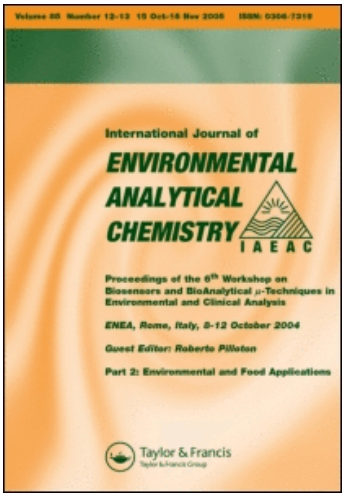

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A Method for the Determination of Particulate Organic Nitrogen in Natural Waters

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A Method for the Determination of Particulate Organic Nitrogen in Natural Waters

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A procedure is described for the determination of particulate organic nitrogen using Kjeldahl digestion in conjunction with measurement of ammonia by an indophenol-blue reaction. The method has a coefficient of variation of 1.5% at the 100-mcg nitrogen level. The detection limit is 3.5 mcg nitrogen. Beer's law is obeyed up to 100 mcg nitrogen. Quantitative recoveries of ammonia were obtained from glycine and glucosamine, but lower recoveries (77% to 87%) were obtained from arginine and histidine.

INTRODUCTION

The methods which have been used previously for the determination of particulate organic nitrogen (PON) in sea water were either elaborate[1,2] or required specialized equipment.[3] Strickland and Parsons[4] described in detail a micro-Dumas method and a Kjeldahl method for PON; the latter after Holm-Hansen[5] is suitable for low concentrations of PON. The simpler and more direct procedure discussed in this note has been adapted from a method developed for blood analysis by Andrews.[6] It was found suitable for eutrophic waters.

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EXPERIMENTAL

Initial experiments were made with a standard ammonium sulphate solution, using the conditions given by Andrews,[6] which are as follows. To aliquots of the solution 1 ml acid digestion mixture (0.04% SeO_2 + 20% H_2SO_4) was added, followed by 10 ml alkali-phenol (25 g phenol + 56 g KOH per litre) and 10 ml chloramine-T (45 g chloramine-T† + 213.3 g K_3PO_4 per litre); the solution was heated at 100°C for 20 min and the optical density was measured in a 1-cm cell at 630 nm. It was found that the order of the addition of the alkali-phenol and chloramine-T reagents was not critical, in contrast with the results of Bolleter *et al.*[7] who used hypochlorite. If the period between addition of these reagents exceeded 5 min, some loss of ammonia occurred, but the loss did not exceed 15% even with a 1-hr interval during which the solution was heated and shaken.

The effect of the period of heating after the addition of both reagents on the colour development was examined and the results are shown below:

Period of heating (min)	5	10	15	20	30	60
Optical density with 100 mcg nitrogen (as NH_4^+, corrected for blank)	.320	.420	.460	.475	.480	.460

In all subsequent work, a heating period of 20 min was used. After cooling, the colour was stable for at least 2 hr and it had decreased by only 5% after 24 hr in the dark.

The effect of varying the concentrations of acid, alkali, phenol, phosphate, and chloramine-T on colour development was investigated. The concentration of each reagent was varied in turn while keeping the concentrations of the other reagents at the levels given above. The results (Table I) show that the concentration of potassium hydroxide could be reduced to 80% of that originally used without reducing the sensitivity, and this modification was made in subsequent work. Hence, the concentration of sulphuric acid could also have been reduced, but the original concentration was used in order to maintain the digestion efficiency. The concentration of the other reagents appeared to be at optimal levels.

PRECISION, LIMIT OF DETECTION, BEER'S LAW AND ACCURACY

Replicate analyses, including digestion of blank filters and aliquots of the ammonium sulphate standard (containing 70 mcg nitrogen), gave a coefficient

† Sodium N-chloro-*p*-toluene sulphonamide.

TABLE I

Optical densities with 50 mcg nitrogen (as NH_4^+, corrected for the blank)

Reagent	Per cent of the original reagent concentration				
	20	60	80	100	120
H_2SO_4	0.060	0.180	0.220	0.210	0.080
KOH	0.190	0.220	0.220	0.210	0.155
Phenol	0.080	0.145	0.165	0.210	0.235
K_3PO_4	—	0.055	0.140	0.210	0.160
Chloramine-T	0.130	0.180	0.210	0.210	0.200

of variation of 1.5%. For blanks the corresponding value was 5%. The detection limit, as defined by Wilson [8], at the 99% probability level is 3.5 mcg nitrogen. Beer's law is obeyed up to at least 100 mcg nitrogen. Recoveries from four amino acids were tested as measures of accuracy and the following results were obtained: glycine, 100%; glucosamine, 100%; arginine, 77–87%; histidine, 87%.

METHOD

All measurements were made on a Unicam SP500 spectrophotometer.

Reagents

Ammonia-free distilled water Distilled water was passed through a 3 $cm^2 \times$ 20 cm column of Zeo-Karb (14–52 mesh) in the H form immediately before use for the preparation of reagents, blanks and standards.

Digestion mixture 200 ml of analytical-reagent-grade conc. sulphuric acid (s.g. 1.98) was added to 600 ml water and 0.4 g selenium dioxide. This mixture was cooled, made up to 1 litre with water, and kept in an amber glass bottle.

Alkaline-phenol 25 g analytical-reagent-grade phenol was dissolved in 1 litre of water together with 45 g analytical-reagent-grade potassium hydroxide. This reagent should be kept in an amber glass bottle.

Chloramine-T 212 g anhydrous tri-potassium orthophosphate together with 45 g chloramine-T (iodometric assay 98–103%) was dissolved and made up to 1 litre with water. This reagent should be filtered through a glass-fibre filter and kept in an amber glass bottle.

Stock standard solution (500 *mcg* NH_4-*N*/*ml*) 1.18 g of the dried (105°C for 2 hr) analytical-reagent-grade ammonium sulphate was dissolved in 500 ml water. This solution should be kept in a dark glass bottle to which 1 ml chloroform has been added.

Working standard solution (100 *mcg* NH_4-*N*/*ml*) This solution was freshly prepared from the stock solution.

Recommended Method

An appropriate volume of water to give 20–100 mcg nitrogen was filtered under a vacuum of one third atmosphere (to decrease cellular damage and release soluble contents), through a Whatman glass-fibre filter GF/C (Schleicher & Schull No. 6 was found to be of equal quality) which had been combusted at 500°C for 2 hr. Higher temperatures should be avoided as the filter sinters and alters its porosity. The residues should be preserved under deep-freeze if not analyzed within a few hours. The filter was transferred to a 2.5 × 20 cm thick-walled hard glass test tube, 1 ml of the digestion mixture was added, the tube was covered with a glass stopper and the contents were digested on an aluminium heating block for 2 hr at about 300°C. After cooling, 10 ml alkaline phenol reagent was added, followed by 10 ml chloramine-T reagent, shaking well after each addition. The tube was transferred to a water-bath at 90–100°C for 20 min. The solution was either centrifuged or filtered through a glass-fibre filter to remove suspended fibres. The solution was made up to volume and its extinction was measured at 630 nm in a cell of appropriate path-length. In waters with a high load of particulate organic matter, charring may occur during digestion. This may be overcome by careful addition of 30% hydrogen peroxide (1 ml) and further digestion for 1 hr. Calibrate the method using 25, 50, and 100-mcg amounts of NH_4-N. The sensitivity of the method may vary slightly and a standard should be run with each batch of samples. Run a blank on ammonia-free distilled water.

DISCUSSION

The method proved satisfactory in routine use in this laboratory over a period of two years. During this time Holm-Hansen[5] described a ninhydrin-hydrindantin method, which was compared with the present method and found to be about 10 times more sensitive. This has advantages for very oligotrophic waters but in most situations the simpler method given here has adequate sensitivity.

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