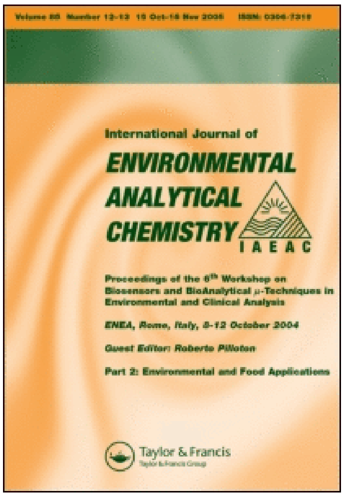

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DETERMINATION OF NITRATE IN EUTROPHIC COASTAL SEAWATER BY REDUCTION TO NITRITE WITH HYDRAZINE

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The determination of nitrate as nitrite in seawater remains the method of choice. The widely differing conditions recommended hitherto for the reduction of nitrate to nitrite with hydrazine sulphate (HS), and the consequent erratic results, are shown to be due primarily to the sensitivity of the reduction to uncontrolled non-equilibrium fluctuations in the concentration of oxygen in samples, standards and dilution water. Ca^{2+} and Mg^{2+} ions, also responsible for complete suppression of the reduction, were precipitated and further inactivated by pyrophosphate. Reduction by HS compares well with the cadmium reduction column and avoids the generation of cadmium-rich column effluent.

KEY WORDS: Nitrate determination, nitrate reduction, eutrophic seawater.

INTRODUCTION

Nitrate in (sea)water is a useful pollution control parameter and probably forms the principal limiting factor controlling the primary productivity of phytoplankton in euphotic zones.

The intensely coloured azo derivative formed on diazotisation of sulphanilamide followed by coupling with N-(1-naphthyl)ethylenediammonium dichloride has often been used for the determination of low concentrations of nitrate. The preliminary reduction stage of nitrate to nitrite is usually performed with cadmium[1–3]. Reduction with hydrazine sulphate (HS) is a less frequently applied procedure[4–6] owing to the capriciousness of the reduction efficiency. The reduction is based on the conversion of nitrate to nitrite by HS, catalysed by copper ions, under alkaline conditions and at elevated temperatures. Concentrations of the reagents, pH, reduction time and temperature strongly influence the conversion rate and can sometimes lead to destruction of the nitrite, probably by encouraging reduction beyond the nitrite stage. In a previous investigation[7] the factors responsible for the capriciousness of the homogeneous catalysed reduction of nitrate by HS were identified, resulting in a reliable and accurate manual analytical procedure. The purpose of the present investigation was to assess the possibility of determining nitrate in eutrophic seawater by means of the improved HS procedure, in order to present a manual technique which is comparable and complementary to the automated reduction by cadmium.

In addition, the use of HS constitutes an alternative in laboratories where Cd pollution due to the generation of Cd-rich effluent from Cd columns is undesirable.

EXPERIMENTAL

Reagents

All chemicals were of analytical reagent grade. The following reagent solutions were prepared using doubly distilled water.

1) Alkaline sodium carbonate solution. Sodium carbonate (3 g) and sodium hydroxide (11.20 g) were dissolved in water and diluted to 100.0 ml.

2) Copper-pyrophosphate solution. Sodium pyrophosphate decahydrate (1 g) was dissolved in water to which was added sodium hydroxide (988 mg), followed by 0.02 M copper(II) sulphate solution (142 μl). This solution was diluted to 100.0 ml and stored in a refrigerator.

3) Stock hydrazine sulphate (HS) solution. HS (108 mg of $N_2H_4.H_2SO_4$) was dissolved in 10.0 ml of water in a 10-ml glass tube; during the dissolution, a slow stream of N_2 gas (*ca.* 1 ml s^{-1}) was bubbled through the solution by means of a Pasteur pipette. Since HS attacks cork and rubber, these materials are not to be recommended as stoppers. The solution was stored in a refrigerator and the air above the solution was kept oxygen-free by introducing N_2 gas each time the glass tube was opened.

4) Sulphanilamide solution. Sulphanilamide (400 mg) was dissolved in water containing concentrated hydrochloric acid (36%, 35 ml) and diluted to 100 ml.

5) N-(1-Naphthyl)ethylenediamine dihydrochloride solution (NED). NED (120 mg) was dissolved in water (100 ml) and stored in a refrigerator.

Procedure

Determination of the optimum concentration of HS: 100.0 ml of doubly distilled water was transferred to a centrifuge tube and de-oxygenated for about 30 min by the introduction of N_2 gas (*ca.* 7 ml s^{-1}). Solutions (0.6 ml containing 60 nmol of nitrate) were pipetted, in duplicate, into a series of 10 glass tubes (volume 10 ml). Reagent 2 (200 μl) was added (while avoiding an increase in the oxygen content by shaking or vortexing). Reagent 3 (300 μl) was pipetted into a separate 10-ml glass tube, diluted to 3.00 ml with de-oxygenated water and mixed by a reduced stream of N_2. The air above the nitrate containing solutions in the glass tubes was replaced by N_2, and 2.9 ml of de-oxygenated water was added followed by the diluted HS (80 μl in the first tube, with successive 7 μl increments for the following tubes, resulting in a total of 143 μl for the tenth tube). Immediate mixing took place by the passage of N_2 gas (*ca.* 7 ml s^{-1}) for a period of *ca.* 6 s. The tubes were then placed in a water bath (47 ± 3°C) within 30 min after mixing, and maintained at this temperature for a period of at least 90 min. After removal from the bath the solutions were treated

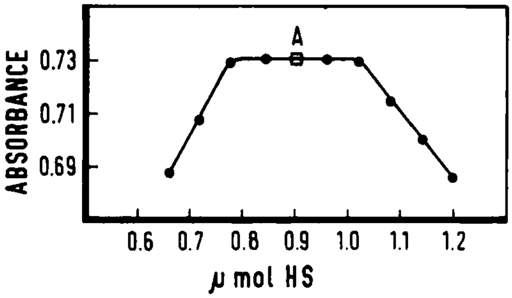

Figure 1 Absorbance curve of 15 μM nitrate versus HS concentration. The optimal amount of HS is indicated by A.

with reagent 4 (100 μl; direct vortexing) and reagent 5 (100 μl). The absorbance was measured at 540 nm in a 1-cm cell (Shimadzu UV-120 spectrophotometer) at least 10 min after vortexing of reagent 5. The coloured complex remained stable for at least 6 h. The optimum range of HS concentrations is that which gives the highest possible reduction of nitrate to nitrite and hence the highest possible absorbance (Figure 1). The amount of HS to be used for the determination of nitrate in seawater samples, standards and blanks is given by the centre (A) of the optimum range (the optimum amount of HS appeared to be the same using a nitrate standard in distilled water or seawater). The determination of this optimum amount of HS should be repeated each month and upon renewal of reagent 3. Sub-optimum concentrations of HS are indicated by a decrease in the reproducibility and absorbance values of a standard nitrate solution.

Determination of nitrate in seawater

Ca^{++} and Mg^{++} (which interfere with the reduction) were separated from the sample by adding 200 μl of reagent 1 to 5.00 ml seawater in a 10-ml centrifuge tube. After mixing, the carbonates and hydroxides of calcium and magnesium were precipitated upon standing or by centrifuging. Standards or samples, up to 0.60 ml in volume and containing not more than 80 nmol of nitrate, were pipetted into 10-ml glass tubes and diluted with water to give a volume 0.60 ml (any transfer of Ca-Mg precipitate was avoided). Reagent 2 (200 μl) was added (no mixing). The air above the solutions in the glass tubes was replaced by N_2 and 3.00 ml of de-oxygenated distilled water (containing the optimum amount of HS) was added. Then the procedure described above was followed.

RESULTS AND DISCUSSION

Calibration graph and absorbance

The absorbance measured using the conditions described in the Experimental section was 0.73 (SD = 0.009, RSD = 1.2%; n = 8) for 15 μM nitrate standard in the final volume, with a detection limit (defined as twice the standard deviation of the blank multiplied by the obligatory dilution factor of the sample in the final solution) of 0.9 μM in seawater samples. A calibration graph is presented in Figure 2. The procedure allows for 100–150 determinations per day.

Influence of oxygen and HS concentration

The tolerance for variations in the redox potential required for optimal reduction of nitrate to nitrite was small: variations should not exceed a maximum of 15% of the optimum HS concentration described in the procedural section. A lower HS concentration results in a decrease in the conversion of nitrate to nitrite, while a higher concentration reduces nitrite further to a lower oxidation state. Owing to the reaction

$$N_2H_4 + O_2 \rightarrow N_2 + 2H_2O$$

it was found that an uncontrolled fluctuation of more than 0.1 μmol O_2 during the reduction (see also Figure 1) results in a decrease in the formation of nitrite. Fluctuations in oxygen content originate from e.g. the sample volume, and in general are held within a limit of 0.1 μmol., which presupposes a high level of reproducibility in analytical execution. The volume of 3.00 ml de-oxygenated water (containing the optimum amount of HS) was introduced as a compromise between a minimum required amount of buffer volume, decreasing the percentage of HS which is subject to uncontrolled fluctuations in oxygen content in the sample volume (0.6 ml), and a restriction in the dilution of the sample.

Other interferences

Mg^{++} and Ca^{++} strongly influence the reduction and their abundance in seawater requires their removal from samples. Up to a maximum of 53.5 mM magnesium and 11.3 mM calcium (representing the average concentration in seawater with 35‰ salinity[8]) in a 5 ml sample can be precipitated by 0.2 ml of reagent 1. Samples with

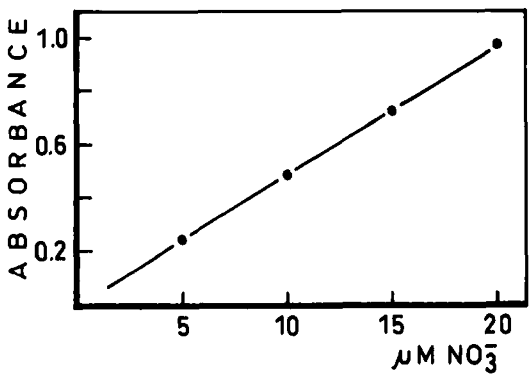

Figure 2 Nitrate concentration in the final solution versus absorbance (1 cm cuvet).

higher concentrations of magnesium and calcium should be treated with proportionally increased amounts of, respectively, hydroxide and carbonate. Magnesium and calcium remaining in solution after the pretreatment with reagent 1 are complexed with pyrophosphate (reagent 2) up to a limit of 40 μg magnesium or 100 μg calcium. A combination of 30 μg magnesium and 80 μg calcium was found not to interfere with the reduction. Other mixtures were not investigated. However, the pH range within which the reduction of nitrate is optimal is restricted and is equivalent to NaOH concentrations between 12 and 30 mM. During the reduction the concentration of hydroxide originating from reagent 2 is 13 mM. Therefore, the maximum tolerated concentration of hydroxide originating from reagent 1 and remaining in solution after precipitation of magnesium, is restricted to 17 mM during the reduction (the highest amount of NaOH remains only in fresh water samples low in magnesium content). The pretreatment of fresh water samples with reagent 1 can even be omitted if the amount of magnesium present during the reduction is <65 μg or that of calcium is <220 μg, provided the amount of pyrophosphate in reagent 2 is raised to 2.4 g. Higher amounts of pyrophosphate interfere with the reduction.

A minor decrease in the optimum concentration of HS takes place when the concentration of NaOH is raised from 13 to 30 mM. If required, more precision in the determination of nitrate may be obtained by adjusting the amount of reagent 1 according to the salinity of the sample (a linear relationship exists between the salinity and the required amount of reagent 1, which rises to 200 μl for samples with 35‰ salinity containing 53.5 mM magnesium and 11.3 mM calcium).

Copper complexation

The optimum concentration of copper, which acts as a catalyst in the reduction of nitrate, ranges from 1.27 μM to 1.72 μM. 1.5 μM was chosen during the reduction. Since the tolerance for variations in copper concentration is restricted and since the copper concentration in the 0.02 M stock solution (see reagent 2) might be subject to changes in time, it is advisable to redefine the optimal copper concentration in reagent 2 bimonthly by means of a copper curve. In general, the amounts of chelating compounds in fresh water and seawater are so small that the added amount of copper in reagent 2 is not affected.

In general, it is recommended to control regularly reagents and procedure by means of spiking.

Comparison with the nitrate reduction by cadmium, and spiking of samples

Nitrate reduction with HS was compared with the copperized cadmium reduction column technique in use at the Delta Institute for Hydrobiological Research in Yerseke (the Netherlands). Samples originating from the Westerschelde estuary and North Sea were determined and both methods showed excellent agreement (Table 1). The same variety of samples was spiked with a fixed amount of nitrate (the natural concentration of nitrate in the samples was artificially increased with 100 μM) and the results are shown in Table 1.

Table 1 Comparison of nitrate concentration (in μM) in seawater samples (SS) determined with the cadmium reduction method (Cd) and the HS reduction method (HS), and total nitrate concentration (TN) after spiking with 100 μM. Salinity (SAL) in ‰

SS	*SAL*	*Cd*	*HS*	*TN*	*SS*	*SAL*	*Cd*	*HS*	*TN*	*SS*	*SAL*	*Cd*	*HS*	*TN*
1	20.0	197	207	314	9	8.0	223	224	319	17	24.6	89	95	193
2	22.9	144	150	254	10	5.9	206	205	306	18	19.9	220	222	332
3	25.2	119	123	218	11	2.8	177	170	272	19	18.1	231	237	336
4	24.2	102	97	201	12	19.0	165	175	283	20	14.1	309	321	419
5	21.2	108	119	215	13	14.2	320	342	430	21	11.0	350	348	457
6	15.3	174	175	284	14	17.6	251	235	322	22	7.4	414	401	514
7	12.6	194	198	291	15	21.6	137	144	236	23	6.3	314	308	404
8	10.7	197	200	311	16	24.9	64	72	175	24	34.0	1	1	103

The proposed method of determination of nitrate in seawater can be regarded as a manual alternative where the automated cadmium reduction technique is not directly available, and is applicable in the more eutrophic areas of the marine environment. The proposed method avoids cadmium pollution, which amounts to 0.5 mM in the column effluent, due to the complexation of cadmium by chloride[1], while the reaction products of HS are N_2, water and sulphate.

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