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Field measurement of nitrate in marine and estuarine waters with a flow analysis system utilizing on-line zinc reduction

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ABSTRACT

A sensitive reagent-injection flow analysis method for the spectrophotometric determination of nitrate in marine, estuarine and fresh water samples is described. The method is based on the reduction of nitrate in a micro column containing zinc granules at pH 6.5. The nitrite formed is reacted with sulfanilamide and *N*-(1-naphthyl)ethylene diamine (Griess reagent), and the resulting azo compound is quantified spectrophotometrically at 520 nm. Water samples in the range of 3–700 μg L^{-1} NO_3^-–N can be processed with a throughput of up to 40 samples per hour, a detection limit of 1.3 μg L^{-1} and reproducibility of 1.2% RSD (50 μg L^{-1} NO_3^-–N, $n = 10$). The proposed method was successfully applied for the determination of nitrate in estuarine waters and the reliability was assessed by the analyses of certified reference materials and recovery experiments. The method is suitable for waters with a wide range of salinities, and was successfully used for more than 3200 underway nitrate measurements aboard *SV Pelican1* in the "Two Bays" cruise in January 2010.

1. Introduction

Nitrate is one of the principal nutrients that stimulates the growth of macrophytes and phytoplankton causing so-called eutrophication of aquatic systems [1,2], and it is frequently the limiting nutrient in marine systems. Increases in nitrate concentrations in coastal and inland waters are mainly due to anthropogenic sources such as agriculture, and domestic and industrial wastewater discharges. In aquatic environments, the most common ionic forms of inorganic nitrogen are ammonium, nitrite, and nitrate. Ammonium tends to oxidize to nitrate in a two step process ($NH_4^+ \rightarrow NO^+ \rightarrow NO_3^-$) which is mediated by aerobic chemoautotrophic bacteria (*Nitrosomonas* and *Nitrobacter*), even if levels of dissolved oxygen decline to value as low as 1.0 mg O_2 L^{-1} [3]. Therefore, the concentration of nitrate in well-oxygenated surface waters is generally much higher than that of ammonium and nitrite.

Effective management, preservation and restoration of fresh, estuarine and marine waters relies on the ability to measure the spatial and temporal variations of nutrient species in a manner that provides a clear picture of the sources, transport and transformation of nutrient species in aquatic ecosystems. Analytical methods that are portable and which enable rapid, sensitive and robust measurements of nutrients in the field are therefore highly desirable as a means of collecting spatial and temporal data.

Many analytical methods have been proposed for determination of nitrate in waters, including ion chromatography [4], gas chromatography–mass spectrometry [5], capillary electrophoresis [6], potentiometry [7], polarography [8], and spectrophotometry [9–11]. Of these, some cannot cope with the high ionic strengths of marine and estuarine water samples, while others involve instrumentation that is either bulky, or delicate, and therefore generally unsuitable for field application. Some may require the use of a separation step or the use of large volumes of reagents, be capable of only low sample throughput, or suffer from poor sensitivity and precision. These considerations, and the increasing demand for rapid and sensitive environmental analytical methods have led to the development of flow-based analytical methods for nutrient determinations in various samples [12–17].

Most flow injection analysis methods for the determination of nitrate determination are based on either homogeneous or heterogeneous reduction of nitrate to nitrite and subsequent colorimetric determination of the nitrite by Griess reaction, in which diazotization with sulfanilamide is followed by coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED) to form a pink azo dye [18]. Titanium (III) and hydrazine have been used as soluble reductants for nitrate reduction (c.f. Table 1). Both approaches have limitations: the hydrazine method requires lengthy reaction times for reduction and color development in order to achieve adequate sensitivity [19], while titanium (III) chloride solution is violet and

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Table 1
Comparison of the analytical performance of the proposed method with other reported spectrophotometric flow analysis methods that utilize the Griess reagent for determination of nitrate in water samples.

Matrix	Reduction agent	Type of flow analysis system	Dynamic range (μg L^{-1} $NO_3{}^-$–N)	LOD (μg L^{-1} $NO_3{}^-$–N)	RSD (%)	Sample throughput (h^{-1})	Reference
Water samples	Zinc column	FIA	10–600	1.3	1.2	40	*This work*
Rain water samples	Hydrazine	FIA	226–2259		<3	40	[19]
Potable waters	Titanium (III) chloride	FIA	20–5000	20		30	[20]
Natural waters	Copperized cadmium column	FIA	14–9800	1.4	1	30	[21]
Water and soil samples	Copperized cadmium column	FIA	226–1129		1.5	90	[22]
River water samples	Copperized cadmium column	FIA	0–12000	24	1.4		[23]
Dairy samples	Copperized cadmium column	SIA	113–903	34	3.4	21	[24]
Soil samples	Copperized cadmium column	FIA	50–7000	44	1.02	35–40	[25]
Waste water samples	Copperized cadmium column	SIA	11–3390	2.2	<0.7	24	[26]
Water samples	Copperized cadmium–silver alloy	FIA	25–3000	13	0.1–6.7	100	[27]
Natural waters	Copperized cadmium column	FIA	0–7900	113	0.9	22	[28]
Water samples	Copperized cadmium column	FIA	0–5000		1.96	72	[29]
Water samples	Photo-induced conversion	FIA	0–1400	0.7	3	10	[30]
Biological samples	Cadmium column	FIA	0.35–280	0.35			[31]

FIA, flow injection analysis; SIA, sequential injection analysis.

absorbs light in the same range as the pink azo dye [20]. A photoreduction method has also been reported for nitrate determination in flow injection analysis systems (c.f. Table 1).

However, long irradiation times are required to achieve total nitrate reduction, and the conversion rate has also been found to increase with pH in the range 5–7, with a conversion efficiency of 50% achieved at pH 7 [30]. At this pH there is the potential for UV photo-oxidation of ammonium, urea, and other N compounds to occur in the presence of dissolved oxygen [32].

The most widely used segmented continuous flow [33] and flow injection analysis methods for nitrate determination [21–25], utilize heterogeneous reduction of nitrate to nitrite with a column of copperized cadmium granules or a Cd tubular reductor. Use of the cadmium column is often unreliable because of over-reduction to hydroxylamine and ammonia [34], or because of the loss of reduction efficiency that occurs due to poisoning of the column during the course of an analytical run. Use of cadmium is also undesirable because of the potential occupational health issues and the production of toxic waste [30,35,36]. Therefore, the use of an environmentally friendly reductant is highly desirable. Cardoso de Faria and Pasquini reported a flow injection method that utilised a copperized zinc column instead of Cd reduction [37]. This method was effectively applied to the determination of nitrate in tap and mineral waters using similar reduction conditions to that employed in the Cd reduction method, and it is surprising that this modification has not been more widely adopted. Another alternative approach using an environmentally innocuous reagent involves the quantitative conversion of nitrate to nitrite with corn leaf nitrate reductase. However, even under optimal conditions, the reaction kinetics are slow, and sample throughput using a segmented continuous flow analyser is limited to 5–12 samples h^{-1} [35,38].

Nitrate can be converted to ammonia in an alkaline medium using zinc or Devarda's alloy reductant [39] and this approach has been reported for determination of nitrate with a gas-sensing probe [40] and a bulk acoustic impedance detector [41]. Zinc has also been used as a heterogeneous reducing agent for conversion of nitrate to nitrite in batch mode [42,43] which is optimal under neutral or slightly acidic conditions [44].

In this work, a simple hybrid reagent-injection flow analysis method that utilizes on-line zinc reduction in the spectrophotometric determination of nitrate is described. The consumption of Griess reagent is minimized by the use of reagent-injection, and the use of zinc entirely eliminates the use of cadmium and its associated toxic waste. The method, which involves reduction under weakly acidic conditions, has been shown to be suitable for on-line measurement of nitrate in marine, estuarine and fresh waters, and has been deployed in extended field trials aboard the *SV Pelican 1*.

2. Experimental

2.1. Reagents

All chemicals were of analytical-reagent grade, and ultra-pure water (Millipore Milli-Q water system) was used to prepare all solutions. A nitrate stock solution (100.0 mg L^{-1} $NO_3{}^-$–N) was prepared by dissolving 0.1517 g of sodium nitrate (BDH, Pool, England), dried at 110 °C for 3 h, in water and diluting to 250.00 mL. The solution was stored at 4 °C. Working standards were prepared daily by serial dilution of the stock solution.

The color forming reagent was prepared daily by dissolving 1.000 g of sulfanilamide (E. Merck, Darmstadt, Germany) and 0.025 g of *N*-(1-naphthyl)ethylenediamine dihydrochloride (Sigma–Aldrich, Steinheim, Germany) and 7.35 mL of concentrated hydrochloric acid and diluting to 50.0 mL with ultra-pure water.

The 0.1 mol L^{-1} citrate buffer solution (pH = 6.5) was prepared by dissolving 7.35 g trisodium citrate dihydrate (Sigma–Aldrich, Steinheim, Germany) in 150 mL of water, and the pH adjusted to 6.5 with 0.1 mol L^{-1} hydrochloric acid before dilution to 250 mL with ultra-pure water.

2.2. Preparation of the zinc reduction column

The column was made of a length of Tygon® tubing (8 cm × 2.3 mm i.d.) filled with metallic zinc granules (0.15–0.40 mm), which were retained by disks of 25 μm nylon mesh (Swiss Screens (Aust) Pty Ltd., Huntingwood, NSW, Australia). Before use, the column was activated by flushing it with 0.1 mol L^{-1} hydrochloric acid at 2 mL min^{-1} for 3 min followed by 0.4 mg L^{-1} $NO_3{}^-$–N for 3 min at the same flow rate.

2.3. Instrumentation

The proposed method was developed and tested in the laboratory using a reagent-injection flow analysis system similar to that previously described for phosphate determination [17] and shown in Fig. 1. The sampling system consisted of a 12 V DC peristaltic pump (SP) (7518-00, Masterflex, Cole-Parmer Instrument Co, Vernon Hills, IL 60061, USA). This was used to collect sample from a water intake in the hull one metre below the surface and pump it through a coarse filter (25 μm mesh nylon screen (Swiss Screens (Aust) Pty Ltd., Huntingwood, NSW, Australia), held in a membrane filter assembly (Swinnex® 47, Millipore Corp, Billerica, MA 01821, USA) before passing through a 0.2 μm pore-size tangential flow filtration (TFF) unit (Vivaflow® 50, Gelman, Pall Life Sciences, Ann Arbor, MI 48103, USA) at a flow rate of ca. 100 mL min^{-1}. A sin-

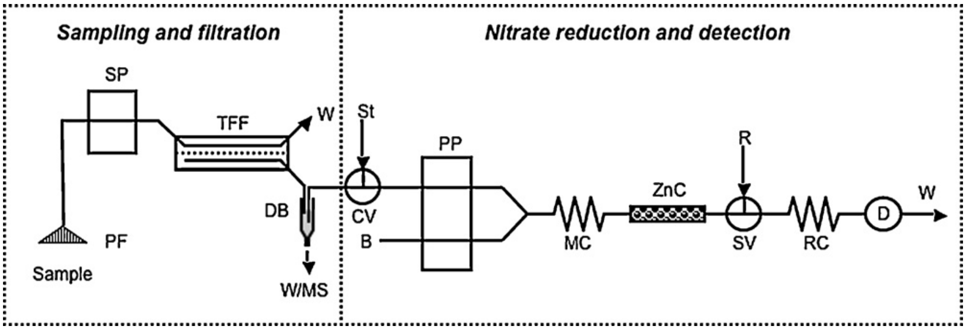

Fig. 1. Reagent-injection flow analysis manifold used for the determination of nitrate. Key: PF, prefilter; SP, sampling pump; TFF, tangential flow filter; W, waste line; DB, debubbler; W/MS, waste/manual sampling line; St, standard; CV, calibration valve; PP, peristaltic pump; B, buffer line; MC, mixing coil; ZnC, zinc column; R, Griess reagent; SV, solenoid valve; RC, reaction coil; D, detector.

gle Vivaflow® TFF unit was found to operate effectively with little reduction in membrane flux for at least 2 days even in when used in turbid estuarine waters. The filtrate flow from the TFF was typically between 4 and 2 mL min^{-1}, and the excess was allowed to drain through the waste aperture of the debubbler, which was designed to maintain a constant head of sample under the lowest filter flow conditions. When filter flux was high, such as immediately following replacement of a TFF unit, the surplus filtrate was allowed to run to waste out of the top of the debubbling unit.

Filtrate from the TFF (or a standard solution) and buffer (pH 6.5) were each continuously pumped at 1.0 mL min^{-1} and mixed in a serpentine mixing coil (coil length 300 mm, 0.5 mm i.d.) before passing through the zinc granule column. Griess reagent was contained in a machined polymethylmethacrylate reservoir (volume ca. 15 mL) that was pressurized with argon to 50 kPa. The reagent was injected using a miniature solenoid injection valve (LFVA series, Lee Company, Westbrook, CT, USA), which is rated for at least 10×10^6 cycles. This was mounted in a machined Delrin® manifold block such that on activation for 100 ms, ca. 15 μL or reagent was injected directly into the common flow line as shown in Fig. 1. The sample-injected reagent zone was then mixed in a knotted reaction coil (coil length 300 mm, 0.5 mm i.d.) before passing through a multi-reflection flow cell (effective optical path length of ca. 17 mm [45]) where the absorbance was monitored. The light source used for absorbance measurements was a green LED (λ_{max} = 520 nm, Kirkbright RS components, P/N 4663510), and the transmitted signal was detected using a solid state photodiode, and log amplified to give a signal output in the range of ±10 V. All pump and valve switching and data acquisition was performed using a Lab VIEW 8.5 program (National Instruments, Austin, TX, USA).

A two-way valve (CV) was used to switch from continuous pumping of the filtered sample to continuous induction of a standard solution of (St), used for the purpose of calibrating the nitrate response.

2.4. Sample collection

A number of samples were collected from riverine, estuarine and coastal waters around Melbourne, SE Australia for the purposes of validating the proposed method.

Field validation of the instrument and method was performed in Port Philip and Western Port Bays, and Bass Strait in Victoria, SE Australia, as part of the *SV Pelican 1* “Two Bays cruise” in January 2010. More than 3200 nitrate analyses were performed on-line during eight days of extended operation, over a cruise path of 285 km. Samples for batch analysis were also collected periodically from the manual sampling line (W/MS, Fig. 1), refrigerated and transported to the laboratory for comparative analysis.

3. Results and discussion

Preliminary tests were carried out with the aid of different flow analysis assemblies to select the most suitable manifold configuration, which is depicted in Fig. 1. In order to optimize the portable flow analysis system, the influence of chemical and hydrodynamic parameters on the nitrate reduction efficiency as well as the magnitude of peak height was investigated.

3.1. Optimization of manifold and reagent variables

To establish the best conditions for determination of nitrate the procedure was optimized by a univariate method.

The experimental results showed that pH is an important parameter for reduction of nitrate to nitrite by a zinc column. The effect of pH on the peak height was investigated with different buffer solutions (acetate, tris, citrate, ammonium and phosphate). The peak heights obtained with acetate and citrate were higher than the rest of buffers. It was also observed that there is an increase in the peak height when the pH increased up to 6.5 and then decreased at higher pHs (Fig. 2). The citrate buffer, besides contributing to the control of pH, can act as masking agent for some of metal ions like iron that interfere when present in large quantities in sample solution. Therefore, citrate buffer (0.1 M, pH = 6.5) was selected for subsequent studies.

The use of two chromogenic reagents (sulfanilamide and NED) when combined gave better results [26] than when added sequentially because it made the determination faster, with less dispersion.

The effect of sulfanilamide concentration in the range of 2.5–15 g L^{-1} on the peak height was studied, and the sensitivity of determination was observed to strongly increase up to 10 g L^{-1} before leveling off. Therefore, the 10 g L^{-1} of sulfanilamide was chosen for all subsequent work.

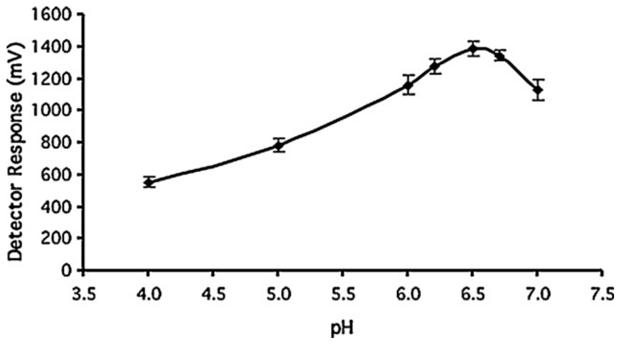

Fig. 2. Effect of pH on the peak height (100 μg L^{-1} NO_3^-–N; citrate buffer).

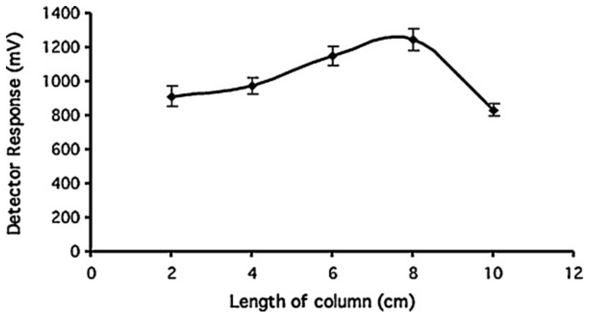

Fig. 3. Effect of the length of reduction column on the peak height (100 µg L^{-1}, NO_3^-–N; citrate buffer, pH 6.5).

The influence of NED concentration on the peak height was also investigated in the range of 0.05–1 g L^{-1}. The peak height increased for all concentrations up to 0.25 g L^{-1} where it reached a plateau; this concentration of 0.25 g L^{-1} of NED was chosen to ensure maximum sensitivity.

The effect on the absorbance of the azo dye, of the hydrochloric acid concentration (0.2–1 M) in the chromogenic reagent, was also studied; a maximum sensitivity was observed to occur when the hydrochloric acid concentration reached 0.75 M.

The chromogenic volume injected into the sample stream, which in this system was controlled by the solenoid injection valve timing, had a marked effect on the peak height. When the injection time of the reagent was varied from 10 to 200 ms (corresponding to reagent volumes of 1.5 to 30 µL). It was observed that there was no improvement in sensitivity for injection times of greater than 100 ms (injection volume ~15 µL), and consequently this injection time was selected for all subsequent determinations.

The optimum length of the Zn reduction column was established with a 100 µg L^{-1} nitrate standard by using reduction column lengths of 2.0, 4.0, 6.0, 8.0, and 10.0 cm (Fig. 3). The result shows that as the length of column is increased to 8 cm, the sensitivity increases, but that there is a decrease in peak height response with additional band broadening thereafter, possibly due to increased back pressure. A column length of 8 cm was therefore selected for all subsequent work because the sensitivity and reproducibility were acceptable, and the column back pressure was comparatively low.

The efficiency of the zinc column for conversion of nitrate to nitrite was investigated by passing the solutions of these ions through the column and gave complete conversion over the whole range tested. As a routine procedure, the efficiency of any new zinc column was measured by determining the ratio of the peak heights of 100 µg L^{-1} NO_3^-–N and 100 µg L^{-1} NO_2–N, which was always greater than 95%.

Table 2
Calibration range and detection limit of nitrate in two different flow rates (sample + buffer) under optimum conditions.

Characteristics	Total flow rate (2.1 mL min^{-1})	Total flow rate (5.0 ml min^{-1})
Linear range (µg L^{-1})	3–700	8–1200
Correlation coefficient	0.9998	0.9991
Slope	13.775	5.131
Detection limit (µg L^{-1})	1.3	3.9
Sample throughput (h^{-1})	40	90

Table 3
Tolerance limit of foreign ions in the determination of 100 µg L^{-1} NO_3^-–N, where $W_{ion}/W_{nitrate}$ is the weight ratio of interferent to nitrate.

Foreign ion	Tolerance limit ($W_{ion}/W_{nitrate}$)
K^+, Na^+, Mg^{2+}, SO_4^{2-}, EDTA, CO_3^{2-}, NH_4^+, Cl^-, Ca^{2+}, CH_3COO^-	10,000
Br^-, Mn^{2+}	5000
PO_4^{3-}	1000
Co^{2+}, Fe^{2+}	200
Fe^{3+}	100
Cu^{2+}	20

The lifetime of the zinc column depends on the amount of oxidizable material passing through it. Under continuous flow operation, as described here, the effective lifetime of column was found to be approximately 9 h with a ≤10% decrease in signal being observed during that time. While this is not ideal, regular injection of a calibrating standard enabled back correction to compensate for slight decreases in sensitivity. Reactivation of the column *in situ*, e.g. with 0.1 mol L^{-1} hydrochloric acid or 0.05% EDTA solution, which is used to regenerate copperized cadmium columns, did not prove satisfactory, and installation and activation of a fresh column was usually performed after a day of use.

The analytical sensitivity is also affected by the total flow rate as shown in Table 2. At the lower combined flow rate of 2.1 mL min^{-1}, the sensitivity of method increased, with a corresponding improvement in the detection limit, which can be attributed to either or a combination of lower hydrodynamic dispersion and increased residence time. The improved sensitivity and lower buffer consumption favours the use of combined flow rate of 2.1 mL min^{-1} except in situations where a higher sample throughput is required.

Table 4
Determination of nitrate using the proposed method for various riverine (R), estuarine (E) and marine (M) waters in SE Australia.

Sample	Salinity [48]	Added (µg L^{-1} NO_3^-–N)	Found (µg L^{-1} NO_3^-–N)[a]	Recovery (%)
Dandenong Creek, High Street Road (R)[b]	0.2	0	631.8 ± 1.7	
		100.0	729.9 ± 1.3	98.1
Mile Creek, Keysborough (R)	0.8	0	186.5 ± 1.3	
		100.0	288.8 ± 2.5	102.3
Yarra River, Fairfield Park (R)[b]	0.2	0	530.2 ± 7.8	
		100.0	633.4 ± 4.7	103.2
Yarra River, Hawthorn Bridge (E)	0.2	0	461.9 ± 2.7	
		100.0	560.3 ± 7.1	98.4
Herring Island, Yarra River (E)	3.3	0	457.7 ± 0.7	
		100.0	555.0 ± 1.3	97.3
Yarra River, Morell Bridge (E)	4.5	0	399.8 ± 1.1	
		100.0	498.7 ± 1.8	98.9
Port Phillip Bay, Williamstown Jetty (E/M)	18.3	0	4.8 ± 0.4	
		100.0	105.0 ± 1.3	100.2
Port Phillip Bay, Rosebud Pier (M)	31.3	0	3.3 ± 0.3	
		100.0	104.2 ± 4.3	100.9

[a] Mean and standard deviation of three determinations.
[b] Sample analysed after dilution with ultrapure water.

3.2. Analytical characteristics

Under the optimized conditions, the analytical characteristics of the proposed method were evaluated by examining the linear calibration range, detection limit, precision and sample throughput. A linear calibration relationship between peak height and concentration of NO_3^-–N was observed for the range of 3–700 $\mu g\,L^{-1}$. A detection limit of was 1.3 $\mu g\,L^{-1}$ NO_3^-–N (S/N of 3:1) was determined using the linear regression method described by Miller and Miller [46], while the relative standard deviation ($n = 10$) was 1.2% at 50 $\mu g\,L^{-1}$ NO_3^-–N. The sampling frequency was ca. 40 samples per hour.

3.3. Effect of interfering ions

The influence of foreign ions in the determination of 100 $\mu g\,L^{-1}$ of NO_3^-–N was studied under the optimum conditions. The tolerance limit was defined as the concentration of a foreign ion (W_{ion}) causing a relative error of less than ±5% with respect to the concentration of nitrate (W_{nitrate}). The results are summarized in Table 3, and show that at the given concentration, no significant interference was observed in the determination of nitrate.

3.4. Laboratory and field application

The proposed method was applied to the determination of nitrate in various water samples with a range of salinities and nitrate concentrations. The reliability of the method for analysis of these real samples was checked by recovery experiments and analysis of a certified reference material (Analytical Products Group Inc., Lot Number 40720-40721). Table 4 shows that the recovery of the spiked sample was always in the range of 97–101%, and at the 5% confidence level, there is satisfactory agreement between the result obtained using the proposed method (7.85 ± 0.12 NO_3^-–N) and certified value (7.69 ± 0.0448 mg/L NO_3^-–N).

The suitability of this nitrate method was also was tested using the combine sampling and flow analysis system onboard *SV Pelican1* in the “Two Bays Study” that was conducted during January 2010.

Fig. 4(a)–(c) shows concentration plots for about 75% of the >3000 measurements made during the cruise. It is evident that for much of the cruise, the measured nitrate concentrations were close to, or less than the detection limit. This is in accord with previous studies [47], that showed that Port Phillip Bay frequently had NO_x concentrations of much less than 5 $\mu g/L$ NO_3^-–N, and that as a consequence, photosynthetic production is nitrogen limited in this system. This plot also clearly illustrates the advantages of mapping nutrient concentrations in the manner described, in that it shows that there are major sources of oxidized nitrogen from the Yarra River estuary, the Eastern Treatment Plant ocean outfall into Bass Strait, which had a maximum concentration in excess of 600 $\mu g/L$ NO_3^-–N), and several locations near the Western Treatment Plant. Such features would most likely be missed, or at best, underestimated using traditional sampling and laboratory based analysis.

Fig. 4 also shows that manually collected samples gave similar results to those determined using underway flow analysis. Comparison of these data is complicated by the difficulty of properly pairing the results from the flow analysis system with those obtained by hand sampling and laboratory analysis. This arises because there was delay, often of 2–3 min from the commencement of filtrate collection from the manual sampling line (W/MS, Fig. 1) until there was sufficient sample (ca. 30–40 mL) for laboratory analysis. Thus the comparison is really one of a high frequency sequence of measurements from the flow analysis system with a single measurement of an aggregated sample collected over a period of several minutes. This effect was most pronounced when the sampling vessel was passing through an estuarine gradient (ca. 08:50 h, Fig. 4(a)) or outfall plumes (ca 13:00 h Fig. 4(b); ca. 10:00, 10:20 h, Fig. 4(c)). Furthermore, the sample refrigeration facilities aboard *SV Pelican1* were unable to cope adequately with daily temperatures in excess of 40 °C, and freezing of samples was very slow. As a consequence, some loss of sample integrity may have occurred prior to laboratory analysis. Despite these limitations in the validation procedure, it is clear that data from the comparative method track the underway measurements from the flow analysis system quite closely (Fig. 4), and there is no significant difference between the two sets of data at the 95% level of confidence ($t_{\text{Stat}} = 0.1049$, $t_{\text{Critical, Two Tail)}} = 2.0518$, df = 27).

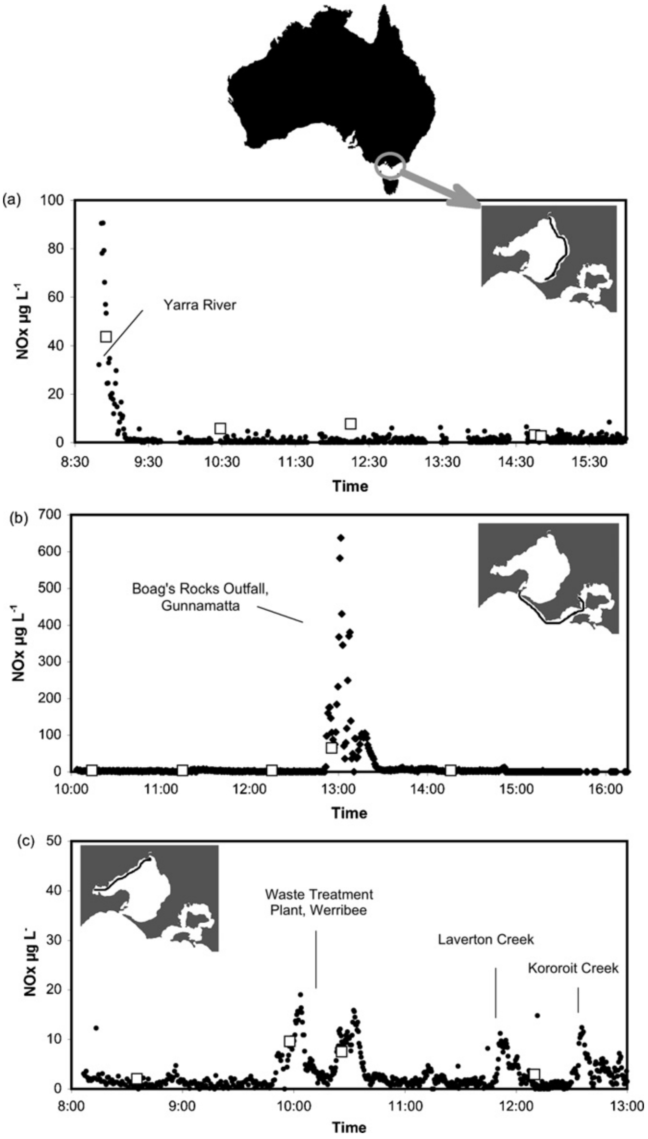

Fig. 4. Results for “Two Bays” underway nitrate measurements in Port Phillip, Bass Strait and Western Port bays on (a) 11 January 2010, (b) 12 January 2010 and (c) 23 January 2010. Elevated concentrations can be seen in the Yarra River estuary (Plot (a) between 08:45 and 09:15), the South Eastern Treatment plant outfall at Boag’s Rocks (Plot (b) between 12:40 and13:30) and the Western Treatment Plant outfall and lavender and Kororoit Creeks (Plot (c) between 09:45 and 10:45, and 11:45 and 13:00). (●) are data measured in the field using reverse FIA, while (□) are comparative data obtained from hand sampling and laboratory analysis.

4. Conclusion

A simple and sensitive spectrophotometric flow analysis method for determination of nitrate in waters with a wide range of salinities is presented. The use of a zinc column offers a promising means for reduction of nitrate to nitrite because of its simplicity, low cost, lack of toxicity, excellent reproducibility and high nitrate reduction efficiency. The proposed method may be used as an alternative to other FIA procedures involving the copperized cadmium column for the determination of nitrate in different water samples. This method allows the determination of nitrate with a detection limit of 1 μg L^{-1} which is comparable with or even better than some previously reported FIA methods using the Griess reagent (Table 1). The method has successfully been applied in extensive underway determinations of nitrate in estuarine and coastal marine waters.

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