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Underway analysis of nanomolar dissolved reactive phosphorus in oligotrophic seawater with automated on-line solid phase extraction and spectrophotometric system

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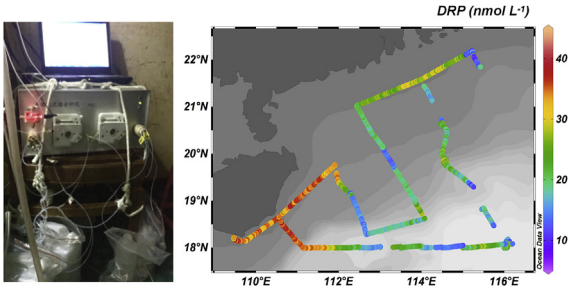
HIGHLIGHTS

- On-line solid phase extraction of phosphomolybdenum blue without common interference.
- Automated flow analyzer with laboratory-made hardware and software.
- 2-week continuous underway determination of nanomolar phosphate in the South China Sea.
- Accurate and high resolution field seawater analysis with occasional maintenance.

GRAPHICAL ABSTRACT



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ABSTRACT

The automated in-field determination of trace phosphate (and other nutrients) is highly valuable for studying nutrient dynamics and cycling in oligotrophic oceans. Here, we report an automated portable analyzer for week-long underway analysis of nanomolar dissolved reactive phosphorus (DRP) in seawater. The method is based on classic phosphomolybdenum blue (PMB) chemistry combined with on-line solid phase extraction (SPE) and flow analysis. Under optimized conditions, the formed PMB from sample is automatically concentrated on a hydrophilic lipophilic balanced (HLB) copolymer SPE. The PMB compound can be eluted with NaOH solution and measured in a flow-through detection system. All the components of the analyzer are computer controlled using laboratory-programmed software based on LabVIEW. The system exhibited advantages of high sensitivity (detection limit of 1.0 nmol L^{-1}) and reproducibility (relative standard deviation of 5.4%, n = 180), insignificant carry-over effect and no interferences from salinity, silicate, arsenate and other P-containing compounds (concentrations at environmental level). The analytical time was 4–7 min/sample, depending on the DRP concentration. The accuracy of the method was validated through the analysis of reference materials and comparison with two other published methods (slope of 0.986 ± 0.027, intercept of 0.39 ± 0.64 nmol L^{-1}, R^2 of 0.9608, range of 0–80 nmol L^{-1}, n = 57). The system has been successfully applied for a two-week continuous underway determination of DRP in surface seawater during a cruise in the South China Sea. Based on the laboratory and field evaluations, it is concluded that this system is suitable for accurate and high resolution underway DRP measurements in oligotrophic areas.

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1. Introduction

Phosphorus is one of the most essential nutrients for all organisms, therefore its distribution, transport, and chemical/biological transformations are important aspects in both marine and fresh water bodies [1–3]. Due to the constant biological uptake, low solubility of phosphorus minerals and processes such as adsorption by particulate material, sedimentation, etc., the concentrations of phosphate are at nanomolar levels in some oligotrophic areas (e.g. Refs. [4–6]). Therefore, it is highly desirable for the marine scientist to measure phosphate (and other nutrients) at nanomolar levels. The applicable methods for seawater analysis have been comprehensively reviewed [7,8]. Although there are a number of methods capable of measuring micromolar levels of phosphorus in fresh waters ([9] and references therein), only a few methods have been widely used for trace analysis of dissolved reactive phosphorus (DRP) in saline samples [8]. These methods are mainly based on pre-concentration of the dilute samples or increasing the optical length of detection cell, as will be briefly discussed below.

In the well-known Magnesium Induced Co-precipitation (MAGIC) method [10–12], a small amount of NaOH is first added into the seawater sample. After mixing, the formed $Mg(OH)_2$ can co-precipitate phosphate, which can then be concentrated by centrifugation, dissolved in dilute acid and measured with the classic phosphomolybdenum blue (PMB) method [13,14]. Besides concentrating phosphate using MAGIC method, some solid phase extraction (SPE) methods are also available, which are based on the pre-concentration of the derivatization products of analytes (not the analytes themselves). These SPE methods have been extensively developed and applied for the determination of trace nutrients and metals in natural waters [15–30]. These methods are sensitive (with different enrichment factors), selective (analyte reacts with specific reagents) and easily combined with different detectors [[31] and references therein]. For DRP measurements, two sorbents (C18 and HLB, hydrophilic-lipophilic balance) have been used. The successful replacement of C18 with HLB reduces the reaction time and organic eluent consumption. Moreover, temperature control (water batch) and preparation of "phosphate-free" seawater are not needed for the HLB method [19,30]. However, an organic solvent such as ethanol is still needed for activating the cartridge, which complicates the analytical system and increases the possibility of bubble formation. In the long-path length liquid waveguide capillary cell (LWCC) method, the sensitivity can be amplified using a longer optical cell, normally made of Teflon AF I/II [[32] and references therein, [33] and references therein]. Since the LWCC is commercially available (www.wpiinc.com), it has been combined with different flow analysis methods (normally the conventional nutrient auto-analyzers) and been widely used for trace DRP analysis, as summarized before [7,8,34].

Flow analysis techniques are widely used by marine scientists because of their preciseness, robustness, simplicity, low risk of contamination and ability to be automated [31,35–37]. For trace nutrient samples, there might be potential contaminations and/or losses during the sample storage and transportation [8]. The phosphate distribution in surface marine waters is variable, therefore, a large number of data is highly desired to achieve an adequate spatial representation of phosphate in aquatic system [38]. Several underway and *in situ* analytical system for continuous determination of nanomolar DRP have been reported, which can provide real-time data and minimize contamination artifacts [34,38–41]. Our knowledge of biogeochemical dynamics of nutrients in the ocean can be greatly advanced because of these in field observations [41].

To the best of our knowledge, there is no report to date on the week-long continuous underway monitoring of DRP using SPE method in oligotrophic areas. In this paper, we firstly describe the laboratory optimization of our previously developed HLB method [19,30]. On-line SPE has been combined with flow techniques for automating the system and reducing the reagent consumption. To simplify the system, the activation step of the cartridge using organic solvent was eliminated without affecting the performance of the cartridge, which also shortens analytical cycle time and eliminates environmental and operator health safety concerns. All the automated procedures were controlled by a laboratory-made universal flow analyzer and laboratory-programmed software written by LabVIEW. After comprehensive evaluation of its performance, this sensitive, relatively low-cost, automated analyzer was successfully applied for two-week continuous high-resolution underway measurements of nanomolar DRP in the South China Sea (SCS).

2. Experimental

2.1. Reagents and standards

Ultra-pure water was collected from a Millipore water purification system (www.merckmillipore.com) and used during the research. The chemicals were purchased from Sinopharm Chemical Reagent Co., China, unless stated otherwise. These chemicals were reagent grade or better and used without further purification.

A stock mixed reagent (MR) solution for color development was prepared by mixing 100 mL of 130 g L^{-1} $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ solution with 300 mL of 9 mol L^{-1} H_2SO_4, then adding 100 mL of 3.5 g L^{-1} potassium antimony tartrate [20]. The MR solution was stored at 4 C in a refrigerator and diluted 3 times with pure water to prepare the working solution. An ascorbic acid (AA) solution of 25 g L^{-1} was prepared every other day. The stock rinsing solution of 2.0 mol L^{-1} NaCl and eluent solution of 2.0 mol L^{-1} NaOH were prepared by dissolving appropriate amounts of solid NaCl and NaOH in water, respectively. These stock solutions were diluted carefully just before use.

The phosphate and silicate stock solutions were prepared by dissolving oven-dried (105 C for 2 h) KH_2PO_4 and Na_2SiF_6 in pure water, respectively, and stored at 4 C. An arsenate stock solution of 13.33 mmol L^{-1} was purchased from ChemService Co., USA. Working standards were prepared after stepwise dilutions. National standard seawater samples (GBW08623) and environmental phosphate reference material (GSBZ500028-94) were used for the validation of the method.

All reagents (except for AA) were prepared 72 h before the cruise. Ascorbic acid was weighed and packaged at our shore based laboratory and dissolved in high purity water at sea as needed, typically at 2-day intervals. The standard solutions were prepared during the cruise according to the range of concentrations found in the real samples. All the glassware, containers and bottles used during the experiments were pre-cleaned by soaking in 2 mol L^{-1} HCl solution for at least 24 h, followed by triple rinsing with high purity water, air drying in a clean room, and packaging in zip lock plastic bags.

2.2. Instruments

Two laboratory-made universal flow analyzers were used in this study. The components of the analyzers have been described in one pending patent and our previous work [42]. In brief, the analyzers consisted of two dual/four-channel peristaltic pumps (www.lgpump.com.cn), one 6-port two-position injection valve (www.vici.com) and one 8-position selection valve (www.vici.com). All the analytical procedures, including pump speed, pump rotation direction, valve position, and duration of each step, were

automatically controlled using specially designed hardware and in-house graphical user software programmed in LabVIEW (www.ni.com). During preliminary experiments, silicone tubing was used and then PharMed BPT tubing (www.coleparmer.com) of 0.89 mm (orange/orange color band) and 2.06 mm (purple/purple color band) was equipped to the peristaltic pumps. PTFE tubing of 0.8 and 1.6 mm i.d. and standard connector fittings (1/4″-28 tpi, www.vici.com) were used throughout the system for all liquid connections.

The detection module consisted of a custom-made "Z" shaped flow cell of 2-cm path length, a red LED as light source (C5SMF www.cree.com, luminous intensity of 2200 mcd, peak wavelength of 621 nm), a USB 2000 + miniature fibre optic CCD spectrophotometer (www.oceanoptics.com) as detector, and two fiber optics connecting the flow cell with light source and spectrophotometer. The Oasis HLB cartridge (3 mL/60 mg, P/N: WAT094226, www.waters.com) was used for concentrating PMB. The connection of the cartridge to system has been described earlier [30].

2.3. Description of the workflow

A schematic illustration of the flow analysis workflow is shown in Fig. 1. During laboratory experiments, the underway sampling system-indicated in the lower left corner of Fig. 1 by a green-dash border-was replaced by standard solutions or discrete samples. Table 1 provides timing and flow rate details for the four-step operational cycle. During step 1, new samples merged with the mixture of MR and AA (pre-mixed in mixing coil 1, MC1, 0.5 m long, 0.75 mm i.d.) in MC2 (5.5 m long, 1.6 mm i.d.) and the previous samples were flushed at high speed with pump 1 (P1). meanwhile, NaCl solution was used to rinse the HLB cartridge to reduce the Schlieren effect [19]. During step 2, the multi-position valve switched from NaCl solution to NaOH solution, and the extracted PMB of the previous sample was eluted from the cartridge into the detection system, where its absorbance was measured. Then in step 3, pure water was used to clean the detection system and cartridge. During step 4, the 6-port valve switched from position "B" to "A", and the newly formed PMB was concentrated on the cartridge. The duration of step 4 varied depending on the sample concentrations.

It should be noted that this procedure only has 4 steps, making it much simpler than the previous study (11 steps [19]), because of the elimination of HLB activation with ethanol and combination of on-line SPE with integrated flow system. Prior to the system shutdown, the entire manifold tubing was flushed with pure water to prevent accumulation of salts and other contaminants.

2.4. Field application

Underway measurements of DRP were made during a cruise in the South China Sea (SCS) during May 17-June 2, 2016. Fig. 2 shows the cruise route in the SCS, plotted with Ocean Data View [43]. As shown in Fig. 1, sub-surface seawater (~4.5 m) was pumped into a YSI (YSI 6600d, www.ysi.com) flow-through cell using a submersible pump (100QJ1.2-59/8, ww.ruiron.com) at ~30 L/min. The YSI sensor was used to measure both temperature and salinity. From the YSI flow cell, seawater was pumped through an in-line polyether sulfone 0.45 μm filter into the sampling bottle (~100 mL), which was also used as a de-bubbler. Samples were aspirated from the bottle to the flow analyzer system, and analyzed as described in Section 2.3.

During the cruise, 22 samples were manually collected in pre-washed HDPE bottles from the same underway system. These samples were frozen at −20 °C [20,44] and returned to the laboratory for comparative analysis using a manual SPE method [30] after the cruise. Discrete seawater samples were collected using Niskin bottles attached to a Rosette sampler during the cruise. Within 1 h after collection, these samples were analyzed on board using a semi-automated SPE method [19]. If immediate measurement was not possible, samples were stored at 4 °C in the dark until analysis [6]. The data of discrete surface samples were used for the comparison with underway data in the same station.

3. Results and discussion

In the previous research [19,30,45], several parameters related to this research have been comprehensively studied, such as the kinetics of PMB reaction with reagents at different concentrations, the loading of PMB on the HLB cartridge, the elution of PMB from HLB cartridge, etc. In the preliminary work of this study, these optimized parameters were verified and adjusted slightly as shown in Section 2. Only parameters related to underway analysis were

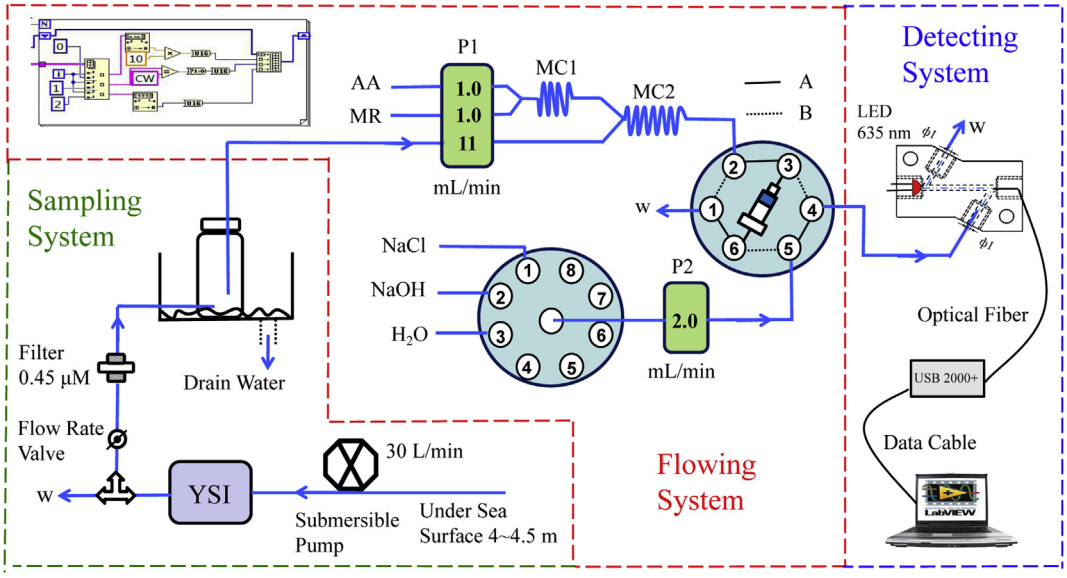

Fig. 1. Schematic illustration of the work flow in laboratory and on ship with underway sampling system. AA, ascorbic acid; MR, mixed reagent; P1/2, pump 1/2; MC1/2, mixing coil 1/2; W, waste; LED, light emitting diode.

Table 1
Flow analysis program and valve position descriptions.

Step	P1 (mL min^{-1}/rpm)	P2 (mL min^{-1}/rpm)	8-position valve	6-port valve	Time (s)	Description
1	23/70	2.0/40	1	B	40	Flush the previous sample and full the reaction coil; rinse HLB cartridge with NaCl solution
2	0/0	2.0/40	2	B	40	Elute the extracted PMB of previous sample from cartridge with NaOH solution; stopped-flow color development of PMB in MC2 proceeds
3	0/0	2.0/40	3	B	40	Clean the cartridge and detection channel with H_2O; stopped-flow color development of PMB in MC2 continues now for a total reaction time 80 s
4	13/40	0/0	3	A	300	Pre-concentrate PMB on HLB cartridge

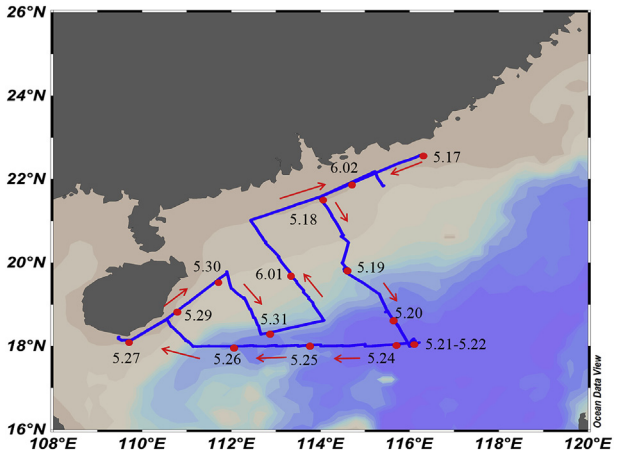

Fig. 2. Cruise track of the R/V *Dongfanghong* II in the SCS, May 17-June 2, 2016. The figure was created with the software Ocean Data View.

evaluated in the following experiments.

3.1. Effect of temperature on interference from arsenate and silicate

Silicate and arsenate are two of the main interfering species for the determination of DRP using the PMB method, because these two ions can also form similar blue compounds with molybdate [46,47]. The interference is affected by reagent chemistry, reaction, temperature, sample preservation, etc. [48]. As shown in Fig. 3, under the protocol of this method, the effects of silicate and arsenate on DRP determination at environmental relevant concentrations were insignificant at room temperature. However, at elevated temperatures, these interferences (as well as the sensitivity of DRP determination) increased and had serious effects on the analysis, which is consistent with other research (e.g. Ref. [49]). Moreover, heating at higher temperature also affected the color formation of PMB [50]. Therefore, the system was kept at room temperature to eliminate the interference from silicate and arsenate. A water bath should be used if the temperature of samples are too low (e.g. at polar areas).

3.2. Effect of salinity

Salinity has two effects on flow analysis: physical influence of refractive index difference between sample and carrier solution (Schlieren effect), and chemical influence of ionic strength difference [45]. The Schlieren effect was overcome by rinsing the cartridge using NaCl solution at the same concentration as the NaOH solution used as eluent [19]. Here, the effect of salinity on the chemical reaction was evaluated. Calibration curves (0–100 nM, six concentrations) were plotted at different salinity values (0, 7, 14, 21, 28 and 35) and measured according to Section 2.3. As shown in Fig. 4, no significant differences were observed in the slope and

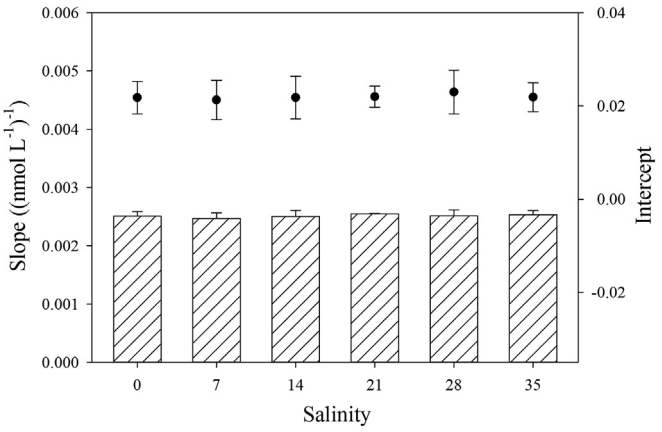

Fig. 4. Effect of salinity.

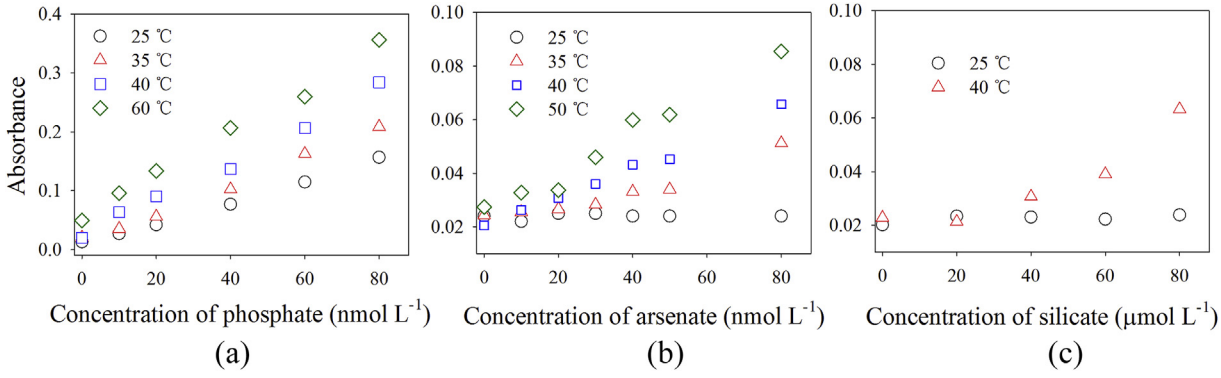

Fig. 3. Effect of temperature on the detection of (a) phosphate, (b) arsenate and (c) silicate.

intercept of the calibration curves at different salinity levels. Therefore, pure water can be used for preparing standard solutions without bothering the time-consuming preparation of "phosphate-free" seawater.

3.3. Effect of wash time and carry-over effect

Normally the DRP concentration does not change significantly between two subsequent determinations in an underway system, however, there could be drastic changes in the DRP concentration at the edges of cold/warm eddy currents. Therefore, it is desirable to study the carry-over effect, which describes how the analyte in a sample is "carried" by an analytical system "over" to the next sample [51]. For any flow based analyzer, this effect is highly dependent upon the presence of poorly flushed "dead volumes" in the flow stream. The carry-over effect can be quantified using different equations. In this experiment, samples of low concentration (10 nmol L^{-1}, *i-2*), high concentration (80 nmol L^{-1}, *i-1*) and low concentration (10 nmol L^{-1}, *i*) were determined sequentially as per the method proposed by Zhang [49]. The carry-over coefficient (k_{CO}) can be calculated as:

$$k_{CO} = (A_i - A_{i-2})/A_{i-1}$$

where A_{i-2}, A_{i-1} and A_i are the measured absorbance values of samples *i-2*, *i-1* and *i*, respectively.

As shown in Fig. 5, the coefficients decreased exponentially with the increase of washing time (i.e., step 1 in Table 1), and became constant when the washing time was more than 35 s. Therefore, 40 s was chosen as the washing time to minimize the carry-over effect. With this washing, the carry-over effect was negligible when going from a high concentration sample to a low concentration sample (inset of Fig. 5).

3.4. Method performance

The typical signal output under the optimized conditions is shown in Fig. 6. There are regular pulses because of the switch between samples and NaOH solution. However, the signal of the analyte was not affected and showed a good linear relationship with concentration of the analyte (inset of Fig. 6). The sensitivity and determination range could be easily adjusted by changing the sample loading volumes, if needed. The detection limit, calculated as three times the standard deviation of the measured blanks

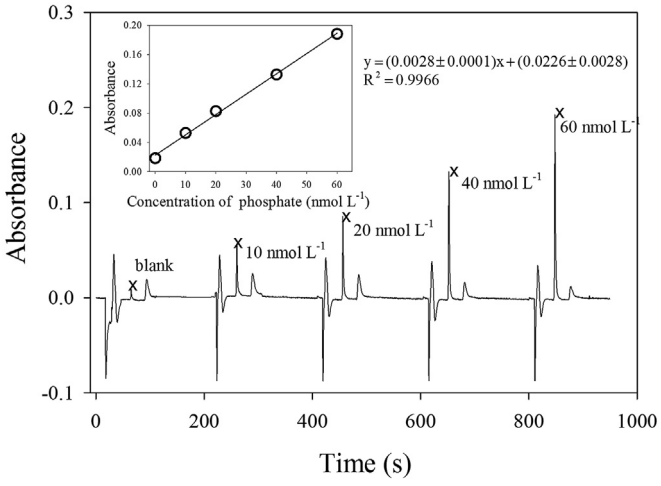

Fig. 6. Typical output signal and calibration curve.

(n = 5), was 1.0 nmol L^{-1} for 50 mL sample. The analytical range and sensitivity can be adjusted through concentrating variable volume of sample by adjusting the time in Step 4 of Table 1. The stability of the SPE cartridge was investigated through continuous analysis of water samples containing 60 nmol L^{-1} phosphate over ~30 h, until the cartridge was damaged. It was found that the cartridge performed well for at least 180 times without any sensitivity loss (relative standard deviation of 5.4%). For in-field application, the cartridge was changed after analyzing ~100 seawater samples to ensure good data quality.

During the cruise, calibration curves were measured every 2 days after changing the reagents. The data quality control (QC) sample of 30 nmol L^{-1} was analyzed twice every day after changing the cartridge. As shown in Fig. 7, the slopes of inter-day calibration curves during the cruise were stable over the two weeks, even with the use of different batches of reagents and cartridges. The measured value of QC samples was 29.6 ± 1.9 nmol L^{-1} (n = 27), which agreed well with the theoretical value of 30 nmol L^{-1}, demonstrating that the system was reliable and stable. The deviation included inevitable errors, such as errors in preparing the standard solution and/or the influence of land and shipboard laboratory conditions on instruments' performance. After the cruise, the performance of the cartridges used during the cruise (n = 28)

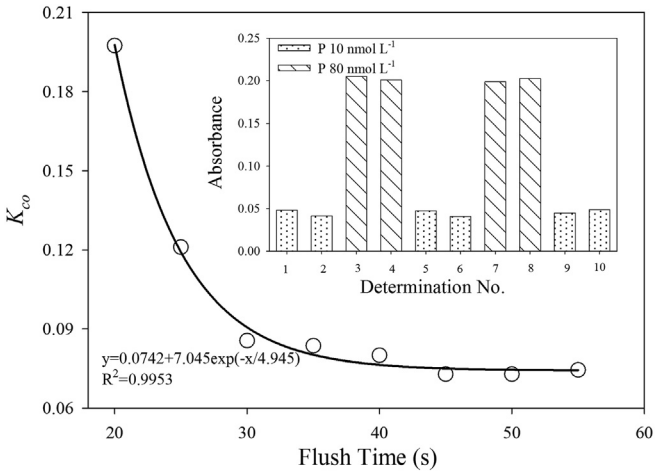

Fig. 5. Carry-over effect.

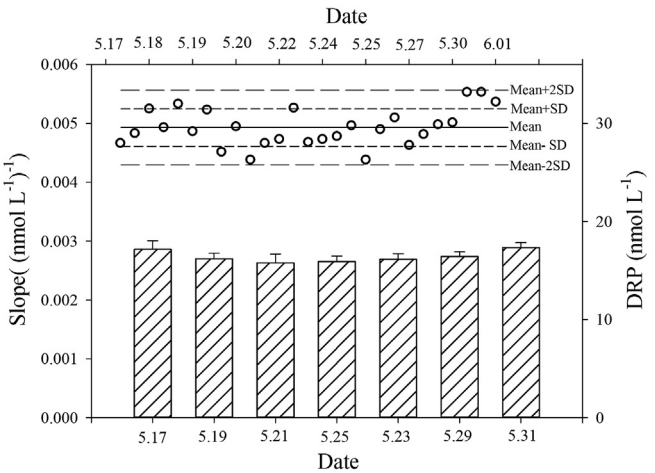

Fig. 7. Calibrations plots and QC samples measured at sea during two-week cruise.

was re-evaluated through the analysis of phosphate samples using a manual SPE method [30]. The measured value was 45.6 ± 1.9 nmol L^{-1}, which is almost the same as the theoretical value of 45 nmol L^{-1}. The highly reproducible results obtained with the different cartridges illustrate the stability of the analytical system in both land-based and shipboard laboratories under rough, in-field conditions. Frequent recalibration to account for signal drift was not necessary.

3.5. Interferences from P-containing compounds

It is possible for the determination of DRP to be affected by the hydrolysis of various forms of phosphorus in the seawater, such as dissolved organic phosphorus [3]. Therefore, it is highly desirable to evaluate the interferences from P-containing compounds for DRP analysis [52]. Using a similar procedure as our previous study [30], a set of samples consisting of P-containing compounds (0–1000 nmol L^{-1}) was prepared and analyzed as "DRP sample" according to Section 2.3. It was assumed that the measured DRP was due to the degradation of these P-containing compounds. The ratios of calibration curve slopes of the P-containing compounds and phosphate were calculated as degradation percentage. As shown in Table 2, most of the tested compounds had undetectable degradation. Only a few of the P-containing compounds showed some degradation behavior which could be detected, but was still insignificant. These interferences were less serious than those observed during the previous similar study [30] due to the shorter reaction time. Besides the reaction parameters, there could be a relationship between the degradation percentage and the physicochemical properties and structures of these compounds, which needs to be confirmed using more model compounds.

3.6. Validation of the method: accuracy and recovery

Since there is no available certified nanomolar phosphate solution [8], phosphate standard/certified solutions at the μmol L^{-1} level were diluted and measured using this method. In addition, the method was also validated through the analysis of a set of samples collected in the SCS and comparison with data measured using other previously published methods [19,30]. There was excellent agreement between the values from the proposed method and the values from reference solutions/methods, with a slope of 0.986 ± 0.027, intercept of 0.39 ± 0.64 nmol L^{-1}, R^2 of 0.9608 and range of 0–80 nmol L^{-1}, n = 57. Some discrepancies were observed for several data pairs, which could be due to lack of synchronicity between the discrete sampling and continuous sampling.

The recovery was estimated as the ratio of the slopes of standard curves prepared in seawater collected from the SCS and pure water [20]. The average recovery was (104 ± 3.8)% (n = 6), indicating that this method is not affected by the seawater matrix.

3.7. In field application

The automated DRP analysis system was operated continuously on the underway seawater supply for two weeks during the cruise in the SCS. A total of 2250 data points for DRP concentration in surface seawater were continuously obtained with minimal maintenance. Measurements were only interrupted during the beginning of the cruise for analytical system performance checks and sampling system maintenance. After data qualification, about 7.9% of the data was discarded because of occasional bubble formation and other laboratory issues causing errors in the detection. The distribution of surface DRP concentrations over the two weeks is presented in Fig. 8. The DRP concentrations exhibited a strong spatial variation, with concentrations ranging from several nmol L^{-1} to more than 40 nmol L^{-1}, which is in accordance with the published data of this area [53–55]. The variation in DRP concentrations could be attributed to the varied biological activities and

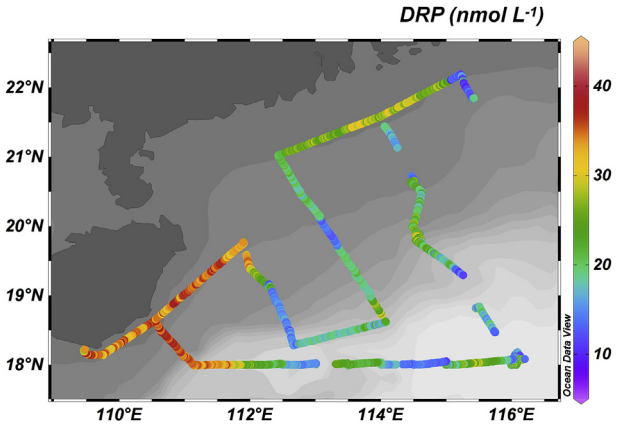

Fig. 8. Field observation of DRP concentrations in the SCS.

Table 2
Information and degradation percentage for a series of phosphorus compounds.

Name of compound	Compound group	Phosphorus bonding	Manufacture	Degradation percentage (%), this/previous method [30]
2-Aminoethylphosphonic acid	phosphonate	C–P	Sigma	0.8/-
Glyphosate	phosphonate	C–P	Adamas	ND*/-
Sodium Phosphonoformate tribasic hexahydrate	phosphonate	C–P	Adamas	ND*/ND*
Adenosine 5′-monophosphate monohydrate	monoester	C–O–P	Alfa	ND*/ND*
2′-Deoxyadenosine 5′-monophosphate	monoester	C–O–P	Alfa	ND*/41.0
DL-α-Glycerol phosphate magnesium salt hydrate	monoester	C–O–P	Sigma	0.9/64.6
Guanosine 5'-monophosphate disodium salt hydrate	monoester	C–O–P	Acros	1.6/70.2
Glycerol phosphate disodium salt hydrate	monoester	C–O–P	Sigma	ND*/57.9
4-Nitrophenyl phosphate disodium salt hexahydrate	monoester	C–O–P	Alfa	ND*/-
O-Phosphorylethanolamine	monoester	C–O–P	TCI	ND*/-
Phytic acid dipotassium salt	monoester	C–O–P	Sigma	1.6/-
Phytic acid sodium salt hydrate	monoester	C–O–P	Sigma	2.7/-
Riboflavin 5′-monophosphate sodium salt hydrate	monoester	C–O–P	TCI	ND*/-
Phospho(enol)pyruvic acid monopotassium salt	monoester	C–O–P	Adamas	ND*/-
Pyridoxal 5′-phosphate hydrate	monoester	C–O–P	Sinopharm	ND*/-
Adenosine 5′-triphosphate disodium salt hydrate	tri-polyphosphate	P–O–P	Acros	ND*/-
Sodium pyrophosphate	pyrophosphate	P–O–P	Alfa	ND*/34.5
Sodium tripolyphosphate	tri-polyphosphate	P–O–P	Adamas	ND*/ND*

ND*: not detected.

environmental conditions in different regions. We will not focus on the biogeochemical cycle of P in this paper and further explanations of the dataset will be discussed in our future publications.

4. Conclusions

The determination of trace nutrients is extremely important but also very challenging for both marine scientists and analytical chemists. Based on the enrichment of PMB on HLB cartridge, we have developed several methods for the determination of nanomolar DRP in oligotrophic seawater [19,20,30]. Here, we have reported a modified method using an integrated analyzer for automatic 2-week underway analysis. Compared with other similar research [7,8], this method has several advantages, such as no interferences from salinity, silicate, arsenate and P-containing compounds, no need for "phosphate-free" seawater, no baseline shift from PMB coating, no necessity for organic solution to activate the cartridge, high accuracy and stability under harsh conditions, and most importantly, long-term continuous application with minimal maintenance.

The reliability and practicability of this prototype for underway analysis of DRP have been tested and confirmed in both land-based and shipboard laboratories. This analyzer can be further improved with some modifications, such as: 1) using integrated LED-photodiode detection to simplify the system; 2) designing dual-cartridge manifold to increase the analysis throughput; and 3) replacing multi-position valve with three-way solenoid valve to reduce the cost of the instrument. These and other improvements will be discussed in our future reports.

Acknowledgments

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