COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION

DIVISION of FISHERIES and OCEANOGRAPHY

Report No. 55

LABORATORY TECHNIQUES IN MARINE CHEMISTRY

II. Determination of ammonia in sea water and the preservation of samples for nitrate analysis

By G. Dal Pont, M. Hogan and B. Newell

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II. DETERMINATION OF AMMONIA IN SEA WATER AND THE PRESERVATION OF SAMPLES FOR NITRATE ANALYSIS

by

G. Dal Pont, M. Hogan and B.S. Newell

A. Determination of ammonia in sea water

INTRODUCTION

In our previous manual (Major *et al.* 1972) we recommended the use of the Solorzano (1969) method for assaying ammonia in sea water. Experience over 2-3 years with several operators, however, has shown this method to possess several disadvantages.

(a) Contrary to the claims of the author, the method displays a salt error. This may be due to the quality of sodium hypochlorite available in Australia or the different pH obtained in fresh and salt water samples. Whatever the cause, the differing yields in fresh and salt water produces difficulties with reagent blanks and with samples of varying salinity. In general, increasing dosage of hypochlorite in fresh water samples decreases yield and increases blanks. Increasing dosage of hypochlorite in sea water samples increases yield to a plateau.

(b) Yields tend to be erratic, varying with operator (presumably because of slight differences in timing and manner of addition of reagents) and with batches of reagent. The yield factor for 1 µg atom/l NH_3-N varies from 0.084 to 0.154 absorbance in a 10 cm curvette.

(c) Sometimes artificially high results are obtained through discolouration or precipitation of magnesium hydroxide.

To overcome these problems, other, more recent, published methods have been subjected to trials. That of Truesdale (1971) gave only brown discoloured samples with little or no yield of azo dye. The method of Benesch and Mangelsdorf (1972) gave poor sensitivity, although sodium dichloro-iso-cyanurate seemed a more stable and reliable reagent than sodium hypochlorite. Finally the method of Grasshof and Johanssen (1972) was tried. This method proved more reliable, but still exhibited some disadvantages. At the dosage recommended by the authors (private communication) sensitivity was very low. With the dosage of reagents given in the published method for

automatic analysis, sensitivity improved but occasional high results were obtained. These were traced to slight and variable turbidity caused by magnesium hydroxide precipitation. An examination of the effects of the individual reagents showed that low sensitivity resulted from too low a pH. Increase in pH to the optimum for colour yield resulted in precipitation of magnesium hydroxide, the citrate present being inadequate.

By increasing the dosage of citrate and sodium hydroxide, both problems were overcome. In fact it was found possible to reduce the dosage of sodium dichloro-iso-cyanurate without loss of yield. In addition, the trisodium citrate was found to be the biggest contributor to the reagent blank. A procedure of boiling this reagent has been introduced.

In conclusion we would like to state that results obtained by the Solorzano method on local sea waters should be interpreted with caution. At best they are likely to be inaccurate and at worst erroneously high, especially with inexperienced operators.

REAGENTS AND APPARATUS

1. Buffer — Dissolve 200 g trisodium citrate $|C_3H_4(COO\ Na)_3.2H_2O|$ A.R. in ca- 700 ml deionised distilled water in a 2 litre graduated beaker. Add 11 g sodium hydroxide (NaOH) A.R. and dissolve. Boil until volume is reduced to just under 500 ml. Cool, transfer to 500 ml volumetric flask. Make up to mark with washings from the beaker. Prepare weekly.

2. Phenol-nitroprusside reagent — Dissolve 17.5 g phenol (C_6H_5OH) A.R. grade (preferably preserved with hypophosphorous acid e.g. Mallinckrodt A.R.) and 0.2 g sodium nitroprusside $|Na_2Fe(CN)_5NO.2H_2O|$ in 250 ml deionised distilled water. Prepare weekly.

3. Oxidising reagent — Dissolve 5 g sodium hydroxide (NaOH) A.R. and 0.5 g sodium dichloro-cyanurate ($C_3N_3O_3HCl_2$) in 250 ml deionised distilled water. Prepare weekly.

4. Standard ammonia solution — Dissolve 535 mg ammonium chloride (NH_4Cl) A.R. in 1 litre of deionised distilled water. Dilute 5 ml to 1 litre for a working standard containing 0.7 µg ammonia nitrogen per ml.

5. Screw-cap bottles — Tubes or bottles of at least 100 ml capacity with airtight caps.

6. Pipettes

7. Waterbath — Thermostatically controlled. (For distilled water calibration curves or fresh-water samples.)

8. Spectrophotometer with at least 5 cm optical path length curvettes.

PROCEDURE

1. Rinse screw-top bottles with sample. Dispense 50 ml of sample into bottle.

2. Add 5 ml Reagent 1 (buffer solution). Mix.

3. Add 2 ml Reagent 2 (phenol-nitroprusside solution). Mix.

4. Add 2 ml Reagent 3 (oxidising solution). Mix.

5. Replace cap. Stand at room temperature for at least 60 minutes.

6. Read absorbance at 630 nm in 5 cm curvette.

7. Read absorbance of original sample at 630 nm. Subtract this from reading made under (6) |Turbidity correction|.

STANDARDS AND BLANK

Because of salt error effects (see notes below) it is recommended that calibration be carried out by "spiking" two or more replicate samples with known amounts of ammonia standard. For the range of ammonia nitrogen commonly encountered in sea water (0 to 30 µg/l) two replicates spiked with 1 and 2 ml of working standard (14 and 28 µg ammonia nitrogen per litre) are usually sufficient.

Great care must be exercised in preparing and using the deionised distilled water. A column about 2 x 20 cm of cation-exchange resin (e.g. Zeo Carb 225) in hydrogen form, is required. After acid treatment, the column should be washed with at least 2 litres of distilled water. In subsequent use after standing, at least 500 ml of distilled water should be run through and discarded before samples are taken. Samples must always be withdrawn directly into the vessel to be used and the vessels capped or stoppered as soon as possible.

A reagent blank may be obtained by subjecting 50 ml of deionised distilled water to the above procedure. The salt error has negligible effect at the low levels of reagent blank absorbance (0.020-0.030).

If it is desired to set up a standard series in distilled water, then step 5 under Procedure should be amended, and all standards incubated in a water bath for 30 minutes at 70°C, after standing at room temperature for 5-10 minutes (see notes below).

NOTES

1. The yield of colour in distilled or fresh water samples is less than that in sea water samples (about 80%) where the reaction is carried out at room temperature. It is thought that this may be due to the differing pH resulting from the addition of reagents to distilled water

(pH 11.3) and sea water (pH 10.2). The difference is largely abolished by warming at 70°C for 30 minutes. However, even then, distilled or fresh water samples sometimes give slightly lower yields (in the order of 2-5% low). The reason for this is not known, but it may be related to the greater volatility of ammonia in fresh water, particularly at the higher pH obtaining, some ammonia escaping into the air space above the sample.

2. Distilled or fresh water samples display a slight yellow colouration at room temperature whereas sea water samples are clear blue. This yellow colouration gives a slight absorbance (not more than 0.005) at 630 nm and in effect counterbalances the salt error in distilled water reagent blanks at room temperature. After standing at room temperature for 5-10 minutes and warming at 70°C this yellow colour disappears. Hence batches of standards and samples in fresh water should always be run at the same temperature.

3. At zero time (i.e. before any colour has developed) both distilled and sea water samples show identical absorbance when turbidity and yellowing are subtracted. There is, therefore, no "optical blank" caused by the presence of the reagents in sea water.

4. The colour once formed in sea water (either at room temperature or at 70°C) remains constant for at least 68 hours, although turbidity due to magnesium hydroxide precipitation may occur in some samples which have been warmed. On the other hand, the colour formed in distilled water tends to fade slowly after about 4 hours.

5. Samples should not be treated longer than 30 minutes at 70°C. In the case of sea water samples, prolonged heating tends to precipitate magnesium hydroxide. With fresh water samples, prolonged heating decreases yield and increases the reagent blank.

6. The sensitivity of this method is higher than that of the method given in our manual (Major *et al.* 1972). The absorbance factor for 1 µg atom/l NH_3-N in a 10 cm curvette is at least 0.170. The precision is also much better. Within any one batch, this factor does not fluctuate beyond ±0.002. There is slightly more fluctuation between batches, but this diminishes with experience.

7. Analysis of samples should be carried out within 2 hours of collection. Since filtering usually introduces contamination, unfiltered samples must be used. Hence the need for a turbidity correction (Procedure, Step 7). If the samples cannot be analysed within 2 hours, they should be stored in a deep freeze (Degobbis 1972).

Reagents 1 and 2 may be added in reverse order without any adverse effect on sensitivity or precision. Hence it is possible to utilise the preservative effect of phenol as suggested by Degobbis (1972). In this case, Reagent 2 must be made up in two solutions, 7% phenol and 0.08% sodium nitroprusside. 2 ml of phenol solution per 50 ml of sample is added in the field. In the laboratory, a 50 ml aliquot is taken for analysis, and to this is added 2 ml of nitroprusside solution, 5 ml of

Reagent 1 and 2 ml of Reagent 3. The colour is then developed and read as usual.

Neither copper nor mercuric ions proved suitable as preservatives. Copper ions produce massive absorbance at 630 nm, whilst mercuric ions produce a yellow precipitate (at any dosage).

8. Precautions must be taken to avoid contamination with ammonia. No ammonia containing reagent should be kept in the laboratory, nor any cleansing agents. Samples should be exposed to the air as little as practicable.

9. The sodium dichloro-cyanurate reagent (dichloro-s-triazine-2, 4, 6 (1H, 3W, 5H)-trione, sodium form) is difficult to obtain as a pure reagent. We have found the technical grade of this compound, sold for disinfecting swimming pools, to be quite adequate. It is cheap and readily available.

10. This method is applicable to fresh water, sea water and brackish water down to about 17‰ salinity. Below this salinity magnesium hydroxide precipitates and the reaction is inhibited.

11. Sampling bottles must be scrupulously clean. If bacteria or organic matter are allowed to accumulate on the walls, substances giving an ammonia reaction are released by the reagents, and high results are obtained. It is recommended to wash the sample bottles thoroughly with detergent or dilute HCl after use, and to rinse them well with the water to be sampled before drawing samples for analysis.

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B. Preservation of samples for nitrate analysis

INTRODUCTION

Sea water samples collected and analysed for nitrate in this laboratory by the strychnidine method (Major *et al.* 1972) are preserved by the addition of 50 mg Hg Cl_2 to each 60 ml sample.

In view of the toxicity hazard of mercury, other means of preservation were investigated, in particular, lower levels of mercuric ion, freezing, cupric ions and the ammonia-ammonium chloride buffer described by Grasshof (1964).

EXPERIMENTAL

A bulk sample (18 l) was "spiked" with potassium nitrate to a concentration of 325 µg N/l. 200 ml aliquots from this bulk sample were then dispensed into a number of 250 ml capacity, all polythene, screw-cap bottles. These bottles were then divided into 7 batches and given the treatment indicated in Table 1. On day 7, 3 bottles of untreated sample were dosed to 2.5 mg % $CuSO_4.2H_2O$. By this time, the nitrate content of these untreated samples had fallen to less than half the original (and by 12 days had fallen almost to zero).

At intervals of 5, 12, 24, 34 and 181 days (9 and 12 days storage, 16 and 19 days from start, for the copper preserved samples) one bottle was selected at random from each batch and analysed for nitrate content by the cadmium-copper couple method described in Major *et al.* (1972). Each sample was passed through 2 of the 4 columns in use to obtain duplicate readings. The absorbances obtained are shown in Table 1. The overall precision was poorer than usually obtained with this method, in fact 4 of the 75 preserved samples showed quite aberrant readings. This is ascribed to the impairment of column function by the mercuric ions. Although the original cadmium method (Grasshof 1964) employed a cadmium-mercury couple, the presence of both copper and mercury in a column seems to cause erratic reduction. It is therefore not recommended to use mercuric ions as a preservative if the cadmium-copper couple method is used.

RESULTS

It may be seen that levels of mercuric ion 33 times lower (2.5 mg % v. 83 mg %) than those currently used are quite adequate to prevent loss of nitrate on storage.

For analysis by the cadmium-copper couple method, preservation by freezing, cupric ions or ammonia-ammonium chloride buffer is equally effective up to 5 months storage. Use of the Grasshof buffer is especially advantageous since it is a stable reagent, is not critical

in dosage, and simply transfers one step of the analysis to the field. The Grasshof buffer is interchangeable with the ammonium chloride buffer described in Major *et al.* (1972). (In sea water, the ammonia component is redundant and was omitted to minimise contamination of the laboratory atmosphere.)

Grasshof Buffer. Dissolve 250 g of NH_4Cl (A.R.) in 500 ml of deionised distilled water. Add 100 ml 6% W/V NH_4OH (A.R.) and mix. Make up to 1 litre.

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TABLE 1

Absorbances of samples preserved as described and analysed for nitrate content at various intervals.

Days	0	5	9	12	24	34	181
Treatment:							
None	.508 .511	.280 .264		.009 .009			
2.5 mg % $CuSo_4.2H_2O$	.232		.235	.250			
2.5 ml % Grasshof Buffer	.506 .506	.522 .514		.518 .528	.520 .528	.520 .520	.515 .510
Frozen	.514 .516	.506 .488		.506 .508	.523 .529	.522 .525	.515 .497
2.5 mg % $Hg\ Cl_2$	.505 .506	.520 .525		.520 .510	.522 .523	.520 .520	.515 .510
5.0 mg % $Hg\ Cl_2$	.505 .514	.520 .526		.521 .520	.521 .521	.520 .520	.520 .515
10 mg % $Hg\ Cl_2$	.512 .506	.526 .520		.515 .512	.520 .521	.520 .525	.525 .483
25 mg % $Hg\ Cl_2$	.509 .509	.518 .529		.520 .520	.530 .523	.510 .520	.517 .487