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COMPARISON OF OPEN TUBULAR CADMIUM REACTOR AND PACKED CADMIUM COLUMN IN AUTOMATED GAS-SEGMENTED CONTINUOUS FLOW NITRATE ANALYSIS

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Detailed procedures are provided for preparing packed cadmium columns to reduce nitrate to nitrite. Experiments demonstrated the importance of conditioning both open tubular cadmium reactor (OTCR) and packed copper-coated cadmium columns to achieve 100% reduction efficiency. The effects of segmentation bubbles in the OTCR upon reduction efficiency and baseline noise in nitrate analysis are investigated using an auto-analyzer. Metal particles derived from segmentation bubbles in OTCR result in interference with continuous flow analyses. Therefore packed columns are recommended for determination of low level nitrate in natural waters.

Keywords: Cadmium column; nitrate; nitrite; continuous flow analysis

INTRODUCTION

There are few selective methods to directly determine nitrate concentration in natural waters. The most widely used method of nitrate determination relies upon the conversion of nitrate to nitrite. The resulting nitrite is determined by a diazotization reaction^[1-3]. The colorimetric technique for measuring the diazotization product is both sensitive and reliable. The overall precision and accuracy of nitrate analysis is therefore limited primarily by the reduction step. Various reductants have been proposed and tested, but their reduction efficiencies^[4-5] proved to be too low. Copper-coated cadmium columns were introduced for

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nitrate analysis in seawater by Wood *et al.*^[6] more than thirty years ago. Since then they have become the most commonly used reductant for the determination of nitrate in seawater^[7–15].

However, preparation of packed columns is laborious, time consuming and requires considerable manual skill. It involves cutting cadmium sticks into filings and sieving them to obtain an appropriate uniform size then packing the filings in a uniform consistent manner into glass or plastic tubing. Despite much effort, many analytical chemists have experienced unsatisfactory column performance, *i.e.*, low reduction efficiency and short lifetimes.

To incorporate such columns into gas-segmented continuous flow nitrate analysis with an autoanalyzer requires some modification in the flow system. Since packed columns do not permit gas bubbles free passage, the segmentation gas bubbles must be removed before passage through the cadmium column and reintroduced subsequently. This unavoidably results in enhanced mixing between samples and wash solution. In an attempt to reduce carry-over and sample dispersion an open tubular cadmium reductor (OTCR) was developed for gas-segmented continuous flow nitrate analysis^[16]. It reduces nitrate to nitrite while samples flow through it in a series of gas-segmented slugs. Commercial OTCRs are readily available (Alpkem, Oregon; Bran+Luebbe, Illinois; Irama, Oregon, USA). However, many autoanalyzer operators have experienced frustrations similar to those with packed columns, *i.e.*, low reduction efficiency and short lifetimes. There are few systematic studies of the performance and mechanism of cadmium reductors^[17–20]. Procedures reported in literature for the preparation of cadmium columns are different in detail and none of them seems to guarantee columns with consistently high reduction efficiencies and reasonably long lifetimes. Most importantly no direct comparisons have been published on the relative performance of packed columns versus OTCR. In attempt to optimize the performance of cadmium reductors for low level nitrate analysis in oligotrophic seawater, techniques of preparation, charging and maintenance of both packed columns and OTCRs have been refined and critical evaluations and comparisons of their advantages and limitations in conjunction with gas-segmented continuous flow nitrate analysis were conducted in our laboratory. This paper reports the results of our systematic investigation.

EXPERIMENTAL

Reagents and solutions

Standard and reagent solutions were prepared using water that was fresh distilled then run through a Millipore Super-Q Plus Water System (Millipore Corporation,

Bedford, Massachusetts, USA) increasing resistivity to 18 meg-ohms. To avoid any contamination, it was used the same day it was purified. Nitrate stock standard solutions (10 mM) were prepared from high purity grade (99.99%, Aldrich, Milwaukee, Wisconsin, USA) pre-dried (105°C for 1 hour) potassium nitrate (KNO_3). Nitrite standard stock solutions (10 mM) were prepared from analytical reagent-grade pre-dried (105°C for 1 hour) sodium nitrite (NaNO_2). The stock solutions were stored in polyethylene bottles at 4°C in a refrigerator. Working standard solutions were prepared by diluting these stock solutions with water or Low Nutrient Sea Water (LNSW). LNSW was collected from surface of the Sargasso Sea after the spring phytoplankton bloom, aged for one month and then filtered through 0.3-micron pore size glass fiber filters. Sulfanilamide stock solutions were prepared by dissolving 10 g of sulfanilamide ($\text{C}_6\text{H}_8\text{N}_2\text{O}_2\text{S}$) in 1 liter of 10% (v/v) HCl solution. 1 ml of Brij-35 surfactant (polyoxyethylene(23) lauryl ether ($\text{C}_{12}\text{H}_{25}(\text{OCH}_2\text{CH}_2)_{23}\text{OH}$, ICI Americas Inc.) was mixed with 200 ml of the stock sulfanilamide solution daily and used as a working solution. 1 g of NED (n-1-naphthylethylenediamine dihydrochloride, $\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$) was dissolved in 1 liter of water as a NED solution on a daily basis. Imidazole buffer solution was prepared by dissolving 13.6 g of imidazole ($\text{C}_3\text{H}_4\text{N}_2$) in 4 liters of water and adjusting the pH to 7.8 with diluted HCl. 2% copper sulfate solution (20 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 liter of water) was used for coating copper on the OTCR and cadmium fillings.

Activation of the open tubular cadmium reactor

A commercial OTCR (Alpkem, Oregon, Irama, Oregon, or Brun+Lubee, Illinois, USA) is a hollowed cadmium tube covered with a plastic sleeve for safety and wound into a 5 or 10-turn coil. It needs to be cleaned with HCl solution and coated with copper before it can be used for nitrate reduction. The OTCR was connected to a 10-ml plastic syringe and flushed sequentially with 10-ml of water, then 10-ml of 0.5N HCl solution, then a few more syringes of water. Flushing with HCl must be completed within a few seconds and followed immediately by flushing with water since prolonged contact of cadmium with HCl solution can dissolve too much cadmium. The clean OTCR was then slowly flushed with the CuSO_4 solution until a large amount of black precipitated copper results, indicating completion of coating. Flushing was then stopped. Prolonged flushing with copper solution can cause excessive dissolution of cadmium. Last the copper-coated OTCR was filled with the imidazole buffer solution which permits storage for at least one month with no measurable loss in activity.

Preparation of the packed cadmium reduction column

Although cadmium granules are commercially available, freshly prepared cadmium filings with cadmium sticks (J.T. Baker Chemical Co. Phillipsburg, New Jersey, USA) are usually more reactive since surface of the cadmium granules is often covered with an oxidation product, CdO. Fresh filings were sieved and the fraction between 25 and 60-mesh size (0.25–0.71 mm) retained. The filings were initially washed with acetone to remove any organic contamination on the surface. Approximately 50 g of these filings were then washed twice with 10% HCl immediately followed by water. The water was then decanted and 50 ml of 2% CuSO₄ solution added. Upon contacting the copper solution, the surface of the cadmium filings was coated with copper and the silvery filings turned gray. Gentle swirling was necessary to achieve a uniform coating. While swirling, brown flakes of colloidal copper appeared and the blue color of the solution faded. The faded solution was then decanted, fresh CuSO₄ solution added and swirled again. This procedure was repeated until the blue color of solution did not fade. During this process do not use a stirring bar to vigorously stir the cadmium filings. Excessive disturbance can remove the copper coating from the cadmium filings and expose new cadmium surfaces to the copper solution. Continuous exposure of new cadmium surfaces to the copper solution will eventually dissolve all the cadmium filings resulting in a useless lump of brownish copper mud.

The copper-coated cadmium filings were then washed with water until the supernatant was clear and free of fine particles. The filings were then kept under water to avoid air exposure prior to packing. 2-mm ID glass tubes were used as columns. The lower end was plugged with glass wool and the upper end connected to a 10-ml pipette tip. The column was filled with water and Cd filings transferred in suspension through the pipette tip into the column. By gently tapping the tube and pipette tip Cd filings can be packed tightly and uniformly in the column. Caution must be exercised to avoid entraining any air bubbles in the column during packing. When filled the top of column was plugged with glass wool and plastic tubing filled with buffer solution connected to both ends of the column to form a closed loop.

Stabilization and reduction efficiency of the OTCR and packed cadmium columns

Before installing a cadmium column or OTCR on the analytical manifold, a 40- μ M nitrite solution was pumped continuously through the sample line and the absorbance signal recorded. A column was then installed in the analytical mani-

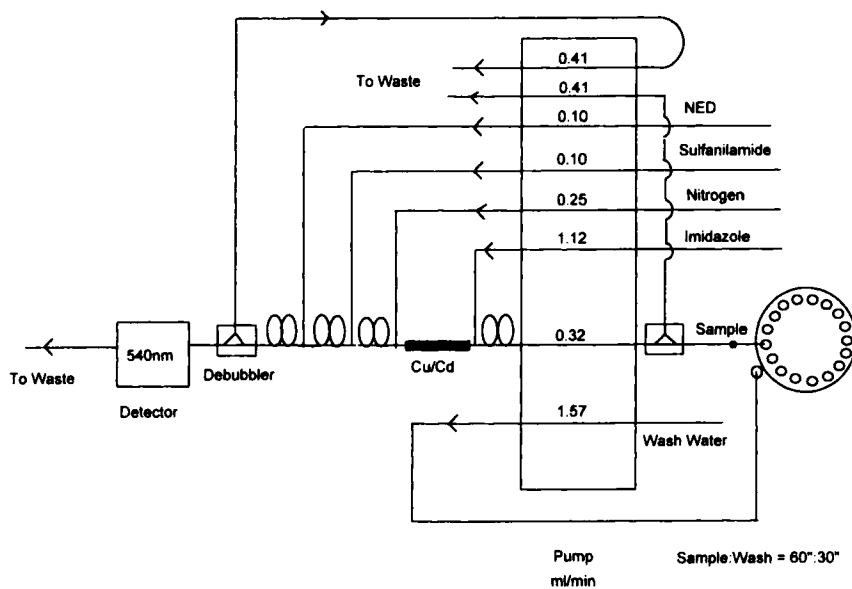


FIGURE 1 Flow diagram of gas-segmented continuous flow nitrate analysis using a packed copper-coated cadmium column

fold and a 40- μ M nitrate solution was pumped continuously through the sample line while recording the output signal. The absorbance signal initially increased with time and reached a steady state in 10–15 minutes. This steady state signal from nitrate was compared to the signal obtained from same concentration of nitrite solution without the packed cadmium column on-line. The reduction efficiency of this column is calculated from the ratio of the absorbance of the nitrate solution to the absorbance of same concentration of nitrite solution and has been previously found to be better than 99%^[20,21]. The reduction efficiency of the cadmium column must be checked periodically by analyzing a nitrate standard solution followed by a nitrite solution of the same concentration. Better than 99% reduction efficiencies are essential to analyze the trace levels of nitrate typical of oceanic near surface waters.

Automated analysis of nitrite and nitrate

An Alpkem Flow Solution autoanalyzer (Alpkem, Oregon, USA) was used for gas-segmented continuous flow analysis of nitrate and nitrite concentrations in seawater samples^[21]. The manifold configuration and flow diagram for the

gas-segmented continuous flow nitrate analysis used is shown in Figure 1. To analyze nitrate, samples must first be mixed with imidazole buffer solutions (pH=7.8) and then passed through a copper-coated cadmium reduction column. Nitrate in the samples is thereby reduced to nitrite. The total nitrite (the sum of the nitrite present in the original sample and nitrite derived from the reduction of nitrate) is determined by diazotizing with sulfanilamide and coupling with N-1-naphthylethylenediamine dihydrochloride to form a pink color azodye. Absorbance of dye is measured at 540 nm. Nitrite concentrations in the original samples are determined separately using same system but without cadmium reduction. Nitrate concentrations are then obtained by difference (subtracting nitrite values from the sum of nitrite and nitrate values). If desired nitrite and nitrate can be determined simultaneously by splitting the samples into two parallel analytical systems. In such a multi-channel system it is critically important, however, to precisely match the flow rates of each subsystem to obtain synchronous output signals.

RESULTS AND DISCUSSION

Reactivity of cadmium filings without copper coating

To study the role of copper coating on the cadmium surface upon the reductive reactivity of a column, a bare cadmium column was prepared using cadmium filings without a copper coating to compare with the cadmium column with a copper coating. First we examined the reactivity of the bare cadmium column on the reduction of nitrite. As mentioned above, control experiment was run first by pumping 2.4 and 20 μM nitrite solutions through the analytical system with no column on line (run 1 and 2 in Table I). The absorbance measured in this control experiment provides a reference to which column reduction efficiencies can be compared. Absorbances approximately proportional to the concentrations (0.011 and 0.092 from 2.4 and 20 μM nitrite solutions, respectively) were obtained. The bare cadmium column was then connected on line and the same nitrite solutions passed through the system. Absorbances observed from the same nitrite solutions that had been passed through the bare cadmium column were similar to those obtained with no cadmium column online (compare run 1 and 2 with run 3 and 4 in Table I). Since no change in nitrite signal was observed after the nitrite solution passed a bare cadmium column it was concluded that no nitrite reduction occurred when the nitrite solution was in contact with bare cadmium.

Secondly, we examined the reactivity of a bare cadmium column upon the reduction of nitrate. No signal was observed from pumping 2.4 and 20 μM nitrate

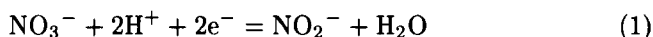
solutions through the analytical system with no column on line (run 5 and 6 in Table I) as expected. The bare cadmium column was then connected and the same nitrate solutions were passed through the system. No signal was observed from pumping different concentrations of nitrate solutions through the bare cadmium column (run 7 and 8 in Table I). It was concluded that no nitrite from reduction of nitrate results when nitrate solutions were passed over bare cadmium filings. Since nitrite is the only reaction product detected in the experiments, the absence of a nitrite signal does not rule out the possibility of reduction of nitrate to a product with a lower oxidation state than nitrite. To test this possibility, an ordinary copper-coated cadmium column was used. Test of 20 μM nitrite and nitrate solutions (run 9 and 10) indicated that the nitrate reduction efficiency of the column was satisfactory (close to 100%). A 20 μM nitrate solution was then passed through a bare cadmium column and the effluent collected. This effluent was then analyzed for nitrate using the copper-coated cadmium column (run 11 in Table I). No significant difference in absorbance was obtained between the original nitrate solution and the effluent that had passed through the bare cadmium column. This result indicates that no significant further reduction of nitrate beyond nitrite had occurred on the cadmium surface. In summary, these data show that the bare cadmium surface is nonreactive to either nitrate or nitrite at least on time scales of continuous flow analysis (a few minutes).

TABLE I Reductive reactivity of cadmium column with no copper coating as compared to copper-coated cadmium column on the reduction of nitrate and nitrite at various concentrations in aqueous solutions

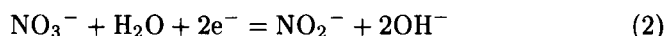
run	column type	test solution	Concentration (μM)	absorbance	reduction efficiency (%)
1	—	NO_2^-	2.4	0.011	—
2	—	NO_2^-	20.0	0.092	—
3	Cd	NO_2^-	2.4	0.011	0
4	Cd	NO_2^-	20.0	0.092	0
5	—	NO_3^-	2.4	0.000	—
6	—	NO_3^-	20.0	0.000	—
7	Cd	NO_3^-	2.4	0.000	0
8	Cd	NO_3^-	20.0	0.000	0
9	Cd-Cu	NO_2^-	20.0	0.092	0
10	Cd-Cu	NO_3^-	20.0	0.091	99
11	Cd-Cu	NO_3^-	20.0 ^a	0.090	98

a. This sample was collected from effluent of a bare cadmium column that has been passed through with a 20 μM nitrate solution.

Thermodynamically nitrate is a strong oxidizing reagent in acidic solution. The reduction of nitrate ion to nitrite ion has a standard potential of 0.94V in acidic solution,

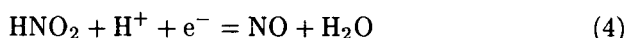
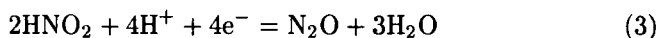


The reduction potential is a function of hydrogen ion concentration and it decreases to 0.015V in basic solution,



In neutral or mild alkaline solution as seawater (pH = 8) the reduction potential is about 0.4V.

Nitrite is also a strong oxidizing reagent in acidic solution. The reductions of nitrite ion to N_2O or NO have standard potentials of 1.297V and 0.983 V in acidic solution, respectively



In neutral or mild alkaline solutions like seawater (pH = 8) the reduction potentials are 0.8 and 0.5V, respectively.

On the other hand the reduction potential of Cd^{2+} to metal Cd is only -0.403V ,



There are therefore about 0.8V and 0.9V of E.M.F. to drive the reduction of nitrate and nitrite by cadmium in neutral solutions. Even in basic solutions the reduction of both nitrate and nitrite by cadmium is thermodynamically favored. Since we observed no reduction of either nitrate or nitrite in sample solution that had been in contact with the cadmium surface, despite those thermodynamics, the reduction reaction must be hindered by reaction kinetics.

Stabilization of OTCR and packed copper-coated cadmium reduction columns

A series of experiments were designed to test the reactivity of freshly charged OTCR or newly packed columns in reducing nitrate and nitrite (Table II). The first experiment involved pumping a nitrite solution through an analytical system that had no OTCR or a copper-coated cadmium column on-line. A solution of 40 μM nitrite yielded an absorbance of 0.185 AU. Upon connecting a newly activated OTCR an absorbance of 0.080 AU was obtained with the same concentration (40 μM) of nitrite solution. An absorbance of 0.110 AU was obtained from 40 μM nitrate solution. This implies a reduction efficiency of 59.5% for nitrate

and a nitrite yield of only 43.2%, i.e., more than half of the nitrite must be reduced by the OTCR to even lower oxidation state nitrogen compounds such as NO, N₂O, N₂, NH₂OH and NH₃. The lower reduction efficiency for nitrate is not due to passivity of the Cd surface. In fact the copper-coated Cd surface is over-reactive. It reduced all nitrate to nitrite and further reduced 40.5% of the nitrite formed to even lower oxidation state nitrogen compounds. That the nitrite yield from nitrate solution (59.5%) is higher than that from nitrite solution (43.2%) is most probably due to the time lag required for the reduction of nitrate to nitrite inhibiting further reduction

TABLE II Effect of conditioning on the reduction efficiencies of freshly prepared copper-coated cadmium column on the reduction of nitrate and nitrite at various concentrations in aqueous solutions

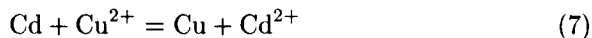
run	column type	test solution	concentration (μM)	absorbance	reduction efficiency (%)
1	—	NO ₂ ⁻	40	0.185	—
2	Cd-Cu ^a	NO ₂ ⁻	40	0.080	43.2
3	Cd-Cu ^a	NO ₃ ⁻	40	0.110	59.5
5	Cd-Cu	NO ₂ ⁻	40	0.185	0
6	Cd-Cu	NO ₃ ⁻	40	0.185	100
7	Cd-Cu	NO ₂ ⁻	8.0	0.037	0
8	Cd-Cu	NO ₃ ⁻	8.0	0.037	100

a. This was freshly prepared copper-coated cadmium column without conditioning

The reduction potential for copper and its divalent ion couple is 0.337V,



If the cadmium surface first reacts with CuSO₄ solution the cadmium will be oxidized to the cation Cd²⁺ and Cu²⁺ will be reduced to metallic copper that will precipitate on the cadmium surface as a layer of porous coating.



A galvanic cell is then established with copper as the cathode and cadmium as the anode. The EMF of this galvanic cell is 0.740 V. The reducing power of the copper coated cadmium surface is in fact lower than pure cadmium since copper is a less reductive metal than cadmium. However, the EMF established in this microcell drives electrons from the anode (cadmium) to the cathode (copper). Electrons thereby pass from the copper surface to nitrate ion in aqueous solution and the redox reaction is facilitated by copper bridging between cadmium and aqueous nitrate ion. The catalytic effect of the mixed metal (or a metal alloy)

accelerates the redox reaction. It has been reported that the oxidation rate of cadmium by dissolved oxygen in aqueous solution is 10 times higher with Cu-Cd alloy surface than with metallic cadmium^[22]. In this case, electron transfer from Cd to dissolved oxygen is facilitated through a microcell using a Cu cathode.

To achieve a 100% recovery from sample nitrate one must obtain both 100% efficiency of reduction of nitrate to nitrite and no further reduction (0%) of nitrite to lower nitrogen compounds. Stabilization or conditioning of OTCR or copper-coated cadmium columns is essential to approach this optimum. To condition OTCRs and packed columns, a new column was put on line and a 40 μM nitrate standard solution pumped continuously through the sample line while absorbance is recorded. Absorbance increased with time from an initial value of 0.110 to a steady state value of 0.185 in about 10–15 minutes. The nitrate solution was then passed through the column for another 10 minutes to ensure a constant output signal (see Figure 2). Although the detailed mechanism of this conditioning process is not clear it probably involves re-arranging the reactive surface site to create a uniform reactive surface with selective reduction of nitrate to nitrite. After conditioning different concentrations of nitrate solutions were passed and 100% yields were obtained (run 6 and 8 in Table II). A 100% yield of nitrite were also obtained from pumping different concentrations of nitrite solution through the column, which verifies that with proper conditioning there is no further reduction of nitrite to lower oxidation state compounds (run 5 and 7 in Table II).

In summary, we found that newly activated OTCR or copper-coated Cd column is over reactive in respect to the reduction of nitrate to nitrite. A stabilization process, or conditioning, is necessary to obtain 100% nitrate reduction efficiencies. During daily analysis the reduction efficiency of OTCR or packed column should be periodically checked by measuring absorbance of a nominal concentration of nitrate solution followed by a nitrite solution of same concentration. Reduction efficiency can be calculated from the ratio of absorbance of the nitrate solution to that of the nitrite solution of same concentration. If it is below 95% the column should be recharged by pumping CuSO_4 solution through the column for 10 minutes and followed by conditioning with a 40 μM nitrate solution.

Comparison of OTCR and packed copper-coated cadmium columns

The OTCR was designed to reduce carry-over in continuous flow analysis. In order to avoid oxidation of cadmium by atmospheric oxygen, nitrogen gas is usually used as segmentation gas instead of air. Intersample air bubbles generated by the auto-sampler must be removed, however, to avoid oxidation of cadmium by atmospheric oxygen. If intersample air segmentation is removed, there is signifi-

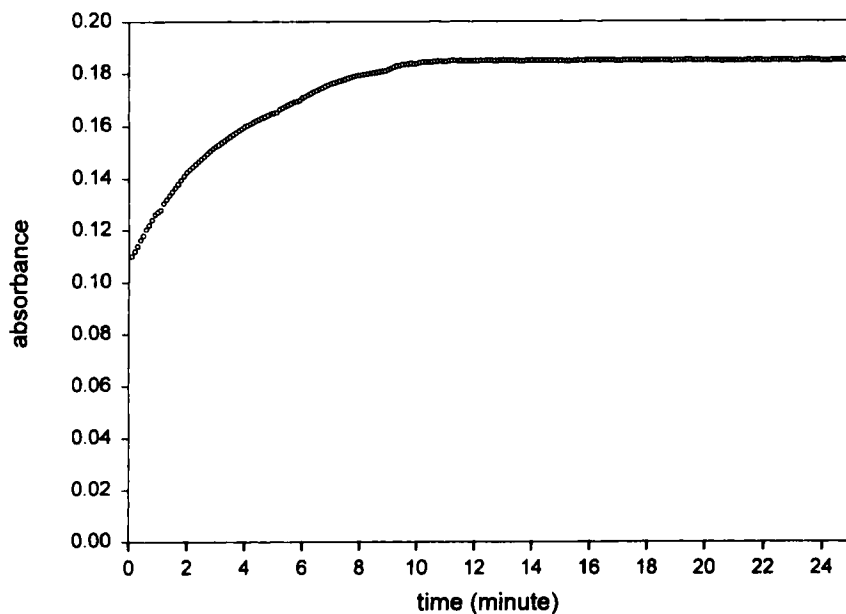


FIGURE 2 Effect of conditioning on the reduction efficiency of a copper-coated cadmium column

cant carry-over in the flow system^[23]. With removal of intersample air segmentation the OTCR has no advantage over packed columns. We have used OTCR to measure nitrate concentrations in seawater samples on several cross-basin cruises. Over two thousand samples were analyzed on each cruise by a shipboard auto-analyzer equipped with an OTCR. From time to time noisy nitrate peaks were observed. Close examination revealed that black particles were accumulating in the debubbler located between the OTCR and the detector. When the gas-segmented flow streams passed through the OTCR, fine black particles flowed out of the OTCR; some were trapped in the debubbler and some flowed into the detector. Apparently the reaction of sample nitrate with cadmium resulted in the loss of loosely bounded Cu particles. This signal noise could be reduced by rapidly flushing the OTCR with a syringe of buffer solution. The high-speed flow apparently removed copper coating loosely attached to the inner surface of OTCR. Unfortunately this treatment reduced the lifetime of the OTCR apparently accelerating the loss of the necessary copper coating on the cadmium surface.

To use OTCR for measuring low nitrate concentrations in oligotrophic waters it is necessary to lower the detection limit by increasing the signal to noise ratio.

Since the detector we used is sensitive to low analyte signals (the most sensitive scale is 0.0005 AUFS) base line noise becomes critical. A steady state baseline can be achieved with the flow system without an OTCR on-line at a setting of 0.0005 AUFS. The base line noise of the whole analytical system is less than 5% of AUFS – i.e. an absorbance of 0.00002. However the base line noise increases to 0.001 if an OTCR was put on-line. The base line noises associated with the OTCR then becomes the limiting factor in achieving low detection limits for nitrate analysis. Although few particles accumulated in the debubbler after first flushing off the loosely attached copper particles very fine particles were still flowing out of the OTCR and into the detector. Even though no reaction is taking place when reagent blank solution flows through the OTCR, nitrogen gas segmentation bubbles are likely physically removing more loosely attached copper particles from cadmium surface into the solution. When these particles enter in the light path within the flow cell, absorption and scattering by these particles results in severe output signal noise at low AUFS. To verify this hypothesis the nitrogen injection site was changed from upstream of the OTCR to downstream. With no bubbles passing through OTCR the baseline noise was greatly reduced. When samples were analyzed using such modified flow configuration, however, the nitrate signal was found to decrease to about 70% compared with pumping the same concentration nitrate solution with nitrogen-segmented flow through a 61-cm long OTCR. Different nitrate concentrations (0.08, 10 and 40 μM) gave a similar reduction efficiency (70%) as shown in Table III. This puzzling phenomenon was explained when we connected two OTCRs in series. The signals were increased to 93%. The signal achieved 100% only when three OTCRs (total length of 183 cm) were connected together in series. Clearly a much longer flow path is required for complete reaction by the OTCR when no segmentation bubbles are employed. Segmentation gas bubbles enhance mixing within flow slugs and hence increase contact between the sample solution and the copper-coated cadmium surface. A flow stream without gas segmentation is more laminar. Longer times are therefore needed for complete mixing and hence complete reduction of nitrate by the copper-coated cadmium surface.

On the other hand, when packed columns are used, a steady state reagent baseline could be readily obtained at the lowest AUFS setting (0.0005). In preparing the packed columns, the copper coating process was carried out in a beaker and all fine copper particles washed out before packing. Moreover, glasswool plugs on both sides of the packed column would prevent any particles dislodged from the column and entering the detector. The advantage of packed columns over OTCR in regards to background noise is appreciable.

TABLE III Effect of N₂ segmentation bubbles on the reduction efficiencies of OTCR at various concentrations of nitrate in aqueous solutions

run	length of OTCR (cm)	N ₂ segmentation in the OTCR	test solution	concentration (μ M)	absorbance	reduction efficiency (%)
1	—		NO ₂ ⁻	40	0.185	—
2	61	yes	NO ₃ ⁻	40	0.185	100
3	61	yes	NO ₂ ⁻	40	0.185	0
4	61	yes	NO ₃ ⁻	10	0.045	100
5	61	yes	NO ₂ ⁻	10	0.045	0
6	61	no	NO ₃ ⁻	10	0.032	72
7	61	no	NO ₂ ⁻	0.08	0.00036	0
8	61	no	NO ₃ ⁻	0.08	0.00026	73
9	61	no	NO ₂ ⁻	40	0.185	0
10	61	no	NO ₃ ⁻	40	0.135	73
11	61+61	no	NO ₂ ⁻	40	0.185	0
12	61+61	no	NO ₃ ⁻	40	0.174	93
13	61+61+61	no	NO ₂ ⁻	40	0.185	0
14	61+61+61	no	NO ₃ ⁻	40	0.185	100

In terms of reduction efficiency and lifetime, packed columns are also better than OTCR. Packed columns have much larger surface area to volume ratios. When a packed column is used, the flow is channeled through small gaps between cadmium filings and disrupted. The greater turbulence induced means the contact probability and consequent reaction rate is greatly enhanced. A packed copper-coated cadmium column as short as 3 cm length with only a 3 mm diameter could reduce a sample of 60 μ M nitrate with 100 % reduction efficiency. One might suspect that carry-over and sample dispersion are potentially problems with packed columns. In fact in our system carryovers with packed columns were minimal. Sample peaks from packed columns were as symmetrical as typical chromatographic peaks generated by separation columns. Using short wash times (30 seconds) the sample dispersion into the wash solution was apparent in the recorder trace as a shallow valley between sample peaks. Sample dispersion can be minimized by increasing sample time. Sample signals reached their maximum (a steady state signal) at sample times of 150 seconds. Carry-over did increase slightly as the length of column was increased. At 60-second sample times and 30 second wash times the carryover coefficient was

0.24% for a 3-cm column and 0.50% when a 26 cm column was used (Table IV). Although short columns had less carry-over, the lifetime of the columns is proportional to their length. A 26-cm packed copper-coated cadmium column had an acceptable carry-over and could maintain 100% reduction efficiency for more than 1000 seawater samples.

TABLE IV Effect of column length on the carry-over coefficients of packed copper-coated cadmium column

<i>Length (cm)</i>	<i>test solution</i>	<i>concentration (μM)</i>	<i>absorbance</i>	<i>reduction efficiency (%)</i>	<i>carry-over coefficient (%)</i>
26	NO_3^-	60	0.278	100	0.53
26	NO_3^-	40	0.185	100	0.50
3	NO_3^-	40	0.186	100	0.24
3	NO_3^-	2.4	0.011	100	0.22

Based on the comparative performance characteristics of OTCR and packed columns (Table V), packed cadmium columns are, therefore, recommended for use with auto-analyzers when determining low concentrations nitrate in oligotrophic waters. Using the procedures we recommended for packed Cd column we were able to achieve a method detection limit of 5 nM of nitrate with a conventional commercial auto-analyzer. The mean absorbance of seven replicate of 20 nM nitrate samples was 0.0001 with a coefficient of variation of 8% only.

TABLE V Comparative performance characteristics of OTCR and packed copper-coated cadmium column

	<i>OTCR</i>	<i>Packed column</i>
Reduction efficiency	>95%	>99%
Lifetime (day)	7–15	30–60
Detection limit (nM)	50–100	5–10

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