Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes
Edited by Jamie Bartram and Richard Ballance
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Chapter 7 - PHYSICAL AND CHEMICAL ANALYSES

This chapter was prepared by R. Ballance

In compiling this chapter, care has been taken to avoid procedures that require delicate or sophisticated equipment. For many of the variables for which methods of analysis are presented here, further information relating to their selection and inclusion in water quality monitoring and assessment programmes (such as their environmental significance, normal ranges of concentrations, and behaviour in the aquatic environment) can be found in the companion guidebook *Water Quality Assessments*.

7.1 Preparation and use of chemical reagents

The following general rules should be followed in the preparation and use of chemical reagents. The best quality chemical reagents available should be used - normally “analytical reagent grade”. For most laboratory purposes, water distilled in a borosilicate glass still or a tin still will be satisfactory. For preparing some reagents, dilution water requires special treatment, such as a second distillation, boiling to drive off CO_2 or passing through a mixed bed ion exchanger. Where such special treatment is necessary, this is stated.

Recipes for the preparation of reagents usually give directions for the preparation of a 1-litre volume. For those reagents that are not used often, smaller volumes should be prepared by mixing proportionally smaller quantities than those given in the recipe. Where a working standard or working solution is to be made by dilution of a stock solution, no more of the stock solution should be prepared than will be used within the next six months. Furthermore, only the amount of stock solution necessary to meet the immediate need for a working or standard solution should be diluted at one time.

Reagent solutions should be kept in tightly stoppered glass bottles (except where they are incompatible with glass, as with silica solutions). Rubber or neoprene stoppers or screw tops with gaskets are suitable, provided that the reagents do not react with these materials. For short-term storage, for example during a field trip of a week or two, small quantities of reagent may be transported in plastic bottles with plastic screw caps.

Reagent containers should always be accurately labeled with the name of the reagent, its concentration, the date that it was prepared and the name or initials of the person who prepared it.

Table 7.1 Characteristics of three common acids

Characteristic	Hydrochloric acid (HCl)	Sulphuric acid (H_2SO_4)	Nitric acid (HNO_3)
Relative density of reagent grade concentrated acid	1.174-1.189	1.834-1.836	1.409-1.418
Percentage of active ingredient in concentrated acid	36-37	96-98	69-70
Molarity of concentrated acid (mol l^{-1})	11-12	18	15-16

Table 7.2 Volume (ml) of concentrated acid needed to prepare 1 litre of dilute acid

Desired strength (mol l^{-1})	HCl	H_2SO_4	HNO_3
6 mol l^{-1}	500	333.3	380
1 mol l^{-1}	83	55.5	63.3
0.1 mol l^{-1}	8.3	5.6	6.3

To prepare a 0.1 mol l^{-1} solution, measure 16.6 ml of 6 mol l^{-1} solution and dilute it to 1 litre.

To prepare a 0.02 mol l^{-1} solution, measure 20.0 ml of 1 mol l^{-1} solution and dilute it to 1 litre.

Hydrochloric, sulphuric and nitric acids and sodium hydroxide are in common use in the analytical laboratory. The characteristics of the acids are given in Table 7.1, and directions for preparing dilutions that are frequently needed in Table 7.2. Preparation of four different concentrations of sodium hydroxide is detailed in Table 7.3. Other concentrations may be made by appropriate dilution with distilled water.

7.2 Alkalinity

The alkalinity of water is its capacity to neutralise acid. The amount of a strong acid needed to neutralise the alkalinity is called the total alkalinity, *T*, and is reported in mg l^{-1} as $CaCO_3$ The alkalinity of some waters is due only to the bicarbonates of calcium and magnesium. The pH of such water does not exceed 8.3 and its total alkalinity is practically identical with its bicarbonate alkalinity.

Table 7.3 Preparation of uniform solutions of sodium hydroxide

Desired concentration of NaOH solution (mol l^{-1})	Weight (g) of NaOH to prepare 1 litre of solution	Volume (ml) of NaOH (15 mol l^{-1}) to prepare 1 litre of solution
15	600	1,000
6	240	400
1	40	67
0.1	4	6.7

Water having a pH above 8.3 contains carbonates and possibly hydroxides in addition to bicarbonates. The alkalinity fraction equivalent to the amount of acid needed to lower the pH value of the sample to 8.3 is called phenolphthalein alkalinity, *P* This fraction is contributed

by the hydroxide, if present, and half of the carbonate (the pH range of 8.3 is approximately that of a dilute bicarbonate solution).

The stoichiometric relationships between hydroxide, carbonate and bicarbonate are valid only in the absence of significant concentrations of other weak anions. This applies especially to the alkalinity (and acidity) of polluted waters and wastewaters.

Principle

Alkalinity is determined by titration of the sample with a standard solution of a strong mineral acid. The procedure given uses two colour indicators to determine the end-points in a titration. It is satisfactory for most routine applications. If high levels of accuracy are essential, electrometric titration is preferred, and must also be used when the colour, turbidity or suspended matter in a sample interferes seriously with the determination by the indicator method. Low alkalinities (below approximately 10 mg l^{-1}) are also best determined by electrometric titration.

Titration to the end-point of pH 8.3 determines the phenolphthalein alkalinity and to the end-point of pH 4.5 the total alkalinity. The pH to which the titration for total alkalinity should be taken lies between 4 and 5, depending on the amount of the alkalinity and free carbon dioxide in the sample. For practical purposes the end-point of pH 4.5 (indicated by methyl orange) gives sufficiently accurate results.

Wherever possible, the titration should be carried out on filtered water at the point of sampling. If this is not possible, the sampling bottle must be completely filled and the alkalinity determined within 24 hours.

Interferences

Colour, turbidity and suspended matter may interfere with the visual titration by masking the colour change of an indicator. Turbidity and suspended matter can be eliminated by filtration. The colour of the sample can be reduced by activated carbon and filtration. Free chlorine may affect the indicator colour response and should be removed by the addition of a small amount (usually one drop) of 0.1 mol l^{-1} sodium thiosulphate solution.

The presence of finely divided calcium carbonate suspensions in some natural waters may cause a fading end-point and should be removed by filtration. Silicate, phosphate, borate, sulphide and other anions of weak inorganic and organic acids (e.g. humic acids) will be included in the total alkalinity estimate. They do not interfere with the titration but can influence the validity of stoichiometric relationships.

Apparatus

√ White porcelain dish, 200-ml capacity, or conical flask.

√ Burette, 25 ml or 50 ml.

Reagents

√ Carbon dioxide-free distilled water must be used for the preparation of all stock and standard solutions. If the distilled water has a pH lower than 6.0, it should be freshly boiled for 15 minutes and cooled to room temperature. Deionised water may be substituted for distilled water provided that it has a conductance of less than 0.2 mS m^{-1} and a pH greater than 6.0.

√ Sodium carbonate, 0.05 mol l^{-1} Dissolve in water 5.300 g anhydrous sodium carbonate previously oven-dried for 1 hour at 250-300 °C and make up to 1 litre.

√ Sulphuric acid, 0.05 mol l^{-1} Dilute 3.1 ml sulphuric acid (density 1.84) to 1 litre. Standardise against 0.05 mol l^{-1} sodium carbonate using methyl orange indicator. If required, this solution may be diluted to 0.01 mol l^{-1}

√ Phenolphthalein indicator. Dissolve 0.5 g of phenolphthalein in 50 ml of 95 per cent ethanol, and add 50 ml of distilled water. Add a dilute (e.g. 0.01 to 0.05 mol l^{-1}) carbon dioxide-free solution of sodium hydroxide one drop at a time, until the indicator turns faintly pink.

√ Methyl orange indicator. Dissolve 0.05 g of methyl orange in 100 ml water.

√ Mixed indicator. Dissolve 0.02 g of methyl red and 0.1 g of bromocresol green in 100 ml of 95 per cent ethanol. This indicator is suitable over the pH range 4.6-5.2.

Procedure

1. Mix 100 ml of the sample with two or three drops of phenolphthalein indicator in the porcelain basin (or in a conical flask over a white surface). If no colour is produced, the phenolphthalein alkalinity is zero. If the sample turns pink or red, determine the alkalinity by titrating with standard acid until the pink colour just disappears. In either case, continue the determination using the sample to which phenolphthalein has been added.

2a. Add a few drops of methyl orange indicator. If the sample is orange without the addition of acid, the total alkalinity is zero. If the sample turns yellow, titrate with standard acid until the first perceptible colour change towards orange is observed.

2b. The determination by means of mixed indicator is done in the same way as with methyl orange. The mixed indicator yields the following colour responses: above pH 5.2, greenish blue; pH 5.0, light blue with lavender grey; pH 4.8, light pink-grey with a bluish cast; pH 4.6, light pink.

Any difficulty experienced in detecting the end-point may be reduced by placing a second 100-ml sample with the same amount of indicator (phenolphthalein, methyl orange or mixed indicator) in a similar container alongside that in which the titration is being carried out. Another way to provide a standard end-point is to prepare buffer solutions to which are added indicators in the same amount as in an alkalinity titration.

Calculation

Phenolphthalein alkalinity as $CaCO_3$

$$P = \frac{100{,}000 \times A \times M}{V} \text{ mg l}^{-1}$$

Total alkalinity as $CaCO_3$

$$T = \frac{100{,}000 \times A \times M}{V} \text{ mg l}^{-1}$$

$$T = \frac{100{,}000 \times B \times M}{V} \text{mg l}^{-1}$$

where A = volume of standard acid solution (ml) to reach the phenolphthalein end-point of pH 8.3
B = volume of standard acid solution (ml) to reach the end-point of methyl orange or mixed indicator
M = concentration of acid (mol l^{-1})
V = volume of sample (ml)

Using 100 ml of sample and 0.01 mol l^{-1} standard acid solution, the numerical value of alkalinity as mg l^{-1} $CaCO_3$ is 10 times the number of millilitres of titrant consumed.

Precision

The precision of visual titration is estimated at 2-10 per cent for alkalinity between 50 and 5 mg l^{-1}.

7.3 Aluminium

Although aluminium is among the most abundant elements in the earth's crust, it is present in only trace concentrations in natural waters. Because it occurs in many rocks, minerals and clays, aluminium is present in practically all surface waters, but its concentration in waters at nearly neutral pH rarely exceeds a few tenths of a milligram per litre. In addition, in treated water or wastewater, it may be present as a residual from the alum coagulation process. The median concentration of aluminium in river water is reported to be 0.24 mg l^{-1} with a range of 0.01 to 2.5 mg l^{-1}.

Sample handling

Because aluminium may be lost from solution to the walls of sample containers, samples should be acidified with 1.5 ml of concentrated nitric acid per litre of sample before storage in plastic containers. If the pH is not less than 2 after the addition of acid, more nitric acid should be added. If only soluble aluminium is to be determined, filter a portion of unacidified sample through a 0.45 µm membrane filter, discard the first 50 ml of filtrate and use the succeeding filtrate, after acidification, for the determination. Do not use filter paper, absorbent cotton or glass wool for filtering any solution that is to be tested for aluminium because these materials will remove most of the soluble aluminium.

Principle

Dilute aluminium solutions, buffered to a pH of 6.0 and with Eriochrome cyanine R dye added, produce a red to pink complex with a maximum absorption at 535 nm. The intensity of the developed colour is influenced by the aluminium concentration, reaction time, temperature, pH, alkalinity and the concentration of other colours in the sample. To compensate for colour and turbidity, the aluminium in one portion of the sample is complexed with EDTA to provide a blank. Interference by iron and manganese is eliminated by adding ascorbic acid. The limit of detection in the absence of fluoride and polyphosphates is approximately 6 mg l^{-1}.

Interferences

Negative errors are caused by both fluoride and polyphosphates because of their complexation with aluminium. When the fluoride concentration is constant, the percentage error decreases with increasing amounts of aluminium. The fluoride concentration is often known or can be readily determined, and fairly accurate results can therefore be obtained by adding the known amount of fluoride to a set of standards. A procedure is given for the removal of complex phosphate interference. Orthophosphate under 10 mg l^{-1} does not interfere. The interference caused by even small amounts of alkalinity is removed by acidifying the sample just beyond the neutral point of methyl orange. Sulphate does not interfere up to a concentration of 2,000 mg l^{-1}.

Apparatus

√ Colorimetric equipment. One of the following is required:
- Spectrophotometer: for use at 535 nm with a light path of 1 cm or longer.

- Filter photometer: equipped with a green filter, with maximum transmittance between 525 and 535 nm and with a light path of 1 cm or longer.

- Nessler tubes: matched set, tall form, 50-ml capacity.

√ Glassware: all glassware should be treated with warm 1+1 HCl and rinsed with aluminium-free distilled water to avoid errors due to materials adsorbed on the glass. The glassware should be well rinsed to remove all traces of the acid.

Reagents

√ Stock aluminium solution. Use either metal or salt to prepare a solution in which 1 ml contains 500 µg Al. Dissolve 500.0 mg aluminium metal in 10 ml concentrated HCl and dilute to 1,000 ml with distilled water. Alternatively, dissolve 8.791 g aluminium potassium sulphate, $AlK(SO_4).12H_2O$, in water and dilute to 1,000 ml. Adjust the weight of the chemical (8.791 g) by dividing it by the decimal fraction of assayed aluminium potassium sulphate in the reagent used.

√ Standard aluminium solution. Dilute 10.00 ml stock aluminium solution to 1,000 ml with distilled water (1.00 ml = 5.00 µg Al). Prepare fresh daily.

√ Sulphuric acid, H_2SO_4, 3 mol l^{-1} and 0.01 mol l^{-1}

√ Ascorbic acid solution. Dissolve 0.1 g ascorbic acid in water and make up to 100 ml in a volumetric flask. Prepare fresh daily.

√ Buffer reagent. Dissolve 136 g sodium acetate, $NaC_2H_3O_2.3H_2O$, in water, add 40 ml of 1 mol l^{-1} acetic acid and make up to 1 litre.

√ Stock dye solution. The stock dye solution is stable for at least a year and can be prepared from any one of several commercially available dyes. Suitable dyes, their suppliers and directions for preparing a solution are:

Solochrome cyanine R 200 (*Arnold Hoffman and Co, Providence, RI, USA*), or Eriochrome cyanine (*K & K Laboratories, Plainview, NY, USA*). Dissolve 100 mg in water and dilute to 100 ml in a volumetric flask. This solution should have a pH of about 2.9.

Eriochrome cyanine R (*Pfaltz & Bauer Inc., Stamford, CT, USA*). Dissolve 300 mg dye in about 50 ml water. Adjust pH from about 9 to about 2.9 with 1+1 acetic acid (approximately 3 ml will be required). Dilute with water to 100 ml.

Eriochrome cyanine R (*EM Science, Gibbstown, NJ, USA*). Dissolve 150 mg dye in about 50 ml water. Adjust pH from about 9 to about 2.9 with 1+1 acetic acid (approximately 2 ml will be required). Dilute with water to 100 ml.

√ Working dye solution. Dilute 10.0 ml of stock dye solution to 100 ml with distilled water in a volumetric flask. Working solution is stable for at least six months.

√ Methyl orange indicator solution. Dissolve 50 mg methyl orange powder in distilled water and dilute to 100 ml.

√ EDTA. Dissolve 3.7 g of the sodium salt of ethylenediaminetetraacetic acid dihydrate in water and dilute to 1 litre.

√ Sodium hydroxide, NaOH, 1 mol l^{-1} and 0.1 mol l^{-1}

Procedure

Preparation of calibration graph

1. Prepare standards and a blank by diluting 0 ml to 7.0 ml portions (0 to 7.0 µg Al) of the aluminium working standard to approximately 25 ml in 50-ml volumetric flasks. Add 1 ml of 0.01 mol l^{-1} H_2SO_4, and mix. Add 1 ml ascorbic acid solution and mix. Add 10 ml buffer solution and mix.

2. With a volumetric pipette add 5.00 ml working dye solution and mix. Immediately make up to 50 ml with distilled water. Mix and let stand for 5 to 10 minutes. The colour begins to fade after 15 minutes.

3. Read transmittance or absorbance on a spectrophotometer using a wavelength of 535 nm or a green filter providing maximum transmittance between 525 and 535 nm. Adjust the instrument to zero absorbance with the standard containing no aluminium. Plot the concentration of aluminium (µg Al in 50 ml final volume) against absorbance.

Sample treatment when there is no interference by fluoride or phosphate

4. Pour 25.0 ml of sample or a measured portion of sample diluted to 25 ml into a porcelain dish or flask, add a few drops of methyl orange indicator and titrate with 0.01 mol l^{-1} H_2SO_4 to a faint pink colour. Record the amount of acid used and discard the sample.

5. Pour 25 ml of sample into each of two 50-ml volumetric flasks. To each of these, add the amount of 0.01 mol l^{-1} sulphuric acid that was used in the titration plus 1 ml excess. To one of the samples add 1 ml EDTA solution; this will serve as a blank by complexing any aluminium present and compensating for colour and turbidity. To both samples add 1 ml ascorbic acid solution and mix. Add 10 ml buffer solution and mix.

6. With a volumetric pipette add 5.00 ml working dye solution and mix. Immediately make up to 50 ml with distilled water. Mix and let stand for 5-10 minutes. Set the instrument to zero absorbance or 100 per cent transmittance using the EDTA blank. Read transmittance or absorbance of the sample and determine aluminium concentration from the calibration curve.

Visual comparison

7. If photometric equipment is not available, prepare and treat standards and a sample, as described above, in 50-ml Nessler tubes. Make up to the mark with water and compare sample colour with the standards after a contact time of 5-10 minutes. A sample treated with EDTA is not needed when Nessler tubes are used. If the sample contains turbidity or colour, the Nessler tube method may result in considerable error.

Removal of phosphate interference

8. Add 1.7 ml of 3 mol l^{-1} H_2SO_4 to 100 ml of sample in a 200-ml Erlenmeyer flask. Heat on a hotplate for at least 90 minutes, keeping the temperature of the solution just below the boiling point. At the end of the heating period the volume of the solution should be about 25 ml. Add distilled water if necessary to keep it at, or slightly above, that volume.

9. Cool the solution and then bring the pH to 4.3 to 4.5 with NaOH (use 1 mol l^{-1} NaOH and then 0.1 mol l^{-1} as the end-point is approached). Monitor with a pH meter. Make up to 100 ml with distilled water, mix, and use a 25-ml portion for the test. Treat a blank in the same manner using 100 ml distilled water and 1.7 ml of 3 mol l^{-1} H_2SO_4 Subtract the blank reading from the sample reading or use it to set the instrument to zero absorbance before taking the sample reading.

Correction for samples containing fluoride

10. Measure the fluoride concentration in the sample by either the SPADNS or electrode method (see section 7.10, Fluoride). Add the measured amount of fluoride to each of the samples used for preparing the calibration curve or used in the visual comparison.

Calculation

$$\text{Aluminium} = \frac{\mu\text{g Al (in 50 ml final volume)}}{\text{ml sample}} \text{ mg l}^{-1}$$

7.4 Biochemical oxygen demand

The biochemical oxygen demand (BOD) is an empirical test, in which standardised laboratory procedures are used to estimate the relative oxygen requirements of wastewaters, effluents and polluted waters. Micro- organisms use the atmospheric oxygen dissolved in the water for biochemical oxidation of organic matter, which is their source of carbon. The BOD is used as an approximate measure of the amount of biochemically degradable organic matter present in a sample. The 5-day incubation period has been accepted as the standard for this test (although other incubation periods are occasionally used).

The BOD test was originally devised by the United Kingdom Royal Commission on Sewage Disposal as a means of assessing the rate of biochemical oxidation that would occur in a natural water body to which a polluting effluent was discharged. Predicting the effect of pollution on a water body is by no means straightforward, however, and requires the consideration of many factors not involved in the determination of BOD, such as the actual temperature of the water body, water movements, sunlight, oxygen concentrations, biological populations (including planktonic algae and rooted plants) and the effects of bottom deposits. As determined experimentally by incubation in the dark, BOD includes oxygen consumed by the respiration of algae. The polluting effect of an effluent on a water body may be considerably altered by the photosynthetic action of plants and algae present, but it is

impossible to determine this effect quantitatively in 5-day BOD experiments. Consequently, no general ruling can be given on the BOD of samples containing algae, and each case should be considered on its merits. Suspended organic matter in an effluent is frequently deposited over a short distance immediately downstream of an outfall, where it may result in a very considerable decrease in the local dissolved oxygen concentration.

A further complication in the BOD test is that much of the oxygen- consuming capacity of samples may be due to ammonia and organically bound nitrogen, which will eventually be oxidized to nitrite and nitrate if nitrifying bacteria are present. Furthermore, the ammonia added in the dilution water used for the method presented here may also be nitrified so that, to this extent, the BOD value is not representative of the sample alone. Nitrifying bacteria are extremely sensitive to trace elements that may be present, and the occurrence of nitrification is sporadic and unpredictable even with samples known to contain nitrifying bacteria. Moreover, because of the slow growth of nitrifying bacteria, the degree of nitrification will depend on the number of these organisms initially present. Nitrification does not occur to any detectable extent during the 5-day BOD determination of crude and settled sewage and almost all industrial effluents. The BOD test is thus useful for determining the relative waste loadings to treatment plants and the degree of oxygen demand removal provided by primary treatment. Occurrence of nitrification during the 5-day incubation is almost always confined to treated effluents and river waters, which have already been partially nitrified. Only these cases need special attention, presenting the question of whether or not to use the method incorporating an inhibitor of nitrification. Determination of the degree of nitrification is tedious but, unless it is known, the BOD value may be misleading in assessing treatment plant performance or in calculating the effect of an effluent on a river. In some instances, nitrification has been shown to account for more than 70 per cent of the BOD of a well purified sewage effluent. Nevertheless, procedures in which nitrification may occur have been in use for many years and no attempt is made in the following method to eliminate nitrification.

The BOD determined by the dilution method presented here has come to be used as an approximate measure of the amount of biochemically degradable organic matter in a sample. For this purpose the dilution test, applied skilfully to samples in which nitrification does not occur, remains probably the most suitable single test, although manometric methods may warrant consideration in some cases. The analyst should also consider whether the information required could be obtained in some other way. For example, the chemical oxygen demand (COD) test will result in virtually complete oxidation of most organic substances, thereby indicating the amount of oxygen required for complete oxidation of the sample. In other circumstances, and particularly in research work, determination of the organic carbon content may be more appropriate. In any case, results obtained by the BOD test should never be considered in isolation but only in the context of local conditions and the results of other tests.

Complete oxidation of some wastes may require too long a period of incubation for practical purposes. For certain industrial wastes, and for waters polluted by them, it may be advisable to determine the oxidation curve obtained. Calculations of ultimate BOD from 5-day BOD values (e.g. based on calculations using exponential first-order rate expressions) are not correct. Conversion of data from one incubation period to another can be made only if the course of the oxidation curve has been determined for the individual case by a series of BOD tests carried out for different incubation periods.

The dilution method of determining BOD described below is the one most generally used. The dissolved oxygen content of the liquid is determined before and after incubation for 5 days at 20 °C. The difference gives the BOD of the sample after allowance has been made for the dilution, if any, of the sample.

Sample handling

The test should be carried out as soon as possible after samples have been taken. If samples are kept at room temperature for several hours, the BOD may change significantly, depending on the character of the samples. In some instances it may decrease and in others it may increase. The decrease at room temperature has occasionally been found to be as much as 40 per cent during the first 8 hours of storage. If samples cannot be dealt with at once they should, whenever practicable, be stored at about 5 °C. In the case of individual samples collected over a long period, it is desirable to keep all the samples at about 5 °C until the composite sample can be made up for the BOD determination.

Samples must be free from all added preservatives and stored in glass bottles.

It is necessary that excess dissolved oxygen be present during the whole period of incubation, and desirable that at least 30 per cent of the saturation value remains after 5 days. Since the solubility of atmospheric oxygen at the temperature of incubation is only 9 mg l^{-1}, samples that absorb more than about 6 mg l^{-1} during incubation for 5 days will not fulfil this condition. This is the case with sewage, nearly all sewage effluents, and many other waste liquids. The additional oxygen is supplied by diluting the sample with clean, well aerated water. The amount of dilution depends upon the nature of the sample.

Interferences

If the pH of the sample is not between 6.5 and 8.5, add sufficient alkali or acid to bring it within that range. Determine the amount of acid and alkali to be added by neutralising a separate portion of the sample to about pH 7.0 with a 1 mol l^{-1} solution of acid or alkali, using an appropriate indicator (e.g. bromothymol blue), or pH meter. Add a calculated aliquot volume of acid or alkali to the sample for the BOD test.

Some samples may be sterile, and will need seeding. The purpose of seeding is to introduce into the sample a biological population capable of oxidising the organic matter in the wastewater. Where such micro-organisms are already present, as in domestic sewage or unchlorinated effluents and surface waters, seeding is unnecessary and should not be carried out.

When there is reason to believe that the sample contains very few micro-organisms, for example as a result of chlorination, high temperature, extreme pH or the specific composition of some industrial wastes, the dilution water should be seeded.

For seeding, to each litre of dilution water add 5 ml of a fresh sewage effluent of good quality obtained from a settling tank following an aerobic biological process of purification. If necessary, settle (not filter) the effluent in a glass cylinder for about 30 minutes.

If such effluent is not available, use settled domestic sewage that has been stored at 20 °C for 24 hours; for seeding, add 1-2 ml of the supernatant to each litre of dilution water.

The special difficulties in choosing a seed for industrial effluents that are toxic, or that are not broken down by sewage bacteria, are dealt with in the following sub-section on “Seeding samples of industrial effluents”. If the samples are analysed in different laboratories, better agreement between test results will be achieved by using the same type of seed or, preferably, the same seed.

Some samples may be supersaturated with dissolved oxygen, especially waters containing algae. If such samples are to be incubated without dilution, the dissolved oxygen

concentration should be lowered to saturation to prevent loss of oxygen during incubation. The sample should be brought to about 20 °C in a partly filled bottle and well shaken.

A few sewage effluents and certain industrial effluents contain either residual chlorine or the products of the action of chlorine on certain constituents. Such liquids cannot be used directly for the determination of BOD because of the bactericidal effect of the chlorine or of its products and also because chlorine would introduce an error into the determination of dissolved oxygen. If the samples are allowed to stand for 1 to 2 hours, the residual chlorine will often be dissipated. Dilutions for BOD can then be prepared with properly seeded standard dilution water.

Higher concentrations of chlorine, and of many compounds containing available chlorine, may be removed by treating a portion of the sample with sodium bisulphite. The treated portion is then used for the BOD test. This procedure will probably give reasonably good results for domestic sewage effluents that have been chlorinated, since the chlorine will be present chiefly as chloramines formed by combination of chlorine with the ammonia present. However, in the case of other effluents consisting of, or containing, industrial wastes, the chlorine may have combined with organic compounds present to produce substances which, although giving no reaction for chlorine with the starch-iodide test described below, are inhibitory to biochemical oxidation or are even bactericidal. The BOD, as determined in these circumstances, is generally lower than would be expected for the organic content as measured by other tests.

Should a value for BOD of a chlorinated effluent be required, notwithstanding the uncertainty of the interpretation of the test, the following procedure should be used:

1. If the sample is alkaline to phenolphthalein bring it to a pH of 5.0 by the addition of dilute sulphuric acid. Add a crystal of potassium iodide to a convenient measured volume of sample (e.g. 100 ml) and titrate it with approximately 0.0l25 mol l^{-1} or 0.025 mol l^{-1} sodium bisulphite (or sulphite) solution, using a few drops of starch solution as an indicator.

2. To another portion of sample, sufficient to carry out the BOD test, add the requisite amount of dilute sulphuric acid to adjust the pH to 5.0, followed by the volume of sodium bisulphite solution determined by the previous titration. After thorough mixing allow to stand for several minutes, then check the absence of chlorine by testing a small portion of the treated sample with neutral starch-iodide.

3. Confirm the absence or excess of bisulphite on another portion by means of starch solution and a drop of 0.0125 mol l^{-1} iodine, which should develop a blue colour. Adjust the pH to about 7.3 before proceeding with the test.

4. Make up the dilution with seeded dilution water and proceed as for unchlorinated samples.

Note: Some wastewaters contain substances reacting with iodine, which precludes the determination of dissolved oxygen by iodometric titration. An instrumental method should be used (see determination of dissolved oxygen, section 6.5.)

Seeding samples of industrial effluents

A seed of sewage effluent, as described above, is satisfactory for many industrial effluents. However, if the BOD of such effluents as found by the standard test is substantially less than the chemical oxygen demand (COD) it may be for one of the following reasons:

(i) the sample contains compounds resistant to biochemical breakdown,

(ii) the seeding organisms are of an unsuitable type or require acclimatisation, or

(iii) toxic or bacteriostatic compounds are present, exerting an inhibiting effect at the concentration employed for the test.

Compounds constitutionally resistant to breakdown will not exert an oxygen demand on the receiving waters, but substances amenable to breakdown will generally contribute to the pollution load, even if the BOD test fails for reasons (ii) and (iii) above. Before embarking on the tedious, and sometimes impossible, task of preparing a seed by the method described below, the analyst should decide whether sufficient information about the sample may be given by alternative methods such as determinations of COD and organic carbon. Sometimes, if the difficulty is the result of condition (iii), it is possible to obtain reliable BOD values merely by increasing the dilution until the toxic constituents of the sample are below the inhibitory threshold concentrations. If this procedure fails, or if condition (ii) applies, the following method should be used:

1. Neutralise the sample if necessary, then add about 10 per cent of the threshold toxic concentration of the sample (if known; otherwise add a concentration that is thought unlikely to kill activated sludge organisms) to a mixture of settled sewage and activated sludge (2,000 mg l^{-1} suspended solids) and aerate by diffused air or by stirring.

2. After one day, allow the sludge to settle and decant the supernatant liquid, top up to the same volume with sewage and sample as before. Repeat daily. After 3 or 4 days measure the BOD of the sample using dilution water seeded with the settled mixture, then increase the proportion of sample in the mixture by a factor of 2.

3. Continue the procedure, doubling the proportion in 3- or 4-day intervals, until a maximum BOD has been reached.

If a laboratory-scale, continuously-fed, activated sludge unit is available, this can be used in a similar way to produce a seed acclimatised to the sample. Sometimes, adapted seed is available from the effluent of a biological treatment process receiving the waste in question, or the seed might be developed from the receiving water below the point of discharge of this waste, if it is not being treated.

Apparatus

√ Incubation bottles. It is recommended that narrow-mouthed, glass-stoppered bottles of a nominal capacity of 250 ml be used, and it is essential that the bottles are clean. New bottles should be cleaned with either 5 mol l^{-1} hydrochloric or sulphuric acid and thoroughly rinsed. In normal use, bottles are kept clean by the acidic iodine solution of the Winkler procedure and require no treatment apart from thorough rinsing with tap water and distilled water. Special cleaning may be necessary in some cases, but the use of chromic acid is not recommended because traces of chromium may remain in the bottle.

Some analysts prefer to use bottles of about 125 ml capacity, thus reducing the incubator space required. There is evidence, however, that with samples of some types the size of bottles (i.e. the ratio of the glass surface to the volume of liquid) may influence the result. The analyst wishing to use small bottles must, therefore, be satisfied that such a procedure gives results similar to those obtained by use of bottles of standard size.

As a precaution against drawing air into the dilution bottle during incubation, a water seal is recommended. Satisfactory water seals are obtained by inverting the bottles in a water-bath or adding water to the flared mouth of special BOD bottles.

√ Incubator or water-bath. The temperature of incubation should be 20 ± 0.5 °C. A water-bath, or constant temperature room is usually employed. Incubation must be carried out in the dark. Some samples may contain algae which, if incubated in the light, would give off oxygen by photosynthetic action, and thus interfere with the BOD determination.

Reagents
√ Dilution water. The logical diluent for a sewage effluent is the river water into which the effluent is discharged, but this method can be adopted only in special cases and is obviously unsuitable where effluents from widely differing localities are dealt with in one laboratory. Moreover, the river water may itself have a considerable BOD.

Distilled water alone is unsatisfactory as a diluent, and it is recommended that a synthetic dilution water be employed. This is prepared by adding reagents to good quality distilled water. Water from copper stills should not be used since copper inhibits biochemical oxidation (0.01 mg l^{-1} is the maximum safe concentration). Some commercial vapour-compression stills have also been shown to produce water containing copper.

Deionised water produced in some commercial units has been found satisfactory, but deionising columns in hard-water areas require frequent regeneration. It may be convenient, however, to run two deionising columns in series, or to deionise the water from a vapour compression still. Water from a new or freshly regenerated column should always be shown to give similar BOD values to distilled water, bearing in mind that the resins may introduce or fail to remove undesirable organic matter.

Stock solutions of the following pure chemicals are required; any solutions showing signs of precipitates or growths should be discarded.
√ Ferric chloride solution: dissolve 0.125 g ferric chloride, $FeCl_3.6H_2O$, in 1 litre water.

√ Calcium chloride solution: dissolve 27.5 g anhydrous calcium chloride, $CaCl_2$, (or equivalent if hydrated calcium chloride is used), in 1 litre water.

√ Magnesium sulphate solution: dissolve 25 g magnesium sulphate, $MgSO_4.7H_2O$, in 1 litre water.

√ Phosphate buffer stock solution: dissolve 42.5 g potassium dihydrogen phosphate, KH_2PO_4, in 700 ml water and add 8.8 g sodium hydroxide. This should give a solution of pH 7.2 which should be checked. Add 2 g ammonium sulphate, and dilute to 1 litre.

Add 1 ml of each reagent to each litre of freshly distilled (or deionised) water. Bring the water to incubation temperature 20 ± 1 °C and saturate with oxygen by bubbling air through it or by shaking the partially filled bottle, and use as soon as possible. Discard any dilution water remaining unused and clean the bottle, preferably with a sterilising agent. Thoroughly wash and rinse free from residual traces of the agent, and store out of direct sunlight. Stocks of dilution water should never be “topped up” with fresh solution.

A satisfactory dilution water, when incubated with or without a seed under standard conditions should not absorb more than 0.2 mg l^{-1} of oxygen, and in any case must not absorb more than 0.5 mg l^{-1} A high oxygen uptake may sometimes be associated with the presence of water-soluble organic vapours in the laboratory atmosphere. Water for dilution should therefore be distilled (or deionised) and used in a room from which volatile organic compounds are excluded. Air used for aeration must be as clean as possible.

Procedure

1. Pretreatment of dilution water by seeding is sometimes necessary (see above). Pretreatment of sample (see “Interferences”) is needed if the sample is supersaturated with oxygen or if the sample contains residual chlorine. If the pH of the sample is not between 6.5 and 8.5, it should be brought within this range.

2. Samples that have been stored in a refrigerator should be allowed to reach room temperature before dilutions are made. All samples must be well mixed just before dilution.

3. In some wastes, suspended matter may cause difficulty because the distribution of the solids may be uneven when the sample is made up into dilutions. This may cause discrepancies in the results from different dilutions or duplicate dilutions. In such cases, shake the sample vigorously immediately before the dilutions are made. Artificial homogenising procedures may cause an increased oxygen demand, and cannot be recommended.

4. Sometimes, the BOD determination in settled or filtered samples is needed. In such cases a settling time of 30 minutes is usually applied. For the BOD test of filterable substances, membrane filter, glass-fibre filter or paper filter may be used. The type of filter should be indicated in reporting the result. If determinations other than the BOD test are carried out on the filtered sample (e.g. residue, COD), it is recommended that filters of the same type and porosity be used for all of those procedures.

Dilution

5. Unless the approximate BOD of the sample is already known, the required degree of dilution will not be known and more than one dilution will have to be made. Recommended dilutions are given in Table 7.4. With experience, the analyst will often be able to use the COD as a guide to the dilution required. As low a dilution as possible should be used consistent with at least 30 per cent of the oxygen remaining after 5 days. It should be noted that some metals, e.g. copper, chromium, lead, will partially inhibit oxygen consumption.

6. In preparing dilutions for the BOD test, siphon or pour carefully the standard dilution water (seeded if necessary) into a graduated cylinder of capacity 1,000-2,000 ml, filling the cylinder half-way without entrainment of air. Add the quantity of carefully mixed sample to make the desired dilution and dilute to the desired level with dilution water. Mix well. Each analyst will have a preferred detailed procedure for preparing dilutions. Nevertheless, the following principles must be strictly adhered to:

(i) The sample and dilution water must be mixed thoroughly, but violent agitation leading to the formation of minute air bubbles must be avoided. Mixing may be accomplished by careful repeated inversion of a bottle or stoppered measuring cylinder containing the sample and dilution water, or by use of a magnetic stirrer in a completely filled bottle.

(ii) Dilutions involving the measurement of less than 5 ml of sample should be made by first diluting the sample in a volumetric flask (e.g. 10 dilution) and then using the appropriate volume of this mixture for final dilution to the required strength.

(iii) The diluted mixture is transferred to two incubation bottles (more if replicate results are required) by siphoning or by careful pouring. If a siphon is used, at least 50 ml of mixture must be discarded before the first bottle is filled. Bottles must be filled completely, allowed to stand for a few minutes and then tapped gently to remove bubbles. The stoppers are then inserted firmly without trapping air bubbles in the bottle.

(iv) On any one occasion, exactly the same mixing and transfer techniques must be used for all dilutions and samples.

(v) Bottles of the dilution water used in the test must be prepared at the same time as the sample dilutions to permit a determination of the blank.

Table 7.4 Recommended dilutions for the BOD test

Range of BOD values to be determined (mg l^{-1})	Sample volume (ml)	Dilution water volume (ml)	Dilution factor "d"	Report to nearest mg l^{-1}	Source of sample
0 to 6	undiluted	0	1	0.1	R
4 to 12	500	500	2	0.2	R, E
10 to 30	200	800	5	0.5	R, E
20 to 60	100	900	10	1	E, S
40 to 120	50	950	20	2	S
100 to 300	20	980	50	5	S, C
200 to 600	10	990	100	10	S, C
400 to 1,200	5	995	200	20	I, C
1,000 to 3,000	2	998	500	50	I
2,000 to 6,000	1	999	1,000	100	I

R River water
E Biologically purified sewage effluent
S Settled sewage or weak industrial wastewater
C Crude (raw) sewage
I Strong industrial wastewater

Determination of dissolved oxygen and incubation

7. Determine the initial concentration of dissolved oxygen in one bottle of the mixture of sample and dilution water, and in one of the bottles containing only dilution water. Place the other bottles in the incubator (those containing the sample, or the mixture of sample and dilution water, and that containing the plain dilution water to act as a blank, unseeded or seeded in accord with previous steps).

8. Incubate the blank dilution water and the diluted samples for 5 days in the dark at 20 °C. The BOD bottles should be water-sealed by inversion in a tray of water in the incubator or by use of a special water-seal bottle. Although it is known that the BOD of some samples is increased if the liquid is agitated during the incubation, it is not at present suggested that agitation should be provided.

9. After 5 days determine the dissolved oxygen in the diluted samples and the blank using the azide modification of the iodometric method or an electrometric method. (for particulars see "Dissolved oxygen", section 6.5.) Those dilutions showing a residual dissolved oxygen of

at least 30 per cent of the initial value and a depletion of at least 2 mg l^{-1} should be considered the most reliable.

Independent check of the technique

10. It might be thought desirable, from time to time, to check the technique. For this purpose, pure organic compounds of known or determinable BOD are used. If a particular organic compound is known to be present in a given waste, it may well serve as a control on the seed used. A number of organic compounds have been proposed, such as glucose and glutamic acid. In exceptional cases, a given component of a particular waste may be the best choice to test the efficacy of a particular seed. For general use, a mixture of glucose and glutamic acid has certain advantages. Glucose has an exceptionally high and variable oxidation rate with relatively simple seeds. When glucose is used with glutamic acid, the oxidation rate is stabilised and is similar to that obtained with many municipal wastes.

11. For the check, dissolve 150 mg each of glucose and glutamic acid (both dried at 103 °C for 1 hour) in 1 litre of water. This solution should be freshly prepared.

12. Make up a 1 in 50 dilution using seeded dilution water and determine the BOD in the usual way. The BOD should be approximately 220 mg l^{-1} If the result obtained is less than 200 mg l^{-1} or more than 240 mg l^{-1}, some defect in the seed, dilution water or experimental techniques should be suspected.

Immediate dissolved oxygen demand

Substances oxidisable by molecular oxygen, such as ferrous iron, sulphite, sulphide and aldehyde, impose a load on the receiving water and must be taken into consideration. The total oxygen demand of such a substrate may be determined by using a calculated initial dissolved oxygen (DO) or by using the sum of the immediate dissolved oxygen demand (IDOD) and the 5-day BOD.

Where a differentiation of the two components is desired, the IDOD should be determined. It should be understood that the IDOD does not necessarily represent the immediate oxidation by molecular dissolved oxygen but may represent an oxidation by the iodine liberated in the acidification step of the iodometric method.

The depletion of dissolved oxygen in a standard water dilution of the sample in 15 minutes has been arbitrarily selected as the IDOD. To determine the IDOD, the dissolved oxygen of the sample (which in most cases is zero) and of the dilution water are determined separately. An appropriate dilution of the sample and dilution water is prepared, and the dissolved oxygen of the sample dilution minus the observed dissolved oxygen after 15 minutes is the IDOD (mg l^{-1}) of the sample dilution.

Calculation

(1) When BOD has been determined in an undiluted sample

BOD (mg l^{-1}) = DO before incubation (mg l^{-1}) - DO after incubation (mg l^{-1})

(2) When BOD has been determined in a diluted sample

A. Without correction for blank (i.e. for the BOD of the dilution water itself)

When seeding is not required:

$$BOD = \frac{D_1 - D_2}{P} \text{ mg l}^{-1}$$

When using seeded dilution water:

$$BOD = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

Including IDOD if small or not determined:

$$BOD = \frac{D_c - D_2}{P} \text{ mg l}^{-1}$$

$$IDOD = \frac{D_c - D_1}{P} \text{ mg l}^{-1}$$

where:

D_0 = DO of original dilution water
D_1 = DO of diluted sample immediately after preparation (mg l^{-1})
D_2 = DO of diluted sample after 5 days' incubation
D_c = DO available in dilution at zero time = $D_o p + D_s P$
D_s = DO of original undiluted sample
p = decimal fraction of dilution water used
P = decimal fraction of sample used: ($P + p = 1.00$)
B_1 = DO of dilution of seed control* before incubation;
B_2 = DO of dilution of seed control* after incubation;

$$f = \frac{\text{\% of seed in } D_1}{\text{\% of seed in } B_1}$$

Seed correction = $(B_1 - B_2)f$

* The seed control refers to a separate test to check the BOD attributable to the seed added to the sample. For this purpose, measure the oxygen depletion of a series of seed dilutions and use the one giving 40-70 per cent oxygen depletion.

The DO determined on the unseeded dilution water after incubation is not used in the BOD calculations because this practice would overcorrect for the dilution water. In all the above calculations, corrections are not made for small losses of DO in the dilution water during incubation. If the dilution water is unsatisfactory, proper corrections are difficult and the results are questionable.

B. With correction for the BOD of the dilution water

If the BOD of the dilution water reaches the limit of 0.5 mg l^{-1} or approaches it, the correction may be of importance, especially for samples of water having a low BOD but requiring a dilution. In such cases, correction for BOD may be used. The calculation is then:

$$BOD = \frac{1{,}000}{V} \times (BOD_m - BOD_d) + BOD_d \text{ mg l}^{-1}$$

$$BOD = \frac{1{,}000}{V}\left[(S_m - S_t) - (D_m - D_t)\right] \text{mg l}^{-1}$$

where:

BOD = BOD of the sample
BOD_m = BOD of the mixture (sample + dilution water)
BOD_d = BOD of the dilution water (blank)
V = volume (ml) of sample in 1 litre of the mixture
S_m = DO of the mixture before incubation
S_t = DO of the mixture after incubation (after *t* days)
D_m = DO of the dilution water before incubation
D_t = DO of the dilution water after incubation for *t* days

Expression of results

BOD_t in mg l^{-1}, where *t* indicates the number of days in incubation.

Precision and accuracy

Using a procedure very similar to the above, 78 analysts in 55 laboratories analysed natural water samples plus an exact increment of biodegradable organic compounds. At a mean value of 2.1 and 175 mg l^{-1} BOD, the standard deviations were ± 0.7 and ± 26 mg l^{-1} respectively. There is no acceptable procedure for determining the accuracy of the BOD test.

7.5 Chemical oxygen demand

The chemical oxygen demand (COD) is the amount of oxygen consumed by organic matter from boiling acid potassium dichromate solution. It provides a measure of the oxygen equivalent of that portion of the organic matter in a water sample that is susceptible to oxidation under the conditions of the test. It is an important and rapidly measured variable for characterising water bodies, sewage, industrial wastes and treatment plant effluents.

In the absence of a catalyst, however, the method fails to include some organic compounds, such as acetic acid, that are biologically available to the aquatic organisms but does include some biological compounds, such as cellulose, that are not part of the immediate biochemical demand on the available oxygen of the receiving water. The carbonaceous portion of nitrogen compounds can be determined but the dichromate is not reduced by any ammonia in a waste or by any ammonia liberated from the proteinaceous matter. With certain wastes containing toxic substances, COD or a total organic carbon determination may be the only method for determining the organic load. It should be noted that the COD is not a measure of organic carbon, although the same chemical reactions are involved. Where wastes contain only readily available organic bacterial nutrients and no toxic matter, the results can be used to obtain an approximate estimate of the ultimate carbonaceous BOD values.

The use of exactly the same technique each time is important because only a part of the organic matter is included, the proportion depending on the chemical oxidant used, the structure of the organic compounds and the manipulative procedure.

The dichromate method has been selected as a reference method for the COD determination because it has advantages over other oxidants owing to its oxidising power, its applicability to a wide variety of samples and its ease of manipulation. The test will have most value for

monitoring and control of effluents and receiving waters after correlation with other variables has been established.

Principle

The sample is boiled under reflux with potassium dichromate and silver sulphate catalyst in strong sulphuric acid. Part of the dichromate is reduced by organic matter and the remainder is titrated with ferrous ammonium sulphate.

Interferences

Straight-chain aliphatic compounds, aromatic hydrocarbons and pyridine are not oxidised to any appreciable extent, although this method gives more nearly complete oxidation than a permanganate method. The straight-chain compounds are more effectively oxidised when silver sulphate is added as a catalyst. However, silver sulphate reacts with chlorides, bromides or iodides to produce precipitates that are only partially oxidised. There is no advantage in using the catalyst in the oxidation of aromatic hydrocarbons, but it is essential to the oxidation of straight-chain alcohols and acids.

The oxidation and other difficulties caused by the presence of chlorides in the sample may be overcome by adding mercuric sulphate before refluxing, in order to bind the chloride ion as a soluble mercuric chloride complex, which greatly reduces its ability to react further.

Nitrite nitrogen exerts a COD of 1.14 mg mg^{-1} of nitrite nitrogen. To eliminate significant interference due to nitrites, 10 mg of sulphamic acid for every 1 mg of nitrite nitrogen in the refluxing flask may be added. If a series of samples containing nitrite is analysed, the sulphamic acid may be added to the standard dichromate solution, since it must be included in the distilled water blank. Thus, 120 mg of sulphamic acid per litre of dichromate solution will eliminate the interference of up to 6 mg of nitrite nitrogen per litre in the sample if a 20-ml sample is used. An aliquot volume of the sample diluted to 20 ml should be used to eliminate the interference of higher concentrations of nitrite.

Ferrous iron and hydrogen sulphide exert COD of 0.14 mg mg^{-1} Fe^{2+} and 0.47 mg mg^{-1} H_2S respectively. Appropriate corrections can be calculated and subtracted from the result or both interferences can be removed by bubbling air through the sample, if easily volatile organic matter is not present.

The procedure can be used to determine COD values of 50 mg l^{-1} with the standard dichromate solution (0.0417 mol l^{-1}). With the dilute dichromate, values are less accurate, especially below 10 mg l^{-1}, but may be used to indicate an order of magnitude.

Sample handling

Samples should be taken with bottles that do not release organic substances into water; glass-stoppered glass bottles are satisfactory. Unstable samples should be tested without delay, especially wastewater and polluted water samples. Natural, not heavily polluted, water should be analysed on the same day or at least within 24 hours and the sample should be kept cold before analysis.

If there is to be a delay before analysis the sample may be preserved by adding sulphuric acid, about 2 ml H_2SO_4 (d = 1.84) diluted 1+2 to each 100 ml of sample. If samples are to be stored for longer than 24 hours, deep freezing is recommended.

Depending on the aim of the analysis, COD can be determined on unfiltered and/or filtered samples. When both determinations are carried out, the difference gives the COD of the particulate matter. Samples containing settleable solids should be homogenised sufficiently by means of a blender to permit representative sampling for the COD determination in unfiltered samples. For the analysis of filtrate, the original (not homogenised) sample is used. Filtration through glass-fibre filters is recommended, but hard paper filters may be used if the sample has a high COD. The filters should be pre-rinsed with water.

Apparatus

√ A reflux apparatus consisting of a 250-ml Erlenmeyer flask (500 ml if large samples are used) with ground-glass neck, and a 300-mm double surface condenser (Liebig, Friedrichs, West or equivalent) with a ground-glass joint. Since absolute cleanliness is essential, flasks and condensers should be protected from dust by inverted cups when not in use. The glassware must be used exclusively for COD determinations.

√ A heating mantle or hotplate.

√ A hotplate producing at least 1.5 W cm^{-2} of heating surface to ensure adequate boiling of the liquid in the flask. Heating mantles are preferred because they prevent the problem of overheating.

Reagents

√ Sulphuric acid (*d* =1.84).

√ Standard potassium dichromate solution, 0.0417 mol l^{-1} Dissolve 12.259 g of $K_2Cr_2O_7$ primary standard grade, dried at 103 °C for 2 hours, in distilled water and dilute to 1.000 litre.

√ Dilute standard potassium dichromate solution, 0.00417 mol l^{-1} Dilute 100 ml of the standard potassium dichromate solution to 1.000 litre.

√ Standard ferrous ammonium sulphate solution, 0.250 mol l^{-1} Dissolve 98 g of $Fe(NH_4)_2(SO_4)_2.6H_2O$ analytical grade crystals in distilled water. Add 20 ml of H_2SO_4 (*d* = 1.84), cool and dilute to 1.000 litre. This solution may be standardised against the standard potassium dichromate solution as follows:

√ Dilute 10.0 ml of standard potassium dichromate solution, 0.0417 mol l^{-1}, to about 100 ml. Add 30 ml H_2SO_4 (*d* = 1.84) and allow to cool. Titrate with the ferrous ammonium titrant, using 2 or 3 drops of ferroin indicator.

$$\text{Concentration (mol } l^{-1}) = \frac{V_1 \times 0.25}{V_2}$$

where:

V_1 = volume (ml) of $K_2Cr_2O_7$
V_2 = volume (ml) of $Fe(NH_4)_2(SO_4)_2$

√ Dilute standard ferrous ammonium sulphate solution, 0.025 mol l^{-1} Dilute 100 ml of the standard ferrous ammonium sulphate solution to 1.000 litre. Standardise daily against the dilute standard potassium dichromate, 0.00417 mol l^{-1}

√ Silver sulphate, reagent powder. This reagent may be used either directly in powder form or in saturated solution, or it may be added to the sulphuric acid (about 5 g of Ag_2SO_4 to 1 litre of H_2SO_4; 1-2 days are required for dissolution).

√ Mercuric sulphate, analytical grade crystals.

√ Ferroin indicator solution. Dissolve 0.695 g of ferrous sulphate, $FeSO_4.7H_2O$, in water. Add 1.485 g of 1, 10-phenanthroline monohydrate, shaking until dissolved. Dilute to 100 ml. This solution is also commercially available.

√ Sulphamic acid, analytical grade (required only if the interference of nitrites is to be eliminated).

√ Anti-bumping granules that have been previously heated to 600°C for 1 hour.

Procedure

Initial dilutions in volumetric flasks should be made on waste with a high COD value to reduce the error that is inherent in measuring small samples.

A. Samples with low chloride concentrations

If the sample contains less than 100 mg l^{-1} chloride after evaporation, proceed as follows:

1. Place in an Erlenmeyer flask 20.0 ml of the sample or an aliquot diluted to 20.0 ml with distilled water.

2. Add 10.0 ml of standard potassium dichromate solution, 0.0417 mol l^{-1}, and a few anti-bumping granules. Mix well.

3. Add slowly, with caution, 30 ml of concentrated H_2SO_4 containing silver sulphate, mixing thoroughly by swirling while adding the acid. If H_2SO_4 containing silver sulphate is not used, add 0.15 g of dry silver sulphate and then, slowly, 30 ml of concentrated H_2SO_4

Note: If the liquid has not been well mixed local heating may occur on the bottom of the flask and the mixture may be blown out of the flask.

4. Attach the condenser to the flask and reflux the mixture for 2 hours. Allow to cool and then wash the condenser with distilled water.

5. Dilute the mixture to about 150 ml with distilled water, cool to room temperature, and titrate the excess dichromate with standard ammonium ferrous sulphate using 2-3 drops of ferroin indicator. Although the quantity of ferroin is not critical, do not vary it among different samples even when analysed at different times. The end-point is when the colour changes sharply from blue-green to reddish-brown, even though the blue-green may reappear within several minutes.

6. Reflux in the same manner a blank consisting of 20 ml of distilled water together with the reagents and titrate as in step 5, above.

B. Samples with high chloride concentration

If the sample contains more than 100 mg l^{-1} chloride after evaporation or dilution, proceed as follows:

To 20.0 ml of sample or aliquot in the flask add 0.5 g of mercuric sulphate and shake thoroughly. This addition is sufficient to complex 40 mg of chloride ion or 2000 mg l^{-1} when 20.0 ml of sample are used. If more chloride is present, add more $HgSO_4$ to maintain a HgSO4: Cl^- ratio of 10:1. It is not important if a slight precipitate develops because it will not affect the determination. Continue with steps 2 to 6, as above.

Adjustments for other sample sizes

If a water is expected to have a higher or lower than normal COD, a sample ranging in size from 10.0 ml to 50.0 ml may be used with the volumes, weights and concentrations adjusted accordingly. Table 7.5 gives the appropriate reagent quantities for different sample sizes. Use these quantities when following the procedure given above. When using large samples, increase the size of the Erlenmeyer flask to 500 ml to permit titration within the refluxing flask.

C. Samples with low COD

Follow one of the procedures given above for high and low chloride concentrations with the following differences:

1. Use dilute standard potassium dichromate, 0.00417 mol l^{-1}

2. Perform the back titration with either 0.025 mol l^{-1} or 0.01 mol l^{-1} ferrous ammonium sulphate.

3. Use redistilled water for the preparation of all reagents and blanks.

Exercise extreme care with this procedure because a trace of organic matter in the glassware or the atmosphere may cause a gross error. If a further increase in sensitivity is required, reduce a larger sample to 20 ml (final total volume 60 ml) by boiling in the refluxing flask on a hotplate in the presence of all the reagents. Carry a blank through the same procedure. The ratio of water to sulphuric acid must not fall much below 1.0 or a high blank will result because of the thermal decomposition of potassium dichromate. This technique has the advantage of concentrating without significant loss of easily digested volatile materials. Hard-to-digest volatile materials, such as volatile acids, are lost but an improvement is gained over ordinary evaporative concentration methods. Moreover, as sample volume increases, correspondingly lower concentrations of chlorides will be complexed by 0.4 g of $HgSO_4$.

Table 7.5 Reagent quantities for different sample sizes

Sample size (ml)	Standard potassium dichromate (ml)	H_2SO_4 with Ag_2SO_4 (ml)	$HgSO_4$ (g)	Ferrous ammonium sulphate (mol l^{-1})	Final volume before titration (ml)
10.0	5.0	15	0.2	0.05	70
20.0	10.0	30	0.4	0.10	140
30.0	15.0	45	0.6	0.15	210
40.0	20.0	60	0.8	0.20	280
50.0	25.0	75	1.0	0.25	350

Test of the technique and reagents

The technique and the quality of the reagents may be tested using potassium acid phthalate as a standard substance. Potassium acid phthalate has a theoretical COD of 1.176 g g^{-1} Dissolve 425.1 mg of potassium acid phthalate in distilled water and dilute to 1,000 ml for a 500 mg l^{-1} COD solution. A recovery of 98-100 per cent of the theoretical oxygen demand can be expected.

Calculation

$$\text{Concentration of COD} = \frac{(a-b) \times c \times 8{,}000}{v} \text{ mg l}^{-1}$$

where:

a = ferrous ammonium sulphate (ml) used for blank
b = ferrous ammonium sulphate (ml) used for sample
c = molarity (mol l^{-1}) of ferrous ammonium sulphate
v = volume of sample (ml)

Precision and accuracy

A set of synthetic unknown samples containing potassium acid phthalate and sodium chloride was tested by 74 laboratories. At a COD of 200 mg l^{-1} in the absence of chloride, the standard deviation was 6.5 per cent. At 160 mg l^{-1} COD and 100 mg l^{-1} chloride the standard deviation was 6.3 per cent, while at 150 mg l^{-1} COD and 1,000 mg l^{-1} chloride it was 9.3 per cent. These standard deviations refer to the distribution of results from all laboratories.

For most organic compounds the oxidation is 95-100 per cent of the theoretical value. Benzene, toluene and pyridine are not completely oxidised.

7.6 Boron

In most natural waters boron is rarely found in concentrations greater than 1 mg l^{-1}, but even this low concentration can have deleterious effects on certain agricultural products, including

citrus fruits, walnuts and beans. Water having boron concentrations in excess of 2 mg l^{-1} can adversely affect many of the more common crops. Groundwater may have greater concentrations of boron, particularly in areas where the water comes in contact with igneous rocks or other boron-containing strata. The boron content in many waters has been increasing as a result of the introduction of industrial waste and of the use of boric acid and its salts in cleaning compounds.

Ingestion of boron at concentrations usually found in natural water will have no adverse effects on humans. Ingestion of large quantities of boron, however, can affect the central nervous system, while extended consumption of water containing boron can lead to a condition known as borism.

The photometric curcumin method described below for determining boron concentrations is applicable in the 0.10-1.00 mg l^{-1} range and can be extended by selection of an appropriate sample volume. The curcumin method of analysis is a reference method.

Sample handling

Many types of glass contain boron and their use should therefore be avoided. Samples should be stored in polyethylene bottles or alkali-resistant, boron-free glassware.

Principle

When a sample of water containing boron is acidified and evaporated in the presence of curcumin, a red-coloured product called rosocyanine is formed. The rosocyanine is taken up in ethanol, and the red colour is compared photometrically with standards. The minimum detectable amount of boron is 0.2 µg.

Interferences

The method is not applicable if more than 20 mg of NO_3-N per litre is present. When the total "hardness" of the sample exceeds 100 mg l^{-1} as $CaCO_3$, the colour measurement may be affected by the formation of an opalescence in the alcoholic solution of rosocyanine. This interference may be prevented by passage of the sample through a cation exchange column.

Apparatus

√ Colorimetric equipment. Either spectrophotometer or absorptiometer for measurement at 540 nm, providing a light path of at least 1 cm.

√ Evaporating dishes: capacity 100-150 ml, of low boron content. Glass, platinum or other material found by experience to be suitable may be used. All dishes must be of a similar size, shape and composition.

√ Water-bath: set at 55 ± 2°C.

√ Glass-stoppered volumetric flasks: capacity 25 and 50 ml.

√ Ion-exchange column: 50 cm long × 1.3 cm in diameter.

Reagents

√ Curcumin reagent. Dissolve 40 mg of finely ground curcumin and 5.0 g of oxalic acid in 80 ml of 950 ml l^{-1} ethanol. Add 4.2 ml of concentrated HCl and make the solution up to 100 ml

with ethanol in a 100-ml volumetric flask. This reagent will be stable for several days if stored in a refrigerator.

√ Strongly acidic cation exchange resin.

√ Hydrochloric acid: 1+5.

√ Ethanol: 95 per cent.

√ Boron stock standard. Dissolve 571.6 mg of anhydrous boric acid, H_3BO_3, in distilled water and dilute to 1 litre (1.00 ml (100 µg B).

√ Boron working standard. Dilute 10.00 ml of stock boron solution to 1 litre with distilled water (1.00 ml (1.00 µg B).

Procedure

Preparation of calibration graph

1. Pipette 0 (blank), 0.25, 0.50, 0.75 and 1.00 ml of the boron working standard into evaporating dishes of the same shape, size and composition. Add distilled water to each standard to bring the total volume to 1.0 ml. Treat these standards as described in step 4, below, beginning with the addition of the 4.0 ml of curcumin.

2. Subtract the absorbance of the blank from the absorbances of the standards and prepare a calibration graph relating the net absorbance to the amount of boron (µg).

Ion-exchange removal of calcium and magnesium

3. If the “hardness” is greater than 100 mg l^{-1}, as $CaCO^3$, the sample should be treated by ion exchange before analysis as follows. Charge the column with 20 g of a strongly acidic cation exchange resin. Backwash the column with distilled water to remove the entrained air bubbles. Thereafter, make certain that the resin remains covered with liquid at all times. Pass 50 ml of 1+5 hydrochloric acid through the column and then wash the column free of chloride ion with distilled water. Pipette a 25 ml sample, or a smaller aliquot of a sample of known high boron content, onto the resin column. Adjust the rate of flow through the column to about 2 drops per second and collect the effluent in a 50-ml volumetric flask. Wash the column with small portions of distilled water until the flask is full to the mark. Mix the contents of the flask and transfer 2.00 ml into the evaporating dish. Add 4.0 ml of curcumin reagent and complete the analysis as described in the following section.

4. For water containing 0.10-1.00 mg of boron per litre, use 1.00 ml of sample. For water containing more than 1.00 ml of boron per litre, make an appropriate dilution with boron-free distilled water, so that a 1.00-ml aliquot contains approximately 0.50 µg of boron. Pipette the 1.00 ml of sample or dilution into an evaporating dish.

5. Add 4.0 ml of curcumin reagent to each evaporating dish and swirl the dish gently to mix the contents thoroughly. Float the dishes on a water-bath set at 55 ± 2 °C and evaporate the contents to complete dryness. Remove each dish from the water-bath 15 minutes after the contents appear dry and the odour of HCl has disappeared.

6. After the dishes cool to room temperature, add 10.0 ml of 95 per cent ethanol to each dish, stirring gently with a polyethylene rod to ensure complete dissolution of the red-coloured product. Wash the contents of each dish into a 25-ml volumetric flask, using 95 per cent

ethanol. Make up to the mark with 95 per cent ethanol and mix thoroughly by inverting the flask.

7. Measure the absorbance at 540 nm within 1 hour of drying the samples. Subtract the absorbance of a boron-free blank to obtain the net absorbance. If the final solution is turbid, it should be filtered through filter paper before the absorbance is measured.

Precautions

Variables such as volume and concentration of reagents, as well as the time and temperature of drying, must be carefully controlled for a successful determination. Evaporating dishes must be identical in shape, size and composition to ensure equal evaporation times. Increasing the time of evaporation results in intensification of the resulting colour.

Calculation

Determine the amount of boron (µg) equivalent to the net absorbance from the calibration graph. The concentration of boron in mg l^{-1} is obtained by dividing this weight by the volume of the sample in millilitres.

If hardness ions were removed by ion exchange, the volume of sample applied to the resin column should be divided by 25, i.e.:

$$\text{Concentration of boron} = \frac{\mu g\,B}{\text{ml sample}/25}\ \text{mg l}^{-1}$$

Precision and accuracy

A synthetic unknown sample containing B, 240 µg l^{-1}; As, 40 µg l^{-1}; Be, 250 µg l^{-1}; Se, 20 µg l^{-1}; and V, 6 µg l^{-1} in distilled water was analysed in 30 laboratories by the curcumin method with an overall relative standard deviation of 22.8 per cent and a difference of 0 per cent between the overall mean and the true value.

7.7 Calcium

Calcium dissolves out of almost all rocks and is, consequently, detected in many waters. Waters associated with granite or siliceous sand will usually contain less than 10 mg of calcium per litre. Many waters from limestone areas may contain 30-100 mg l^{-1} and those associated with gypsiferous shale may contain several hundred milligrams per litre.

Calcium contributes to the total hardness of water. On heating, calcium salts precipitate to cause boiler scale. Some calcium carbonate is desirable for domestic waters because it provides a coating in the pipes which protects them against corrosion.

The method described here is the EDTA titrimetric method.

Sample handling

Samples should be collected in plastic or borosilicate glass bottles without the addition of preservative. If any calcium carbonate is formed during sample storage, it must be redissolved by the addition of nitric acid before analysis.

Principle

When EDTA is added to water containing calcium and magnesium ions, it reacts with the calcium before the magnesium. Calcium can be determined in the presence of magnesium by EDTA titration; the indicator used is one that reacts with calcium only. Murexide indicator gives a colour change when all of the calcium has been complexed by EDTA at a pH of 12-13.

Interferences

Orthophosphate precipitates calcium at the pH of the test. Strontium and barium interfere with the calcium determination, and alkalinity in excess of 300 mg l^{-1} may cause an indistinct end-point with hard waters. Under the conditions of the test, normal concentrations of the following ions cause no interference with the calcium determination: Cu^{2+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Zn^{2+}, Al^{3+}, Pb^{2+} and Sn^{4+}

Apparatus

√ Porcelain dishes, 100-ml capacity.
√ Burette, 25 or 50 ml.
√ Pipettes.
√ Stirring rods.
√ Graduated cylinder, 50 ml.
Reagents
√ Sodium hydroxide, 1 mol l^{-1} Cautiously dissolve 40 g of NaOH in 600 ml of distilled water. Make up to 1 litre in a volumetric flask and allow to stand for at least 48 hours to permit sodium carbonate to precipitate. Decant the supernatant and store in a rigid polyethylene bottle, tightly sealed. When needed, withdraw solution by siphoning to avoid opening the bottle.

√ Murexide indicator. The indicator changes from pink to purple at the end-point. Prepare by dissolving 150 mg of the dye in 100 g of absolute ethylene glycol. Alternatively, a dry mixture of the dye with sodium chloride is a stable form of the indicator. Prepare by mixing 200 mg of dye with 100 g of solid NaCl and grinding the mixture to a fine powder (i.e. one that passes through a 40-mesh sieve).

√ Standard EDTA titrant, 0.01 mol l^{-1} Weigh 3.723 g of disodium ethylenediaminetetraacetate dihydrate, dissolve in distilled water and make up to 1 litre in a volumetric flask. Standardise against standard calcium solution, 1,000 mg l^{-1}, and adjust so that 1 ml of standard EDTA is equivalent to 1 ml of standard calcium solution.

√ Standard calcium solution. Weigh 1.000 g of $CaCO_3$ (primary standard grade) that has been dried at 105 °C and place it in a 500-ml Erlenmeyer flask. Place a funnel in the neck of the flask and add small volumes of 6 mol l^{-1} HCl until all the $CaCO_3$ has dissolved. Add 200 ml of distilled water and boil for a few minutes to expel CO_2. Cool, add a few drops of methyl red indicator, and adjust to intermediate orange colour by adding 3 mol l^{-1} NH_4OH or 6 mol l^{-1} HCl as necessary. Transfer the contents of the flask and rinsings to a 1-litre volumetric flask and make up to the mark with distilled water (1.0 ml is equivalent to 1 mg $CaCO_3$, 0.4008 mg Ca^{2+}, 0.2431 mg Mg^{2+} and 1 ml standard EDTA titrant).

Sample pretreatment

Heavily polluted water containing considerable organic matter should be treated by nitric acid digestion as follows. Mix the sample and transfer 50 ml to a 125-ml Erlenmeyer flask. Add 5 ml of concentrated HNO_3 and a few boiling chips or glass beads. Bring to a slow boil and evaporate until about 10 ml remain. Continue heating and add HNO_3 in 1-ml portions. The solution will be clear and light in colour when digestion is complete. Do not let any dry areas appear on the bottom of the flask during digestion.

Remove from heat and cool. Wash down the walls of the flask with distilled water and transfer the contents to a 50-ml volumetric flask. Rinse the Erlenmeyer flask two or three times with a little distilled water and add the rinsings to the volumetric flask. Cool to ambient temperature before filling the volumetric flask to the mark with distilled water.

Procedure

1. Prepare a colour comparison blank by placing 50 ml of distilled water in a white porcelain dish.

2. Prepare the sample for titration by placing 50 ml of sample in a white porcelain dish. If the sample is high in calcium, use a measured portion of the sample containing 5-10 mg of calcium and make up to 50 ml with distilled water.

3. Add 2 ml of NaOH solution to both the sample and the comparison blank and stir. The resulting pH should be between 12 and 13.

4. Add 0.1-0.2 mg of Murexide indicator mixture (or 1-2 drops of indicator solution) to the blank. Stir, then add 1-2 drops of EDTA titrant from the burette. Stir until the colour turns from red to an orchid purple. Record the burette reading. Keep the blank for a colour reference comparison.

5. Add 0.1-0.2 mg of indicator mixture (or 1-2 drops of indicator solution) to the sample.

6. If the sample turns red, add EDTA titrant slowly, with constant stirring. Continue to add EDTA until the colour turns from red to faint purple. Add EDTA drop by drop until the colour matches the colour comparison blank. The change from faint purple to orchid purple usually takes place with the addition of 5 or 6 drops of EDTA titrant.

7. Read the burette and determine the volume of EDTA titrant used by subtracting the burette reading obtained at step 4.

Calculation

$$\text{Concentration of Ca} = \frac{A \times C \times 400.8}{\text{ml sample}} \text{mg l}^{-1}$$

where

A = volume of EDTA titrant used for titration of sample (ml)

C = is calculated from the standardisation of the EDTA titrant:

$$C = \frac{\text{ml of standard calcium solution}}{\text{ml of EDTA titrant}}$$

7.8 Chloride

Chloride anions are usually present in natural waters. A high concentration occurs in waters that have been in contact with chloride-containing geological formations. Otherwise, a high chloride content may indicate pollution by sewage or industrial wastes or by the intrusion of seawater or saline water into a freshwater body or aquifer.

A salty taste in water depends on the ions with which the chlorides are associated. With sodium ions the taste is detectable at about 250 mg l^{-1} Cl^-, but with calcium or magnesium the taste may be undetectable at 1,000 mg l^{-1}

A high chloride content has a corrosive effect on metal pipes and structures and is harmful to most trees and plants.

7.8.1 Silver nitrate method

Principle

Chloride is determined in a neutral or slightly alkaline solution by titration with standard silver nitrate, using potassium chromate as indicator. Silver chloride is quantitatively precipitated before red silver chromate is formed.

Interferences

Bromide, iodide and cyanide are measured as equivalents of chloride. Thiosulphate, sulphite and sulphide interfere and the end-point may be difficult to detect in highly coloured or very turbid samples.

Apparatus

√ Porcelain dish, 200-ml capacity.
√ Graduated cylinder, 100 ml.
√ Stirring rods.
√ Burette, 25 or 50 ml. pH meter.
Reagents
√ Silver nitrate standard solution. Dissolve 4.791 g silver nitrate, $AgNO_3$, in distilled water and dilute to 1 litre. Store in a brown glass bottle (1.0 ml - 1.0 mg Cl^-).

√ Sodium chloride standard solution. Dissolve 1.648 g dried sodium chloride, NaCl, in about 200 ml of distilled water in a beaker. Transfer to a 1-litre volumetric flask. Rinse the beaker twice with distilled water and pour the rinsings into the volumetric flask. Make up to the mark with distilled water (1.0 ml - 1.0 mg Cl^-).

√ Potassium chromate indicator. Dissolve 5 g potassium chromate, K_2CrO_4, in 100 ml distilled water. Add silver nitrate solution drop by drop to produce a slight red precipitate of silver chromate, and filter.

Procedure
1. Measure 100 ml of sample into a porcelain dish. Check the pH; it must be between 5.0 and 9.5 in this procedure. If the pH of the sample is below 5.0, add a small amount of calcium carbonate and stir. If the pH is above 9.5, add 0.1 mol l^{-1} nitric acid drop by drop to bring the pH to about 8. Stir, and add a small amount of calcium carbonate.

2. Add 1 ml potassium chromate indicator solution and stir. The solution should turn a reddish colour.

3. Titrate with silver nitrate solution with constant stirring until only the slightest perceptible reddish coloration persists. If more than 25 ml is required, take 50 ml of the sample and dilute it to 100 ml before titration.

4. Repeat steps 1 to 3 on a 100-ml distilled water blank to allow for the presence of chloride in any of the reagents and for the solubility of silver chromate.

Calculation

$$\text{Chloride as } Cl^- = \frac{1,000(V_1 - V_2)}{\text{Volume of sample}} \text{ mg l}^{-1}$$

where

$V1$ = volume of silver nitrate required by the sample (ml)
$V2$ = volume of silver nitrate required by the blank (ml)

When the sample contains less chloride than 50 mg l^{-1}, and the amount of silver nitrate required is consequently small, an appreciable error may be introduced because of the difficulty of determining the exact point at which the reddish colour becomes perceptible. It is helpful to compare the colour with that of a similar solution that has not been taken to the end-point of the titration and, therefore, contains no reddish component in its colour. Alternatively, the titration can be taken to match the colour of a liquid that has been titrated to a small, but known, amount beyond its end-point, subtracting this amount from that required by the sample.

Reporting

Record the Cl- results in mg l^{-1} The results should be rounded off as follows:

Concentration range (mg l^{-1})	<50	50-100	100-200	200-500
Record to nearest (mg l^{-1})	1	2	5	10

7.8.2 Mercuric nitrate method

Principle

Chloride can be titrated with mercuric nitrate, $Hg(NO_3)_2$, because of the formation of soluble, slightly dissociated mercuric chloride. In the pH range 2.3 to 2.8, diphenylcarbazone indicates the titration end-point by formation of a purple complex indicator and an end-point

enhancer. Increasing the strength of the titrant and modifying the indicator mixtures extend the range of measurable chloride concentrations.

Interferences

Bromide and iodide are titrated with $Hg(NO_3)_2$ in the same manner as chloride. Chromate, ferric and sulphite ions interfere when present in concentrations that exceed 10 mg l^{-1}

Apparatus

√ Erlenmeyer flask, 250 ml.
√ Microburette, 5 ml with 0.01-ml graduations.
Reagents
√ Sodium chloride standard solution. Dissolve 0.824 g dried sodium chloride, NaCl, in about 200 ml of distilled water in a beaker. Transfer to a 1-litre volumetric flask. Rinse the beaker twice with distilled water and pour the rinsings into the volumetric flask. Make up to the mark with distilled water (1.0 ml - 500 µg Cl^-).

√ Nitric acid, HNO3, 0.1 mol l^{-1}

√ Sodium hydroxide, NaOH, 0.1 mol l^{-1}

A. Reagents for chloride concentration below 100 mg l^{-1}

√ Indicator-acidifier reagent: The HNO_3 concentration of this reagent is an important factor in the success of the determination and can be varied as indicated in (i) or (ii) below to suit the alkalinity range of the sample. Reagent (i) contains enough HNO_3 to neutralise a total alkalinity of 150 mg l^{-1} as $CaCO_3$ to the proper pH in a 100-ml sample. Adjust the amount of HNO_3 to accommodate samples with alkalinity other than 150 mg l^{-1}

Reagent (i). Dissolve, in the order named, 250 mg *s*-diphenylcarbazone, 4.0 ml concentrated HNO_3 and 30 mg xylene cyanol FF in 100 ml 95 per cent ethyl alcohol or isopropyl alcohol. Store in a dark bottle in a refrigerator. This reagent is not stable indefinitely. Deterioration causes a slow end-point and high results.

Reagent (ii). Because pH control is critical, adjust pH of highly alkaline or acid samples to 2.5 ± 0.1 with 0.1 mol l^{-1} HNO_3 or NaOH, not with sodium carbonate. Use a pH meter with a non-chloride type of reference electrode for pH adjustment. If only the usual chloride-type reference electrode is available for pH adjustment, determine the amount of acid or alkali required to obtain the desired pH and discard this sample portion. Treat a separate sample portion with the determined amount of acid or alkali and continue the analysis. Under these circumstances, omit HNO_3 from the indicator reagent.

√ Standard mercuric nitrate titrant, 0.00705 mol l^{-1} Dissolve 2.3 g $Hg(NO_3).2H_2O$ in 100 ml distilled water containing 0.25 ml concentrated HNO_3 Dilute to just under 1 litre. Make a preliminary standardisation by titrating against standard sodium chloride solution. Use replicates containing 5.00 ml standard NaCl solution and 10 mg sodium bicarbonate, $NaHCO_3$, diluted to 100 ml with distilled water. Adjust titrant to 0.00705 mol l^{-1} and make a final standardisation: 1.00 ml (300 µg Cl- Store away from light in a dark bottle.

B. Reagent for chloride concentrations greater than 100 mg l^{-1}

√ Mixed indicator reagent. Dissolve 0.50 g *s*-diphenylcarbazone powder and 0.05 g bromophenol blue powder in 75 ml of 95 per cent ethyl or isopropyl alcohol and dilute to 100 ml with the same alcohol.

√ Strong standard mercuric nitrate titrant, 0.0705 mol l^{-1} Dissolve 25 g $Hg(NO_3)\ 2H_2O$ in 900 ml distilled water containing 5.0 ml concentrated HNO_3 Dilute to just under 1 litre and standardise against standard sodium chloride solution. Use replicates containing 25.00 ml standard NaCl solution and 25 ml distilled water. Adjust titrant to 0.0705 mol l^{-1} and make a final standardisation: 1.00 ml = 5.00 mg Cl^-

Procedure

A. Titration of chloride concentrations less than 100 mg l^{-1}

1. Use a 100-ml sample or smaller portion so that the chloride content is less than 10 mg.

2. Add 1.0 ml indicator-acidifier reagent. The colour of the solution should be green-blue at this point. A light green indicates pH less than 2.0, a pure blue indicates pH more than 3.8. For most potable waters, the pH after this addition will be 2.5 ± 0.1. For highly acid or alkaline waters adjust pH to 8 before adding indicator-acidifier reagent.

3. Titrate with 0.00705 mol l^{-1} $Hg(NO_3)_2$ titrant to a definite purple end-point. The solution turns from green-blue to blue a few drops before the end-point.

4. Determine the blank by titrating 100 ml distilled water containing 10 mg $NaHCO_3$

B. Titration of chloride concentrations greater than 100 mg l^{-1}

1. Use a sample portion (5 to 50 ml) requiring less than 5 ml titrant to reach the end-point. Measure into a 150-ml beaker.

2. Add approximately 0.5 ml mixed indicator reagent and mix well. The colour should be purple.

3. Add 0.1 mol l^{-1} HNO_3 drop by drop until the colour just turns yellow.

4. Titrate with strong $Hg(NO_3)_2$ titrant to first permanent dark purple.

5. Titrate a distilled water blank using the same procedure.

Calculation

$$\text{Chloride} = \frac{(A - B) \times M \times 70{,}900}{\text{ml sample}} \text{ mg l}^{-1}$$

where:

A = ml titration for sample
B = ml titration for blank
M = molarity of $Hg(NO_3)_2$

Precision

A synthetic sample containing Cl^-, 241 mg l^{-1}; Ca, 108 mg l^{-1}; Mg, 82 mg l^{-1}; NO_3^--N, 1.1 mg l^{-1}; NO_2^--N, 0.25 mg l^{-1}; K, 3.1 mg l^{-1}; Na, 19.9 mg l^{-1}; SO_4^{2-}, 259 mg l^{-1} and 42.5 mg total alkalinity (contributed by $NaHCO_3$) in distilled water was analysed in 10 laboratories by the mercuric nitrate method with a relative standard deviation of 3.3 per cent and a relative error of 2.9 per cent.

7.9 Chlorophyll a

Analysis of the photosynthetic chlorophyll pigment present in aquatic algae is an important biological measurement which is commonly used to assess the total biomass of algae present in water samples (see section 11.3).

Sampling

Samples should be taken with an appropriate sampler, such as a depth or grab sampler (see section 5.3.2), a submersible pump or a hose-pipe sampler (see section 5.2.1). For nutrient-poor (high transparency) water up to 6 litres will be required. For eutrophic waters, 1-2 litres are usually adequate.

Principle

Three types of chlorophyll (chlorophyll *a*, *b*, and *c*) are found in phytoplankton and may be extracted with acetone. Each type has a characteristic light absorption spectrum with a particular peak absorbance. The acetone extract is analysed in a spectrophotometer at these peaks. The peak height indicates chlorophyll concentration.

When samples are concentrated by filtration for the purposes of analysis, the phytoplankton cells die. Consequently, the chlorophyll immediately starts to degrade and its concentration is thus reduced. The degradation product of chlorophyll *a*, phaeophytin *a*, fluoresces in the same spectral region, and this can lead to errors in results. It is therefore essential to measure the concentration of phaeophytin *a* and to make appropriate corrections to analytical results.

Apparatus

√ Spectrophotometer, with a spectral band width between 0.5 and 2 nm.
√ Cuvettes, 1 cm; longer path-length cuvettes may be used (usually 4 cm or 10 cm).
√ Centrifuge.
√ Tissue-grinder.
√ Centrifuge tubes, 15 ml, graduated, screw-tops.
√ Filters, glass fibre GF/C, 4.7 cm diameter.
√ Filtration cup and pump.
Note: As far as possible, all apparatus should be acid- and alkali-free.

Reagents

√ Magnesium carbonate suspension, 1.0 g $MgCO_3$ in 100 ml distilled water. Shake before use.

√ Acetone solution, 90 per cent acetone.

√ Hydrochloric acid, 1 mol l^{-1}

Procedure

1. After recording the initial water volume, separate the cells from the water by filtration. Filter continuously and do not allow the filter to dry during filtration of a single sample. As filtration ends, add 0.2 ml of $MgCO_3$ suspension to the final few millilitres of water in the filter cup. If extraction is delayed at this point, filters should be placed in individual labelled bags or plastic Petri dishes and stored at -20 °C in darkness. Samples may be transported in this form.

2. Place the filter in the tissue-grinder, add 2-3 ml of 90 per cent acetone, and grind until the filter fibres are separated. Pour the acetone and ground filter into a centrifuge tube; rinse out the grinding tube with another 2 ml of 90 per cent acetone and add this to the centrifuge tube. Make up the total volume in the centrifuge tube to 10 ml with 90 per cent acetone. Place top on tube, label, and store in darkness at 4 °C for 10-12 hours. Samples may also be transported in this form.

3. Centrifuge closed tubes for 15 minutes at 3,000 rev/min to clarify samples. Decant the clear supernatant into a clean centrifuge tube and record the volume.

4. Fill a cuvette with 90 per cent acetone. Record absorbance on the spectrophotometer at 750 nm and 663 nm. Zero on this blank if possible; otherwise record the absorbance and subtract it from sample readings.

5. Place sample in the cuvette and record absorbance at 750 nm and 663 nm (750a and 663a).

6. Add two drops of 1 mol l^{-1} HCl to sample in 1-cm cuvette (increase acid in proportion to volume for larger cuvettes). Agitate gently for 1 minute and record absorbance at 750 nm and 665 nm (750b and 665b).

7. Repeat the procedure for all samples. Some preliminary samples may need to be taken to assess the best sample volume.

Calculation

1. Subtract absorbance:

663a - 750a = corrected 663a absorbance
665b - 750b = corrected 665b absorbance

2. Use these corrected 663a and 665b absorbances to calculate:

$$\text{Chlorophyll } a = \frac{26.73(663a - 665b) \times V_e}{V_s \times l} \text{ mg m}^{-3}$$

$$\text{Phaeophytin } a = \frac{26.73[1.7(665_b) - 663_a] \times V_e}{V_s \times l} \text{ mg m}^{-3}$$

where

V_e = volume of acetone extract (litres)
V_s = volume of water sample (m^3)
l = path length of cuvette (cm)

Chlorophyll *a* concentrations should be recorded. The ratio of chlorophyll *a* to phaeophytin *a* gives an indication of the effectiveness of sample preservation, as well as of the condition of the algal population.

7.10 Fluoride

While fluoride is considered to be one of the major ions of seawater, its concentration in seawater, 1.3 mg kg^{-1}, is indicative of most natural water concentrations. Rarely, natural waters (mainly groundwaters of arid regions) may contain fluoride concentrations greater than 10 mg l^{-1} Fluoride may be added to drinking water to assist in control of dental caries. Such additions require close control of fluoride concentrations to roughly 1.0 mg l^{-1}, as higher levels can cause mottling of the teeth. The guideline value of 1.5 mg l^{-1} in drinking water has been proposed by WHO. The local application of this value must take into account the climatic conditions and levels of water consumption. Mottling of teeth has been classed, in the USA, as a cosmetic effect, but a maximum limit of 4 mg l^{-1} fluoride has been set to prevent skeletal fluorosis (a crippling condition that can result from excessive fluoride intake).

Fluoride is used in certain industrial processes and consequently occurs in the resulting wastewaters. Significant industrial sources of fluoride are the production of coke, glass and ceramics, electronics, steel and aluminium processing, pesticides and fertilisers, and electroplating operations. Waste levels may range from several hundred to several thousand milligrams per litre in untreated wastewaters. It is worthy of note that conventional treatment (lime) seldom reduces fluoride concentrations below 8-15 mg l^{-1} without dilution.

The reference method for the analysis of fluoride ions is potentiometric, using the lanthanum fluoride, solid state, selective ion electrode. The secondary method is a photometric procedure employing the lanthanum alizarin complex.

Sample handling

Generally, clean polyethylene bottles are preferred for collection and storage of samples for fluoride analysis, provided that long-term evaporative loss is not encountered. Glass and borosilicate glass bottles should be avoided; however, they may be used provided that low pH is not maintained, and that the containers have been thoroughly cleaned and have not previously been in contact with solutions of high fluoride concentration. Pretreatment with high levels of sodium thiosulphate (more than 100 mg l^{-1}) should be avoided.

7.10.1 Selective ion electrode method

Principle

The fluoride electrode consists of a single lanthanum fluoride crystal, the internal portion of which is in contact with a constant concentration of fluoride ion and an internal reference electrode. Upon contact of the external electrode surface with the test solution (standard or unknown) a potential difference is set up across the crystal which is related to the fluoride ion concentrations in contact with the crystal surfaces. An external reference electrode in the test solution completes the circuit and allows measurement of the membrane or crystal potential:

Internal reference electrode	F^- (internal)	LaF_3	Test solution	External reference electrode

Since the relationship between potential and fluoride ion concentration is described by a form of the Nernst equation ($E = E° - RT \ln a_{F^-}$), it is the log fluoride ion activity that is related to change in measured potential. Consequently, variations in ionic strength between samples and standards and among samples must be prevented. Similarly, since it is the free fluoride ion activity that yields the electrode response, formation of complex species (Al, Fe) or undissociated hydrofluoric acid must be prevented. The procedure is designed to maintain control of these problems.

Interferences

Without the addition of a suitable complexing agent, polyvalent cations such as Al(III), Fe(III), Si(IV) will remove free fluoride ion from solution as soluble complexes. Similarly, if pH is not maintained above 5.0, the presence of molecular hydrogen fluoride and HF_2^- reduces the free fluoride ion concentration.

Fluoride ion selectivity is extremely high with respect to most aqueous cations. However, if solution pH is not maintained below 8, hydroxide ion will begin to yield sufficient electrode response to interfere with the fluoride measurement since the relative response, $F^-:OH^-$ is about 10:1. The electrode does not respond to fluoroborate (BF_4^-) directly.

Apparatus

√ High impedance millivoltmeter. An expanded-scale or digital pH meter or ion-selective meter or any high impedance millivolt potentiometer.

√ Reference electrode. Sleeve type rather than fibre-tip reference electrode should be used, especially for very dilute solutions.

√ Fluoride electrode. Commercial electrode.

√ Magnetic stirrer. Proper insulation should be provided to prevent heat transfer to sample solution during measurement. Stirring bars should be Teflon-coated.

√ Stopwatch or timer.

Reagents

√ Deionised or distilled water. All solutions should be prepared from high-quality distilled or deionised water.

√ Fluoride standard solution. Dissolve 0.221 g of analytical grade anhydrous sodium fluoride, NaF, in distilled water and dilute to 1 litre (1 ml = 100 µg F).

√ A working standard of 10 µg F- per 1.0 ml should be prepared just before starting the analysis.

√ Total ionic strength adjustment buffer. The total ionic strength adjustment buffer solution (TISAB) may be prepared by the following procedure:

Dissolve 57 ml glacial acetic acid, 58 g sodium chloride and 4.0 g 1, 2-cyclohexylene-diaminetetraacetic acid in 500 ml distilled water. Adjust pH to 5.0-5.5 with 6 mol l^{-1} sodium hydroxide. Stir and cool during NaOH addition (about 125 ml will be required). Dilute to final volume of 1 litre with distilled water.

Procedure

1. Preparation of calibration graph. Prepare a series of standards over the appropriate concentration range, such that the TISAB constitutes 50 per cent of the solution by volume. Linear millivolt response versus log of concentration should be obtained from roughly 0.2 to 2,000 mg l^{-1} In the linear region, three standards should suffice to determine the standard curve. In non-linear regions, i.e. low levels, more data points are necessary.

2. Instrument operation. Follow manufacturer's recommendation for obtaining millivolt readings. Some instruments read in absolute millivolts while others operate with relative millivolt scales.

3. Sample measurement. Mix sample to be analysed with an equal volume of TISAB in a beaker. If high levels of Al (>3 mg l^{-1}) or Fe (>200 mg l^{-1}) are present, follow the procedure for distillation. Ensure that the sample has achieved room temperature (or that measurement temperature is the same as that of standards). Place beaker on an insulated magnetic stirrer, add a stirring bar, immerse the electrodes and allow 3 minutes for equilibration. Determine fluoride concentration from the calibration curve. The electrodes should be rinsed and dried between samples. Frequent recalibration should be made with an intermediate standard. The detection limit is about 0.02 mg l^{-1} fluoride.

Calculation

The concentration of fluoride ion can be determined directly from the calibration curve if the samples and standards are treated alike and the concentration axis is constructed in terms of initial standard concentration before addition of TISAB.

Precision and accuracy

Precision is limited by variations in temperature, instrument drift and scale reading errors (if the instrument has a needle read-out). With proper recalibration (roughly once per hour), relative precision of ± 2 per cent has been reported by one manufacturer, independent of concentration.

7.10.2 SPADNS method

Principle

The SPADNS colorimetric method is based on the reaction between fluoride and a zirconium-dye lake. Fluoride reacts with the dye lake, dissociating a portion of it into a colourless complex anion, ZrF_6^{2-}, and the dye. As the amount of fluoride increases, the colour produced becomes progressively lighter.

The reaction rate between fluoride and zirconium ions is greatly influenced by the acidity of the reaction mixture. If the proportion of acid in the reagent is increased, the reaction can be made almost instantaneous. Under such conditions, however, the effect of various ions differs from that in the conventional alizarin methods. The selection of dye for this rapid fluoride method is governed largely by the resulting tolerance to these ions.

Interferences

Chlorine, colour and turbidity interfere and must be removed by distillation. Interference caused by alkalinity, chloride, iron, phosphate and sulphate is not linear and so cannot be accounted for mathematically. Whenever any substance is present in quantities large enough to produce an error of 0.1 mg l^{-1}, or if the total interfering effect is in doubt, distil the sample. If alkalinity is the only interference it can be neutralised with either hydrochloric or nitric acid.

Volumetric measurements of sample and reagent are extremely important to analytical accuracy. Samples and standards should be at the same temperature or at most have a 2 °C temperature differential. Maintain temperature as nearly constant as possible throughout colour development. Different calibration curves are required for different temperature ranges.

Apparatus

√ Colorimetric equipment. One of the following is required:
- Spectrophotometer for measurement at 570 nm and capable of providing a light path of 1 cm or longer.

- Filter photometer providing a light path of at least 1 cm and equipped with a greenish yellow filter having maximum transmittance at 550 to 580 nm.

Reagents
√ Stock fluoride solution. Dissolve 0.221 g of analytical grade anhydrous sodium fluoride, NaF, in distilled water and dilute to 1 litre (1 ml ≡100 µg F-).

√ Working standard of 10 µg F- per 1.0 ml, which should be prepared just before the analysis.

√ SPADNS solution. Dissolve 0.958 g of SPADNS, sodium 2-(parasulphophenylazo)-1, 8-dihydroxy-3, 6-naphthalene disulphonate, also called 4, 5-dihydroxy-3-(parasulphophenylazo)-2, 7-naphalene disulphonic acid trisodium salt, in distilled water and dilute to 500 ml. This solution is stable for at least one year if protected from direct sunlight.

√ Acid-zirconyl reagent. Dissolve 130 mg zirconyl chloride octahydrate, $ZrOCl_2.8H_2O$, in about 25 ml of distilled water. Add 350 ml of concentrated hydrochloric acid and dilute to 500 ml with distilled water.

√ Acid-zirconyl/SPADNS reagent. Mix equal quantities of SPADNS solution and acid- zirconyl reagent. The combined reagent is stable for at least two years.

√ Reference solution. Add 10 ml of SPADNS solution to 100 ml distilled water. Dilute 7 ml of concentrated HCl to 10 ml and add to the diluted SPADNS solution. The resulting solution is used for setting the instrument reference point (at zero) and is stable for at least one year. Alternatively, a prepared standard of 0 mg F- l^{-1} may be used as a reference.

√ Sodium arsenite solution. Dissolve 5.0 g of sodium arsenite, $NaAsO_2$, in distilled water and dilute to 1 litre.

Procedure
1. Preparation of standard curve. Prepare fluoride standards in the range of 0 to 1.4 mg F- l^{-1} by diluting appropriate quantities of standard fluoride solution to 50 ml with distilled water.

Pipette 5.00 ml each of the SPADNS solution and the acid- zirconyl reagent or 10.00 ml of the mixed acid-zirconyl/SPADNS reagent to each standard and mix well. Avoid contamination. Set the photometer to zero absorbance with the reference solution and obtain absorbance readings of the standards. Plot a curve of the relationship between mg fluoride and absorbance. Prepare a new standard curve whenever a fresh reagent is made or a different standard temperature is desired.

As an alternative to using a reference, set the photometer at a convenient point (0.300 or 0.500 absorbance) with the prepared 0 mg F- l^{-1} standard.

2. Sample pretreatment. If the sample contains residual chlorine, remove it by adding 1 drop (0.05 ml) $NaAsO_2$ solution per 0.1 mg residual chlorine and mix. (Sodium arsenite concentrations of 1,300 mg l^{-1} produce an error of 0.1 mg l^{-1} when the fluoride concentration is 1.0 mg F- l^{-1})

3. Colour development. Use a 50-ml sample or a portion diluted to 50 ml with distilled water. Adjust temperature to that used for the standard curve. Add 5.00 ml each of SPADNS solution and acid-zirconyl reagent (or 10.00 ml of acid-zirconyl/SPADNS reagent), mix well and read absorbance, after setting the reference point of the photometer at zero as described in step 1 above. If the absorbance falls beyond the range of the standard curve, repeat the procedure using a diluted sample.

Calculation

$$\text{Fluroride} = \frac{A}{\text{ml sample}} \times \frac{B}{C} \text{ mg l}^{-1}$$

where

A = F- determined from plotted curve (µg)
B = final volume of diluted sample (ml)
C = volume of diluted sample used for colour development (ml).

7.10.3 Fluoride distillation

Principle

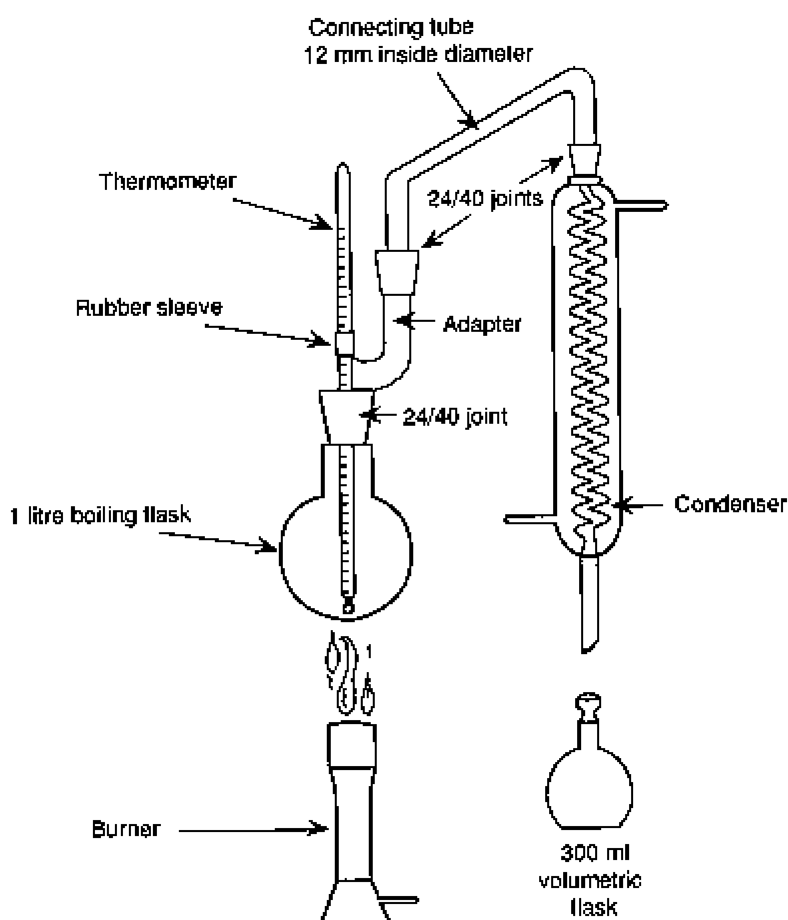
In the event of interferences that cannot be controlled in the direct fluoride analysis procedures, it is possible to remove the fluoride from solution selectively. Distillation of either fluorosilicic or hydrofluoric acid from an acid solution of higher boiling point separates the fluoride from other constituents in the original sample.

Apparatus

Distillation apparatus (shown in Figure 7.1), consisting of a 1-litre round-bottomed, long-necked boiling flask (of borosilicate glass), a connecting tube, an efficient condenser, a thermometer adapter, and a thermometer reading to 200 °C. Any comparable apparatus with the essential design features may be used. The critical points to observe are those that could affect complete fluoride recovery, such as obstruction in the vapour path and trapping of liquid in the adapter and condenser, and conditions that might enhance sulphate carry-over. An asbestos shield or similar device should be used to protect the upper part of the boiling

flask from the burner flame. If desired, this apparatus can be modified so that the heat is automatically shut off when distillation is completed.

Figure 7.1 Direct distillation apparatus for fluoride



Reagents

√ Sulphuric acid, H_2SO_4, concentrated.

√ Silver sulphate, Ag_2SO_4, crystals.

Procedure

1. Place 400 ml distilled water in the boiling flask and carefully add 200 ml concentrated H_2SO_4 Swirl until the flask contents are homogeneous. Add 25-35 glass beads and connect the apparatus as shown in Figure 7.1, making sure that all joints are tight. Begin heating slowly at first, then as rapidly as the efficiency of the condenser will permit (the distillate must be cool) until the temperature of the flask contents reaches exactly 180 °C. Discard the distillate. The process removes fluoride contamination and adjusts the acid:water ratio for subsequent distillations.

2. After cooling the acid mixture remaining after step 1 (to 120 °C or below), add 300 ml of sample, mix thoroughly, and distil as before until the temperature reaches 180 °C. To prevent sulphate carry-over, do not heat above 180 °C.

3. When high-chloride samples are distilled, add Ag_2SO_4 to the boiling flask at the rate of 5 mg for each mg of Cl^-

4. Use the sulphuric acid solution in the flask repeatedly until the contaminants from the samples accumulate to such an extent that recovery is affected or interferences appear in the distillate. Check suitability of the acid periodically by distilling standard fluoride samples. After the distillation of high-fluoride samples, flush the still with 300 ml distilled water and combine two fluoride distillates. If necessary, repeat the flushing operation until the fluoride content of the distillates is at a minimum. Include the additional fluoride recovered with that of the first distillation. After periods of inactivity, similarly flush the still and discard the distillate. Use the distillate to conduct fluoride analysis by one of the prescribed methods. Correct the volume relationship if the distillate volume differs from that of the original sample.

7.11 Iron

Iron is an abundant element in the earth's crust, but exists generally in minor concentrations in natural water systems. The form and solubility of iron in natural waters are strongly dependent upon the pH and the oxidation- reduction potential of the water. Iron is found in the +2 and +3 oxidation states. In a reducing environment, ferrous (+2) iron is relatively soluble. An increase in the oxidation-reduction potential of the water readily converts ferrous ions to ferric (+3) and allows ferric iron to hydrolyse and precipitate as hydrated ferric oxide. The precipitate is highly insoluble. Consequently, ferric iron is found in solution only at a pH of less than 3. The presence of inorganic or organic complex-forming ions in the natural water system can enhance the solubility of both ferrous and ferric iron.

Surface waters in a normal pH range of 6 to 9 rarely carry more than 1 mg of dissolved iron per litre. However, subsurface water removed from atmospheric oxidative conditions and in contact with iron-bearing minerals may readily contain elevated amounts of ferrous iron. For example, in ground-water systems affected by mining, the quantities of iron routinely measured may be several hundred milligrams per litre.

It is the formation of hydrated ferric oxide that makes iron-laden waters objectionable. This ferric precipitate imparts an orange stain to any settling surfaces, including laundry articles, cooking and eating utensils and plumbing fixtures. Additionally, colloidal suspensions of the ferric precipitate can give the water a uniformly yellow-orange, murky cast. This coloration, along with associated tastes and odour, can make the water undesirable for domestic use when levels exceed 0.3 mg l^{-1}

The reference method of analysis is a photometric method described here in which iron is bound into a colour-forming complex with 1, 10-phenanthroline.

Sample handling

In the sampling and storage process, iron in solution may undergo changes in oxidation form and it can readily precipitate on the sample container walls or as a partially settleable solid suspension. For total iron measurements, precipitation can be controlled in the sample containers by the addition of 1.5-2.0 ml of concentrated HNO_3 per litre of sample immediately after collection. If the pH is not less than 2 after the addition of acid, more HNO_3 should be added.

Principle

For total iron determinations, precipitated iron is brought into solution by boiling with acid. Ferric iron is reduced to the ferrous state by the addition of hydroxylamine hydrochloride.

Ferrous iron is chelated with 1, 10-phenanthroline to form an orange-red complex. Colour intensity is proportional to iron concentration. Absorbance can be measured spectrophotometrically at 510 nm or photometrically using a green filter having maximum transmittance near 510 nm. For cell lengths of 1 cm, Beer's law is obeyed in iron solutions containing 0.1-5 mg l^{-1} The most rapid colour development occurs between pH 2.9 and pH 3.5, while colour intensity is unaffected by a pH between 3 and 9.

If large amounts of organic materials are present, the sample must first be digested with H_2SO_4 to destroy organic structures and to bring all the iron into solution. Hydrochloric acid is then added until the HCl concentration is between 7 and 8 mol l^{-1} and the iron is extracted as $FeCl_3$ into diisopropyl ether. Iron is then re-extracted into water and reduced with hydroxylamine.

Interferences

Strongly oxidising substances may interfere. Cyanide, nitrite, phosphates, chromium, zinc, cobalt and copper interfere if concentrations exceed 10 times that of iron. Additionally, cobalt or copper present in excess of 5 mg l^{-1} and nickel in excess of 2 mg l^{-1} result in interferences. Bismuth, cadmium, mercury, molybdate and silver cannot be present because they precipitate phenanthroline.

Cyanide and nitrite may be removed by boiling with acid. The same procedure converts polyphosphates into orthophosphates, which cause less interference. Excess hydroxylamine addition will reduce strongly oxidising agents, and excess phenanthroline is required to guarantee complete iron complexation if large concentrations of interfering metal ions are present. The milky solution produced from molybdate interference can be overcome by adjusting the pH to greater than 5.5.

For samples that are highly coloured or that contain large amounts of organic material, ashing procedures should precede analysis. The sample may be wet-ashed with sulphuric acid and nitric acid or dry-ashed at temperatures not exceeding 700 °C.

Preparation for the analysis of highly contaminated water and industrial wastewater must include careful consideration of possible interferences. The general procedures for correcting the interferences described above will aid in dealing with specific interferences. The ultimate choice may be to eliminate interferences by extracting the iron with diisopropyl ether from a hydrochloric acid solution and then back-extracting the iron with water.

Apparatus

√ Colorimetric equipment. One of the following is required:
- Spectrophotometer, for use at 510 nm, providing a light path of 1 cm or longer.

- Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 510 nm.

- Nessler tubes, matched, 100 ml, tall form.

√ Acid-washed glassware. Wash all glassware with concentrated hydrochloric acid and rinse with distilled water before use to remove deposits of iron oxide.

√ Separatory funnels, 125 ml, Squibb form, with ground-glass or TFE stopcocks and stoppers.

Table 7.6 Selection of light path for various iron concentrations

Final volume (µg Fe)		Light path (cm)
50 ml	**100 ml**	
50-200	100-400	1
25-500	50-200	2
10-40	20-80	5
5-20	10-40	10

Reagents

Use reagents low in iron. Use iron-free distilled water in preparing standards and reagent solutions. Store reagents in glass-stoppered bottles. The HCl and ammonium acetate solutions are stable indefinitely if tightly stoppered. The hydroxylamine, phenanthroline and stock iron solutions are stable for several months. The standard iron solutions are not stable; prepare daily as needed by diluting the stock solution. Visual standards in Nessler tubes are stable for several months if sealed and protected from light.

√ Hydrochloric acid, HCl, concentrated, containing less that 0.00005 per cent iron.

√ Hydroxylamine hydrochloride solution. Dissolve 10 g $NH_2OH.HCl$ in 100 ml water.

√ Ammonium acetate buffer solution. Dissolve 250 g $NH_4C_2H_3O_2$ in 150 ml water. Add 700 ml concentrated (glacial) acetic acid. Prepare new reference standards with each buffer preparation because even a good grade of $NH_4C_2H_3O_2$ contains a significant amount of iron.

√ Phenanthroline solution. Dissolve 100 mg 1, 10-phenanthroline monohydrate, $C_{12}H_8N_2H_2O$, in 100 ml water by stirring and heating to 80 °C. Do not boil. Discard the solution if it darkens. Heating is unnecessary if two drops of concentrated HCl are added to the water. One ml of this reagent is sufficient for no more than 100 µg Fe.

√ Stock iron solution. Use metal or salt for preparing the stock solution as described below.

√ Method (i). Use electrolytic iron wire or "iron wire for standardising" to prepare the solution. If necessary clean the wire with fine sandpaper to remove any oxide coating and to produce a bright surface. Weigh 200.0 mg wire and place in a 1,000-ml volumetric flask. Dissolve in 20 ml of 3 mol l^{-1} H_2SO_4 and dilute to the mark with distilled water (1.00 ml (200 µg Fe).

√ Method (ii). If ferrous ammonium sulphate is preferred, slowly add 20 ml concentrated H_2SO_4 to 50 ml distilled water and dissolve 1.404 g of $Fe(NH_4)_2(SO_4)_2\ 6H_2O$. Add 0.1 mol l^{-1} potassium permanganate ($KMnO_4$) drop by drop until a faint pink colour persists. Dilute to 1,000 ml with distilled water and mix (1.00 ml (200 µg Fe).

√ Standard iron solutions. Prepare fresh solutions the day they are to be used. Pipette 50.00 ml stock solution into a 1,000-ml volumetric flask and dilute to the mark with distilled water (1.00 ml (10.0 µg Fe). Pipette 5.00 ml stock solution into a 1,000-ml volumetric flask and dilute to the mark with distilled water (1.00 ml (1.00 µg Fe).

Preparation of calibration graph

1. Prepare a blank and a series of standards to encompass the range of concentrations of iron in the samples.

2. For photometric measurement Table 7.6 can be used as a rough guide for selecting the proper light path at 510 nm. Treat the blank and the standards as indicated in the procedure. Zero the instrument against distilled water and measure the absorbance of the standards and the blank.

3. Subtract the absorbance of the blank from the absorbances of the standards to obtain the net absorbance. Prepare a calibration graph relating net absorbance to the amount of iron.

Procedure
1. Mix the sample thoroughly and measure out a volume of solution containing not more than 0.5 mg of iron into a 125-ml Erlenmeyer flask. If necessary, dilute to 50 ml and add 2 ml of concentrated HCl and 1 ml of hydroxylamine hydrochloride solution.

2. Drop in a few glass beads and boil until the volume is reduced to 10-20 ml. Cool to room temperature.

3. Transfer to a 50-ml or 100-ml volumetric flask, add 10 ml of ammonium acetate buffer solution and 2 ml of phenanthroline solution, and dilute to the mark with distilled water. Mix thoroughly and set aside for 10-15 minutes for full colour development.

4. Measure the colour intensity spectrometrically at 510 nm. Subtract the absorbance of the blank from that of the sample to determine the net absorbance.

Calculation

Determine the weight of iron (µg Fe) equivalent to the net absorbance from the calibration graph.

$$\text{Concentration of Fe} = \frac{\mu g\ \text{Fe}}{\text{ml sample}}\ \text{mg l}^{-1}$$

Precision and accuracy

An overall relative standard deviation of 25.5 per cent and a difference of 13.3 per cent between the overall mean and the true value among 44 laboratories examining iron by the phenanthroline method have been reported. The sample contained 300 µg Fe, 500 µg Al, 50 µg Cd, 110 µg Cr, 470 µg Cu, 70 µg Pb, 120 µg Mn, 150 µg Ag and 650 µg Zn per litre of distilled water.

7.12 Magnesium

Magnesium is a relatively abundant element in the earth's crust and hence a common constituent of natural water. Waters associated with granite or siliceous sand may contain less than 5 mg of magnesium per litre. Water in contact with dolomite or magnesium-rich limestone may contain 10-50 mg l^{-1} and several hundred milligrams per litre may be present in water that has been in contact with deposits containing sulphates and chlorides of magnesium.

By a similar action to that of calcium, magnesium imparts hardness to water. This may be reduced by chemical softening or by ion exchange. It should be noted that the difference between total hardness and the calcium concentration can be used to calculate the magnesium concentration.

Sample handling

Samples should be collected in plastic or borosilicate glass bottles without the addition of preservative. If any calcium carbonate is formed during sample storage, it must be redissolved before analysis.

7.12.1 Titrimetric method

Principle

When EDTA is added in a titration to water containing calcium and magnesium ions, it reacts with the calcium before the magnesium. In this procedure calcium is precipitated as its oxalate and is removed by filtration before titration. A small amount of Eriochrome Black T is added to the solution which, when buffered at pH 10.0 ± 0.1, becomes wine-red in colour. When the solution is titrated with EDTA, the magnesium is complexed; at the end-point the solution changes from wine-red to blue. A small amount of the compleximetrically neutral salt of EDTA with magnesium is added to the buffer because magnesium ions must be present to obtain a satisfactory end-point. This introduces sufficient magnesium and eliminates the need for a blank correction.

It should be noted that the procedure subsequent to the precipitation of calcium oxalate (and its removal from solution by filtration) is the same as that for hardness.

Interferences

Several metal ions can interfere with the titration by producing fading or indistinct end-points. To minimise these interferences, sodium sulphide solution is added. The sodium sulphide inhibitor precipitates insoluble sulphides and, if these are dark in colour and present in appreciable quantities, they may tend to mask the end-point or alter the colour change.

Not all samples require the use of the inhibitor. To determine whether addition of the inhibitor is necessary, the analyst should compare the results of two titrations, one with and one without inhibitor.

Apparatus

√ Porcelain dishes, 100-ml capacity.
√ Burette, 25 or 50 ml.
√ Pipettes.
√ Stirring rods.
√ Graduated cylinder, 50 ml.

Reagents

√ Ammonium oxalate solution, 5 per cent. Dissolve 10 g of ammonium oxalate monohydrate, $(NH_4)_2C_2O_4.H_2O$, in 250 ml of distilled water. Filter the solution if it contains a precipitate.

√ Buffer solution. Add 55 ml of concentrated hydrochloric acid to 400 ml of distilled water. Slowly, with stirring, add 310 ml of 2-aminoethanol (also called ethanolamine). Add 5.0 g of magnesium disodium ethylenediaminetetraacetate and dilute to 1 litre with distilled water. This buffer has a normal shelf-life of about 1 month. Discard the buffer when addition of 1 or 2 ml to a sample fails to produce a pH of 10.0 ± 0.1 at the end-point of a titration.

√ Sodium sulphide inhibitor. Dissolve 5.0 g of sodium sulphide, $Na_2S.9H_2O$, in 100 ml of distilled water. Store in a glass container with a tightly fitting rubber stopper to reduce deterioration through air oxidation.

√ Eriochrome Black T indicator. Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-napthol-4-sulphonic acid; No. 203 in the Colour Index. Dissolve 0.5 g of dye in 100 ml of triethanolamine (also known as 2, 2', 2"-nitrilotriethanol). This may also be prepared by grinding 0.5 g of Eriochrome Black T together with 100 g of NaCl.

√ Standard EDTA titrant, 0.01 mol l^{-1} (see section 7.7, Calcium).

√ Standard calcium solution (see section 7.7, Calcium).

Sample pretreatment

Samples containing high concentrations of organic material may give indistinct end-points. If this cannot be overcome by dilution, pretreat samples by nitric acid digestion (see section 7.7, Calcium).

Procedure

1. Removal of calcium. Measure 75 ml of the sample into a beaker or similar vessel. Add 2 ml of buffer solution and 1 ml of 5 per cent ammonium oxalate solution. Mix and allow to stand for 5-10 minutes. Filter through Whatman No. 5 filter paper or equivalent. Discard the first 5-10 ml of the filtrate.

2. Titration of sample. Dilute 25.0 ml of the filtered sample to about 50 ml with distilled water in a porcelain dish. Determine whether the pH is 10.0 ± 0.1. If it is not, but the deviation is small, additional buffer may be sufficient to adjust it. If the deviation is large, the pH must be adjusted before the addition of the ammonium oxalate. If the inhibitor is being used, it must be added at this point in the procedure.

3. Add 2 drops of indicator solution or a small amount of dry-powder indicator mixture.

4. Add the standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears from the solution, adding the last few drops at intervals of 3-5 seconds. At the end-point the solution is normally blue. Working in daylight or under a daylight fluorescent lamp is highly recommended. Ordinary incandescent light tends to produce a reddish tinge in the blue at the end-point. If the end-point is indistinct, either the indicator has deteriorated or the inhibitor must be added before titration begins.

5. If sufficient sample is available and there is no interference, improve the accuracy by increasing the sample size. If a large sample volume is taken, add proportionally larger amounts of buffer, inhibitor and indicator.

6. Titrate a distilled water blank of the same volume as the sample. Add identical amounts of buffer, inhibitor and indicator solution.

Calculation

$$\text{Concentration of Mg} = \frac{(A - B) \times C \times 243.1}{\text{ml sample}} \text{mg l}^{-1}$$

where

A = volume of EDTA for titration of sample (ml)
B = volume of EDTA for titration of blank (ml)
C is calculated from the standardisation of the EDTA titrant:

$$C = \frac{\text{ml of standard calcium solution}}{\text{ml of EDTA titrant}}$$

7.12.2 Magnesium hardness method

Principle

Magnesium may also be determined by calculating the difference between the total hardness and the calcium hardness of the sample. This yields the value of magnesium hardness as mg l^{-1} $CaCO_3$ which, when multiplied by 243.1, will be the concentration of magnesium.

Apparatus

√ Porcelain dishes, 100-ml capacity.
√ Burette, 25 or 50 ml.
√ Pipettes.
√ Stirring rods.
√ Graduated cylinder, 50 ml.
Reagents
√ Buffer solution. Add 55 ml of concentrated hydrochloric acid to 400 ml of distilled water. Slowly, with stirring, add 310 ml of 2-aminoethanol (also called ethanolamine). Add 5.0 g of magnesium disodium ethylenediaminetetraacetate and dilute to 1 litre with distilled water. This buffer has a normal shelf-life of about 1 month. Discard the buffer when addition of 1 or 2 ml to a sample fails to produce a pH of 10.0 ± 0.1 at the end-point of a titration.

√ Sodium sulphide inhibitor. Dissolve 5.0 g of sodium sulphide, $Na_2S.9H_2O$, in 100 ml of distilled water. Store in a glass container with a tightly fitting rubber stopper to reduce deterioration through air oxidation.

√ Eriochrome Black T indicator. Sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-napthol-4-sulphonic acid; No. 203 in the Colour Index. Dissolve 0.5 g of dye in 100 ml of triethanolamine (also known as 2, 2', 2"-nitrilotriethanol). This may also be prepared by grinding 0.5 g of Eriochrome Black T together with 100 g of NaCl.

√ Standard EDTA titrant, 0.01 mol l^{-1} (see section 7.7, Calcium).

√ Standard calcium solution (see section 7.7, Calcium).

Sample pretreatment

Samples containing high concentrations of organic material may give indistinct end-points. If this cannot be overcome by dilution, follow the nitric acid digestion procedure described under "Sample pretreatment" in section 7.7, Calcium.

Procedure

1. Titration of sample. Dilute 25.0 ml of the sample to about 50 ml with distilled water in a porcelain dish. Determine whether the pH is 10.0 ± 0.1. If it is not, but the deviation is small, additional buffer may be sufficient to adjust it. If the deviation is large, the pH must be adjusted before the addition of the ammonium oxalate. If the inhibitor is being used, it must be added at this point in the procedure.

2. Add 2 drops of indicator solution or a small amount of dry-powder indicator mixture.

3. Add the standard EDTA titrant slowly, with continuous stirring, until the last reddish tinge disappears from the solution, adding the last few drops at intervals of 3-5 seconds. At the end-point, the solution is normally blue. Working in daylight or under a daylight fluorescent lamp is highly recommended. Ordinary incandescent light tends to produce a reddish tinge in the blue at the end-point. If the end-point is indistinct, either the indicator has deteriorated or the inhibitor must be added before titration begins.

4. If sufficient sample is available and there is no interference, improve the accuracy by increasing the sample size. If a large sample volume is taken, add proportionally larger amounts of buffer, inhibitor and indicator.

5. Titrate a distilled water blank of the same volume as the sample. Add identical amounts of buffer, inhibitor and indicator solution.

6. From the results obtained when following the procedure for the determination of calcium, record as amount "*A*" the number of millilitres of standard EDTA titrant required to reach the orchid purple colour end-point.

Calculation

$$\text{Total hardness} = \frac{(T - S) \times C \times 1{,}000}{\text{Volume of sample (ml)}} \text{ mg l}^{-1} \text{ as } CaCO_3$$

$$\text{Calcium hardness} = \frac{A \times C \times 1{,}000}{\text{Volume of sample (ml)}} \text{ mg l}^{-1} \text{ as } CaCO_3$$

Magnesium hardness = total hardness - calcium hardness

Magnesium hardness (as mg $CaCO_3$ l^{-1}) × 0.2431 = mg Mg l^{-1}

where

T = volume of EDTA for titration of the total hardness sample (ml)
S = volume of EDTA for titration of the blank (ml)
A = volume of EDTA for titration in the calcium procedure (ml)
C is calculated from the standardisation of the EDTA titrant:

$$C = \frac{\text{ml of standard calcium solution}}{\text{ml of EDTA titrant}}$$

7.13 Manganese

Although manganese in groundwater is generally present in the soluble divalent ionic form because of the absence of oxygen, part or all of the manganese in surface waters (or water from other sources) may be in a higher valence state. Determination of total manganese does not differentiate the various valence states. The heptavalent permanganate ion is used to oxidise manganese and/or any organic matter that causes taste. Excess permanganate, complexed trivalent manganese, or a suspension of quadrivalent manganese must be detected with great sensitivity to control treatment processes and to prevent their discharge into a water distribution system. There is evidence that manganese occurs in surface waters both in suspension in the quadrivalent state and in the trivalent state in a relatively stable, soluble complex. Although rarely present at concentrations in excess of 1 mg l^{-1}, manganese imparts objectionable and tenacious stains to laundry and plumbing fixtures. The low manganese limits imposed on an acceptable water stem from these, rather than toxicological, considerations. Special means of removal are often necessary, such as chemical precipitation, pH adjustment, aeration and use of special ion exchange materials. Manganese occurs in domestic wastewater, industrial effluents and receiving water bodies.

The analytical method described here is the persulphate method.

Principle

Persulphate oxidation of soluble manganese compounds to permanganate is carried out in the presence of silver nitrate. The resulting colour is stable for at least 24 hours if excess persulphate is present and organic matter is absent.

Interferences

As much as 0.1 g chloride, Cl-, in a 50-ml sample can be prevented from interfering by adding 1 g mercuric sulphate, $HgSO_4$, to form slightly dissociated complexes. Bromide and iodide will still interfere and only trace amounts may be present. The persulphate procedure can be used for potable water with trace to small amounts of organic matter if the period of heating is increased after more persulphate has been added. For wastewaters containing organic matter, use preliminary digestion with nitric and sulphuric acids, HNO_3 and H_2SO_4 respectively. If large amounts of Cl^- are also present, boiling with HNO_3 helps remove them. Interfering traces of Cl^- are eliminated by $HgSO_4$ in the special reagent.

Coloured solutions from other inorganic ions are compensated for in the final colorimetric step.

Samples that have been exposed to air may give low results because of precipitation of manganese dioxide, MnO_2 Add 1 drop of 30 per cent hydrogen peroxide, H_2O_2, to the sample (after adding the special reagent) to redissolve precipitated manganese.

Minimum detectable concentration

The molar absorptivity of the permanganate ion is about 2,300 l g^{-1} cm^{-1} This corresponds to a minimum detectable concentration (98 per cent transmittance) of 210 µg Mn l^{-1} when a 1-cm cell is used or 42 µg Mn l^{-1} when a 5-cm cell is used.

Apparatus

√ Colorimetric equipment. One of the following is required:

- Spectrophotometer, for use at 525 nm, providing a light path of 1 cm or longer.

- Filter photometer, providing a light path of 1 cm or longer and equipped with a green filter having maximum transmittance near 525 nm.

- Nessler tubes, matched, 100 ml, tall form.

Reagents
√ Special reagent. Dissolve 75 g $HgSO_4$ in 400 ml concentrated HNO_3 and 200 ml distilled water. Add 200 ml 85 per cent phosphoric acid, H_3PO_4, and 35 mg silver nitrate, $AgNO_3$ Dilute the cooled solution to 1 litre.

√ Ammonium persulphate, $(NH_4)_2S_2O_8$, solid.

√ Standard manganese solution. Prepare a 0.1 mol l^{-1} potassium permanganate, $KMnO_4$, solution by dissolving 3.2 g $KMnO_4$ in distilled water and making up to 1 litre. Age for several weeks in sunlight or heat for several hours near the boiling point, then filter through a fine fritted-glass filter crucible and standardise against sodium oxalate, $Na_2C_2O_4$, as follows:

Weigh several 100- to 200-mg samples of $Na_2C_2O_4$ to ± 0.1 mg and transfer to 400-ml beakers. To each beaker add 100 ml distilled water and stir to dissolve. Add 10 ml of 1+1 H_2SO_4 and heat rapidly to 90-95 °C. Titrate rapidly with the $KMnO_4$ solution to be standardised, while stirring, to a slight pink end-point colour that persists for at least 1 minute. Do not let temperature fall below 85 °C. If necessary, warm beaker contents during titration; 100 mg $Na_2C_2O_4$ will consume about 15 ml permanganate solution. Run a blank on distilled water and H_2SO_4

$$\text{Molarity of } KMnO_4 = \frac{g Na_2C_2O_4}{(A - B) \times 0.06701}$$

where

A = ml titrant for sample
B = ml titrant for blank

Average the results of several titrations. Calculate the volume of this solution necessary to prepare 1 litre of solution so that 1.00 ml = 50.0 µg Mn as follows:

$$\text{ml } KMnO_4 = \frac{4.55}{\text{molarity } KMnO_4}$$

To this volume add 2-3 ml concentrated H_2SO_4 and $NaHSO_3$ solution drop by drop, with stirring, until the permanganate colour disappears. Boil to remove excess SO_2, cool, and dilute to 1,000 ml with distilled water. Dilute this solution further to measure small amounts of manganese.

√ Standard manganese solution (alternative). Dissolve 1.000 g manganese metal (99.8 per cent min.) in 10 ml redistilled HNO_3 Dilute to 1,000 ml with 1 per cent (*v/v*) HCl (1 ml (1.000 mg Mn). Dilute 10 ml to 200 ml with distilled water (1 ml (0.05 mg Mn). Prepare dilute solution daily.

√ Hydrogen peroxide, H_2O_2, 30 per cent.

√ Nitric acid, HNO_3, concentrated.

√ Sulphuric acid, H_2SO_4, concentrated.

√ Sodium nitrite solution. Dissolve 5.0 g $NaNO_2$ in 95 ml distilled water.

√ Sodium oxalate, $Na_2C_2O_4$, primary standard.

√ Sodium bisulphite. Dissolve 10 g $NaHSO_3$ in 100 ml distilled water.

Procedure

Treatment of sample

1. If a digested sample has been prepared according to directions for reducing organic matter and/or excessive chlorides, pipette a portion containing 0.05-2.0 mg Mn into a 250-ml conical flask. Add distilled water, if necessary, to 90 ml, and proceed as in step 2 below.

2. To a suitable sample portion, add 5 ml special reagent and 1 drop H_2O_2 Concentrate to 90 ml by boiling or dilute to 90 ml. Add 1 g $(NH_4)_2S_2O_8$, bring to a boil, and boil for 1 minute. Do not heat on a water-bath. Remove from heat source, let stand for 1 minute, then cool under the tap. (Boiling too long results in decomposition of excess persulphate and subsequent loss of permanganate colour; cooling too slowly has the same effect.) Dilute to 100 ml with distilled water free from reducing substances, and mix. Prepare standards containing 0, 5.00, ... 1, 500 µg Mn by treating various amounts of standard Mn solution in the same way.

Nessler tube comparison
3. Use standards prepared as in step 2 above and containing 5 to 100 µg Mn per 100 ml final volume. Compare samples and standards visually.
Photometric determination
4. Use a series of standards from 0 to 1,500 µg Mn per 100 ml final volume. Make photometric measurements against a distilled water blank. The following table shows the light path length appropriate for various amounts of manganese in 100 ml final volume:

Mn range (µg)	**Light path (cm)**
5-200	15
20-400	5
50-1,000	2
100-1,500	1

5. Prepare a calibration curve of manganese concentration v. absorbance from the standards and determine Mn in the samples from the curve. If turbidity or interfering colour is present, make corrections as in step 6 below.
Correction for turbidity or interfering colour
6. Avoid filtration because of possible retention of some permanganate on the filter paper. If visual comparison of colour is used, the effect of turbidity can only be estimated and no correction can be made for interfering colour ions. When photometric measurements are made, use the following "bleaching" method, which also corrects for interfering colour. As soon as the photometer reading has been made, add 0.05 ml H_2O_2 solution directly to the sample in the optical cell. Mix and, as soon as the permanganate colour has faded

completely and no bubbles remain, read again. Deduct absorbance of bleached solution from initial absorbance to obtain absorbance due to Mn.

Calculation

A. When all of the original sample is taken for analysis:

$$Mn = \frac{\mu g\ Mn \quad (\text{in } 100\ ml\ \text{final volume})}{ml\ sample} mg\ l^{-1}$$

B. When a portion of the digested sample (100 ml final volume) is taken for analysis:

$$Mn = \frac{\mu g\ Mn/100\ ml}{ml\ sample} \times \frac{100}{ml\ portion} \quad mg\ l^{-1}$$

Precision and accuracy

A synthetic sample containing Mn, 120 µg l^{-1}; Al, 500 µg l^{-1}; Cd, 50 µg l^{-1}; Cr, 110 µg l^{-1}; Cu, 470 µg l^{-1}; Fe, 300 µg l^{-1}; Pb, 70 µg l^{-1}; Ag, 150 µg l^{-1} and Zn, 650 µg l^{-1} in distilled water was analysed in 33 laboratories by the persulphate method, with a relative standard deviation of 26.3 per cent and a relative error of 0 per cent.

A second synthetic sample, similar in all respects except for 50 µg Mn l^{-1} and 1,000 µg Cu l^{-1}, was analysed in 17 laboratories by the persulphate method, with a relative standard deviation of 50.3 per cent and a relative error of 7.2 per cent.

7.14 Nitrogen, ammonia

When nitrogenous organic matter is destroyed by microbiological activity, ammonia is produced and is therefore found in many surface and groundwaters. Higher concentrations occur in water polluted by sewage, fertilisers, agricultural wastes or industrial wastes containing organic nitrogen, free ammonia or ammonium salts.

Certain aerobic bacteria convert ammonia into nitrites and then into nitrates. Nitrogen compounds, as nutrients for aquatic micro-organisms, may be partially responsible for the eutrophication of lakes and rivers. Ammonia can result from natural reduction processes under anaerobic conditions.

The proportions of the two forms of ammonia nitrogen, i.e. free ammonia and ammonium ions, depend on the pH:

pH	6	7	8	9	10	11
% NH_3	0	1	4	25	78	96
% NH_4	100	99	96	75	22	

Sample handling

The preferred procedure is to remove ammonia from the sample by distillation. The ammonia may then be determined either by titration or colorimetrically using Nessler's reagent. Direct nesslerisation of the sample is quicker but is subject to considerable interference. The procedure given is the distillation and titration method.

If it is not possible to carry out the determination very soon after sampling, the sample should be refrigerated at 4 °C. Chemical preservation may be achieved by adding either 20-40 mg $HgCl_2$ or 1 ml H_2SO_4 to 1 litre of sample.

Principle

Ammonia can be quantitatively recovered from a sample by distillation under alkaline conditions into a solution of boric acid followed by titration with standard acid. The method is particularly suitable for the analysis of polluted surface and ground waters that contain sufficient ammonia to neutralise at least 1 ml of 0.00714 mol l^{-1} HCl.

Interferences

Volatile amines, if present, interfere with the acid titration. Generally, however, this method is less subject to interferences than other methods.

Apparatus

√ Distillation apparatus, consisting of a 1-litre, round-bottomed, heat-resistant glass flask fitted with a splash head, together with a suitable vertical condenser of either the spiral tube or double surface type. The condenser must be arranged so that the outlet tip can be submerged in the liquid in the receiver.

√ Usual laboratory glassware.

Reagents

√ Ammonia-free water. This should be prepared fresh for each batch of samples, since it is virtually impossible to store ammonia-free water in the laboratory without contamination from ammonia fumes.

(i) Distillation. To each litre of tap water add 2 ml of a solution of ferrous sulphate (100 g l^{-1} $FeSO_4.7H_2O$) and sufficient sulphuric acid to give a slight acid reaction to methyl orange. Distil with care, preferably in an all-glass distillation apparatus provided with a splash head. Reject the first 50 ml of distillate and then proceed until three- quarters of the volume of water has distilled over. Test for the absence of ammonia in the distillate with Nessler's reagent in the manner described below.

(ii) Ion exchange. As an alternative, ammonia may be removed from distilled water by the use of a strongly acidic cation exchange resin (hydrogen form). If only a small quantity of ammonia-free water is needed, add about 3 g of the cation exchange resin to each litre of distilled water and shake for a few minutes. If a regular supply of ammonia-free distilled water is needed, it is convenient to pass distilled water slowly down a column of the resin enclosed in a glass tube (250 mm long and about 25 mm in diameter is suitable). In either case, check that the water is free from ammonia by testing with Nessler's reagent.

√ Light magnesium oxide.

√ Indicating boric acid solution. Dissolve 20 g pure boric acid, H_3BO_3, in warm water and dilute to approximately 1 litre. Add 20 ml methyl red solution (0.5 g l^{-1}) and 0.4 ml methylene blue solution (15 g l^{-1}) and mix well. One drop of 0.1 mol l^{-1} NaOH should change the colour of 20 ml of the solution from purple to green. The solution is stable for several months.

√ Hydrochloric acid, 0.00714 mol l^{-1} Prepare approximately 0.714 ml l^{-1} HCl by diluting 65 ml of hydrochloric acid (*d* = 1.18) to 1 litre. Standardise by a suitable method and dilute with water to give a solution of the required strength.

Procedure

1. Before assembling the apparatus, thoroughly clean the distillation flask, splash head and condenser. In order to free the apparatus from possible contamination by ammonia, add to the flask about 350 ml water (preferably ammonia-free) and distil until the distillate is shown to be ammonia-free by testing with Nessler's reagent. Empty the distillation flask and allow it to cool.

2. Place a suitable volume of the sample in the flask; 100 ml should be sufficient for a purified effluent, while 200 to 400 ml of surface water may be necessary to give a final titration of reasonable magnitude. Use a 400-ml beaker as a receiver and keep the lower end of the delivery tube from the condenser below the surface of the absorbent liquid throughout the distillation.

3. Neutralise the measured volume of sample with NaOH, if necessary.

4. Dilute the measured volume of sample, if necessary, to 400 ml with ammonia-free water in the distillation flask and add about 0.25 g magnesium oxide.

5. Place 50 ml of the indicating H_3BO_3 solution in the 400-ml receiving beaker.

6. Distil at a rate of about 10 ml per minute. As the indicating H_3BO_3 solution changes colour, titrate with 0.00714 mol l^{-1} HCl, continuing the distillation until the addition of one drop of the standard acid produces a permanent pink colour in the solution.

7. At the completion of the titration, remove the receiver from the apparatus before the source of heat is withdrawn.

8. Carry out a blank determination and correct the final titration values for samples to compensate for any ammonia in the reagent used.

Calculation

$$\text{Ammonia nitrogen (as N)} = \frac{100V_2}{V_1} \text{ mg l}^{-1}$$

where

V_1 = volume of sample taken (ml)
V_2 = volume of 0.00714 mol l^{-1} acid used (ml).

7.15 Nitrogen, Kjeldahl

Kjeldahl nitrogen is defined as the sum of ammonia nitrogen and those organic nitrogen compounds converted to ammonium sulphate under the conditions of the digestion procedure described below. The organic Kjeldahl nitrogen is obtained by subtracting the value of ammonia nitrogen from the Kjeldahl nitrogen value.

Sample handling

Conversion of organic nitrogen to ammonia may occur in samples between collection and analysis. This effect may be decreased by the addition of 2 ml of sulphuric acid (*d* = 1.84) per litre of sample and storage at 4 °C, but it is prudent to analyse all samples as soon as possible after collection.

Mercuric salts sometimes used for conservation interfere in the colorimetric method.

Principle

The sample is heated in the presence of sulphuric acid and a catalyst, alcohol also being added to ensure removal of oxidised nitrogen. After digestion, the solution is diluted. The ammonia is determined by photometry or, if sufficiently large amounts are present, distilled from the solution and titrated.

Interferences

Neither this nor any other single method can guarantee that every organic nitrogen compound will be broken down to ammonia. This is unlikely to lead to serious difficulty, but analysts should be watchful for exceptional cases. Nitrate and nitrite are removed in the procedure described below and so cause no important errors.

7.15.1 Titrimetric determination following mineralisation and distillation

Sample handling

Samples for laboratory analysis must be cooled as soon as possible after being taken and must be kept at a temperature around 4 °C until the time of analysis.

The analysis must be carried out as soon as possible after taking the sample, but if it cannot be carried out within 24 hours the sample may be acidified at the sampling point to pH 2 with concentrated sulphuric acid (in general 2 ml of acid is sufficient for 1 litre of sample).

Note: Attention is drawn to the fact that sulphuric acid is capable of entrapping ammonia vapour.

Principle

Mineralisation of the organic matter in an acid medium and in the presence of a catalyst. Interference due to oxidised forms of mineral nitrogen may be eliminated by the addition of ethyl alcohol. Steam distillation in an alkaline medium of the ammonia nitrogen obtained. Titrimetric determination (direct or back titration).

Apparatus

√ Usual laboratory equipment. The glassware used for the preparation of the reagents and for determination should not be used for other determinations, and should not come into contact with ammonia or ammonium salts in high concentrations. When using new equipment it is a good idea to carry out the blank test twice in graduated flasks. Between analyses, the flasks or tubes should be filled with water, stoppered and kept in the dark.

√ Kjeldahl flask, 500-ml capacity, and flask heater (or mineralisation burners).

√ Distillation or steam distillation apparatus. It is recommended that the base of the refrigerant be fitted with a glass cap to prevent possible condensation running into the flask in which the distillate is collected. When distillation equipment is exposed to the atmosphere of the laboratory, its walls may pick up traces of ammonia nitrogen that cannot be removed by rinsing in water. For the determination of low nitrogen contents it is essential to clean the apparatus by carrying out one or two "blank" distillations of a sodium hydroxide solution before every series of measurements. Between tests the equipment should be kept protected from the laboratory atmosphere.

√ Burette, 20 ml, 0.1-ml graduations.

Reagents
√ Only water recently demineralised on strong cationic resin, or water of equivalent purity with an insignificant nitrogen content, should be used in the analysis and for preparation of the reagents. The reagents themselves must be of analytical purity.

√ Concentrated sulphuric acid (d = 1.84 g ml^{-1}).

√ Sulphuric acid, solution at roughly 5 g l^{-1}

√ Sodium hydroxide, solution at 400 g l^{-1} (10 mol l^{-1}).

√ Boric acid, solution at 10 g l^{-1}

√ Sulphuric acid, standard solution, 0.05 mol l^{-1}

√ Sulphuric acid, standard solution, 0.01 mol l^{-1}

√ Mineralisation catalyst: Prepare a homogeneous mixture of 995 g of potassium sulphate, K_2SO_4, and 5 g of powdered selenium.

√ Indicator: solution of methyl red and bromocresol green in 95 per cent ethyl alcohol (by volume).

√ Solution of methyl red in water, 10 g l^{-1}

√ Ethyl alcohol.

Procedure
1. Take at least 50 ml of sample so as to have between 0.2 and 20 mg of nitrogen expressed as N.
Mineralisation
2. Place the sample for analysis in a Kjeldahl flask containing a boiling regulator (glass beads, ceramic chips or pumice). Add 1 g of catalyst and, if appropriate, an antifoam agent. Add 10 ml of ethyl alcohol. Add 10 ml of concentrated sulphuric acid. Fit a funnel to the flask, inserting the stem into the neck of the flask.

3. Bring slowly to the boil and evaporate until a white vapour is given off. Thereafter increase the heat and continue to heat for 2 hours. Leave to cool to room temperature.

Note: If mineralisation is incomplete (turbid or highly coloured liquid), the operation must be repeated, reducing the size of sample for analysis or carrying out preliminary dilution (in the case of samples with a heavy organic load).

Distillation and determination
4. If necessary, decant the contents of the flask and the washing water (about 200 ml to 250 ml) into the distillation apparatus.

5. Add 50 ml of 10 mol l^{-1} sodium hydroxide. Admit steam for distillation of the ammonia nitrogen.

6. Collect about 200 ml of distillate in a receptacle containing 10 ml of boric acid solution and 3 or 4 drops of indicator. Add sufficient water, if required, to ensure that the end of the extension tube of the refrigerant is submerged in the solution.

7. Carry out titration, using:

(i) the 0.05 mol l^{-1} standard solution of sulphuric acid if the sample for analysis contains between 2 and 20 mg of nitrogen, expressed as N, or

(ii) the 0.01 mol l^{-1} standard solution of sulphuric acid if the sample for analysis contains between 0.2 and 2 mg of nitrogen, expressed as N.

8. Note the volume V_1 required.
Blank test
9. Carry out a blank test under the same conditions of mineralisation and determination as for the sample being analysed. Let V_0 be the required volume of 0.05 or 0.01 mol l^{-1} sulphuric acid in millilitres.
Calculation

The Kjeldahl nitrogen content, expressed as mg N l^{-1}, is given by:

$$N = \frac{(V_1 - V_0)c \times 1{,}000 \times 28}{V} \text{ mg } l^{-1}$$

where:

V_1 = the volume of 0.05 or 0.01 mol l^{-1} sulphuric acid (ml) used for the determination
V_0 = the volume of 0.05 or 0.01 mol l^{-1} sulphuric acid (ml) used for the blank test
c = the molarity (0.05 or 0.01 mol l^{-1}) of the standard solution of sulphuric acid used for the determination
V = the volume of the sample taken for analysis (ml)

7.15.2 Spectrophotometric method after mineralisation

Sample handling

Samples for the analytical laboratory must be cooled to a temperature of around 4 °C as soon as possible after being taken. It is recommended that the analysis be carried out as soon as possible and that acidification of the sample be avoided for the determination of low concentrations because sulphuric acid is capable of entrapping ammonia vapour.

Principle

Mineralisation of the organic matter in an acid medium in the presence of a catalyst. Determination by indophenol blue spectrophotometry at a wavelength around 630 nm.

Apparatus

√ Usual laboratory equipment. The glassware used to prepare the reagents and carry out the determination must not be used for other determinations, nor must it come into contact with ammonia or ammonium salts in high concentration. When using new equipment it is a good idea to carry out the blank test twice in the tubes in which the colour will be developed. Between analyses the flasks or tubes should be filled with water, stoppered, and kept in the dark.

√ Kjeldahl flasks, 500-ml capacity, and flask heater (or mineralisation burners).

√ Spectrophotometer, 630 nm, fitted with measuring tubes 10 mm deep.

Reagents
√ Concentrated sulphuric acid (d = 1.84 g ml^{-1}).

√ Sodium hydroxide, 400 g l^{-1} solution.

√ Alkaline solution, complexing and chlorinated: Dissolve 20 g of sodium hydroxide tablets and 380 g of dihydrated trisodium citrate ($C_6H_5Na_3O_7.2H_2O$) in about 800 ml of water. Raise to boiling point and keep simmering gently for 20 minutes. Cool, add 4 g of dihydrated dichloro-1, 3-isocyanuric acid sodium salt ($C_3NaCl_2N_3O_3.2H_2O$) and make up the volume to 1,000 ml with water. Store this solution at around 4 ° C.

√ Phenol and nitroprusside solution. Dissolve 35 g of phenol and 0.4 g of sodium nitroprusside ($Na_2[Fe(CN)_5NO]. 2H_2O$) in water and make up the volume to 1,000 ml with water. Store this solution in a brown glass flask at around 4 ° C. Never place it in direct sunlight. Handle in subdued light.

√ Mineralisation catalyst. Prepare a homogeneous mixture of 995 g of potassium sulphate (K_2SO_4) and 5 g of powdered selenium.

√ Ethyl alcohol.

√ Stock nitrogen solution corresponding to 100 mg N per litre. Dissolve 381.9 mg of anhydrous ammonium chloride in water and make up to 1,000 ml in a volumetric flask.

√ Standard nitrogen solution corresponding to 10 mg N per litre. At the time of use, dilute the stock solution to one-tenth.

√ 0.1 paranitrophenol solution in water (*m/v*).

Procedure
1. Sample for analysis. Take at least 50 ml from the sample so that the sample for analysis contains between 20 and 200 µg of nitrogen expressed as N.

2. Preparation of calibration solutions. For spectrophotometric measurements carried out in tubes 10 mm deep, place increasing volumes (e.g. 0, 1, 2, 5, 10, 15, 20 ml) of the standard 10 mg N l^{-1} solution in a series of 100-ml volumetric flasks. Make up to the volume and homogenise.

3. Mineralisation. Place the sample for analysis and each of the standard solutions in a series of Kjeldahl flasks containing a boiling regulator (glass beads, ceramic chips or pumice). Add 0.2-0.5 g of catalyst followed respectively by 2 to 5 ml of concentrated H_2SO_4 and 5 ml of ethyl alcohol. Fit a funnel to the flask, inserting the stem into the neck of the flask. Bring slowly to the boil and evaporate until white vapour appears. Thereafter increase the heat and continue to heat for 1 hour. Cool to room temperature.

Note: If mineralisation is incomplete (turbid or highly coloured liquid), recommence the operation, reducing the size of the sample for analysis or carrying out preliminary dilution.
4. Determination. Collect the mineralisates obtained in step 3 and the wash water in a series of 200-ml volumetric flasks to obtain a volume of about 150 ml. Neutralise with sodium hydroxide solution in the presence of a drop of paranitrophenol. Make up to the volume with water. Homogenise and allow to settle if necessary. Take 20 ml of each solution and place in a series of test-tubes. Add in sequence, without waiting between each addition:
1.0 ml of the phenol and nitroprusside solution, stir;
1.0 ml of the complexing and chlorinated alkaline solution.
5. Stir and place in the dark for at least 6 hours.

6. Carry out the spectrophotometric measurements at the maximum of the absorption curve (wavelength generally around 630 nm) after having adjusted the apparatus to zero absorbance relative to the control.

7. Control. Carry out a control test alongside the determination, replacing the sample by water.

Calculation

Taking the concentrations of the calibration solutions as a starting point, establish a calibration curve and use it to deduce the Kjeldahl nitrogen content of the sample being analysed, having regard to any possible dilutions. Express the result in milligrams of N per litre.

7.16 Nitrogen, nitrate

Nitrate, the most highly oxidized form of nitrogen compounds, is commonly present in surface and ground waters, because it is the end product of the aerobic decomposition of organic nitrogenous matter. Significant sources of nitrate are chemical fertilisers from cultivated land and drainage from livestock feedlots, as well as domestic and some industrial waters.

The determination of nitrate helps the assessment of the character and degree of oxidation in surface waters, in groundwater penetrating through soil layers, in biological processes and in the advanced treatment of wastewater.

Unpolluted natural waters usually contain only minute amounts of nitrate. In surface water, nitrate is a nutrient taken up by plants and assimilated into cell protein. Stimulation of plant growth, especially of algae, may cause water quality problems associated with eutrophication. The subsequent death and decay of algae produces secondary effects on water quality, which may also be undesirable. High concentrations of nitrate in drinking water

may present a risk to bottle-fed babies under three months of age because the low acidity of their stomachs favours the reduction of nitrates to nitrites by microbial action. Nitrite is readily absorbed into the blood where it combines irreversibly with haemoglobin to form methaemoglobin, which is ineffective as an oxygen carrier in the blood. In severe cases a condition known as infantile methaemoglobinaemia may occur which can be fatal for young babies.

The determination of nitrate in water is difficult because of interferences, and much more difficult in wastewaters because of higher concentrations of numerous interfering substances.

The first method given here, Devarda's alloy method, involves oxidation, distillation and titration. One of its attractive features is that it can be performed on the residue remaining in the flask after the distillation process required in the determination of ammonia nitrogen. The distillation step in the method also eliminates many interferences and is often used for the analysis of wastewater samples. In the second method given, nitrate is reduced to nitrite in a cadmium column and nitrite is then determined by the method given in section 7.17.

Sample handling

To prevent any change in the nitrogen balance through biological activity, the nitrate determination should be started as soon as possible after sampling. If storage is necessary, samples should be kept at a temperature just above the freezing point, with or without preservatives, such as 0.8 ml of concentrated sulphuric acid (d = 1.84) or 40 mg of mercury (as mercuric chloride) per litre of sample. If acid preservation is employed, the sample should be neutralised to about pH 7 immediately before the analysis is begun.

7.16.1 Devarda's alloy method (reduction to ammonia)

Principle

This method is suitable for nitrate concentrations exceeding 1 mg l^{-1}, especially for wastewater and polluted surface water. The analysis may be carried out either on the original sample or on the residue from the determination of ammonia.

Nitrate is reduced to ammonia by nascent hydrogen, by the use of Devarda's alloy (59 per cent Al, 39 per cent Cu, 2 per cent Zn). The resulting ammonia is distilled and its concentration determined by titration.

Nitrites are also reduced by Devarda's alloy and their separate determination can be carried out rapidly and readily. The nitrate concentration can therefore be satisfactorily determined by subtracting the nitrite fraction from the total oxidised nitrogen.

Sometimes, especially when the proportion of nitrogen present as nitrite is small, the report of the analysis is confined to total oxidised nitrogen.

Interferences

Ammonia must be removed from the sample before the main procedure is started. This is achieved either by pretreatment or by using the distillation residue from the determination of ammonia. The air in the laboratory and the distilled water used for solutions and during the procedure should be free of ammonia. If the sample or any reagent needs to be filtered, only nitrogen-free filters should be used. Nitrite is determined separately and subtracted from the result.

Apparatus

√ The same glassware and distilling apparatus as for the ammonia nitrogen determination (see section 7.14).

Reagents

The following reagents are required in addition to those used for the ammonia nitrogen determination:

√ Devarda's alloy, powdered. If fine powder is not available, the material should be ground to pass through a 0.07-0.1 mm sieve (200-140 mesh). It should be as free as possible from nitrogen. It can be purchased as such but a blank determination of its nitrogen content should always be carried out under the conditions of the test.

√ Sodium hydroxide, 10 mol l^{-1} (needed only if an original sample is used - see procedure).

Procedure

Determination on an original sample

1. Before assembling the apparatus, thoroughly clean the distillation flask, splash head and condenser. In order to free the apparatus from possible contamination by ammonia, add to the flask about 350 ml water, preferably ammonia-free, and distil until the distillate is shown to be free from ammonia by testing with Nessler's reagent. Empty the distillation flask and allow it to cool.

2. Measure 200 ml of the sample into the flask.

3. Add 10 ml of 10 mol l^{-1} sodium hydroxide. Evaporate in the distillation flask to 100 ml. Allow the residue to cool.

4. Continue as indicated in step 6, below.

Determination on the residue from the analysis for ammonia

5. At the end of the distillation procedure for the analysis for ammonia, allow the residue to cool.

6. To the cooled residue add sufficient ammonia-free water to bring the volume in the distillation flask to about 350 ml. Add 1 g Devarda's alloy, and immediately connect the flask to the condenser.

7. After some minutes, start the distillation, keeping the lower end of the delivery tube from the condenser below the surface of the liquid in the receiver throughout the distillation.

8. Place 50 ml of the indicating boric acid solution in the receiver and distil at a rate of about 10 ml per minute.

9. As the absorbent solution changes colour, titrate with 0.00714 mol l^{-1} hydrochloric acid, continuing the distillation until the addition of one drop of the standard acid produces a permanent pink colour in the solution.

10. At the completion of the titration, remove the receiver from the apparatus before the source of heat is withdrawn.

11. Carry out blank determinations as appropriate. For each sample, correct the final titration figure for any ammonia in the reagent used.

Calculation

$$\text{Nitrate nitrogen (as N)} = \frac{(a-b)\times 100}{V} - n \text{ mg l}^{-1}$$

where

a = volume of 0.00714 mol l^{-1} acid used for titration of the distillate of the sample (ml)
b = volume of 0.00714 mol l^{-1} acid used for titration of the distillate of the blank (ml)
V = volume of the undiluted sample (ml)
n = concentration of nitrite nitrogen in mg l^{-1} N, determined separately

Note: The same calculation without subtracting nitrite gives the result for total oxidised nitrogen.

The result is reported as nitrate nitrogen (as N) mg l^{-1} and should be rounded to two significant figures.

7.16.2 Cadmium reduction method

Principle

Nitrate is reduced to nitrite when a sample is passed through a column containing amalgamated cadmium filings. Nitrite, that originally present plus that reduced from nitrate, is then determined. A separate determination for the concentration of nitrite alone is necessary. The applicable range of this method is 0.01 to 1.0 mg l^{-1} of nitrate plus nitrite nitrogen.

Interferences

Build-up of suspended material in the reduction column will restrict sample flow. Since nitrate nitrogen occurs in a soluble state, the sample may be filtered through a glass-fibre filter or a 0.45 µm pore diameter membrane filter. Highly turbid samples may be treated with zinc sulphate before filtration to remove the bulk of the particulate matter present in the sample.

Low results may be obtained for samples that contain high concentrations of iron, copper or other metals. Addition of EDTA to the samples will eliminate this interference. Samples containing oil and/or grease will coat the surface of the cadmium and inhibit the reduction process. This interference may be eliminated by pre-extracting the sample with an organic solvent.

Apparatus

√ Reduction column. This can be constructed from three pieces of tubing joined together as shown in Figure 7.2: 100 mm of 50 mm diameter tubing is joined to 300 mm of 10 mm diameter tubing which is, in turn, joined to 350 mm of 2 mm diameter tubing (all diameters are internal). The 2 mm diameter tubing is bent at its lower end so that it will be parallel to the 10 mm diameter tube and also bent at its upper end to form a siphon. This last bend should be just level with the top of the 10 mm diameter tube as shown in Figure 7.2. This

arrangement allows liquid placed in the top reservoir to flow out of the system but to stop when the liquid level reaches the top of the cadmium filings. Place a mark on the reservoir to indicate the level that will be reached when 80 ml of liquid is added to the system.

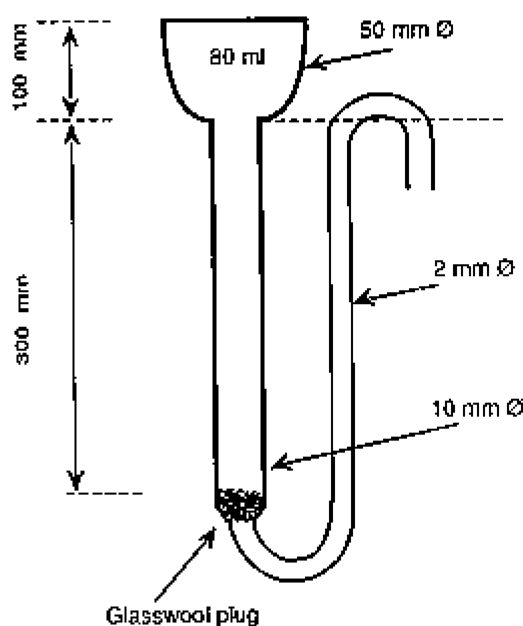
√ Nessler tubes, 50 ml, or volumetric flasks, 50 ml.

√ Erlenmeyer flasks, 125 ml.

√ Graduated cylinders, 50 ml.

√ Colorimetric equipment, either a spectrophotometer equipped with 10 mm or larger cells for use at 540 nm or a filter photometer with light path 1 cm or longer and equipped with a filter having maximum transmittance near 540 nm.

Figure 7.2 Cadmium reduction column



Reagents

√ Ammonium chloride solution, concentrated. Dissolve 100 g of NH_4Cl in 500 ml of distilled water and store in a glass bottle.

√ Dilute ammonium chloride solution. Dilute 50 ml of concentrated ammonium chloride solution to 2 litres with distilled water. Store in a glass or plastic bottle.

√ Amalgamated cadmium filings. File sticks of pure cadmium metal (reagent grade, 99.9 per cent Cd) with a coarse metal hand file and collect the fraction that passes a sieve with 5 mm openings but is retained on a sieve with 0.2 mm openings. Stir about 300 g of filings with 300 ml of 10 g l^{-1} mercuric chloride solution for 3 minutes. This amount should be sufficient to charge six reduction columns. Allow the metal to settle and decant off the liquid. Wash the amalgamated filings several times with distilled water then briefly with dilute (1+99) nitric acid followed by several washings with dilute (1+99) hydrochloric acid. Wash thoroughly with distilled water until no nitrite can be detected in the supernatant fluid. Store the filings in the dark under dilute ammonium chloride solution.

√ Stock nitrate solution. Dissolve 7.218 g of potassium nitrate, KNO_3, in distilled water and dilute to 1 litre. Preserve with 2 ml of chloroform per litre. This solution is stable for about 6 months (1.0 ml (1.0 mg nitrate N).

√ Working nitrate solution. Dilute 10.0 ml of stock nitrate solution to 1 litre with distilled water (1.0 ml (0.01 mg nitrate N).

The reagents required for the nitrite nitrogen procedure are described in section 7.17.

Procedure

1. Preparation of reduction columns. Pack a plug of glass wool in the bottom of a reduction column and fill the column with distilled water. Pour in sufficient amalgamated cadmium filings to produce a column 300 mm in length. Use the column size specified because columns of smaller length and diameter give erratic results and show rapid deterioration. Wash the column thoroughly with dilute ammonium chloride solution. Use a flow rate no greater than 8 ml per minute. If the flow rate is too fast, slow it by constricting the end of the outlet siphon or by packing more glass wool at the base of the column. On the other hand, flow rates less than 5 ml per minute unnecessarily increase the time required for an analysis and may give low results. When not in use, cover the metal in the column with dilute ammonium chloride solution. Prepare several reduction columns similarly.

2. Sample pretreatment:

(i) If turbidity or suspended solids are present, filter the sample through a glass fibre filter or a membrane filter.

(ii) If the sample contains oil or grease, adjust the pH to 2 by the addition of hydrochloric acid to 100 ml of filtered sample. Extract the oil and grease from the filtrate with two 25-ml portions of a non-polar solvent (chloroform or equivalent).

(iii) Adjust the pH of the sample to between 7 and 9 using a pH meter and by adding either ammonia or hydrochloric acid as appropriate.

3. Place 80-90 ml of sample in a 125-ml Erlenmeyer flask and add 2.0 ml of concentrated ammonium chloride solution. Mix and pour the sample from the flask onto the column until the marked level in the top of the column is reached. Place a 50-ml graduated cylinder under the outlet to collect the effluent and discard any of the sample remaining in the Erlenmeyer flask. Shake the flask as dry as possible and retain it for collecting the main portion of the effluent.

4. Allow between 25 and 30 ml of effluent to collect in the cylinder and then replace the cylinder with the Erlenmeyer flask that contained the sample. Discard the contents of the cylinder. The passage of 25 to 30 ml of sample through the column removes the ammonium chloride solution or traces of a previous sample from the voids in the column. The volume flushed through is not critical provided that it exceeds 25 ml, but sufficient sample should be left in the column so that 50 ml will be available for subsequent analysis. The volume of flushing effluent should therefore not exceed 30 ml. A maximum of about eight columns can be handled conveniently at one time by one technician. The technician should experiment to find a suitable time delay between adding samples to successive columns so that there will be time to reject the flushing liquid from one column and replace the cylinder by the Erlenmeyer flask before too much flushing liquid has escaped from the next column in line.

5. Allow the remainder of the reduced sample to collect in the flask. When the flow of the sample from the column has ceased, pour exactly 50 ml from the flask into the 50-ml measuring cylinder. Drain and discard the remaining reduced sample from the Erlenmeyer flask, shake the flask as dry as possible and then pour the 50 ml of reduced sample back into the flask.

Note: There is no need to wash columns between samples, but if columns are not to be re-used for several hours or longer, pour 50 ml of dilute ammonium chloride solution into the top and allow it to pass through the system. Store the cadmium filings in this ammonium chloride solution and never allow them to get dry. The cadmium filings can be regenerated by washing them briefly with HNO_3 (1+99), rinsing them thoroughly with distilled water, drying, re-sieving and re-amalgamating.

6. Prepare a series of standards in 50-ml Nessler tubes as follows:

Volume of working nitrate solution (ml)	Concentration when diluted to 100 ml (mg l^{-1} NO_2-N)
0.0 (blank)	0.0
0.5	0.05
1.0	0.10
2.0	0.20
5.0	0.50
10.0	1.00

7. Colour development. Proceed as described in steps 4 and 5 of section 7.17.

Calculation

Obtain a standard curve by plotting absorbance of standards against NO_3-N concentration. Compute sample concentrations directly from the standard curve. Report the results as milligrams of oxidised N per litre (the sum NO_2-N plus NO_3-N). If the concentration of NO_2-N has been determined separately, subtract it from the value obtained for oxidised N to obtain mg l^{-1} NO_3-N.

7.17 Nitrogen, nitrite

Nitrite is an unstable, intermediate stage in the nitrogen cycle and is formed in water either by the oxidation of ammonia or by the reduction of nitrate. Thus, biochemical processes can cause a rapid change in the nitrite concentration in a water sample. In natural waters nitrite is normally present only in low concentrations (a few tenths of a milligram per litre). Higher concentrations may be present in sewage and industrial wastes, in treated sewage effluents and in polluted waters.

Sample handling

The determination should be made promptly on fresh samples to prevent bacterial conversion of the nitrite to nitrate or ammonia. In no case should acid preservation be used for samples to be analysed for nitrite. Short-term preservation for 1 to 2 days is possible by the addition of 40 mg mercuric ion as $HgCl_2$ per litre of sample, with storage at 4 C.

Principle

Nitrite reacts, in strongly acid medium, with sulphanilamide. The resulting diazo compound is coupled with *N*-(1-naphthyl)-ethylenediamine dihydrochloride to form an intensely red-coloured azo-compound. The absorbance of the dye is proportional to the concentration of nitrite present.

The method is applicable in the range of 0.01-1.0 mg l^{-1} nitrite nitrogen. Samples containing higher concentrations must be diluted.

Interferences

There are very few known interferences at concentrations less than 1,000 times that of the nitrite. However, the presence of strong oxidants or reductants in the samples will readily affect the nitrite concentrations. High alkalinity (>600 mg l^{-1} as $CaCO_3$) will give low results owing to a shift in pH.

Apparatus

√ Spectrophotometer equipped with 10-mm or larger cells for use at 540 nm.
√ Nessler tubes, 50 ml, or volumetric flasks, 50 ml.
Reagents
√ Hydrochloric acid, 3 mol l^{-1} Combine 1 part concentrated HCl with 3 parts distilled water.

√ Nitrite- and nitrate-free distilled water. Add 1 ml of concentrated sulphuric acid and 0.2 ml manganous sulphate ($MnSO_4$) solution to 1 litre of distilled water and make it pink with 1-3 ml potassium permanganate solution (440 mg $KMnO_4$ in 100 ml distilled water). Redistil in an all borosilicate glass still. Discard the first 50 ml of distillate. Test each subsequent 100 ml fraction of distillate by the addition of DPD indicator, discarding those with a reddish colour that indicates the presence of permanganate.

√ DPD indicator solution. Dissolve 1 g of DPD oxalate or 1.5 g of *p*-amino-*N*, *N*-diethyl-aniline sulphate in chlorine-free distilled water containing 8 ml of H_2SO_4, 18 mol l^{-1}, and 200 mg of disodium ethylenediaminetetraacetate dihydrate (EDTA). Make up to 1 litre and store in a brown bottle and discard the solution when it becomes discoloured.

Note: DPD indicator solution is commercially available.

√ Buffer-colour reagent. Distilled water free of nitrite and nitrate must be used in the preparation of this reagent. To 250 ml of distilled water add 105 ml concentrated hydrochloric acid, 5.0 g sulphanilamide and 0.5 g *N*-(1-naphthyl)-ethylenediamine dihydrochloride. Stir until dissolved. Add 136 g of sodium acetate ($CH_3COONa.3H_2O$) and again stir until dissolved. Dilute to 500 ml with distilled water. This solution is stable for several weeks if stored in the dark.

√ Stock nitrite solution. Distilled water free of nitrite and nitrate must be used in the preparation of this solution. Dissolve 0.4926 g of dried anhydrous sodium nitrite (24 hours in desiccator) in distilled water and dilute to 1 litre. Preserve with 2 ml chloroform per litre (1.0 ml (100 µg nitrite N).

√ Working nitrite solution. Distilled water free of nitrite and nitrate must be used in the preparation of this solution. Dilute 10.0 ml of the stock solution to 1 litre (1.0 ml (1.0 µg nitrite N).

Procedure

1. If the sample has a pH greater than 10 or a total alkalinity in excess of 600 mg l^{-1} (expressed as $CaCO_3$), adjust to approximately pH 6 with 3 mol l^{-1} HCl.

2. If necessary, pass the sample through a filter of pore size 0.45 µm, using the first portion of filtrate to rinse the filter flask.

3. Place 50 ml of sample, or an aliquot diluted to 50 ml, in a 50-ml Nessler tube and set aside until preparation of standards is completed. At the same time, prepare a series of standards in 50-ml Nessler tubes as follows:

Volume of working nitrate solution (ml)	Concentration when diluted to 50 ml (mg l^{-1} of NO_2-N)
0.0 (blank)	0.0
0.5	0.01
1.0	0.02
1.5	0.03
2.0	0.04
3.0	0.06
4.0	0.08
5.0	0.10
10.0	0.20

4. Add 2 ml of buffer-colour reagent to each standard and sample, mix, and allow colour to develop for at least 15 minutes. The pH values of the solutions at this stage should be between 1.5 and 2.0.

5. Measure the absorbance of the standards and samples at 540 nm. Prepare a standard curve by plotting the absorbance of the standards against the concentration of NO_2-N.

Calculation

Read the concentration of NO_2-N in samples directly from the calibration curve. If less than 50 ml of sample is taken, calculate the concentrations as follows:

$$\text{Nitrite nitrogen (as N)} = \frac{\text{mg l}^{-1}\text{ from standard curve} \times 50}{\text{ml sample}}$$

7.18 Phosphorus

Groundwaters rarely contain more than 0.1 mg l^{-1} phosphorus unless they have passed through soil containing phosphate or have been polluted by organic matter.

Phosphorus compounds are present in fertilisers and in many detergents. Consequently, they are carried into both ground and surface waters with sewage, industrial wastes and storm run-off. High concentrations of phosphorus compounds may produce a secondary problem in water bodies where algal growth is normally limited by phosphorus. In such situations the presence of additional phosphorus compounds can stimulate algal productivity and enhance eutrophication processes.

Principle

Organically combined phosphorus and all phosphates are first converted to orthophosphate. To release phosphorus from combination with organic matter, a digestion or wet oxidation technique is necessary. The least tedious method, wet oxidation with potassium peroxydisulphate, is recommended.

Orthophosphate reacts with ammonium molybdate to form molybdophosphoric acid. This is transformed by reductants to the intensely coloured complex known as molybdenum blue. The method based on reduction with ascorbic acid is preferable. Addition of potassium antimonyl tartrate increases the coloration and the reaction velocity at room temperature.

For concentrations of phosphate below 20 µg l^{-1}, the recommended procedure involves extraction of the molybdenum blue complex from up to 200 ml of water into a relatively small volume of hexanol, so that a considerable increase in sensitivity is obtained.

Interferences

The method is relatively free from interferences. Changes in temperature of ± 10 °C do not affect the result.

Apparatus

√ Heating equipment. One of the following is required:

√ Hotplate, a 30 cm × 50 cm heating surface is adequate.

√ Autoclave. An autoclave or pressure-cooker capable of developing 1.1-1.4 kPa cm^{-2} may be used in place of a hotplate.

√ Colorimetric equipment. One of the following is required:

√ Spectrophotometer, with infrared phototube, for use at 880 nm, providing a light path of 40 mm or longer. If this wavelength is not obtainable on the spectrophotometer, a wavelength of 710 nm may be used but there will be some loss of sensitivity.

√ Absorptiometer, equipped with a red colour filter and a light path of 40 mm or longer.

√ Acid-washed glassware. The glassware used, including sample bottles, should be reserved for the determination of phosphate, should not be used for any other purpose and should be left full of sulphuric acid (4.5 mol l^{-1}) until required for use. If necessary, glassware may be cleaned with chromic acid, equal mixtures of nitric and hydrochloric acids, or pure sulphuric acid. Detergents containing phosphate compounds must not be used.

Reagents

√ Phenolphthalein indicator solution. Dissolve 0.5 g of phenolphthalein in 50 ml of 95 per cent ethyl alcohol, and add 50 ml of distilled water. Add a dilute (e.g. 0.01 or 0.05 mol l^{-1}) carbon dioxide-free solution of sodium hydroxide, a drop at a time, until the indicator turns faintly pink.

√ Potassium peroxydisulphate solution. Dissolve 5 g $K_2S_2O_8$ in 100 ml distilled water. Prepare daily.

√ Phosphate stock solution. Dissolve 4.390 g potassium dihydrogen phosphate, KH_2PO_4, in 1 litre of water. Add one or two drops of toluene as a preservative (1.0 ml stock solution is equivalent to 1.0 mg P).

√ Phosphate working solution. Dilute 10 ml of stock solution to 100 ml with distilled water and mix well. Then dilute 10 ml of this solution to 1 litre and mix. The dilute solution thus obtained will not keep very long and should be freshly prepared when required (1.0 ml working solution is equivalent to 1.0 µg P).

√ Ammonium molybdate tetrahydrate solution, 40 g l^{-1}

√ Sodium hydroxide, approximately 5 mol l^{-1}

√ Sulphuric acid, approximately 2.5 mol l^{-1} Add carefully, with mixing, 140 ml sulphuric acid (*d* = 1.84) to water, cool and make up to 1 litre.

√ Potassium antimonyl tartrate solution. Dissolve 2.7 g potassium antimonyl tartrate in water and make up to 1 litre.

√ Reducing agent. Mix together 250 ml of 2.5 mol l^{-1} sulphuric acid, 75 ml ammonium molybdate solution and 150 ml distilled water. Add 25 ml potassium antimonyl tartrate solution and again mix well. The solution should be kept in a refrigerator and is stable for several weeks. Immediately before use of the reagent in step 9 of the procedure, pour an aliquot into an Erlenmeyer flask and add 1.73 g ascorbic acid for each 100 ml of reagent. After the addition of ascorbic acid the solution is unstable and cannot be stored. For each standard and each sample, 8 ml of solution is required.

Procedure

1. Take 100 ml of thoroughly mixed sample.

2. Add 1 drop (0.05 ml) of phenolphthalein indicator solution. If a red colour develops, add sulphuric acid solution drop by drop to just discharge the colour.

3. Add 2 ml sulphuric acid solution and 15 ml potassium peroxydisulphate solution.

4. Boil gently for at least 90 minutes, adding distilled water to keep the volume between 25 and 50 ml. Alternatively, heat for 30 minutes in an autoclave or pressure-cooker at 1.1-1.4 kPa cm^{-2}

5. Cool, add 1 drop (0.05 ml) phenolphthalein indicator solution, and neutralise to a faint pink colour with sodium hydroxide solution.

6. Restore the volume to 100 ml with distilled water and set aside.

7. Prepare a series of standards in 50-ml volumetric flasks as follows:

Volume of working phosphate solution (ml)	Concentration, when diluted to 40 ml ($\mu g\ l^{-1}$ of phosphorus)
0.0 (blank)	0
1.0	25
2.0	50
3.0	75
4.0	100
8.0	200
12.0	300
16.0	400

8. Place 40 ml of sample in a stoppered 50-ml volumetric flask.

9. Add 8 ml of the mixed reducing agent to the standards and samples, make up to 50 ml with distilled water and mix. Allow to stand for 10 minutes.

10. Measure the absorbance of the blank and each of the standards. Prepare a calibration graph by plotting absorbance against the concentration of phosphorus in $\mu g\ l^{-1}$

11. Measure the absorbance of the samples and, from the calibration graph, read the number of $\mu g\ l^{-1}$ of phosphorus in the samples.

7.19 Potassium

Although potassium is a relatively abundant element, its concentration in natural fresh waters is usually less than 20 mg l^{-1} Brines and seawater, however, may contain as much as 400 mg l^{-1} of potassium or more. The colorimetric method of analysis given here avoids the need for sophisticated laboratory equipment but does require the use of a centrifuge. Potassium concentrations may also be determined by flame photometry (see section 8.3).

Principle

Potassium is determined by precipitating it with sodium cobaltinitrite, oxidising the dipotassium sodium cobaltinitrite with standard potassium dichromate solution in the presence of sulphuric acid, and measuring the excess dichromate colorimetrically. A series of standards with known concentrations of potassium must be carried through the procedure with each set of samples because the temperature and the time of preparation can significantly affect the results.

Interferences

Ammonium ions interfere and should not be present. Silica may interfere if a turbid silica gel is formed as a result of either evaporation or the addition of the reagent, and this must be removed by filtration of the coloured sample. The procedure permits measurement of potassium with an accuracy of ± 0.5 mg l^{-1}.

Apparatus

√ Colorimetric equipment. One of the following is required:
- Spectrophotometer, for use at 425 nm and providing a light path of 1 cm or longer.

- Filter photometer, equipped with a violet filter, with a maximum transmittance of 425 nm and providing a light path of 1 cm or longer (for concentrations below 20 mg l^{-1}, a longer light path is desirable).

- Nessler tubes, matched, 100 ml tall form.

√ Centrifuge with 25-ml centrifuge tubes.

√ Small-diameter glass stirring rods to stir the precipitate in the centrifuge tubes.

Reagents
√ Nitric acid, HNO_3, 1 mol l^{-1}

√ Nitric acid, 0.01 mol l^{-1} Dilute 10 ml of the 1 mol l^{-1} nitric acid to 1 litre with distilled water.

√ Trisodium cobaltinitrite solution. Dissolve 10 mg $Na_3Co(NO_2)_6$ in 50 ml distilled water. Prepare fresh daily and filter before use.

√ Standard potassium dichromate solution. Dissolve 4.904 g anhydrous $K_2Cr_2O_7$ in distilled water and make up to 1 litre in a volumetric flask. This solution is stable for at least one year.

√ Sulphuric acid, H_2SO_4, concentrated.

√ Standard potassium solution. Dissolve 1.907 g KCl, dried at 110 °C, and dilute to 1 litre with deionised distilled water (1 ml ≡ 1.00 mg K).

Procedure
1. For normal surface and groundwaters, take a 100-ml sample and concentrate its potassium content by evaporation until about only 5 ml remain. Transfer this concentrated sample to a 25-ml centrifuge tube and make up to 10.0 ml with deionised distilled water.

2. The reaction is dependent on time and temperature, so both of these should be kept reasonably constant for all samples and standards in a series of tests: ± 15 minutes and ± 5 °C.

3. At room temperature add, with mixing, 1 ml of the 1 mol l^{-1} nitric acid and 5 ml of the trisodium cobaltinitrite solution. Let stand for 2 hours.

4. Centrifuge for 10 minutes. Carefully pour off the liquid and wash the precipitate with 15 ml of the 0.01 mol l^{-1} nitric acid. Mix with a small glass stirring rod to ensure contact between the precipitate and the wash solution.

5. Centrifuge again for 10 minutes. Pour off the liquid and add, with mixing, 10.00 ml of standard potassium dichromate solution and 5 ml concentrated sulphuric acid.

6. Cool to room temperature. Make up to 100 ml with deionised distilled water. If the solution is turbid, filter it into a Nessler tube and make up to 100 ml.

7. Preparation of standards. Pipette portions of 1, 2, 3, 4, 5, 6 and 7 ml of the standard potassium solution into a series of 25-ml centrifuge tubes, and make up to 10 ml with deionised distilled water. Treat all tubes in the manner described for the sample in steps 3 to 6 above to obtain colour standards containing 1.00 to 7.00 mg K.

8. If a spectrophotometer is being used, prepare a calibration curve with absorbance plotted against mg K. Measure the absorbance of the sample and determine the concentration of potassium from the calibration curve.

9. If visual colour comparison is being made, compare the colour of the sample with the colour of the standards and select the standard that is the closest.

Calculation

$$\text{Potassium} = \frac{\text{mg K} \times 1{,}000}{\text{ml sample}} \text{mg l}^{-1}$$

7.20 Selenium

The chemistry of selenium is similar in many respects to that of sulphur, but selenium is a much less common element. The selenium concentrations usually found in water are of the order of a few micrograms per litre, but may reach 50-300 µg l^{-1} in seleniferous areas and have been reported to reach 1 mg l^{-1} in drainage water from irrigated seleniferous soil. Well water containing 9 mg of selenium per litre has been reported.

Little is known about the oxidation state of selenium in water. Selenium appears in the soil as basic ferric selenite, as calcium selenate and as elemental selenium. Although the solubility of elemental selenium is limited, selenium may be present in water in the elemental form as well as the selenate (SeO_4^{2-}), selenite (SeO_3^{2-}) and selenide (Se^{2-}) anions. In addition, many organic compounds of selenium are known. The geochemical control of selenium concentrations in water is not understood, but adsorption by sediments and suspended materials appears to be of importance.

Selenium is an essential, beneficial element required by animals in trace amounts but toxic when ingested at higher levels. A guideline value of 0.01 mg l^{-1} for selenium in drinking water has been recommended by WHO on the basis of long-term health effects. In humans, the symptoms of selenium toxicity are similar to those of arsenic and the toxic effects of long-term exposure are manifested in nails, hair and liver. Selenium poisoning has occurred in animals grazing exclusively in areas where the vegetation contains toxic levels of selenium because of highly seleniferous soils. In general, such soils are found in arid or semi-arid areas of limited agricultural activity. Selenium deficiency in animals occurs in many areas of the world and causes large losses in animal production.

Two methods of selenium analysis are given and both are secondary methods of analysis. The photometric diaminobenzidine method is less sensitive than the fluorometric, but with sample preconcentration the limit of detection is generally acceptable.

Sample handling

Selenium in concentrations of around 1 µg l^{-1} has been found to be adsorbed on Pyrex glass and on polyethylene containers. Collect the sample in a polyethylene bottle and acidify by the addition of 1.5 ml of concentrated HNO_3 per litre if the sample is to be stored.

Principle

Oxidation by acid permanganate converts all selenium compounds to selenate. Many carbon compounds are not completely oxidised by acid permanganate, but it is unlikely that the selenium-carbon bond will remain intact through this treatment. Experiments demonstrate that inorganic forms of selenium are oxidised by acid permanganate in the presence of much greater concentrations of organic matter than would be expected in water supplies. There is substantial loss of selenium when solutions of sodium selenate are evaporated to complete dryness, but in the presence of calcium all the selenium is recovered. An excess of calcium over the selenate is not necessary.

Table 7.7 Percentage selenium recovery in the presence of iodide and bromide interference

Iodide (mg)	Br^- 0 mg	Br^- 1.25 mg	Br^- 2.50 mg
0	100	100	96
0.5	95	94	95
1.25	84	80	-
2.50	75	-	70

Selenate is reduced to selenite in warm 4 mol l^{-1} HCl. Temperature, time and acid concentrations are specified to obtain quantitative reduction without loss of selenium. The optimum pH for the formation of piazselenol is approximately 1.5. Above pH 2, the rate of formation of the coloured compound is critically dependent on the pH. When indicators are used to adjust the pH, the results are frequently erratic. Extraction of piazselenol is not quantitative, but equilibrium is attained rapidly. Above pH 6, the partition ratio of piazselenol between water and toluene is almost independent of the hydrogen ion concentration.

Interferences

No inorganic compounds give a positive interference. It is possible that coloured organic compounds exist that are extracted by cyclohexane but it seems unlikely that interference of this nature will resist the initial acid oxidation or other treatment to remove dissolved organics. Negative interference results from compounds that lower the concentration of diaminonaphthalene by oxidising this reagent. The addition of EDTA eliminates negative interference from at least 2.5 mg ferric iron. Manganese has no effect in any reasonable concentration, probably because it is reduced along with the selenate. Iodide and, to a lesser extent, bromide cause low results. The recovery of selenium from a standard containing 25 µg Se in the presence of varying amounts of iodide and bromide is shown in Table 7.7. The percentage recovery improves slightly as the amount of selenium is decreased. The minimum detectable concentration is 10 µg Se l^{-1}

Apparatus

√ Colorimetric equipment. A spectrophotometer, for use at 480 nm, providing a light path of 1 cm or longer.

√ Separatory funnel 250 ml, preferably with a fluorocarbon stopcock.

√ Thermostatically controlled water-bath (50 °C), with cover, pH meter.

√ Centrifuge, for 50-ml tubes (optional).

√ Centrifuge bottles, 60 ml, screw-capped, fluorocarbon.

Reagents
√ Stock selenium solution. Dissolve 1.633 g selenious acid, H_2SeO_3, in distilled water and dilute to 1 litre (1.00 ml ≡ 1.00 mg Se(IV)).

√ Standard selenium solution. Dilute an appropriate volume of stock selenium solution with distilled water to produce a series of working standards spanning the concentration range of interest.

√ Methyl orange indicator solution.

√ Calcium chloride solution. Dissolve 30 g $CaCl_2.2H_2O$ in distilled water and dilute to 1 litre.

√ Potassium permanganate, 0.1 mol l^{-1} Dissolve 3.2 g $KMnO_4$ in 1,000 ml distilled water.

√ Sodium hydroxide, 0.1 mol l^{-1}

√ Hydrochloric acid, concentrated and 0.1 mol l^{-1}

√ Ammonium chloride solution. Dissolve 250 g NH_4Cl in 1 litre distilled water.

√ EDTA-sulphate reagent. Dissolve 100 g disodium ethylenediaminetetraacetate dihydrate (also called ethylenedinitrilotetraacetic acid disodium salt) and 200 g sodium sulphate in 1 litre distilled water. Add concentrated ammonium hydroxide drop by drop while stirring until the dissolution is complete.

√ 2, 3-diaminonaphthalene (DAN) solution. Dissolve 200 mg DAN in 200 ml 0.1 mol l^{-1} HCl. Shake for 5 minutes. Extract three times with 25-ml portions of cyclohexane, retaining the aqueous phase and discarding organic portions. Filter through a Whatman No. 42 or equivalent filter paper and store in a cool dark place for no longer than 8 hours.

CAUTION: This reagent is TOXIC. Handle it with extreme care.
√ Cyclohexane, C_6H_{12}
√ Toluene.
√ Sodium sulphate, anhydrous (required if no centrifuge is available).
√ Ammonium hydroxide, NH_4OH, 50 per cent strength by volume.
Procedure

Oxidation to selenate

1. Prepare standards containing 0, 10.0, 25.0 and 50.0 μg Se in 500-ml Erlenmeyer flasks. Dilute to approximately 250 ml, add 10 drops methyl orange indicator solution, 2 ml 0.1 mol l^{-1} HCl, 5 ml $CaCl_2$ solution, 3 drops 0.1 mol l^{-1} $KMnO_4$, and a 5-ml measure of glass beads to prevent bumping. Boil vigorously for approximately 5 minutes.

2. To a 1,000-ml sample in a 2-litre beaker add 10 drops methyl orange indicator solution. Titrate to the methyl orange end-point with 0.1 mol l^{-1} HCl and add 2 ml excess.

3. Add 3 drops $KMnO_4$, 5 ml $CaCl_2$ solution and a 5 ml measure of glass beads to prevent bumping. Heat to boiling, adding $KMnO_4$ as required to maintain a purple tint. Ignore a precipitate of MnO_2 because it will have no adverse effect.

4. After the volume has been reduced to approximately 250 ml, transfer the solution to a 500-ml Erlenmeyer flask.

Evaporation
5. Add 5 ml 0.1 mol l^{-1} NaOH to each flask and evaporate to dryness. Avoid prolonged heating of the residue.
Reduction to selenite
6. Cool the flask, add 5 ml concentrated HCl and 10 ml NH_4Cl solution. Heat in a boiling water-bath for 10 ± 0.5 minutes.
Formation of piazselenol
7. Transfer the warm solution and ammonium chloride precipitate, if present, from the flask to a beaker suitable for pH adjustment, washing the flask with 5 ml EDTA- sulphate reagent and 3 ml 50 per cent NH_4OH. Adjust the pH to 1.5 ± 0.3 with NH_4OH, using a pH meter. The precipitate of EDTA will not interfere. Add 1 ml diaminobenzidine solution and heat in a thermostatically controlled water-bath at 50 C for approximately 30 min.
Extraction of piazselenol
8. Cool and then add NH_4OH to adjust the pH to 8 ± 1; the precipitate of EDTA will dissolve. Pour the sample into a 50-ml graduated cylinder and adjust the volume to 50 ± 1 ml with washings from the beaker.

9. Pour the contents of the graduated cylinder into a 250-ml separatory funnel. Add 10 ml toluene and shake for 30 ± 5 seconds. Discard the aqueous layer and transfer the organic phase to a centrifuge tube. Centrifuge briefly to clear the toluene from water droplets. If a centrifuge is not available, filter the organic phase through a dry filter paper to which approximately 0.1 g anhydrous Na_2SO_4 has been added.

Determination of absorbance
10. Read the absorbance at approximately 420 nm, using toluene to establish zero absorbance. The piazselenol colour is stable, but evaporation of toluene concentrates the colour to a marked degree in a few hours. Beer's law is obeyed up to 50 µg.
Calculation

$$\text{Concentration of selenium} = \frac{\mu\text{g Se}}{\text{ml sample}} \text{ mg l}^{-1}$$

Precision and accuracy

A synthetic unknown sample containing 20 µg l^{-1} Se, 40 µg l^{-1} As, 250 µg l^{-1} Be, 240 µg l^{-1} B and 6 µg l^{-1} V in distilled water was determined by the diaminobenzidine method, with a relative standard deviation of 21.2 per cent and a relative error of 5.0 per cent in 35 laboratories.

7.21 Reactive silica

After oxygen, silicon is the most abundant element in the earth's crust. It is a major constituent of igneous and metamorphic rocks, of clay minerals such as kaolin, and of feldspars and quartz. Although crystalline silica is a major constituent of many igneous rocks and sandstones, it has low solubility and is therefore of limited importance as a source of

silica in water. It is likely that most of the dissolved silica in water originates from the chemical breakdown of silicates in the processes of metamorphism or weathering.

The concentration of silica in most natural waters is in the range 1-30 mg l^{-1} Up to 100 mg l^{-1} is not uncommon. Over 100 mg l^{-1} is relatively rare, although more than 1,000 mg l^{-1} is occasionally found in some brackish waters and brines.

Sample handling

Samples should be stored in plastic bottles to prevent leaching of silica from glass. Samples should be passed through a membrane filter of 0.45 μm pore size as soon as possible after sample collection and should be stored at 4 °C without preservatives. Analysis should be performed within 1 week of sample collection.

Principle

Ammonium molybdate at a pH of approximately 1.2 reacts with silica and phosphate to form heteropoly acids. Addition of oxalic acid destroys any molybdophosphoric acid but not the molybdosilicic acid. The yellow molybdosilicic acid is reduced by aminonaphtholsulphonic acid to heteropoly blue. The blue colour of the heteropoly blue is more intense than the yellow colour of the molybdosilicic acid, so that this reaction increases the sensitivity of the method.

Silica can be measured at either 815 nm or 650 nm. The sensitivity at 650 nm is approximately half that at 815 nm.

Interferences

Both the apparatus and the reagents may contribute silica. The use of glassware should be avoided as far as possible and only reagents low in silica should be used. A blank determination should be carried out to correct for silica introduced from these sources. Tannin, large amounts of iron, colour, turbidity, sulphide and phosphate are potential sources of interference. The treatment with oxalic acid eliminates the interference from phosphate and decreases the interference from tannin. Photometric compensation may be used to cancel interference from colour or turbidity in the sample.

Apparatus

√ Colorimetric equipment. One of the following is required:
- Spectrophotometer for measurement at 815 nm, providing a light path of at least 1 cm.

- Absorptiometer for measurement at a wavelength of 815 nm.

Note: If no instrument is available for measurement at this wavelength, an instrument capable of measuring at 650 nm can be used, but this leads to a decrease in sensitivity.

Reagents

√ Hydrochloric acid, 6 mol l^{-1} Combine 1 volume of concentrated HCl with an equal volume of water.

√ Ammonium molybdate reagent. Dissolve 10 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in distilled water, with stirring and gentle warming, and dilute to 100 ml.

Filter if necessary. Adjust to pH 7-8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle. If the pH is not adjusted, a precipitate gradually forms. If the solution is stored in glass, silica may leach out, causing high blanks. If necessary, prepare silica-free NH_4OH by passing gaseous NH_3 into distilled water contained in a plastic bottle.

√ Oxalic acid solution. Dissolve 10 g of oxalic acid dihydrate, $H_2C_2O_4.2H_2O$, in distilled water and dilute to 100 ml.

√ Reducing agent. Dissolve 0.5 g of 1-amino-2-naphthol-4-sulphonic acid and 1 g of Na_2SO_3 in 50 ml of distilled water, with gentle warming if necessary. Add this solution to a solution of 30 g of $NaHSO_3$ in 150 ml of distilled water. Filter into a plastic bottle and store in a refrigerator away from light. Discard the solution when it becomes dark. Do not use aminonaphtholsulphonic acid that is incompletely soluble or that produces reagents that are dark even when freshly prepared; such material is not suitable for silica determinations.

√ Silica standard stock solution. Dissolve 313.0 mg of sodium hexafluorosilicate, Na_2SiF_6, in 1,000 ml of distilled water (1 ml (0.1 mg SiO_2).

Silica standard working solution. Dilute 100 ml of standard stock solution to 1,000 ml (1 ml (10 µg SiO_2).

Procedure

1. Prepare standards and a blank by pipetting portions of the silica working standard into tall-form 50-ml Nessler tubes and make up to 50 ml with distilled water. If measurement is to be made at 650 nm, use portions of 0-30 ml (giving 0-300 µg l^{-1} concentrations in the Nessler tubes); if measurement is to be made at 815 nm use portions of 0-10 ml (0-100 µg l^{-1}). If cells with a light path longer than 1 cm are to be used, the silica concentrations should be proportionally reduced.
Note: Reduction by aminonaphtholsulphonic acid is temperature-dependent. The best results will be obtained if tubes are immersed in a water-bath, thermostatically controlled at a temperature near 25 or 30 °C.
2. To each standard add, in rapid succession, 1.0 ml of HCl, 6 mol l^{-1}, and 2.0 ml of ammonium molybdate reagent. Mix by inverting the tubes at least six times.

3. Add 1.5 ml of oxalic acid solution and mix thoroughly. At least 2 minutes, but less than 15 minutes, after addition of the oxalic acid, add 2.0 ml of reducing agent and mix thoroughly.

4. After 5 minutes, measure the absorbance at 650 or 815 nm.

5. Subtract the absorbance of the blank from the absorbances of the standards to obtain the net absorbances.

6. Prepare a calibration graph relating the net absorbance to the amount of silica (SiO_2).

7. Measure 50 ml of sample into a tall-form Nessler tube and add, in rapid succession, 1.0 ml of HCl, 6 mol l^{-1}, and 2.0 ml of ammonium molybdate reagent. Mix by inverting the tube at least six times.

8. Add 1.5 ml of oxalic acid solution and mix thoroughly. At least 2 minutes, but less than 15 minutes, after addition of the oxalic acid, add 2.0 ml of reducing agent and mix thoroughly.

9. After 5 minutes, measure the absorbance at 650 or 815 nm.

10. Subtract the absorbance of the blank from the absorbance of the sample to obtain the net absorbance.

Calculation

Determine the amount of silica (SiO_2) equivalent to the net absorbance from the calibration graph and divide by the volume of the sample (ml) to obtain the concentration of SiO_2 in mg l^{-1}

$$\text{Concentration of silica} = \frac{SiO_2 \text{ (from graph)}}{\text{ml of sample}} \text{mg } l^{-1}$$

7.22 Sodium

Sodium is a common element, the sixth most abundant, and present to some extent in most natural waters. Concentrations vary from negligible in freshwater to considerable in seawater and brackish water. The permeability of agricultural soil is harmed by a high ratio of sodium ions to total cations. Sodium concentrations higher than a few milligrams per litre are undesirable in feed water for high-pressure boilers. When compounded with certain anions (e.g. chloride), sodium imparts a salty taste to drinking water and, if the concentration is sufficiently high, consumers may not be willing to drink it.

Sodium concentrations can be determined by flame photometry (see section 8.3) but the method presented below does not require any sophisticated laboratory equipment.

Principle

Sodium is precipitated as sodium zinc uranyl acetate hexahydrate, $NaC_2H_3O_2.Zn(C_2H_3O_2)_2.3UO_2(C_2H_3O_2)_2.6H_2O$, by adding a large volume of zinc uranyl acetate reagent, previously saturated with the sodium salt, to a small volume of concentrated sample. At least 10 ml of reagent must be used for each millilitre of sample and the mixture must be allowed to stand for 60 minutes or more. After collection in a filter crucible, the precipitate is washed with successive small portions of the salt-saturated reagent, then with 95 per cent ethyl alcohol, also saturated with the triple salt. It is then washed with diethyl ether to remove the alcohol, and the ether is evaporated by drawing a stream of air through the sample. The air-dried sample is then weighed.

Apparatus

√ Beakers, 20 or 50 ml, borosilicate glass.
√ Fritted glass crucibles, 30 ml, borosilicate glass or porcelain of medium porosity.
√ Vacuum pump or aspirator, with manifold and individual petcocks.

Reagents

√ Zinc uranyl acetate reagent. Mix 2 ml concentrated acetic acid with 100 ml distilled water. Add 10 g uranyl acetate dihydrate, $UO_2(C_2H_3O_2)_2.2H_2O$, and 30 g zinc acetate dihydrate, $Zn(C_2H_3O_2)_2.2H_2O$, and warm to dissolve. Cool, add 2-3 mg NaCl, let stand for 24 hours and filter off the precipitate of zinc uranyl acetate. The filtrate is the triple salt saturated reagent and should be stored in a borosilicate glass bottle.

√ Pure sodium zinc uranyl acetate. Add 25 ml of zinc uranyl acetate solution to 2 ml saturated sodium chloride solution. Stir. Collect the precipitate by filtering through a fritted glass crucible. Wash the precipitate three times with concentrated acetic acid, then three times with diethyl ether.

√ Ethyl alcohol wash solution. Saturate 95 per cent ethyl alcohol with pure sodium zinc uranyl acetate. Decant or filter the solution just before use.

√ Diethyl ether.

Procedure

1. If necessary, remove any suspended matter from 50-100 ml of the sample by filtration.

2. Select a portion of the clear sample containing less than 8 mg sodium. This could be as little as 2 ml of a very salty water such as seawater or as much as 30 ml of a relatively salt-free “fresh” water. Pipette the measured portion into a borosilicate glass beaker of appropriate size.

3. Evaporate to dryness over a steam bath or hot water-bath.

4. Cool the residue to room temperature. Add 1.0 ml distilled water and stir with a glass rod to dissolve the residue. If all residue does not dissolve, add distilled water in 1.0 ml increments until all of the residue is dissolved.

5. Add zinc uranyl acetate reagent in the ratio of 10 ml of reagent for each 1.0 ml of distilled water used to dissolve the residue. Mix, cover the beaker and let stand for 1 hour, stirring periodically to prevent the formation of a supersaturated solution.

6. Arrange an apparatus for suction filtration using a medium porosity fritted glass (or porous-bottomed porcelain) crucible that has been previously weighed.

7. Pour the contents of the beaker into the crucible and filter under suction. Rinse the beaker at least five times with 2-ml portions of the zinc uranyl acetate reagent, pouring the rinsings into the crucible. After the last rinse, maintain suction for several minutes to remove all possible traces of the reagent.

8. Maintain suction while washing the contents of the crucible five times with 2-ml portions of the ethyl alcohol wash solution. Then conclude the washing with three small portions of diethyl ether.

9. Continue suction for a few minutes until the ether has evaporated and the precipitate in the crucible is dry.

10. If salts have crystallised on the outside of the crucible, wipe it clean with a soft cloth or tissue.

11. Weigh the crucible and contents. Repeat the weighing after a 15-minute interval and a third time after a further 15 minutes to check on the constancy of the weight.

12. Return the crucible to the suction apparatus, apply suction and add warmed distilled water in portions of approximately 10 ml, using a total of about 100 ml to dissolve all traces of the sodium zinc uranyl acetate. Dry the crucible with ethyl alcohol wash solution and diethyl ether as in step 8 above, and reweigh the crucible as in step 11.

13. Subtract the weight obtained in step 12 from that obtained in step 11. This represents the weight of the sodium zinc uranyl acetate.

Calculation

$$\text{Concentration of sodium} = \frac{A \times 14.95}{\text{ml sample}} \text{mg l}^{-1}$$

where

A = the weight of the sodium zinc uranyl acetate (mg)

7.23 Sulphate

Sulphate is an abundant ion in the earth's crust and its concentration in water can range from a few milligrams to several thousand milligrams per litre. Industrial wastes and mine drainage may contain high concentrations of sulphate. Sulphate also results from the breakdown of sulphur-containing organic compounds.

Sulphate is one of the least toxic anions and WHO does not recommend any guideline value for it in drinking water. However, catharsis, dehydration and gastrointestinal irritation have been observed at high concentrations in drinking water and WHO therefore suggests that health authorities should be notified when concentrations of sulphate in drinking water exceed 500 mg l^{-1}.

Sample handling

Samples may be stored, refrigerated, in either plastic or glass containers for not more than seven days.

7.23.1 Titrimetric method

Principle

Barium ions in water are titrated as hardness in the EDTA hardness determination. Sulphate can therefore be indirectly determined on the basis of the difference in hardness of the sample before and after the addition of barium ions in excess of that required for sulphate precipitation.

Apparatus

√ Porcelain dishes, 100-ml capacity.
√ Burette, 25 or 50 ml.
Reagents
√ Hydrochloric acid, approximately 1 mol l^{-1} Carefully add 83 ml HCl (*d* = 1.18) to about 500 ml distilled water in a large beaker. Stir and cool to room temperature before transferring, with rinsings, to a 1-litre volumetric flask. Make up to the mark with distilled water. Store in a tightly stoppered glass bottle.

√ Standard EDTA titrant, 0.01 mol l^{-1} (see section 7.7, Calcium).

√ Eriochrome Black T indicator (see section 7.7, Calcium).

√ Buffer solution (see section 7.12, Magnesium).

√ Barium chloride solution, approximately 0.01 mol l^{-1} Dissolve 2.443 g of barium chloride dihydrate, $BaCl.2H_2O$, in 500 ml of distilled water. Transfer, with rinsings, to a 1-litre

volumetric flask. Add 2.0 ml concentrated HCl and make up to the mark with distilled water. Store in a polyethylene bottle. The solution is stable for six months.

Procedure
1. Place 50 ml of sample in a porcelain dish. Add 1 to 2 ml of buffer solution. The pH should be 10.0 ± 0.1. Adjust as necessary.

2. Add 2 drops of indicator solution (or a small portion of dry powder indicator mixture).

3. Titrate slowly with EDTA standard titrant, stirring continuously, until the last reddish tinge disappears and the sample becomes blue. The titration should be completed within 5 minutes of the addition of buffer. Record the amount of EDTA titrant used.

4. If more than 15 ml of titrant are used, repeat steps 1 to 3 using 25 ml of sample diluted to 50 ml with distilled water. If less than 1 ml is used, repeat using 100 ml of sample, or more if necessary.

5. Calculate hardness as $CaCO_3$ mg l^{-1} (see calculation, below).

6. Measure 100 ml of sample and pour into a beaker. Neutralise the alkalinity to pH 4.5 with 1 mol l^{-1} HCl or HNO_3 Add 1 ml more of the acid.

7. Bring the sample to the boil to expel carbon dioxide. Add 10 ml of barium chloride standard solution to the boiling sample. After the volume has been reduced to less than 100 ml, remove from heat and allow to cool.

8. Transfer with rinsings to a 100-ml graduated cylinder and make up to the 100-ml mark with distilled water. Allow any precipitate to settle.

9. Pour 50 ml of the clear supernatant into a porcelain dish. Add 2 ml of buffer solution; the pH should be 10.0 ± 0.1. Adjust as necessary.

10. Add 2 drops of the indicator solution (or a small portion of the dry powder indicator mixture).

11. Titrate slowly with EDTA standard titrant, stirring continuously, until the last reddish tinge disappears and the sample becomes blue. The titration should be completed within 5 minutes of the addition of buffer. Record the amount of EDTA titrant used.

12. If more than 15 ml of titrant are used, repeat steps 9 to 11 using 25 ml of sample diluted to 50 ml with distilled water. If less than 1 ml is used, repeat using 100 ml of sample, or more if necessary.

Calculation

The calculation is in three parts. First, the hardness is calculated from the results of the titration in step 3 of the procedure.

$$\text{Hardness} = \frac{1{,}000 \times H}{V_1} \text{ mg } l^{-1} \text{ as } CaCO_3$$

where

H = volume of titrant used in step 3 of procedure (ml)
V_1 = volume of sample used in the titration at step 3 (ml)

The second part of the calculation yields a value that is the hardness plus the amount of barium chloride that did not combine with sulphate in the sample. This is determined from the results of the titration in step 11 of the procedure.

$$\text{Hardness} + \text{BaCl}_u = \frac{1{,}000 \times T}{V_2} \text{mg l}^{-1} \text{ as CaCO}_3$$

where

T = volume of titrant used in step 11 of the procedure (ml)
V_2 = volume of sample used in the titration in step 11 (ml)
$BaCl_u$ = volume of barium chloride that did not combine with SO_4 (ml)

The final part of the calculation is to add the result of the first titration to the concentration of the barium chloride solution (1,000 mg l^{-1}), to subtract the result of the second titration and to convert the result to mg l^{-1} SO_4

$$\{\text{Hardness (mg l}^{-1}) + 1{,}000 - [\text{Hardness} + \text{BaCl}_u]\} \times 0.96 = \text{mg l}^{-1} \text{ SO}_4$$

7.23.2 Gravimetric method with drying of residue

Principle

Sulphate is precipitated in a hydrochloric acid solution as barium sulphate ($BaSO_4$) by the addition of barium chloride ($BaCl_2$). The precipitation is carried out near the boiling temperature and after a period of digestion the precipitate is filtered, washed with water until free of Cl^-, dried and weighed as $BaSO_4$

The gravimetric determination of SO_4^{2-} is subject to many errors, both positive and negative. Interferences that lead to high results are suspended matter, silica, $BaCl_2$ precipitant, NO_3^-, SO_3^{2-} and water occluded in the precipitant. Interferences leading to low results are alkali metal sulphates, heavy metals (especially chromium and iron) and the solubility of the $BaSO_4$ precipitant, especially in acid solution.

Apparatus

Steam bath.

√ Drying oven equipped with thermostatic control.

√ Desiccator.

√ Analytical balance capable of weighing to 0.1 mg.

√ Filter: either fritted glass filter, fine porosity with a maximum pore size of 5 µm, or membrane filter with a pore size of about 0.45 µm.

√ Vacuum oven.

Reagents
√ Methyl red indicator solution. Dissolve 100 mg methyl red sodium salt in distilled water and dilute to 100 ml.

√ Hydrochloric acid, HCl 1+1.

√ Barium chloride solution. Dissolve 100 g $BaCl_2.2H_2O$ in 1 litre distilled water. Filter through a membrane filter or hard-finish filter paper before use. One millilitre is capable of precipitating approximately 40 mg SO_4^{2-}

√ Silver nitrate-nitric acid reagent. Dissolve 8.5 g $AgNO_3$ and 0.5 ml concentrated HNO_3 in 500 ml distilled water.

√ Silicone fluid.

Procedure

Removal of silica

1. If the silica concentration exceeds 25 g l^{-1}, evaporate sample nearly to dryness in a platinum dish on a steam bath. Add 1 ml of HCl, tilt and rotate dish until acid comes in complete contact with the residue. Continue evaporation to dryness. Complete drying in an oven at 180 °C and, if organic matter is present, char over the flame of a burner. Moisten residue with 2 ml of distilled water and 1 ml of HCl and evaporate to dryness on a steam bath. Add 2 ml of HCl, take up soluble residue in hot water and filter. Wash insoluble silica with several small portions of hot distilled water. Combine filtration and washing. Discard residue.

Precipitation of barium sulphate
2. Adjust volume of clarified sample to contain approximately 50 mg SO_4^{2-} in a 250-ml volume. Lower concentrations of SO_4^{2-} may be tolerated if it is impracticable to concentrate the sample to the optimum level, but in such cases limit the total volume to 150 ml. Adjust pH with HCl to pH 4.5 to 5.0 using a pH meter or the orange colour of methyl red indicator. Add 1 to 2 ml of HCl. Heat to boiling and, while stirring gently, slowly add warm $BaCl_2$ solution until precipitation appears to be complete, then add 2 ml excess. If amount of precipitate is small, add a total of 5 ml $BaCl_2$ solution. Digest precipitate at 80-90 ºC, preferably overnight but for not less than 2 hours.

Preparation of filters
3. (i) Fritted glass filter: dry to constant weight in an oven maintained at 105 ºC or higher, cool in a desiccator and weigh.

(ii) Membrane filter: place filter on a piece of filter paper or a watch glass and dry to constant weight in a vacuum oven at 80 ºC while maintaining a vacuum of at least 85 kPa or in a conventional oven at 103-105 ºC. Cool in a desiccator and weigh membrane only.

Filtration and weighing
4. Filter $BaSO_4$ at room temperature. Wash precipitate with several small portions of warm distilled water until washings are free of Cl^- as indicated by testing with $AgNO_3.HNO_3$ reagent. If a membrane filter is used, add a few drops of silicone fluid to the suspension before filtering to prevent adherence of precipitate to holder. Dry filter and precipitate by the same procedure used in preparing the filter. Cool in a desiccator and weigh.

Calculation

$$\text{Concentration of sulphate} = \frac{\text{mg BaSO}_4 \times 411.6}{\text{ml sample}} \text{mg l}^{-1}$$

7.24 Total dissolved solids

The substances remaining after evaporation and drying of a water sample are termed the “residue”. The residue is approximately equivalent to the total content of the dissolved and suspended matter in the water sample. Non- filterable residue corresponds to the total suspended solids (see section 7.25) and the filterable residue is the total dissolved solids (TDS).

The results of a determination of total dissolved solids (TDS) can be used to check the accuracy of analyses when relatively complete analyses have been made on a water sample. This is accomplished by comparing the value of calculated TDS with the measured value. Ion concentrations in mg l^{-1} of constituents required to calculate the TDS are as follows:

Calculated TDS = 0.6 (alkalinity) + Na + K + Ca + Mg + Cl + SO_4 + SiO_3 + (NO_3-N) + F

The measured TDS concentration should be higher than the calculated value, because a significant contributor may not be included in the calculation. If the measured value is less than the calculated value, all values are suspect. If the measured value is higher than the calculated value, the low ion sum is suspect and selected constituents should be reanalysed. The acceptable ratio is:

$$1.0 < \frac{\text{measured TDS}}{\text{calculated TDS}} < 1.2$$

Principle

A well mixed, measured portion of a sample is filtered through a standard glass-fibre filter and the filtrate is evaporated to dryness in a weighed dish and dried to constant weight at 180 ºC. The increase in dish weight represents the total dissolved solids.

Apparatus

√ Evaporating dishes made of porcelain (90 mm), platinum or high-silica glass.

√ Steam bath.

√ Desiccator provided with a desiccant containing a colour indicator for moisture concentration.

√ Analytical balance capable of weighing to 0.1 mg.

√ Glass-fibre filter discs without organic binder.

√ Filtration apparatus. All of the following are suitable:

- Membrane filter funnel.

- Gooch crucible, capacity 25-40 ml, with Gooch crucible adapter.

- Filtration apparatus with reservoir and coarse (40-60 µm) fritted disc as filter support.

√ Suction flask, size large enough for chosen sample size.

√ Drying oven, for operation at 180 ± 2 ºC.

Procedure
1. Prepare the glass-fibre filter disc by placing it, wrinkled side up, in the filtration apparatus. Apply vacuum and wash the disc with three successive 20 ml washings of distilled water. Continue suction to remove all traces of water. Discard washings.

2. Prepare the evaporating dish by heating a clean dish to 180 ± 2 ºC in an oven for 1 hour. Cool and store in desiccator until needed. Weigh immediately before use.

3. Choose a sample volume to yield between 2.5 and 200 mg dried residue. If more than 10 minutes are required to complete filtration, increase filter size or decrease sample volume but do not produce less than 2.5 mg of residue.

4. Filter measured volume of well mixed sample through the glass-fibre filter, wash with three successive 10-ml volumes of distilled water, allowing complete draining between washings. Continue suction for about 3 minutes after filtration is complete.

5. Transfer filtrate to a weighed evaporating dish and evaporate to dryness on a steam bath. If filtrate volume exceeds dish capacity, add successive portions to the same dish after evaporation.

6. Dry for at least 1 hour in an oven at 180 ± 2 ºC, cool in a desiccator to balance temperature and weigh.

7. Repeat cycle of drying, cooling, desiccating and weighing until a constant weight is obtained or until weight loss between successive weighings is less than 4 per cent or 0.5 mg, whichever is less.

Calculation

$$\text{Total dissolved solids} = \frac{(A - B) \times 1{,}000}{\text{sample volume in ml}} \text{ mg l}^{-1}$$

where

A = weight of dish + solids (mg)
B = weight of dish before use (mg)

7.25 Total suspended solids

The term “total suspended solids” (TSS) applies to the dry weight of the material that is removed from a measured volume of water sample by filtration through a standard filter. The test is basically empirical and is not subject to the usual criteria of accuracy. To achieve

reproducibility and comparability of results requires close attention to procedural details, especially filter characteristics and time and temperature of drying. The method described is based on the following conditions: filtering by glass fibre filter (Whatman GF/C grade or equivalent) and drying at a temperature of 103-105 °C for 2 hours to a constant weight, i.e. a variability of not more than 0.5 mg. If other filters (paper, membrane, etc.) or other temperatures are used, it is necessary to report the specifications followed (e.g. total suspended solids at .... °C, type of filter and pore size or number).

Results may be of questionable value if the total suspended solids include oils, grease or other volatile material. It is obvious that the result of a test cannot include materials that are volatile under the conditions of the procedure. Possible sources of error should be recognised when results are interpreted.

Sample handling

Non-homogeneous particulates such as leaves, sticks, fish and lumps of faecal matter should be excluded from the sample. Too much residue on the filter will retain water and may require prolonged drying.

Long-term preservation of a sample is not practical; analysis should therefore begin as soon as possible. Transportation and short-term storage of a sample will not normally affect the results of the test.

Interferences

Volatile material in a sample will distort results.

Apparatus

√ Filter holder. All of the following types of filter holder are suitable:

√ Membrane filter funnel.

√ Buchner funnel, with adapter to fit suction flask.

√ Gooch crucible, capacity 25-40 ml size, with Gooch crucible adapter.

√ Glass-fibre filter discs, Whatman GF/C or equivalent, of a size compatible with the filter holder.

√ Suction flask, 500-ml capacity.

√ Drying oven, 103-105 °C.

√ Desiccator.

√ Analytical balance, capacity 200 g (or more), accuracy 0.1 mg.

√ Vacuum pump or aspirator.

Procedure

Preparation of glass-fibre filter discs

1. Place a filter disc on the filter holder. Assemble filter holder in suction flask apparatus, connect to vacuum source and apply vacuum.

2. Wash the filter disc with three successive 20-ml portions of distilled water. Continue to apply vacuum for 2-3 minutes after the water has passed through the filter. Discard the filtrate.

3. Remove the filter paper from the membrane filter funnel or the Buchner funnel and place it on a supporting surface in a drying oven. If the Gooch crucible/filter combination is being used, place it in the drying oven. The oven should be maintained at 103-105 °C and drying should be continued for at least 1 hour.

4. Cool the filter or Gooch combination in a desiccator and weigh it on an analytical balance.

5. Repeat the cycle of drying, desiccating and weighing until the weight loss between two successive series of operations is less than 0.5 mg.

6. Store filter(s) or Gooch crucible(s) in the desiccator until required.

Sample analysis

1. Remove the filter disc or Gooch crucible from the desiccator, weigh it and record its weight.

2. Place the filter in the filter holder and assemble the filter holder in the suction flask apparatus. Connect to the vacuum source and apply vacuum.

3. Wet the filter with a few drops of distilled water to seat the filter.

4. Shake the sample vigorously and measure out 100 ml in a 100-ml graduated cylinder or volumetric flask. Pour this portion of the sample into the filter funnel, being careful not to disturb the seating of the filter disc.

5. Rinse out the measuring flask or cylinder with a small quantity of distilled water. If the sample is very low in suspended material, a larger volume of sample may be used.

6. When filtration is complete, carefully remove the filter disc from the filter holder with tweezers (or remove the Gooch crucible from its supporting socket with a pair of tongs) and place it in the drying oven. Dry for at least 1 hour at 103-105 °C. Cool in a desiccator and weigh.

7. Repeat the drying, desiccating and weighing cycle until the weight loss between two successive weighings is less than 0.5 mg.

8. Record the final weight obtained.

Calculation

$$\text{Total suspended solids} = \frac{A - B}{C} \times 10^6 \quad \text{mg l}^{-1}$$

where

A = weight of filter + solids (g)
B = weight of filter (g)
C = volume of sample filtered (ml)

Report the results as:

Total suspended solids dried at ....°C, ... mg l^{-1}

Precision and accuracy

Precision data are not available. Accuracy data on actual samples cannot be obtained.

7.26 Source literature and further reading

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