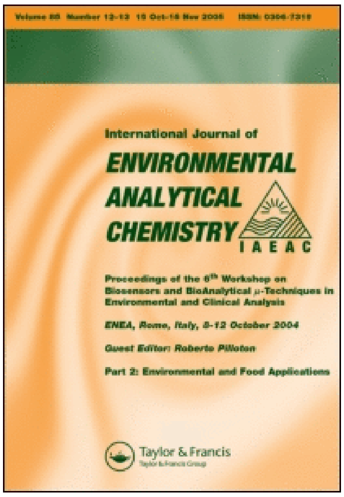

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DETERMINATION OF NITRATE IN THE PRESENCE OF NITRITE IN NATURAL WATERS BY FLOW INJECTION ANALYSIS WITH A NON-QUANTITATIVE ON-LINE CADMIUM REDUCTOR

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Several analytical problems in the determination of nitrate using flow injection analysis (FIA) coupled with an on-line Cd reductor have been studied. It was found difficult to prepare a nearly 100%-efficient copperized Cd reductor which maintains its efficiency over a lengthy period. Instead, the use of a narrow and lower efficiency Cd coil is recommended because it is more stable and therefore more suitable for FIA. Since the conversion of nitrate to nitrite is not quantitative, results for nitrate tend to be over-estimates when nitrite is also present. This problem has been solved by using a simple correction scheme to compensate for the effect of nitrite, thus enabling the correct nitrate concentration to be evaluated. The validity of the correction procedure has been confirmed by running a series of known standards containing both nitrate and nitrite with three types of FIA manifolds. Results for nitrate were accurate for fresh and saline waters even when the co-existing nitrite concentrations were high.

KEY WORDS: Nitrate, nitrite, cadmium-reductor, pink azo dye, interference.

INTRODUCTION

The most widely adopted method for the determination of nitrate in natural waters, including seawater, involves passage of the sample through a copperized cadmium reductor column to reduce nitrate to nitrite as proposed by Grasshoff[1]. The nitrite is subsequently determined by the pink azo dye method[2,3]. Although numerous versions of this technique (both manual and automatic) have been published in past years, there is still considerable controversy about the efficiency of the Cd reductor. Findings fall into three categories: (a) A reduction efficiency of 100% has been claimed by several investigated[4,5], (b) The reduction may not be quantitative, so results should be termed "nitrite plus nitrate"[6-8]. If the nitrate concentration is needed, it is obtained by simply ignoring the efficiency problem and subtracting the determined nitrite concentration from the primary result[9], (c) The reduction is not quantitative, but nitrate can be evaluated using a correction equation[10,11]. In the authors' experience it is very difficult to

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achieve a 100% reduction rate with either a large column filled with copperized Cd filings or a narrow-bore coil containing copper coated Cd wire in automated analysis.

The incompleteness of the reduction may not be important as nitrate predominates over nitrite in most natural waters. However, when nitrite is also present at appreciable levels quantification of nitrate may become doubtful. Such conditions can occur in, for example, aquarium tanks, maricultural ponds, sewage, estuarine waters, and surface seawater in upwelling areas. The results obtained by this method will include both nitrite originally-present and that newly-generated from partial reduction of nitrate, but will exclude nitrite lost by reduction to other nitrogen species. In addition, reaction products from the reductor may interfere with the subsequent color formation reaction causing the absorbance to be low, and leading to a mis-calculation of the reduction rate. As a consequence, the results for nitrate would be over-estimates if the calibration curve was made for nitrate standards only. A correction procedure would be necessary if the "true" concentration of nitrate is to be measured.

In the present work no attempt was made to prepare a Cd reductor having an efficiency of 100%. Attention was concentrated on the feasibility of making a reproducible and stable non-quantitative on-line reductor that is suitable for the routine analysis of nitrate using flow injection analysis (FIA). A novel correction scheme is proposed to enable correction to be made for the effect of nitrite, so that the concentration of nitrate can be accurately evaluated.

EXPERIMENTAL

Reagents

The reagents were prepared having the following concentrations; 2% w/v ammonium chloride, 2% w/v sulphanilamide (SUL) in 15% v/v hydrochloric acid, and 0.3% w/v of N-1-naphthylethylenediamine hydrochloride (NED).

Standards

Stock standard 2.50 mM solutions of nitrite and nitrate were prepared from sodium nitrite and potassium nitrate respectively. Working standards and mixed standards of the required concentrations were prepared daily by diluting and mixing the stock standards.

Cadmium reductor

The use of Cd wire to form a reductor coil as suggested by Stainton[4] was adopted in this study. A 1 m-length cadmium wire (1 mm diameter) was cleaned by dipping it in a 1 M hydrochloric acid for no more than 30 s, and then rinsed with distilled water. The wire was copperized by immersing it in a 1% copper (II) nitrate solution for 10 – 20 s to form a black coating on the surface. A soft wet tissue paper was used to wipe away any loosely-bound black filaments, leaving a very thin but firmly-bound layer on the surface. The coated Cd wire was then threaded into a Teflon tube (1 m long; 1.5 mm ID) and coiled to form a reductor coil. A small loose plug of nylon wool was inserted at both ends of the coil to prevent any residual filaments from entering the main stream and the detector. The reductor was installed in a f.i.a. manifold for nitrate and tested by continuous passage of a 20 µM nitrate solution through the reductor until a steady output was attained. If the reductor was not going to be used for a few days, the Cd wire was removed from the coil and stored in dry condition.

Instrumental layout and manifolds

Three flow injection analysis manifolds (Figure 1) were used in this study. The main stream paths of all the manifolds were constructed from 0.8 mm ID Teflon tubing. The coiled Cd reductors were installed in the appropriate positions. The ratios of the flow rates for the carrier(s) and three reagents (ammonium chloride, SUL, and NED) were 2.5:0.1:0.1:0.1 respectively with Manifold I; 1.2:1.2:0.1:0.1:0.1 with Manifold II; and 2.5:2.5:0.1:0.1:0.1 with Manifold III. The flow rates were induced with an Ismatec IPS-12 peristaltic pump, which was operated at the pumping speed corresponding with a dial setting of 30. The sample loops on the injectors all had a capacity of 0.3 mL. Each manifold was connected to a double-beam Shimadzu 160-A spectrophotometer fitted with one or two 1 cm Hellma flow cuvettes (capacity 71 µL) as appropriate. The absorbance of the pink azo dye was measured at 543 nm.

Operation

Manifold I was of a conventional single-channel design which measures "original plus reduced nitrite" only, and was operated in the normal way. Manifolds II and III, each containing two injectors, were designed for the determinations of both species. With Manifold II the sample was loaded to fill both sample loops, but the injections were made ca. 30 s apart. The flows from the two injectors were merged into one main stream, and the reagents (SUL and NED) were added sequentially. The first sample segment was injected by turning the injector V_1 for the nitrite measurement. When the first peak appeared on the recorder (ca. 30s after the injection), a second segment of sample was injected *via* the valve V_2, mixed with ammonium chloride, passed through the Cd-reductor, and then merged with the other flow. The second peak on the recorder corresponded to the combined results of the original nitrite plus nitrite formed by reduction of the nitrate (Figure 2). The time span for the dual-injection determination was ca. 90s, corresponding to a throughput rate of 30 – 35 samples per hr. With Manifold III the sample was also injected in two consecutive steps, as with Manifold II, but the injected sample segments each went to individual streams leading to different flow cuvettes installed in a double-beam spectrophotometer. The flow cuvette for nitrite measurement was placed in the "reference" position so that the sample injected through injector valve V_1 gave a negative absorption peak. This was followed by a positive signal produced by the second sample segment injected through V_2 and measured in the "sample" position of the cuvette chamber. The time span for measuring a sample with Manifold III was ca. 80 s. The recorder outputs for the three manifolds are demonstrated in Figure 2.

Calibration procedure

With Manifold I, nitrite and nitrate standards were injected separately and the resultant peaks were used to construct two calibration curves (Slope S_1 for nitrite and S_2 for

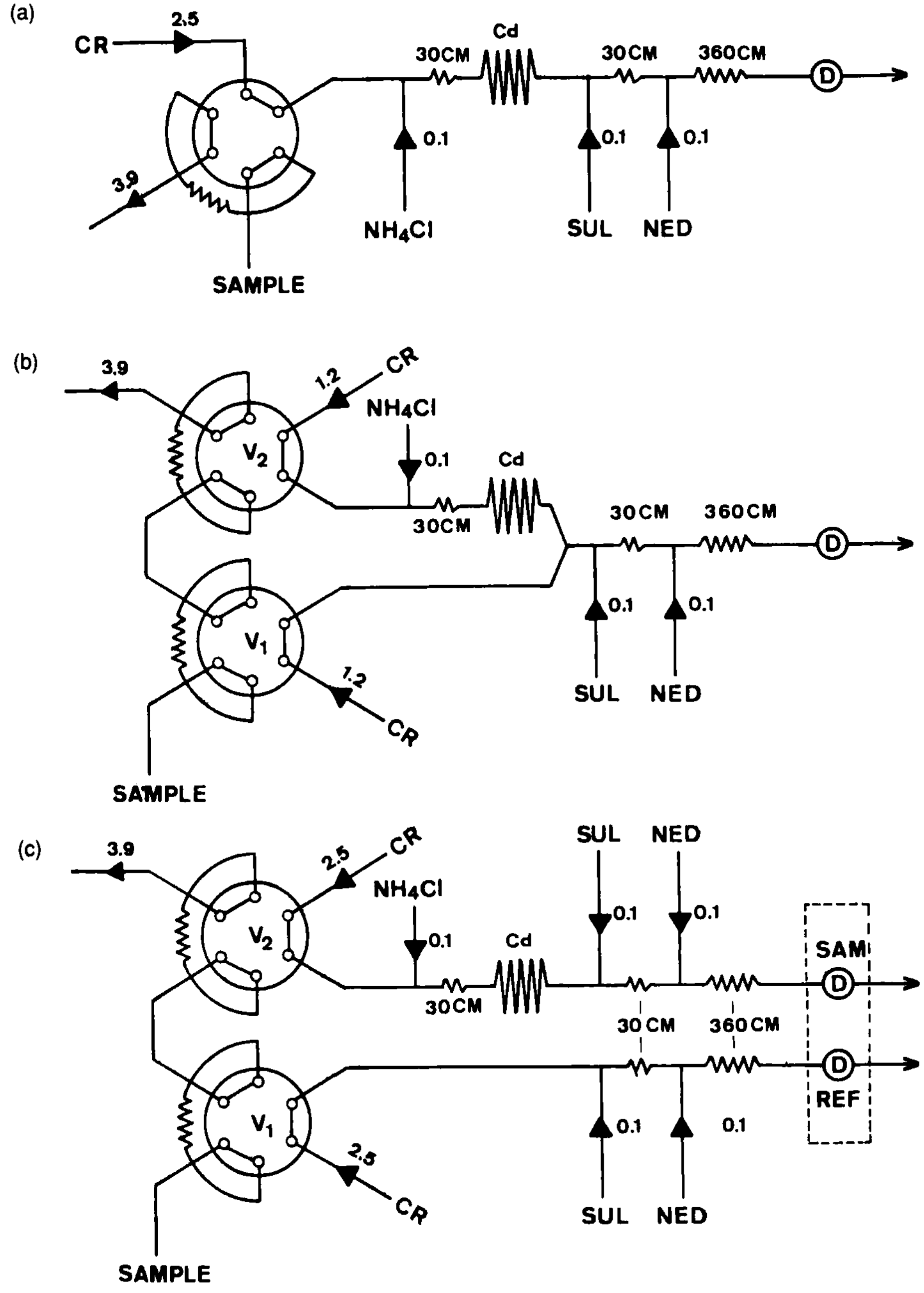

Figure 1 The three FIA manifolds used in this study (CR: carrier flow; D: detector; Cd: reductor). The triangles on the manifolds represent the position of pumping tubes marked with flow rate ratios; the actual flow rates (mL/min) correspond to double the ratio values when the Ismatec IPS-12 pump is operated at a dial setting of 30. (a) Manifold I is a single channeled system for measurement of "original plus reduced" nitrite; (b) Manifold II is a dual-injection merged-flow system, which measures nitrite through injector V_1 and "original plus reduced" nitrite through injector V_2; (c) Manifold III is a dual-injection dual-channel system with two flow cuvettes installed in one double-beam spectrophotometer.

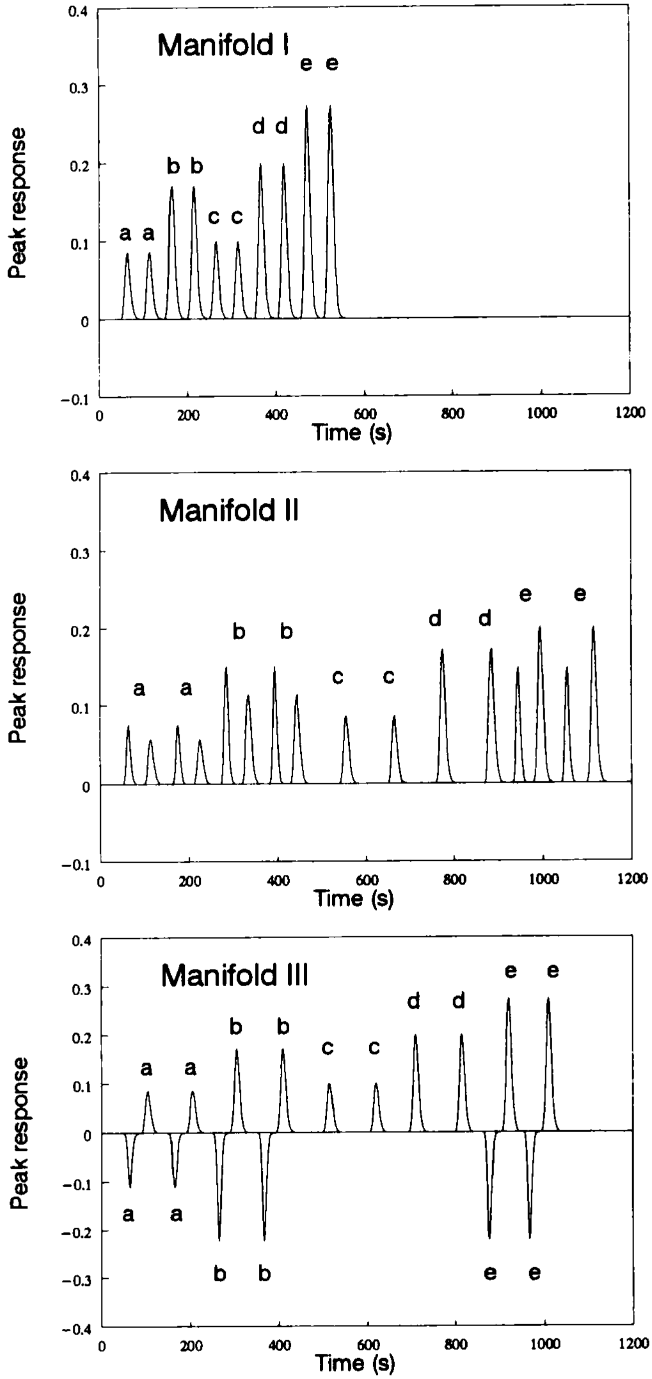

Figure 2 Signal outputs for the three manifolds shown in Figure 1. The concentrations in the tested samples were: (a) 5 µM NO_2^- (b) 10 µM NO_2^- (c) 10 µM NO_3^- (d) 20 µM NO_3^- (e) 10 µM NO_2^- + 10 µM NO_3^-. All samples were analyzed in duplicate.

nitrate). The concentrations of nitrite in all the standard samples were measured by a manual procedure[15]. For Manifold II and III, standard nitrite and nitrate solutions were also injected separately. Each 2-step injection of nitrite standard solution (one through the reductor and the other not) produced two peaks. These when plotted gave two straight lines of slopes S and S_1. Each dual injection of a nitrate solution gave only one signal at the position of the second peak, and the slope of the calibration curve for nitrate was named S_2. These slope values were used to calculate the concentration of nitrate in the sample. Details will be described in the later sections.

RESULTS AND DISCUSSION

Problems in the copperization

The reduction rate of nitrate on a pure Cd surface is slow, but can be much improved by coating with a layer of copper. In most published work reductor columns have been prepared by stirring Cd granules with a copper (II) sulphate solution, washing thoroughly with distilled water, and then packing them into a column or coil. On-line coating procedures have not proved satisfactory because large amounts of coating substances could be trapped in the column. The efficiency of a newly-prepared reductor is not high, and it needs to be activated using an extensive wash with a nitrate-rich solution[8,12]. According to the previous reports the washing procedure could take more than 30 min. The exact reason for this is not clear. In the authors' experience a newly coated Cd wire reductor may still contain some loosely-bound coating substances, as if it was snapped by the fingers, black filaments could be seen to fall off, and might sometimes cause a blockage in the flow injection analysis system. A later batch experiment showed that the washing procedure may simply serve to remove loosely-bound filaments which strongly interfere with the subsequent colorimetry.

Interference caused by the coating material

A sample of the black coating substance was prepared by immersing a length of Cd wire in a 1% w/v copper (II) sulphate solution for two days and collecting the precipitate by filtration. It was dried in an oven at 90°C and ground into a fine powder. This was found by atomic absorption spectrometry to contain 60% Cu and 22% Cd. To examine its effect on the azo dye formation, milligram amounts of the power were added to 25 mL of 20 µM nitrite solution, 1 mL aliquots of the color forming reagents were added with shaking. After 10 minutes the absorbance of the pink color was measured at 543 nm and compared with that of an untreated standard. The results are shown in Figure 3A. It can be seen that the addition of the black powder before addition of the colorimetric reagents stopped the formation of the pink color. If the black powder was added after the pink azo dye had developed, the color faded rapidly within a few minutes. These findings clearly demonstrate that if any filaments become detached from the Cd reductor and later come into contact with the acid reagents in the manifold, they will inhibit the formation of the pink azo dye. Addition of filaments to preformed azo dye led to destruction of the dye.

In another series of experiment it was found that when copper (II) sulphate was added to a 20 µM nitrate solution before adding the color forming reagents, there was considerable suppression of the production of the azo dye (Figure 3B). The absorbance

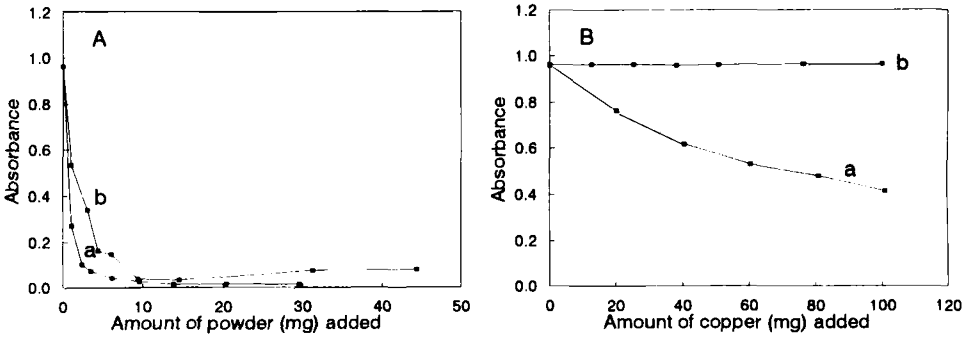

Figure 3 Interference of the (A) black coating powder and (B) cupric sulphate in the colorimetry of the pink azo dye. Sample: 25 mL of 20 μM nitrite solution added with 1 mL aliquots of SUL and NED reagents. Interfering substances were added (a) before adding the reagents; (b) after the pink azo dye colour had fully developed.

decreased as copper concentrations increased, and the rate of the color formation was slowed down. This phenomenon is probably the result of the formation of a strong chelate between the copper (II) and the ethylenediamine reagent[13]. However, if the color formation reaction was completed before the addition of the copper (II), the absorbance was no longer affected. It should also be noted that the interference caused by the black powder was more severe than that caused by the same amount of copper as copper (II) sulphate. Evidently the cupric ions were not the only source of interference. There must have been some other interfering factors, possibly sulphur species, which also leached from the black powder under acidic condition. For this reason the copper nitrate, instead of copper sulphate, was used as the solution for preparing of the coating.

Batch coating procedure

Cadmium wire has considerable advantages over Cd powder or granules as a support phase for the reductor because any loosely-bound Cu coating can be easily wiped away with a moist tissue. It was found that when the coated Cd wire was threaded into the coil and installed in a FIA manifold, the reduction rate reached a steady state within a few minutes. The extensive wash with a concentrated nitrate solution used with Cd powder or granules was not necessary. A stability test was made on a newly prepared Cd-wire reductor by using a 20 μ M NO_3^- solution as the carrier and monitoring the resultant signal for 24 hr. No significant change was observed. The efficiency of the column was also found to be stable for at least four days.

Reduction efficiency

Although almost quantitative reduction of nitrate to nitrite under careful optimized conditions has been reported by Nydahl[14], many handbooks for freshwater or seawater analysis have admitted that in routine work it is difficult to achieve this efficiency even in careful manual work with large columns. The minimum suggested criteria performance in manual operation are: 75% by APHA Standard Methods[6], and 90% by Grasshoff *et al.*[9]. A reduction efficiency of 93% was suggested by Strickland and Parsons[10] for a newly prepared manual column. In automated analysis, either using air-segmented flow analyzer or flow injection analysis, the reduction capacity of an on-line reductor has been found to depend upon the experimental conditions. In principle, such systems would be expected to be less efficient than manual operation because of the lower contact time and faster flow rate.

However, the "true" reduction efficiency is difficult to define and measure. Several factors may influence it: (1) The pH of the effluent from the reductor is subject to change; this causes variations in the molar extinction coefficient of the pink azo dye. (2) The effluent may contain interfering substances that interfere with the subsequent formation of the pink azo dye. (3) Reduction beyond nitrite may occur; importance of this reaction is difficult to assess; for example, if a nitrite solution is divided into two fractions, one being passed through the Cd reductor and the other not, a comparison on the resultant colors does not give a true measure of the extent of the over-reduction rate because the former fraction may contain interfering substances.

For these reasons, the conventional way to measure the efficiency, that obtained by running an equal concentration of nitrite and nitrate standard solution separately through the same Cd reductor and comparing the results, should be termed as the "apparent" efficiency (E%). The E% value obtained in this way confirms, although not with the absolute certainty, that over-reduction does occur.

Correction for nitrite

The determination of the reduced nitrite is usually referred to a calibration curve made with standard nitrate solution alone. The data obtained this way should not be regarded as a measure of nitrate. APHA Standard Methods[6] suggested that the primary results should be termed as the sum of NO_3^--N plus NO_2^--N plus unless the concentration of nitrite is determined separately and corrected for. Such a definition is imprecise because it does not give the correct value for the total sum of nitrite plus nitrate if the conversion of nitrate to nitrite is not quantitative. The direct subtraction of the nitrite value from the primary result of "nitrite plus nitrate" as suggested by several investigators[8,9] is also not the correct way to evaluate the nitrate concentration. The correction equation given by Strickland and Parsons[10] and Parsons *et al.*[11] with a multiplication factor of 0.95 to compensate for the 5% over-reduction is questionable, because the effect of incomplete reduction (E% = 93%) has been ignored, and the factor given could be variable as the column becomes deactivated. A simple test was made to evaluate the validity of these correction procedures. A mixed standard solution containing equal concentrations (10 µM) of nitrate and nitrite was analyzed on a conventional FIA system (Manifold I). The apparent efficiency (E%) of the on-line Cd reductor was estimated to be ca. 58% with a flow rate of 5.2 mL/min. The primary result, calculated from a calibration curve made with nitrate standards alone, was 27 µM. In this case it is not appropriate to term the result "nitrite plus nitrate" (the correct total value should be 20 µM). The nitrate concentration in this mixture could not be obtained either by direct subtraction of nitrite concentration, or by any other correction procedure. To solve this problem, a correction scheme is proposed for both single-channel and dual-injection systems.

Correction for single-channel system

In practice, when a nitrite and a nitrate standard solution were run separately through the single-channel manifold (Manifold I), the resultant peak heights (or absorbance) are:

$$\mathrm{Abs} = S_1 \times [NO_2^-] \qquad (1)$$

$$\mathrm{Abs} = S_2 \times [NO_3^-] \qquad (2)$$

where S_1 and S_2 are sloped of the calibration curves for nitrite and nitrate respectively. If a sample contains both species, then the measured absorbance can be interpreted as the accumulative sum of both signals:

$$\mathrm{Abs} = S_1 \times [NO_2^-] + S_2 \times [NO_3^-] \qquad (3)$$

Therefore, the nitrate concentration can be calculated using the slope for nitrate after deduction of a correction term.

$$[NO_3^-] = \frac{\mathrm{Abs}}{S_2} - \frac{S_1}{S_2} \times [NO_2^-] \qquad (4)$$

where S_1/S_2 is the reciprocal of the apparent efficiency (E%) of the reductor. The nitrite concentration can be obtained either manually, or by running on the same FIA system but omitting the Cd reductor. Theoretically, the reduction efficiency will never exceed 100%, and therefore the value for S_1/S_2 should always be no less than 1.

Correction for dual-injection systems

Manifolds II and III were designed for the determination of both nitrite and nitrate. For each sample, the two injections result in two peaks, Abs(1) and Abs(2), on the recorder. The calibration with the nitrite standards can be calculated into two slopes: $S = \mathrm{Abs(1)}/[NO_2^-]$ and $S_1 = \mathrm{Abs(2)}/[NO_2^-]$. In contrast, the injection of nitrate standards would only produce one signal corresponding to the second peak, and would yield a calibration curve for nitrate with a slope $S_2 = \mathrm{Abs(2)}/[NO_3^-]$. For a sample containing both species, the individual concentrations can be calculated from the following equations:

$$[NO_2^-] = \frac{\mathrm{Abs(1)}}{S} \qquad (5)$$

$$[NO_3^-] = \frac{\mathrm{Abs(2)}}{S_2} - \frac{S_1}{S_2} \times \frac{\mathrm{Abs(1)}}{S} \qquad (6)$$

The first peak signal, Abs(1), should have a positive value with Manifold II and a negative value with Manifold III.

Laboratory test

To test the validity of these equations, experiments were carried out with the three FIA manifolds using known mixed standards. The concentration ranges tested were 1 – 10

Table 1 Laboratory tests on nitrite + nitrate mixtures with Manifold I.

Conc. added $[NO_2^-]$ *(µM)*	*Conc. added* $[NO_3^-]$ *(µM)*	*Peak height Abs. ± sd (n = 3)*	*Primary results (µM)*	*Conc. found* $[NO_3^-]$ *(µM)*
5	0	0.087 ± 0.000	–	
10	0	0.173 ± 0.001	–	
0	10	0.100 ± 0.000	–	
0	20	0.200 ± 0.000	–	
1	1	0.027 ± 0.000	2.7	1.0
5	1	0.098 ± 0.000	9.8	1.1
10	1	0.182 ± 0.001	18.2	0.9
1	5	0.067 ± 0.001	6.7	5.0
5	5	0.137 ± 0.001	13.7	5.0
10	5	0.222 ± 0.001	22.2	4.9
1	10	0.118 ± 0.000	11.8	10.1
5	10	0.186± 0.002	18.6	9.9
10	10	0.272 ± 0.003	27.2	9.9
1	20	0.216 ± 0.002	21.6	19.9
5	20	0.288 ± 0.001	28.8	20.1
10	20	0.372 ± 0.003	37.2	19.9

Primary results were calculated directly from the slope for nitrate. Reported nitrate concentrations were calculations were calculated by Eq.(4).

µM for NO_2^- and 1 – 20 µM for NO_3^-. With the speed dial setting of 30 on the Ismatec pump, the flow rates for the carrier(s) were 5 mL/min on the Manifolds I and III, and were 2.4 mL/min on the Manifold II. After merging with the flow of the ammonium chloride reagent, the rates at which the sample passes through the reductor were 5.2 and 2.6 mL/min respectively. The contact times in the Cd reductor were ca. 11 s with Manifold I/III and ca. 22 s with Manifold II. The time for the formation of the pink azo dye in all the manifolds was ca. 25 s, allowing ca. 90% of the color to develop at 25°C[15].

The results for Manifold I are shown in Table 1. The mean slopes (S_1 and S_2) for nitrite and nitrate solutions were 0.0173 and 0.0100 $A\mu M^{-1}$ respectively (where A is the absorbance), indicating an apparent reduction rate of 58%. When S_2 was used for calculating the concentration of the test samples, the primary results for the "original + reduced" nitrite were all higher than the true total concentrations. The over-estimation of nitrate was roughly equivalent to $[NO_2^-]/[NO_3^-]/E\%$. Using Eq.(4) the correct nitrate concentrations were obtained for all the tested samples. For example, the calculated nitrate concentrations for a 1 µM NO_3^- prepare in 1,5, and 10 µM NO_2^- solution were 1.0, 1.1, and 0.9 µM respectively.

The results for the dual-injection manifolds are listed in Table 2 and 3. The three calibration slopes (S, S_1, and S_2) for the Manifold II were 0.0152, 0.0116, and 0.086 $A\mu M^{-1}$ respectively. The apparent reduction rate (E%) was 74%, slightly higher than that with the Manifolds I and III due to the longer residence time of the sample in the Cd reductor. However, the over-all response was lower due to the dilution with the other stream. The sensitivities given by the Manifold III were equivalent to those given by the one-channel or two individual-channel system, and the three calibration slopes were - 0.0227, 0.0173, and 0.0101 $A\mu M^{-1}$ respectively. The primary results for nitrate were all over-estimates if only S_2 was used for the calculation. However, the nitrate concentrations that were calculated using Eq.(6) corresponded closely with the original values.

Table 2 Laboratory tests on nitrite + nitrate mixtures with Manifold II.

Conc. added		*First peak*	*Second peak*	*Primary*	*Conc. found*	
$[NO_2^-]$ (μM)	*$[NO_3^-]$ (μM)*	*Abs(1) ± sd (n = 3)*	*Abs(2) ± sd (n = 3)*	*results (μM)*	*$[NO_2^-]$ (μM)*	*$[NO_3^-]$ (μM)*
5	0	0.076 ± 0.000	0.058 ± 0.000	–		
10	0	0.152 ± 0.000	0.116 ± 0.000	–		
0	10	0.000 ± 0.000	0.086 ± 0.000	–		
0	20	0.000 ± 0.000	0.172 ± 0.001	–		
1	1	0.015 ± 0.000	0.019 ± 0.000	2.2	1.0	0.9
5	1	0.077 ± 0.000	0.067 ± 0.001	7.8	5.1	0.9
10	1	0.152 ± 0.001	0.125 ± 0.002	14.5	10.0	1.0
1	5	0.015 ± 0.001	0.054 ± 0.000	6.3	1.0	4.9
5	5	0.077 ± 0.000	0.101 ± 0.001	11.7	5.1	4.9
10	5	0.151 ± 0.001	0.159 ± 0.000	18.5	9.9	5.1
1	10	0.015 ± 0.001	0.099 ± 0.001	11.5	1.0	10.2
5	10	0.075 ± 0.001	0.145 ± 0.000	16.9	4.9	10.2
10	10	0.150 ± 0.002	0.201 ± 0.002	23.4	9.9	10.0
1	20	0.015 ± 0.001	0.183 ± 0.002	21.3	1.0	19.9
5	20	0.077 ± 0.002	0.228 ± 0.002	26.5	5.1	19.7
10	20	0.151 ± 0.002	0.290 ± 0.003	33.7	9.9	20.3

Reported concentrations for nitrite and nitrate were calculated by Eq.(5) and Eq.(6).

Table 3 Laboratory tests on nitrite + nitrate mixtures with Manifold III.

Conc. added		*First peak*	*Second peak*	*Primary*	*Conc. found*	
$[NO_2^-]$ (μM)	*$[NO_3^-]$ (μM)*	*Abs(1) ± sd (n = 3)*	*Abs(2) ± sd (n = 3)*	*results (μM)*	*$[NO_2^-]$ (μM)*	*$[NO_3^-]$ (μM)*
5	0	–0.114 ± 0.000	0.087 ± 0.001	–		
10	0	–0.227 ± 0.001	0.173 ± 0.000	–		
0	10	0.000 ± 0.000	0.101 ± 0.001	–		
0	20	0.000 ± 0.000	0.202 ± 0.000	–		
1	1	–0.023 ± 0.001	0.027 ± 0.000	2.7	1.0	1.0
5	1	–0.114 ± 0.001	0.098 ± 0.001	9.7	5.0	1.0
10	1	–0.225 ± 0.002	0.182 ± 0.001	18.0	9.9	0.9
1	5	–0.023 ± 0.001	0.068 ± 0.000	6.7	1.0	5.0
5	5	–0.115 ± 0.000	0.138 ± 0.001	13.7	5.1	4.9
10	5	–0.227 ± 0.000	0.224 ± 0.002	22.2	10.0	4.9
1	10	–0.023 ± 0.001	0.116 ± 0.001	11.5	1.0	9.8
5	10	–0.113 ± 0.001	0.187 ± 0.001	18.5	5.0	9.9
10	10	–0.224 ± 0.002	0.275 ± 0.003	27.2	9.9	10.2
1	20	–0.023 ± 0.000	0.220 ± 0.002	21.8	1.0	20.1
5	20	–0.113 ± 0.001	0.286 ± 0.003	28.3	5.0	19.7
10	20	–0.225 ± 0.001	0.373 ± 0.002	36.9	9.9	19.8

Reported concentrations for nitrite and nitrate were calculated by Eq.(5) and Eq.(6).

Effect of variation of pumping speed

Although all the above experiments were performed at a fixed pumping speed, i.e. at a dial setting of 30 on the Ismatec IPS-12 pump, it is feasible to operate the FIA manifolds at higher flow rates to increase the throughput rate. However, this can affect the calibration in three ways: (1) the physical dispersion increases with the increase of flow rate, but diffusion is minimized because of the shorter residence time, (2) the shorter residence time may lead to incomplete formation of the pink azo dye, and (3) the contact time of the sample with the reductor will be reduced leading to less efficient reduction. The over-all effect on the sensitivity was demonstrated with the Manifold II system (Figure 4). By doubling the pumping speed (changing the dial setting from 30 to 60), the three slope values (S, S_1, and S_2) were changed from 0.0152, 0.0116, and 0.0086 $A\mu M^{-1}$ to 0.0115, 0.0086, and 0.0048 $A\mu M^{-1}$ respectively. The apparent efficiency (E%) was changed from 74% to 57% (Figure 5) when the contact time was reduced from 22 s to 11 s.

Precision and detection limit

An over-all relative standard deviation of ca. 1% was achieved by the three manifolds at 10 – 20 μM levels. The detection limits (calculated as 3 times the standard deviation of the baseline variation of the blank) for the three manifolds were 0.05 μM for nitrite and ca. 0.1 μM for nitrate.

Comparison of the three manifolds

For a sample which contains both nitrate and nitrite, the data produced by the Manifold I should not be reported alone. The nitrite concentration must be measured separately either by a manual procedure or by using another FIA channel (or by disconnecting the reductor). With Manifolds II and III both species in a sample can be measured almost simultaneously by the dual-injection operation. These have advantages over the conventional two-channel systems, because only one spectrophotometer is needed. They are also versatile for measuring single species, by simply loading the sample through one injector and ignoring the other. In the authors' experience, the Manifold II system, which requires the fewest installations (only one flow cuvette, one detector, and one set of

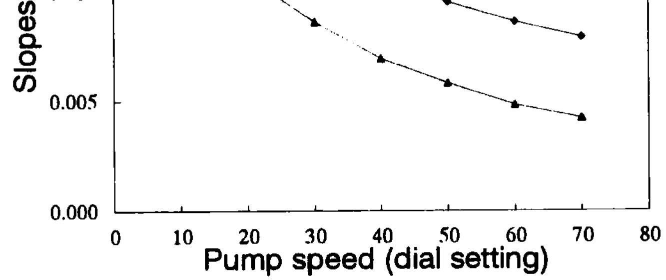

Figure 4 Effect of pumping speed on the sensitivity of the Manifold II. The pumping speed dial setting of 30 on the Ismatec IPS-12 peristaltic pump gave a flow rate of 2.4 mL/min for the carrier flow.

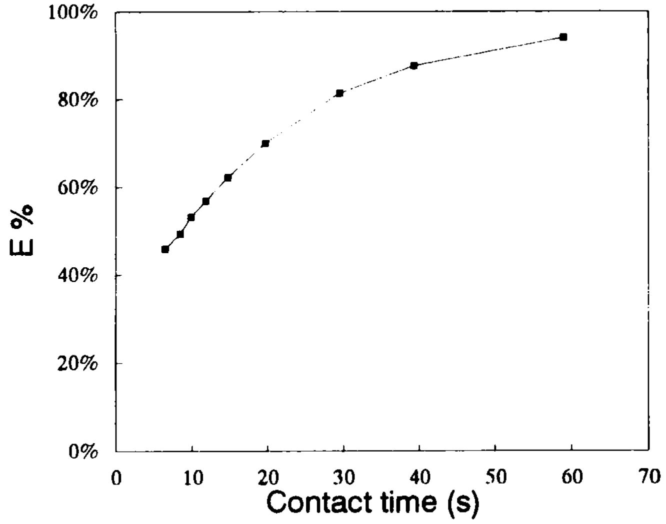

Figure 5 Effect of contact time on the apparent reduction efficiency of the no-line Cd reductor. The contact time was defined as the mean residence time of the sample in the reductor, equivalent to (void volume in the reductor)/(flow rate).

reagents), is probably the most useful system for routine analysis of both nitrite and nitrate. Although the throughput rate of this manifold (ca. 30 samples/hr) was less than that obtained with a similar design suggested by van Staden[16], it provided a better reduction efficiency and a higher sensitivity.

Measurement of seawater

Manifold II was applied to the measurement of the concentrations of nitrite and nitrate in a series of spiked seawater. A surface seawater from the East China Sea was collected at a location ca. 10 km offshore of Keelung (Taiwan). It was filtered with a Whatman GF/C filter and stored in a polypropylene bottle. Neither nitrite, nor nitrate was detectable in this seawater. In the f.i.a. system a 0.55 M NaCl solution was used as the carrier(s) to reduce the refractive index interference caused by the salt gradient in the flow. This almost eliminated the interfering signal (nor more than 0.001 A on the recorder chart). The system was calibrated by making measurements on this seawater after spiking with known amounts of standards. The three slope values (S, S_1, and S_2) were found to be 0.0145, 0.0111, and 0.0064 $A\mu M^{-1}$ respectively for nitrite and nitrate through the two injection channels. The apparent reduction efficiency (E%) of the Cd reductor was ca. 57%, slightly lower than that found for the freshwater medium under the same conditions. A series of spiked seawater was prepared by adding 100 mL aliquots of seawater with both nitrite and nitrate standard solutions. The peaks obtained with Manifold II were evaluated using Eq. (6), and the calculated concentrations of nitrite and nitrate are reported in Table 4. The recoveries for both species were found to be satisfactory (98 – 102%) for all samples within the tested range of 1.98 – 19.42 μM for nitrite and 9.71 – 47.39 μM for nitrate. The small variations of salinity in the spiked samples (due to the dilution of standard solution) did not significantly influence the recovery.

Table 4 Measurement of spiked seawater with Manifold II.

Spiked conc.		*Volume**	*Conc. found (n=3)*		*Recovery (%)*	
$[NO_2^-]$ (μM)	*$[NO_3^-]$ (μM)*	*of sample (mL)*	*$[NO_2^-]$ (μM)*	*$[NO_3^-]$ (μM)*	*NO_2^-*	*NO_3^-*
0	0	100.0	0.0 ± 0.0	0.0 ± 0.0	–	–
1.98	9.88	101.2	2.0 ± 0.0	9.9± 0.1	101%	100%
4.83	28.99	103.5	4.8 ± 0.0	28.5 ± 0.2	99%	98%
9.71	19.42	103.0	9.7 ± 0.1	19.7 ± 0.1	100%	101%
4.74	47.39	105.5	4.7 ± 0.0	47.0 ± 0.1	99%	99%
19.42	9.71	103.0	19.5 ± 0.1	9.9 ± 0.0	100%	102%

* Aliquot of 100 mL filtered seawater was added with x mL of 1 mM nitrite standard and y mL of 1 mM nitrate standard. (x = 0.2, 0.5, 1, 2 mL; y = 1, 2, 3, 5 mL)

CONCLUSION

Flow injection analysis incorporating an on-line Cd reductor is probably the most convenient method for the determination of nitrate in natural waters, especially seawater. However, a reductor with an efficiency of 100% is almost impossible to prepare, and in addition the decrease in its efficiency which can occur during a series of measurements leads to uncertainties in the results obtained. It was found easier to prepare a stable, reproducible, and relatively low efficiency reductor than one that acts almost quantitatively, but soon loses its efficiency. Directions are given for the preparation of such a reductor and the need for cleaning loose materials from the Cu plated Cd has been demonstrated. A correction scheme for FIA systems equipped with a non-quantitative reductor to correct the effect of nitrite on nitrate measurement has been devised. With this method only the stability of the Cd reductor is a major concern, and the non-quantitative reduction of nitrate ceases to be a problem. Using a stable but low efficiency reductor, the concentration of nitrate could be accurately obtained even if the co-existing nitrite concentration is at least equal to that of nitrate.

A dual-injection merged-flow manifold with an on-line Cd coiled reductor has been proposed for the determinations of nitrite and nitrate in natural fresh and saline waters. With this system both species in a sample can be measured almost simultaneously at a high precision (< 1% at 20 μM) and at a reasonable throughput rate. A detection limit of 0.1 μM can be readily achieved. It has the advantages over other systems in terms of simplicity and versatility, and is therefore recommended for routine analysis.

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