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High resolution determination of nanomolar concentrations of dissolved reactive phosphate in ocean surface waters using long path liquid waveguide capillary cells (LWCC) and spectrometric detection

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Abstract

In the last decade, long path length, low volume, liquid waveguide capillary cells (LWCC) in conjunction with conventional nutrient auto-analyzers have been applied to determinations of nanomolar levels of phosphate, nitrate, and nitrite in oligotrophic waters. This article reports a high resolution, real-time, continuous method for nanomolar dissolved reactive phosphate measurements in ocean surface waters with data logging every 30 seconds for up to 16 consecutive hours. Surface seawater is pumped continuously from a shipboard underway tow-fish unit to a helium gas-segmented, continuous-flow, nutrient auto-analyzer modified with a 250 cm LWCC. To circumvent baseline instability due to reagents, a parallel channel with deionized water (DI) and reagents is run and later subtracted from the sample absorbances. The detection limit is 0.8 nmol/L. The precision (as relative standard deviation) at 5 nmol/L phosphate is 6.1% (n = 5) and 0.8% (n = 5) at 50 nmol/L. We also report an optimized method for discrete samples using a 200 cm LWCC. To minimize any background phosphate concentration in low nutrient seawater used as wash water solution, we use DI water, but increase sample and wash times to achieve plateau-shaped peaks after the transient wash/sample mixing period. The detection limit is 0.5 nmol/L. The precision at 10 nmol/L phosphate is 1.8% (n = 8) and 0.9% (n = 9) at 60 nmol/L. The two systems have successfully been deployed on the U.S. GEOTRACES 2010 cruise, transecting the upwelling area northwest of Africa and the highly stratified, oligotrophic, subtropical North Atlantic gyre.

Phosphorus is an essential nutrient for phytoplankton growth and its major inorganic form, dissolved orthophosphate, is directly bioavailable and plays a key role in photosynthesis (Falkowski 1997; Estela and Cerdà 2005; Motomizu and Li 2005; Paytan and McLaughlin 2007). Due to physical and biological processes, large spatial and temporal variations in phosphate concentrations exist in the oceans. In surface ocean waters, phosphate typically is found in nanomolar concentrations, increasing in deeper waters to micromolar concentrations as remineralization takes place. Physical processes such as upwelling, mesoscale eddies, and storm mixing also affect the surface phosphate distributions (Wu et al. 2000; Li and Hansell 2008a). Nitrogen has traditionally been viewed as the nutrient limiting primary production in marine waters (Ryther and Dunstan 1971; Patey et al. 2008). However, with an increased understanding of the nitrogen cycling in the ocean, it appears that phosphate also has the potential to limit primary production in oligotrophic regions such as the North Atlantic Subtropical gyre (Wu et al. 2000; Li and Hansell 2008a; Lomas et al. 2010). These findings are supported by high activities of bacterial and phytoplankton alkaline phosphatases and a nitrate: phosphate ratio greater than the Redfield Ratio (16:1), indicating that the availability of phosphate to some extent may be controlling the primary production in the system (Li and Hansell 2008a; Lomas et al. 2010). Hence, accurate quantification of phosphate at low concentrations in oligotrophic waters is essential.

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The spectrometric method based on the formation of the blue form of reduced phosphomolybdate (molybdenum blue) is widely used for determining dissolved (or soluble) reactive phosphate at micromolar concentrations (Murphy and Riley 1962; Mee 1986; Alvarez-Salgado et al. 1992; Zhang and Chi 2002; Estela and Cerdà 2005; Paytan and McLaughlin 2007). For determining phosphate at nanomolar concentrations, preconcentration by magnesium-induced co-precipitation (MAGIC) has proven useful (Karl and Tien 1992; Rimmelin and Moutin 2005). After addition of NaOH, phosphate is scavenged into the $Mg(OH)_2$ precipitate. The precipitate is centrifuged, then dissolved in dilute HCl and subsequently quantified via the molybdenum blue method. During the last decade, long path length, low volume, liquid waveguide capillary cells (LWCC), with spectrophotometric detection, have gained foothold as a simple, easily automated, and reliable technique for nanomolar phosphate measurements (Zhang 2000; Zhang and Chi 2002; Gimbert et al. 2007; Li et al. 2008; Patey et al. 2008; Ma et al. 2009). The standard wavelength for the molybdenum blue method is 880 nm, but due to the large absorption of far-red wavelengths by water, light transmitted in a LWCC is negligible at this wavelength (Patey et al. 2008). For this reason, the second absorption maximum of molybdenum blue at 700 nm (Murphy and Riley 1962) is used for determining phosphate when working with the LWCC technique.

The molybdenum blue method does suffer from a number of interferences, which potentially can result in an overestimation of phosphate (Karl and Tien 1992; Anagnostou and Sherrell 2008; Ma et al. 2009). This is more likely to be significant when phosphate is present at nanomolar concentrations. Hydrolysis of pyrophosphates and organic phosphorus compounds due to the reaction's acidic conditions (pH < 1) also can form phosphomolybdate complexes, and hence "soluble reactive phosphate" is a common operational term used to describe the dissolved (<0.2–0.7 µm) phosphate measured with the molybdenum blue method (Benitez-Nelson 2000; Paytan and McLaughlin 2007). Li and Hansell (2008b) investigated dissolved organic phosphate (DOP) interference for a LWCC method and found DOP interference to be low and similar to the DOP interference for the MAGIC method (Karl and Tien 1992). Arsenate [As(V)], typically ranging from 12 to 21 nmol/L in the open ocean (Cutter et al. 2001; Cutter and Cutter 2006), forms a similar blue complex of an equivalent molar absorptivity to that of phosphate, but reacts more slowly with the reagents forming the arsenomolybdate complex (Johnson 1971; Downes 1978; Karl and Björkman 2002). The reduction of arsenate to arsenite [As(III)] with thiosulphate can be employed as a preliminary step for phosphate measurements because arsenite is nonreactive to the molybdate reagent, but is rarely applied for automated nanomolar phosphate measurements (Karl and Björkman 2002). Silicate [$Si(OH_4)$] is another potential interferent with the molybdenum blue method, but can be minimized with optimized reaction conditions. Reducing pH < 1 in the final solution, reacting at room temperature, and the addition of antimony as a catalyst reduces silicate molybdenum blue formation (Zhang et al. 1999). Gimbert et al. (2007) reported that interference from silicate can be effectively masked by addition of tartaric acid. These finding are supported by Ma et al. (2009) who used similar conditions for LWCC phosphate experiments adding silicate in concentrations between 0 and 240 µmol/L to samples containing 0 or 83 nmol/L phosphate. The results did not show any significant difference in absorbance between samples with or without silicate (Ma et al. 2009).

Variations in baseline over time due to instability of reagents and coating of the walls of the LWCC and sample lines with colloidal molybdenum blue are also well known (Li et al. 2008; Ma et al. 2009). These baseline variations can become a challenge for long-term continuous measurements (hours), since an increase in the baseline can mask the analyte concentration signal.

Low nutrient seawater (LNSW) has routinely been used in oceanography for preparation of standards, blanks, and wash solution to match the salinity of the samples, and thereby, improve accuracy (Zhang 2000). However, in oligotrophic waters, phosphate concentrations are of the same order of magnitude as that in LNSW, and its determination is therefore inherently blank-limited. Zhang and Chi (2002) recommended "phosphate-free" seawater prepared from the supernatant from the MAGIC procedure for wash solution and for nanomolar level phosphate standards. However, by adding NaOH large amounts of Mg are removed, increasing the alkalinity and changing the matrix of the seawater. Alternatively, Li et al. (2008) eliminated phosphate from seawater by adding ferric chloride and subsequent co-precipitation of phosphate with ferric hydroxide, producing a clear solution at final pH of ~ 6. Hence, it is difficult to prepare a blank for nanomolar phosphate measurements with the same physical properties as seawater samples (Froelich and Pilson 1978). In addition to the chemistry of the water for blanks and standards, differences in the physical properties of high and low salinity seawater samples, combined with non-ideal optical characteristics of flow cell design in continuous-flow analysis, change how light is transmitted in the flow cell. This causes changes in the refractive index (Schlieren effect) when the wash water and sample mixing zone passes through the flow cell, with resultant absorbance errors (Froelich and Pilson 1978; Alvarez-Salgado et al. 1992; Motomizu and Li 2005; Ma et al. 2009). Such differences can be a problem when using deionized water (DI) as wash solution in between seawater samples in continuous-flow analysis, resulting in an analytical error up to 13 nmol/L in North Sea water for phosphate (Ormaza-González and Statham 1991). Thus, the choice of water type for wash solution in discrete sample analysis, or for standards and blanks is a critical aspect of nanomolar phosphate determinations.

In automated, gas-segmented, continuous-flow analysis (SCFA), deliberate introduction, and later removal, of gas bub-

bles to the sample stream significantly reduce carry-over and sample dispersion and thereby improve precision (Zhang and Chi 2002). However, the large internal surface-to-volume ratio of a LWCC tends to allow bubbles and micro-bubbles to stick to the internal surface of LWCC (Patey et al. 2008), and can pose a major challenge compared with smaller, conventional flow cells. To prevent bubble breaking in the sample stream, and hence bubbles entering the LWCC, a regular bubble pattern must be obtained by addition of a suitable surfactant (Zhang and Chi 2002). The segmentation gas solubility in water must also be taken into consideration to prevent bubble formation via oversaturation.

In this article, we report two optimized gas-segmented, continuous-flow spectrometric methods to determine dissolved reactive phosphate in surface seawater at nanomolar concentrations using LWCC (Note: "soluble" used in the older literature is ambiguous – could be soluble on particulate matter or truly dissolved. The dissolved ion is the most bioavailable to phytoplankton, so filtration is necessary to measure this fraction. Hereafter, this dissolved reactive phosphate will be referred to simply as "phosphate"). The first method has a continuous flow of seawater from an underway sampling unit, allowing real time monitoring for up to 16 consecutive hours. This analytical system minimizes any baseline drift influences on the output signal by running a reference channel with only DI water and reagents parallel to the seawater channel. The second method is for discrete samples from depth profiles and uses DI water as wash water solution to minimize errors due to the wash water having higher phosphate concentrations than the samples. To prevent absorbance errors due to differences in refractive index between DI water and seawater, sample and wash times are sufficiently increased to achieve plateau-shaped peaks typical for gas-segmented continuous-flow analysis. For both analytical systems, helium is used as segmentation gas to prevent bubbles forming in the LWCC. The two systems have successfully been deployed on the 2010 U.S. GEOTRACES cruise transecting the upwelling area northwest of Africa and the highly stratified, oligotrophic, subtropical North Atlantic gyre.

Materials and procedures

Shipboard underway system

The 2010 U.S. GEOTRACES expedition used the vessel R/V *Knorr* and left Lisbon, Portugal, on 15 Oct 2010 and arrived in Charleston, South Carolina, USA, on 26 Nov 2010. For underway surface sampling a shipboard trace metal clean tow-fish unit (e.g., Vink et al. 2000) was towed ~ 5 m off the starboard side at 1–3 m depth depending on the ship's roll. Seawater was pumped continuously using a 13 mm (1/2 inch) Wilden P1 PFA Teflon air-driven, dual-diaphragm pump into the analytical laboratory through a 1/2-inch Teflon tube at 4 L/min. In the analytical laboratory an outlet from a "T" in this line was connected to a diaphragm metering pump (KNF Stepdos) to circulate filtered seawater [0.2 µm hollow fiber filter (MediaKap®-5)] from the outlet to an intake "T" for the auto-analyzer sample line and then to waste at a flow rate of 30 mL/min. The lag time from when the surface seawater entered the mouth of the underway system until it reached the light detector of the auto-analyzer was 11 min. Discrete surface samples were acquired from the shipboard underway system 3-6 times per day during transect and filtered through a 0.2 µm capsule filter (Pall Corporation, PN 12122). These samples were placed in 125 mL, acid-cleaned polyethylene bottles and refrigerated until analysis within 12–36 h.

Liquid waveguide capillary cell

Commercially available Liquid Waveguide Capillary Cell (World Precision Instruments) with either a 200 cm or a 250 cm path length and a 550 µm i.d. are used. The LWCC is made of quartz capillary tubing coated with a low refractive index polymer, coiled three times, and placed in a protective 30 cm × 30 cm metal box. There are two fiber optical connections (input and output) and two connections for sample inflow and outflow. The sample flow and light pass are connected inside the LWCC box by "T" connectors.

Continuous system

An Alpkem gas-segmented Rapid Flow Analyzer (RFA-300™) for phosphate determinations is modified with two identical analytical channels. One channel is for the continuous flow of seawater samples. The other channel is for a continuous flow of DI water used as a reference channel for monitoring any baseline drifts due to instability of reagents over time. Each channel is connected to an individual 250 cm LWCC. The analytical manifold configuration and flow diagram for the continuous system are shown in Fig. 1. Standard pump tubes are used (Astoria-Pacific, Clackamas, Oregon, USA). The sample stream is debubbled before being bubble-segmented with helium gas (analytical grade, 2 psi). To bypass the seawater sample line for calibrating or cleaning the system, a three-way Luer Lok™ valve is connected before the debubbler. After merging with the ammonium molybdate and ascorbic acid reagents, the sample stream passes through a 25 turn glass reaction coil, continuing to a 4 mL heat bath at 37°C, and then mixing in another 25 turn glass reaction coil before entering the LWCC. A debubbler is placed before the LWCC to remove the segmentation gas and any micro air bubbles in the sample stream. To get a synchronous output, the total sample flow rate of each channel is matched with a 7 min time delay between reagent addition and measurements. Light transmission through the LWCC is measured by a LED-based photometric detection system (LEDSpec, World Precision Instruments) with a 700 nm LEDSpec wavelength module designed for LWCC use. The LWCC and LEDSpec are connected with fiber optic cables. The LEDSpec is then connected to a computer with a USB cable, recording the raw data (.txt-file) every 30 s with a typical run of 12 to 16 h long. To quantify the concentration of phosphate, the absorbance at 700 nm is measured using the 700 nm LEDSpec wavelength module; this applies to both channels.

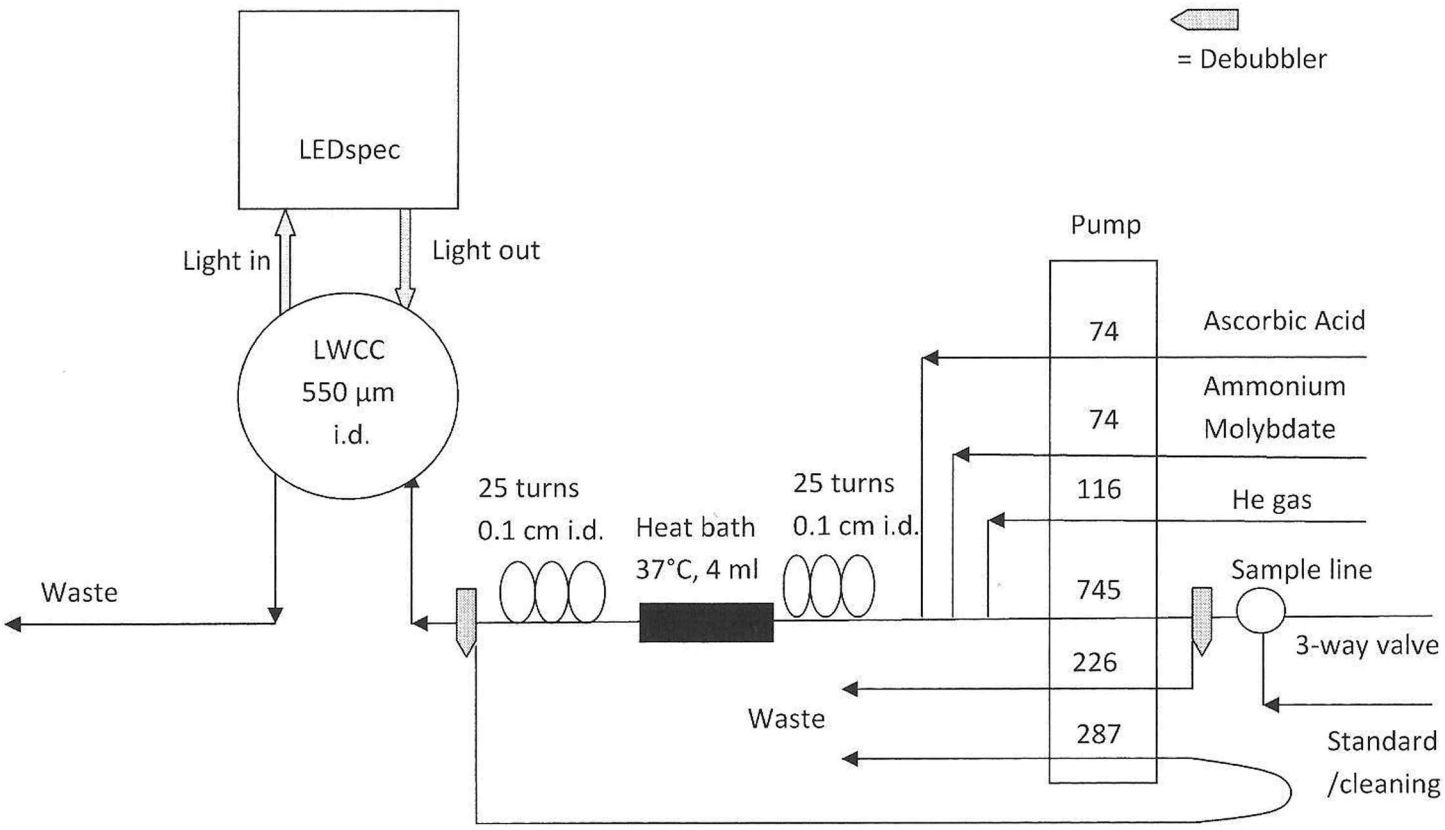

Fig. 1. Flow diagram of the underway continuous system for phosphate. For this application, a conventional gas-segmented, continuous-flow nutrient analyzer is combined with a 250 cm LWCC and a LED-based photometric detection system (LEDSpec). Standard pump tubing from Astoria-Pacific (Clackamas, Oregon, USA); rates are in µL/min.

At the beginning of an analytical run, DI water and reagents are introduced to both channels until stable baselines are obtained; the LEDSpec is then zeroed to establish the baseline. The three-way Luer Lok™ valve is turned so that seawater is introduced and the signal recorded. The system can then be run for 12–16 hours. To test for any differences in sensitivities between the two LWCC channels or within a LWCC during a run, and correct for any refractive index offset, identical standards in seawater and DI water (*See* "Reagents and standards") are run in the beginning and at the end of the analytical run. If any differences in sensitivity between channels are observed, a sensitivity correction factor (SCF) is applied to obtain identical absorbances between channels (SCF = $([Abs]_{seawater\ std} - [Abs]_{seawater})/([Abs]_{DI\ std} - [Abs]_{DI})$). If any baseline drift within a given channel is observed, a drift correction factor (DCF) is applied to correct sample absorbance at a given time t (DCF = $([Abs]_{std,\ start} - [Abs]_{std,\ end})$/run time, then $[Corrected\ Sample\ Abs]_t$ = [Sample Abs] – DCF * t). All calculations are done off-line using Microsoft Excel.

Discrete sample system

An Astoria-Pacific (Clackamas, Oregon, USA) gas-segmented, continuous flow nutrient auto-analyzer (300 series) modified with a 200 cm LWCC is used for measuring discrete phosphate samples at nanomolar levels. The manifold configuration and flow rates for phosphate are identical to the continuous system (Fig. 1). Light from the analyzer's broad spectrum tungsten lamp is connected to LWCC and from it to the detector with fiber optic cables. Using the Astoria-Pacific light detector, the absorbance at 690 nm is measured to quantify the concentration of phosphate. DI water is used as blank and wash water solutions in between samples with a wash time of 90 s and a sample time of 120 s. Samples are introduced to the instrument using an auto sampler, and all samples are measured in triplicate. Because the reagents are pumped through the system continuously, blank signals from the reagents are set to zero during analysis using the Astoria-Pacific software (FASPac™) baseline correction feature. In this way any potential baseline shift during the analysis and coating effects are taken into account and corrected. To test for the refractive index offset due only to salinity changes between DI wash water and seawater sample, seawater samples that bracket the expected salinities and no reagents are run before starting reagents and analytical runs. The absorbance of this refractive index offset is subtracted from the sample absorbance before calculating the final phosphate concentration. To check the calibration (*See* "Reagents and standards"), a 0.2 µm filtered

LNSW sample collected in the oligotrophic North Atlantic Gyre, and kept in a 20 L polyethylene cubitainer in the dark, is used as an internal data quality control sample throughout the analytical runs (every 10 samples). If the measured concentration varies by more than 10% from its long term average of 9.5 ± 0.8 nmol/L value, the run is stopped and the calibration redone. Any suitable LNSW that is 0.2 µm filtered and kept in the dark can be used for this quality control sample after monitoring its phosphate concentration over 2-4 weeks to verify its concentration and stability.

Cleaning and maintenance

LWCC and the analytical manifolds are cleaned every 15 to 18 h by pumping Chemwash (KOH, ethanol and Triton detergent, Astoria-Pacific) and DI water (Barnstead E pure, 18 MΩ) through the system for 10 min, then 5% (v/v) HCl, and then DI water for 10 min. Tubing for the RFA peristaltic pump is changed every 100 running hours. When pump tubing is replaced, a more thorough cleaning of LWCC is also performed: the LWCC is filled with 10% Contrad(R) NF (NaOH and sodium polyacrylate, Astoria Pacific) for an hour and then flushed with DI water. In severe cases where blank absorbances are unstable, World Precision Instrument's cleaning kit (part # 501609, dilute triethanolamine, methanol and HCl) is used.

Reagents and standards

All polyethylene bottles used for samples and storage of reagents and standards are soaked in 5% (v/v) HCl overnight and rinsed three times with DI water to remove trace nutrients. A 0.5 mmol/L phosphate standard is prepared (high-purity grade KH_2PO_4, Alfa Aesar) in DI water and stored in a polyethylene bottle and refrigerated for up to 3 months. Working standards in the range 5–70 nmol/L are prepared daily from serial dilutions of the phosphate stock solution in 0.2 µm filtered, freshly collected (i.e., immediately before calibration) seawater from the shipboard underway unit. These are essentially standard additions for calibrating the systems. Equivalent standards are also prepared in DI water. For the continuous system, each of 5 working standard additions is run for 20 min via bypassing the seawater line using the three-way Luer Lok™ valve. Calculations of unknown phosphate seawater concentrations are done using the slope from the standard additions calibration in offline Microsoft Excel. For the discrete system, calibration blank and working standards equivalent to the continuous system are run to calculate calibration slope and concentrations using the Astoria-Pacific software (FASPac).

All reagents are analytical grade and their solutions are prepared daily for the discrete system and before every run (12–16 h) for the continuous system. Molybdate solution: 0.5 g of ammonium molybdate(IV) tetrahydrate $[(NH_4)_6Mo_7O_{24}\cdot 4H_2O]$ is dissolved in 40 mL of 2.5 M H_2SO_4, then diluted with 150 mL DI water plus 10 mL antimony potassium tartrate stock solution [1.5 g of antimony potassium tartrate $(C_4H_4K_2O_{12}Sb_2\cdot 3H_2O)$ dissolved in 500 mL DI water]. This solution is kept in an acid-cleaned polyethylene bottle. Reducing solution: 1.0 g ascorbic acid is dissolved in 100 mL DI water, then 100 mL 15% sodium dodecyl sulfate (SDS) as surfactant is added. This solution is kept in an acid-cleaned, brown, polyethylene bottle. The reagent solution bottles are covered with Parafilm ("M" Pechiney) to avoid potential contamination while in use.

Assessment

Refractive index

In oligotrophic waters nutrient concentrations are of the same order of magnitude as the low nutrient seawater (LNSW) used as wash solution in micromolar nutrient determinations (Zhang 2000). This means that the use of LNSW as blank or wash water for nanomolar measurements is problematic (e.g., if the sample's concentration is below blank and wash water concentrations, negative absorbance readings are found). Thus, we use DI water as wash solution for the discrete system. Ultrapure (>18 mΩ) DI water is available onboard all large research vessels, and easily replaced if contaminated. This also makes this method less labor-intensive compared to the use of filtered LNSW, or "phosphate free" seawater from MAGIC supernatants (Zhang and Chi 2002; Patey et al. 2010), as a wash water solution. Because the Schlieren effect is more pronounced at the sample and wash solution interface where the salinity, and hence the refractive index gradient, is greatest (Zhang 2000), we increased sample/wash times to stabilize the optical signal from 90s/60s, used typically (e.g., Zhang and Chi 2002), to 120s/90s. Furthermore, gas segmentation reduces sample dispersion and carry-over (Zhang and Chi 2002), and we found that 120 s is sufficient time for the sample to generate the typical plateau-shaped peak, and a 90 s wash time adequately cleans out the LWCC between samples (Fig. 2). Nevertheless, in the stable region of the optical signal, there is a potential offset in the refractive index due to salinity differences between DI water and seawater. To test this, we ran seawater samples with salinities ranging from 0 to 37 without reagents. In the normal seawater salinity range of 28–37, we found a constant offset of –0.005 ± 0.0003 Abs units, which at typical calibration curve slopes equates to ca. 2 nmol/L. This value emphasizes the importance of measuring and applying this offset to obtain the final phosphate concentration for seawater samples. Including the refractive index offset, the longer sample and wash times mean that the system can only analyze 6 samples in triplicate per hour.

The continuous system has a constant flow of seawater from the shipboard underway sampling unit, so no wash water is used, and hence no issues with differences in refractive index are present during the run. However, it is important to account for the refractive index offset between the DI channel and the seawater channel just like the discrete system. Quantifying this offset is included in measuring the sensitivity correction factor so no additional correction is needed like those in the discrete system.

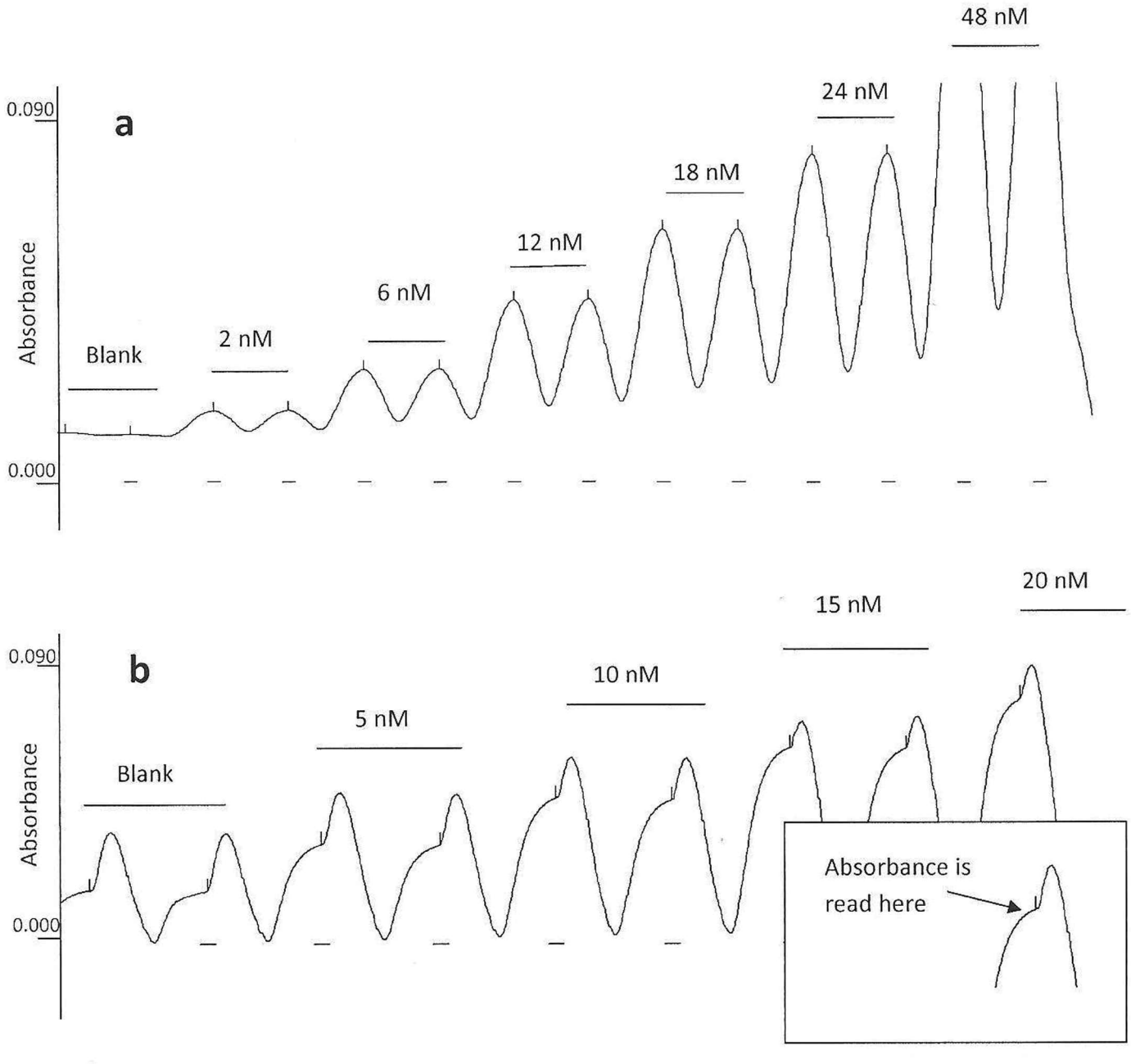

Fig. 2. Comparisons of different wash water solutions for the discrete system. Standards run in duplicate. (a) Low nutrient seawater from the Sargasso Sea with a phosphate concentration of 7 nmol/L used as wash water solution and for standards. If samples are below 7 nmol/L, negative peaks will appear (not shown). (b) DI water is used as wash water solution and standards are prepared in seawater with a phosphate concentration of 7 nmol/L. Blank is seawater with no standard addition. Absorbances are read at the vertical ticks (see insert).

Baseline stability and segmentation gas

Variations in the baseline over time due to instability of reagents and coating of the LWCC and sample tubing walls are well known (Li et al. 2008; Ma et al. 2009) and can become a challenge in long-term measurements (hours), decreasing the sensitivity of the LWCC method. For discrete analyses, the Astoria-Pacific software's (FASPac) baseline correction feature is used to zero the blank signal from the reagents + DI wash water at a fixed interval (every 35 min). For the continuous system, baseline variations are monitored by running a parallel reference channel with only DI water and reagents. To ensure the same sensitivity in both LWCC channels during an

analytical run, identical standards in the respective matrix are analyzed at the start and end of the run. The correction factors for sensitivity and drift described above are then applied. The maximum drift (DCF) we have found in 2 years of use was 0.01 Abs/hour, and the maximum difference in sensitivity was 12%. In addition, to verify that salinity changes do not affect the responses of the two channels, identical standards in seawater and DI water were analyzed. The test of homogeneity of the two calibration slopes revealed no statistical difference between them (ANCOVA, df = 8, *F*-ratio = 0.83, *P* = 0.41).

For both the discrete and continuous systems, it is essential to keep a regular cleaning schedule to minimize coating of the LWCC walls with colloidal molybdenum blue, ensure low baseline noise, and establish a regular bubble pattern. Microbubbles are occasionally produced in the sample stream and can escape the debubbler and enter LWCC, causing spikes in the absorbance signal from the reflection of light on bubble surfaces (Zhang 2000). Sodium dodecyl sulfate is added as a surfactant to minimize bubble breaking and to prevent microbubbles sticking to the walls in LWCC (Zhang and Chi 2002; Patey et al. 2010). Additionally, helium is used as segmentation gas instead of air or nitrogen for the discrete and continuous systems. Changes in pressure gradients within the system due to variations in tube and fitting diameters, and gas solubility in water, can cause dissolved gases to come out in solution in the LWCC, causing noise in the absorbance signal. Hence, to avoid supersaturation and potential degassing, low solubility helium rather than nitrogen is used. It may seem counterintuitive, but using a less soluble segmentation gas minimizes the gas from going into solution in the first place and then degassing before or in the LWCC.

Interferences with the molybdenum blue method

Both silicate and arsenate [As(V)] can interfere with the molybdenum blue method, potentially resulting in an overestimation of phosphate concentration (Karl and Tien 1992; Zhang et al. 1999; Gimbert et al. 2007; Anagnostou and Sherrell 2008; Ma et al. 2009). Silicate interference is minimized under reaction conditions of pH = 1.3 and addition of potassium antimony tartrate as catalyst (Zhang et al. 1999; Gimbert et al. 2007; Ma et al. 2009). Arsenate interference was investigated by Ma et al. (2009). Using reversed flow injection analysis (rFIA) in connection with LWCC and a 2 min reaction time, they didn't find any significant interference from arsenate up to 53 nmol/L for samples containing 0 and 82.5 nmol/L phosphate. This lack of interference is probably due to the slower reaction rate of the arsenomolybdate complex relative to the phosphomolybdate complex (Johnson 1971; Downes 1978; Ormaza-González and Statham 1991; Karl and Björkman 2002) and the very short reaction time for color development with this method. Using SCFA LWCC, Li and Hansell (2008b) reported no significant differences for LNSW samples before and after reduction of arsenate to arsenite with potassium iodide, suggesting insignificant arsenic interference with their method. Patey et al. (2010) conducted SCFA LWCC arsenate interference experiments with a 10 min color formation time following addition of reagents. Based on a 20 nmol/L arsenate seawater concentration, a soluble reactive phosphate overestimate of 4.6 ± 1.4% for samples containing 5.00 nmol/L phosphate was calculated. Since color development time is 7 min after addition of reagents in the systems described here, we spiked DI water and LNSW with varying concentrations of arsenate to test the responses. For DI water samples spiked with 13, 20, and 27 nmol/L As(V), the apparent phosphate concentration was below the systems' detection limits of 0.5–0.8 nmol/L (see below). The same arsenate concentrations were added to a LNSW sample with 10.5 nmol/L phosphate concentration. Increases in apparent phosphate of 7.1 ± 6.1%, 6.8 ± 5.1%, and 9.7 ± 4.8%, respectively, were found. Similar increases were measured for the continuous system. While arsenate interference is present in our systems, it has large errors and is thus right at the detection limits, even with the highest possible arsenate concentration for oligotrophic seawater (Cutter et al. 2001; Cutter and Cutter 2006). Thus, our findings are in accordance with what was previously reported (Li and Hansell 2008b; Ma et al. 2009; Patey et al. 2010); arsenate interference is minimal.

Sample handling

To prevent particles from entering the LWCC and clogging in the capillaries or generating spikes in the absorbance signal, samples must be < 0.4 µm filtered before analysis. Furthermore, cell lysis and hydrolysis of particulate organic phosphate in unfiltered seawater can change the dissolved nutrient composition during storage, in effect contaminating the dissolved sample (Patey et al. 2010). However, it is important to also recognize that filter type can result in losses of dissolved phosphate concentration (Mee 1986; Li and Hansell 2008b; Patey et al. 2010). For example, Li and Hansell (2008b) found adsorptive loss between 2% and 66% depending on filter type for a 50 nmol/L phosphate sample, with higher losses for seawater than DI water. Because the method reported here is intended for the determination of dissolved phosphate, the fraction that is directly available to phytoplankton, and not total phosphate, we recommend the use of a low adsorptive-loss filter type. Based on the above work of Li and Hansell (2008b), we chose a filter with cellulose acetate membrane. However, we recommend equilibrating the capsule filter by running liters (>1 L) of seawater through it before sampling to minimize the effect of potential adsorption.

It is desirable to analyze seawater nutrient samples onboard ship as soon as possible after sampling to eliminate any storage-related problems. This is not an issue for the continuous LWCC system, but could create problems for the discrete LWCC. To address the stability of nanomolar phosphate concentration in seawater, six 1 L samples taken at six different depths (50 to 285 m) were collected on the US GEOTRACES 2011 North Atlantic Cruise at 26°N 47°W (1 L acid-cleaned polyethylene bottles, 4°C, stored in the dark). Samples were analyzed by the discrete LWCC system within 2 h of sample

collection (t = 0), and then on day 3, day 5, and day 9 (Fig. 3). At day 9 recovery for a 8.3 nmol/L sample (50 m) relative to t = 0 was 100 ± 8.1% (n = 4), for a 8.8 nmol/L sample (95 m) relative to t = 0 was 100 ± 5.6% (n = 4) and for a 20.4 nmol/L sample (136 m) relative to t = 0 was 100 ± 13.2% (n = 4). At depths below 185 m phosphate concentrations were > 100 nmol/L and the recovery relative to t = 0 were 100 ± 2.8%. Patey et al. (2010) found no significant changes in phosphate concentration following a 24 h storage period (LNSW, 4°C, stored in the dark). Krom et al. (2005) did a comparison of nutrient data from the analysis of seawater samples analyzed on-board within 2 h of collection and frozen samples. They concluded that freezing samples as acceptable for phosphate concentrations above 20 nmol/L; samples with lower nutrient concentration were poorly preserved. Based on our results and those in the literature for seawater with nanomolar phosphate concentrations, it is recommended that discrete samples stored at 4°C in the dark be analyzed within 24 to 36 h.

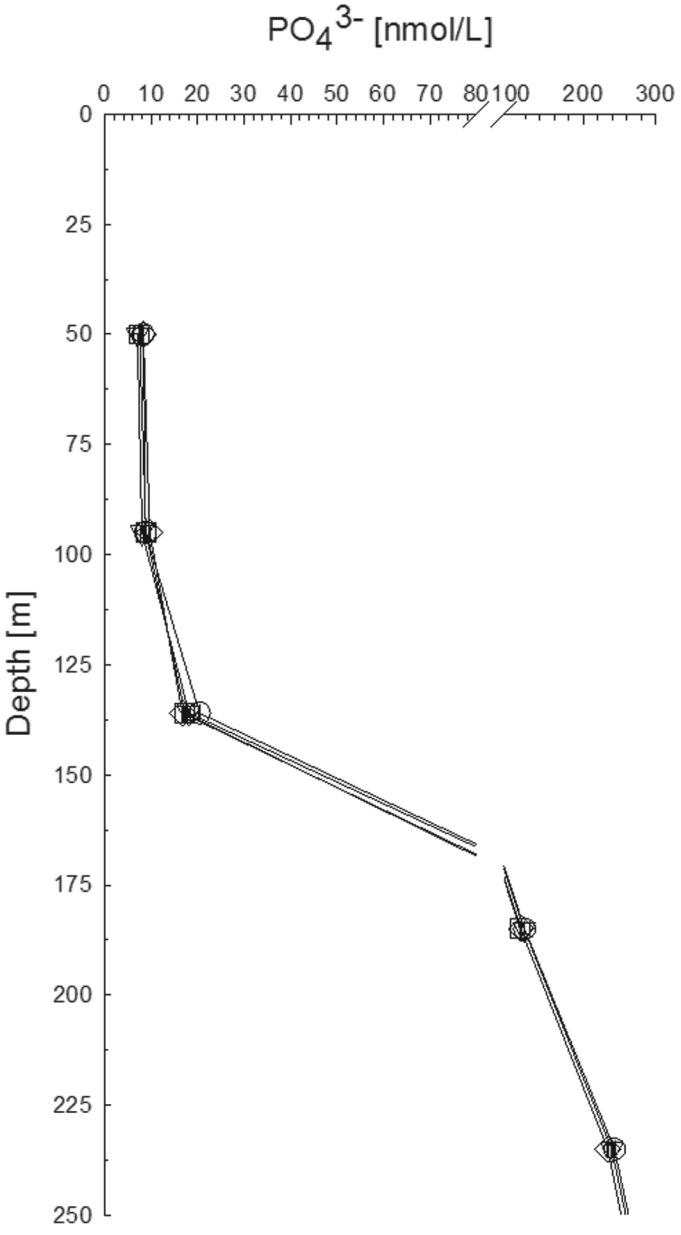

Fig. 3. Samples from 26°N, 47°W were collected for a storage experiment during the US GEOTRACES 2011 cruise. Samples were 0.2 µm filtered and analyzed by the discrete LWCC system at day 0 (t = 0), day 3, day 5, and day 9.

Analytical figures of merit

The precision for the analytical systems was evaluated by analyzing replicate seawater samples. For the discrete sample system, the precision as relative standard deviation (RSD) for phosphate at 10 nmol/L is 1.8% (n = 8) and at 60 nmol/L is 0.9% (n = 9). For the continuous system, the relative standard deviation for phosphate at 5 nmol/L is 6.1% (n = 5, 20 min measurements) and at 50 nmol/L is 0.8% (n = 5). Zhang and Chi (2002) found the RSD for phosphate at 10 nmol/L was 2%, whereas precision found by Patey et al. (2008) at the same phosphate concentration was 4.8%. Ma et al. (2009) found RSDs for phosphate at 25 nmol/L and 83 nmol/L were 1.5% and 1.9% (n = 9), respectively. Thus, the systems described here have equivalent precision to those of other workers.

The detection limit (3σ of reagent blank) for the discrete system (200 cm LWCC) for phosphate is 0.5 nmol/L (n = 9) and 0.8 nmol/L (n = 10) for the continuous system (250 cm LWCC). These detection limits are in agreement with other studies (Table 1). The low detection limits make the LWCC method suitable for phosphate determinations in oligotrophic waters such as the subtropical North Atlantic gyre. With respect to the upper working limit, the LWCC methods described here have linear responses for phosphate concentrations between 0.5 and 700 nmol/L for the discrete system (200 cm LWCC, Fig. 4a) and between 0.8 and 300 nmol/L for the continuous system (250 cm LWCC, Fig. 4b). Above these concentration ranges the absorbance-concentration curves asymtope. Zhang and Chi (2002) found a linear response in absorbance to phosphate concentrations below 200 nmol/L, whereas Patey et al. (2008) found a linear relationship for phosphate concentrations between 5 to 100 nmol/L. By diluting the sample with DI water or using a shorter LWCC, the linear dynamic range of phosphate analysis can easily be extended (Zhang and Chi 2002).

To evaluate accuracy, two approaches were used. The first involved analyzing the commercially-available Japanese reference seawater (General Environmental Technos), with a certified phosphate concentration of 0.08 ± 0.01 µmol/kg using the discrete LWCC system. Unfortunately, we found a poor recovery, < 45% (Table 2). This reference material was produced 4–5 y before analysis and close to expiration date, so the poor recovery could be due to the long storage time. However, it was not really intended as a nanomolar nutrient reference material. Interestingly, we found the same concentrations for two different lots of this reference material in 2009 and 2010 (Mann-

Table 1. Overview of reported phosphate detection limits for LWCC method.

Technique	Length of LWCC	Detection limit PO_4^{3-} (nmol/L)	Reference
SCFA	200 cm	0.5 nmol/L	This study, discrete system
SCFA	250 cm	0.8 nmol/L	This study, continuous system
SCFA	200 cm	0.5 nmol/L	Patey et al. 2010
Reverse FIA	200 cm	0.5 nmol/L	Ma et al. 2009
FIA	200 cm	1.5 nmol/L	Li et al. 2008
SCFA	200 cm	0.8 nmol/L	Patey et al. 2008
FIA	100 cm	10.0 nmol/L	Gimbert et al. 2007
SCFA	200 cm	2.0 nmol/L	Krom et al. 2005
SCFA	200 cm	0.5 nmol/L	Zhang and Chi 2002

SCFA = segmented, continuous-flow analysis; FIA = flow-injection analysis.

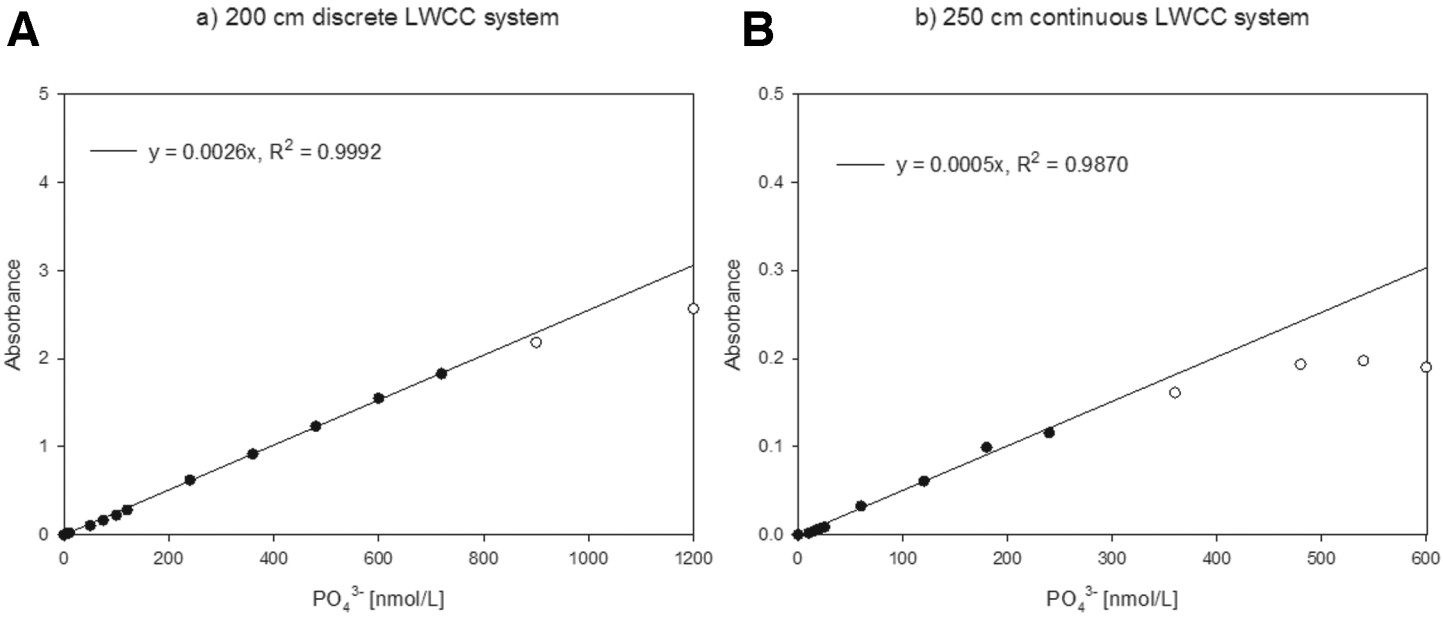

Fig. 4. Linearity of phosphate determination in (a) 200 cm discrete LWCC system and (b) 250 cm LWCC continuous system. Open circles indicate the absorbance of phosphate concentration are beyond the linear dynamic range and are not used for linear regressions.

Table 2. Recoveries from Certified Reference Standards (General Environmental Technos, Japan) using the discrete LWCC system.

Sample	PO_4^{3-} (nmol/L)	*n*	Certified µmol/kg
RMNS BA 2010	32.8 ± 5.3	10	0.08 ± 0.01
RMNS BA 2009	33.7 ± 3.6	8	0.08 ± 0.01

Whitney U test, df = 28, NS, Table 2) on two different GEOTRACES cruises, at least supporting LWCC's high precision.

The second evaluation of LWCC accuracy was to run parallel samples with the established, high-sensitivity MAGIC technique (Karl and Tien 1992; Rimmelin and Moutin 2005). Four sets of samples from five different depths were collected on the U.S. GEOTRACES 2009 Intercalibration Cruise at 30°N 140°W. One set of samples was analyzed on-board by the discrete LWCC system. The second set of samples was pre-concentrated on-board using the MAGIC method. The precipitate was then frozen and analyzed at the land-based ODU laboratory (ODU) after the cruise with the optimized MAGIC method (Rimmelin and Moutin 2005). The third set of water samples (1 L, acid cleaned polyethylene bottle) was immediately frozen on-board, and then thawed, pre-concentrated using the MAGIC method and analyzed in the ODU laboratory 2 months after the cruise. The last set of samples (1 L, acid cleaned polyethylene bottle) was also immediately frozen on-

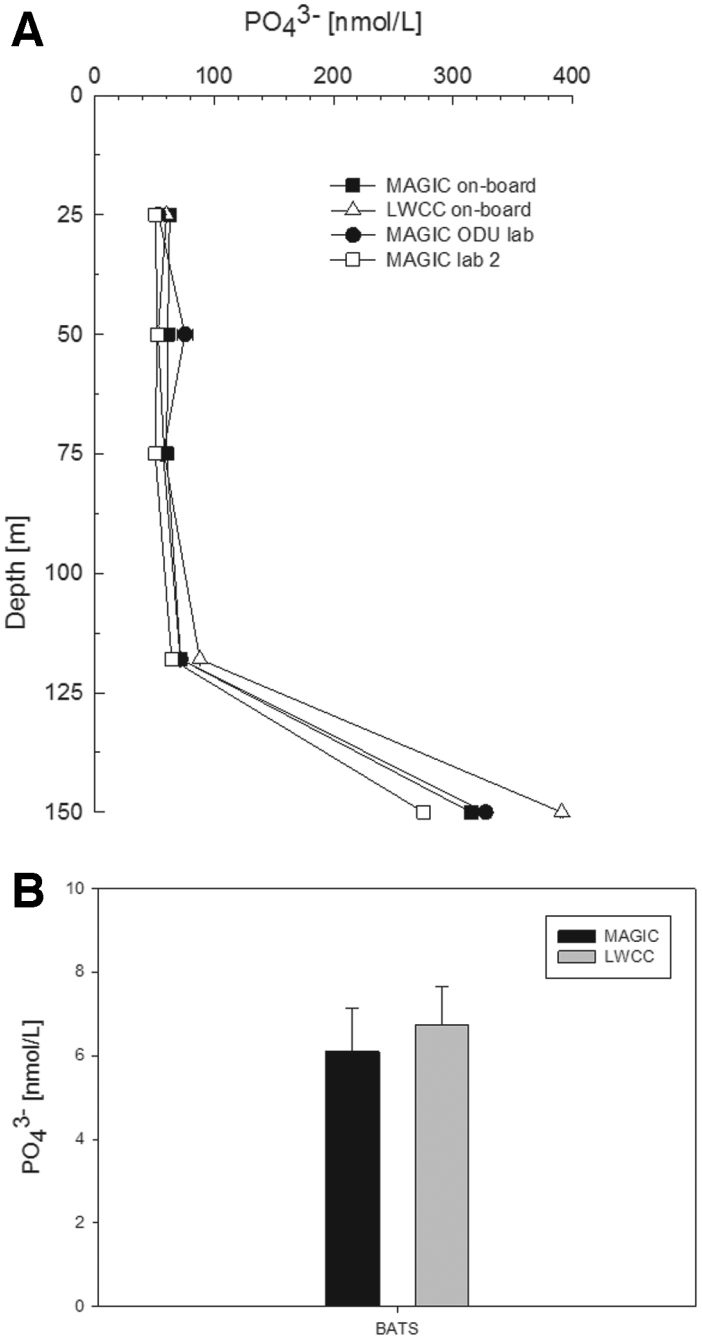

Fig. 5. Comparisons of the LWCC method with the MAGIC method for phosphate determinations in seawater. All samples were 0.2 µm filtered. (a) Pacific seawater samples were collected in four sets from 12 L GoFlo bottles at the SAFe station (30°N, 140°W). (b) Atlantic seawater samples were collected at BATS station (31°N, 64°W).

board and sent to an experienced shore laboratory (lab 2) and analyzed using the MAGIC method. Results in Fig. 5a show the recovery for LWCC relative to the MAGIC method is 100 ± 17% (n = 16) for concentrations below 100 nmol/L. The 150 m data set was excluded due to exceeding the working range limit for the MAGIC method (Rimmelin and Moutin 2005). A second set of MAGICs were prepared with Atlantic seawater samples collected at the BATS station (31°N 64°W) in 2008 and analyzed at our land-based ODU laboratory and by the discrete LWCC system within 2 days; results are shown in Fig. 5b. No significant difference was found between the LWCC and MAGIC methods (t-test, df = 17, $P < 0.05$). Overall, our results suggest excellent accuracy based on the agreement between the two methods for phosphate concentrations below 100 nmol/L, supporting Li and Hansell's (2008b) similar findings.

Demonstration of the systems' performance

The discrete and the continuous systems were deployed on the 2010 U.S. GEOTRACES cruise that crossed the upwelling area northwest of Africa and the highly-stratified, oligotrophic, subtropical North Atlantic gyre in Oct–Nov 2010 (Fig. 6). Fig. 7a shows underway phosphate concentrations measurements in the upwelling area north of Africa using the continuous and discrete sample systems. The continuous measurements display a considerable amount of structure due to the heterogeneous nature of upwelling, with phosphate concentrations fluctuating between 5 and 100 nmol/L. Discrete sampling was not able to capture this high variability (Fig. 7a). Fig. 7b shows phosphate concentrations in the oligotrophic open ocean during a 16 h transect from 19°49′ N, 56°47′ W to 20°06′ N, 59°02′ W. In this region the continuous measurements were as uniform as the discrete sampling, with phosphate concentrations ranging between 3 and 7 nmol/L. Due to the nature of continuous measurements (every 30 s), no replicate data are available. However, if we average the continuous data ± 2 min around the sampling time for the discrete samples for the data in Figs. 7a and 7b, we found a ratio between the two systems (continuous/discrete) = 100 ± 3% (n = 10). This excellent agreement shows that both independent systems respond equivalently to dissolved reactive phosphate concentrations.

Discussion

High resolution measurements make a continuous system able to record near real-time changes in phosphate concentrations, which the discrete sample system (6 samples per hour) or manual methods such as MAGIC cannot do. Using a continuous intake of seawater in combination with the LEDSpec LWCC system, it is possible to record data every second if desired. To meet the computer capacity and data processing time, the 30 s sampling rate was chosen for the 2010 U.S. GEOTRACES cruise. Maintenance, change of new reagents, and calibration of the instrument take 2 to 3 h, causing gaps in the data sets (Figs. 7a and 7b). To avoid gaps, two independent phosphate channels could be operated with different times for maintenance and calibrations. Li et al. (2008) developed a shipboard underway system for monitoring nanomolar nutrient concentrations using flow injection analysis (FIA) in combination with LWCC, processing 20 samples per hour. While excellent, this FIA system has one sixth the temporal resolution than the continuous method reported here.

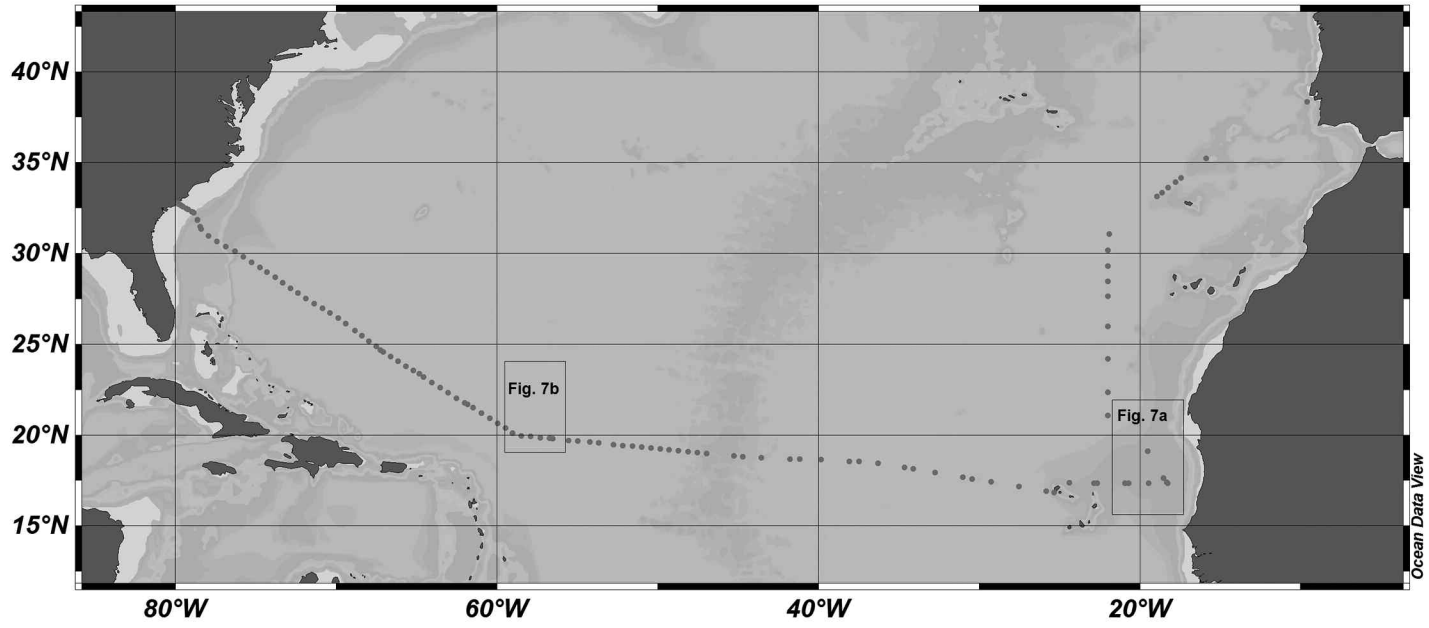

Fig. 6. Ship's track for surface sampling during the U.S. GEOTRACES North Atlantic Transect, 15 Oct to 26 Nov 2010.

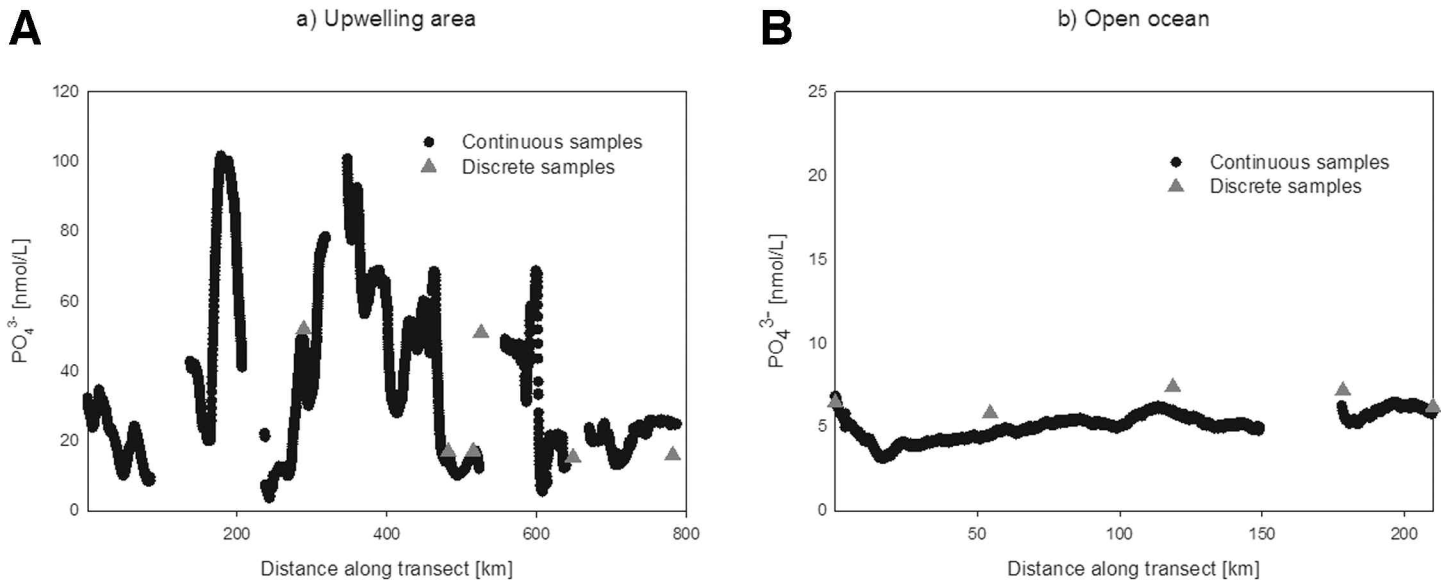

Fig. 7. Underway phosphate determinations by a continuous and a discrete system in (a) a 800 km transect in the upwelling area northwest of Africa (section from 20°45′ N, 21°37′ W to 17°21′ N, 18°12′ W), and (b) a 210 km transect in the oligotrophic, subtropical North Atlantic gyre (19°49′ N, 56°47′ W to 20°06′ N, 59°02′ W). See Fig. 6 for graphical track. Gaps in continuous measurements are due to calibration and maintenance periods.

The use of continuous nanomolar measurements of nutrients like phosphate during surface transects, and equivalent discrete measurements in depth profiles of the upper water column are an essential part of international programs such as GEOTRACES. To fully interpret the biogeochemistries of trace elements and isotopes at nanomolar and below concentrations requires measurements of essential nutrients like phosphate at equivalently low concentrations. Studying trace element biogeochemistries in physically dynamic waters such as upwelling regions is particularly difficult due to rapid changes from horizontal and vertical advection and biological uptake. In these waters, higher data resolution with continuous nutrient measurements can record changes in surface nutrient concentrations on the kilometer scale, revealing temporal vari-

ability and quantifying horizontal gradients, and hence fluxes. Pumping while on station could even quantify temporal variability in such regions to the minute time scale. Having nanomolar detection limits on discrete samples allows the same type of evaluations on the vertical scale, particularly needed for calculating vertical advective/diffusive fluxes.

References

Alvarez-Salgado, X. A., F. Fraga, and F. F. Pérez. 1992. Determination of nutrient salts by automatic methods both in seawater and brackish water: the phosphate blank. Mar. Chem. 39:311-319 [doi:10.1016/0304-4203(92)90016-4].

Anagnostou, E., and R. M. Sherrell. 2008. MAGIC method for subnanomolar orthophosphate determination in freshwater. Limnol. Oceanogr. Methods 6:64-74 [doi:10.4319/lom.2008.6.64].

Benitez-Nelson, C. R. 2000. The biogeochemical cycling of phosphorus in marine systems. Earth Sci. Rev. 51:109-135 [doi:10.1016/S0012-8252(00)00018-0].

Cutter, G. A., L. S. Cutter, A. M. Featherstone, and S. E. Lohrenz. 2001. Antimony and arsenic biogeochemistry in the western Atlantic Ocean. Deep-Sea Res. II 48:2895-2915 [doi:10.1016/S0967-0645(01)00023-6].

Cutter, G. A., and L. S. Cutter. 2006. Biogeochemistry of arsenic and antimony in the North Pacific Ocean. Geochem. Geophys. Geosys. 7:1-12.

Downes, M. T. 1978. An automated determination of low reactive phosphorus concentrations in natural waters in the presence of arsenic, silicon and mercuric chloride. Water Res. 12:743-745 [doi:10.1016/0043-1354(78)90022-2].

Estela, J. M., and V. Cerdà. 2005. Flow analysis techniques for phosphorus: an overview. Talanta 66:307-331 [doi:10.1016/j.talanta.2004.12.029].

Falkowski, P. G. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO2 in the ocean. Nature 387:272-275 [doi:10.1038/387272a0].

Froelich, P. N., and M. E. Q. Pilson. 1978. Systematic absorbance errors with Technicon Autoanalyzer II colorimeters. Water Res. 12:599-603 [doi:10.1016/0043-1354(78)90139-2].

Gimbert, L. J., P. M. Haygarth, and P. J. Worsfold. 2007. Determination of nanomolar concentrations of phosphate in natural waters using flow injection with a long path length liquid waveguide capillary cell and solid-state spectrophotometric detection. Talanta 71:1624-1628 [doi:10.1016/j.talanta.2006.07.044].

Johnson, D. L. 1971. Simultaneous determination of arsenate and phosphate in natural waters. Environ. Sci. Technol. 5:411-414 [doi:10.1021/es60052a005].

Karl, D. M., and G. Tien. 1992. MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments. Limnol. Oceanogr. 37:105-116 [doi:10.4319/lo.1992.37.1.0105].

———, and K. M. Björkman. 2002. Dynamics of DOP, p. 249-366. *In* D. A. Hansell and C. A. Carlson [eds.], Biogeochemistry of marine dissolved organic matter. Elsevier [doi:10.1016/B978-012323841-2/50008-7].

Krom, M. D., E. M. S. Woodward, B. Herut, N. Kress, P. Carbo, R. F. C. Mantoura, G. Spyres, T. F. Thingstand, P. Wassmann, C. Wexels-Riser, V. Kitidis, C. S. Law, and G. Zodiatis. 2005. Nutrient cycling in the south east Levantine basin of the eastern Mediterranean: Results from a phosphorus starved system. Deep-Sea Res. II 52:2879-2896.

Li, Q. P., D. A. Hansell, and J.-Z. Zhang. 2008. Underway monitoring of nanomolar nitrate plus nitrite and phosphate in oligotrophic seawater. Limnol. Oceanogr. Methods 6:319-326 [doi:10.4319/lom.2008.6.319].

———, and D. A. Hansell. 2008a. Nutrient distributions in baroclinic eddies of the oligotrophic North Atlantic and inferred impacts on biology. Deep-Sea Res. II 55:1291-1299 [doi:10.1016/j.dsr2.2008.01.009].

———,and ———. 2008b. Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater. Anal. Chim. Acta 611:68-72 [doi:10.1016/j.aca.2008.01.074].

Lomas, M. W., A. L. Burke, D. A. Lomas, S. W. Bell, C. Shen, S. T. Dyhrman, and J. W. Ammerman. 2010. Sargasso Sea phosphorus biogeochemistry: an important role for dissolved organic phosphorus (DOP). Biogeosciences 7:695-710 [doi:10.5194/bg-7-695-2010].

Ma, J., D. Yuan, M. Zhang, and Y. Liang. 2009. Reverse flow injection analysis of nanomolar soluble reactive phosphorus in seawater with a long path length liquid waveguide capillary cell and spectrophotometric detection. Talanta 78:315-320 [doi:10.1016/j.talanta.2008.11.017].

Mee, L. D. 1986. Continuous flow analysis in chemical oceanography: Principles, applications and perspectives. Sci. Total Environ. 49:27-87 [doi:10.1016/0048-9697(86)90232-9].

Motomizu, S., and Z.-H. Li. 2005. Trace and ultratrace analysis methods for the determination of phosphorus by flow-injection techniques. Talanta 66:332-340 [doi:10.1016/j.talanta.2004.12.056].

Murphy, J., and J. P. Riley. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27:31-36 [doi:10.1016/S0003-2670(00)88444-5].

Ormaza-González, F. I., and P. J. Statham. 1991. Determination of dissolved inorganic phosphorus in natural waters at nanomolar concentrations using a long capillary cell detector. Anal. Chim. Acta 244:63-70 [doi:10.1016/S0003-2670(00)82479-4].

Patey, M. D., M. J. A. Rijkenberg, P. J. Statham, M. C. Stinchcombe, E. P. Achterberg, and M. Mowlem. 2008. Determination of nitrate and phosphate in seawater at nanomolar concentrations. Trends Anal. Chem. 27:169-182 [doi:10.1016/j.trac.2007.12.006].

———, E. P. Achterberg, M. J. A. Rijkenberg, P. J. Statham, and M. Mowlem. 2010. Interferences in the analysis of nanomolar concentrations of nitrate and phosphate in oceanic waters. Anal. Chim. Acta 673:109-116 [doi:10.1016/j.aca.2010.05.029].

Paytan, A., and K. McLaughlin. 2007. The oceanic phosphorus cycle. Chem. Rev. 107:563-576 [doi:10.1021/cr0503613].

Rimmelin, P., and T. Moutin. 2005. Re-examination of the MAGIC method to determine low orthophosphate concentration in seawater. Anal. Chim. Acta 548:174-182 [doi:10.1016/j.aca.2005.05.071].

Ryther, J. H., and W. M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. Science 171:1008-1013 [doi:10.1126/science.171.3975.1008].

Vink, S., E. A. Boyle, C. I. Measures, and J. Yuan. 2000. Automated high resolution determination of the trace elements iron and aluminium in the surface ocean using a towed fish coupled to flow injection analysis. Deep-Sea Res. I 47:1141-1156 [doi:10.1016/S0967-0637(99)00074-6].

Wu, J., W. Sunda, E. A. Boyle, and D. M. Karl. 2000. Phosphate depletion in the Western North Atlantic Ocean. Science 289:759-762 [doi:10.1126/science.289.5480.759].

Zhang, J. -Z. 2000. Shipboard automated determination of trace concentrations of nitrite and nitrate in oligotrophic water by gas-segmented continuous flow analysis with a liquid waveguide capillary flow cell. Deep-Sea Res. I 47:1157-1171 [doi:10.1016/S0967-0637(99)00085-0].

———, C. J. Fischer, and P. B. Ortner. 1999. Optimization of performance and minimization of silicate interference in continuous flow phosphate analysis. Talanta 49:293-304 [doi:10.1016/S0039-9140(98)00377-4].

———, and J. Chi. 2002. Automated analysis of nanomolar concentrations of phosphate in natural waters with liquid waveguide. Environ. Sci. Technol. 36:1048-1053 [doi:10.1021/es011094v].

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