Kjeldahl Nitrogen in Waters 1987

Methods for the Examination of Waters and Associated Materials

London: Her Majesty's Stationery Office

First Published 1988

ISBN 0 11 752129 9

Her Majesty's Stationery Office

Standing order Service

Placing a standing order with HMSO BOOKS enables a customer to receive other titles in this series automatically as published.

This saves the time, trouble and expense of placing individual orders and avoids the problem of knowing when to do so.

For details please write to HMSO BOOKS (PC 13A/1), Publications Centre, PO Box 276, London SW8 5DT quoting reference

X22.04.22.

The standing order service also enables customers to receive automatically as published all material of their choice which additionally saves extensive catalogue research. The scope and selectivity of the service has been extended by new techniques, and there are more than 3,500 classifications to choose from. A special leaflet describing the service in detail may be obtained on request.

About This Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as a series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being issued when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has 9 Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage Works Control Methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee.

The names of those associated with this method are listed inside the back cover. Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No. 5

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary

1 July 1987

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

Lone working, whether in the laboratory or field, should be discouraged.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Guide to Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection' Public Health Laboratory Service Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient give information on the type of injury, especially is poisoning is suspected, as the patient may be taken directly to a specialized hospital.

A General Information

A1 Introduction to the Methods

A method for the determination of nitrogen in organic compounds was published by J G C T Kjeldahl in 1883[1]. It became the starting point for the development of methods for the determination of organic nitrogen in a wide variety of materials, using an ever widening range of variations upon Kjeldahl's original method[2,3]. However, the general description, 'Kjeldahl method' has remained associated with all of these, irrespective of the purpose they serve and the exact nature of the method.

Basically, the method consists in the digestion of the material under analysis in concentrated sulphuric acid in the presence of a catalyst and very often in the presence of added potassium or sodium sulphate to raise the boiling point of the digestion mixture and so to increase the efficacy of digestion. Ammonium sulphate is formed by reaction of organically bound nitrogen with the acid, and ammonia in the digest is subsequently determined by standard methods.

The digestion allows the quantitative determination of organic nitrogen in the trivalent state. Nitrogen in the form of azide, azine, azo, hydrazone, nitrate, nitrite, nitrile, nitro, nitroso, oxime and semicarbazone is not quantitatively recovered, although the inclusion of a preliminary reduction step may achieve better recoveries. Numerous references to reducing agents for this purpose will be found in the chemical literature. Amongst those of fairly general application are the use of titanium (III) salts[4], preliminary digestion with sulphuric acid and salicylic and followed by the addition of thiosulphate or zinc dust[12], or preliminary treatment with a mixture of hydriodic acid and red phosphorus[13]. The method using Devarda's alloy for the determination of oxidized nitrogen (in this series of publications[14]) may be used as the preliminary reduction stage. However, it is considered that there is little call in the UK water industry for a detailed method incorporating preliminary reduction although such a method is under study internationally[15]. An alternative method of determining ammonia, oxidized nitrogen and most forms of organic nitrogen in raw and potable waters involves the oxidation of the sample with peroxodisulphate in akaline conditions in an autoclave and subsequent determination of nitrate[20]. However, this cannot be described as a 'Kjeldahl', method since the principle is quite different.

A variety of catalysts have been used in Kjeldahl methods. Studies on the recovery of nitrogen from pure organic compounds indicate that mercury is the preferred catalyst. Recovery of nitrogen from the heterocyclic compound nicotinic acid is generally regarded as the 'acid' test of a catalyst and mercury has been shown to achieve quantitative performance. However, other catalyst systems have been shown to achieve similar performance on nicotinic acid and, in view of environmental objections to mercury, occupy an important place in the range of Kjeldahl methods available to the analyst. Nevertheless, the mercury-catalysed method (method B in this booklet) is generally regarded as the reference method against which others are judged. Selenium-catalysed methods are sometimes regarded as providing a less toxic alternative reference method[17], but are particularly prone to loss of ammonia during digestion[16] and the toxic properties of selenium are considerable.

Catalysts are often used in conjunction with the addition of potassium or sodium sulphate in order to raise the boiling point of the sulphuric acid during digestion so as to increase the rate of digestion and obtain maximum recovery. In such methods, maintenance of the sulphate/sulphuric acid ratio on the final digest is a critical factor in the successful application of the method and additional sulphuric acid may be required in order to compensate for its consumption by the oxidation demand of samples containing large amounts of organic matter[5,7]. However, too high a digestion temperature, resulting from poorly-controlled heating, will cause loss of ammonia from the digest by volatilization, as will prolonged heating of any digest, irrespective of the catalyst.

Any ammoniacal nitrogen present in the sample will be retained during digestion and the final determined nitrogen concentration will thus be the sum of nitrogen from organic compounds responsive to the digestion and from ammonia. The term 'Kjeldahl nitrogen' is applied to this result. The following definition applies internationally[17]:

> Kjeldahl nitrogen: The content of organic nitrogen and ammoniacal nitrogen in a sample determined after mineralization.
>
> It does not include nitrate and nitrite nitrogen, and does not necessarily include all organically bound nitrogen.

All the methods in this booklet determine Kjeldahl nitrogen according to this definition. However, nitrate and nitrite may cause interference effects which may or may not be quantitative in their effect upon the final Kjeldahl nitrogen result. Separate determination of organic nitrogen, if required, may be achieved by distilling off the ammonia from the sample aliquot, made slightly alkaline, before the application of these methods.

Four methods for the determination of Kjeldahl nitrogen are presented in this booklet. They have been selected on the basis of current UK water industry practice and in response to particular needs.

Method B is the reference digestion, mercury-catalysed method, for application to all types of sample where a limit of detection of 1 mg N/L is acceptable. The method includes a procedure for the titrimetric determination of ammonia after distillation from the digest; alternatively, the colorimetric methods in Section F might be applied to the distillate.

Method C has copper as catalyst. Ethanol is added before digestion in order to eliminate possible interference by oxidized nitrogen. Ammonia is determined directly in the digest solution. The method is applicable to the same types of samples as Method B, although the detection limit is 3 mg N/L.

Method D is suited for the analysis of raw and potable waters in fulfilment of EEC Directive monitoring requirements. The method has a limit of detection of 0.09 mg N/L. Hydrogen peroxide is used as an additional oxidizing agent, but it has not yet proved possible to demonstrate reliably quantitative recovery of nitrogen from nicotinic acid in the concentration range of the method. However, good recoveries have been achieved from other organic nitrogen compounds and it is considered that the method is sufficient in this respect for the sample types for which it is intended.

Method E uses a mixed catalyst of copper and titanium. Inclusion of titanium allows a longer digestion period before possible onset of ammonia loss[18], but its presence in the digest requires distillation of ammonia prior to colorimetric determination. Rapid steam distillation is specified for this purpose. The method is applicable to the same types of sample as method B. The limit of detection is 0.85 mg N/L.

Section F sets out automated colorimetric procedures for the determination of ammonia in digests or distillates. These procedures are based on those described in another publication in this series[24].

Table A1 summarizes the main features of Methods B, C, D and E.

A2 Sample Collection and Preservation

These instructions are common to all the methods in this booklet.

A2.1 Samples should be collected in either glass or polyethylene containers. They should be analysed as soon as possible after sampling, but if this should not be possible, storage at 4 °C is recommended; the analyst must establish the maximum storage period which can be tolerated before concentration changes become significant.

A2.2 As an alternative to refrigeration, samples may be preserved by bringing them to pH 2 with sulphuric acid.

Table A1 Comparison of Methods on this booklet

Catalyst	Mercury (Method B)	Copper (Method C)	None (peroxide added to promote oxidation of organic material) (Method D)	Copper/Titanium (Method E)
Analysis of Digest	Distillation followed by titration or automated colorimetry according to Method F	Automated colorimetry directly on the diluted digest.	Manual colorimetry after neutralization or automated colorimetry according to Method F directly on the digest.	Distillation followed by automated colorimetry according to Method F.
Range of Application (mg Nin the maximum sample aliquot)	Up to 10	Up to 5	Up to 0.025	Up to 12.5
Limit of Detection (mg N/L using the maximum sample aliquot)	1.0	3.0	0.093	0.85
Remarks	The reference digestion method for Kjeldahl determinations	Alcohol incorporated to overcome possible interference from oxidized nitrogen. May not recover nitrogen quantatively from nicotinic acid.	Method primarily intended for potable water analysis. May not recover nitrogen quantitatively from nicotinic acid.	

A2.3 Samples should be well shaken immediately prior to analysis in order to distribute any solids evenly. In the event that a measure only of soluble nitrogen concentration is required, samples should be allowed to settle prior to analysis, or preferably be filtered through a glass fibre or membrane filter. However, note that organic nitrogen compounds can be absorbed during filtration. The filter medium must first be checked in this respect.

A3 Standard Solutions and Reagents Common to the Methods

Analytical Reagent grade chemicals should be used, unless otherwise stated.

A3.1 Ammonia-free water

There are two methods for preparing such water:

Method A: Pass distilled water through a bed of strongly acidic cation exchange resin (in the hydrogen form). Collect the eluate and store in a glass-stoppered bottle containing about 10 g/L of the same ion exchange resin.

Method B: Acidify distilled water with 0.1 mL/L of sulphuric acid (d_{20} 1.84) and redistill in an all glass apparatus. Discard the first 50 ml of distillate, then collect and store the rest of the distillate as described in method A above. Do not allow to go to dryness, nor allow solid to form on the walls of the distillation flask.

A3.2 Stock Standard Nitrogen Solution A. 1 mL contains 1 mg N

Dissolve 3.819 ± 0.005 g of ammonium chloride (dried at 105 °C for a least two hours) in about 800 ml of ammonia-free water (Section A3.1), quantitatively transfer to a 1 litre calibrated flask and make up to the mark with ammonia-free water, mix well. The solution may be stored in a stoppered glass bottle for not more than four weeks.

A3.3 Standard Nitrogen Solution B. 1 mL contains 0.1 mg N

Add 50.0 ± 0.1 mL of stock standard solution (A3.2) to a 500 mL calibrated flask. Make up to volume with ammonia-free water (Section A3.1) and mix well. Store the solution in a stoppered glass bottle for not more than four weeks.

Note that when standards are used for calibrating ammonia determination directly on acid digests (Methods C and D), the calibration procedure specifies the addition of sulphuric acid to the calibration standards to provide comparable concenrations, unless it can be shown that there is no practical important difference between acidified and non-acidified standard solutions.

Table A2 Preparation of Standard Nitrogen Solutions A3.5

Standard Solution	Volume taken (ml) for dilution to 100 mL	Nitrogen Concentration of resulting solution mgN/L
A3.3	50	50
A3.3	40	40
A3.3	30	30
A3.3	20	20
A3.3	10	10
A3.3	5	5
A3.3	2.5	2.5
A3.4	10	1
A3.4	8	0.8
A3.4	6	0.6
A3.4	5	0.5
A3.4	4	0.4
A3.4	2	0.2
A3.4	1	0.1

A3.4 Standard Nitrogen Solution C. 1 mL contains 0.01 mg N

Add 50.0 ± 0.1 ml of standard solution B (A3.3) to a 500 mL calibrated flask. Make up to volume with ammonia-free water (A3.1) and mix well. Store the solution in a stoppered glass bottle for not more than one week.

A3.5 Standard Nitrogen Solutions

Table A2 gives details for the preparation of working standard solutions which may be required for calibration of methods. Use pipettes or burettes and calibrated flasks for all dilutions, and store the solutions in stoppered glass bottles, for not more than two days, for solutions up to 5 mg N/L; or for up to one week for more concentrated solutions.

A3.6 Cleaning Solution

Dissolve 100 ± 2 g of potassium hydroxide in 100 ± 2 mL of water. Cool the solution and add 900 ± 50 mL of industrial methylated spirits. Store in a polyethylene bottle. THIS REAGENT IS TOXIC. It is stable for at least six months.

B Manual Determination of Kjeldahl Nitrogen (Mercury Catalysed Method)

B1 Performance Characteristics of the Method

B1.1	Substance determined	Nitrogen in the trivalent state.
B1.2	Type of sample	Waste waters and effluents.
B1.3	Basis of the method	Rigorous sulphuric acid digestion of the sample in the presence of a mercury catalyst to convert nitrogen compounds present (but see Section B2) to ammonium sulphate; distillation of the digest under alkaline conditions and titration of the liberated ammonia.
B1.4	Range of Application	Up to 10 mg N in the maximum sample aliquot taken.
B1.5	Calibration Curve	Not applicable.
B1.6	Standard Deviation (with titrimetric finish)	

Sample	Nitrogen concentration	Total standard deviation	Degrees of freedom
	mg N/L	mg N/L	
Nicotinic acid solutions	10	0.2	8 (a)
	100	1.7	10 (a)
	200	10.3	23 (b)
	400	9.2	23 (b)
Settled sewage	48	0.6	8 (a)

B1.7	Limit of detection (with titrimetric finish)	1.0 mg N/L using a 100 mL sample aliquot.
B1.8	Sensitivity	1.0 mL of 0.02 M hydrochloric acid solution is equivalent to 0.28 mg of nitrogen equivalent to 2.8 mg N/L using 100 mL of sample.
B1.9	Bias	No information apart from that arising from the presence of interfering or incompletely recovered species.
B1.10	Interferences	See Section B3.

B1.11 Time required for analysis

	Time (hours) for 6 determinations			
	Flasks (a)		Tubes/heating block (b)	
	Operator	Total	Operator	Total
Sample digestion	1.5	2.5	1.5	4.0
Complete analysis	1.5	4.0	1.5	5.25

Note: When using a heating block the digestion times will be of a similar order even if the number of digestions is increased to 20.

a) Data obtained by the North West Water Authority, using flask digestion.

b) Data obtained by the Anglian Water Authority, using tube digestion in a heated metal block.

B2 Principle
(See also Section A1)

By digestion of the sample in the presence of sulphuric acid, potassium sulphate and mercuric oxide as catalyst, organic nitrogen compounds, with certain exceptions, (See Section A1) are converted to ammonium ion, together with any free ammonia or ammonium nitrogen present.

Potassium sulphate is included in the digestion mixture in sufficient amount, relative to sulphuric acid, to raise the boiling point to 365 ± 10°C. This temperature is sufficiently high to ensure complete conversion of organic nitrogen to ammonium ion, but not so high as to cause loss of ammonia by volatilization during digestion[5,6,7].

B3 Interferences

B3.1 Halides

Halides may interfere as a result of the loss of mercury catalyst by volatilization as mercury halide and consequent incomplete conversion of organic nitrogen to ammonia. As a guide, more than 60 mg of chloride in the sample volume taken for digestion may cause difficulty, but quantitative data are lacking.

B3.2 Nitrate/Nitrite (see Table B1)

The presence of nitrate and/or nitrite may lead to erratic results which are dependent on the other substances present in the sample. A variety of organic compounds form nitro and nitroso derivatives, primary amines give nitrogen with consequent loss of potential Kjeldahl Nitrogen but secondary and many tertiary amines give N-nitrosamines.

B4 Elimination of Interferences

B4.1 Halides

After the sample has been added to the digestion vessel as given in Section B9.1 omit the addition of catalyst in step B9.2 until steps B9.3 to B9.5 have been carried out. If necessary extend the heating period of step B9.5 to ensure that the bulk of the halide present is removed. Cool the vessel, add 6.8 ± 0.1 g of catalyst mixture (B6.2.2) and proceed as given in step B9.6.

B4.2 Oxidized nitrogen

If the concentration of oxidized nitrogen in the sample aliquot is likely to cause unacceptable bias or loss of precision, it may be removed by distillation as ammonia after preliminary reduction with Devarda's alloy in alkaline solution[14]. It is necessary first to determine ammonia in the sample separately so that the ammonia nitrogen can be summed with the organic nitrogen (only) which will be determined on the sample after reduction in order to arrive at the Kjeldahl nitrogen concentration. See Section A1. However, volatile amines may be lost from the sample during preliminary distillation. An alternative approach is reduction with titanium (III) in acid solution[4], but in this case, nitrogen may be lost as a result of reactions between primary amines and nitrous acid. (Note Ref 23).

Table B1 Interference by oxidized nitrogen on the determination of Kjeldahl nitrogen on settled sewage

Nitrate Nitrogen Added		Nitrite Nitrogen Added		Kjeldahl Nitrogen Found	
Concentration in sample	Mass of Nitrate Nitrogen	Concentration in sample	Mass of Nitrite Nitrogen		
mg/L	mg	mg/L	mg	mg/L (duplicate results)	
nil	nil	nil	nil	31.4	31.9
10	1	nil	nil	31.6	31.4
100	10	nil	nil	31.6	32.5
200	20	nil	nil	27.1	37.0
300	30	nil	nil	30.0	29.7
1,000	100	nil	nil	12.9	6.7
10,000	1,000	nil	nil	1.7	19.9
nil	nil	5	0.5	31.6	—
nil	nil	10	1	32.5	35.6
nil	nil	20	2	32.5	—
nil	nil	30	3	33.6	31.4
nil	nil	100	10	42.0	30.0

Using the standard deviation from B1.6 the 95% confidence interval on a single analytical result is ± 1.5 mg N/L.*

* The use of a confidence interval to detect bias due to the presence of interfering substances usually involves the assumption that the interfering substance or substances do not affect the precision of the method. This condition is probably not satisfied for the effect of oxidized nitrogen on the Kjeldahl determination.

B5 Hazards

B5.1 Concentrated sulphuric acid should only be added to samples inside a fume cupboard since toxic volatile substances such as hydrogen cyanide or hydrogen sulphide may be evolved from some samples.

B5.2 During the digestion of the sample sulphur trioxide will be evolved. Therefore the digestion procedure must be carried out inside a well ventilated fume cupboard and the flasks containing hot concentrated sulphuric acid must be shielded from the operator who should wear protective clothing, gloves and a full face shield. Alternatively, the digestion apparatus may incorporate equipment to prevent the escape of harmful reaction products into the laboratory atmosphere and/or render the reaction products harmless, by neutralization, before discharge to the laboratory's main drainage system.

The distillation step following digestion requires the use of hot concentrated sodium hydroxide solution and a full face shield should be worn.

B5.3 The flasks in which the digest is made alkaline and from which ammonia is distilled may suffer from pin-holing and/or general alkaline etching of the glass[21]. Flasks should be regularly inspected before use and replaced if there is any doubt about their soundness.

B5.4 Mercury, the catalyst, is toxic; consequently digest solutions **must not be pipetted by mouth**. All residues obtained from any of the procedures given in Section B9 should be treated as described in Section B12 of this booklet.

B6 Reagents and Standards

Analytical reagent grade materials should be used except where otherwise stated.

B6.1 Water

The water used for blank determinations, for preparing digest solutions, reagent and standard solutions and for dilution purposes, should have a nitrogen content that is negligible compared with the smallest concentrations to be determined in the samples. Water prepared as described in Section A3 will be suitable, but in most cases distilled or deionized water may also be acceptable.

B6.2 Digestion reagents

B6.2.1 Sulphuric acid (d_{20}1.84), low in nitrogen.

B6.2.2 Potassium sulphate/mercuric oxide—catalyst

Mix by grinding together 138 ± 2 g of potassium sulphate and 2 ± 0.1 g of mercuric oxide. Alternatively use equivalent commercially available catalyst tablets. Whichever is used, the catalyst mix should be kept in a container labelled 'HIGHLY TOXIC' which is stored in a locked outer receptacle when not in use.

B6.2.3 30% m/v Hydrogen peroxide solution

B6.2.4 Anti-bumping granules

eg pumice stone, 1 mm mesh.

B6.3 Reagents for the titrimetric determination of ammonia

B6.3.1 Sodium hydroxide—sodium thiosulphate reagent

Cautiously dissolve 400 ± 10 g of sodium hydroxide pellets and 25 ± 1 g of sodium thiosulphate in about 800 mL of water. Cool and dilute to 1000 ± 10 mL with water. Mix well and store in a polyethylene bottle. This reagent is stable for at least one month.

B6.3.2 0.05% m/v Methyl red indicator solution

Dissolve 0.5 ± 0.1 g of methyl red, water soluble grade, in about 900 mL of water and dilute with water to 1000 ± 10 mL. Mix well and store in a glass or polyethylene container. This reagent is stable for at least six months.

B6.3.3 0.15% m/v Methylene blue solution

Dissolve 1.5 ± 0.1 g of methylene blue in about 900 mL of water and dilute with water to 1000 ± 10 mL. Mix well and store in a glass or polyethylene container. This reagent is stable for at least one month.

B6.3.4 Indicating boric acid solution

Dissolve 20 ± 1 g of boric acid in about 900 mL of warm water. Cool to room temperature. Add 10 ± 1 mL of methyl red solution (B6.3.2) and 2.0 ± 0.2 mL of methylene blue solution (B6.3.3) and dilute to 1000 ± 10 mL. Mix well and store in a glass or polyethylene container. 0.1 mL of 0.01 M sodium hydroxide solution added to 20 mL of this solution should be sufficient to change the colour from purple to green; if not, discard the indicating boric acid solution and prepare freshly. This solution is stable for at least three months.

B6.3.5 0.02 M Hydrochloric acid solution

Prepare and standardize this solution according to standard analytical procedures. Adjust the concentration to exactly 0.02 M. Maintain a check of the strength of this hydrochloric acid solution whilst in use. An alternative to hydrochloric acid is sulphamic acid which is a primary standard.

B7 Apparatus

B7.1 Digestion vessels

Kjeldahl digestion flasks, 300 mL capacity. Alternatively digestion tubes, 250 ml capacity, may be used with a heating block.

B7.2 Heating device for digestion vessels

Kjeldahl digestion flasks may be heated singly over a Bunsen flame or by some form of electric heating. It is usually convenient to carry out several digestions simultaneously and the flasks may be mounted on a suitable rack heated either by gas or by electricity.

Whilst the heating device must be capable of producing the digestion conditions given in Section B9, the surface of the flask above the level of the liquid contents must not be subjected to overheating.

Digestion tubes are most conveniently heated using a compatible heating block (see Appendix), provided that the digestion conditions chosen offer precision and accuracy of result closely similar to the flask method or which are acceptable to the operator.

B7.3 Distillation apparatus for nitrogen determination

Assemble as given in Figure 1 from the following commercially available components:

Round-bottomed flask, capacity 500 mL. The neck should be fitted with a PTFE sleeve to prevent possible seizure with the condenser due to contact with the alkaline reagent introduced in Section B9, step 14. See also Section B5.4.

Condenser

Splash head

Delivery adaptor

Conical 'receiver' flask, capacity 250 mL

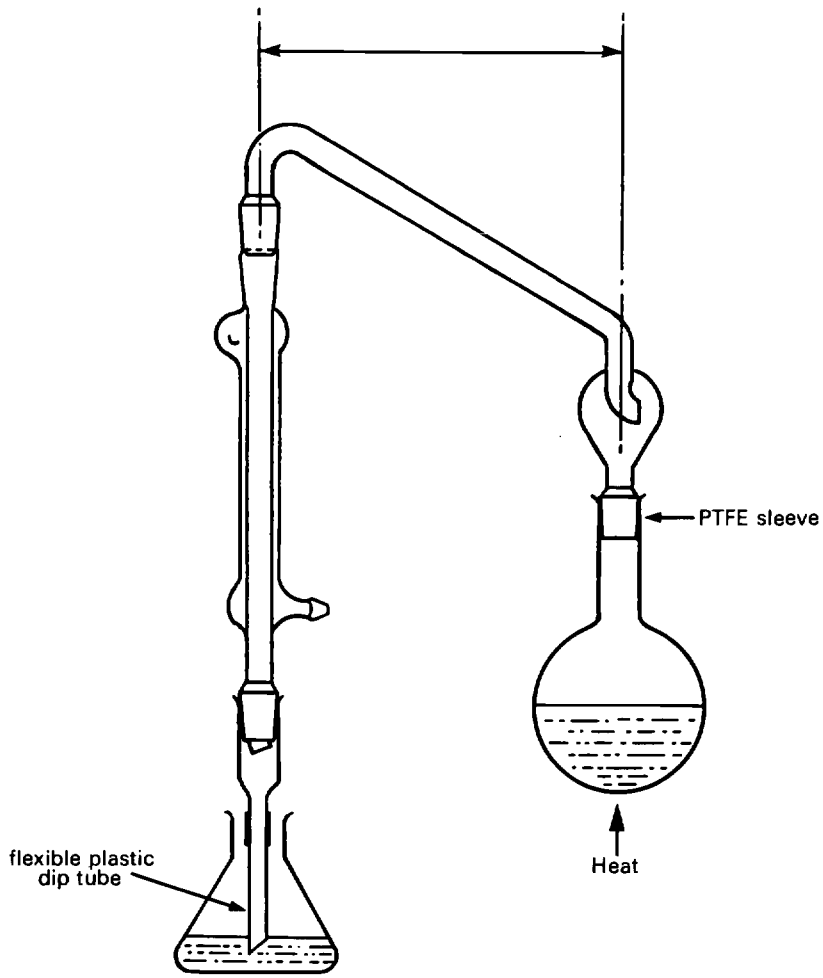


Figure 1 DISTILLATION APPARATUS

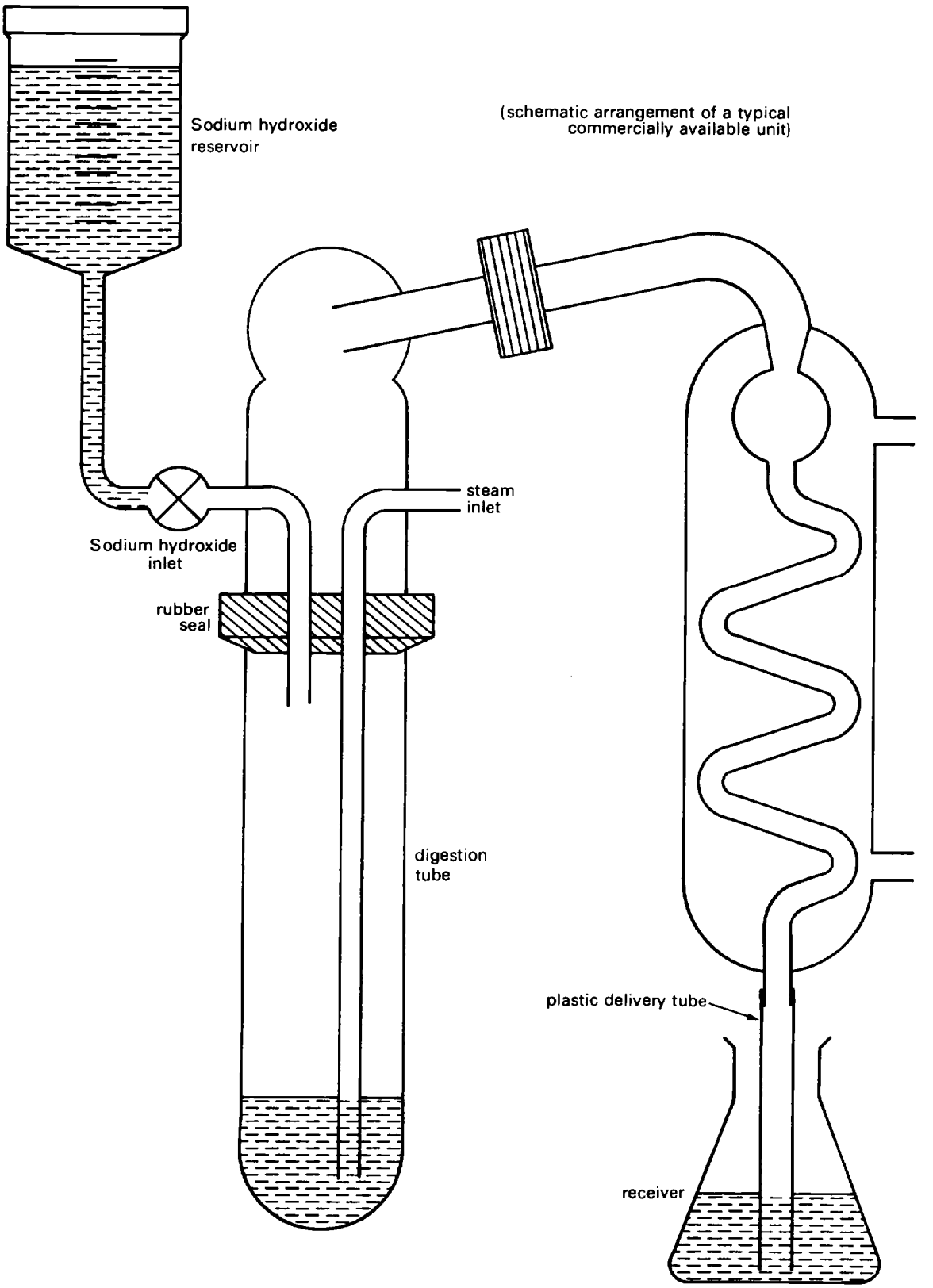


Figure 2 STEAM DISTILLATION APPARATUS

Commercially available distillation (usually steam distillation as depicted in Fig 2) units may be used if the operator is satisfied that no loss of ammonia occurs during the distillation procedure (see also section E8). Apparatus performance may be checked by using standard ammonia solutions in the place of digest solutions.

The apparatus should be purged with ammonia free steam before use.

B8 Sample Collection and Preservation See Section A2.

B9 Analytical Procedure

Read Hazards, Section B5, before starting this procedure.

Step	Procedure	Notes
	Digestion Procedure	
B9.1	Place a measured volume of sample (note a) in the flask or digestion tube (B7.1) (note b). Make the volume up to 100 mL with ammonia-free water (A3.1) if the sample volume is less than 100 mL.	(a) The following sample volumes should be used, according to the anticipated Kjeldahl nitrogen concentration of the sample:
B9.2	Add 6.8 ± 0.1 g of catalyst mixture (B6.2.2) and 2–3 anti-bumping granules (B6.2.4). (note c)	(b) Digestion tubes of similar capacity may be used as an alternative to flasks.
B9.3	Cautiously add 10 ±0.5 mL of sulphuric acid (B6.2.1) whilst gently swirling the digestion vessel (note d).	(c) See Section B3.1 and B4.1 if potential halide interference is a concern.
B9.4	Stand the flask on a digestion rack inside a fume cupboard, or insert the tube on a heating block.	(d) If the sample aliquot contains more than 0.5 g of organic matter, add an extra 4.5 ± 0.1 mL of acid per additional gram, or an extra 9.0 ± 0.1 mL of acid per additional gram if the organic matter is fatty in nature.
B9.5	Heat the digestion vessel gently to evaporate the water present.	(e) Avoid overheating the upper sections of digestion flasks or tubes.
B9.6	Heat the digestion vessel more strongly to sustain brisk boiling (note e). The sulphuric acid should reflux strongly up to about halfway along the neck of the digestion vessel. White fumes of sulphur trioxide will be visible above the clear reflux area (note f).	(f) Strong heating is needed to achieve decomposition of refractory nitrogen compounds. Cooling by fume extraction systems and draughts could reduce the digestion temperature and therefore should be prevented. Confirmation of correct digestion conditions may be shown by the recovery of nitrogen from nicotinic acid control standards (see Section B13).
B9.7	When the contents of the digestion vessel have become clear or no more than a pale straw colour (note g), continue to reflux as in step B9.6 for 60 ± 10 min (note h).	(g) The digestion mixture chars initially and usually becomes a clear, pale straw or colourless solution within about 30 minutes. Silica present in the sample may cause turbidity and certain metals may contribute colours.
		(h) Reflux must not be prolonged because ammonia may be lost by volatilization from the digest.

Concentration mg N/L	Volume mL
Up to 50	100
50–200	50
200	25

Step	Procedure	Notes
B9.8	Allow the solution to cool to 40 ± 5°C and cautiously add 40 ± 5 mL of water whilst swirling the flask (note i). Retain this solution for the determination of ammonia (note j) either by the distillation and titration procedure (step B9.10) or by the automatic colorimetric procedure given in Section F applied to the distillate collected in 0.02 M hydrochloric acid (B6.3.5) at step B9.12 instead of the boric acid solution.	(i) Water is added to prevent crystallization of the digestion solution. Any crystals that appear may be dissolved by gentle warming and further dilution of the solution with water. (j) The solution may be stored at room temperature in a polyethylene bottle for at least 2 weeks without significant loss of ammonia.
	Blank Digestion Procedure	
B9.9	Carry out steps B9.1 to B9.8 as given above, replacing the sample (B9.1) by a corresponding volume of water (note a) to give a total volume of 100 mL.	
	Distillation procedure for ammonia determination	
B9.10	Assemble the distillation apparatus as shown in figure 1.	
B9.11	Quantitatively transfer the digest solution (B9.8) to a distillation flask (B7.3 and B5.4) and make up the flask contents to 250 ± 25 mL with water. Add a few anti-bumping granules (B6.2.4).	
B9.12	Add 50 ± 0.5 mL of indicating boric acid solution (B6.3.4) to the receiver flask.	
B9.13	Reassemble the apparatus (note k).	(k) Ensure that the tip of the delivery adaptor is below the surface of the indicating boric acid solution.
B9.14	Add 40 ±2 mL of sodium hydroxide/sodium thiosulphate reagent (B6.3.1) to the distillation flask and quickly reassemble the apparatus.	
B9.15	Heat the flask so that distillation proceeds at about 10 mL/minute (notes 1 and m).	(l) Use commercially available distillation units according to the manufacturer's instructions. The appearance of a black precipitate of mercuric sulphide signifies that the correct distillation conditions have been achieved.
B9.16	Collect about 120 mL of distillate then lower the receiver so that the delivery adaptor is above the surface of the solution.	(m) The receiving boric acid solution becomes green during the course of the distillation.
B9.17	Continue to collect a further 30 mL of distillate.	
B9.18	Stop the distillation and rinse the delivery adaptor with about 5 mL of water.	

Step	Procedure	Notes
B9.19	Titrate the solution in the receiver flask with 0.02 M hydrochloric acid solution (B6.3.5) to the appearance of a permanent grey-purple colour. Note the titre and let this be T_1 mL.	
	Blank Distillation	
B9.20	Carry out steps B9.10 to B9.19, but using the blank digest solution (B9.9) at step B9.11. Let the titre obtained at step B9.19 be T_2 mL.	
	Calculation	
B9.21	Kjeldahl nitrogen concentration, mg N/L $= \frac{(T_1 - T_2) \times 0.28 \times 1000 \text{ mgN/L}}{V}$	
	T_1 = sample titre (mL 0.02 M hydrochloric acid solution)	
	T_2 = blank titre (mL 0.02 M hydrochloric acid solution)	
	V = volume of sample taken for digestion (B9.1)	
	0.28 = mg of nitrogen equivalent to 1.0 mL of 0.02 M hydrochloric acid solution	

B10 Extension of the Range of the Method

If required, the range of the method can be extended to higher concentrations of nitrogen by the use of stronger standard hydrochloric acid solution for the titration of ammonia in the distillate. Alternatively smaller volumes of digest solution may be distilled. In either case appropriate modifications to the calculation of results will be necessary.

If lower concentrations of ammonia need to be determined suitable volumes of digest solution may be analysed colorimetrically using the continuous flow procedure given in Section F of this booklet. Note that at all concentrations of nitrogen it is possible to use colorimetry both before or after distillation instead of distillation followed by titrimetry to determine the ammonia content of the digestion solution.

B11 Sources of Error

B11.1 Digestion Procedure

Sample foaming and spattering, particularly in the initial steps of the digestion procedure can lead to errors. Close control of digestion temperature and of the duration of the digestion is also necessary to avoid errors (see Section B2). The continued 'digestion' period (step B9.7) after the digest has cleared is an essential part of the process to ensure complete recovery of nitrogen, particularly that present as part of a heterocyclic system. However over-prolonged digestion may lead to falsely low results.

Excessive heating of the digestion flask or tube above the liquid level may lead to the loss of nitrogen from the digest.

B11.2 Distillation Procedure for ammonia determination

The distillation rate and time of distillation must be closely controlled otherwise a complete recovery of evolved ammonia may not be obtained.

B11.3 Ammonia content of water used for blank determinations

The blank titre should not exceed 0.3 mL of 0.02 M hydrochloric acid solution (B6.3.5). If it does exceed this value, check the preparation of the indicator boric acid solution (B6.3.4). If a high value persists then all the reagents and working conditions should be critically examined.

B12 Disposal of Mercury-Containing Residues from the Distillation Flask

In order to avoid risks to the environment, eg sewage treatment works and watercourses, and to recover some of the cost of expensive reagents, the following procedure is recommended.

As the residual contents of the distillation flask are strongly alkaline and may be hot, a safety vizor and gloves must be worn during the operation when the flask is dismantled from the distillation apparatus. This procedure and the decantation of the supernatant liquid should be carried out under a fume extraction hood.

B12.1 Procedure

Cool the residual contents of the distillation flask and transfer to a suitable container eg a 2.5 litre glass bottle (a Winchester quart bottle). Allow the precipitate of mercuric sulphide to settle. The supernatant alkaline liquor should be oxidized or diluted 500 to 1 with water before disposal.

Mercuric Sulphide is a notifiable waste and must not be disposed to land without consent

Precious metal refiners may accept mercuric sulphide for recovery. For information on mercury recovery for reuse if no repurchaser is available, see reference 11.

B13 Checking the Accuracy of the Analytical Result

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of analytical results. It is recommended that experimental tests to check sources of inaccuracy should be made regularly. Many types of test are possible and should be used as appropriate.

As a minimum control, it is suggested that a standard solution of nicotinic acid (pyridine-3-carboxylic acid) eg 10 mg N/L (ie 100 mL of a solution containing 0.879 g nicotinic acid/litre) should be analysed at the same time and in exactly the same way as normal samples. The results obtained may then be evaluated by control chart techniques such as those recommended[10].

Note that nicotinic acid does not char easily. It may be necessary for complete recovery of nitrogen to add a small amount of a nitrogen-free organic substance that does char easily to create the reducing conditions necessary at the beginning of a digestion process[7]. Sucrose (about 0.1 g) or washed filter paper may be suitable but it is advisable to check that these do not contribute to the blank value. When digesting nicotinic acid it is particularly important to reflux the digest mixture as given in steps B9.6 and B9.7[5,6,7].

C Semi-automated Determination of Kjeldahl Nitrogen (Copper Catalysed, Multiple Tube, Block Digestion Method followed by Air Segmented Continuous Flow Colorimetry)

C1 Performance Characteristics of the Method

C1.1	Substance determined	Nitrogen in the trivalent state.
C1.2	Type of sample	Waste waters and effluents.
C1.3	Basis of the method	Digestion of the sample with concentrated sulphuric acid in the presence of sodium sulphate and a copper catalyst to convert nitrogen compounds present (see Sections A1 and B2) to ammonium sulphate. The ammonia content of the digest solution is determined by continuous flow colorimetry to enable the Kjeldahl nitrogen concentration of the sample to be calculated.
C1.4	Range of Application	Up to 5 mg N in the digest solution, equivalent to up to 100 mg N/L in the original sample when using a 50 mL sample aliquot.
C1.5	Calibration Curve (of the continuous flow ammonia chemistry)	Linear up to 20 mg N/L

C1.6 Standard Deviation (within-batch, 9 degrees of freedom)

Sample	Concentration	Standard Deviation
	mg N/L	mg N/L
Sewage	60.3	1.8
Sewage effluent	48.1	1.5

C1.7	Limit of detection	3 mg N/L, when using the maximum sample volume, 50 mL.

C1.8 Time required for analysis (20 determinations)

Procedure	Total time (h)	Operator time (h)
Digestion	3.0	0.5
Total (and analysis)	4.0	0.5

The data presented in the above table were obtained by the Water Research Centre (Stevenage Laboratory). The analysis of the digest solutions was carried out using the procedure given in Ref 19. (See Section C5.3).

C2 Principle

C2.1 Digestion of the sample

By hot digestion with a mixture of sulphuric acid, sodium sulphate and a copper catalyst, the nitrogen of many organic substances is converted to ammonium sulphate. For scope of the conversion, see Section A1. Ethanol is added to overcome potential interference from oxidized nitrogen (see Section B3.2). See also Ref 23.

C2.2 Analysis of digestion solution

The ammonia content of the digest solution is determined using the automated procedure given in Ref 19 and then related to the Kjeldahl nitrogen concentration of the original sample. However the procedures given in Section F of this booklet should give comparable performance. (See also Section C5.3).

C3 Interferences

See Section B3.2.

C4 Hazards

C4.1 Concentrated sulphuric acid should only be added to samples inside a fume cupboard since toxic volatile compounds such as hydrogen cyanide or hydrogen sulphide may be evolved from some samples.

C4.2 During the digestion of the sample, sulphur trioxide will be evolved. The procedures therefore must be carried out inside a fume cupboard fitted with an efficient extraction system. Alternatively, the digestion unit may incorporate equipment to prevent the escape of harmful reaction products into the laboratory atmosphere and/or render the reaction products harmless, by neutralization, before discharge to the laboratory's main drainage system.

C5 Reagents

Analytical reagent grade chemicals should be used except where stated otherwise.

C5.1 Water

The water used for blank determinations and for preparing digest solutions should have a nitrogen content that is negligible compared with the smallest concentrations to be determined in the samples. Water prepared as described in Section A3 will be suitable; but in most cases distilled or deionized water may also be acceptable.

C5.2 Digestion reagents

C5.2.1 Sulphuric acid (d_{20}1.84).

C5.2.2 Pumice stone Granular, 8 to 20 mesh, as anti-bumping granules.

C5.2.3 Digestion catalyst.

Mix together 600 ± 5 g of sodium sulphate, anhydrous, and 18 ± 0.2 g of copper sulphate, pentahydrate.

C5.2.4 Absolute alcohol (99.7% ethanol)

C5.3 Reagents for the automatic analysis of digest solutions

The preparation of the reagents and the analytical procedure used to obtain the performance data in Section C1 are described in Ref 19. The procedures given in Section F of this booklet, should give comparable performance data. Analysts are advised to make their own checks.

C6 Apparatus

C6.1 Digestion

C6.1.1 A heating block suitably drilled to accommodate the digestion tubes (C6.1.2), and which can be thermostatically controlled at a temperature sufficient to maintain the digest mixture at the boil under reflux conditions (see Appendix).

C6.1.2 Digestion tubes, 250 mL capacity.

C6.1.3 Stand for digestion tubes.

C6.1.4 Fume cupboard fitted with an efficient extraction system capable of safely removing heavy toxic fumes that are generated during digestion. Also refer to Section C4.

C6.2 Analysis of digestion solutions

Air-segmented continuous flow apparatus assembled as given in Ref 19, or in Section F6, for range Fa. (See also Section C5.3).

C7 Sample Collection and Preservation

As given in Section A2.

C8 Analytical Procedure Read Hazards, Section C4, before starting this procedure.

Step	Procedure	Notes
	Digestion Procedure	
C8.1	Measure 50.0 ± 0.5 mL of sample into a digestion tube (note a) and add to it 10.0 ± 0.5 mL of absolute alcohol (C5.2.4) and 15–20 granules of pumice stone (C5.2.2).	(a) Repeat steps C8.1–C8.4 inclusive for the number of samples to be processed as one batch.
C8.2	Add 1.00 ± 0.05 g of catalyst mixture (C5.2.3).	
C8.3	Cautiously add 10.0 ± 0.5 mL of sulphuric acid (d_{20}1.84) (note b) with swirling to mix the contents	(b) The acid may be delivered using a dispenser. The digestion tube must be pointed away from every one.
C8.4	Preheat the heating block to 200 ± 10°C. Insert the digestion tubes after step C8.3 into the heating block (note c). Maintain the temperature of the block at this temperature for 30 ± 2 minutes (note d).	(c) After note (a) above, if a single extraction hood is used for the maximum number of digestion tube spaces available in the heating block and this maximum number exceeds the number of samples in the batch, make up to the maximum with blank digestions containing reagents only (C8.9). See also the Appendix.
		(d) During this initial heating, water evaporates and any oxidised nitrogen present is driven off as volatile ethyl nitrate or nitrite. (See also Ref 23).
C8.5	Increase the thermostat setting of the block to increase the block temperature to 370 ± 5°C and maintain this temperature for 30 ± 2 minutes (note e).	(e) During this time the sulphuric acid should reflux smoothly at a height 15–25 mm below the top of the digestion tube.
C8.6	Switch off the heating block and when fumes cease to evolve from the tubes, remove them from the block (note f).	(f) Usually 15 minutes after the end of the digestion period (C8.5).
C8.7	Allow the tube to cool for a further 10 minutes then with continuous swirling add about 100 mL of water to the contents of the tube (note g).	(g) The tube should be held pointing away from the operator.
C8.8	Cool the contents of the tube to room temperature. Quantitatively transfer the contents of the tube to a 250 mL calibrated flask and dilute to volume with water. Mix thoroughly and then determine ammonia in the solution by either the procedure given in Ref 19 or in Section F (note h).	(h) The solution may be stored at room temperature in a polyethylene bottle for at least 2 weeks without significant loss of determinand. See Section C5.3
	Blank digestion procedure	
C8.9	Repeat step C8.1 but substitute the sample with the same volume of water. Carry out steps C8.2 to C8.8 to obtain a blank digest solution for ammonia determination using the same procedure as that used for samples.	

Step	Procedure	Notes
	Calculation of result (note i)	
C8.10	Let the ammonia nitrogen content of the digest solution corrected for any blank value (C8.9) = A mg N/L. Thus the Kheldahl nitrogen content of the sample. $= \frac{A \times 250}{V}$ mgN/L where V is the volume in mL of the sample taken for digestion.	(i) This calculation is carried out after a mg/L value for N is obtained from one of the chosen analytical procedures (See Section C8.8).

C9 Recovery of Organic Nitrogen

The recovery of nitrogen from organic compounds common to the sample type has been investigated. The data is tabulated below. Table C1 shows the measured nitrogen content compared to the theoretical and Table C2 shows the percent recoveries of known amounts of nitrogen added to sewage samples. These data are considered representative of the method's performance when analysing waste water and effluent samples.

Table C1(a) Comparison of measured content of nitrogen in organic compounds with theoretical value, when using this Method's experimental conditions.

Sample	Measured %N	Theoretical %N
Urea	46.67	46.62
Glycine	18.10	18.65
Glutamic acid	9.52	9.52

Table C2(a) Recovery of added nitrogen from samples.

	Initial Nitrogen mg N/L	Conc of Nitrogen Added mg N/L	Source of Added Nitrogen	Conc of Nitrogen Measured mg N/L	Recovery of Nitrogen Added (%)
Sewage	59.0	20.0	Ammonium sulphate	79.5	101
	59.0	20.0	Urea	78.5	99
	59.0	20.0	Glycine	79.0	100
	59.0	20.0	Glutamic acid	80.0	101
Sewage Effluent	55.0	20.0	Ammonium sulphate	76.0	101
	55.0	20.0	Urea	76.0	101
	55.0	20.0	Glycine	76.0	101
	55.0	20.0	Glutamic acid	74.5	99

(a) Data supplied by the Water Research Centre (Stevenage Laboratory). Ammonia in the digests was determined using the phenol/hypochlorite chemistry given in Ref 19 (See Section C5.3).

C.10 Extension of the Procedure

If the content of Kjeldahl nitrogen in the sample is expected to be greater than 100 mg N/L an appropriate dilution of the digest solution should be made. A reduced volume should be taken for digestion of samples known to contain a concentration of Kjeldahl nitrogen greater than 500 mg N/L.

C.11 Sources of Error

C.11.1 Careful attention to the temperature of the heating block is vital. See the Appendix.

C.12 Checking the Accuracy of the Analytical Result

The method given in Section B is considered to be the Reference method because of the rigorous digestion conditions. Method C may not be able to digest all heterocyclic nitrogen compounds if these are present in the sample. However Method C does give quantitative recoveries of nitrogen from substances most likely to be present in samples (see Section C9); the method is therefore considered suitable for general monitoring.

D Determination of Kjeldahl Nitrogen in Raw and Potable Water (Hydrogen Peroxide, Multiple tube, Block Digestion Method followed by Manual or Air-Segmented Continuous Flow Colorimetry)

D1 Performance Characteristics of the Method

D1.1	Substances determined	Nitrogen in the trivalent state.
D1.2	Type of sample	Raw and potable water.
D1.3	Basis of the method	Digestion of the sample with concentrated sulphuric acid and 50% m/v hydrogen peroxide (see Sections A1 and B2). After dilution of the digest, ammonia is determined by manual colorimetry as given in this section or by air segmented continuous flow colorimetry as given in Section F.
D1.4	Range of Application	Up to 1 mg N/L (using a 25 ml sample volume). The range may be extended by varying the sample volume.
D1.5	Calibration Curve (for the manual ammonia determination)	Linear.

D1.6 Total standard deviation (with manual colorimetric finish)—all with 9 degrees of freedom (c)

Sample Type	Concentration	Standard Deviation
	mg N/L	mg N/L
Standard solution (a)	1.00	0.053
Standard solution (a)	0.50	0.032
Standard solution (b)	0.50	0.023
Tap water	0.32	0.036
Tap water	0.75	0.027

D1.7	Limit of detection (with manual colorimetric finish) (c)	0.093 mg N/L. (25 ml sample volume)

D1.8 Time required for analysis (batch of 20 samples, manual colorimetric finish) (c), (which may increase, see Step D8.9 note e).

Total analytical time: 3.5 h
Operator time: 2.0 h

Notes: (a) Solution of ammonium chloride
(b) Solution of sodium glutamate
(c) Data from Thames Water Authority (New River Head Laboratories)

D2 Principle

D2.1 Digestion of the sample

The sample portion is digested with concentrated sulphuric acid and 50% hydrogen peroxide in a heated aluminium block (see Appendix). When cool, the digest is diluted prior to subsequent analysis.

D2.2 Analysis of digest solution

The ammonia content of the digest solution is determined using a manual colorimetric procedure taken from another publication in this series[8]. Alternatively, the automated procedure described in Section F may be employed, using concentration range Fb.

D3 Interferences

D3.1 For general information, see Section B3.2.

D3.2 The effects of nitrate, nitrite and alkalinity are shown in Table D1.

Table D1(d)

Other Substances (Expressed in terms of substance in brackets)	Concentration	Effect in mg N/L of other substance at a nitrogen (e) concentration of		
	mg/L	0.000 mg/L mg/L	0.500 mg/L mg/L	1.00 mg/L mg/L
Nitrate (N)	10	+0.0	+0.008	-
	15	+0.039	+0.026	-
	20	+0.082	+0.035	-
	25	+0.095	-	+0.10
Nitrite (N)	1	-	-	+0.01
Alkalinity ($CaCO_3$)	1000	−0.006	+0.018	-
Alkalinity ($CaCO_3$)	1000	0.000	+0.019 (f)	-

If the other substances did not interfere, the effect would be expected (95% confidence) to lie between:

± 0.044 at 0.000 mg N/L
± 0.050 at 0.500 mg N/L
± 0.10 at 1.00 mg N/L

(d) Data from Thames Water Authority, New River Head Laboratories.
(e) Solutions of ammonium chloride, except where stated.
(f) Solution of sodium glutamate.

D4 Hazards

D4.1 Hydrogen peroxide causes burns if it contacts the skin or eyes. Any spillages must be washed off with copious amounts of water and, for eyes, medical attention should be obtained.

D4.2 For other hazards, see section C4 and Reference 8.

D5 Reagents

Use Analytical reagent grade chemicals unless stated otherwise.

D5.1 Water

The Water used for blank determinations and for preparing digest solutions should have a nitrogen content that is negligible compared with the smallest concentrations to be determined in the samples. Water prepared as described in Section A3 will be suitable, but in most cases distilled or deionized water will be acceptable.

D5.2 Digestion reagents

D5.2.1 Sulphuric acid (d_{20}1.84), low in nitrogen.

D5.2.2 Pumice stone, Granular, 8 to 20 mesh, as anti-bumping granules.

D5.2.3 Hydrogen peroxide 50% m/m (d_{20}1.18).

Each new batch of this reagent should be checked to ensure it gives an acceptable blank.

D5.3 Reagents for the manual colorimetric analysis of digest solutions

D5.3.1 0.4% m/V Phenolphthalein Indicator Solution

Dissolve 0.40 ± 0.01 g of phenolphthalein in 50 ± 5 mL of ethanol and dilute to 100 mL with water in a measuring cylinder. This solution is stable for at least three months.

D5.3.2 50% m/V Sodium hydroxide solution

Dissolve 500 ± 20 g of sodium hydroxide in about 800 mL of water. Carry out this operation in a fume cupboard. Cool to room temperature and dilute to 1 litre with water in a measuring cylinder. Store this solution in a polyethylene bottle. This solution is stable indefinitely.

D5.3.3 25% V/V Sulphuric acid solution

Slowly and cautiously, with stirring, add 25 ± 1 mL of sulphuric acid (D5.2.1) to 50 ± 5 mL of water. Cool and then dilute to 100 mL with water in a measuring cylinder. This solution is stable indefinitely.

D5.3.4 Salicylate Reagent

Dissolve 130 ± 1 g of sodium salicylate and 130 ± 1 g of tri-sodium citrate in about 950 ml of water contained in a 1 litre calibrated flask. Then add 0.970 ± 0.005 g of sodium nitroprusside, first ensuring that the pH value of the solution is not greater than 8.0 units. Dissolve and then dilute to 1 litre with water. Stored in an amber glass bottle the reagent is stable for at least two weeks.

D5.3.5 Sodium dichloroisocyanurate ('DIC') reagent

Dissolve 32.0 ± 0.1 g of sodium hydroxide in 500 ± 50 mL of water. Cool the solution to room temperature and add 2.00 ± 0.02 g of dichloro-s-triazine 2, 4, 6, (1H, 3H, 5H)—trione, sodium salt (sodium dichloroiso-cyanurate) to the solution. Dissolve and transfer the solution quantitatively to a 1 litre calibrated flask. Dilute to 1 litre with water. Stored in an amber glass bottle this reagent is stable for at least two weeks.

D5.4 Reagents for the automatic analysis of digest solutions (if used as an alternative)

See Section F3.

D6 Apparatus

D6.1 Digestion

D6.1.1 A heating block suitably drilled to accommodate the digestion tubes (D6.1.2), and which can be thermostatically controlled at a temperature sufficient to maintain the digest mixture at the boil under reflux conditions (see Appendix).

D6.1.2 Digestion tubes, 250 mL capacity.

D6.1.3 Stand for digestion tubes.

D6.1.4 Fume cupboard fitted with an efficient extraction system capable of safely removing heavy toxic fumes that are generated during digestion. Also refer to Section D4.

D6.2 Analysis of digest solutions (manual colorimetric procedure)

D6.2.1 Spectrophotometer, capable of operating at a wavelength of 655 nm and equipped with 10 mm path-length cells.

D6.3 Analysis of digest solutions (automatic procedure)

Air-segmented continuous flow apparatus, assembled as given in Section F6 using range Fb.

D6.4 General

All glassware must be carefully cleaned using the cleaning solution (A3.6) followed by thorough rinsing with water in order to minimize the possibility of contamination in this low-level method.

D7 Sample Collection and Preservation

As given in Section A2.

D8 Analytical Procedure

Read Hazards, Section D4, before starting this procedure.

Step	Procedure	Notes
	Digestion Procedure	
D8.1	Add 25.00 ± 0.03 mL of sample into a digestion tube (note a).	(a) A 50 ml sample volume may be used for extra sensitivity. See Section D9. Sample volumes smaller than 25 mL may be taken for samples having a Kjeldahl nitrogen concentration greater than 1 mg N/L.
D8.2	Add 2.00 ± 0.02 mL of sulphuric acid (D5.2.1) and two anti-bumping granules (D5.2.2) to the tube.	
D8.3	Insert the tube into the heating block (note b).	(b) Repeat steps D8.1 to D8.3 inclusive for the number of samples to be processed as one batch.
D8.4	Cautiously heat the tube at 200°C to evaporate the water present (note c).	(c) Refer to Appendix.
D8.5	Increase the thermostat setting of the block to increase the temperature of the tube contents to boil under reflux (note c).	
D8.6	Remove the tube from the heating block and allow to cool to a temperature at which the hydrogen peroxide (D5.2.3) can be safely added without excessive foaming (note d).	(d) After 3 min is normally adequate.
D8.7	Carefully add 0.200 ± 0.005 mL of the hydrogen peroxide (D5.2.3) to the tube, allowing it to run down the side of the tube. Mix by gentle swirling.	
D8.8	Replace the tube in the block and reheat until all traces of water have disappeared and the solution is again boiling under reflux.	
D8.9	Repeat steps D8.6 to D8.8 twice. (note e).	(e) Most samples will require only a total of 0.6 mL of hydrogen peroxide for complete digestion. However, up to a total of 2.4 mL may be added to samples containing a large amount of organic matter but the effect upon the blank value must be checked.
D8.10	Remove the tube from the heating block and allow to cool (note f).	(f) Ensure that all reaction fumes have ceased to evolve before removing the tubes.
D8.11	Cautiously add 20 ± 2 mL of water to the digest in the tube and then quantitatively transfer th digest solution to a 50 mL calibrated flask for determination of ammonia (notes g and h).	(g) Do **not** make up to volume at this stage. Use no more than 15 ml of water in the transfer. (h) The solution may be stored at room temperature in a cleaned polyethylene bottle for up to one week without loss of ammonia.

Step	Procedure	Notes
	Blank digestion procedure	
D8.12	Carry out steps D8.2 to D8.11 to obtain a reagent blank digest solution (note i).	(i) Omission of water in the blank digestion procedure makes possible the establishment of a true reagent blank value, unaffected by possible contribution of organic nitrogen from even high quality laboratory water. However, this contribution may have to be determined and allowed for when standard solutions are to be carried through digestion or when samples with Kjeldahl nitrogen greater than 1 mg N/L are to be diluted prior to digestion.
	Automatic colorimetric determination of ammonia	
D8.13	Dilute the digest to 50 ml with water in the calibrated flask (D8.11), mix well and carry out the determination of ammonia given in Section F using range Fb. Obtain the concentration of ammonia, in mg N/L, of each solution and multiply it by 2 to arrive at the Kjeldahl nitrogen concentration of the original sample, unless 50 ml of sample was taken at step D8.1 in which case the digest concentration equates directly to the sample concentration. (Note j).	(j) If a volume smaller than 25 mL was taken at step D8.1, appropriate multiplication of the ammonia result must be made. See also note (i).
	Manual colorimetric determination of ammonia	
	Calibration	
D8.14	Into a series of 50 mL calibrated flasks, pipette 25 mL of working standard ammonia solutions of concentrations 0.2, 0.4, 0.6, 0.8 and 1.0 mg N/L, prepared as described in Section A3.4. Use one portion of each solution.	
D8.15	To one of the calibrated flasks, add 0.1 mL of phenophthalein indictor (D5.3.1) (note k).	(k) It is **essential** to proceed with steps D8.15–D8.17 sequentially for each flask before starting at D8.15 for the next, in order to avoid less of ammonia from alkaline solutions.
D8.16	Add 50% m/v sodium hydroxide solution (D5.3.2) to the flask until the contents just turns pink (note l).	(l) About 2.2 mL will be required. Approach the appearance of the pink colour with additions of about 0.05 mL at a time to avoid adding an excess of alkali.
D8.17	Immediately add 25% v/v sulphuric acid (D5.3.3) in about 0.05 mL additions until the pink colour is just discharged (note m).	(m) It is essential to keep to a minimum the time the solution is alkaline.
D8.18	Repeat steps D8.15 to D8.17 for each of the remaining flasks in turn (note k).	
D8.19	Add 4.00 ± 0.05 mL of salicylate reagent (D5.3.4) to each flask and mix well.	
D8.20	Add 4.00 ± 0.05 mL of DIC reagent (D5.3.5) to each flask and mix well.	

Step	Procedure	Notes
D8.21	Dilute with water to 50 mL and mix well. Allow the colour to develop for at least 30 minutes (note n).	(n) All calibration solutions and samples in each batch of determinations must be allowed to develop their colour at the same temperature (± 0.05°C). Use a water bath or incubator if necessary.
D8.22	Measure the absorbance of each solution at 655 ± 2 nm (note o) in a cell of optical path length 10 mm against water in a reference cell. Let the absorbance be A_c units.	(o) The wavelength of maximum absorbance should be checked for each individual instrument and used for all subsequent measurements.
D8.23	Carry out a blank determination using the blank digest solution from step D8.12 and carrying it through steps D8.15 to D8.22. Let the absorbance measured be A_B units (note p).	(p) Typical blank absorbance values lie in the range 0.08 is 0.14. Higher values would probably indicate an unsatisfactory quality of reagents D5.2.1 or D5.2.3.
D8.24	Subtract the absorbance of the blank, A_B units, from the absorbance, A_c units, of each calibration solution and plot a graph of absorbance concentration of ammonia against nitrogen in the calibration solutions (mg N/L). This graph should be linear and should pass through the origin (note q).	(q) Having initially established the linearity of the calibration graph, it suffices thereafter to run only one calibration standard and its associated blank with each batch of analyses as a check on performance. See Section D11.
	Analysis of Samples	
D8.25	Carry each digest solution (from step D8.11) through steps D8.15 to D8.22. Let the absorbance measured be A_s units.	
D8.26	Calculate the absorbance due to ammonia nitrogen formed by digestion from Kjeldahl nitrogen), A_R units, from $A_R = A_s - A_B$	
D8.27	Read off the ammonia nitrogen concentration corresponding to A_R from the calibration graph (D8.24). This is equal to the Kjeldahl nitrogen concentration in mg N/L of the original sample.	

D9 Change in Concentration Range for the Method

D9.1 If greater sensitivity is required, 50 mL sample volumes may be used at step D8.1. The analytical procedure remains otherwise unchanged. The range of application will thus be restricted to less than 0.5 mg N/L and appropriate recallibration should be made.

D9.2 Outline of performance characteristics (for 50 mL samples) are given in Table D2.

Table D2(a)

Total standard Deviation on standard solutions:—	
Concentration mg N/L	Total standard deviation mg N/L
0.05	0.0077
0.50	0.0332
Limit of Detection: 0.026 mg N/L	
Sensitivity: 0.05 mg N/L gives an absorbance of about 0.50 units in 10 mm cells after correction for the blank.	

(a) Data from the Laboratory of the Government Chemist.

D9.3 Concentrations of greater than 1 mg N/L can be dealt with by taking a smaller sample volume. However, performance characteristics for this modification are lacking.

Table D3(a)

Substances	% Recovery
Asparagine	97
Glycine	79
Leucine	94
Proline	92
Tryptophan	100
Valine	89
Creatinine	72
2, 2'Dipyridyl	45
EDTA (disodium salt)	53
Guanidine carbonate	71
Hexamine	98
8-Hydroxyquinoline	79
Methyl orange	64
Nicotinic acid	62
p-Nitroaniline	44
Sulphamic acid	89
Thioacetamide	100
Urea	102

(a) Data fron the laboratory of the Government Chemist. Recovery was tested on solutions containing each substance at a concentration equivalent to 1 mg N/L.

D10 Recovery of Organic Nitrogen

The recovery of nitrogen, using this method, from a variety of organic nitrogen compounds has been investigated. Table D3 lists the percentage recoveries obtained, exemplifying the method's scope in respect of certain classes of compound which are 'refractory' in relation to the Kjeldahl digestion. However, if recovery from such compounds is regarded as critical, the analyst should conduct recovery trials to characterise more fully the method's performance.

D11 Sources of Errors

D11.1 Careful attention to the temperature of the heating block is vital. See the Appendix.

D11.2 Contamination of samples, reagents or apparatus with traces of ammonia or organic nitrogen must be avoided in this low-level method.

D11.3 Prolonged retention of the digest in an alkaline condition during neutralization must be avoided.

D12 Checking the Accuracy of the Analytical Result

The method given in Section B is considered to be the Reference Method because of its rigorous digestion conditions. Method D may not be able to digest all heterocyclic nitrogen compounds if these are present in the sample. Table D3 gives typical recovery data which should be taken into account when applying this method.

E Semi-automated Determination of Kjeldahl Nitrogen (Copper/Titanium Catalysed, Multiple Tube, Block Digestion Method followed by Distillation and Air Segmented Continuous Flow Colorimetry)

E1 Performance Characteristics of the Method

E1.1	Substance determined	Nitrogen in the trivalent state.
E1.2	Type of sample	Wastewaters and effluents.
E1.3	Basis of the method	Digestion of the sample with concentrated sulphuric acid in the presence of potassium sulphate and copper/titanium catalyst to convert nitrogen compounds present (see Sections A1 and B2) to ammonium sulphate. The ammonia content of the digest solution is determined by distillation followed by continuous flow colorimetry to enable the Kjeldahl nitrogen concentration of the sample to be calculated.
E1.4	Range of Application	Up to 12.5 mg N in the maximum sample aliquot.
E1.5	Calibration Curve (for the continuous flow ammonia chemistry)	Linear.

E1.6 Standard deviation

Sample	Concentration	Standard Deviation	Degrees of Freedom
	mg N/L	mg N/L	
Standard solution(a)	20	1.3(d)	3
Standard solution(a)	200	5.4(c)	12
Standard solution(b)	200	4.9(c)	10
Settled Sewage	37	1.3(d)	2
Settled sewage	86	1.1(d)	2
Laundry effluent	11	1.2(d)	2
Abbatoir effluent	180	1.6(d)	2

(a) Standard solution of nicotinic acid.
(b) Standard solution of ammonium chloride.
(c) Total standard deviation.
(d) Within-batch standard deviation.

E1.7	Limit of detection	0.85 mg N/L (estimated from between-batch standard deviations of blanks)[18].

E1.8 Time required for analysis — For 6 samples simultaneously in a heating block:

Procedure	Total time (h)	Operator time (h)
Total (and analysis)	ca.4	ca.1

The data presented in the above table were obtained by the Anglian Water Authority (Regional Standards Laboratory). The analysis of the digest solutions were carried out using Section F of this booklet.

E2 Principle

E2.1 Digestion of the sample

By hot digestion with a mixture of sulphuric acid, potassium sulphate and a copper/titanium catalyst, the nitrogen of many organic substances is converted to ammonium sulphate. For scope of the conversion see Section A1. This method is capable of achieving quantitative recovery from nicotinic acid. Inclusion of titanium minimizes risk of loss of ammonia by volatilization during digestion.

E2.2 Analysis of digestion solution

After steam distillation from the digest made alkaline, ammonia is determined using the automated procedure given in Section F and then related to the Kjeldahl nitrogen concentration of the original sample.

E3 Interferences

See Section B3.1.

E4 Hazards

E4.1 The digestion tubes may be susceptible to alkaline etching or pin-holing over a period of time. See Section B5.4.

E4.2 For other hazards, see Section B5.1 and B5.2.

E5 Reagents

Analytical reagent grade chemicals should be used except where stated otherwise.

E5.1 Water

The water used for blank determinations and for preparing digest solutions should have a nitrogen content that is negligible compared with the smallest concentrations to be determined in the samples. Water prepared as described in Section A3 will be suitable, but in most cases distilled or deionized water will also be acceptable.

E5.2 Digestion reagents

E5.2.1 Sulphuric acid (d_{20}1.84), low in nitrogen.

E5.2.2 Pumice stone Granular, 8 to 20 mesh, as anti-bumping granules.

E5.2.3 Digestion catalyst mixture

Thoroughly mix together 10.5 ± 0.5 g of titanium dioxide, 10.5 ± 0.5 g of copper sulphate, pentahydrate and 350 ± 5 g potassium sulphate. This mixture is stable indefinitely.

This mixture is available commercially in the form of tablets, two of which are required for each digestion.

E5.3 Distillation reagents

E5.3.1 0.5% m/v phenolphthalein indicator solution

Dissolve 0.50 ± 0.01 g of phenolphthalein in 60 ± 1 mL of industrial methylated spirits. Add 40 ± 1 mL of water. Mix well and store in a glass bottle. This solution is stable for at least three months.

E5.3.2 40% m/v sodium hydroxide solution

Cautiously dissolve 400 ± 20 g of sodium hydroxide pellets in about 800 mL of water in a 2-litre beaker immersed in cold water. Allow the solution to cool to room temperature and then dilute to 1 litre with water in a measuring cylinder. Store in a polyethylene bottle. This solution is stable indefinitely.

E5.3.3 20% v/v sulphuric acid solution

Add slowly and cautiously, with stirring, 20.0 ± 0.5 mL of sulphuric acid (d_{20}1.84) to 80 ± 1 mL of water in a 200 mL beaker immersed in cold water. Allow to cool and dilute with water to 100 ± 1 mL. Store in a glass bottle. This reagent is stable indefinitely.

E5.4 Reagents for the automatic determination of ammonia in distillates

See Section F5. Note that calibration standards must be prepared as described in Section F5.11.

E6 Apparatus

E6.1 Digestion

E6.1.1 A heating block suitably drilled to accommodate the digestion tubes (E6.1.2) which can be thermostatically controlled at a temperature sufficient to maintain the digest mixture at the boil under reflux conditions (see Appendix).

E6.1.2 Digestion tubes, 250 mL capacity. See also Section E4.2.

E6.1.3 Stand for digestion tubes.

E6.1.4 Filter funnels, borosilicate glass, 55 mm in diameter.

E6.1.5 Thermometer, glass, maximum temperature 480°C.

E6.1.6 Powder funnel, borosilicate glass, 55 mm in diameter, stem capable of accepting the thermometer (E.6.1.5).

E6.1.7 Fume cupboard fitted with an efficient extraction system capable of safely removing heavy toxic fumes that are generated during digestion. Also refer to Section E4.

E6.2 Distillation

Rapid distillation system. Various systems are available commercially. They involve steam distillation and are configured along the lines of the apparatus shown in Figure 2 (Section B7). The system must be operated according to the manufacturers' instructions. However, some units may generate significant amounts of ammonia in the steam from electrolyte solutions used in the steam boiler and some modification may need to be discussed with the manufacturers.

E6.3 Analysis of distillates

Air segmented continuous flow apparatus assembled as given in Section F6 using range Fa.

E7 Sample Collection and Preservation

As given in Section A2.

E8 Analytical Procedure

Read Hazards, Section E4, before starting this procedure.

Step	Procedure	Notes
	Digestion Procedure	
E8.1	Turn on the block digestor and set the thermostat control to give a block temperature of 250°C ± 10°C by the time the samples are ready for digestion. (note a)	(a) When first using this method, check that this temperature would not cause the tube contents to boil over at step E8.7. If it would, use a lower temperature at that step.

Step	Procedure	Notes
E8.2	Add an appropriate volume of sample (up to 50 mL) into a digestion tube (note b).	(b) The sample taken should contain no more than 12.5 mg N.
E8.3	Make up to 50 ± 2 mL with water.	
E8.4	Carefully add 10 ± 0.05 mL sulphuric acid down the side of the tube and swirl to mix well.	
E8.5	Add 5.40 ± 0.05 g of catalyst mixture (E5.2.3) or 2 catalyst tablets then 2 anti-bumping granules to each tube.	
E8.6	Repeat steps E8.2 to E8.5 for all samples and standards to be processed as one batch.	
E8.7	Place the tubes in the block digestor (note a) and raise the block temperature to 350 ± 10°C.	
E8.8	After 20 ± 5 mins alter the thermostat setting to give a block temperature of 440° ± 5°C (note c).	(c) The actual final temperature of the mixture should be 390 ± 10°C; the block temperature required to obtain this may depend on the particular block being used and on fume cupboard draught conditions (see Section B9.6, note e). Use the thermometer (E6.1.5) and power funnel (E6.1.6) to check digest temperature.
E8.9	Check the tubes periodically to determine when fuming occurs (thick, white fumes swirling around in the tube), and note the time (note d).	(d) Approx 15 mins after increasing the block temperature.
E8.10	After 40 ± 5 mins (from fuming) fit funnels, E6.1.4, stem downwards, into the tops of the tubs.	
E8.11	After a further 50 ± 5 mins (1½ hrs from fuming) remove all tubes from the block and allow them to cool for at least 10 mins in the stand.	
E8.12	Add 75 ± 5 mL deionized distilled water to each tube; the digests are then ready for distillation.	
	Distillation	
	NOTE. The following distillation procedure (steps E8.13 to E8.18) was used during performance testing using a Kjeltech rapid distillation apparatus).	
E8.13	Fit a tube containing a cooled diluted digestion mixture into the rapid distillation apparatus.	
E8.14	Place a 250 mL conical flask containing 15 ± 5 mL water, about 0.05 mL of 20% sulphuric acid (E5.3.3) and about 0.05 mL of phenolphthalein solution, beneath the collecting tube (note e).	(e) It has been found unnecessary to have the tip of the collecting tube beneath liquid surface, provided that acid conditions are maintained in the collection flask.
E8.15	Add 70 ± 10 mL of 40% (w/v) sodium hydroxide solution (E5.3.2) to the distillation tube.	

Step	Procedure	Notes
E8.16	Set the timer according to the equipment's instructions and start the distillation. Ensure that the distillate in the conical flask does not turn pink; if it does, swirl the solution well and, if necessary, add 20% sulphuric acid (E5.3.3) in 0.05 mL increments until the pink colour just disappears (note f).	(f) This careful addition of dilute acid is necessary because the pH is critical for auto analysis.
E8.17	After 5 mins distillation time (note g) lower the conical flask, containing the distillate, so that the delivery tube is clear of the liquid surface; leave for 15 secs for more distillate to rinse the tube, then switch off the steam.	(g) A longer distillation time, eg 7 mins, should be used if solid lumps of digestion mixture remain in the tube.
E8.18	Quantitatively transfer the distillate to a 250 mL calibrated flask and make up to the mark with water. Mix well. This solution is then ready for determination of ammonia. Determine the ammonia concentration (C_s mg N/L) using the automated procedure given in Section F.	
	Preparation of Blank	
E8.19	In place of the sample in step, E8.2 use 50 mL of water. Let the concentration of ammonia in the final diluted distillate be C_b mg N/L.	
	Calculation of Results	
E8.20	Blank corrected concentration of ammonia in the diluted distillate is $C_s - C_b$ mg N/L. Therefore the Kjeldahl nitrogen concentration in the original sample is $\frac{250\ (C_s - C_b)}{V}$ mg N/L. Where V (mL) is the volume of sample taken.	

E9 Sources of Error See Sections B11.1, B11.3 and F8.

E10 Checking the Accuracy of the Analytical Result See Section B13.

F Air-segmented Continuous Flow Colorimetric Analysis of Digest Solutions for Ammonia

Note: Except where modified to accommodate the relatively high acidity of the digest solutions, this method is similar to the S chemistry of method F of the 'Ammonia in Waters 1981' booklet in this series[8].

F1 Performance Characteristics of the Method

F1.1	Substance determined	Ammonia and ammonium ions.
F1.2	Type of sample	Digest solutions from Section B, D, E or C (see Section C5.3) procedures.
F1.3	Basis of the method	Air-segmented continuous flow colorimetry
F1.4	Range of Application	Fa: Up to 50 mg N/L (with dialysis). Fb: Up to 0.5 mg N/L (without dialysis).
F1.5	Calibration Curve	Fa: linear to 50 mg N/L. Fb: linear to 0.5 mg N/L.

F1.6 Total standard deviation on standard solutions of ammonia

Range	Concentration	Standard Deviation	Degrees of Freedom
	mg N/L	mg N/L	
Fa	20	0.3	19
Fa	30	0.3	19
Fa	40	0.5	19
Fb	0.1	0.007	9
Fb	0.4	0.038	9

F1.7	Limit of detection	Fa: 3 mg N/L. Fb: 0.009 mg N/L.
F1.8	Time required for analysis	For 40 sample digest solutions the analysis time is approx 2 h, 1 h of which is operator time.

The data presented in the above table were obtained jointly by the Severn Trent Water Authority (Coalport Analytical Laboratory), the Yorkshire Water Authority (Doncaster Laboratory) (Fa) and Thames Water Authority (New River Head Laboratories) (Fb). The limit of detection values were obtained visually from the recorder traces.

F2 Principle

The solution containing ammonium ions resulting from the digestion of nitrogen compounds present in the original sample is analysed automatically by the technique of air-segmented continuous flow colorimetry.

Ammonia (and hence total nitrogen in the original sample) is determined by forming an indophenol blue type compound using the reaction between the ammonia, hypochlorite and salicylate[8]. Calibration standards are prepared incorporating an amount of sulphuric acid corresponding to that used in digestion where direct analysis of the digests is prescribed (Methods C and D) (see Section A3.3). Analysis of distillates (Method B and E) requires calibration standards without added acid.

F3 Interferences

See Reference 8. Only the presence of substances which may remain in digests intended for direct analysis need be considered. No interferences will occur in the analysis of the distillates provided that their pH is controlled as described in the methods.

F4 Hazards

The precautions given in the essay review on continuous flow analysis[9] should be observed.

Normal laboratory safety precautions must be observed. Concentrated sulphuric acid (d_{20} 1.84) must be handled cautiously.

F5 Reagents and Standards

Analytical reagent grade chemicals are used except where stated otherwise.

F5.1 Water

The water used for blank determinations and for preparing reagent and standard solutions should have an ammonia content that is negligible compared with the smallest concentrations to be determined in the samples. Distilled or deionized water is usually suitable.

F5.2 Citrate reagent 1

Dissolve 40.0 ± 0.5 g of trisodium citrate and 34.0 ± 0.1 g of sodium hydroxide in about 800 mL of water in a 1 litre beaker. Dilute to 1000 ± 10 mL with water and mix well.

Stored in a glass or plastic bottle this reagent is stable for at least 3 weeks.

F5.3 Citrate reagent 2

Dissolve 40.0 ± 0.5 g of trisodium citrate in about 950 mL of water. Dilute to 1000 ± 10 mL with water and mix well. Stored in a glass or plastic bottle this reagent is stable for at least 3 weeks.

F5.4 Salicylate reagent

Dissolve 34.0 ± 0.5 g of sodium salicylate in about 500 mL of water in a 1 litre calibrated flask. Add 0.400 ± 0.005 g of sodium nitroprusside (ensure that the pH value of the salicylate solution is not greater than 8.0 before making this addition). Swirl to dissolve the solid and dilute to volume with water and mix well. Stored in an amber glass bottle this reagent is stable for at least 2 weeks.

F5.5 Sodium dichloroisocyanurate reagent

Dissolve 10.00 ± 0.05 g of sodium hydroxide in 500 ± 50 mL of water. Cool the solution to room temperature and add 0.800 ±0.008 g of sodium dichloroisocyanurate (dichloro-s-triazine 2, 4, 6 (1H, 3H, 5H) trione sodium salt). When dissolution is complete, quantitatively transfer the solution to a 1 litre calibrated flask, dilute to volume with water and mix well. Stored in an amber glass bottle between 1 and 5°C, this reagent is stable for at least 2 weeks.

F5.6 Salicylate/Citrate mixed reagent (for range Fb)

Dissolve 34.0 ± 0.5 g of sodium salicylate and 40.0 ± 0.5 g of trisodium citrate in about 950 mL of water contained in a 1 litre calibrated flask. Then add 0.400 ± 0.005 g of sodium nitroprusside. Swirl to disolve the solid and then make up to volume with water. Stored in an amber glass bottle, this reagent is stable for at least 2 weeks.

F5.7 10% v/v Sulphuric acid

Cautiously add with stirring 100 ± 1 mL sulphuric acid (d_{20} 1.84) to about 800 mL of water in a 2 litre beaker immersed in cold water. Allow to cool and dilute with water to 1000 ± 10 mL. Stored in a glass or plastic bottle this solution is stable for at least 3 months.

F5.8 1.5% v/v Sulphuric acid solution

Cautiously add with stirring 15.0 ± 0.5 mL of sulphuric acid (d_{20} 1.84) to about 800 ml of water in a 2 litre beaker immersed in cold water. Allow to cool and dilute with water to 1000 ± 10 mL. Stored in a glass or plastic bottle this solution is stable for at least 3 months.

F5.9 Ammonia calibration standard solutions for range Fa

Add 15 ± 0.5 mL of 10% v/v sulphuric acid solution (F5.7) to each of six 100 mL calibrated flasks. Add 5.00, 10.00, 20.00, 30.00, 40.00 and 50.00 mL of ammonia standard solution B (A3.3) into the series of flasks and dilute to volume with water. Mix well. These solutions contain ammonia at concentrations of 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mg N/L respectively. These calibration standard solutions are stable for at least one week.

F5.10 Ammonia calibration standard solutions for range Fb

Add 15 ± 0.5 mL of 10% v/v sulphuric acid solution (F5.7) to each of six 100 mL calibrated flasks. Add 1.00, 2.00, 3.00, 4.00 and 5.00 mL of ammonia working standard solution (A3.4) into the series of flasks and dilute to volume with water.

Mix well. These solutions contain ammonia at concentrations of 0.10, 0.20, 0.30, 0.40 and 0.50 mg N/L respectively. These calibration standard solutions are stable for at least one week.

F5.11 Ammonia calibration standard solutions for use in the analysis of distillates from methods B and E

Prepare as described in F5.9 (or, exceptionally, F5.10) but **omitting** the addition of sulphuric acid.

F6 Apparatus

The following apparatus which is set out in Figures 3 (Range Fa) or 4 (Range Fb) is required:

Sample presentation unit (sampler).

Multichannel peristaltic pump.

Analytical cartridge, incorporating pump tubes, mixing coils and/or delay coils and dialyser unit.

Detector (colorimeter or spectrophotometer), which houses a flow-through cell of path length 15 mm.

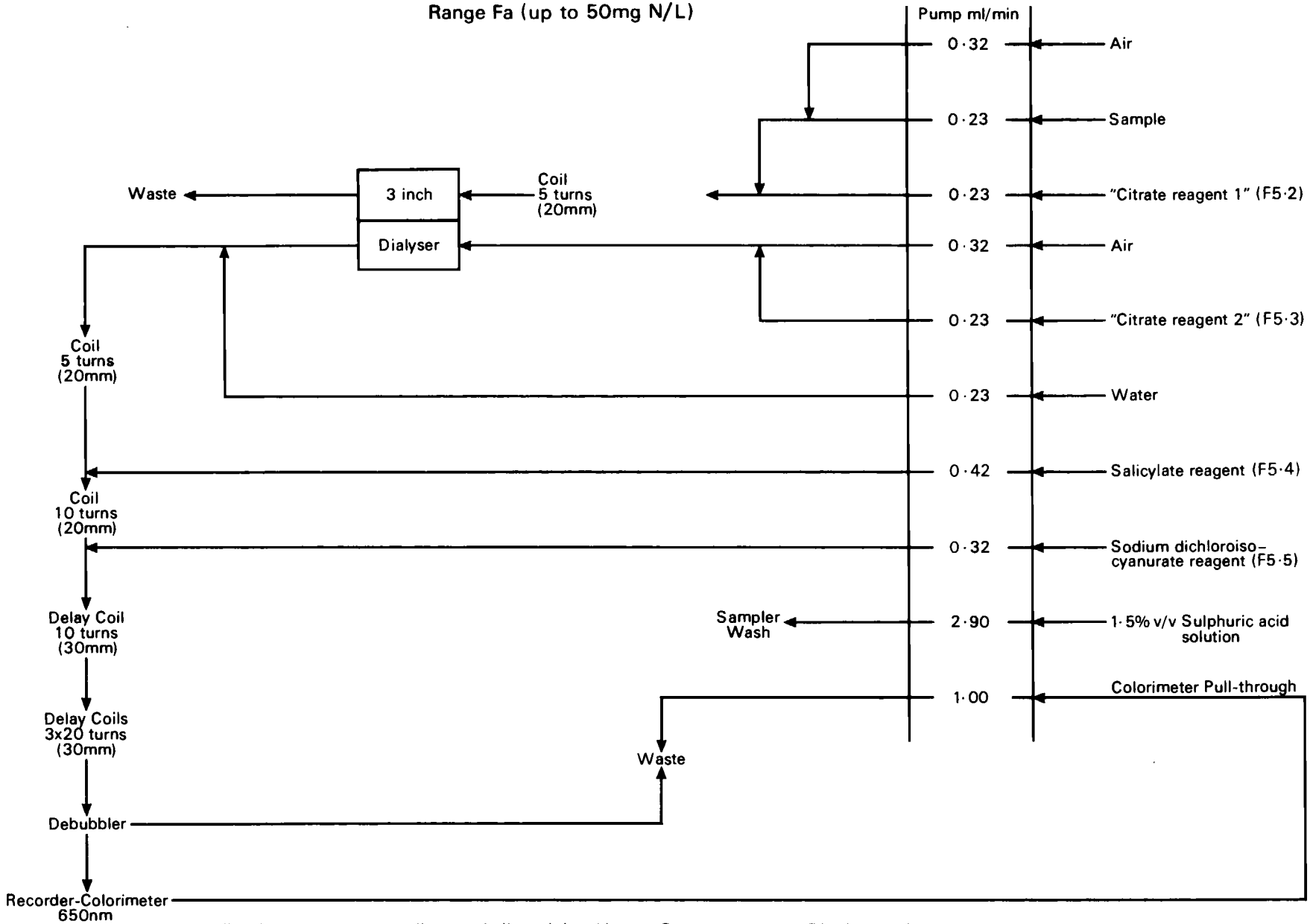
Measurement unit (recorder or printer).

Consult the essay review[9] on continuous flow analysis for further information.

F7 Analytical Procedure

Step	Procedure	Notes
	Starting operating	
F7.1	Connect the system as shown in Figures 3 (Range Fa) or 4 (Range Fb) as appropriate (notes a and b).	(a) Follow the manufacturer's general operating instructions. (b) See reference 9.
F7.2	With the sample probe at rest in the wash receptacle solution, place all the reagent lines in their respective reagents (note c). Start pump and switch on detector and measurement unit (note d).	(c) Ensure that there is sufficient of each reagent to avoid 'topping up' during one batch of analysis. (d) Allow the system to equilibrate for at least 20 minutes and during this period check that the bubble pattern and hydraulic behaviour of the system is satisfactory. If not eliminate difficulties before proceeding to step F7.3.

Figure 3 AUTOMATED AMMONIA LINE (FLOW) DIAGRAM

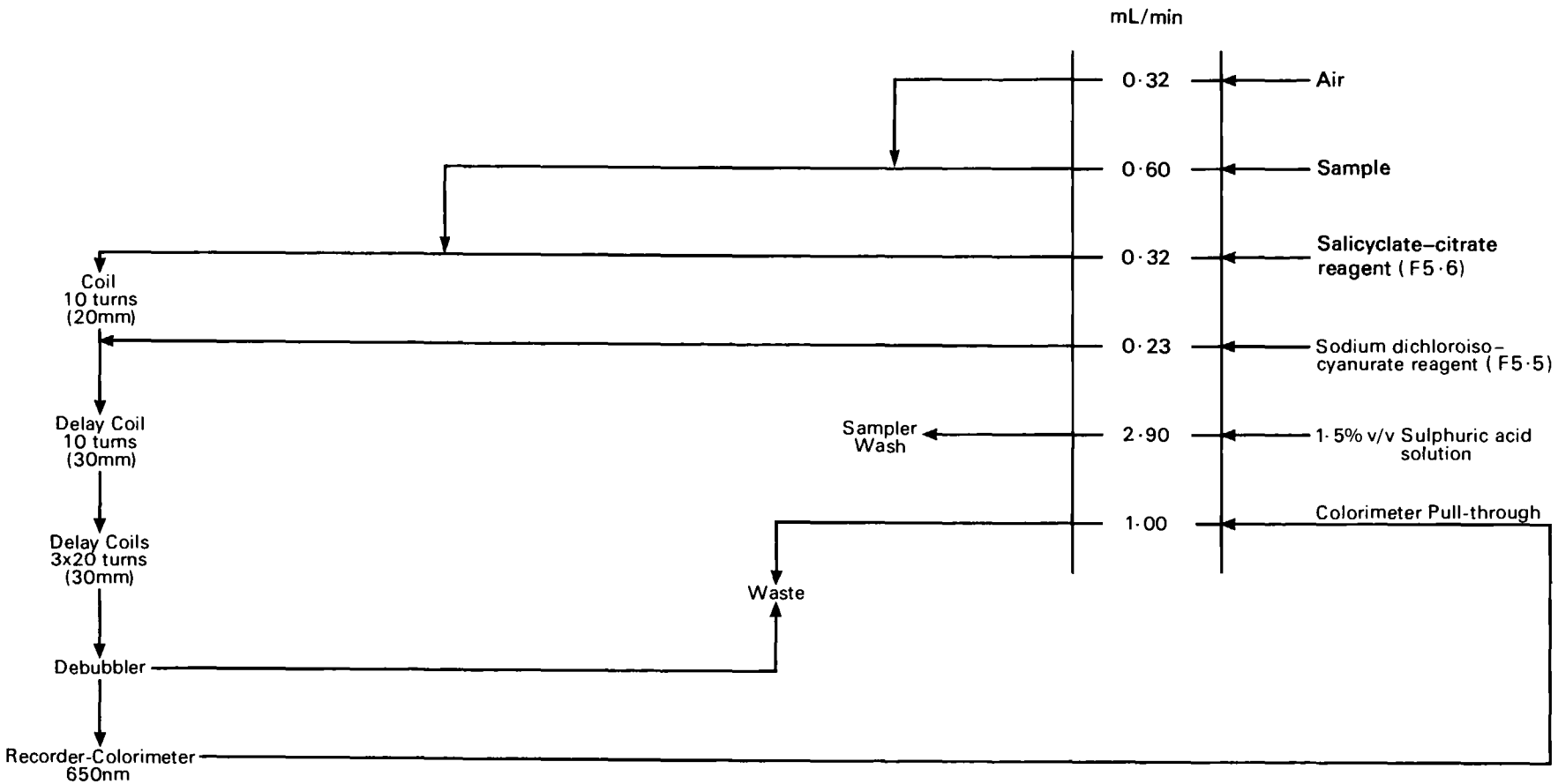


All coils 2mm bore, mean diameter indicated thus ( ) Sample wash ratio 70:30 seconds

Figure 4

AUTOMATED AMMONIA LINE (FLOW) DIAGRAM

Range Fb (up to 0·5mg N/L)



All coils 2mm bore, mean diameter indicated thus ( ) Sample wash ratio 60:30 seconds

Step	Procedure	Notes
	Initial Sensitivity Setting	
F7.3	When an acceptably smooth baseline trace is given on the measurement unit, adjust the baseline response to about 5 per cent of full scale (note e) and then transfer the sample probe into a C_M standard solution (note f).	(e) An elevated setting of the baseline allows for any negative drift that may occur. (f) C_M is the greatest concentration that the calibration is intended to cover.
F7.4	When there is a positive stable response at the measurement unit due to the colour produced from the C_M standard solution (note g), adjust this response to read between 90 and 95 per cent of full scale (notes h and i).	(g) The sample probe need only remain in the C_M standard solution for sufficient time to give a stable reading. (h) A setting 5 to 10 per cent below full scale allows for any increase in sensitivity that may occur. (i) This may be directly possible on some measurement units but others may require range expansion facilities.
F7.5	Return the sample probe to rest in the wash position (note j).	(j) First remove any traces of C_M standard solution from the outside of the sample probe.
	Analysis of Samples	
F7.6	Load the sample turntable in the following order (notes k and l).	(k) The turntable can be loaded during the initial stabilization period (steps F7.2 to F7.4). (l) Alternative arrangements of solution on the turntable are discussed in Reference 9.
	Position No on turntable/Solution 1–6 Calibration standards in ascending order 7–8 Blank (note m) 9–18 Samples (note n) 19 Calibration standard (note o) 20–21 Blank (note m) 22–31 Samples (note n) 32 Calibration standard (note o) 33–34 Blank (note m) 35 Calibration standard (note o)	(m) Blank digest solution. (n) A control standard should occupy one of the sample positions as a check of system control (see Section F9). (o) The standard which occupies position No 5 to check the calibration.
	Repeat the sequence 7–21 until all the samples have been processed (notes p and q).	(p) If cross contamination occurs between two samples (visible on the measurement units trace as incomplete separation of consecutive sample responses) both samples are reanalysed, perhaps in reverse order, separated by a blank solution.
F7.7	When a steady baseline is obtained on the measurement unit (after step F7.5) readjust the baseline to about 5 per cent of full scale if necessary and start the sampling unit.	(q) The complete calibration may be checked at the end of the analytical batch if necessary.
F7.8	When all the system responses due to the processed solutions have appeared on the measurement unit and a final baseline has been obtained, this unit can be switched off.	

Step	Procedure	Notes
	Calculation of Results	
F7.9	Plot a calibration curve of measurement unit responses (y axis) against concentration of standard solutions (x axis) (note r).	(r) The blank corrected responses of the calibration standard analysed at the end of each group of samples and those at the end of the turntable (if used) should be acceptably close to their respective blank corrected initial calibration standard responses. If not, refer to reference 9 for suggested procedures to obtain calibration curves. Note: Providing the linearity of calibration is acceptable to the analyst, a single point calibration might suffice.
F7.10	Using the calibration curve or single point calibration convert the measurement unit responses due to the samples into concentrations of ammonia in the samples (note s).	(s) The measurement unit responses of the samples must first be corrected for any baseline and sensitivity changes. The ammonia results are expressed as mg N/L.
	Shut-down Procedure	
F7.11	Transfer all reagent lines to water and continue to pump for at least 15 minutes. Switch off pump and detection unit.	

F8 Sources of Error

Refer to Reference 9.

F9 Checking the Accuracy of the Analytical Result

Once the method has been put into normal routine operation many factors may subsequently adversely affect the accuracy of the analytical results. It is recommended that experimental tests to check certain sources of inaccuracy should be made regularly. Many types of tests are possible and they should be used as appropriate. As a minimum, however, it is suggested that a standard solution is analysed at the same time and in exactly the same way as normal samples. This standard solution, whose concentration is 90 per cent of the highest concentration that the calibration is intended to cover, is termed the quality control standard [see Section E7.6 (note n)]. It is prepared from a different stock standard solution to that used for preparing the calibration standards, but which has been stored under identical conditions.

The measured concentration of the quality control standard solution should then be plotted on a quality control chart which will facilitate detection of inadequate results, and will also allow the standard deviation of routine analytical results to be estimated.

Further information on accuracy of results can be found in Reference 10.

Appendix The Use of Block Digesters[25]

1 Introduction

Block digesters are finding increasing use in the analytical laboratory to digest samples and convert determinands of interest to a more determinable form. The most common reasons for the increase are that traditional digestion techniques tend to (i) consume much operator time, (ii) use a large area of bench space for multiple digestions and (iii) be hazardous. A block digester approach tends to reduce these three aspects of a digestion procedure. Also, it is claimed that experimental conditions are more controlled with the use of block digesters, resulting in an increase in precision and accuracy of data.

There are numerous factors relating to the use of block digesters, and, before describing them it is appropriate to define what is meant by the term BLOCK DIGESTER.

2 Description of a Block Digester

A block digester is a solid block of metal (usually aluminium) which is so drilled to hold securely a number of straight-sided, round-bottomed glass tubes in which the digestion of the samples takes place. The block is capable of being heated to high temperatures and is equipped with a heat supply which may be thermostatically controlled and time-programmed.

3 Related Factors

3.1 Block digesters of various sizes and different number of drilled holes are commercially available. The limiting factors are the required diameter of the holes and the proviso that the solid fraction of the block surrounding each hole is uniform, capable of retaining uniform heat to ensure that digestion of the sample is complete, independent of the position in the block. Drilled holes on the perimeter of the block tend not to conform with the proviso. This non-conformity is discussed in Section 3.5.

The wide choice of block digester in terms of the number of holes and hence the number of samples that can be digested simultaneously, would suggest that the purchase of a block digester does not need to be justified by the number of samples to be handled, as automation usually has to be. Currently, 6, 12, 16, 20, 24, 35, 36 and 40-holed block digesters are known to be in use and there seems no reason why an appropriate solid block of metal should not be purchased and drilled according to individual requirements.

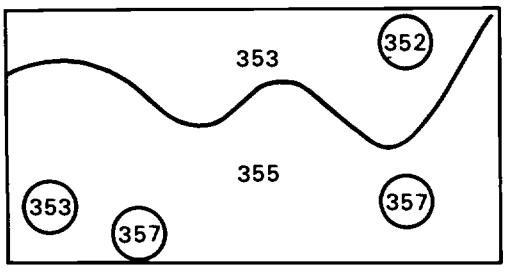
3.2 Most sizes of heating block, as purchased, may either be secured to the surrounding case or be removable. Blocks of the latter type may be interchangeable. There are potential advantages with both arrangements of design and the operator must decide which design is best for his particular application and required accuracy of result. The following design features of both types of block digester are given for the guidance of the potential user.

3.2.1 Secured heating block

The disadvantages are obvious, but a distinct advantage is an increased guarantee that heat is uniformly distributed throughout the block. With a secured heating block, a minimum air-space between block and the case containing the heating elements is assured. In some designs the heating element(s) protrude into the lower quarter of the block. An experiment to determine the temperature variation across a secured heating block has been described[25]. The block measured (in inches) approximately $11 \times 8 \times 7.5$ high and contained 40 drilled holes. A thermocouple was suspended in an empty digestion tube in each of the 40 holes in sequence for a fixed period of time. A mean temperature value of 355 °C was recorded with a standard deviation at that temperature of 1 °C. A temperature profile of the experiment is given in Fig 5. Two

temperature zones were obtained at 353 °C and 355 °C. Only four hole positions were at variance as shown. This variance, however, did not affect the precision of result.

Figure 5 Diagram of measured temperature values (°C)



3.2.2 Removable heating block

Advantages

Subject to constant dimensions of the surrounding heater cases obtained from one manufacturer, the following options (a) to (f) may all be applicable to a block digester design which allows for the block to be removed from the heater case.

(a) One block may be replaced by another of identical dimensions.

(b) With identical dimensions of block, a 6-holed block for example may be interchanged with a 12-holed block.

(c) A single block may be replaced by two or more blocks whose combined dimensions are the same as the single block which they are replacing.

(d) Two or more blocks can have different numbers of drilled holes per block.

(e) Two or more blocks can have the same number of drilled holes but with different diameters.

(f) An undrilled block may be used to complete the total block volume available within the heater case.

Disadvantages

The possibility of not being able to secure a uniform distribution of heat across the whole volume of the available block space may result from any of the following.

(a) The air-space between a single block and the heater case must be sufficient to enable ease of removal of the block and may lead to excessive heat losses.

(b) If one block is replaced by two or more smaller blocks, the additional air-spaces may result in further heat losses.

(c) The temperature profile across the total block volume within the heater case may be affected by the precision of manufacture of the blocks, particularly if two or more small blocks are used in one heater case. Where multiple blocks are used it may prove troublesome if, before routine use, block temperatures are not measured at all possible configurations within the heater case and if the blocks are not then coded with regard to configuration to give desired temperatures.

(d) Temperature monitoring itself may prove more troublesome with the removable block design of block digester where multiple blocks are used. Each block should have its own provision for emplacement of a thermometer or other temperature measuring device.

3.3 Thermostat Control and Programming

These controls are either located on the front panel of the heater case or they are incorporated in a separate control unit.

In general block digesters with only a manual thermostat control have the control mechanism and the temperature display on the front panel of the heater case.

Block digesters which have, in addition, temperature programmable facilities, usually have all the electronics incorporated into a separate control unit.

Both arrangements have their merits but it is essential that electronics should always be protected from potential liquid spillages.

3.4 Confirming the Temperature of the Block

Some temperature adjustment controls have a temperature display whereas others are simply numbered. Even with temperature displays there appears to be no guarantee that the actual temperature of the block is the temperature set by the operator. Initial checks for all block digesters should be made to confirm temperature dial settings or to assess the deviation from the setting. The following procedure for measuring the temperature of the block is suggested.

3.4.1 Procedure for measuring the temperature of the block

1. Select a temperature setting or number on the display.
2. Fill each drilled hole with fine, dry sand.
3. Switch on the heating block.
4. Place a thermometer (or calibrated thermocouple) into the centre of the sand at the mid-depth of a drilled hole in a central position on the block. Keep the thermometer upright.
5. Leave thermometer in-situ until the temperature is observed not to change by more than one degree (or unit) in one minute.
6. Confirm step 5 for a further one minute and record temperature.
7. Repeat steps 4 to 6 for each drilled hole.
8. Repeat steps 1 to 7 for other temperature or numbered settings on the display.

The above procedure may be inconvenient and more precise than is required. Two modifications are offered as possible suitable alternatives:

(a) At stage 2 in the above procedure read: 'Place an appropriate digestion tube into each drilled hole'.

At stage 4 in the above procedure read: 'Place the thermometer into the tube and hold in an upright position such that the bulb of the thermometer is just above the base of the tube'.

(b) At stage 2 repeat (a) above.

At stage 4 read: 'Place a thermocouple between the tube and the side of the drilled hole such that the tip rests between mid-depth and base of the drilled hole'.

Having ascertained the relationship between measured temperature and temperature control setting by either of the previously listed procedures or any other chosen procedure, it is then convenient to refer to the temperature control setting to identify the operating temperature.

3.4.2 Procedure for measuring digest temperatures

The fit of the tube to the block and the height of the liquid in the tube may all cause minor variations in the actual digestion temperature. For some digestions this may

affect the accuracy and precision of the results. If it is necessary to check this, run a set of typical digestions (not required for analysis) and measure the temperature of each with a pre-checked thermometer or sheathed thermocouple, being careful to immerse to the same depth in each tube. If the block contains anomalous holes only check positions being used.

3.5 Digestions at the Perimeter of the Block

On occasions, the operator may observe that there is a reduction in the volume of liquid in the digestion tubes placed in the central holes of the block, as compared with volumes in tubes in holes at its perimeter. The operator should then ascertain whether the reduction of liquid (reagents) results in an incomplete digestion of the sample(s). Ideally, this check is achieved by simultaneously digesting one standard sample at each drilled hole position and calculating any deviation in determinand concentration(s) relative to the position in the block. An exercise in which twelve samples of a solid material were each digested in duplicate in a 40-holed block digester, one in the centre and the other on the perimeter of the block, is detailed in Ref 25. The results of the exercise indicated that digestion was complete, independent of the position in the block.

3.6 Selection of Digestion Tubes

The following points are considered important for the selection of the glass tube used for digestion.

(a) The glass itself must be durable, resistant to high temperatures, have a low coefficient of expansion and of a thickness which allows a rapid transfer of heat to the solution contained within.

(b) Tubes are normally straight-sided and round-bottomed. They should fit snugly into the drilled hole yet must be easily removable.

(c) Invariably, the digested sample is subsequently analysed as a solution. Calibrated digestion tubes are an advantage in the preparation of this solution.

(d) The overall height of the tube is also important. The level of the liquid in the digestion tube should not protrude above the top of the heating block and the remaining height should be such that the air space above the level of the liquid is sufficient to satisfactory reflux of the liquid without loss.

(e) Enquiries to suppliers of digestion tubes regarding guarantees of tolerances of manufacture have revealed a reluctance to supply such a guarantee. To some extent this attitude is understandable but naturally it makes the user's life more difficult. However, a few glass-blowing companies will guarantee tolerance for their manufacture, but it is emphasised that the potential user of the tubes should produce the specification of dimensions and liaise closely with the manufacturer. An increased precision of manufacture is usually associated with increased costs, although, with small, local companies, on a tube to tube basis compared to the tubes offered by the manufacturer of the block digester, the price is usually lower even with the 'increase'. Screw-capped culture tubes, bought in bulk, have been used for small-sale block digestion. It is worth bringing to the attention of potential purchasers that experience has shown as high as 20% rejection on the grounds of inappropriate dimensions.

3.7 Safety Precautions

3.7.1 Removal of toxic fumes

The digestion procedures may result in the generation of toxic fumes. The need for an efficient extraction system to remove the fumes cannot be over emphasized. The operator should consider a custom-built extraction hood which is conveniently seated on top of a collection of digestion tubes in the block. The extraction hood may then be connected at its distal end to a suitable fume removal unit. Advice on extraction systems of this type is usually available from manufacturers.

3.7.2 Safety screens

It is highly recommended that a safety screen is placed between block digester and operator.

3.7.3 Tube failure and spillage

Digestion tubes may crack, or the control mechanism may malfunction and the temperature of the block increase unexpectedly. There is great danger to the operator if digestion reagents come into contact with a heated block. Switch off at the mains before touching the block at all or attempting to remove undamaged tubes. After such an event, the electronics should be thoroughly inspected by a qualified electrician for damage. The drilled holes should be thoroughly cleaned with copious amounts of water or very dilute alkali solution using pads of tissue paper soaked in water or dilute alkali.

References

(1) Kjeldahl J, *Z Anal Chem*, 1883, **22**, 66.

(2) Morries P, 'A Century of Kjeldahl', *J Assoc Publ Analysts*, 1983, **21**, 53.

(3) Thornburn Burns D, *Anal Proc*, 1984, **21**, 210.

(4) Gasser J K R, *Analyst*, 1963, **88**, 237.

(5) Ogg C L and Willits C O, *J A O A C*, 1950, **33**, 100.

(6) APHA-AWWA-WPCF. *Standard Methods for the Examination of Water and Waste Water. 15 Edition* 1980.

(7) Middleton G and Stuckey R E, *J Pharmacy and Pharmacol*, 1951, 829.

(8) *Ammonia in Waters, 1980.* Methods for the Examination of Waters and Associated Materials. London HMSO.

(9) Petts K W. *Air Segmented Continuous Flow Automatic Analysis in the Laboratory, 1979.* Methods for the Examination of Waters and Associated Materials. London HMSO, 1979.

(10) *General Principles of Sampling and Accuracy of Results, 1980.* Methods for the Examination of Waters and Associated Materials. London HMSO.

(11) *Chloride in Waters, Sewage, Effluents and Solutions 1981.* Methods for the Examination of Waters and Associated Materials. London HMSO.

(12) *Official Methods of Analysis of the Association of Official Analytical Chemists. 13th Edition*, 1980, p15.

(13) *Methods of Quantitative Micro-Analysis.* Wilton R F and Waters W A, (Editors), 2nd Edition, 1955, p83.

(14) *Oxidized Nitrogen in Waters, 1981.* Methods for the Examination of Waters and Associated Materials. London HMSO.

(15) International Organization for Standardization. TC 147/SC2, work item 101.

(16) Ogg C L and Willits C O. *J A O A C*, 1949, **32**, 118.

(17) International Organization for Standardization. *International Standard* ISO 5663–1984 (E).

(18) Croll B T, Tomlinson T and Whitfield C R W, *Analyst*, 1985, **110**, 861.

(19) Deal E and Petts K W, *Water Research Centre report 7-S* The Determination of Kjeldahl Nitrogen in Sewage Samples: A Block Digestion Technique followed by Autoanalysis, November 1980.

(20) Smart M M, Reid F A and Jones J R, *Water Res.*, 1981, **15**, 919.

(21) Hatfall, R S, *Chem Br*, 1987, **24**, 431

(22) Ebina J, Tsuyoshi T and Toyozo S, *Water Res.*, 1983, **17**, 1721

(23) Atkinson, G F, *Chem Br.*, 1987 **24**, 434

(24) *Total Nitrogen and Total Phosphorus in Sewage Sludge 1985.* Methods for the Examination of Waters and Associated Materials. London HMSO

(25) Notes for the use of Block Digesters in chemical analysis. K W Petts, Water Research Centre Laboratory Record No. 264-S, 1984.

Address for Correspondence

However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this booklet are requested to write to:

The Secretary
The Standing Committee of Analysts
The Department of the Environment
43 Marsham Street
London SW1P 3PY
England

Printed in the United Kingdom for Her Majesty's Stationery Office
Dd290884 8/88 C10 G443 10170

Department of the Environment

Standing Committee of Analysts

This method took a long time in preparation and testing. It was one of the methods proposed right at the start in 1973. Hence the number of people concerned with its preparation is large. Main Committee members listed are those serving at times when that Committee was consulted.

P Adams	2
G W Ashley	3
H T Barnhoorn	1
Dr G I Barrow	1
F B Basketter	1
D Bennet	2
Dr G A Best	1
D E Bond	1 (deceased)
J Borland	1
Dr J M Carter	1
P A Chave	1
Dr G W Clayfield	1
B E P Clement	1
Dr R L Cooper	1
Dr B T Croll	1
Dr J Dadswell	1
Dr J A W Dalziel	2
J A Davies	1
T A Dick	1
J W R Dutton	1
Dr G E Eden	1
M C Finniear	1
A R Folkard	2
I Fox	2
Dr I Gardiner	1
Dr J Gardiner	1
D Gelsthorpe	2.3
G I Goodfellow	1.2
Dr K Goodhead	1
T R Graham	1
L Hancock	1
Dr N Harkness	1
I Harper	1
W Hepple	3
E Hodges	1
G J Holland	1
A S Holmes	2.3
Dr A J Howard	1
Dr D T E Hunt	1
J S Leahy	1
W M Lewis	1
P J Long	1
F M Longmaid	3
R Mackinson	2
D Mealor	2
J C McCullins	1
Dr D S McIntyre	2
P J Morries	1.2.3
Dr R Mounce	2
C C Musselwhile	2.3
D Myles	1.2.3 (deceased)
A H Neild	1
Dr H A Painter	1
Dr A T Palin	1
Dr S J Patterson	1.2
K W Petts	2.3
L R Pittwell	1.2.3
Dr S R Porter	2
Dr J E Portman	1.2
L D Purdie	1
B D Ravenscroft	1
B Rhodes	1
L A Richards	1
Prof J P Riley	1
D Robinson	2
R Robinson	3
C C Scott	2
R Sinar	1 (deceased)
M Stockley	2.3
Dr D Taylor	1
Dr W H Thomas	3
Dr K C Thompson	1
Dr A M Ure	1
R J Vincent	1.2.3
B T Whitham	1
Dr D A Williams	1
A L Wilson	1 (deceased)
Dr R Wood (LGC)	1
Dr R Wood (MAFF)	1
D Woods	3

1 member of Main Committee
2 member of Working Group
3 member of Panel

HMSO publications are available from:

HMSO Publications Centre
(Mail and telephone orders only)
PO Box 276, London, SW8 5DT
Telephone orders 01-622 3316
General enquiries 01-211 5656
(queuing system in operation for both numbers)

HMSO Bookshops
49 High Holborn, London, WC1V 6HB 01-211 5656 (Counter service only)
258 Broad Street, Birmingham, B1 2HE 021-643 3740
Southey House, 33 Wine Street, Bristol, BS1 2BQ (0272) 264306
9–21 Princess Street, Manchester, M60 8AS 061-834 7201
80 Chichester Street, Belfast, BT1 4JY (0232) 238451
71 Lothian Road, Edinburgh, EH3 9AZ 031-228 4181

HMSO's Accredited Agents
(see Yellow Pages)

and through good booksellers

ISBN 0 11 752129 9