Technical Note

A note of caution on reduction conditions when using the cadmium–copper column for nitrate determinations in aquatic environments of varying salinities

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ABSTRACT

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In a widely used method for nitrate determination in seawater, the reduction of nitrate occurs under acidic conditions because the sample is mixed with pure ammonium chloride. Under these conditions, about 40% of the nitrite formed is reduced further, leading to poor sensitivity and reproducibility. When the pH of ammonium chloride is adjusted to 8.5 with ammonia, the loss of nitrite is only about 5%, the sensitivity is improved by a factor of two, and the precision by a factor of five for seawater as well as freshwater. For samples of varying salinities ($(0\text{–}30)\,S\times10^3$), the use of buffered ammonium chloride obviates the need for salinity corrections.

INTRODUCTION

In several technical reports (Friedrich et al., 1972; Whitledge et al., 1981) and at least two laboratory manuals (Strickland and Parsons, 1968; Parsons et al., 1984) describing a widely used method for nitrate determination in seawater, the reduction of nitrate to nitrite by a cadmium column is carried out with pure ammonium chloride as a buffer. After experiencing persistent difficulties in obtaining acceptable reproducibility with the procedure (typical coefficient of variation of 5% instead of the usual 0.5% as mentioned by

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Parsons et al. (1984)), we examined some aspects of the reduction conditions which were expected to lead to these problems.

After eliminating the column itself as a source of variability, the main source of the problem was hypothesized to be the reduction of nitrate beyond nitrite under acidic conditions (Grasshoff, 1964, 1983). As the pH of pure ammonium chloride (30 g l^{-1}) was about five, this was a definite possibility because the buffering capacity of seawater is not sufficient to keep the sample at a neutral pH during the reduction process. In fact, Grasshoff (1983) stated that "the pH is adjusted to 8.5 with ammonia", but did not explicitly explain why this adjustment is necessary.

RESULTS AND DISCUSSION

First, the changes in pH of various water samples upon addition of pure ammonium chloride were measured by simulating the sample/reagent volume ratio used in the automated analysis. Table 1 shows that pure ammonium chloride can lower the pH of seawater by more than one unit under these conditions and by more than two units for deionized distilled water, whereas ammonium chloride buffered to pH 8.5 brings all samples to a uniform pH of 8.2–8.3. As nitrate reduction must be carried out in the pH range 8.0–8.4 (Solorzano and Sharp, 1980), the use of pure ammonium chloride does not seem appropriate.

A series of nitrite standards were then passed through the nitrate channel of a Technicon (Tarrytown, NY, USA) AutoAnalyzer with the reduction column open or closed to estimate the possible loss of nitrite as a result of the acidic reduction conditions. The same standards were run a second time

TABLE 1

Changes in pH of various water samples upon addition of pure (pH 4.8) or buffered (pH 8.5) ammonium chloride

Sample	Initial pH	Final pH	
		+pure NH$_4$Cl	+buffered NH$_4$Cl
Aged ESAW[a] medium	8.25	6.95	8.3
Used ESAW[b] medium	8.7	7.1	8.3
Freshwater (tapwater)	6.4	5.9	8.3
Deionized distilled water	7.6	5.15	8.2

[a]Artificial seawater (Harrison et al., 1980) stored for 1 week.
[b]Artificial seawater after phytoplankton growth.

through the same channel, but using ammonium chloride buffered to pH 8.5 with ammonia.

The results (Table 2) indicate that about 40% of the nitrite is reduced further when pure ammonium chloride is used. At pH 8.5, the nitrite loss was only about 5%, which is consistent with results of others using different buffers (Morris and Riley, 1963; Wood et al., 1967). Although still significant, this small loss apparently cannot be avoided.

Nitrate standards were then analyzed with and without pH-adjusted ammonium chloride. Table 3 indicates that, in addition to a greater sensitivity at pH 8.5 (peak heights for a given nitrate concentration were almost twice as high as before), the variability is much lower (by a factor of five). These results also rule out the possibility of column deactivation by the slightly alkaline ammonium chloride solution.

Tests with standards prepared in deionized distilled water were included to investigate the impact of this problem on samples of internal nitrate extracts from phytoplankton (Collos, 1982; Dortch, 1982) or samples of freshwater. Although use of pH-adjusted ammonium chloride significantly reduced the variability of nitrate determinations in freshwater, the remaining variability was still greater than for seawater samples (probably because of the lack of buffering capacity in freshwater).

Finally, we looked at the effect of salinity on nitrate analyses which is encountered when dealing with samples of different salinities in an estuary or a river plume. Artificial seawater (Harrison et al., 1980) with a salinity of 30

TABLE 2

Peak heights (in cm) of 50 μM nitrite standards analyzed on the nitrate channel under various conditions (mean values of five replicates ± 2 SD)

Reduction column	Ammonium chloride pH	
	4.8	8.5
Open	14.4±0.6	22.7±0.5
Closed	22.0±0.5	23.9±0.2

TABLE 3

Coefficients of variation of replicate 50 μM nitrate standards under varying reduction conditions and in different matrices (number of samples in parentheses)

Matrix	Ammonium chloride pH	
	4.8	8.5
Seawater	4.9 (12)	0.7 (12)
Freshwater	5.4 (8)	1.2 (8)

was used to make a range of salinities: $S \times 10^3$ was 0, 5, 10, 15, 20, 25 and 30 (except 30 which was made with 30×10^{-3} NaCl) by dilution with DDW (deionized distilled water, for which S was zero). From the water of each salinity, standard concentrations ranging from 0 to 20 μM of nitrate were made. Samples were analyzed with a Technicon AutoAnalyzer and peak heights were recorded. Linear regressions between a set of standard concentrations (0–20 μM) and their corresponding peak heights were calculated for $S \times 10^3$ of 0, 5, 10, 15, 20, 25 and 30. Thus, five linear regressions were run and five slopes and constants were derived. Because we use water of $S = 30$ for our laboratory standards, only comparisons between a slope for $S \times 10^3$ of 0, 5, 10, 15, 20 and 25 and a slope for $S = 30$ were made with a t-test. The computed t values are presented in Table 4, together with slopes and standard errors of regression coefficients. At the significance level (P) of 0.05, only the slopes for S of 0, 5 and 10 are statistically different from the slope for $S = 30$ when ammonium chloride was not buffered; this result indicates that salinities below 15 have an effect on nitrate analysis. However, when ammonium chloride was buffered, t-tests show no significant differences between salinities of 0, 5, 10, 15, 20 and 25 and salinity of 30 (at $P = 0.05$); this indicates that no salinity corrections are necessary for nitrate analyses when using buffered ammonium chloride.

As this modification is easy to make, it is recommended that, when ammonium chloride is used as a buffer, its pH should be adjusted to 8.5. Although this may not affect the overall accuracy if standards and samples have the same buffering capacity, it certainly improves the sensitivity (by a factor of two) and precision (by a factor of five) of the procedure. For samples of varying salinities, the use of buffered ammonium chloride obviates the need for a salinity correction.

TABLE 4

Salinity effects on nitrate analyses; impact of ammonium chloride pH on the slope of regression lines between nitrate concentrations and recorded peak heights

Salinity (10^{-3})	With pure NH₄Cl			With buffered NH₄Cl		
	Slope	SE	t-value	Slope	SE	t-value
0	35.20	0.50	−22.10	53.70	0.74	0.89
5	40.51	0.48	−15.17	53.70	0.10	1.00
10	43.22	0.47	−11.53	53.08	0.32	0.55
15	50.22	0.45	−1.70	55.25	0.78	1.82
20	51.58	0.61	0.21	53.15	0.49	0.59
25	52.10	0.57	0.89	51.88	0.88	−0.22
30	51.41	0.54	0.00	52.25	1.45	0.00

SE — standard error of the regression coefficient.

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