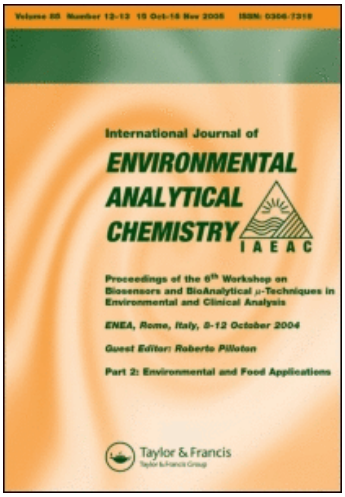

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DETERMINATION OF NITRATE IN SMALL WATER SAMPLES (100 μL) BY THE CADMIUM–COPPER REDUCTION METHOD: A MANUAL TECHNIQUE WITH APPLICATION TO THE INTERSTITIAL WATERS OF MARINE SEDIMENTS

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The standard technique for the analysis of NO_3^- in seawater and the interstitial waters of marine sediments is the cadmium–copper reduction method. The manual NO_3^- method presented here provides a technique that is simple, cost-effective and capable of analysing small volumes (100 μL). The technique is also suitable for analysing NO_3^- in DET (diffusive equilibration in thin films) gel samples. Reduction of NO_3^- to NO_2^- is achieved by drawing the sample into contact with a copper-coated cadmium wire using a micrometer burette. The reduced sample is then expelled and NO_2^- is determined colorimetrically. The method has a concentration range of 0–100 μM NO_3^- and a detection limit ($2s_b$) of 0.4 μM NO_3^-. Eight samples can be processed per hour.

Keywords: Marine; Interstitial water; Nitrate; Colorimetric method; Small volume

INTRODUCTION

Sampling nitrate at high resolution in the marine environment, for example in sediment interstitial waters using DET (diffusive equilibration in thin films) [1], can require the analysis of small volume (100 μL) saline samples.

The most sensitive and widely used methods for the analysis of NO_3^- in seawater are based on the heterogeneous reduction of NO_3^- to NO_2^-, which is then determined by the formation of a highly coloured azo dye [2,3]. Any NO_2^- present originally in the sample must be corrected for. The reduction, which is achieved using either metallic cadmium granules or powder, has been used by a number of workers [4–7]. Text books detailing methods of seawater analysis routinely quote the cadmium–copper reduction method [8,9]. Salt effects using the cadmium–copper reduction method are negligible and interference from other constituents of seawater (excluding NO_2^-) does not occur. Although samples containing high sulphide concentration

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will increase the rate of column deterioration as a result of Cd and Cu sulphide precipitates forming on the surface of the reductor [7].

The application of the cadmium–copper technique to small volumes has been achieved using flow injection analysis (FIA). For example, the FIA system of Daniel *et al*. [6] uses a sample volume of 100 μL and gives a working range of 0–100 μM, a reduction efficiency of close to 100%, a detection limit ($2\sigma_b$) of 0.3 μM and a sampling rate of 45 per hour. However, due to the relatively complex nature of the manifold, the construction and maintenance of FIA systems can be time consuming. Furthermore, FIA methods are not low cost because they require the use of a peristaltic pump and computer software. These methods are also problematic when determining NO_3^- in DET gel samples. Since it is not possible to separate the gel from the solution into which the sample must be back-equilibrated (separation techniques result in loss of sample volume and increase the likelihood of contamination), it is inevitable that small fragments of gel are occasionally introduced alongside the solution. From the authors' experience of using FIA to detect Fe in DET gel samples, analysis can be seriously disrupted by the accidental injection of gel fragments. For example, blockages caused by gel fragments within the manifold result in instabilities in flow rate, and if a gel fragment enters the spectrophotometer cell, absorbance readings can be directly affected. Attempts to employ in-line traps or filters have proved unsuccessful. These either detrimentally reduce the sample volume entering the manifold, or become clogged, affecting analytical reproducibility and necessitating cleaning.

Manual non-FIA systems are comparatively low-cost, and are simpler to construct and maintain than FIA systems and manual systems are also better able to cope with the occasional introduction of DET gel fragments. Typically, manual methods have a detection limit of 0.04–0.1 μM, precision of 0.04–0.5 μM, reduction yields of 70–100%, and a working range of 0.04–60 μM [4,5,7]. However the systems are designed to work using large (> 25 mL) sample volumes.

The method presented here is based on the manual method of Hansen and Koroleff [7], with modifications to enable high precision results to be obtained using a sample volume of 100 μL, without the relatively high cost and high maintenance problems associated with the FIA methods described above.

The main modifications applied to the Hansen and Koroleff [7] method are as follows: (1) In order to reduce sample volume, the glass reductor column of 3–5 mm i.d. (internal diameter) is replaced with a plastic (pump) tube of 1.3 mm i.d. (2). The cadmium granule reductor is replaced with a 1 mm diameter cadmium wire reductor because the i.d. of the column was considered too small to enable packing with Cd granules (3). In order to preserve sample volume, the sample is not mixed with buffer solution prior to entering the reductor column. Instead the sample is mixed with buffer during and after the reduction step (4). Small volumes (100 μL) of sample are precisely drawn into, and then out of, the reductor column using a micrometer burette rather than large (50 mL) volumes of sample being passed through the column using a pump (5). Instead of the sample moving continuously though the reductor column in a single direction, the sample remains in stationary contact with the reductor for 45 s before being expelled into the reaction vessel via a single inlet/outlet (6). The concentrations of sulphanilamide (SAN) reagent and naphthylethylenediamine dihydrochloride (NED) reagent were reduced by 50% in order to increase the volume thus enabling more precise volumes to be pipetted.

EXPERIMENTAL

Preparation of Reagents

Reagents were prepared as follows using analytical grade chemicals and deionized water (Millipore Milli-Q water system):

Ammonium chloride buffer was made up by dissolving 10 g of ammonium chloride in 0.9 L of deionized water. The solution was adjusted to pH 8.5 using 30% ammonia solution and made up to 1 L using deionized water.

Sulphanilamide (SAN) reagent was made up by dissolving 5 g of sulphanilamide in 0.8 L of deionized water, then 50 mL of concentrated HCl was added and the solution was make up to 1 L with deionized water and 2 mL of Brij 35 (wetting agent, sigma). Naphthylethylenediamine dihydrochloride (NED) reagent was made up by dissolving 0.5 g of NED in 1 L of deionized water.

100 mL of copper sulphate solution was made up by dissolving 2 g of $CuSO_4 \cdot 7H_2O$ in deionized water.

1 L of artificial seawater (ASW) was made up by dissolving 31.31 g NaCl, 6.82 g $MgSO_4 \cdot 7H_2O$ and 0.0412 g of $NaHCO_3$ in deionized water.

1 L of 2 M HCl was made up using concentrated HCl and deionized water.

Standards were prepared from 1000 μM stock solutions: stock solutions of NO_3^- and NO_2^- were prepared using $NaNO_3$ and $NaNO_2$ respectively, dissolved in either deionized water or ASW.

Construction of Nitrate Reduction System

The nitrate reduction system (Fig. 1) consists of a Cu-Cd reductor column, a 200 μL micrometer burette for drawing up precise small volumes of sample, a sample inlet/outlet, and a 10 mL syringe for flushing the system. Connections between system parts were constructed using three-way valves (Cole-Parmer, manual manifold valves) and sealed using cyclohexanone.

The reductor column used was a plastic (pump) tube with an i.d. of 1.3 mm and a length of about 30 cm. One end of the column was connected via a three-way valve

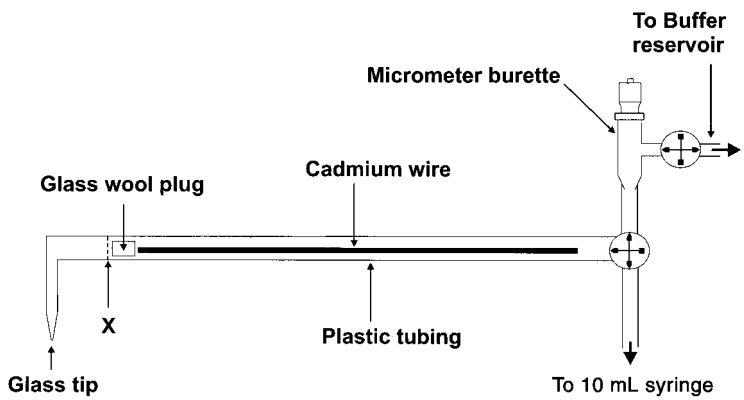

FIGURE 1 Schematic representation of micro-nitrate reduction system. Location *X* shows the position of the meniscus when the sample is drawn into the reductor column.

to the micrometer burette and a detachable 10 mL syringe, the other end of the column was left open (Fig. 1). The micrometer burette was additionally connected to a reservoir of buffer solution via a second three-way valve. The reductor column was filled with a cadmium wire (1 mm diameter, Newmetals and Chemicals, about 26 cm length), and a small (5 mm) plug of glass wool was inserted in the open end of the tube. A curved glass tip of i.d. 1.3 mm and length 5 cm was inserted against the glass wool plug to provide the sample inlet/outlet. The end of the tip was narrowed to < 0.1 mm i.d. to limit the introduction of DET gel fragments. When the construction of the system was complete, the column was taped to a solid horizontal surface in order to prevent movement.

The cadmium wire was treated with 2 M HCl by slowly drawing 6 mL of acid through the column (approximately 1 mL per minute) using the 10 mL syringe. The column was then flushed with deionized water until the solution leaving the column was no longer acidic, the wire was then treated with 1.5 mL of $CuSO_4$ solution and flushed with buffer solution. The reductor was finally activated by passing about 3 mL of 100 μM NO_3^- solution through the column.

Determination

100 μL of sample was drawn in through the glass tip using the micrometer burette. This sample aliquot was then drawn up further so that the meniscus of the sample was positioned immediately in front of the glass wool plug (position X, Fig. 1). The sample was left in contact with the Cd wire for 45 s then ejected, via the inlet/outlet glass tip, into the reaction tube. Immediately following the expulsion of the reacted sample, 100 μL of buffer from the buffer reservoir was added to the sample, again via the glass tip. Between samples, the column was slowly flushed with 2 mL of buffer solution using the syringe.

The reduced sample was mixed with 12.5 μL of SAN reagent, and after about 1 min, 12.5 μL of NED reagent was added. Using a pipette, the sample was transferred into a spectrophotometer cell (internal dimensions of 2 mm width, 28 mm height) of path length 10 mm. Absorbance was measured within 2 h at a wavelength of 536.5 nm. The cell was washed several times with deionized water between samples.

Total volume in contact with the Cd wire within the reductor column was calculated to be ~ 135 μL. The volume taken up by the glass wool plug (~ 6 μL) together with the estimated volume of stationary buffer pools in contact with the Cd wire (~ 5 μL), gave a total dead-volume of 11 μL, i.e. $\sim 10\%$ of the sample volume. However, this value is likely to vary slightly according to the exact positioning of the Cd wire and glass wool plug within the tube.

A calibration was performed whenever a new reductor was used. Following this initial calibration, blanks and check standards of NO_3^- made up in ASW were run after every 6–10 samples during analysis in order to check for baseline drift and changes in column sensitivity. Depending on the sample absorbance values, low (1.5 μM) and higher (20 μM) standards were employed.

Test for Detection Limit and Determination of Blanks

Nitrate concentrations were determined for ten consecutive blank samples. From the results, the detection limit ($2s_b$) was calculated. The level of contamination in the

blank samples was determined by subtracting the absorbance value of the sample matrix from that of the procedural blank.

Test for Precision

Precision (s) was determined at two levels, 1.5 and 20 μM NO_3^-. Ten determinations were performed at each level.

Test for Stability and Range

Stability was determined for standards in the range 10–100 μM NO_3^-. Absorbance values were taken at time intervals over a period of 3 h for standards made up in both ASW and deionized water.

Test for Carry-over

Carryover was determined by running one high concentration (100 μM) NO_3^- sample followed by a blank (B1) followed by a second blank (B2). This sequence of high standard, blank, blank, was repeated six times. The 10 mL syringe was used to slowly (at approximately 1 mL per minute) flush the column with buffer solution. Carryover was determined for flush volumes of 1, 2 and 3 mL of buffer solution. A *t*-test was employed to determine the significance of the difference in the means of the B1 set and the B2 set. If the difference was significant, the amount of carryover was calculated as shown:

$$\text{Carryover} = [(\text{B2}_\text{A} - \text{B1}_\text{A})/(100\,\mu\text{M } NO_3^-{}_\text{A} - \text{Bl}_\text{A})] \times 100\%$$

Test for Efficiency

Column efficiency was determined by comparing the absorbance of a standard of 20 μM NO_3^- (minus the blank) analysed as described above, with a 20 μM NO_2^- solution and corresponding blank analysed without the column. The cadmium treatment and activation procedure was repeated if efficiency dropped below 70%.

$$\text{Column efficiency} = \{[20\,\mu\text{M } NO_3^-)_\text{A} - (NO_3^- \text{ blank})_\text{A}]/[(20\,\mu\text{M } NO_2^-)_\text{A} - (NO_2^- \text{ blank}_\text{A}]\} \times 100\%$$

Sampling of a DET Gel Probe

A DET gel probe was deployed in a sea loch (Loch Duich) as described in Mortimer *et al.* [10]. Prior to analysis, individual gel samples of 15–25 μL were back equilibrated into 220 μL of artificial seawater for 24 h and the solutions analysed for NO_3^- as described above.

RESULTS AND DISCUSSION

Detection Limit and Blank Values

The detection limit ($2s_b$) was determined to be 0.4 μM NO_3^-. The level of contamination determined for the procedural blank was ~0.2 μM NO_3^- (blank absorbance value ~0.094, matrix absorbance value 0.091). A major source of contamination was identified as latex powdered gloves and blue nitrile gloves. Their use was therefore discontinued.

Precision, Range and Sample Stability

Precision(s) at the 1.5 μM NO_3^- level was ±0.2 μM (relative standard deviation (RSD) = 12%) and precision at the 20 μM NO_3^- level was ±0.4 μM (RSD = 2%). An example of a calibration plot is given in Fig. 2. Absorbance values for standards in the range 10–100 μM NO_3^- made up in both deionized water and artificial seawater remained stable for three hours.

Rate of Determinations

The number of samples that can be processed in one hour is about eight. Using a microplate spectrophotometer rather than a manual spectrophotometer would reduce the amount of time required for analysis somewhat.

Carryover

Carryover was determined to be 0.3% for flush volumes of 1 mL, but not significant ($P < 0.05$) for flush volumes of 2 mL.

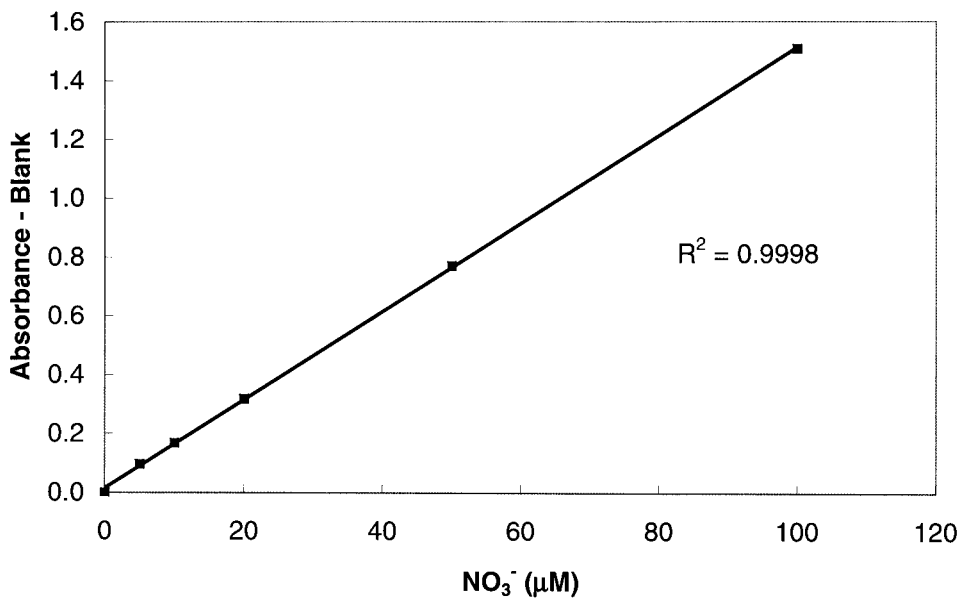

FIGURE 2 Example of a calibration plot.

Efficiency

During column operation efficiency was maintained at 70–90%, below 70% efficiency the column was reactivated. The dead-volume of ~10% (see method section) within the reductor column is likely to contribute towards the comparatively low reduction efficiency of the technique [7]. The column performed best when in continual use; over 300 samples in the low to medium NO_3^- concentration range were processed before column reactivation was required.

Sensitivity Maintenance

Occasionally decreases in the sensitivity of the column were followed by gradual increases in column sensitivity. Column reactivation was therefore not undertaken unless the sensitivity remained permanently low. Such changes in column sensitivity may have been caused by sulphide present in the anoxic pore water samples. If the column had not been in use for several hours (e.g. overnight), reactivated was performed by passing through 3 mL of 100 μM NO_3^- solution.

Application

Determinations of nitrate plus nitrite (ΣNO_3^-) were made for samples from a DET probe that had been deployed in a sea loch. The abundance of nitrate in marine pore waters is typically much greater than that of nitrite [11,12], therefore any significant variations in ΣNO_3^- with depth are likely to be due to changes in the concentration of nitrate rather than nitrite. The results, shown in Fig. 3, clearly show a sharp sub-surface increase in ΣNO_3^- from 0 to 4 mm followed by a more gradual decrease in concentration over the interval 4–20 mm. This profile reflects the net results of microbial nitrification and denitrification processes driven by the bacterial oxidation of organic matter [13]. Micro-electrode results indicate that the depth of the surface oxic layer for sediment cores at this sampling site is 2–4 mm (Anschutz, personal communication). The sub-surface increase in ΣNO_3^- (0–4 mm) therefore results from the aerobic oxidation of NH_4^+ (a product of organic matter degradation) to NO_3^- by nitrifying bacteria living in the upper oxic zone [14]. The decrease in ΣNO_3^- below 4 mm then reflects the sub-oxic process of denitrification where bacteria use NO_3^- in place of O_2 to degrade organic matter, reducing it N_2 in the process [14]. The detection limit (dashed line in Fig. 3) for the gel technique is not constant due to variations in individual gel volume, and is elevated due to the necessity of sample dilution.

The application of the high-resolution DET techniques to measuring NO_3^- in marine sediment has potential in terms of calculating NO_3^- fluxes. A more exciting potential use, however, is in the investigation of new biogeochemical interactions [15,16] which may produce boundary effects between the conventional diagenetic reaction zones. In particular DET has been used to provide evidence for 'sub-oxic nitrification' [17].

Summary

The method provides a simple, reliable, cost-effective means of analysing nitrate in small volume samples of saline water. Analysis of DET gel samples has been shown to be one successful application of this method.

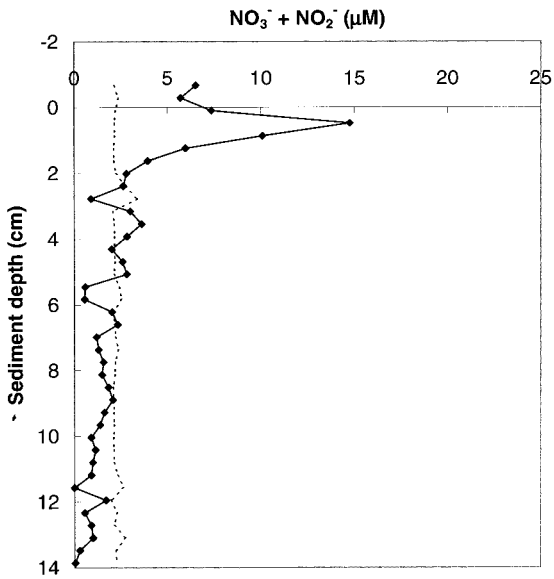

FIGURE 3 DET pore water profile of nitrate plus nitrite (ΣNO_3^-) in a sediment core taken from Loch Duich. The dashed line indicates the detection limit ($2s_b$).

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References

[1] W. Davison, G.W. Grime, J.A.W. Morgan and K. Clarke, *Nature*, **352**, 323–325 (1991).
[2] M.B. Shinn, *Ind. Eng. Chem. Anal. Ed.*, **13**, 33–35 (1941).
[3] K. Bendschneider and R.J. Robinson, *J. Mar. Res.*, **11**, 87–96 (1952).
[4] A.W. Morris and J.P. Riley, *Anal. Chim. Acta*, **29**, 272–279 (1963).
[5] E.D. Wood, F.A.J. Armstrong and F.A. Richards, *J. Mar. Biol. Ass. U.K.*, **47**, 23–31 (1967).
[6] A. Daniel, D. Birot, M. Lehaitre and J. Poncin, *Anal. Chim. Acta*, **308**, 413–424 (1995).
[7] H.P. Hansen and F. Koroleff, In: K. Grasshoff, K. Kremling and M. Enhrhardt, (Eds.), *Method of Seawater Analysis*, 3rd Edn., pp.159–228, Wiley-VCH, Weinheim (1999).
[8] J.D.H. Strickland and T.R. Parsons, *A Practial Handbook of Seawater Analysis*, 2nd Edn. 311pp. Fisheries Research Board of Canada, Ottawa (1972).
[9] T.R. Parsons, Y. Maita and C.M. Lalli, *A Manual of Chemical and Biological Methods for Seawater Analysis*, 173 pp. Pergamon Press Ltd., Oxford (1984).
[10] R.J.G. Mortimer, M.D. Krom, P.O.J. Hall, S. Hulth and H. Stahl, *Mar. Chem.*, **63**, 119–129 (1998).
[11] U. Toshihiro, I. Koike and N. Ogura, *Mar. Chem.*, **59**, 253–270 (1998).
[12] I. Koike and K. Terauchi, *Mar. Chem.*, **52**, 185–193 (1996).
[13] P.N. Froelich, G.P. Klinkhammer, M.L. Bender, N.A. Luedtke,G.R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartman and V. Maynard, *Geochim. Cosmochim. Acta*, **43**, 1075–1090 (1979).
[14] T. Fenchel, G.M. King and T.H. Blackburn, *Bacterial Biogeochemistry: The Ecophysiology of Mineral Cycling*, 2nd Edn., pp. 307. Academic Press: San Diego (1998).
[15] G.W. Luther III, B. Sundby, B.L. Lewis, P.J. Brendel and N. Silverberg, *Geochim. Cosmochim. Acta*, **61**, 4043–4052, (1997).
[16] P. Anschutz, B. Sundly, L. Lefrancois, G.W. Luther III and A. Mucci, *Geochim. Cosmochim. Acta*, **64**, 2751–2763 (2000).
[17] R.G.J. Mortimer, M.D. Krom, S.J. Harris, P.J. Hayes, I.M. Davies, W. Davison and H. Zhang. *Mar. Ecol. Prog. Ser.*, **236**, 31–35 (2002).