Sequential injection analysis of nanomolar soluble reactive phosphorus in seawater with HLB solid phase extraction

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Abstract

A cartridge of solid phase extraction (SPE), hydrophilic–lipophilic balance (HLB), has been used to enrich phosphomolybdenum blue (PMB) from water samples without any other additives. Based on this, the previous on-line SPE method established for the determination of nanomolar soluble reactive phosphorus (SRP) in seawater has been greatly improved. Cetyltrimethylammonium bromide (CTAB), the cationic surfactant needed for the formation of the PMB-CTAB paired compound that could be extracted on a Sep-Pak C18 cartridge using the previous method [Liang, Y., Yuan, D.X., Li, Q.L., Lin, Q.M., 2007. Flow injection analysis of nanomolar level orthophosphate in seawater with solid phase enrichment and colorimetric detection. Marine Chemistry 103, 122–130.], was not necessary. Thus the longer time and higher temperature required for the complete formation of the PMB-CTAB compound were no longer needed. In addition, with application of the sequential injection analysis technique the proposed method showed the advantages of being much faster, simpler, sample and reagent saving, as well as more convenient in operation. The PMB compound formed under room temperature was efficiently extracted on an in-line HLB cartridge, rapidly eluted by 0.15 mol/L NaOH solution, and finally determined with a laboratory-made spectrophotometer at 740 nm. Experimental parameters, including the volume of reagents added, sample loading flow rate, and eluting flow rate, were optimized. Time and temperature for the PMB reaction, and salinity effect were also studied, and these were found to have no severe effect on the detection. With variation of sample loading time at a fixed flow rate, a broadened determination range of 3.4 to 1134 nmol/L phosphate could be obtained. The recovery and the method detection limit of the proposed method were found to be 94.4% and 1.4 nmol/L, respectively. The relative standard deviation (n=7) was 2.50% for the sample at a concentration of 31 nmol/L phosphate. Two typical seawater samples were analyzed with both the proposed method and the magnesium hydroxide-induced coprecipitation method and, using the t-test, the results of the two methods showed no significant difference.

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Keywords: Hydrophilic–lipophilic balance cartridge On-line solid phase extraction Soluble reactive phosphorus Phosphomolybdenum blue

1. Introduction

Phosphorus is an essential nutrient for living organisms in both terrestrial and aquatic environments, and its major inorganic form, orthophosphate, plays a key role in photo-

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coprecipitation (MAGIC) method that has been widely accepted in recent years. Long path length absorbance spectrophotometry can be achieved through the application of a flexible fluoropolymer material (AF-2400, DuPont) with a refractive index (1.29) less than water and seawater (1.33 and 1.34, respectively), and shipboard or in situ analysis is practicable (Zhang and Chi, 2002; Adornato et al., 2007). Recently, the feasibility of combining MAGIC preconcentration and long path length absorbance spectrophotometry for determination of nanomolar/sub-nanomolar concentration of phosphate was demonstrated and the limit of detection was down to 0.3 nmol/L (Adornato et al., 2007). From the standpoint of being simple and shipboard method, the PMB method acts as the foundation for various novel methods. Concentrating the analyte (Karle and Tien, 1992; Heckemann, 2000) and extending the detection cell path length (Zhang and Chi, 2002; Adornato et al., 2007; Gimbert et al., 2007) are the two main efficient techniques. The typical enrichment method is the magnesium hydroxide-induced coprecipitation (MAGIC) method that has been widely accepted by oceanographers (Thomson-Buildris and Karl, 1998; Wu et al., 2000). The MAGIC method is an unavoidable labor-intensive and time-consuming, and its applicability for shipboard usage is limited, although many modifications have been made to simplify the procedure and minimize the analytical time (Rimmelin and Mountin, 2005). Increasing the detection cell path length is another alternative method developed in recent years. Long path length absorbance spectrophotometry can be achieved through the application of a flexible fluoropolymer material (AF-2400, DuPont) with a refractive index (1.29) less than water and seawater (1.33 and 1.34, respectively), and shipboard or in situ analysis is practicable (Zhang and Chi, 2002; Adornato et al., 2007). Recently, the feasibility of combining MAGIC preconcentration and long path length absorbance spectrophotometry for determination of nanomolar/sub-nanomolar concentration of phosphate was demonstrated and the limit of detection was down to 0.3 nmol/L, which could be particularly useful for phosphate sample studies in some ultraaligotrophic lakes or seawater where phosphate concentration were lower than 1 nmol/L (Li and Hansell, 2008).

In our previous work (Liang et al., 2007), flow injection analysis (FIA) with Sep-Pak C18 preconcentration and colorimetric detection were established to determine nanomolar concentrations of SRP in seawater samples. In order to retain PMB on the C18 cartridge, CTAB was used as an ion-pair reagent. The formation of PMB-CTAB paired ions was temperature dependent and time consuming, which made the determination system more complicated and the analysis time longer than expected. Moreover, due to the limitations of the FIA manifold, the reagents were wasted during the FIA operation because the peristaltic pump carrying the reagents had to run throughout the entire procedure. The goal of further research has been to seek other SPE materials that could effectively extract PMB without using the ion-pair reagent, and a more advanced method for manifold control that would be both flexible and suitable.

Among various sorbent materials, the Waters Oasis hydrophilic–lipophilic balance (HLB) sorbent is a macro porous copolymer made using a balanced ratio of two monomers, lipophilic divinylbenzene and hydrophilic N-vinypyrrolidone. The HLB provides reverse-phase capability with a special “polar-hook” for enhanced capture of polar analytes (Waters Corporation, 2004).

As an alternative flow process technique, sequential injection (SI) analysis has shown strong ability to manage multiple solutions. SI analysis is a computer compatible technique with multi-position selection valve and allows automatic handling of samples and reagent solutions with high precision, low contamination and less consumption of reagents (Economou, 2005).

In this study, a method for rapid determination of nanomolar SRP was established. An HLB cartridge was adopted to directly concentrate PMB from the water sample added using molybdate in acidic solution and subsequently ascorbic acid solution. The absence of CTAB made temperature control and the reaction time needed for ion-pair formation unnecessary. Additionally, an FI analyzer coupled with an 8-port selection valve and a laboratory-made spectrophotometer was adopted to ensure automatic reagent adding, solid phase extraction, eluting and determination, without any reagent waste. Various parameters affecting the determination of nanomolar SRP in seawater samples were investigated and optimized, and the interference of silicate and arsenate with SRP determination was also studied.

2. Experimental

2.1. Reagents and solutions

All the chemicals used in this study were of analytical grade, and supplied by Sinopharm Chemical Reagent Co., China, unless stated otherwise. Ammonium hexamolybdate tetrahydrate [(NH₄)₆Mo₇O₂₄·4H₂O] was re-crystallized from ethanol to remove possible remaining phosphate and other impurities. The other chemicals were used as purchased without further purification. All solutions were prepared in Milli-Q water obtained from a Millipore water purification system (Millipore Co., MA, USA).

A mixed reagent (MR) for color development was prepared by mixing 100 mL of 130 g/L (NH₄)₆Mo₇O₂₄·4H₂O solution, 100 mL of 3.5 g/L potassium antimony tartrate and 300 mL of 9 mol/L H₂SO₄ (G.R.). The solution was stored at 4 °C in a refrigerator while not in use. 100 g/L ascorbic acid solution (AA) was prepared daily.

The eluent was 0.15 mol/L NaOH solution prepared by dissolving the appropriate amount of NaOH (G.R.) in water. NaCl solution of 0.15 mol/L was prepared by dissolving the appropriate amount of NaCl (G.R.) in water. Silicate stock solution of 10 mmol/L was prepared from Na₂SiF₆ dried at 105 °C for 1 h. Phosphate stock solution (10.31 mmol/L) and arsenate stock solution (1000 mg/L) were purchased from the National Research Center for Certified Reference Materials (Beijing, China). Working standards were prepared as required by suitable dilution.

Low-nutrient seawater was collected from the surface of the South China Sea and phosphate was removed using the MAGIC method (Karle and Tien, 1992; Zhang and Chi, 2002). This phosphate-free seawater was used as the matrix for preparing standard curve solutions.

2.2. Apparatus

All flasks used in the experiments were cleaned as follows: soaked in 3 mol/L HCl for 2 h, cleaned with Milli-Q water in an ultrasonic bath for 0.5 h, and rinsed thoroughly with Milli-Q water.

The SI analysis system in the study included the following parts: an FIA 3110 flow injection analysis processor (Beijing Titan Instruments Co., China) including two 6-port peristaltic pumps and a 8-way rotary valve; a Vici Valco 8-position valve with electrical actuator (Valco Instruments Co., Inc., TN, USA); a laboratory-made spectrophotometer with a light emitting diode (740 nm) as light source; an Oasis HLB cartridge (60 mg/3 mL, Waters Associates, Milford, MA, USA) with in-line adaptor; and a 20 mm optical path SMA-Z-prxl Cell (FIAlab Instruments, Inc. WA, USA).
A 723 PC spectrophotometer (Shanghai Spectrum Instruments Co., Shanghai, China) with 1 cm cell was used for the study of extraction and elution efficiencies of PMB on the HLB cartridge.

2.3. Extraction and elution efficiencies for PMB on the HLB cartridge

A series of 25 mL samples spiked with phosphate concentrations of 0, 2.06, 3.12, 6.18, 8.24 and 10.30 μmol/L had 0.5 mL solutions of MR and AA added to allow the formation of PMB. Two min later, the absorbance of the sample was measured at 740 nm with the 723 PC spectrophotometer and recorded as \( A_{\text{before}} \). The PMB compound retained on the cartridge was eluted with 2.0 mL of 0.15 mol/L NaOH solution, and combined with another 0.04 mL MR solution. The absorbencies of the eluted solutions were recorded as \( A_{\text{after}} \) (corrected for volume differences).

It was found that without adding the additional MR solution, the eluted PMB in NaOH solution did not present the optimal color. In order to quantitatively investigate this phenomenon, two sets of samples with phosphate concentration at 10.30 μmol/L had 0.5 mL solutions of MR and AA added, the acidity was the same. The SI program is shown in Table 1. During steps 1 and 2, appropriate amounts of MR and AA were simultaneously added to a 150 mL sample solution; 5 pump tubing was of silicon-latex and other tubings of PTFE. After adding the reagents, the pump was stopped for 2 min and 10.30 min after the elution. For each pair of two samples with same volume of either MR solution or acid solution added, the acidity was the same.

2.4. Sequential injection manifold and procedures

Fig. 1 illustrates the sequential injection manifold used for this work. The pump tubing was of silicon-latex and other tubings of PTFE.

The SI program is shown in Table 1. During steps 1 and 2, appropriate amounts of MR and AA were simultaneously added to a 150 mL sample solution that had been exactly measured into a wide-mouth bottle in amounts of MR and AA were simultaneously added to a 150 mL sample solution; 5 pump tubing was of silicon-latex and other tubings of PTFE. After adding the reagents, the pump was stopped for 2 min and 10.30 min after the elution. For each pair of two samples with same volume of either MR solution or acid solution added, the acidity was the same.

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The SI program is shown in Table 1. During steps 1 and 2, appropriate amounts of MR and AA were simultaneously added to a 150 mL sample solution that had been exactly measured into a wide-mouth bottle in advance. The concentrations of \( \left( \text{NH}_4 \right)_6 \text{Mo}_7 \text{O}_{24} \cdot 4 \text{H}_2 \text{O}, \text{H}_2 \text{SO}_4 \) and ascorbic acid solution were 0.0773 mol/L, and 1.4 g/L, respectively. After adding the reagents, the pump was stopped for 2 min and 10.30 min after the elution. For each pair of two samples with same volume of either MR solution or acid solution added, the acidity was the same.

3. Results and discussion

3.1. Extraction and elution efficiencies for PMB on the HLB cartridge

The extraction efficiency of the HLB cartridge for the PMB compound was shown by the comparison of absorbencies of \( A_{\text{before}} \) (PMB solution before elution) and \( A_{\text{after}} \) (PMB solution after elution). The absorbencies of the PMB solutions (before and after passing through the HLB cartridge) were measured at 740 nm with the 723 PC spectrophotometer and delivered to the spectrophotometer for detection at 740 nm. The detector output was recorded using a computer for the quantification of phosphate in the samples. The blank and a 31 nmol/L phosphate solution were used as data quality control samples throughout the experiments in order to check the measurement deviation and provide sound data.

It should be noted that with the SI technique, no reagent or sample solution was wasted.

<table>
<thead>
<tr>
<th>Step Time (s)</th>
<th>8 position valve</th>
<th>Pump 1 flow rate (mL/min)</th>
<th>Pump 2 flow rate (mL/min)</th>
<th>8 way rotary valve</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>1</td>
<td>6.3</td>
<td>0</td>
<td>Fill</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2</td>
<td>6.3</td>
<td>0</td>
<td>Fill</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>3</td>
<td>6.3</td>
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<td>Fill</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>Fill</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>4</td>
<td>0</td>
<td>21.0</td>
<td>Inject</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>4</td>
<td>0</td>
<td>21.0</td>
<td>Fill</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>4</td>
<td>5.6</td>
<td>0</td>
<td>Inject</td>
</tr>
<tr>
<td>8</td>
<td>60</td>
<td>5</td>
<td>5.6</td>
<td>0</td>
<td>Inject</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>6</td>
<td>5.6</td>
<td>0</td>
<td>Inject</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>7</td>
<td>5.6</td>
<td>0</td>
<td>Inject</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>8</td>
<td>8.5</td>
<td>−8.5</td>
<td>Inject</td>
</tr>
</tbody>
</table>

* Time depended on the concentration of phosphate.

3.2. Sequential injection program and valve position description

Table 1

<table>
<thead>
<tr>
<th>Sequential injection program and valve position description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step</td>
</tr>
<tr>
<td>------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
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<tr>
<td>6</td>
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<td>7</td>
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<tr>
<td>8</td>
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<tr>
<td>9</td>
</tr>
<tr>
<td>10</td>
</tr>
<tr>
<td>11</td>
</tr>
</tbody>
</table>

* Time depended on the concentration of phosphate.

Table 2

The absorbances of the PMB solutions (before and after passing through the cartridge) and the eluents

<table>
<thead>
<tr>
<th>( C_p ) (μmol/L)</th>
<th>( A_{\text{before}} )</th>
<th>( A_{\text{after}} )</th>
<th>( A_{\text{eluted}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td>2.06</td>
<td>0.028</td>
<td>−0.001</td>
<td>0.025</td>
</tr>
<tr>
<td>4.12</td>
<td>0.059</td>
<td>0</td>
<td>0.052</td>
</tr>
<tr>
<td>6.18</td>
<td>0.086</td>
<td>0</td>
<td>0.080</td>
</tr>
<tr>
<td>8.24</td>
<td>0.119</td>
<td>0</td>
<td>0.115</td>
</tr>
<tr>
<td>10.30</td>
<td>0.151</td>
<td>0</td>
<td>0.143</td>
</tr>
</tbody>
</table>

Calibration curves

\( A_{\text{before}} = 0.0146 C_p \) (μmol/L)−0.0015

\( R^2 = 0.9911 \)

\( A_{\text{after}} = 0.0138 C_p \) (μmol/L)−0.0015

\( R^2 = 0.9955 \)
passing through the HLB cartridge) and $A_{\text{before}}$ (signal leaving the cartridge). The elution efficiency of the NaOH solution rinsing the retained PMB off the HLB cartridge was determined by the comparison of the absorbencies of the eluent $A_{\text{eluted}}$ and the original PMB solution $A_{\text{before}}$. In order to investigate these two efficiencies, the experiments described in Section 2.3 were carried out. As shown in Table 2, $A_{\text{before}}$ values were almost zero, showing that almost all the PMB was extracted and remained on the HLB cartridge. After elution with the NaOH solution, the eluent had additional MR solution added and was found to have $A_{\text{eluted}}$ values almost the same as their corresponding original $A_{\text{before}}$ values. This result revealed that the PMB on the HLB was eluted by NaOH solution at a high efficiency. The calibration curves of absorbance vs. phosphate concentration are also shown in Table 2. The ratio of the slopes of the two curves taken before and after the SPE procedure on the HLB could approximately represent the combined effect of the extraction and elution efficiencies. The ratio of 94.5% suggests the high extraction efficiency for PMB on the HLB and also high elution efficiency for PMB off the HLB.

It was considered that adjusting the eluent pH with acid solution would increase the $[\text{H}^+] / [\text{MoO}_4^{2-}]$ ratio and optimize the eluent color developing. However, it was found that additional MR solution was needed. Table 3 shows the absorbencies of the eluents with phosphate concentration at 10.30 μmol/L after adding additional MR or acid solution. The data clearly indicate that adding acid solution to the alkaline eluent obtained from NaOH elution could not return the eluent color back to normal. Only if 0.02 mL MR added into a 2 mL eluent, did the eluent color change back to the original. Obviously not only the acidity affected the color. The extra amount of molybdate in additional solution would shift the equilibrium towards the formation of PMB.

### 3.2. Schlieren effect minimization

The Schlieren effect occurs because of the parabolic geometry of the sample zone under laminar-flow conditions and the refractive index difference that exists among the sample, reagent and carrier solutions (McKelvie et al., 1997), and the Schlieren signal can cause large errors in quantification, especially when the analyte concentration is low. It was concluded that pre-rinsing the cartridge with solvents having similar refractive index to the eluent before eluting could be an effective way of minimizing the Schlieren effect. In the experiment, NaCl solution at the same concentration as the eluent NaOH was chosen as the pre-rinsing solvent, and the Schlieren effect could be to a great extent eliminated.

The Schlieren effect is so important in spectrophotometric detection with flow systems for trace analysis that sometimes the other parameters have to be changed to meet the requirements of minimization of this effect. As described in Section 2.3, the eluted PMB in the NaOH solution had lower absorbance before adding additional MR solution, which suggested that the method sensitivity could be lost without the post-cartridge MR addition. Although adding MR into the eluted PMB solution was possible in the manifold design, the serious Schlieren effect due to the post-cartridge addition of MR led to many failed attempts. By comparing the sensitivity loss caused by the lack of MR solution with the increase of the Schlieren effect, obviously the latter had priority. Therefore, no additional MR solution was added post-cartridge in the studies which followed.

### 3.3. Parameter optimization

In order to obtain the lowest detection limit, the effect of various parameters was investigated. These included the volume of reagents added, time and temperature for PMB formation, salinity effect, sample loading flow rate, and eluting flow rate. During optimization of these parameters a 31 nmol/L standard solution was used and the results were evaluated as the highest signal to noise ratio.

#### 3.3.1. Effect of volume of the color developing reagents (MR and AA)

The amount of color developing reagents (MR and AA) added to the sample solution affected the degree of PMB formation. The $[\text{H}^+] / [\text{MoO}_4^{2-}]$ ratio was found to be a very important factor affecting the formation of PMB, and the optimum $[\text{H}^+] / [\text{MoO}_4^{2-}]$ ratio was between 50 and 80 (Murphy and Riley, 1962). Based on this, the ratio was fixed at 74 in this work, and the volumes of MR and AA added to 150 mL sample solution were varied in order to study their effect on the signal. As shown in Fig. 2, when the addition of 2.1 mL AA was kept constant, the signal absorbance was enhanced when added MR in a 150 mL sample increased from 0.7 to 2.1 mL. However, the signal absorbance slightly decreased if the MR added was more than 2.1 mL. When the addition of MR was kept at 2.1 mL, the signal absorbance was almost constant with the addition of AA over the range 0.7 to 2.8. Therefore, 2.1 mL was selected as the optimized volume for both MR and AA reagents in 150 mL samples.

#### 3.3.2. Effect of time and temperature on the formation of PMB

In both FI and SI analysis, the stopped flow technique has been widely applied to improve the sensitivity when longer time is needed for chemical reactions. In our previous study (Liang et al., 2007), a stopped flow time of

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**Table 3**

The absorbencies of eluents with phosphate concentration at 10.30 μmol/L after adding additional MR or acid solution

<table>
<thead>
<tr>
<th>Added volume (mL)</th>
<th>0</th>
<th>0.01</th>
<th>0.02</th>
<th>0.03</th>
<th>0.04</th>
<th>0.05</th>
<th>0.07</th>
<th>0.1</th>
<th>0.15</th>
<th>0.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR</td>
<td>0.068</td>
<td>0.071</td>
<td>0.143</td>
<td>0.144</td>
<td>0.144</td>
<td>0.144</td>
<td>0.144</td>
<td>0.144</td>
<td>0.143</td>
<td>0.143</td>
</tr>
<tr>
<td>Acid</td>
<td>0.068</td>
<td>0.073</td>
<td>0.071</td>
<td>0.071</td>
<td>0.070</td>
<td>0.070</td>
<td>0.071</td>
<td>0.072</td>
<td>0.068</td>
<td>0.069</td>
</tr>
</tbody>
</table>

---

**Fig. 2.** Effect of MR (AA addition kept at 2.1 mL) and AA (MR addition kept at 2.1 mL) volume addition into 150 mL sample on signal. (a) blank (b) 31 nmol/L P.
20 min was needed to complete the formation reaction of PMB-CTAB. In the present work, the stopped flow time was studied in order to optimize the formation time for PMB when CTAB was absent. As shown in Fig. 3, the signal absorbance was almost constant when the stopped flow time was between 0 and 10 min. In other words, the stopped flow time could be greatly reduced in order to save the analysis time in this study. This shorter reaction time was due to the faster formation of PMB and the means of adding MR and AA into the sample; the reagents were added drop by drop, which was favorable for the complete reaction of PMB. The stopped flow time was set as 2 min, which was long enough to confirm completion of the reaction.

In the PMB method, the formation of PMB and the interference are both temperature dependent. Higher temperature increases the detection signal of PMB as well as the reagent blank and thus a suitable temperature is needed to obtain the highest signal to noise ratio. The wide-mouth sample bottle was kept in a water bath with the temperature in the range 5 to 45 °C. As shown in Fig. 4, the absorbance of both 31 and 62 nmol/L phosphate sample solutions increased rapidly when temperature increased from 5 to 10 °C, but was then almost constant from 15 to 45 °C. The reagent blank showed a similar trend, but with only a small increase between 5 and 10 °C. Therefore, the highest signal to noise ratio was found within the temperature range 15 to 45 °C, and so, for convenience, room temperature was chosen as the reaction temperature in the following experiment.

The results of the reaction time and temperature study corresponded with other researchers’ conclusions that at a ratio of [H+]/[Mo] of about 70, in the presence of Sb as a catalyst, the time required for full color development is only 62 nmol/L P.

### 3.3.4. Effect of eluent concentration

Heckemann (2000) found that PMB could be easily recovered from a non-polar polymer (styrene-divinylbenzene, DVB) by alkaline solutions without any need for added organic solvents. HLB had the same monomer, divinylbenzene, and accordingly, the NaOH solution was chosen to serve as the eluent in this study. The concentration of NaOH solution was further examined in the range between 0.01 and 0.4 mol/L. Fig. 6 confirmed that the PMB adsorbed on HLB could be completely eluted by NaOH solution at concentrations higher than 0.1 mol/L. In the subsequent work, 0.15 mol/L NaOH solution was chosen as the eluent.

Other eluents, such as sulphuric acid solution, ethanol, sulphuric acid-ethanol mixed solution, were also tested to compare the elution efficiency. However, only NaOH solution showed the ability to efficiently elute PMB from the HLB cartridge, which was in accordance with the “Retention Map” of HLB provided by the Waters Corporation (2004).
flow rate was chosen as 5.6 mL/min, where the elution was complete and the column pressure was not too high.

3.4. Interference of silicate and arsenate

Silicate and arsenate are two of the main species interfering with the determination of SRP using the PMB method, because of the formation of similar molybdate heteropolyacide compounds that contribute to the detection signal (Levine et al., 1955). The effect of silicate and arsenate in seawater samples on SRP determination using the proposed method was studied, with samples containing 31 nmol/L SRP and various amounts of silicate and arsenate.

As shown in Fig. 9(a), the absorbance showed no significant variation within silicate concentrations between 0 and 160 μmol/L, which are the common concentrations found in different sea waters. The result was in accordance with that obtained by Zhang et al. (1999), where silicate interference could be minimized under optimal reaction conditions including a pH in the final solution of 1.00 (1.11 in the proposed method), room temperature, a [H⁺]/[Mo] ratio of 70 (74 in the proposed method) and the addition of Sb.

With the proposed method, arsenate was more influential than silicate in the determination of SRP, and serious interference could be detected when the arsenate concentration was above 27 nmol/L, as shown in Fig. 9(b). However, since arsenate concentration is at a much low level in most natural waters, for example 20 nmol/L in open ocean (Karl and Tien, 1997), the influence of arsenate could be ignored in most cases. In areas with arsenic pollution or hydrothermal activity, addition of reducing reagents to transform arsenate to arsenite, which is non-reactive to molybdate reagents, was recommended. In this experiment L-cysteine was chosen as the reducing reagent because of its high efficiency at low acid concentration (Chen et al., 1992), which was suitable for the following PMB reaction without any further acidity adjustment. As shown in Table 4, 100 nmol/L arsenate showed no interference after L-cysteine (0.5% in final solution) was added to the sample for 30 min at room temperature and a pH of 1.70.

For a sample of 150 mL, the optimal parameters were selected as the addition of 2.1 mL MR and 2.1 mL AA, room temperature, a stopped flow time of 2 min, a sample loading flow rate of 21.0 mL/min, a sample loading time of 5 min and an eluting flow rate of 5.6 mL/min. The corresponding elution curves for the various phosphate concentrations, which ranged from 0 to 123.7 nmol/L, were obtained together with the signal for the Schlieren effect. The results shown in Fig. 10 indicate that the Schlieren effect had been to a great extent eliminated and would not interfere with detection.

Fig. 11 illustrates 3 calibration curves over a range of different sample loading times and which covered the concentration range between 3.4 and 1134 nmol/L. All the curves were measured 5 times on different days. The determination range could be broadened by choosing SI programs with different sample loading times, depending on the concentration of phosphate in the seawater samples.

The relative standard deviation of repetitive determination of a phosphate sample at a concentration of 31 nmol/L on different days was 2.50%, showing good and reproducible analytical results. The performance of the HLB cartridge remained steady even after the analysis of more than 75 real seawater samples. During the experiment, a 31 nmol/L phosphate solution was inserted as data quality control sample for every 15 samples in order to check the measurement deviation. Once the signal peak was 5% disformed or the peak height deviation was larger than 5%, it was the time for changing the SPE cartridge.

The method detection limit (MDL) was estimated using the method introduced by Berger et al. (1996). Nine aliquots of the phosphate-free seawater sample spiked with 3 nmol/L phosphate were analyzed following the proposed analytical procedures. The MDL was calculated using the following equation:

\[ \text{MDL} = \text{SD} \times t_{0.028} = 1.42 \text{ nmol/L} \]

where \( t_{0.028} \), the Student’s two-tailed \( t \)-statistic at the 98% confidence level with eight degrees of freedom, was 2.896 and SD was the standard deviation.

Table 4

<table>
<thead>
<tr>
<th>Concentration (nmol/L)</th>
<th>0</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before reduction</td>
<td>0.0201</td>
<td>0.1887</td>
<td>0.3309</td>
</tr>
<tr>
<td>After reduction</td>
<td>0.0695</td>
<td>0.0701</td>
<td>0.0724</td>
</tr>
</tbody>
</table>
of nine analyses, 0.49 nmol/L in this experiment. The MDL was low enough to allow the determination of SRP even in oligotrophic regions such as the South China Sea.

3.6. Validation of the method

3.6.1. Recovery

Recovery, the parameter used to evaluate the degree of interference from the matrix, could be represented as the ratio of the slope of standard curves prepared in spiked seawater to that in Milli-Q water. A series of low-nutrient seawater samples were spiked with phosphate at different concentrations and analyzed using the proposed method. The linear equation of calibration curves for matrix spiked samples was $A = 0.0743 + 0.00568C_{p}$ (spiked, nmol/L), and $R^2 = 0.9987$; the curve for Milli-Q water spiked samples was $A = 0.01107 + 0.00606C_{p}$ (spiked, nmol/L), and $R^2 = 0.9995$. The ratio of these two slopes was 93.7%, indicating high overall recovery of the proposed method and little matrix interference in the determination of SRP.

3.6.2. Comparison with the MAGIC method

Two typical seawater samples, obtained from the South China Sea, were analyzed using the proposed method and the MAGIC method (Karl and Tien, 1992). The results are shown in Table 5. There was no statistically significant difference between the proposed and MAGIC methods with the paired Student’s t-test at the 95% confidence level.

3.7. Application

The proposed method has been used to determine the SRP concentrations of seawater samples collected from the South China Sea. The seawater samples were frozen immediately after collection. Before analysis, the samples were completely thawed and mixed, and then 1–3 determinations were performed depending on the sample volume.

Presented in Fig. 12 is an example showing the typical vertical profile of temperature, salinity, nitrate+nitrite and SRP at the South East Asia Time Series (SEATS) station. The temperature and salinity measurement were obtained from SBE911 CTD (Seabird Co.), and concentration of nitrate+nitrite was determined with Tri-223 nutrient analyzer (detection limit equals to 0.2 µmol/L). The overall SRP concentration range was comparable to that reported using the MAGIC method (Wu et al., 2003; Chen et al., 2004). Fig. 12 clearly suggests that the fine structure of SRP was depleted in the mixed layer and the nutricline was located below 50 m.

4. Conclusions

Based on our previous studies, an improved on-line analytical method was established for the determination of nanomolar SRP in seawater samples. PMB was concentrated on an HLB cartridge without any additive reagents. There was no statistically significant difference between the results obtained from the proposed and MAGIC methods. The present method overcame the drawbacks of the previous method, being faster (6–10 h⁻¹ throughput depending on the concentration of samples), sample and reagent saving (maximum 150 mL per analysis and no reagent wasted), the reaction handled at room temperature and less Schlieren effect interference. Of course, quantifying the dissolved organic phosphorus (DOP) interference on SRP determination requires further study. The results have demonstrated the feasibility of the proposed method for determination of nanomolar concentrations of SRP in various natural waters.

### Table 5
Comparison of analytical results of the proposed method and MAGIC method

<table>
<thead>
<tr>
<th>Seawater sample</th>
<th>$C_{p}$±SD (n=4, nmol/L)</th>
<th>Calculated $t$-value</th>
<th>Critical $t$-value (P=0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposed method</td>
<td>34.9±0.5</td>
<td>0.970</td>
<td>2.447</td>
</tr>
<tr>
<td>MAGIC method</td>
<td>33.9±2.0</td>
<td>0.970</td>
<td>2.447</td>
</tr>
<tr>
<td>Sample 1</td>
<td>25.9±0.6</td>
<td>1.040</td>
<td>2.447</td>
</tr>
<tr>
<td>Sample 2</td>
<td>25.0±1.6</td>
<td>1.040</td>
<td>2.447</td>
</tr>
</tbody>
</table>
Furthermore, the method could be applied for shipboard or other in situ analysis in the field.

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