Intercomparison and coupling of magnesium-induced co-precipitation and long-path liquid-waveguide capillary cell techniques for trace analysis of phosphate in seawater

Qian P. Li*, Dennis A. Hansell

Marine and Atmospheric Chemistry, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Key Biscayne, FL 33149, United States

A R T I C L E   I N F O

Article history:
Received 30 October 2007
Received in revised form
21 January 2008
Accepted 24 January 2008
Published on line 7 February 2008

Keywords:
Nanomolar
Phosphate
Seawater

A B S T R A C T

Currently, two common techniques for nanomolar-level phosphate measurements in seawater are magnesium-induced co-precipitation (MAGIC) and long-path liquid-waveguide capillary cell (LWCC) spectrophotometry. These techniques have been applied in the open ocean, and our understanding of phosphate distributions in oligotrophic subtropical gyres is based on those data. However, intercomparison of these methods has not previously been performed at nanomolar levels. Here, we report experimental results directly comparing the MAGIC and LWCC techniques. We also evaluated the impact of various commonly employed filters on phosphate determinations, as well as interferences from dissolved organic phosphorus (DOP) and arsenate. Our results find agreement between these methods at phosphate concentrations <100 nM. We found that filter selection is important for accurate determinations of phosphate, and that DOP hydrolysis affects both techniques similarly. Finally, we demonstrate the advantage of combining MAGIC preconcentration and LWCC spectrophotometry for analysis of very low nanomolar concentrations.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

The role of phosphorus in marine ecosystems is well recognized, as primary production could be controlled by the availability of phosphorus in nutrient-limited oceans, such as the western Sargasso Sea [1] and the eastern Mediterranean Sea [2]. In these regions, concentrations of dissolved inorganic phosphorus (DIP) in the euphotic zone are depleted to below the detection limit of conventional colorimetric methods (~30–50 nM). This analytical disadvantage has led to substantial gaps in understanding the phosphorus cycle and its dynamics in oligotrophic systems [3].

One high-sensitivity technique to determine nanomolar-level phosphate in seawater uses magnesium-induced co-precipitation (MAGIC) to preconcentrate phosphate [4]. In the MAGIC procedure, phosphate is co-precipitated with Mg(OH)₂ at high pH and the resulting precipitate is dissolved in a minimum volume of a hydrochloric acid solution. By carefully setting the volume ratio of sample over final solution, a 5–100-fold increase in analytical sensitivity can be achieved [1,4–6]. The MAGIC procedure has also been successfully incorporated into a chemiluminescence method with a sorption preconcentration for highly sensitive phosphate measurements [7].

A less complicated technique for low phosphate levels employs long-path flow cells [8,9]. To reduce light attenuation, an amorphous polymer material with a refractive index less than that of water was developed [10]. This advance allowed construction of more compact liquid-waveguide cap-

* Corresponding author. Tel.: +1 786 261 4100; fax: +1 858 822 0562.
E-mail addresses: qian@coast.ucsd.edu (Q.P. Li), dhansell@rsmas.miami.edu (D.A. Hansell).
0003-2670/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aca.2008.01.074
illary cells (LWCC) [11]. Over one to two orders of magnitude enhancement in sensitivity for phosphate determination were reported using LWCC in a gas-segmented flow system [12] and in a flow injection system [13].

Both MAGIC and LWCC have been applied to field studies in the open ocean [1,2,14]. However, to our knowledge, comparisons of these methods at nanomolar levels have not been performed, thus leaving some uncertainty in determining the distribution of phosphorus in oligotrophic ecosystems. In this paper, we directly compare the MAGIC technique with the LWCC spectrophotometric method. Our goal is to determine if there is a difference between these two methods for low-level phosphate in seawater. Interference of hydrolyzed dissolved organic phosphorus (DOP) and arsenic on phosphate determinations are discussed. We also compare the DIP adsorptions by various types of nutrient filters as well as their retentions of particulate phosphorus (PP) in seawater. The impact of these filtrations on phosphate determination in seawater is addressed. Finally, we demonstrate the advantage of combining MAGIC and LWCC for better quantifying low nanomolar phosphate in seawater.

2. Methodology and experiments

2.1. LWCC method

Seawater samples were analyzed using a modified continuous flow system with a 2 m LWCC [12]. The optimum conditions for fast color development of phospho-antimony–molybdenum blue complex (0.8–1 min) suggested by Drummond and Maher [15] were slightly modified and applied for phosphate measurement. Reagents for the LWCC method: molybdate reagent was prepared by dissolution of 2.4 g ammonium molybdate ((NH4)6Mo7O24 ·4H2O) in H2SO4 (200 mL 5N) and diluted to 1.0L with deionized water (DIW); hydrazine reagent was prepared by dissolution of 1.0 g hydrazine sulfate (N2H4SO4) in 200 mL DIW. Phosphate concentration was measured at the wavelength of 800 nm. These modifications from a previous LWCC method [12] were intended to reduce the coating effect in the LWCC and to achieve a better net signal for phosphate-molybdate compounds [16]. Phosphate standards were prepared from reagent-grade oven-dried (105 °C for 2 h) KH2PO4. Stock standard solutions of 1.0 mM KH2PO4 were stored in polypropylene bottles at 4 °C. Working standards for phosphate were made by serial dilution of phosphate stock solutions with low phosphate seawater (LPSW). DIW was made in a Millipore Super-Q Plus Water System to a level of 18 MΩ resistance. LPSW was prepared by addition of 20 mL FeCl3 (1 M from analytical grade FeCl3) to 20 L low nutrient seawater at the ratio of 1–1000 (v/v), which removed the trace phosphorus in seawater without significant change of salinity. After settling of the brown particles formed, the supernatant was added to 50 mL seawater in a disposable polypolypropylene centrifuge tube and capped tightly. The tube was inverted several times and then set for 20 min to allow precipitates to fall. The samples were centrifuged at low-speed (1308 × g) by an International Size-2 Centrifuge (Model-R, International Equipment Co.) for 30 min. The supernatant was removed by pipette and the particle pellet dissolved in 2 mL of 0.2N HCl solution. After full dissolution of the particles, deionized water (DIW) was added to a final volume of 10 mL. Phosphate standards for MAGIC5 were prepared in LPSW at 0, 20, 40, 60, 80, and 100 nM. They were treated exactly the same as seawater samples and underwent all the steps described for MAGIC5. We measured the MAGIC5 preconcentration solutions by an automated analyzer [17] or by LWCC method. Solution with five times dilution of LPSW (to match the Mg2+ of the MAGIC5 solution) was used for wash and calibration curve during automated analysis.

2.3. Intercomparison of the two methods

We conducted three intercomparison experiments. The first compared the two techniques in a standard addition series of P concentrations; the second compared the methods on natural seawater without addendums; and the third evaluated the impact of DOP hydrolysis in both procedures.

2.3.1. Experiment 1

Direct comparison between MAGIC and LWCC in a standard addition experiment: A set of samples with 0–80 nM DIP were prepared by adding phosphate stock solution to LPSW. DIP concentrations were then assessed by both LWCC and MAGIC5 methods. Each individual sample analysis was replicated six times (include three times from LWCC and three times from MAGIC5), and the experiments were repeated three times. For each experiment the blank was redone once to check its stability.

2.3.2. Experiment 2

Direct comparison between MAGIC and LWCC in natural seawater sample: Aged seawater (1 year), collected from the surface layer of the western Florida continent shelf (WFCFS) near Dry Tortugas, was analyzed by both methods.

2.3.3. Experiment 3

DOP interference: A set of dissolved organic phosphorus (DOP) standards (100–500 nM) was prepared in LPSW and measured by both MAGIC5 and LWCC methods separately. Increases in DIP as a function of DOP concentrations were assumed due to hydrolysis of the DOP. Two compounds were tested in this study: β-glycerophosphate pentahydrate (β-GP; Alfa Aesar #L03425) and phytic acid (PHY; TCI #P0409). The final results for the two DOP compounds were corrected for their DIP blanks (for 100 nM of DOP, the blank is ~0.6 nM for PHY and ~0.2 nM for β-GP, respectively). β-GP was considered as a labile DOP and PHY was relatively refractory [18].

2.4. Filtration experiments

Six types of filters commonly used for nutrient sampling were tested for interference in phosphate measurements, including Polycarbonate Nuclepore (0.2 μm pore sizes), Whatman GF/F (0.7 μm), Millipore-Capsule (0.2 μm), Millipore-Membrane
DOP and arsenate were removed during precipitation with technique. Here, three experiments were carried out in order to compare the MAGIC technique to an equally sensitivity LWCC method. However, comparisons of MAGIC with other nanomolar concentrations yielded by a modified MAGIC procedure have been explained as reduced interferences from DOP or arsenate. This result excludes interferences by DOP and arsenate. This result suggests that there are no differences in phosphate concentrations measured by these two techniques with interference compounds removed.

2.5. Arsenic interference experiment

Potassium iodide (0.5 mL at 20% from analytical grade KI) and 1.0 mL concentrated HCl were added to 20 mL seawater and allowed a reaction time of 30 min in the dark for arsenic reduction. The arsenic reduction was confirmed by color formation after the addition of starch. I₂ was removed by pumping pure N₂ into the solution (~2 h).

3. Results and discussion

3.1. Intercomparison of MAGIC5 and LWCC methods

Phosphate concentrations measured by molybdate blue chemistry are often termed soluble or dissolved reactive phosphorus (DRP) because the acidic conditions of the method could cause DOP hydrolysis, thus raising DIP above the initial concentrations. The MAGIC procedure is believed to reduce the interference of acid-labile DOP because of the high pH employed in the preconcentration step [4], although sorption of DOP with metal hydroxide has also been evidenced [19]. It had been reported that the MAGIC method could result in higher or lower values when compared to the automated method in measuring seawater samples at Station ALOHA of the Hawaii Ocean Time-series Study [4,14]. Lower DRP concentrations yielded by a modified MAGIC procedure have been explained as reduced interferences from DOP or arsenate [5]. However, comparisons of MAGIC with other nanomolar phosphate methods have not yet been done to test these conjectures. Here, three experiments were carried out in order to compare the MAGIC technique to an equally sensitivity LWCC technique.

3.1.1. Experiment 1

Direct comparison between MAGIC and LWCC in standard addition experiment: As shown in Fig. 1, there is very good agreement between the methods, with \( r^2 = 0.995 \) \((n = 90)\). Because both DOP and arsenate were removed during precipitation with ferric hydroxide (in the production of LPSW), the comparison here excludes interferences by DOP and arsenate. This result suggests that there are no differences in phosphate concentrations measured by these two techniques with interference compounds removed.

3.1.2. Experiment 2

Direct comparison between MAGIC and LWCC in natural seawater sample: This experiment tested for differences in the methods using aged low-nutrient seawater collected from the surface layer of the western Florida continent shelf. The phosphate concentration in this water measured by MAGIC5 was 29.9 ± 1.4 nM \((n = 15)\), slightly lower than 30.4 ± 1.2 nM \((n = 15)\) yielded by LWCC. The difference of 0.6 ± 2.6 nM is small and not statistically significant \((p = 0.25, n = 15)\), suggesting that either the magnitude of interference by DOP is similar for the two high-sensitivity methods or DOP does not seriously interfere with the measurement (i.e., DOP was not significantly hydrolyzed by either method).

3.1.3. Experiment 3

DOP interference: Interference by DOP on DRP determination depends on the concentration of DOP. The mole percent contribution of β-GP to DRP concentration was ~1.4% in LWCC (with linear regression: \( \text{DRP} = 0.0137 \times \text{DOP}; \beta = 0.2164, r^2 = 0.973, n = 15, \text{S.D.} = ±14.5\% \) for DRP at 100 nM DOP; unit for \( \text{DRP} \) and \( \text{DOP} \) is in nM) and ~1.7% in MAGIC5 (with linear regression: \( \text{DRP} = 0.0165 \times \text{DOP}; \beta = 0.3304, r^2 = 0.977, n = 15, \text{S.D.} = ±28.2\% \) at 100 nM). For PHY, the percentage was ~3.2% in LWCC (with linear regression: \( \text{DRP} = 0.0316 \times \text{DOP}; \text{PHY} = 0.0458, r^2 = 0.978, n = 15, \text{S.D.} = ±8.3\% \) at 100 nM) and ~3.0% in MAGIC5 (with linear regression: \( \text{DRP} = 0.0302 \times \text{DOP}; \text{PHY} = 0.5872, r^2 = 0.996, n = 15, \text{S.D.} = ±12.2\% \) at 100 nM). Our results suggest that interference from two selected DOP compounds (β-GP and PHY)
was similarly low between the MAGIC5 and LWCC methods and thus there was not additional hydrolysis in the MAGIC5 procedure, over or above that which occurred in the LWCC DIP measurement. Of course, fully quantifying the DOP interference on DIP measurement requires further experiments to test a wide range of DOP compounds.

### 3.2 Impact of sample filtration

Particulate phosphorus (PP) in seawater is often removed from solution using filters of various pore sizes and composition. Dissolved phosphorus is principally sub-divided into organic (DOP) and inorganic fractions (DIP), and accurate determinations of these require prefiltration of the samples. Besides the removal of PP during filtration, DIP in seawater sample could be changed by filter-adsorption processes [20]. However, these retentions of phosphorus by filtration are not well understood due to the previous lack of high-sensitivity analytical techniques. Here we conducted experiments to assess the influence of filtration techniques on phosphate concentrations using the LWCC method.

DIP adsorption by these filters was first tested using a DIP standard solution (50 nM in DIW). The results of these experiments using the LWCC method. Here we tested the interference of arsenic on MAGIC–LWCC and LWCC methods using potassium iodide in acid condition as a reduction approach [24]. Our results suggest that there was not a sig-

### Table 1 – Impact of various filters on phosphate concentrations in DIW and seawater

<table>
<thead>
<tr>
<th>Filter type</th>
<th>Pore size (µm)</th>
<th>Material</th>
<th>Water</th>
<th>ΔSRP (nM)</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Millipore-Capsule</td>
<td>0.2</td>
<td>Versapor membrane</td>
<td>Seawater</td>
<td>−6.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Nuclepore-Membrane</td>
<td>0.2</td>
<td>Polycarbonate</td>
<td>DIW</td>
<td>−1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Nuclepore-Membrane</td>
<td>0.2</td>
<td>Polycarbonate</td>
<td>Seawater</td>
<td>−7.1</td>
<td>2.0</td>
</tr>
<tr>
<td>Millipore-Membrane</td>
<td>0.45</td>
<td>Cellulose ester</td>
<td>DIW</td>
<td>−0.8</td>
<td>1.2</td>
</tr>
<tr>
<td>Millipore-Membrane</td>
<td>0.45</td>
<td>Cellulose nitrate</td>
<td>Seawater</td>
<td>−4.5</td>
<td>1.4</td>
</tr>
<tr>
<td>MFS Membrane</td>
<td>0.45</td>
<td>Cellulose nitrate</td>
<td>DIW</td>
<td>−1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>MFS Membrane</td>
<td>0.45</td>
<td>Cellulose nitrate</td>
<td>Seawater</td>
<td>−3.1</td>
<td>1.4</td>
</tr>
<tr>
<td>Whatman Syringe Filter</td>
<td>0.45</td>
<td>Glass microfibre</td>
<td>DIW</td>
<td>−33.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Whatman GF/F</td>
<td>0.7</td>
<td>Glass fibre</td>
<td>DIW</td>
<td>−5.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Whatman GF/F</td>
<td>0.7</td>
<td>Glass fibre</td>
<td>Seawater</td>
<td>−8.2</td>
<td>2.2</td>
</tr>
</tbody>
</table>

ΔSRP is the net change of DRP concentration after filtration of a phosphate sample (~50 nM); S.D. is standard deviation; n = 7. See the text for details.

### Table 2 – Phosphate concentrations (nM) in seawater with and without arsenic reduction

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>KI (n = 6)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1 (MAGIC–LWCC)</td>
<td>58.4 ± 0.7</td>
<td>58.6 ± 0.2</td>
<td>100.3</td>
</tr>
<tr>
<td>Exp. 2 (LWCC)</td>
<td>68.9 ± 0.7</td>
<td>68.2 ± 1.3</td>
<td>98.9</td>
</tr>
</tbody>
</table>

Potassium iodide (KI) and hydrochloride (HCl) were added to seawater samples for arsenic reduction (Rupasinghe et al.) [24].
significant arsenic interference in these methods (Table 2). Lack of arsenic interference in long-cell method had been previously suggested [9], probably due to the slow formation of arsenomolybdate at room temperature compared to that of phospho-molybdate [17,21].

3.4. Combining MAGIC5 and LWCC

Here we investigated the feasibility of combining the MAGIC5 and LWCC techniques (MAGIC5–LWCC method) to add further sensitivity in analyses of DRP. Relationships of phosphate concentrations measured by MAGIC5–LWCC and the phosphate additions (in LPSW) are shown in Fig. 2. Each of these samples was concentrated fivefold in the MAGIC procedure, resulting in fivefold increases in DRP relative to DIP added. The coefficient of variation of MAGIC5–LWCC at 1 nM is ∼11% (Fig. 2). The detection limit of this method, estimated as three times the standard deviation of the blank, is ∼0.3 nM, which is lower than the individual MAGIC5 (∼3.5 nM) and LWCC method (∼1.5 nM) with the same set of standards. This combined MAGIC5–LWCC approach could be particularly useful for phosphorus cycle studies in some ultraoligotrophic lakes or seawater where DIP concentrations are <1 nM. On the other hand, this method could also be useful for waters with various salinities, where the LWCC methods may fail because of acute refractive index.

4. Conclusion

We have conducted experiments to compare the two most popular techniques for nanomolar phosphate determination in seawater: MAGIC preconcentration technique and the LWCC spectrophotometry. Our results suggest good agreement between these two methods at phosphate concentrations <100 nM. The feasibility of combining MAGIC5 preconcentration and LWCC for determination of nanomolar/sub-nanomolar concentration of phosphate is demonstrated, lowering the limit of detection to ∼0.3 nM. DOP hydrolysis was found to contribute to DIP, and an absence of interference by arsenate was verified. We found that filtration techniques can significantly influence the phosphate concentrations at nanomolar levels.

Acknowledgements

We would like to thank T.-H. Peng, J.-Z. Zhang, C. Fisher, L. Custals and L. Zamora for help during this work. We acknowledge support from the U.S. National Science Foundation (OCE-0241340) to DAH.

REFERENCES