A High Sensitivity Method for the Determination of Nanomolar Concentrations of Nitrate and Nitrite in Seawater with a Technicon AutoAnalyzer II

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ABSTRACT

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A high sensitivity manifold for the determination of trace quantities (nanomolar concentrations) of nitrate + nitrite and nitrite alone is described. The method uses a classical Technicon AutoAnalyzer II usually employed for shipboard analysis. A reproducibility of ± 1 nmol dm^{-3} for nitrate plus nitrite and nitrite alone was obtained, with an analytical rate of 40 samples h^{-1}.

INTRODUCTION

Nitrate, the most abundant form of inorganic nitrogen, is frequently considered as the limiting factor in the primary productivity of phytoplankton in the oceans, since this form of nitrogen is usually present in concentrations below the detection limit of classical analytical techniques. Nevertheless, Garside (1985) has recently shown that in a well-known oligotrophic tropical area (the Sargasso Sea) it is possible to observe a vertical gradient of nitrate concentrations in the surface mixed layer, generally considered to be exhausted of nitrate. For this he used a high precision chemiluminescence technique (± 2 nmol dm^{-3}) (Garside, 1982) since he considered the colorimetric technique not sensitive enough (detection limit ± 50 nmol dm^{-3}).

Using a commercial Technicon AutoAnalyzer II we succeeded in lowering the determination limit of nitrate and nitrite in seawater to a level comparable to that obtained using Garside's technique, while still preserving a high analytical rate.

This paper describes modifications to the classical automated methods of

determination of nitrate-plus-nitrite and nitrite alone. These modifications consist mostly in lowering the dilution of the sample with the reagents and in amplifying the output signal of the colorimeter.

OUTLINE OF THE METHODS

It should be recalled that nitrate in seawater may be reduced to nitrite by running the sample through a column containing cadmium granules coated with metallic copper (Wood et al. 1967). Following Hansen and Grasshoff (1983) and Strickland and Parsons (1972) we chose ammonium chloride as complexant and buffer.

The nitrite thus produced was determined by diazotizing with sulphanilamide and coupling with N-1-naphthylethylenediamine to form a highly colored azo dye (Bendschneider and Robinson, 1952), the extinction coefficient of which was measured in a colorimeter.

The nitrate concentration was obtained as the difference between the sum nitrate-plus-nitrite and the nitrite alone, and was determined without running the sample through the reduction column.

APPARATUS

The analysis system was a classical Technicon AutoAnalyzer II with three channels (nitrate+nitrite, nitrite, phosphate). It consists of an automatic sampler with a cam (40-2/1, 40 being the sampling rate (h^{-1} and 2/1 the ratio between the sampling time and the rinsing time between two samples), a proportionating pump, a series of coils and fittings (the manifold), a colorimeter (length of the flowcell 50 mm; wavelength of the filter 550 nm) and a strip chart recorder.

Analytical manifolds

Nitrate (+nitrite)
The analytical circuit for the automatic determination of nitrate (+nitrite) is shown in Fig. 1. This manifold consists of two parts. In the first part the sample stream is mixed with ammonium chloride solution and passed through the reduction column. The second part, from the column outlet, is the same as the manifold for the determination of nitrite (Fig. 2).

The first part of the nitrate manifold involves two configurations of the junction of the tubes for the pumping of the sample and the complexant (ammonium chloride). The first configuration (I) is used when low levels of nitrate (range 0–5000 nmol dm^{-3}) are to be determined and has the highest analytical sensitivity. The second configuration (II) is used for the determination of high levels of nitrate (up to 35 μmol dm^{-3}) but with a lower precision.
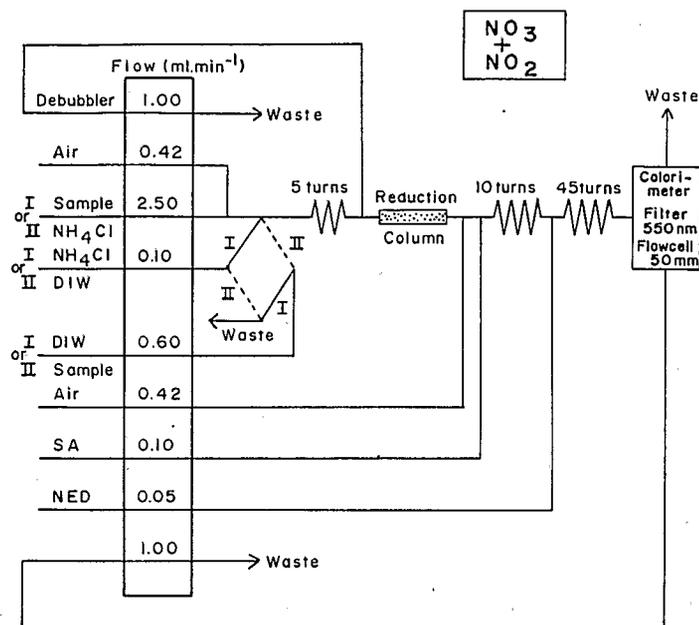
Fig. 1. Schematic diagram of the manifold for determination of nitrate+nitrite: (I) configuration for low analytical range (up to 5000 nmol dm^{-3}); (II) configuration for high analytical range (up to 35 μmol dm^{-3}). DIW, deionized water; SA, sulphanilamide; NED, N-1-naphthylethylenediamine.
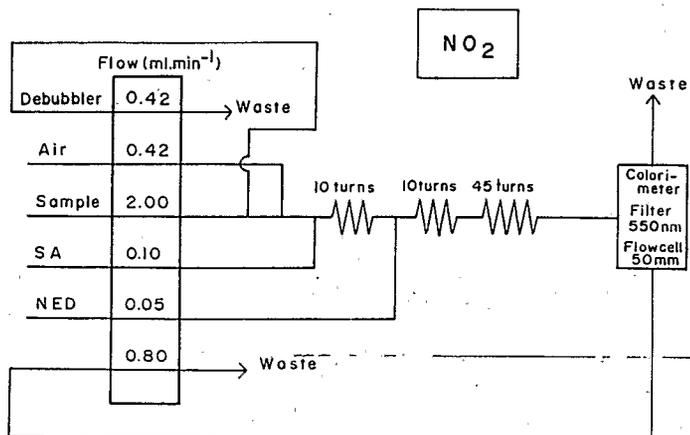
Fig. 2. Schematic diagram of the manifold for determination of nitrite. Abbreviations are as in Fig. 1.

We can already note a difference with the classical manifolds: the pumping rates of sample and reagents. In configuration I the sample is diluted with reagent solutions in the ratio 2.60/2.50 in the first part of the manifold and 2.17/2.02 in the second part (after the column), i.e. a final ratio of 1.117. In the most sensitive procedure range (100–7000 nmol dm^{-3}) of Strickland and Parsons (1972) this ratio was 3.14/2.50 = 1.256, and in the manifold of Hansen and Grasshoff (1983) the dilution ratio was 3.867. The lower dilution of the sample in our method leads to a higher absorbance of the solution measured in the colorimeter.

Nitrite

Nitrite could be determined using the nitrate manifold by simply bypassing the reduction column. Nevertheless, we believe that it is preferable to use two separate manifolds (the one for nitrate + nitrite, the other for nitrite alone) in order to perform the two determinations simultaneously on the same sample. The schematic layout of the manifold for nitrite is illustrated in Fig. 2. The dilution ratio of the sample is lower than that for nitrate at 1.075, and the sensitivity of the nitrite determination should be slightly higher than that for nitrate.

Reduction column

The copperized cadmium granules used as the reduction column filling were prepared exactly as described by Wood et al. (1967). When the column (PVC Technicon tube i.d. 0.254 cm, length 30.5 cm had been filled and the granules well compacted, a small plug of absorbent glass wool was added at each end of the column. When not in use, the column was kept closed by sealing each end with a plastic nipple.

Before use, a new column was activated by passing a nitrate solution (30 μmol dm^{-3}) through the column for 30 min in order to achieve maximum reduction.

REAGENTS

The concentrations of the reagents were changed in comparison with classical automated methods in order to take the modification of the flow rates into account.

Ammonium chloride solutions

Two different ammonium chloride solutions were used according to the chosen configuration (I or II) in order to keep the same ammonium chloride concentration in the stream passing through the reductor. As in the procedure of

Strickland and Parsons (1972), no ammonia was added to adjust the ammonium chloride solutions to pH 8.5.

(1) Configuration I (low analytical range, 0–5000 nmol dm^{-3}) 200 g ammonium chloride were dissolved in 1 dm^3 deionized water.

(2) Configuration II (high analytical range, 0–35 μmol dm^{-3}) 10 g ammonium chloride were dissolved in 1 dm^3 deionized water.

Sulphanilamide (SA)

10 g sulphanilamide and 100 cm^3 concentrated hydrochloric acid were dissolved and made up to 500 cm^3 with deionized water.

N-1-naphthylethylenediamine dihydrochloride (NED)

1 g NED was dissolved in 250 cm^3 deionized water. Five drops of BRIJ 35 (surfactant supplied by Technicon) were then added to the solution.

PROCEDURES

Before and after each series of analyses the analytical circuit was rinsed by pumping dilute hydrochloric acid solution (10% v/v) through the sample tube for 10 min.

In order to limit the risk of contamination arising from sample transfer, the needle of the automatic sampler extracted seawater from the subsampling bottles directly without using the usual plastic sample cups. Measurements were made in duplicate and the operator changed the samples manually every other cycle of sampling–rinsing. The subsampling bottles, 100-ml glass flasks, in which the seawater was withdrawn from the Niskin PVC bottles, were rinsed with dilute hydrochloric acid solution and deionized water before use.

In order to attenuate chemical hydraulic and proportioning pump noise, the colorimeter was operated in the Damp 1 mode. The full capabilities of the range expander of the colorimeter were used by adjusting the standard calibration control. The usual range expander settings are 9.8, 9.0, 6.0, 3.0 and 0.5. The standard calibration control permits the setting of full scale recorder sensitivity from 0.05 absorbance units at a dial setting of 10 to 2.00 absorbance units at a dial setting of 0.0. The 10 setting is difficult to use at sea because of background noise problems; we chose a setting of 9.8 for the highest sensitivity (nitrate range 0–330 nmol dm^{-3}; nitrite range 0–300 nmol dm^{-3}). When the output signal of the colorimeter exceeded the full scale of the recorder, the standard calibration control was adjusted to a lower position. The attenuation ratio of the output signal (height of the peak on the recorder) when the range expander setting was modified from 9.8 to a lower value, was calibrated at regular intervals. The standardization curves (Figs. 3 and 4) were constructed
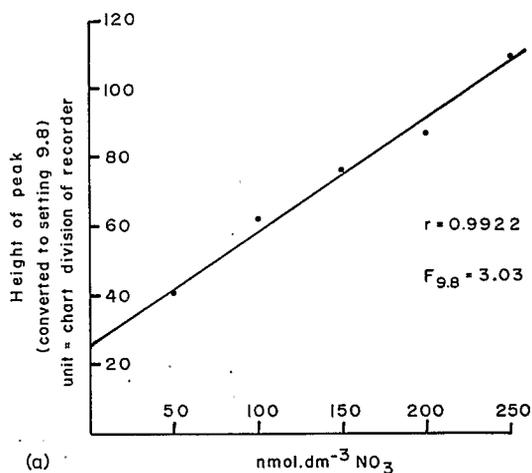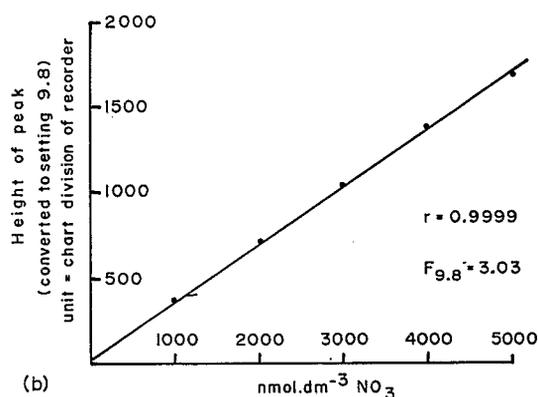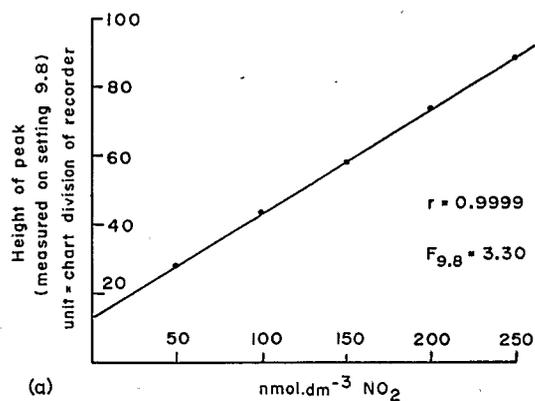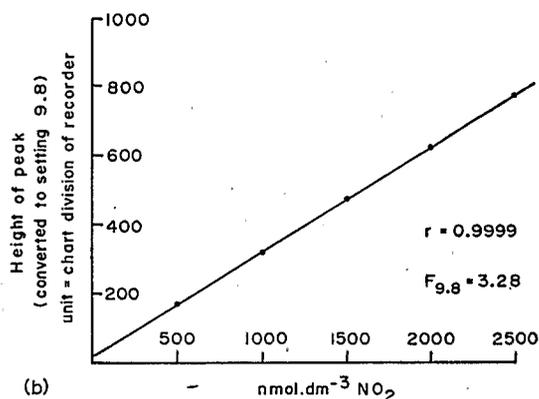
Fig. 3. Calibration curves obtained with the nitrate+nitrite manifold (configuration I) : height of peak (converted for a range expander setting of 9.8) vs. concentration of nitrate added to surface seawater. (a) low concentration range of standards; (b) high concentration range of standards. The calibration factor F is the reciprocal of the slope of the regression line.

with the heights of peaks converted for a range expander setting of 9.8 by taking the attenuation ratio into account.

The pen of the chart recorder was adjusted to zero with the baseline control potentiometer of the colorimeter while deionized water was aspirated via the sample tubing, and reagents via the reagent tubings. The drift of the baseline was estimated using 'rinsing' seawater (the seawater used as a rinsing solution between the samples) which was aspirated for 5 min just before and just after the samples (or standards) (Fig. 5). This seawater was filtered (0.45 μm membrane filter) surface water of low nitrate and nitrite contents; it was also employed for preparing standard solutions.

Fig. 4. Calibration curves obtained with the nitrite manifold : height of peak (converted for a range expander setting of 9.8) vs. concentrations of nitrite added to surface seawater. (a) low concentration range of standards; (b) high concentration range of standards. The calibration factor F is the reciprocal of the slope of the regression line.

According to the expected nitrate concentrations either configuration I or configuration II was chosen; conversion from one to the other was easy by changing the connections of three pumping tubes (Fig. 1: sample/ NH$_4$Cl– NH$_4$Cl/DIW–DIW/sample).

Calibration

Figure 3 shows two calibration curves for nitrate using the high sensitivity manifold (configuration I). The diagrams represent the heights of the peaks (converted to a range expander setting of 9.8) versus concentrations of nitrate added to surface seawater. To run the low range of the standard concentrations (Fig. 3a) the standard calibration control was adjusted to a setting of 9.8 then
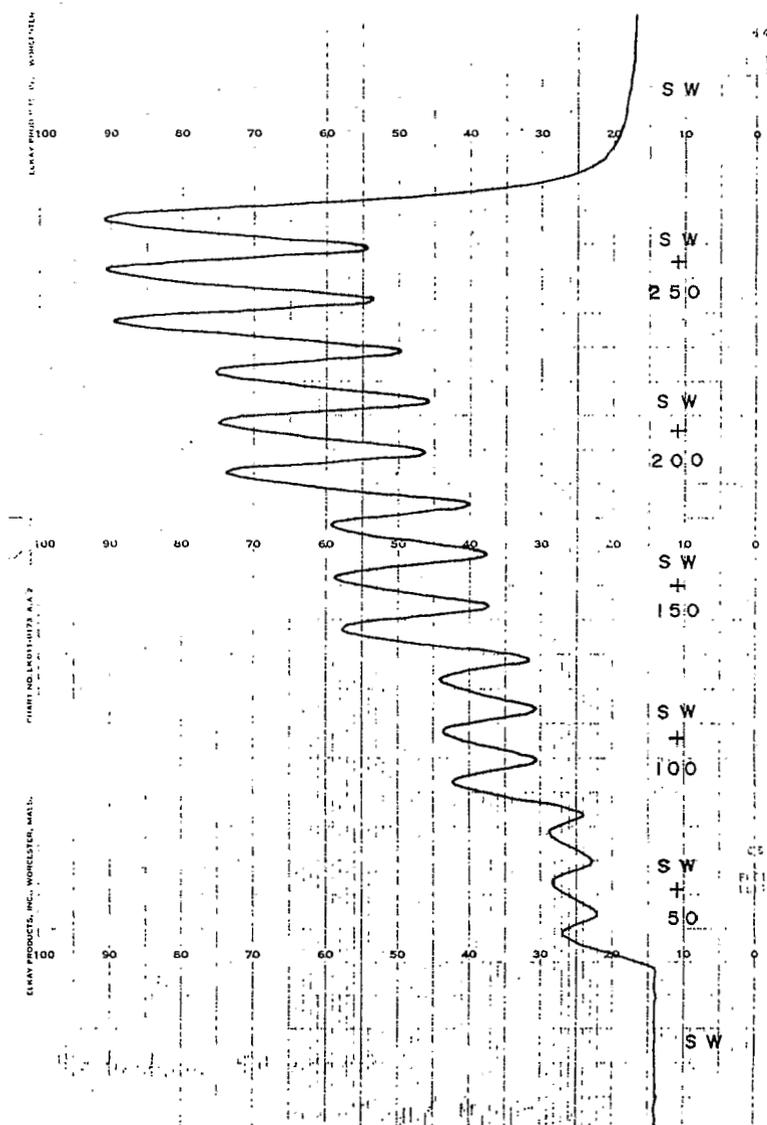
Fig. 5. Recording of analysis of nitrite standards (nmol dm^{-3}) prepared in seawater (SW).

9.0; to run the high range (Fig. 3b) the settings were successively 6.0, 3.0 and 0.5. The standardization factor F, the reciprocal of the slope of the regression line, was the same for the two ranges of concentrations; nevertheless the precision of F was better (higher correlation coefficient r) over the high concentration range than over the low range. Thus the validity of using the range expander settings to measure a large range of concentrations is verified.

Figure 4 shows the standardization curves for nitrite. The low range of standards (0–250 nmol dm^{-3}; Fig. 4a) was performed with a range expander setting of 9.8 and the high range (0–2500 nmol dm^{-3}; Fig. 4b) with settings of 9.0, 6.0 and 3.0. Here again, factor F was unchanged whatever the range expander setting. The value of F would be expected to be lower for nitrite than for nitrate considering the lower dilution ratio of the sample with reagents, as previously
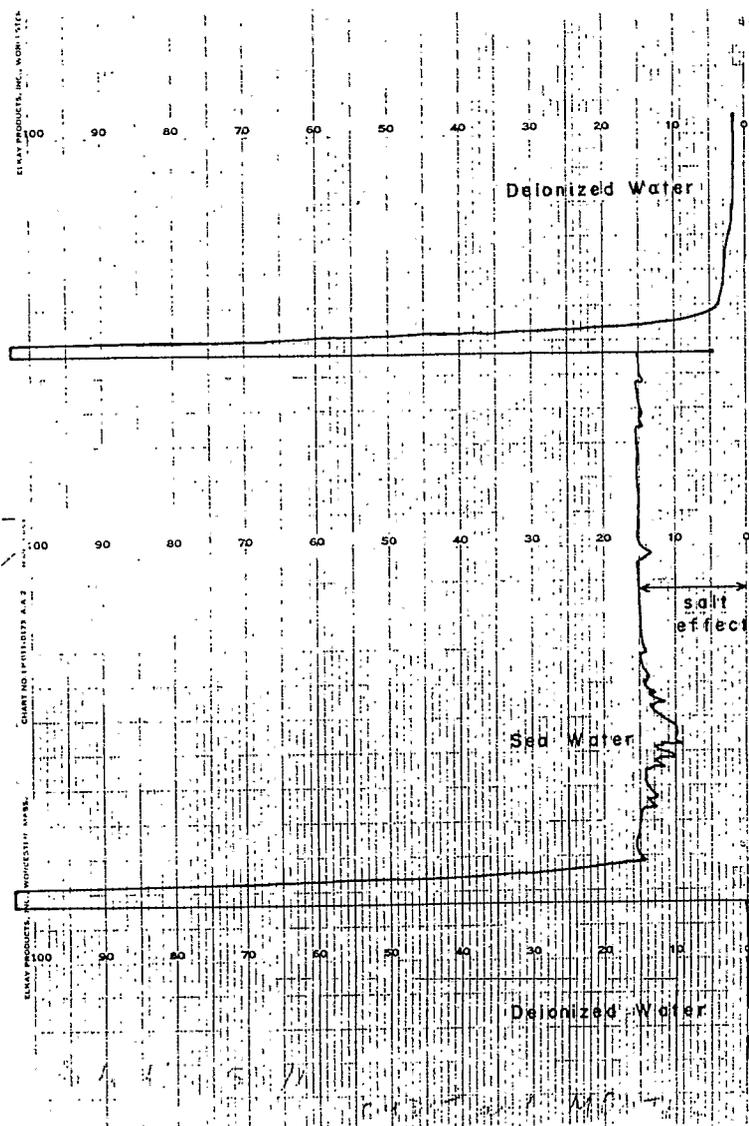
Fig. 6. Recording showing the estimation of the correction for the salt effect. Surface seawater filtered through a 0.45 μm membrane filter was used.

mentioned, however this factor is also a function of the sensitivity of the colorimeter and the recorder.

Fig. 7. Recording showing part of the reproducibility of analysis experiment (run three times) for twelve replicate samples of surface seawater spiked with 100 nmol dm^{-3} nitrite (see Table 2). Note the low drift of the baseline, 2 chart divisions (range expander setting of 9.8) after 60 min of running.

Calculation and precision

Two corrections were applied to the peak heights before converting them into concentrations through multiplication by the standardization factor F. The first correction was necessary because of the drift of the baseline. As previously mentioned, this was estimated using 'rinsing' seawater which was aspirated before the first sample and after the last. For each intermediate sample a linear interpolation was made. The drift of the baseline was generally small: 2–3 chart divisions (range expander setting 9.8) after 60 min running time (Figs. 5–7).

The second correction was for the salt effect. This effect, well-known in connection with flowcells in Technicon colorimeters, (Froelich and Pilson, 1978), was estimated as the difference between the absorbance of the rinsing seawater and that of deionized water without aspirating reagents. The pen of the chart recorder was adjusted to zero with the baseline control potentiometer while deionized water was aspirated via the sample tubing and the reagents tubings (Fig. 6). After that, 'rinsing' seawater was aspirated via the sample tubing. The shift of the signal on the recorder was interpreted as the salt effect. The salt effect was higher for nitrite than for nitrate; it was estimated to be 15 units (50 nmol dm^{-3}) for nitrite (Fig. 6) and 9 units (27 nmol dm^{-3}) for

TABLE 1

Precision using the nitrate manifold

Sample	Peak height[a]			Mean height[b]	Standard deviation
1	42.9	43.3	43.7	(43.3)	0.4
2	42.8	42.7	42.6	42.4	0.1
3	42.2	42.3	42.2	41.6	0.1
4	42.3	42.6	42.6	41.6	0.2
5	42.7	42.8	43.0	41.6	0.2
6	43.2	43.0	43.0	41.6	0.1
7	43.7	43.7	43.9	41.9	0.1
8	44.2	44.5	44.7	42.3	0.3
9	44.7	44.5	44.4	42.1	0.2
10	44.4	44.5	44.5	41.7	0.1
11	45.0	45.0	45.0	42.0	0.0
12	44.8	44.6	44.8	41.4	0.1

Mean peak height (between samples) $=41.8 \pm 0.3$

Mean concentration[c] $=126.7 \pm 0.9$ nmol dm^{-3}

[a]Height of peak (range expander setting 9.8) obtained from analysis of twelve replicate samples of surface seawater spiked with 100 nmol dm^{-3} NO_3; units are divisions of the chart recorder.
[b]Corrected for baseline drift.
[c]With $F=3.03$ (see Fig. 1).

nitrate. This salt effect depends on the salinity of the seawater sample. In the open ocean areas that we usually study, the variation of the salinity is too low to merit a change in the correction of the salt effect, so we adopted constant values for this correction. It was, nevertheless, regularly checked.

The estimation of the precision of the analysis with these manifolds was accomplished by running twelve replicate samples of surface seawater spiked with 100 nmol dm^{-3} of nitrate or nitrite, while the needle of the sampler was introduced three times successively into each sample vessel. Table 1 gives the results of the nitrate determination and Table 2 that of nitrite. Figure 7 shows part of the recording corresponding to Table 2. With two manifolds the reproducibility is ± 1 nmol dm^{-3} at the 100 nmol dm^{-3} level. However, since nitrate is measured as the difference between nitrate + nitrite and nitrite alone, the imprecision of the nitrate determination is larger at ± 2 nmol dm^{-3}.

TABLE 2

Precision using the nitrite manifold

Sample	Peak height[a]			Mean height[b]	Standard deviation
1	(44.8)	(47.5)	48.4	48.4	–
2	48.3	48.4	48.3	48.3	0.1
3	48.1	48.8	48.2	48.3	0.4
4	48.3	48.7	48.4	48.4	0.2
5	48.0	48.3	48.5	48.1	0.3
6	48.4	48.7	48.9	48.5	0.3
7	48.4	48.7	48.5	48.2	0.2
8	48.8	48.6	48.1	48.2	0.4
9	48.2	48.6	48.6	48.1	0.2
10	48.2	48.5	49.3	48.3	0.6
11	48.8	49.0	48.7	48.3	0.2
12	49.1	49.2	49.0	48.5	0.1

Mean peak height (between samples) = 48.3 ± 0.3

Mean concentration[c] = 159.4 ± 1.0 nmol dm^{-3}

[a]Height of peak (range expander setting 9.8) obtained from analysis of twelve replicate samples of surface seawater spiked with 100 nmol dm^{-3} NO_2; units are divisions of the chart recorder.
[b]Corrected for baseline drift.
[c]With $F = 3.30$ (see Fig. 2).

DISCUSSION

The results of these experiments show that it is possible to lower considerably the limit of determination of nitrate of the well-known Technicon AutoAnalyzer II. Using the procedures described herein, the precision level

obtained is comparable to that achieved with more sophisticated equipment. The background noise level and the baseline drift are acceptable in spite of the very large amplification of the output signal from the colorimeter. Continuous sampling of deionized water or 'rinsing' seawater causes an instability of the output signal of ± 0.5 chart division, i.e. ± 2 nmol dm^{-3} (Figs. 5–7). The use of natural seawater as rinsing solution between samples removes the heterogeneous mixing effects due to density differences between the samples and distilled water. Using the high sensitivity manifold for nitrate determination a large dynamic range (three orders of magnitude; 5–5000 nmol dm^{-3}) is possible and, moreover, it is very easy to extend the range to higher concentrations (up to 35 μmol dm^{-3}) by simply disconnecting certain pumping tubes. Another advantage of our method lies in its allowing a high analytical rate (40 samples h^{-1}).

The main difficulty in improving the precision of this method of analysis of nitrate and nitrite in seawater is not increasing the sensitivity or decreasing the determination limit of the method, but rather it is a problem of water contamination at the time of transfer: sampling with Niskin sampler, subsampling with glass bottle and filling the polystyrene sample cup on the rotating plate of the Technicon sampler. Therefore, we decided not to use the small sample cups but to insert the needle of the sampler directly into the subsampling vessel. Although the automated analysis only requires a small volume of water (a few ml) we chose to subsample 100 cm^3 of seawater into glass bottles, since the risks of contamination decrease as the volume of the vessel increases. The glass bottles were filled with dilute HCl solution between the samplings, then rinsed with deionized water immediately before each use. Thus the reproducibility of the measurements was improved to a value of ± 1 nmol dm^{-3}.

It would, however, not be enough to display a high resolution between the very low concentrations of nitrate and nitrite. It is also necessary to know the accuracy of the determinations. This is probably the most difficult feature to evaluate. Accurate measurement of the nitrate or nitrite concentrations depends firstly upon the quality of the distilled or deionized water chosen to adjust the zero level of concentration. There is a major advantage in employing water of the highest purity, as is usually recommended for every analytical technique. Two other factors can be of importance for the accuracy: the presence of particles and the influence of the salt effect. If the interference of the particles can be removed by filtering the seawater samples as we did for the 'rinsing' seawater, the salt effect is an inherent defect in colorimeter measurements with the Technicon AutoAnalyzer II. Froelich and Pilson (1978) showed that the systematic absorbance error with a Technicon colorimeter, due to the refraction processes on the inlet and outlet walls of the cuvette, is proportional to the salinity of a seawater sample. Fortunately, the salinity of our seawater samples did not vary very much ($34.5 < S < 36.5$) and the influence of the salt effect can be considered constant. Nevertheless, attention should be paid to

the determination of this error which is not negligible (27 nmol dm^{-3} for nitrate and 50 nmol dm^{-3} for nitrite). The only solution to suppress the refraction processes would be to use a special cuvette with flat and parallel walls.

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