(OH)_2 + 2H_2SO_4 \rightarrow Mn^{+4}(SO_4)_2 + 3H_2O$

$Mn^{+4}(SO_4)_2 + 2KI \rightarrow Mn^{+2}(SO_4) + K_2SO_4 + I_2$

In effect, oxygen oxidizes Mn^{+2} to Mn^{+4} and Mn^{+4} oxidizes I^- to I_2. One equivalent of oxygen yields one equivalent of iodine. Iodine is then determined titrimetrically with starch as an end point indicator.

Interferences occur from other oxidants in the sample which will liberate iodine. The effect of nitrite can be eliminated with the addition of sodium azide.

REAGENTS

A. Manganese sulphate

Dissolve 480 g of $MnSO_4 \cdot 4H_2O$, 400 g of $MnSO_4 \cdot 2H_2O$, or 364 g $MnSO_4 \cdot H_2O$ in distilled water. Filter and dilute to 1 liter.

B. Alkaline iodide azide

Dissolve 500 g sodium hydroxide, or 700 g potassium hydroxide, and 135 g sodium iodide, or 150 g potassium iodide, in distilled water and dilute to 1 liter. To this add 10 g sodium azide dissolved in 40 ml of distilled water.

C. Sulphuric acid

Concentrated (S.G. 1.84).

D. Starch indicator

Bring 100 ml distilled water to boiling. Prepare a paste of 1 g of potato starch in a few ml of distilled water. Add the boiling water to the paste. Cool and store at 5 C. When a violet or grey tinge is noted discard.

E. Sodium thiosulphate

Prepared from commercially available concentrated standard solutions. Dilute concentrated standard to 0.025 N with distilled water. Prepare fresh weekly. Store at 5 C.

APPARATUS

1. Dissolved oxygen bottles to contain 300 ml. These must be calibrated if second decimal place accuracy is necessary.

2. Dispensing pipettes to deliver 2 ml. One for each of: manganese sulphate reagent (A), alkaline iodide reagent (B), and sulphuric acid (C). It is important that each pipette is used for only one reagent and that the caps for the reagent bottles are not mixed up.

3. Burette - Type used depends on accuracy required and levels to be measured.

4. Magnetic stirring plate with light source in base.

5. 200 ml pipette.

PROCEDURE

1. To the water sample in a calibrated dissolved oxygen bottle add 2 ml of manganese sulphate reagent (A) followed by 2 ml of alkaline-iodide-azide reagent (B). These reagents should be injected well below the liquid surface with sample bottles being open for as short a time as possible.

2. Re-stopper the bottle taking care not to entrap air bubbles, and agitate vigorously to mix reagents.

3. When the precipitate which forms settles, agitate again.

4. When the precipitate has settled to the bottom a second time, remove the stopper and inject 2 ml of sulphuric acid (C). Re-stopper the bottle and mix gently.

   It is essential in the above manipulations that none of the precipitate, or iodine arising from acidification of the precipitate, escapes from the bottle.

5. Remove 200.0 ml of solution from the bottle and add to a 250 ml Erlenmeyer flask.

6. If the colour of the sample indicates much iodine present (deep orange) begin titrating the sample with 0.025 N thiosulphate (E) until colour intensity is reduced to a pale straw yellow colour. Add several drops of starch solution and titrate until first disappearance of the blue colour.

7. If the colour of the sample is pale yellow to begin with, add starch indicator before beginning titration.

CALCULATIONS

A. 1.0 ml of 0.025 N thiosulphate is equivalent to 25×10^{-6} equivalents of iodine which is in turn equivalent to 25×10^{-6} equivalents of oxygen.

$$25 \times 10^{-6} \text{ equivalents of } O_2 = 200 \times 10^{-6} \text{ g } O_2$$

If volume titrated is 200 ml then 1.0 ml of 0.025 N thiosulphate titrant is equivalent to 1.0 mg/liter of O_2 in the original sample.

If oxygen concentrations to 0.1 mg/liter are sufficient the above equality is sufficient to determine dissolved oxygen.

B. If greater accuracy is required one must take into account the dilution effect of the first two reagent additions. This dilution effect is $\frac{V-4}{V}$ where V is the volume in ml of the oxygen bottle used. Oxygen values obtained by assuming 1.00 ml of 0.025 N thiosulphate equivalent to 1.00 mg/liter dissolved oxygen should then be multiplied by $\frac{V}{V-4}$ to correct for dilution.

REFERENCE

1. American Public Health Association (1965). Standard methods for the examination of water and wastewater. 12th ed. APHA, New York, N.Y. 769 p.

TOTAL DISSOLVED SOLIDS - (TDS)

PRECISION

Level	- 500 mg/liter
Standard deviation	- ± 10 mg/liter

OPERATING RANGE - 10 mg per liter to saturation level of salts present.

SAMPLE CONDITION
- Stored at 5 C.
- Less than 30 days old.
- Filter through Whatman GF/C.

METHOD PRINCIPLE - A known volume of water (usually 100 ml) is placed in a pre-weighed evaporating dish and evaporated without boiling. The solid residue is determined by re-weighing the evaporating dish. Since a number of elements form salts which crystallize as hydrates (over-estimate of TDS), or salts which decompose and lose weight during evaporation (under-estimate), this test is of empirical value only and does not correctly measure the weight of dissolved salts present. In waters with specific conductance less than 100 µmhos/cm weighing errors are significant and TDS values become meaningless unless special precautions are taken.

APPARATUS

1. Glass evaporating dishes to contain 100 ml.
2. Oven or infra-red heat lamps to evaporate water samples. Temperature should be just above 100 C.

PROCEDURE

1. Clean evaporating dishes with distilled water and dry 1 hour at 104 C.

2. Cool dishes in desiccator and weigh to obtain tare.

3. Add 100.0 ml of filtered sample to each dish.

4. Place samples in oven at 104 C for 16 hours.

5. Cool dishes in desiccator and re-weigh.

CALCULATIONS

1. Subtract final weight from tare weight and express as mg of dissolved solids.

2. Multiply the above weight by 10 to yield dissolved solids in mg/liter.

REFERENCE

1. American Public Health Association (1965). Standard methods for the examination of water and wastewater. 12th ed. APHA, New York, N.Y. 769 p.

HYDROGEN ION CONCENTRATION - pH

PRECISION

Level	- 8.49
Standard deviation	- $\pm$ 0.02

OPERATING RANGE - 1-14 pH units

SAMPLE CONDITION
- Unfiltered, stored at 5 C.
- Less than 24 hours old.
- Sample brought to room temperature before analysis.

METHOD PRINCIPLE

The pH of an aqueous solution is defined operationally by the following equation:

$$pH = pHs + \frac{(E-E_s)}{0.000198\ T}$$

where pHs is the assigned pH of a buffer used to standardize the system. E and E_s are the EMF's of a suitable cell arrangement with unknown and buffer respectively and T is the temperature (Kelvin). Usually the cell arrangement consists of a glass electrode with an electrolyte bridge to a saturated calomel electrode. Cell EMF is measured with a high impedance voltmeter with usual response being 59 mV per pH unit at 25 C.

The glass electrode assembly is fragile and requires careful use and frequent calibration to obtain meaningful results. Since electrode response may be non-linear it is important to standardize using buffers having a pH close to and preferably bracketing the pH of samples. Suitable time must be allowed for the electrode to come to equilibrium with solutions before a reading is taken. Care must be taken to avoid electrostatic effects such as occur when using Teflon ^{R} or polypropylene laboratory ware, or from nylon clothing worn by the analyst.

REAGENTS

A. Standard buffer solutions

Commercially prepared - to cover range of pH to be measured.

APPARATUS

1. Glass electrode.
2. Calomel reference electrode.
3. Combination electrode (combines 1 and 2 above in a single probe. Either 1 and 2, or 3 are required, not both).
4. pH meter - Orion Model 801, or other instrument suitable for needs.

PROCEDURE

1. Turn on pH meter and allow to warm up.
2. Be sure electrodes have been pre-soaked according to manufacturer's specifications and that reference electrode is filled.
3. Place electrodes in appropriate buffer, measure buffer temperature and adjust temperature compensation on pH meter.
4. Adjust pH meter to read buffer pH level.
5. Rinse electrodes with distilled water and place in a second buffer solution, several pH units different than the first buffer solution, to check linearity of response.
6. Rinse electrodes and immerse in samples taking care to adjust temperature compensation if necessary.
7. Read pH.

CALCULATIONS

No calculations required as instrument reads directly in pH units.

PARTICULATE SOLIDS

PRECISION

Level	- 8 mg/liter
Standard deviation	- 0.5 mg/liter

OPERATING RANGE

- Depends on sample volume available or possible to filter. If possible, volume filtered should be such to give 2.5 mg suspended matter per filter paper.

SAMPLE CONDITION

- Stored at 5 C.
- Less than 48 hours old.
- Particulate collected on either Whatman GF/C (>5 mg/liter) or Millipore matched pair filters (<5 mg/liter).

METHOD PRINCIPLE

Particulate material from a sample is collected on a pre-weighed filter, the pore size of which determines what is considered "Suspended Solids". Particulate and paper are dried and re-weighed, the increase in weight being attributed to suspended solids.

For low levels of suspended matter the fact that a finite amount of sample water (plus its dissolved solids content) remains in the paper and the fact that part of the filter paper may be solubilized or abraded away in the filtration process, necessitates special precautions. These effects can best be overcome using matched weight filter paper pairs (Millipore AAWP047OW). These papers are guaranteed to be within 0.1 mg of each other and are placed in tandem in the filtration assembly. The second paper is used as the tare for the first.

APPARATUS

1. Drying oven set at 104 C.
2. Whatman GF/C 4.25 cm filter papers or Millipore (AAWP047OW) matched pair filters.
3. Millipore filtration assembly.

PROCEDURE

1. Dry filter papers at 104 C for 1 hour. (Only GF/C papers require this step.)

2. Weigh filters to the nearest 0.1 mg. (Only GF/C papers require this step.)

3. Agitate sample and measure suitable volume into a graduated cylinder. Collect particulate on filter with a vacuum less than 15 inches of Hg.

4. Dry filter papers 1 hour at 104 C.

5. Re-weigh papers plus particulate to the nearest 0.1 mg.

CALCULATIONS

1. For GF/C papers* subtract the initial weight from final weight to get weight of suspended matter in 100 ml. Multiply by $\frac{1000}{\text{ml of sample}}$ to get suspended solids in mg/liter.

2. For Millipore matched pair filters use the blank paper weight for the tare value and calculate as above.

NOTE: * The sample collected on this filter paper can be used for suspended phosphorus analysis.

REFERENCES

1. American Public Health Association (1965). Standard methods for the examination of water and waste water. 13th ed. APHA, New York, N.Y. 769 p.

2. Millipore Corp. (1973). Suspended solids analysis. Application Procedure. AP312.

METHODS FOR THE ANALYSIS OF FRESHWATER (1974)

By

M. P. Stainton, M.J. Capel and F.A.J. Armstrong

To receive additions for this publication as they become available, complete this form and send it to:

The Librarian
Department of Environment
Freshwater Institute
501 University Crescent
Winnipeg, Manitoba, Canada
R3T 2N6

NAME _____

ADDRESS _____

_____