Urea in Waters 1984

Methods for the Examination of Waters and Associated Materials

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Contents

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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. Lone working, whether in the laboratory or field, should be discouraged. 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 manufacturer's catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' 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.

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. 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 radio-chemical 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. Known or suspected poisoning cases are usually sent to the nearest hospital having special equipment. To ensure admission to the correct hospital at once, always state whether poisoning is likely when calling on ambulance or arranging for an admission to hospital.

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.

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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 individual methods, 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 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 analytical chemist, biologist, bacteriologist etc. 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 seven 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
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological 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

31 October 1983

A General Information

A1 Introduction to the Methods

This booklet contains (i) a manual method based on the colorimetric determination of the ammonia formed by the enzymic action of urease and (ii) an automated procedure in which chlorination of urea is carried out under very slightly alkaline conditions and the colour developed by subsequent addition of phenol is measured.

Interference from heavy metals in the manual method is minimised by the addition of EDTA. Free and saline ammonia is determined prior to the addition of enzyme and the value thus obtained is subtracted from the total ammonium content after treatment. Alternatively, interference of either kind can be removed by passing the test solution through a column of mixed ion-exchange resins prior to the addition of urease.

Glycine and related amino acids interfere in the automated method, but this interference may be overcome by ion-exchange resin treatment prior to analysis.

The automated method can be used for the analysis of sea water provided steps are taken to counteract the probable effect of the presence of high concentrations of magnesium salts.

Note: Throughout this booklet, both urea and ammonia concentrations are expressed in terms of mg/l of nitrogen.

A2 Sample Collection and Preservation

A2.1 Equipment incorporating urea-formaldehyde plastics must not be used for sampling or storage purposes. Collect samples only in glass containers with glass stoppers.

A2.2 Samples should be analysed as soon as possible after sampling. If this is not possible, they may be stored at 4°C for up to 24 hours.

B Enzymic Spectrophotometric Method for the Determination of Urea in Water (Tentative Method)

B1 Performance Characteristics of the Method

B1.1	Substance Determined	Urea Nitrogen
B1.2	Type of Sample	Raw, Potable and Sea Waters
B1.3	Basis of Method	Urea is converted to ammonia by reaction with the enzyme urease and the resultant total ammonia concentration is determined spectrophotometrically.
B1.4	Range of Application	Up to 1.0 mg/l total urea/ammonia nitrogen using the maximum sample volume of 40 ml. The range can be extended upwards by taking a smaller sample volume.
B1.5	Calibration Curve	Linear up to at least 1 mg/l (with a maximum sample volume of 40 ml).

B1.6 Total Standard Deviation

a) Without using clean-up procedures:
(Section B7.1 and B7.2)

Sample Type	Concentration mg/l	Standard Deviation	Degrees of Freedom
Standard solution	0.100	0.004	9
Standard solution	1.000	0.008	9
Spike Borehole Water	0.507	0.009	9
Spiked River Water	0.489	0.017	9

b) Using clean-up procedure
(Section B7.1 and B7.2)

Sample Type	Concentration mg/l	Standard Deviation	Degrees of Freedom
Standard (0.1 mg/l)	0.117	0.015	9
Borehole Water Spiked with (1 mg/l)	1.02	0.036	9

c) Analysis of Sea Water

The Method, with insignificant minor variations, has been used successfully for the analysis of sea and estuarine waters. Thus at 0.19 mg/l urea a standard deviation of 0.0023 mg/l was obtained. Identical analytical curves were obtained for analysis of sea waters by both this method and the sea water variant of method D with a standard deviation over the range 0.015–0.15 mg/l of 0.0056 mg/l for the calibration points (7).

B1.7 Limit of Detection

(a) (Without using clean-up procedures)
Dependent on the concentration of ammonia in the sample, but typically not less than 0.02 mg/l

(b) (Using clean-up)
0.05 mg/l (9 degrees of freedom)

B1.8 Sensitivity

(a) 0.75 mg/l gives an absorbance of approximately 0.65 units using the maximum 40 ml sample size.

(b) 1 mg/l gives an absorbance of approximately 0.70 units using the maximum 40 ml sample size.

B1.9 Typical Blank Value

(a) (without using clean-up procedures)
0.16 absorbance units

(b) (using clean-up procedures)
0.06 absorbance units

B1.10 Interferences See section B3.

B1.11 Time required for analysis

(a) (without using clean-up procedures)
3 hours total analytical time for 9 samples.
1.5 to 2 hours operator time.

(b) (using clean-up procedures)
6-7 hours total analytical time for 9 samples.
2 to 3 hours operator time.

(a) Data from Yorkshire Authority.

(b) Data from Laboratory of the Government Chemist.

B2 Principle

Urea is broken down by the enzyme urease to form ammonia and carbon dioxide. EDTA is present in the reaction to reduce the inhibitory effect on the urease of various metal ions which may be present in the sample (see section B3). The ammonia formed reacts with hypochlorite ions, generated in situ by the alkaline hydrolysis of sodium dichloroisocyanurate and with sodium salicylate at about pH 12.6, in the presence of sodium nitroprusside, to form a coloured compound (thought to be related to indophenol blue). The compound is blue, but appears green against the yellow colour of the reagent blank. Sodium citrate is used to mask possible interfering cations. The absorbance of the coloured compound is measured spectrophotometrically and related to the total ammonia concentration in the enzyme treated sample by means of a calibration curve. A separate ammonia determination is carried out on the sample without enzyme treatment and the urea nitrogen concentration is obtained by subtracting the preformed ammonia nitrogen concentration from the total urea nitrogen concentration after enzyme treatment. Alternatively, ammonia can be removed from the sample by ion-exchange. This is recommended if the concentration of ammonia is high compared to the concentration of urea.

B3 Interferences

B3.1 The effect of other substances on the activity of the urease is summarised in Table B1.

Table B1 Interference test data for the enzymic spectrophotometric method.

Other Substance (expressed in terms of substance in brackets)	Concentration of other substance mg/l	Breakdown of Urea at 0.5 mg/l (%)
Lead Nitrate (Pb)	414	1.2
	41.4	101
	4.1	100
Cupric chloride (Cu)	635	14
	6.35	97
	0.635	101
Mercuric chloride (Hg)	1.0	21
	0.12	81
	0.012	91

Other Substance (expressed in terms of substance in brackets)	Concentration of other substance mg/l	Breakdown of Urea at 0.5 mg/l (%)
Silver nitrate (Ag)	1.0	1
	0.76	2.0
	0.076	45
Cadmium chloride (Cd)	338	54
	33.8	86
	3.38	99
Zinc chloride (Zn)	10	100
Ferrous sulphate (Fe)	10	105
Calcium chloride (Ca)	1000	45
	400	60
	200	66
	100	76
	50	79
Calcium nitrate (Ca)	1000	12
	400	34
	200	50
	100	64
	50	74
Sodium chloride (Na)	1000	97
Sodium nitrate (Na)	1000	100
Magnesium chloride (Mg)	100	103
Magnesium nitrate (Mg)	100	104
Sodium cyanide (CN)	0.5	99

If no interferences were present, breakdown of urea at this concentration would be expected to lie between 96 and 104% (95% confidence level).

If any of the above are present in sufficient quantity to cause inhibition, they can be removed by using the clean-up procedure, Section B7.1 – B7.2. This has the additional effect of removing any amino acids or ammoniacal nitrogen which may be present in the sample.

The efficiency of the removal of some of these substances is given in Table B2.

Table B2 Efficiency of Interference Removal Procedure

Other Substance (expressed in terms of substance in brackets)			Concentration of other substance mg/l	Removal of other substance (%)
Ammonium chloride	(N)	(a)	1	99
			5	99
			10	99
			15	99
			20	99
			25	99
Zinc chloride	(Zn)	(a)	10	91 – 99
Cupric chloride	(Cn)	(a)	10	89 – 99
Lead nitrate	(Pb)	(a)	10	92 – 99
Ferrous sulphate	(Fe)	(a)	10	84 – 99
Cadmium chloride	(Cd)	(a)	10	95 – 99
Silver nitrate	(Ag)	(b)	1	97
Mercuric chloride	(Hg)	(b)	1	81
Sodium cyanide	(CN-)	(b)	1	107
Sodium hypochlorite	(OCl-)	(b)	1	98
Calcium nitrate	(Ca)	(a)	1000	99

(a) Measured by chemical analysis of influent and eluant.

(b) Estimated from reduction of inhibition of urease.

B3.2 The effect of other substances on the determination of ammonia by this method is shown Table B3. The data were obtained by Thames Water Authority.

Table B3 Interference Effects

Other Substance (expressed in terms of substance in brackets)	Concentration of other substance (mg/l) in a 40 ml sample portion	Effect in mg/l of substance at an ammonia concentration (N) of		
		0.000 mg/l	0.200 mg/l	0.50 mg/l
Sodium Chloride (Cl^-)	1000	+0.002	+0.013	+0.03
Sodium Bicarbonate (HCO^-_3)	1000	+0.002	+0.002	-0.02
Sodium Orthophosphate ($PO_4\equiv$)	100	0.000	-0.001	-0.01
Sodium Sulphate ($SO_4^=$)	500	0.000	+0.001	—
Potassium Fluoride (F^-)	5	+0.002	-0.001	—
Potassium Nitrate (N)	50	+0.006	+0.002	—
Sodium Silicate (SiO_2)	50	0.003	0.000	—
Sodium Thiosulphate ($S_2O_3^=$)	10	-0.001	-0.007	—
Potassium Cyanide (CN^-)	5	+0.002	+0.019	+0.01
Calcium Chloride (Ca)	500	0.000	+0.013	-0.00
Magnesium Acetate (Mg)	50	+0.004	-0.009	+0.00
Iron (III) Sulphate (Fe)	10	+0.001	+0.003	—
Aluminium Sulphate (Cu)	5	0.000	+0.008	—
Copper Sulphate (Cu)	5	+0.003	+0.006	—
Zinc Sulphate (Zn)	5	+0.003	+0.006	—
Lead Acetate (Pb)	10	+0.001	+0.040	+0.01
Aniline ($C_6H_5NH_2$)	1	+0.040	+0.040	—
Ethanolamine ($C_2H_4OH\ NH_2$)	1	+0.164	+0.114	—

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

± 0.003 mg/l at 0.000 mg/l N

± 0.014 mg/l at 0.200 mg/l N

± 0.021 mg/l at 0.500 mg/l N

B4 Hazards

Normal precautions to avoid skin contact and/or ingestion should be taken in the handling of all reagents.

The sodium dichloroisocyanurate solution and particularly the solid reagent should not be allowed to come into contact with acid since this would result in the evolution of highly toxic chlorine gas.

B5 Reagents

(Analytical grade reagents should be used whenever possible. All water used in this method must be ammonia free).

B5.1 Ammonia-free water

Pass distilled water through a column of strongly acidic cation exchange resin (in the hydrogen form). Discard the first few litres of eluate. Store the subsequent eluate in a glass stoppered bottle. The column should be regenerated with hydrochloric acid solution (1 N) at a maximum interval of one month. After regeneration the column must be washed with distilled water until the eluate is no longer acid.

B5.2 Stock Urea Solution (1 ml contains 1 mg urea N)

Dissolve 2.14 ± 0.01 g urea (dried at 105°C for at least 2 hours) in about 800 ml ammonia-free water. Dilute to 1000 ml with ammonia-free water in a calibrated flask. If stored in a refrigerator, this solution is stable for at least a month.

B5.3 Stock Urea Solution (1 ml contains 10 μg urea N)

Pipette 10 ml of stock urea solution into a 1000 ml calibrated flask and make up to the mark with ammonia-free water. If stored in a refrigerator, this solution is stable for at least a week.

B5.4 Stock Standard Ammonia Solution A (1 ml contains 1 mg NH_3 as N)

Dissolve 3.819± 0.005 g ammonium chloride (dried at 105°C for at least 2 hours) in about 800 ml ammonia-free water. Dilute to 1000 ml with ammonia-free water in a calibrated flask. Store the solution in a stoppered glass bottle for not more than one week.

B5.5 Stock Standard Ammonia Solution B (1 ml contains 10 μg NH_3 as N)

Pipette 10 ml of stock standard ammonia solution A into a 1000 ml calibrated flask. Make up to volume with ammonia-free water and mix. Store the solution in a glass stoppered bottle for not more than one week.

B5.6 Cleaning Solution

Dissolve 100±2 g potassium hydroxide in 100±2 ml water. Cool the solution and add 900±50 ml industrial methylated spirits. Store in a polyethylene bottle. THIS REAGENT IS TOXIC.

B5.7 Ion Exchange Resin

A mixed bed resin is used consisting of equivalent amounts of a strong base anion exchanger and a strong acid cation exchanger. Some are described by manufacturers as being suitable for the purification of urea solutions.

B5.8 Buffered Urease Solution

Dissolve 0.5±0.05 g disodium ethylenediamine-tetraacetate and 0.02±0.005 g urease in about 35 ml ammonia-free water. Adjust the pH to 7.0 with a small amount of 2.5 N sodium hydroxide and make up to 50 ml with ammonia-free water in a measuring cylinder.

The urease used is the highly purified or crystalline form with activity of >50,000 units per gram. Less active urease may be used. But it may be necessary to take larger quantities and this may effect blank values. Check each batch of urease by analysing the most concentrated urea standard (prepared as in Section B8.1), using the procedure in steps B7.3 to B7.10, with calculation as prescribed in step B7.14. Quantitative recovery, within ± 5%, of urea should be obtained, otherwise discard the batch of enzyme.

Occasionally a batch of urease is found which, although sufficiently active, gives an unacceptably high ammonia blank value. If no fresh urease is available, such material has been purified by dialysis as follows. Prepare reagent B5.8. Prepare a large volume of a similar batch of reagent from which the urease has been omitted. Place the solution containing urease inside a dialysis membrane and suspend the membrane plus solution in a beaker containing some of the urease free solution. Stir this latter solution slowly. Periodically determine the ammonia content of both solutions. If they are close in ammonia content, replace the urease free solution by a fresh sample of urease free solution. Continue until the ammonia blank value of the urease containing solution is acceptably low. Re-check the urease activity.

B5.9 Salicylate Reagent

Dissolve 130±1 g sodium salicylate and 130±1 g trisodium citrate in about 950 ml ammonia-free water contained in a 1000 ml calibrated flask. Then add 0.970±0.005 g sodium nitroprusside (ensure that the pH value is not greater than 8.0 before making this addition). Swirl to dissolve the solid and then make up to volume with ammonia-free water. Stored in an amber glass bottle, this reagent is stable for at least 2 weeks.

B5.10 Sodium Dichloroisocyanurate Reagent (DIC)

Dissolve 32±0.1 g sodium hydroxide in 500±50 ml ammonia-free water. Cool the solution to room temperature and add 2.00±0.02 g sodium dichloroisocyanurate (dichloro-s-triazine/2, 4, 5 (1H, 3H, 5H) – trione sodium salt) to the solution. When solution is complete transfer to a 1000 ml calibrated flask. Make up to volume with ammonia-free water and mix well. Stored in an amber glass bottle at 4°C this reagent is stable for at least 2 weeks.

B6 Apparatus

In addition to normal laboratory apparatus.

B6.1 A Spectrophotometer – capable of operating at a wavelength of 655 nm and equipped with 10 mm path length cells – is required. Alternatively an absorptiometer with a filter having a transmittance maximum at about 655 nm can be used, but a reduction in sensitivity and precision of determination may result.

B6.2 A water bath capable of operating at 25±0.2°C is preferred but not essential. See section B7, note (c).

B6.3 Glass ion exchange column of 1 cm i.d.

B6.4 All apparatus coming into contact with samples or standards in this method should be free from ammonia. This is particularly important in the determination of low concentrations. All such apparatus should be cleaned using cleaning solution (B5.4), thoroughly rinsed in ammonia-free water and thereafter reserved solely for ammonia or urea determinations.

B7 Analytical Procedure

Step	Procedure	Notes
	Analysis of Samples	
B7.1	Add 5.00±0.01 g of resin (B5.5) in a slurry of water to the column (B6.4), taking care that it beds down evenly. (note a).	(a) This step may be omitted if no enzyme inhibitors (Section B3) are present. However, see step B7.12 et seq. If omitted start at step B7.3.
B7.2	Pass 210 ml of sample through the column at a flow of 1 ml/min. Discard the first 150 ml and collect the last 60 ml for analysis. (note b).	(b) Faster flow rates may result in poorer retention of other substances.
B7.3	Pipette up to 40 ml of the sample eluate from step B7.2 (volume V ml) into a 50 ml calibrated flask and make up to 40.0±0.5 ml with ammonia-free water if necessary. (note c).	(c) It is essential that aliquots less than 40 ml are diluted prior to adding the reagents.
B7.4	Add 1.0±0.1 ml buffered urease solution and mix well. Allow to react in a water bath at 25±0.20C. (note d).	(d) Other temperatures between 20°C and 30°C may be used but all calibrations and determinations should be carried out at the same temperature (within ±0.2°C).
B7.5	Remove the flask from the water after 10–12 minutes.	
B7.6	Add 4.00±0.05 ml of salicylate reagent and mix well.	
B7.7	Add 4.00±0.05 ml of DIC reagent and mix well.	
B7.8	Dilute with ammonia-free water to the mark, mix well, return to the water bath and develop the colour. (note d and e).	(e) The final absorbance decreases by 1.1% per °C when the colour development temperature is increased from 20°C to 30°C.

Step	Procedure	Notes
B7.9	Remove the flask from the water bath after 30–60 minutes and measure the absorbance of the solution at 655±2 nm (note f) in a cell of 10 mm pathlength against water in the reference cell. Let the absorbance be U_s, or T_s if steps B7.1 and B7.2 are omitted.	(f) The wavelength of maximum absorbance should be checked for each individual instrument. This wavelength should be used for all subsequent measurements.
	Determination of blank	
B7.10	Carry about 200 ml of ammonia-free water through steps B7.1 to B7.9 (note g), taking a sample aliquot of 40 ml at step B7.3. Let the absorbance measured in step B7.9 be U_b, or T_b, if steps B7.1 and B7.2 are omitted.	(g) If steps B7.1 and B7.2 are omitted in the analysis of samples, they are omitted here also, and from the preparation of the standards (B8.1.2).
	Calculation (when using ion exchange)	
B7.11	Calculate the absorbance due to urea nitrogen in the sample aliquot, U_r, from: $U_r = U_s - U_b$ Determine the mass of urea nitrogen, M_u µg, in the sample aliquot from U_r and the urea calibration curve (Section B8.1) The urea nitrogen concentration in the sample is given by: $\frac{M_u}{V}$ mg/l as N where V is the volume taken on step B7.3.	
	Separate determination of ammonia (for use when removal of enzyme inhibitors by ion exchange is not required)	
B7.12	Carry the sample through step B7.3 – taking the same volume V ml, for analysis as was used for urea determination – and steps B7.6 to B7.9. Let the measured absorbance be A_s.	
B7.13	Prepare a blank in the same manner described in step B7.12, using 40 ml of ammonia-free water in place of the sample. Let the measured absorbance be A_b.	
	Calculation (when ion exchange is omitted)	
B7.14	Calculate the absorbance due to ammonia nitrogen in the sample aliquot, A_r, from: $A_r = A_s - A_b$ Determine the mass of ammonia nitrogen, M_a µg, in the sample aliquot from A_r and the ammonia calibration curve (Section B8.2).	

Step	Procedure	Notes
	The ammonia nitrogen concentration in the sample, C_a, is given by:	
	$C_a = \frac{M_a}{V}$ mg/l N	
B7.15	Calculate the absorbance due to urea and ammonia nitrogen in the sample aliquot, T_r, from:	
	$T_r = T_s - T_b$	
	where T_s and T_b are the values obtained in steps B7.9 and B7.10 respectively (steps B7.1 and B7.2 having been omitted). Determine the mass of urea and ammonia nitrogen, M_t µg, in the sample aliquot from T_r and the ammonia calibration curve (Section B8.2). The total urea and ammonia nitrogen concentration in the sample C_t, is given by:	
	$C_t = \frac{M_t}{V}$ mg/l N	
	and the urea nitrogen concentration, C_u, is thus given by	
	$C_u = C_t - C_a$ mg/l N	

B8 Preparation of Calibration Curves

To be performed with each new batch of reagents

B8.1 Urea Calibration

B8.1.1. Add, using a burette, the volumes of standard solution B5.3 shown in the table below to a series of 500 ml calibrated flasks.

Volume of Solution B5.3 (ml)	Urea Nitrogen Concentration mg/l	Mass of Nitrogen (µg) for 40 ml
0.0	0.0	0
2.5	0.05	2
5.0	0.10	4
7.5	0.15	6
10.0	0.20	8
20.0	0.40	16
30.0	0.60	24
40.0	0.80	32
50.0	1.00	40

B8.1.2 Carry each solution through steps B7.1 to B7.10, taking 40 ml of the eluate from step B7.2 in step B7.3. If steps B7.1 and B7.2 have been omitted, see B7.10 note g.

B8.1.3 Subtract the absorbance of the blank solution from the absorbances for all the other standards and plot a calibration curve of mass of urea nitrogen in the calibration solution (given in the table above against absorbance). This should be linear and pass through the origin.

B8.2 Ammonia Calibration. (Not required if the clean-up procedure in steps B7.1 and B7.2 is used.)

B8.2.1 Dilute 25 ml of standard solution B5.5 to 250 ml with water in a calibrated flask. Then add, using a burette, volumes of the diluted solution to a series of 50 ml calibrated flasks, as shown in the table below:

Volume of Solution (ml)	Mass of N (μg)
0.0	0
2.0	2
4.0	4
6.0	6
8.0	8
10.0	10
20.0	20
30.0	30
40.0	40

B8.2.2 Add water to each flask to give a volume of 40±0.5 ml.

B8.2.3 Continue as under Procedure, steps B7.12 and B7.13.

B8.2.4 Subtract the absorbance of the blank solution from the absorbances for all the other standards and plot a calibration curve. This is normally linear and passes through the origin.

B9 Sources of Error

Apart from the usual possible manipulative errors inherent in any method, this enzyme method may be prone to errors arising from poor enzyme performance.

Therefore, it is recommended that, in addition to checks on enzyme efficiency with each new batch of urease (Section B5.8), each batch of analyses should incorporate the analysis of single portions of a typical sample and the same sample spiked with a known concentration of urea. A recovery control chart (1) can thus be established and significant deviation of recovery from 100% can be detected as an indication of unsatisfactory performance.

A check on spiking recovery from a real sample will also be a necessary first stage in deciding whether or not removal of interfering substances by means of the clean-up procedure will be required.

C

An Air-Segmented Continuous Flow Method for the Determination of Urea in Waters (Tentative Method)

Note: This method has not been evaluated for the determination of urea in salt water, but see Refs 2, 3.

C1 Performance Characteristics of the Method

C1.1	Substance determined:	Urea nitrogen
C1.2	Type of Sample:	Raw, waste and potable waters, but see Section C2.
C1.3	Basis of method:	Continuous flow colorimetry, using reactions described in Section C2.
C1.4	Range of application:	Up to 10 mg/l
C1.5	Calibration Curve:	Linear up to at least 10 mg/l

C1.6 Standard deviation (c) (within batch)

Sample Type	Urea Concn. (mg/l)	Std. Dev. (mg/l)	Degrees of Freedom
Standard solution (a)	0.2	0.013	9
Standard solution (a)	0.5	0.028	4
Standard solution (a)	1.0	0.045	9
Standard solution (a)	5.0	0.071	9
Standard solution (a)	10.0	0.024	4

C1.7	Limit of detection (b) (c):	0.03 mg/l
C1.8	Bias	None detected.
C1.9	Interference:	No commonly occurring substances interfere, but see Section C3.
C1.10	Sensitivity:	A 10.0 mg/l distilled water standard gives an absorbance of approximately 1.4 using a 10 mm flow-cell
C1.11	Time required for analysis:	The automated system described is operated at 30 determinations per hour. Set up and wash through times are 30 and 15 minutes (total respectively)

(a) Distilled water standards spiked with the stated quantity of urea. These standards were analysed at a rate of 30/h. However, the method can be operated at 40/h by which the following results were obtained for the within-batch standard deviation:

Urea Concentration mg/l	Standard deviation: mg/l	Degrees of freedom
0.2	0.043	9
1.0	0.078	5
5.0	0.266	9

(b) This figure was obtained visually from the recorder trace and was not calculated according to the method described in reference 1.

(c) Data from the Lea Conservancy Catchment Board (Waltham Cross)

C2 Principle

Although this method is intended for the analysis of non-saline waters, reference to its use as a manual method for sea-water analysis has been made (2, 3). The analyst should first check that this method is suitable if sea-water analysis is required.

The sample urea is chlorinated under very slightly alkaline conditions using sodium hypochlorite, sodium hypobromite and hydrochloric acid/magnesium chloride reagents. These reagents are added in quick succession in the order described in reference 3, and as shown in figure 1.

The purpose of the sodium hypobromite is to prevent interference by ammonia. The presence of magnesium chloride in the acid reagent is to increase the sensitivity of the method, and the potassium chloride and hydrogen peroxide are used to increase the rate of colour development.

After initial mixing in the single mixing coil, the pH value of the sample/reagent stream is raised reproducibly with the borate buffer to pH 9.4. The sample is then allowed to react with an aqueous methanolic solution of phenol to form a yellow compound which is measured spectrophotometrically. This yellow compound is thought to be related structurally to the blue indophenol-type compound formed between ammonia. hypochlorite and phenol (3).

The method is strongly pH sensitive; special care should be taken with the reagent preparation so that maximum sensitivity is obtained. The pH value of the mixed reagent stream issuing from the flow-cell should be in the range of 9.35 to 9.5. Initial sample pH values should be in the range 6-8 and adjusted accordingly with acid or alkali using a pH meter.

C3 Interference

The response of other substances on the determination of urea is listed in Table C1.

In addition, if it is required to determine urea in sea-waters, the analyst should be aware of the possible effects of high magnesium concentrations in such samples on the results obtained.

Table C2 shows the recoveries of urea from three types of sample. Samples containing between 0.03 mg/l to 0.13 mg/l urea were spiked with known concentrations of urea in the range of 0.10 to 1.0 mg/l urea. Recoveries ranged from 98.0 to 101.2%.

Table C1 Interference test data for automated urea method

Other substance (expressed in terms of substance in brackets)	Other substance Concentration mg/l	Effect in mg/l at 0.0 mg/l urea
Sodium silicate (SiO_2)	50	<0.03
Sodium chloride (Cl^-)	1000	<0.03
Sodium sulphate (SO_4^{2-})	1000	<0.03
Sodium fluoride (F^-)	100	<0.03
Sodium nitrate (NO_2^-)	50	<0.03
Sodium sulphide (S^{2-})	25	<0.03
Potassium chloride (K)	100	<0.03
Potassium nitrate (NO_3^-)	2000	<0.03
Potassium dihydrogen orthophosphate (PO_4^{3-})	40	<0.03
Calcium chloride (Ca)	200	<0.03
Calcium bicarbonate (HCO_3^-)	305	<0.03
Magnesium chloride (Mg)	50	<0.03
Boric acid (B)	100	<0.03
Ammonium chloride (N)	20	<0.03
Anionic detergent (as Manoxol O.T.)	100	<0.03
Glycine (N)	10	0.07
Synthetic river water containing:		
Na	115	
Ca	76	
Cl^-	64	<0.03
SO_4^{2-}	96	
HCO_3^-	305	
Mixed metal solution containing: iron, copper, zinc, lead, nickel, cadmium, chromium	10 of each metal	<0.03

If the other substance had no effect the result would be expected (95% confidence) to lie between: ±0.026 at 0.000 mg/N/l.

Data obtained by the Lea Conservancy Catchment Board (Waltham Cross).

Table C2 Recovery of Urea from spiked samples
(mean value of duplicate determinations)

Sample	Concentration of urea mg/l			% recovery of added urea
	Initial	Added	Found	
River water	0.05	0.10	0.15	100.0
Sewage works	0.13	0.50	0.63	100.0
effluent	0.13	1.0	1.11	98.0
Borehole water	<0.03	0.20	0.20	100.0
	<0.03	0.80	0.81	101.2

Data obtained by the Lea Conservancy Catchment Board (Waltham Cross)

C4.0 Hazards

C4.1 The precautions given in the essay review on continuous flow analysis should be observed (4).

C4.2 Sodium Hypochlorite, sodium hydroxide and sodium hypobromite are all caustic and should be handled with care, and any spillages washed away with copious amounts of cold water.

C4.3 Phenol is toxic by skin absorption. Gloves and eye protection should be used when handling it, or any reagent or effluent containing it.

C4.4 Methanol is a toxic, volatile and inflammable solvent and should be handled with care ensuring no naked flames are in the vicinity.

C4.5 Hydrogen peroxide causes temporary irritation and whitening of the skin and should be washed off immediately.

C4.6 Bromine is a toxic element with an irritating choking vapour. Gloves and eye protection should be used when handling it. In general, prepare all the reagents required by this method in a well-ventilated fume cupboard fitted with a water supply.

C5.0 Reagents and Standards

Analytical grade reagents should be used unless otherwise stated.

C5.1 Water

The water used for blank determinations and for the the preparation of reagents and standard solutions should have a urea content which is negligible compared with the smallest concentration of urea to be determined in samples. Distilled water is usually suitable.

C5.2 Sodium Hypochlorite, 2.5 – 3.5% m/V available chlorine

Dilute 250±10 ml of sodium hypochlorite solution (10 – 14% m/V available chlorine) to 1 litre with water in a glass stoppered graduated cylinder. Filter the solution and ensure that the pH value is in the range 12.1 to 12.2. Store in an amber glass bottle at a temperature between 1 and 5°C when not in use. This reagent is stable for one week.

Note that other commercially available sodium hypochlorite solutions, which may be more stable, may be preferable to the relatively unstable 10 – 14% m/V grade, providing that an available chlorine concentration of at least 2.5 m/V is available for the working solution.

C5.3 Sodium Hypobromite, 0.2% m/V (as OBr^-)

Dissolve 1.0±0.05g of sodium hydroxide in about 400 ml water contained in a 500 ml calibrated flask. Carefully add, using an all-glass syringe, 0.375±0.005 ml of bromine to the flask. Stir the solution with a magnetic stirrer until all the bromine has dissolved. Check that the pH value is in the range 12.3 – 12.4. Store in an amber glass bottle between 1 and 5°C when not in use. This reagent is stable for at least two weeks.

C5.4 Sodium Hydroxide, approximately 5N

Cautiously dissolve 200±5g of sodium hydroxide in about 800 ml water, cool and dilute to 1 litre with water in a graduated measuring cylinder.

C5.5 Sulphuric Acid, approximately 1N (Air scrubber solution)

Dilute with cooling and stirring, 28±2 ml of sulphuric acid in about 800 ml of water. Make up to the mark with water in a 1 litre graduated measuring cylinder.

C5.6 Combined Hydrochloric Acid 0.25N/Magnesium chloride 3.0% m/V reagent

Dissolve 15±0.1g magnesium chloride hexahydrate in about 200 ml of water contained in a 500 ml calibrated flask. Add 11.3±0.1 ml of concentrated hydrochloric acid and make up to the mark with water.

C5.7 Buffer pH 9.4

Dissolve 10.0±0.1g of disodium ethylenediamine-tetracetic acid, 1.5±0.05g of potassium chloride, 3.0±0.05g of boric acid and 1.25±0.05g of sodium hydroxide in about 450 ml of water contained in a 600 ml beaker. Add 0.5±0.1 ml of hydrogen peroxide (100 volumes). Stir solution with a magnetic stirrer, and place a pH electrode in the solution. The initial pH value should be about 8.5. Cautiously add 5N sodium hydroxide solution dropwise until the pH value is exactly 9.4±0.05. Transfer the solution to a 500 ml calibrated flask and make up to the mark with water. Store in an amber glass bottle between 1 and 5°C when not in use. This reagent is stable for at least two weeks.

C5.8 Phenol reagent

Weigh 11.8±0.1g of phenol (pure white, crystalline) into a 250 ml beaker. Carefully add to this 120±2 ml of methanol and swirl to dissolve the phenol. Transfer the solution to a 500 ml calibrated flask and make up the mark with water, taking care to cool during the operation. Store in an amber glass bottle and keep at a temperature between 1 and 5°C when not in use. This reagent is stable for at least 1 week.

C5.9 Stock Standard urea solution, 1 ml contains 0.1 mg urea/N

Weigh 0.214±0.001g of urea (dried to constant weight at 105°C) and dissolve in about 200 ml of water. Transfer quantitatively to a 1 litre calibrated flask and make up to the mark with water. Store solution in a glass bottle at a temperature between 1 and 5°C when not in use. This solution is stable for at least two weeks.

C5.10 Calibration Standard Urea Solutions

Prepare a series of standard urea solutions containing 0.05, 0.1, 0.5, 1.0, 5.0 and 10.0 mg urea N/l by adding 0.1, 0.2, 1.0, 2.0, 10.0 and 20.0 ml of stock standard urea solution into 200 ml calibrated flasks, making up to the mark with water. These solutions should be prepared fresh on the day of use.

C6 Apparatus

The following apparatus, shown diagrammatically in Figure 1, is required:

Sample presentation unit (sampler)
Multichannel peristaltic pump
Analytical cartridge (manifold) including pump tubes and mixing coils
Colorimeter incorporating a flow-cell (detector unit)
Single pen recorder output, or printer unit (recording unit)

C7 Analytical Procedure

Step	Procedure	Notes
C7.1	**Starting Operation**	
	Arrange the apparatus as depicted in the flow diagram (figure 1) (notes a and b).	(a) Follow the manufacturer's general operating instructions.
		(b) See reference 4.
C7.2	Place the reagent tubes in the respective reagent bottles with the sample probe in wash receptacle solution. Start pump (note c). Switch on recording and detector units (note d), and allow at least 30 minutes for the equipment to warm up.	(c) With a newly constructed manifold, pump-test to ensure hydraulic continuity. Check that bubbles do not accumulate in the flow-cell and eliminate any problems before the next step. Ensure that there is sufficient of each reagent to avoid 'topping-up' during one batch of analyses.
		(d) Ensure that reagents, samples and standards attain room temperature before analysis. Check that the pH value of the stream issuing from the flow-cell is in the range 9.35 – 9.5.
C7.3	**Initial Sensitivity Setting**	
	When a satisfactory baseline has been obtained at the measurement unit with water for 15 minutes, adjust the baseline response to about 5 per cent full scale with the zero control (note e). Aspirate a 10 mg/l standard through the sample probe for about 3 minutes. Return sample probe to rest position (note f).	(e) An elevated baseline setting allows for any negative drift that may occur.
		(f) First remove traces of standard solution from probe.

Step	Procedure	Notes
C7.4	After a period of about 9 minutes from the start of step C7.3, a positive response appears on the chart. Adjust the response to give a reading at about 90-95 per cent full scale (note g).	(g) A setting 5 to 10 per cent below full scale allows for any increase in sensitivity that may occur.

C7.5 Analysis of Samples

Arrange the standards, blanks and samples on the turntable and start the sampler with the sampling rate set at 30/h. (notes h and i).

(h) The turntable can be loaded during the initial setting up period (steps C7.2 to C7.4). Alternative loading patterns are given in reference 4.

(i) Samples' pH value must lie between 6 and 8 (See C2).

Position No. on Turntable	Solution
1 - 6	Calibration standard solutions in ascending order of concentration.
7 - 8	Blank (note j).
9 - 18	Samples (note k).
19	Calibration standard (note l)
20 - 21	Blank
22 - 31	Samples
32	Calibration Std.
33 - 34	Blank

(j) Use the same water as used to prepare the calibration standards.

(k) A control standard should be included in one batch as a check on the system (see reference 1).

(l) The standard which occupies position No. 4, as a check on the calibration.

Repeat the sequence 9 - 34 until all the samples have been processed (note m).

(m) If cross contamination is seen to occur (incomplete peak separation), either separate the samples by blanks and reanalyse them, or run in reverse order.

C7.6 When the last sample or standard has been registered on the recording unit and the final baseline is obtained, this unit can be switched off.

C7.7 Shut down procedure

Remove reagent lines, wipe dry with a tissue and transfer to a beaker of water and allow to pump through for 15 minutes (note n).

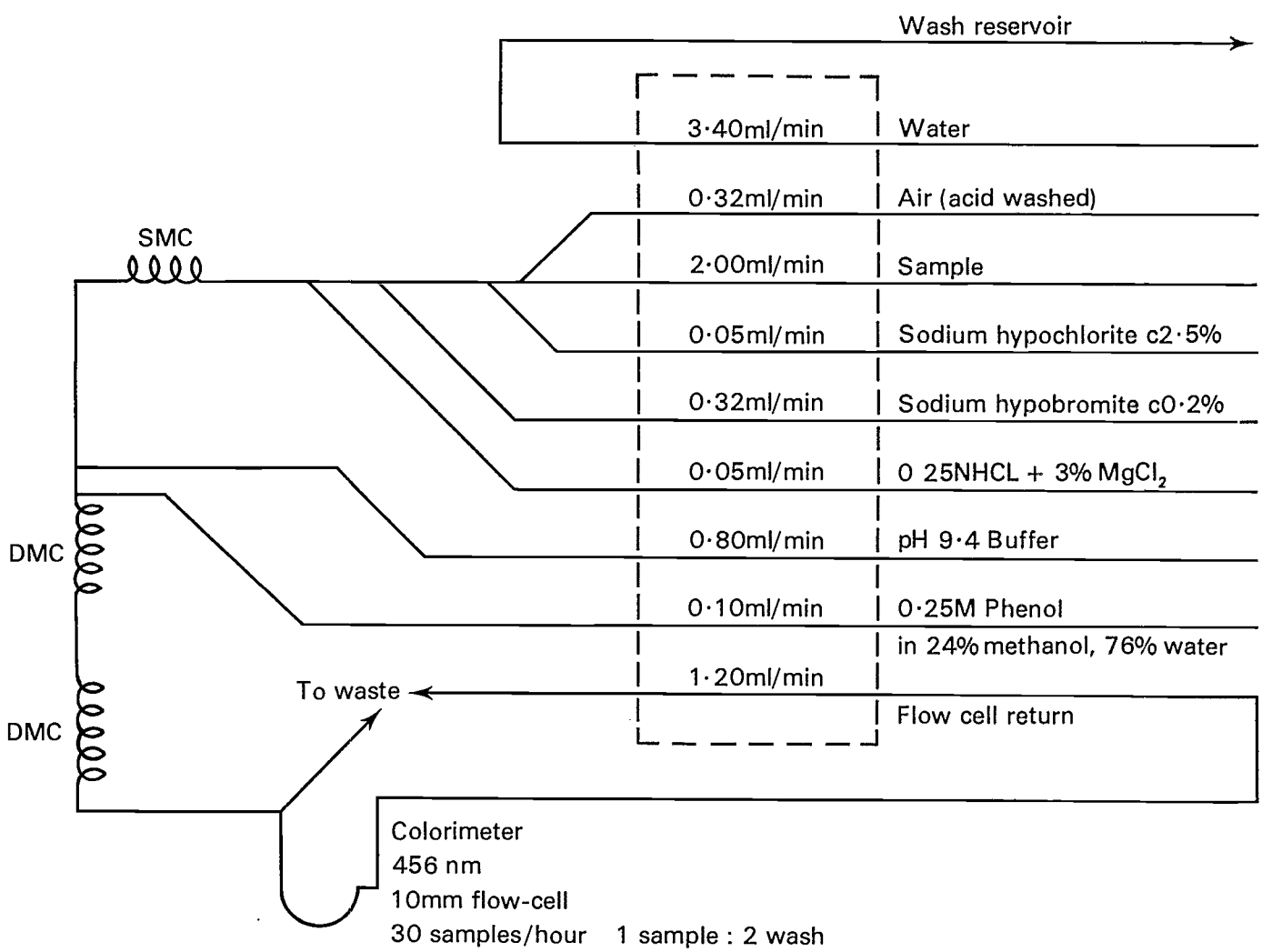
(n) Pumping water through the system removes reagent solutions from the tubing.

C7.8 Switch off pump and detector units.

C7.9 Check all pump tubes for wear, replace any worn tubes with new flow-rated tubes.

Step	Procedure	Notes
C7.10	**Calculation of Results**	
	Plot a calibration curve of measurement unit responses (y axis) against concentration (x axis) of the calibration standard solutions (notes o and p).	(o) Providing that the responses due to the blanks and calibration standards are acceptably close to their respective initial blank corrected values. If not, consult reference 4 for a suggested alternative procedure to obtain calibration curves.
C7.11	Using the calibration curve convert the measurement unit responses due to the samples into concentrations of urea in the samples. The results are expressed as mg/l urea nitrogen.	(p) The measurement unit response of the sample must first be corrected for any baseline or sensitivity changes.

FIGURE 1 AUTOMATIC ANALYSIS FOR UREA – FLOW DIAGRAM



SMC = single mixing coil (10 turns) diameter = 25mm

DMC = double mixing coil (20 turns) diameter = 25mm

D Note on an Alternative Automated Method

D0 Introduction

This method is based on a widely-used clinical method for the determination of blood urea nitrogen (6). Variants have been used for marine and other water samples and dusts (7, 8, 9). It has been optimized for the determination of urea concentrations up to 10 mg/l in water and is used for the analysis of river waters when they are likely to be affected by run-off containing urea from airfields during winter conditions. At the time of drafting this booklet, the method is unsupported by performance data and is therefore appended only as a note.

D1 Principle

Urea reacts in weak acid solution with diacetylmonoxime to produce a colour whose absorbance at 520 nm is intensified in the presence of the thiosemicarbazide and ferric ion and by heating to 95°C. (The marine variant uses manganese ions).

D2 Reagents

D2.1 Sulphuric acid, 20% V/V

D2.2 Stock ferric chloride/phosphoric acid solution

Dissolve 15.0±0.1g of ferric chloride hexahydrate in about 30 ml of water. Cautiously add 300±5 ml of phosphoric acid (d_{20}1.7) to the solution, stirring continuously. Dilute the mixture to 450 ml with water in a measuring cylinder.

D2.3 Stock diacetylmonoxime solution

Dissolve 25.0±0.1g of diacetylmonoxime (2, 3-Butanedione-2-oxime) in about 900 ml of water and dilute to 1 litre with water in a measuring cylinder. Carry out this preparation in a fume cupboard.

D2.4 Stock thiosemicarbazide solution

Dissolve 5.00±0.05g of 2-Thiosemicarbazide in about 900 ml of water and dilute to 1 litre with water in a measuring cylinder.

D2.5 Colour Reagent

Mix together 12.5±0.5 ml of thiosemicarbazide solution, 50±1 ml of diacetylmonoxime stock solution and 1 ml of Brij 35 solution (or other equivalent surfactant) and dilute to 250 ml with water in a measuring cylinder. Prepare this reagent freshly for each occasion on which the method is used.

D2.6 Working acid solution

Mix together 90±1 ml of 20% sulphuric acid solution and 10.0±0.5 ml of stock ferric chloride/phosphoric acid solution.

D3 Apparatus

Apparatus for air-segmented continuous flow analysis is required (4). The flow diagram is shown in Figure 2.

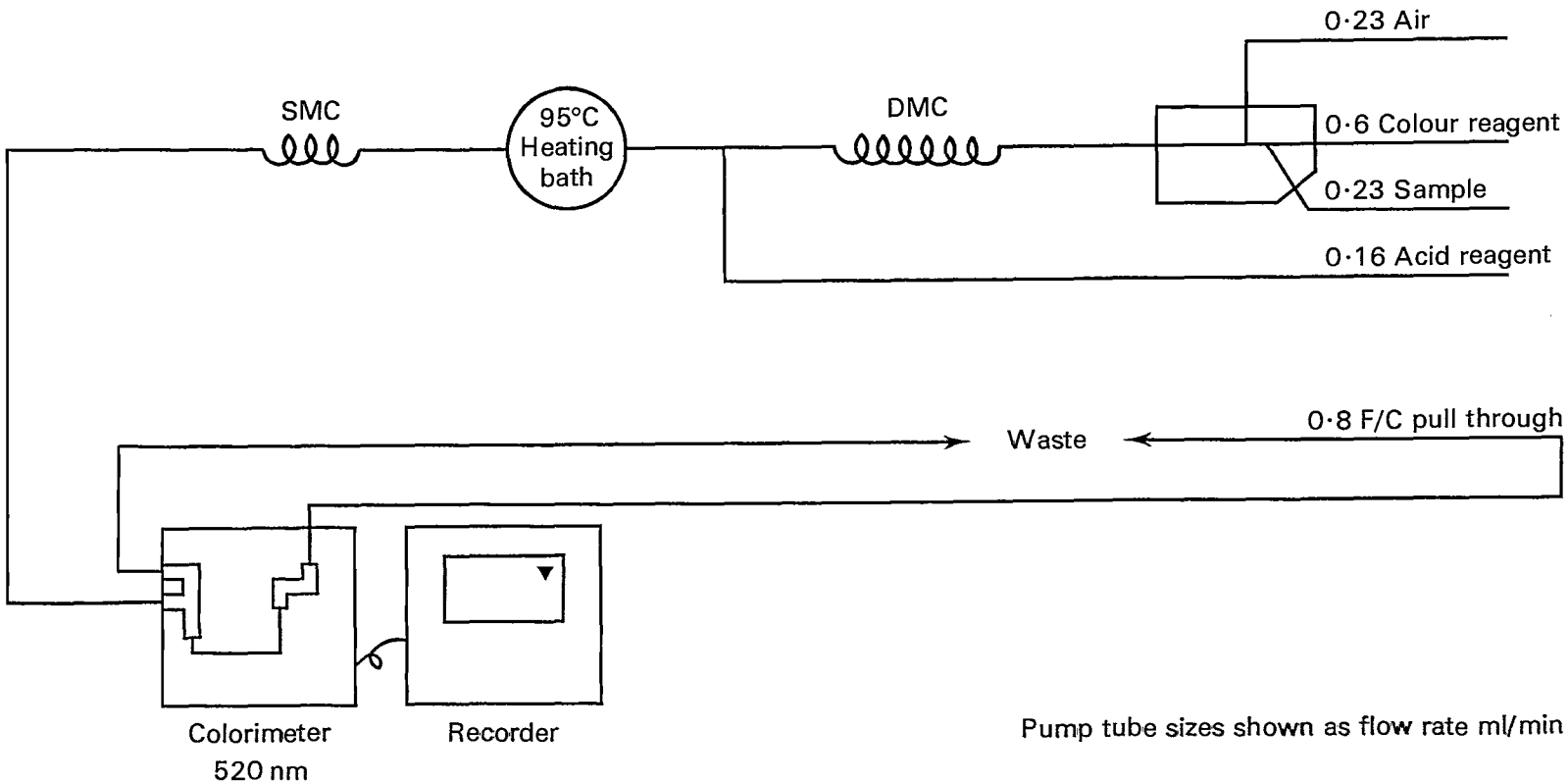
D4 Procedure

The method is carried out in a manner generally similar to the procedure described in Part C of this booklet.

D5 Calibration

The method is calibrated in the range up to 10 mg/l urea nitrogen using standards prepared in similar fashion to those described in parts B and C of this booklet. A more restricted range, up to 2.5 mg/l, can be accommodated if appropriate.

FIGURE 2 UREA (BY OUTLINE METHOD D), UP TO 10mg/L



E Checking the Accuracy of Analytical Results

Once a 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. As a minimum, an analytical quality control standard should be analysed at the same time and in exactly the same way as normal samples. This standard solution is prepared from a different stock standard urea solution to that used to prepare the calibration standards, but which has been stored under identical conditions.

The results obtained for the quality control standard should be plotted on an appropriate quality control chart. In this way, inadequate results will be readily detected, and the standard deviation of routine analytical results may also be estimated. The use of analytical quality control, and the construction of charts is discussed in reference 1.

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G Estimation of the Accuracy of Analytical Results using these Methods

G1 Introduction

Quantitative investigation of the accuracy achievable when these methods are used is limited. Before they can be firmly recommended for general use, it is desirable to know the accuracy achievable in several laboratories. It would, therefore, be of great value if any laboratory using or considering the use of these methods would estimate the accuracy of its own analytical results and report the findings to the Secretary of the Standing Committee of Analysts (for address see below).

The precision achieved and the effects of any interfering substances that may be present in samples are of particular interest. Any information on these aspects would be useful, but the value of such information would be greatly enhanced if it were obtained to a common plan so that the information can be compared and valid conclusions drawn. Accordingly, suggestions for a suitable experimental design and analysis of results are given in the following sections and it is strongly urged that laboratories follow this design whenever possible. The design has been chosen to be as simple as possible; more complex designs are possible and would give more information.

G2 Basis of Suggested Tests

The limit of detection is governed by the within-batch variability of blank determinations. The precision of analytical results may depend on the concentration of urea in the sample analysed and on the type of sample, eg worse precision may be obtained with samples than with standard solutions. For these reasons the basic design recommended is the analysis of one portion of each of the following solutions on each of n days, where n is at least 5 and preferably up to 10.

Solution No.	Description
1	Blank
2	Another blank
3	Standard solution 0.1 mg/l urea
4	Standard solution 1 mg/l urea
5	Typical sample
6	Same sample spiked with 5 mg/l urea

It is essential that these solutions be treated exactly as if they were samples and the procedure specified in the appropriate Analytical Procedure section of the method be rigidly followed. These solutions should be analysed in random order in each batch of analyses. Solutions 1 to 4 should be prepared each day exactly as described in the method and should contain the same amount of hydrochloric acid as is present in the samples. The same batch of water should be used on each day to prepare all four solutions. For solutions 5 and 6 a total of 5 litres of typical samples are required. Prepare solution 6 each day when required by spiking solution 5. When analysing solution 6 it will be necessary to take into account volume change and to take an appropriately sized aliquot. The total period of the tests may be any convenient time so long as the urea concentration in solution 5 does not change appreciably. The results of the analyses of solutions 5 and 6 will provide a check on the effect of sample type on precision. Any deviation of the recovery of spiked sample from 100% may give an indication of the presence of interfering substances.

G3 Evaluation of Results

The raw experimental results should be sent direct to the Department of the Environment for evaluation together with the results obtained from the standards used to establish the calibration curve in each batch of analyses. However, for those laboratories wishing to make the calculations themselves, the details are given below.

G3.1 Convert all results to concentrations as described in the method. Deduct the first of the two blank values (solution 1) from each of the other solution values.

G3.2 Calculate the mean concentration of the n results for each solution.

G3.3 Calculate the standard deviation, s_m of the n results for each solution from:

$$s_m = \sqrt{\frac{\Sigma (X_i - \bar{x})^2}{n - 1}}$$

where x_i = the result from the ith batch
$\bar{x}$ = the mean value of x_i

G3.4 Calculate the within-batch standard deviation, s_w, of the blank from:

$$S_w = \sqrt{\frac{\Sigma (x_{1i} - x_{2i})^2}{2n}}$$

where x_{1i} = the 1st blank result (solution 1) from the ith batch
x_{2i} = the 2nd blank result (solution 2) from the ith batch

G3.5 Calculate the mean percentage recovery, R, of the spiked urea in solution 6 from:

$$R = \frac{\left(\frac{100 + V}{100}\ \bar{X}_6\right) - \bar{X}_5}{S} \times 100$$

where $\bar{x}_5$ = the mean value of the results for solution 5
$\bar{x}_6$ = the mean value of the results for solution 6

V is volume of spike in ml of urea solution added to each 100 ml of solution 5.

S is the final resultant increment in concentration after spiking, in this case S mg/l.

G3.6 Summarise the results as in the following table:

Solution	No of results n	Mean Concentration mg/l	Standard Deviation mg/l	Mean Recovery %
2 Blank				-
3 Standard				-
4 Standard				-
5 Sample				-
6 Solution 5 + spike				

The appropriate sample description should be entered in the space for solution 5. The standard deviation from step G3.4 is entered for the blank solution 2 and the standard deviations from step G3.3 are entered for solutions 3 to 6.

Address for Correspondence

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

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

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