REPORT ON INVESTIGATION INTO THE DETERMINATION OF PARTS PER BILLION OF NITROGEN IN WATER

IAS TECHNICAL REPORT NO. 93/02

by

Consultant to IAS

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The work was carried out on behalf of the Institute of Applied Sciences (IAS) in their laboratories at the University of the South Pacific, Suva between 28/4/93 and 9/6/93.

The brief was,

i) To investigate the development of the indophenol-blue colour in solutions of ammonia which is the basis of the determination of low concentrations of nitrogen following the classical Kjeldahl digestion of the sample.

ii) To modify as necessary the procedure used by IAS for low concentrations of nitrogen in water.

iii) To investigate the factors affecting the blank measurement in the determination of nitrogen in water.
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1. INTRODUCTION

Two methods used by IAS have proved to be unsatisfactory, viz., Method WC4.1, determination of ammonia by formation of indophenol-blue, and Method WC26.2, determination of total nitrogen in water. The method uses a Kjeldahl digestion and distillation followed by determination of ammonia by the formation of indophenol-blue for low levels of nitrogen.

1.1 Method WC4.1 is unsatisfactory because the intensities (absorbance values) of the developed colours are too low. For example, absorbance values reported by IAS workers for an ammonia concentration of 400 parts per billion or micrograms per litre ($\mu$g dm$^{-3}$) are of the order of 0.3 whereas literature values are of the order of 0.5. The low absorbance values greatly reduce the sensitivity of the method and increase the significance of the blank value which should be as low as possible compared to the values obtained for the standard solutions used to calibrate the method.

1.2 Method WC26.2 is unsatisfactory for the same reasons as is Method WC4.1. However in the analysis of water samples other factors play a part as follows,

i) The sample blank as distinct from the calibration blank is affected

   (a) by nitrogen present in the sulphuric acid used in the digestion step
   (b) by the adventitious take-up or loss of nitrogen during the digestion step

ii) There may be loss of ammonia during the distillation step. A small loss is not significant if high nitrogen concentrations are present but is important if trace amounts are being measured.
iii) There is an effective dilution factor in the method which amounts to twenty five. Thus if the indophenol-blue method has a lower detection limit of 10 μg dm\(^{-3}\) the lower detection limit for the sample is 250 μg dm\(^{-3}\) assuming a negligible blank.

2. SUMMARY OF RESULTS

2.1 A method has been developed for the spectrophotometric determination of ammonia via indophenol-blue which gives satisfactory absorbance values for standard solutions and blanks, so that the lower detection limit is of the order of 10 μg dm\(^{-3}\). Details are in Appendix 1.

2.2 A modification of the IAS method for the determination of low concentrations of nitrogen in water has been investigated which omits the distillation step, thereby increasing the sensitivity of the procedure by a factor of five, reducing the analysis time and avoiding possible loss of ammonia in the distillation step. Details are in Appendix 2.

2.3 Some experiments were conducted into the sources and reduction of the blank absorbance values obtained in the analysis of water samples. The investigation was limited by a lack of suitable apparatus but the causes of the high blank values were identified and suggestions are made for their reduction.

3. DETERMINATION OF NITROGEN AS AMMONIA BY INDOPHENOL-BLUE COLORIMETRY

3.1 Preamble

Ammonia reacts with chlorine in alkaline solution to form monochloramine which reacts with phenol in the presence of sodium nitroprusside to form the intensely coloured indophenol-blue. Indophenol-blue is the sodium salt of indophenol; the intense blue colour is due to mesomerism in the anion:
The precipitation of calcium or magnesium salts is prevented by the presence of sodium citrate (calcium and magnesium form soluble citrate complexes). Manganese (II) ion is a catalyst for the formation of indophenol-blue. A very good account of this method is given in "Water and Soil Miscellaneous Publication No. 38, Physical and Chemical Methods for Water Quality Analysis" published for the National Water and Soil Conservation Organisation by the Water and Soil Division, Ministry of Works and Development, Wellington, New Zealand, 1982. This account is more detailed than that given in the Institute of Applied Sciences report on Methods of Analysis of Water, 1992 and was used as a basis for investigating the indophenol-blue method.

3.2 Summary of results

When the procedure given in "Water and Soil Miscellaneous Publication No. 38" (WSMP38) was followed exactly the range of absorbance values obtained in calibration experiments using standard solutions of ammonium chloride were close to those reported by IAS workers using the IAS method, despite the considerable difference in procedure. Thus the absorbance values were too low for the best sensitivity.

A systematic investigation of the WSMP38 method resulted in the following modifications to the recommended procedure,

i) Slightly different reagent solutions and a different mixing procedure for colour development are used. This gives much improved absorbance values, comparable to those in the literature.

ii) Colour development tubes are rinsed, dried and stoppered after use in contract to the WSMP38 procedure. This leads to enhanced absorbance values (see figures 1 and 2).
Fig. 1: Graph of absorbance (A) vs concentration of N in standard solutions: indophenol-blue method, modified WSMP38 procedure using dry tubes.

- ○ Results obtained 5/5/93 (no catalyst)
- ● Results obtained 20/5/93 (catalyst)
- □ Results obtained 24/5/93 (catalyst)
Fig. 2: Graph of absorbance (A) vs concentration of N in standard solutions: indophenol-blue method, modified WSMP procedure using wet tubes.
iii) Manganese (II) sulphate solution is used as a catalyst for colour development which results in full colour development in twenty to thirty minutes instead of ninety minutes (WSMP38) or sixty minutes (IAS Method WC4.1).

Calibration curves of absorbance values against concentration of nitrogen are good - see figures 1 and 2 and Appendix 1, 10.1, note (b).

Reagent blank values can be kept to satisfactorily low levels by the careful preparation of reagents and glassware and by the use throughout of distilled water that has been passed through a column of strongly acidic cation-exchange resin.

Full details of the recommended procedure for the estimation of ammonia by the indophenol-blue method are in Appendix 1.

The sensitivity of the method is as good as or slightly better than that reported in WSMP38 so that the reported performance characteristics can be assumed. These are included in Appendix 1 as are some recommendations from WSMP38 regarding sample collection and preservation and other matters which were not part of the research but are pertinent.
4. DETERMINATION OF LOW CONCENTRATIONS OF NITROGEN IN WATER

4.1 Introduction
As indicated in Section 2.2 the dilution factor of 25 involved in IAS procedure WC4.1 can be reduced by eliminating the distillation step, with the other consequent advantages referred to. For example, if dilution, neutralisation and pH adjustment can be followed by making up to 50 cm³ before the colorimetric step the dilution factor for a 10 cm³ water sample is 5. The determination of nitrogen directly in the Kjeldahl digestion mixture in this way is mentioned in Reference 1 (p.92) but no details are given.

4.2 Neutralisation
Experiments were carried out to see if a 6 cm³ sample of concentrated sulphuric acid (the amount used in the IAS procedure) could be prepared for colour development within a total volume of 50 cm³, i.e., if the sample could be diluted, neutralised, brought to the optimum pH value and diluted to 50 cm³. It was found that, with care, this could be done.

4.3 Colour Development
The indophenol-blue method was applicable to the final solution in the usual way (Appendix 1). Calibration experiments using standard solutions of ammonia containing the same concentration of sodium sulphate as results from the digestion-neutralisation procedure showed that absorbance values are not affected by the high ionic strength (the neutralised solution contains approximately 15.8 g of sodium sulphate per 50 cm³ which is close to the solubility limit).

4.4 Kjeldahl Digestion, blank values
4.4.1 Nitrogen content of AnalaR sulphuric acid
6 cm³ of AR sulphuric acid were neutralised with 50% sodium hydroxide solution, the pH value adjusted to 10-12 and the solution made up to 50 cm³ in a volumetric flask. A 10 cm³ aliquot was taken for colour development as described in Appendix 1.
Four samples of AR sulphuric acid were tested in this way and the concentration of ammonium ion in each was found to be the same, within experimental error, at 0.000107% (approximately half the specified impurity limit).

Thus the dilution of 6 cm$^3$ of sulphuric acid to 50 cm$^3$ will give a solution containing 95 µg dm$^{-3}$ of nitrogen. The experimental blank absorbance should correspond to this concentration of nitrogen (plus a small amount for the standard blank) providing there is no gain or loss of nitrogen during the digestion stage.

4.4.2 Kjeldahl digestion

The digestion-neutralisation-colour development procedure was tested using the IAS digestion method but omitting the catalyst because of its interference in the development of the indophenol-blue colour.

Digestion tubes with and without guard tubes were used (the guard tubes contained glass wool holding 50% sulphuric acid solution). Digestion periods of 2 ¼, 4 and 5 ¾ hours were used with a maximum temperature (as measured by a mercury-in-glass thermometer in a "dummy" tube) of 260-280°C.

Blank samples (6 cm$^3$ sulphuric acid + 0.5 g sodium sulphate + 10 cm$^3$ ammonia-free water) and samples containing known amounts of ammonia were analysed, with disappointing results. The blank absorption values were approximately double the blank values measured on samples of sulphuric acid without digestion, with little obvious benefit from the use of guard tubes. In some cases the blank values were higher than those obtained with solutions containing ammonia. Also, the reproducibility was very poor.
It appears that ammonia is being absorbed from the atmosphere during the digestion process. No reference to this problem has been found in the literature.

5. MODIFICATION OF THE KJELDAHL DIGESTION

5.1 General considerations
As a result of the poor results described above it was decided to consult the literature more thoroughly on the Kjeldahl digestion. A comprehensive study of the factors influencing the efficiency of the Kjeldahl digestion is reported by McKenzie and Wallace (Reference 2). From the point of view of this investigation their most important result is that virtually complete recovery of up to 2 mg of nitrogen can be obtained from a variety of organic nitrogen compounds (including tryptophan, one of the most refractory amino acids) by digestion with 1.5 cm³ sulphuric acid and 3g of potassium sulphate for 2 hours at 390°C.

It was decided to try this procedure on blank water samples since the reduction of the amount of sulphuric acid and a digestion period of two hours should reduce the blank value considerably. Also, because of the smaller amount of acid used, it should be possible to further reduce the dilution factor. (In the event, neither expectation was realised, see below.)

5.2 Method
1.5 cm³ AnalaR sulphuric acid and 3g of potassium sulphate were added to 10 cm³ ammonia-free water in a Kjeldahl tube. The tube was heated to 390°C and held at that temperature for 2 hours. After cooling the contents of the tube were diluted, neutralised, brought to a pH value of 10-12 and made up to 50 cm³ (this volume is necessary because of the limited solubility of potassium sulphate). A 10 cm³ aliquot was used for colour development as detailed in Appendix 1.
5.3 Results
Blank absorbances measured for six samples digested with a guard tube in place gave a mean value of 0.181 absorbance units with a standard deviation of 0.030.

Blank absorbances measured for four samples digested without a guard tube gave a mean value of 0.183 absorbance units with a standard deviation of 0.019.

The mean blank absorbance values correspond to a nitrogen concentration of 130 $\mu$g dm$^{-3}$ in the final solution which is about five times that expected from the nitrogen content of the sulphuric acid.

It is evident that the guard tubes used did not effect an improvement in the experimental blanks.

5.4 Conclusions and recommendations
Results summarised in 5.3 are sufficiently encouraging to justify further work on the procedure outlined in 5.2. Full details are therefore given in Appendix 2.

Further work needs to be done to validate the Kjeldahl digestion-dilution-neutralisation-colour development scheme for parts per billion nitrogen in water. The following investigations should be carried out.

i) Blank experiments with empty guard tubes to get a better idea of the standard deviation

ii) Experiments with standard solutions of ammonia to check the recovery and establish the lower detection limit. This will require a large number of experiments
iii) Experiments to check the efficiency of the digestion process for organic nitrogen in solution. The method is reportedly efficient but should nevertheless be checked. Solutions of urea and/or glutamic acid and/or nicotinic acid are suggested as suitable for this purpose.

iv) Use of modified guard tubes
The guard tubes used for the Kjeldahl digestion experiments (sections 4.4.2 and 5.3) did not reduce the blank measurements as was hoped. It should be possible to reduce uptake of ammonia by the use of a suitable guard tube and it is recommended that one of a bubble-trap design is tried. Fitting a guard tube is easy because of the ground glass fitting on the Kjeldahl tube, but the services of a glass-blower are needed. In designing the trap provision must be made for the 10 cm³ of water that distils during the digestion.
6. GENERAL REMARKS

6.1 High level nitrogen determinations
It might be worth investigating the measurement of high concentrations of nitrogen by the method given in Appendix 2. A sample size appropriate to 1.5 cm$^3$ sulphuric acid would be used. Advantages would be -

i) Saving on chemicals, including catalyst
ii) Shorter digestion period
iii) Saving time by eliminating the distillation step (dilution, neutralisation, etc. is much more convenient and quicker)

6.2 Alternative methods
The traditional Kjeldahl method is attractive because it requires little expensive equipment. However, if the Kjeldahl/indophenol-blue method proves ultimately to be unsatisfactory thought should be given to alternative procedures. Some of these are outlined in the Reference 1 and should be followed up. The method of oxidation by peroxydisulphate seems attractive as it appears not to require expensive equipment. Unfortunately the reference to this method is not available in the library.

Reference 1 was published in 1978 and its latest references are to 1976. The more recent literature should be consulted for advances in the determination of nitrogen.

References

Determination of nitrogen as ammonia by indophenol-blue colorimetry.

1. PRINCIPLE
Ammonia reacts with chlorine in alkaline solution to form monochloramine which reacts with phenol in the presence of sodium nitroprusside to form the intensely coloured indophenol-blue. Indophenol-blue is the sodium salt of indophenol; the intense blue colour is due to mesomerism in the anion:

\[ \text{\begin{tikzpicture}
\draw (0,0) circle (1cm);
\end{tikzpicture}} \quad \text{\begin{tikzpicture}
\draw (0,0) circle (1cm);
\end{tikzpicture}} \]

The reaction is known as the Berthelot reaction. A discussion of factors affecting the reaction is given in Reference 1.

The presence of sodium citrate prevents precipitation of calcium and magnesium salts.

The optimum pH value for colour development is in the range 11.4 to 11.8.

The reaction is catalysed by manganese (II) ions.
2. INTERFERENCES
A number of constituents likely to be present in industrial wastewaters interfere, including nitrite, thiocyanate, cyanide, copper and silver (see Reference 2).

Constituents normally present in relatively unpolluted waters do not interfere but it is reported (Reference 3) that bicarbonate alkalinites interfere in the analysis of preserved water samples. Bicarbonate alkalinites up to at least 500 mg dm$^{-3}$ (as CaCO$_3$) do not interfere in the analysis of fresh samples.

3. SAMPLE PRETREATMENT
The buffer capacity of the reagents is adequate for samples having bicarbonate alkalinity up to 500 mg dm$^{-3}$ (as CaCO$_3$). Samples having higher bicarbonate alkalinites or high acidity should be titrated with sodium hydroxide solution to a pH value of 10.0 to 10.7.

4. APPARATUS
4.1 A spectrophotometer preferably having a precision of $\pm$ 0.001 absorbance units for measurements at 635 nm using a 1 cm path-length cell.

4.2 Glassware and volumetric apparatus
25 cm$^3$ stoppered tubes for colour development.

Automatic dispensers
Two of 0.1 to 1.0 cm$^3$ delivery
Automatic transfer pipette, 10 cm$^3$ size for sample aliquoting

4.3 Cleanliness
Concentrated ammonia solutions should not be stored or handled in the laboratory used for ammonia analysis. Apparatus and reagent bottles should be dedicated to ammonia analysis only and should be stoppered except for the transfer of solutions. After use colour development tubes should be thoroughly rinsed with distilled water, dried and stoppered.
5. HAZARDS
The phenol/ethanol (6.3), sodium nitroprusside (6.4), sodium hydroxide (6.5),
phenate/citrate (6.7) and buffer/chlorine (6.8) solutions are all either toxic or
caustic and none should be pipetted by mouth. Phenol can be absorbed through
the skin and drops or splashes must be washed off immediately. Mercuric chloride
(8.2) is toxic.

6. REAGENTS

6.1 Except where otherwise stated analytical reagent grade chemicals are
to be used.

6.2 Water
In order to keep blank values as low as possible the water used to prepare
reagents, standards and blanks should be distilled water that has been
passed through a strongly acidic cation exchange resin (Amberlite IR 120 or
Dowex 50W 8X(H)} and stored over the same resin.

6.2 Ammonia stock solution
Dissolve 3.8189 g of ammonium chloride (dried at 100°C for 2 hours and
cooled in a desiccator) in water and make up to 1000 cm$^3$ in a volumetric
flask. This solution contains 1000 mg dm$^{-3}$ of nitrogen and is stable for one
month. It is convenient to prepare daily as required a stock solution of 2 mg
dm$^{-3}$ N by diluting 2 cm$^3$ of this stock to 1000 cm$^3$.

Standard solutions of 20, 40 and 80 $\mu$g dm$^{-3}$ N can be prepared by diluting
5 cm$^3$, 10 cm$^3$ and 20 cm$^3$ respectively of the 2 mg dm$^{-3}$ stock to 500 cm$^3$.
Standard solutions of 100, 200, 300 and 400 $\mu$g dm$^{-3}$ N can be prepared by
diluting 5, 10, 15 and 20 cm$^3$ respectively of the 2 mg dm$^{-3}$ stock to 100
cm$^3$. Standard solutions of up to 1000 $\mu$g dm$^{-3}$ N can be prepared by
appropriate dilution as required.

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6.3 Phenol/ethanol solution
Dissolve 72.5 \( \pm \) 0.2 g phenol in 50 cm\(^3\) absolute ethanol. New, uncoloured crystalline phenol is adequate but if any discolouration is evident the phenol must be distilled, collecting the fraction distilling between 178-184\(^\circ\)C. The solution must be kept in a brown glass bottle in the refrigerator and renewed every three weeks or earlier if blank absorbance values increase significantly.

6.4 0.1\% Sodium nitroprusside solution
Dissolve 1 \( \pm \) 0.01 g sodium nitroprusside in water and make up to 100 cm\(^3\) with water (volumetric flask).

Pipette 10 cm\(^3\) of the 1\% sodium nitroprusside into a 100 cm\(^3\) volumetric flask and make up to 100 cm\(^3\) with water. Store in a dark bottle in the refrigerator and renew monthly or if blank absorbance values increase.

6.5 6 mol dm\(^{-3}\) Sodium hydroxide solution
Dissolve 120 \( \pm \) 1 g of sodium hydroxide in water and make up to 500 cm\(^3\).

6.6 Sodium citrate solution
Dissolve 200 \( \pm \) 1 g of trisodium citrate in water and make up to 500 cm\(^3\). The solution is stable for several weeks.

6.7 Phenate/citrate solution
Mix 5 cm\(^3\) phenol/ethanol solution, 6.5 cm\(^3\) of 6 mol dm\(^{-3}\) sodium hydroxide solution and 50 cm\(^3\) of sodium citrate solution. The solution should be prepared freshly every two or three days.

6.8 Buffer/chlorine solution
Dissolve 12.5 \( \pm \) 0.1 g of anhydrous sodium carbonate and 0.8 \( \pm \) 0.05 g of sodium hydrogen carbonate (sodium bicarbonate) in about 100 cm\(^3\) water. Add 35 cm\(^3\) (measuring cylinder) of commercial sodium hypochlorite solution (e.g. Janola) and make up to 200 cm\(^3\). Allow 1 hour before use for
removal of ammonia contamination by the high chlorine concentration. The solution is stable if kept in a tightly stoppered bottle in the refrigerator. The free chlorine content of the reagent must be checked initially and at weekly intervals. The checking procedure is as follows. Dilute 1 cm$^3$ of buffer/chlorine solution with approximately 40 cm$^3$ water and add 10 cm$^3$ (measuring cylinder) of 10% m/v potassium iodide solution and 3 drops of concentrated sulphuric acid. Titrate the liberated iodine with standardised sodium thiosulphate solution of approximately 0.025 mol gm$^{-3}$ using starch indicator. The starch solution should be freshly prepared and added as close to the endpoint as possible. With 0.025 mol dm$^{-3}$ thiosulphate solution the titre should be between 4.5 and 5.6 cm$^3$. If the titre is lower than 4.5 cm$^3$ additional hypochlorite solution must be added to the reagent. If as is likely, the concentration of the thiosulphate solution is slightly different from 0.025 mol dm$^{-3}$ the necessary titre can be calculated by simple proportion.

7. SAMPLE COLLECTION AND PRESERVATION

7.1 Glass or polythene sample containers are satisfactory.

7.2 Ammonia concentrations are particularly susceptible to rapid concentration changes associated with biological activity unless a preservative is used. Unfiltered samples should be refrigerated and/or analysed as soon as possible after collection. The period for which concentrations are substantially unchanged may vary from less than an hour to several days and must be established for each type of sample involved. Samples which have been filtered into sterilised bottles on collection using 0.5 $\mu$m membrane filters can often be kept without refrigeration for several days, but the stability must be established for each sample type.

The preservation of samples, at least by use of mercuric chloride, leads to a decrease in sensitivity, an increase in the lower detection limit and other problems as well as being of doubtful value in the case of seawater samples.
(Reference 3). It is recommended that the preservation of samples in this way is best avoided, if possible.

8. CLEANING OF SAMPLE BOTTLES

8.1 Acid cleaning procedure
Rinse bottles with tap water and then fill with 10% v/v hydrochloric acid and soak overnight or longer. Rinse at least three times using about ten percent of the bottle volume of distilled water for each rinse. Store the clean bottles closed. The acid used for soaking may be reused.

8.2 Cleaning and sterilising procedure
Rinse bottles with tap water and then fill with distilled water to which 0.1% v/v hydrochloric acid and 40 mg dm⁻³ mercuric chloride has been added. Allow to stand for 2 days. Rinse the bottles with distilled water and store closed. Retain the filling solution for reuse.

It is suggested (Reference 3) that the sterilising procedure gives better sample stability than the acid cleaning procedure, especially in filtered samples.

9. PROCEDURE FOR COLOUR DEVELOPMENT AND MEASUREMENT
Read section 5 on hazards before starting this procedure

<table>
<thead>
<tr>
<th>STEP</th>
<th>EXPERIMENTAL PROCEDURE</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.1</td>
<td>Pipette 10 cm³ aliquots into clean, dry tubes. Remove and replace each stopper immediately before and after pipetting</td>
<td></td>
</tr>
</tbody>
</table>
9.2 For each aliquot, remove the stopper and add, in order and as quickly as possible, 1 cm³ phenate/citrate solution 0.6 cm³ sodium nitroprusside solution 0.4 cm³ buffer/chlorine solution 1 drop manganese (II) sulphate solution Replace the stopper and mix the tube contents immediately after reagent addition. Stand for 20 to 30 minutes (note a)

9.3 Measure the absorbance values of the solutions in a 1 cm cell at a wavelength of 635 nm.

10. TREATMENT OF RESULTS

<table>
<thead>
<tr>
<th>STEP</th>
<th>EXPERIMENTAL PROCEDURE</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.1</td>
<td>Calibration curve. Subtract the standard blank absorbance from that of each of the standards and plot a calibration curve of net absorbance value against concentration</td>
<td>b) The standard deviation of replicate determinations of absorbance values is approximately 0.01 absorbance units</td>
</tr>
<tr>
<td>10.2</td>
<td>Calculation of concentration in sample. Subtract the sample blank absorbance from that of each of the samples and read off the concentrations corresponding to the net sample absorbances from the calibration graph</td>
<td>c) It is recommended that the calibration curve be drawn for concentrations of nitrogen up to 400 µg dm⁻³ or 700 µg dm⁻³ as is convenient. Samples giving an absorbance higher than that of the highest standard should be diluted appropriately (section 12)</td>
</tr>
</tbody>
</table>

11. Checking linearity of the calibration curve -
Periodically a series of standards containing 0, 40, 100, 200, 400 and (if appropriate) 600 µg dm⁻³ of nitrogen should be used to check linearity. If linear
curves are obtained consistently two standard solutions should suffice, chosen to suit the concentration range of interest.

12. Samples having a nitrogen concentration higher than that of highest standard should be diluted with the same water used for preparation of the blanks and standards in the batch. The absorbances for the diluted samples should be corrected to net absorbances by subtracting \((x-1)/x\) times the standard blank absorbances and \(1/x\) times the sample blank absorbance, where \(x\) is the dilution. The concentration in the diluted sample is then read from the calibration curve and the concentration in the original sample is obtained by multiplying this by \(x\).

**Explanation of blank corrections**

When the sample is diluted by a factor of \(x\) the sample blank is reduced by a factor \(1/x\) and this must be therefore be subtracted from the measured diluted sample absorbance. But the sample blank includes the standard blank which has therefore also been reduced by a factor \(1/x\). The diluted solution is treated in exactly the same way as all other solutions so the full standard blank absorbance should be deducted from the measured absorbance. Thus an amount equal to \((x-1)/x\) times the standard absorbance must also be subtracted. The full value of the standard blank has now been subtracted, since

\[
\frac{1}{x} + \frac{x - 1}{x} = 1
\]
13. REFERENCES


14. PERFORMANCE CHARACTERISTICS OF THE METHOD

<table>
<thead>
<tr>
<th>14.1</th>
<th>Substance determined</th>
<th>Nitrogen in the form of ammonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.2</td>
<td>Type of sample</td>
<td>Fresh, saline or waste waters</td>
</tr>
<tr>
<td>14.3</td>
<td>Basis of method</td>
<td>Ammonia is treated with chlorine and phenol to give indophenol-blue which is determined spectrophotometrically</td>
</tr>
<tr>
<td>14.4</td>
<td>Range of application</td>
<td>Up to 1000 µg dm⁻³</td>
</tr>
<tr>
<td>14.5</td>
<td>Calibration curve</td>
<td>Linear up to 1000 µg dm⁻³ (but see Sections 10, 12)</td>
</tr>
<tr>
<td>14.6</td>
<td>Standard deviation</td>
<td>Concentration of N/µg dm⁻³</td>
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<tr>
<td></td>
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<td>0</td>
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<td></td>
<td></td>
<td>200</td>
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<tr>
<td></td>
<td></td>
<td>900</td>
</tr>
<tr>
<td>14.7</td>
<td>Lower limit of detection</td>
<td>10 µg dm⁻³</td>
</tr>
<tr>
<td>14.8</td>
<td>Sensitivity</td>
<td>400 µg dm⁻³ gives an absorbance value of approximately 0.5</td>
</tr>
<tr>
<td>14.9</td>
<td>Bias</td>
<td>No evidence of bias in spike recovery tests</td>
</tr>
<tr>
<td>14.10</td>
<td>Interferences</td>
<td>See Section 2</td>
</tr>
<tr>
<td>14.11</td>
<td>Time required for analysis</td>
<td>The analytical time for 25 samples is approximately 2 hours</td>
</tr>
<tr>
<td>14.12</td>
<td>Volume required for analysis</td>
<td>10 cm³ for each determination</td>
</tr>
</tbody>
</table>
Proposed method for the determination of low concentrations of nitrogen in water

1. **PRINCIPLE**
Nitrogen is converted to ammonia by digestion with sulphuric acid (Kjeldahl digestion). The resulting mixture is diluted, neutralised and made up to volume. An aliquot of the resulting solution is used for the determination of ammonia by the indophenol-blue method. An account of the conditions affecting the efficiency of the Kjeldahl digestion is in the Reference.

2. **REFERENCES**
Selenium, copper, silver and other metals interfere.

3. **APPARATUS**

3.1 **Kjeldahl digestion**
- Graduated 50 cm³ Kjeldahl tubes
- Guard tubes to fit Kjeldahl tubes
- 50 cm³ tall-form beaker
- Small glass stirring rod
- Dropping pipettes
- 5 or 10 cm³ measuring cylinder
- 50 cm³ volumetric flasks
- pH meter

3.2 **Indophenol-blue method for the determination of ammonia**
See Appendix 1.

3.3 **Cleanliness**
Concentrated ammonia solutions should not be stored or handled in the laboratory used for ammonia analysis. Apparatus and reagent bottles should
be dedicated to ammonia analysis only and should be stoppered except for the transfer of solutions.

4. HAZARDS
Concentrated sulphuric acid and sodium hydroxide are very corrosive. Mixing of strong acids and alkalis should be carried out with care. Safety glasses should be worn.

See also Appendix 1.

5. REAGENTS
5.1 Kjeldahl digestion
Analytical grade sulphuric acid, sodium hydroxide and potassium sulphate should be used.

The sulphuric acid chosen for nitrogen estimation should be kept in a tightly stoppered bottle and reserved for nitrogen work alone. The ammonia content of the sulphuric acid should be as low as possible and below the manufacturer’s specified limit.

5.1.1 Determination of ammonia in sulphuric acid
Pipette 5 cm$^3$ AnalaR sulphuric acid carefully and with stirring into 20 cm$^3$ nitrogen-free water in a 50 cm$^3$ tall form beaker cooled in ice water. Slowly and with stirring add 12.5 cm$^3$ 50% m/v sodium hydroxide solution from a measuring cylinder (pour the solution down the stirring rod). When the solution is at room temperature adjust the pH value to 10-12 by dropwise addition of the sodium hydroxide solution. If the pH value of 12 is exceeded adjust the value by dropwise addition of 50% sulphuric acid (AR sulphuric acid and nitrogen-free water).

Transfer the solution to a 50 cm$^3$ volumetric flask by pouring down a stirring rod and through a small funnel. Rinse the pH electrode, beaker and rod into
the flask using successive small amounts of nitrogen-free water. Use approximately 1 cm\(^3\) portions from a dropping pipette for rinsing. As many as 10 rinsings can be done in this way. Make the solution up to the mark. Use 10 cm\(^3\) aliquots of the solution for the determination of ammonia by the indophenol-blue method (Appendix 1). Measure the absorbance of the developed colour and read off the concentration of nitrogen in the solution from the calibration curve after subtracting the blank absorbance. The concentration of nitrogen (as ammonia) in the sulphuric acid is 10 times this value.

\[
[NH_4^+] = [N] \times \frac{18}{14}
\]

5.2 Water
Deionised distilled water should be used throughout (see Appendix 1).

5.3 50% m/v Sodium hydroxide solution
This can be prepared as necessary. Use a polythene beaker in ice water for mixing. Store in a polythene container.

5.4 Indophenol-blue method for the determination of ammonia
See Appendix 1.

6. PROCEDURE FOR KJELDAHL DIGESTION
Read section 4 on hazards before starting this procedure.

<table>
<thead>
<tr>
<th>Step</th>
<th>Experimental Procedure</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1</td>
<td>Add 3g samples of AR potassium sulphate to clean, dry Kjeldahl tubes.</td>
<td></td>
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<tr>
<td>6.2</td>
<td>Pipette 10 cm(^3) aliquots of samples and blanks into the Kjeldahl tubes (note a).</td>
<td>a) At least four blanks should be done.</td>
</tr>
<tr>
<td>6.3</td>
<td>Pipette 1.5 cm(^3) sulphuric acid into the Kjeldahl tubes.</td>
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<tr>
<td><strong>6.4</strong></td>
<td>Fit Kjeldahl tubes with empty guard tubes to keep out dust and dirt.</td>
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<tr>
<td><strong>6.5</strong></td>
<td>Place the tubes in the heating block. Heat to 390°C (note b). Maintain the temperature at 390°C for two hours (notes c and d).</td>
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<td></td>
<td>b) With the right hand control knob turned fully clockwise the temperature can be reached in 1 hour.</td>
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<tr>
<td></td>
<td>c) With careful adjustment of the right hand control knob the temperature can be kept between 385 and 395°C. Frequent attention is needed.</td>
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<tr>
<td></td>
<td>d) The temperature can be monitored by use of a 400°C thermometer placed in a mixture of 6 cm³ sulphuric acid and 12g of potassium sulphate in a Kjeldahl tube positioned in the heating block.</td>
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<tr>
<td><strong>6.6</strong></td>
<td>Remove the tubes from the heating block and place in a rack to cool. When the contents of the tube begin to solidify rotate the tubes gently to spread the solid on the sides to facilitate dissolution. Stopper the tubes as soon as possible.</td>
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<tr>
<td><strong>6.7</strong></td>
<td>When the tubes are cool enough add 20 cm³ of water followed by 3.5 cm³ of 50% sodium hydroxide (note e). Swirl the contents of the tube to dissolve as much solid as possible (note f). Stopper the tubes.</td>
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<tr>
<td></td>
<td>e) The temperature should not increase by too much but if necessary cool the tubes under the tap.</td>
<td></td>
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<tr>
<td></td>
<td>f) Some potassium sulphate will remain undissolved.</td>
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</tr>
</tbody>
</table>
When the solutions are at room temperature treat them one at a time as follows. Decant the solution carefully from the solid into a 50 cm³ tall form beaker by pouring down a stirring rod. Rinse the contents of the tube into the beaker using 10 successive 1 cm³ portions of water from a dropping pipette (notes g and h).

Adjust the pH value of the solution to 10-12 by dropwise addition of 50% sodium hydroxide solution. If the pH range is exceeded adjust the value by dropwise addition of 50% v/v sulphuric acid solution.

Transfer the solution to a 50 cm³ volumetric flask by pouring down the stirring rod and through a small funnel. Rinse the pH electrode twice into the beaker with 1 cm³ portions of water (dropping pipette), transferring the washings to the flask. Rinse the beaker with successive 1 cm³ portions of water, transferring them to the flask. About 10 washings will be possible (note i). Make up to the mark with water.

Use a 10 cm³ aliquot of the solution to develop the indophenol-blue colour (Appendix 1) and measure the absorbance at 635 nm using a 1 cm cell.

---

g) The remaining potassium sulphate dissolves readily during rinsing.

h) The same stirring rod and beaker can be used for successive samples.

i) Many small washings are more efficient than a few large ones.
### 6.12 Calculation of concentration in sample

Subtract the sample blank absorbance from that of each of the samples and read off the concentrations corresponding to the net sample absorbances from the calibration curve. Multiply these values by five to get the concentrations of nitrogen in the original samples (notes j and k).

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j) The dilution factor is 5 since the original 10 cm³ sample has effectively been diluted to 50 cm³.

k) If measured absorbances are too high the solutions must be diluted. See Appendix 1, section 12.