Re-examination of the MAGIC method to determine low orthophosphate concentration in seawater

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Received 7 March 2005; received in revised form 24 May 2005; accepted 26 May 2005

Available online 5 July 2005

Abstract

A new and detailed MAGIC25 procedure is proposed to determine low concentration of orthophosphate in seawater. By preconcentrating phosphate 25 times, the procedure allowed detection of nanomolar concentration in seawater with low concentration of particles (detection limit = 0.8 ± 0.5 nM). The centrifugation step was considerably reduced (from 60 to 10 min) in comparison to previous MAGIC method. The calibration coefficient was determined by performing procedural and extra calibration curves and a previously unknown matrix effect in the MAGIC procedure was revealed. Its omission leads to a 12% underestimation of the phosphate concentration. The necessity of pre-filtration was demonstrated and a turbidity blank was measured to avoid overestimation even in the oligotrophic Mediterranean Sea water. The absence of filter reactivity with phosphate (contamination or retention) was verified. A synthetic reagent blank was proposed to simplify procedure in comparison to previous MAGIC method. The slow down of phosphomolybdenum blue complex was observed in the MAGIC concentrate and this results in a minimum reaction time of 30 min. The sensitivity (ratio of signal/noise) showed a three-fold improvement in comparison to the more recent nanomolar methods. Interferences of arsenate and silicate, commonly observed in the classic phosphomolybdic blue spectrophotometry, were undetectable. The MAGIC25 method appears reliable, sensitive, accurate and relatively easy to use during oceanographic cruises.

Keywords: Spectrophotometric analysis; Phosphate; Seawater; Mediterranean sea

1. Introduction

Phosphate availability might control oceanic carbon production in oligotrophic marine seawaters such as the Mediterranean Sea [1–4], the North- and SW Pacific Ocean [5,6] and the North Atlantic Ocean [7,8]. However, phosphate concentrations are typically at or below the detection limit of the blue phosphomolybdic acid assay commonly used during oceanographic cruises [9]. With a current detection limit of 30 nM and an accuracy of 30 nM, this spectrophotometric method suffers from a lack of sensitivity [9,10]. In addition, this method is poorly selective as shown, by interferences of arsenate and silicate and some acid-labile organic phosphate compounds like phosphate-ester and polyphosphates [11–14]. The method determines a fraction referred to soluble reactive phosphorus (SRP) presumed to represent mainly orthophosphate [9,15]. Thus, improving the analytical method to quantify orthophosphate appears warranted.

In principal, two types of methods have been developed for several years in order to decrease the phosphate detection limit below 1 nM in seawater. One type needs complex equipment like laser induced thermal lensing [16], or a gas chromatographic system [17], which are not easy to carry out during oceanographic cruises. Less complex instrumentation is long capillary cell spectrophotometry and more recent reverse phase liquid chromatography, but both these showed...
a detection limit near 1 nM, with a blank equivalent to 8 nM (coefficient of variation = 6–10%) in seawater, including arsenate interference [18,19].

The second type of method is based on a preconcentration step. Some procedures preconcentrate the phosphomolybdenum complex through a liquid–liquid extraction by using organic solvent or through a liquid–solid extraction by trapping the complex on acetate cellulose membranes. The preconcentrated phosphomolybdenum complex is detected by spectrophotometry or chemiluminescence [20–23]. However, these methods suffer from a lack of precision, essentially because of the high blank value. The “MaGnesium Induced Coprecipitation” (MAGIC) method preconcentrates phosphate before molybdic complexation, which minimizes the blank value [24]. It relies upon a 5- to 100-fold phosphate preconcentration step in a magnesium hydroxide precipitate followed by resolubilization in a minimum volume [7,8,25,26]. A reducing reagent (containing disulfite ions) is added to eliminate arsenate spectrophotometric interference, following Johnson [27]. Phosphate is then quantified by the phosphomolybdenium blue procedure, and the measurement is referred as MAGIC-SRP concentration [25]. It permits subsequent determination of nanomolar phosphate concentration.

However, the MAGIC concentration procedure induces some analytical “complications” which must be considered to allow nanomolar concentration determination, including calibration coefficient to be used to convert absorbance into concentration and a possible matrix effect in the MAGIC concentrate. They also concern the reaction rate of the phosphomolybdic reaction time, concentration and a possible matrix effect in the MAGIC procedure (MAGIC25) in order to measure nanomolar phosphate. After addition of 1 mL reagent 3, the concentrates were added to eliminate arsenate spectrophotometric interference, following Johnson [27]. Phosphate is then quantified by the phosphomolybdenum blue procedure, and the measurement is referred as MAGIC-SRP concentration [25]. It permits subsequent determination of nanomolar phosphate concentration.

2. Experimental

2.1. Instrumentation

All instruments used are commercially available. A SIGMA™ (4–15) centrifuge allowed centrifuging simultaneously four 300 mL samples at 1500 × g. A CECIL™ 1011 spectrophotometer (range of measurement from 0.001 to 2; S.D. = 0.001) equipped with a 8 mL-volume 10 cm-path length-cell was used to measure absorbance at room temperature (20–25 °C). A hand-made support was adapted to position precisely the cylindrical cell.

2.2. Preparation of reagent

All reagents were prepared with pro analysis Merck™ Reagent Grade chemicals and with Milli-Q™ high purity demineralised water (DW). All utensils were washed with 10% hydrochloric acid and rinsed three times with DW.

- **Reagent 1:** 1 M NaOH solution (ref. 1.06495.1000; PO₄ < 0.0001%). Storage of this solution is not recommended.
- **Reagent 2:** 0.25 M HCl solution (ref. 1.00317.1000). The reagent can be stored at room temperature.
- **Reagent 3:** Sodium-disulfite reducing reagent was prepared daily by dissolving 1.4 g Na₂O₅S₂ (ref. 1.00731.1000) in 10 mL DW followed by addition of 5 mL of a H₂SO₄ 1.75 M solution prepared by diluting 0.7 g Na₂S₄O₆ (ref. 1.06516.0500) in 50 mL DW [26]. The reagent can be stored at 4 °C for 24 h.
- **Reagent 4:** The ascorbic acid solution was prepared by dissolving 9 g (+) C₆H₈O₆ (ref. 1.00127.0250) in 170 mL DW. The reagent can be stored at 4 °C for several days.
- **Reagent 5:** The molybdic reagent was prepared by mixing 250 mL H₂SO₄ (2.5 M) followed by addition of 75 mL of (NH₄)₆Mo₇O₄·4H₂O, 40 g/L (ref. 1.01182.1000) and 23 mL of K(SbO)C₄H₄O₆·0.5H₂O. 0.5H₂O, 3 g/L and 52 mL of DW. The mixed reagent can be stored at 4 °C for several days.

2.3. MAGIC25 procedure

A minimum of 1 L sample was filtered through a 0.6 μm polycarbonate membrane using a Nalgene™ filtration apparatus. The sample was then divided into four aliquots and poured into 250 mL pre-gauzed polycarbonate centrifuged bottles. After the addition of 1.75 mL of reagent 1, two vigorous homogenizations were successively conducted for 5 min. The four bottles were then centrifuged for 10 min at 1500 × g with a smooth deceleration curve and the supernatant decanted. The precipitate was then solubilized in 6 mL reagent 2 under vigorous homogenization.

From the four concentrates, three were used to quantify phosphate. After addition of 1 mL reagent 3, the concentrates
were maintained at room temperature for 15 min to allow reduction of arsenate into arsenite. The final volume of the MAGIC concentrate was 10 mL, which corresponds to a 25 times preconcentration.

The S&P colorimetric procedure was performed by adding 0.2 mL of reagent 4 and 0.8 mL reagent 5 to the MAGIC concentrate. After a 30 min duration, the absorbance was measured at 880 nm, with a 10 cm-path length cell.

The fourth MAGIC concentrate was used to measure the turbidity blank. In order to fit the volume to 10 mL, 1.2 mL DW and 0.8 mL of reagent 5 were added. Reagents 3 and 4 were omitted to avoid the blue colour formation. The absorbance was also measured at 880 nm and subtracted from the mean phosphate absorbance.

A synthetic reagent blank was determined and subtracted. It was prepared by mixing 1.75 mL reagent 1, 1.7 mL reagent 2, 1 mL reagent 3, 0.25 mL DW (to adjust the volume), 0.2 mL reagent 4 and 0.8 mL reagent 5. The increase in reagent 2 volume in comparison to the sample treatment allows for the same pH and [H+] /[Mg] ratio values as in the MAGIC concentrate (~1.3 and 91, respectively). A [H+] /[Mg] ratio above 200 inhibits the phosphomolybdic reaction whereas below 60 a self-reduction of the molybdate ion occurs [12,30].

2.4. S&P phosphomolybdic blue procedure

The manual colorimetric method conducted on 40 mL sample was similar to the Strickland and Parsons procedure [9]. In three among the four replicates 0.8 mL of reagent 4 and 3.2 mL of reagent 5 were added. After 30 min, the absorbance was measured at 880 nm. Only 3.2 mL of reagent 5 was added to the fourth sub-sample in order to measure the turbidity blank. The reagent blank was prepared by adding 0.8 mL of reagent 4 and 3.2 mL of reagent 5 to 40 mL DW.

2.5. Calibration procedure of the MAGIC25 and the S&P methods

The MAGIC25 calibration was conducted for each new batch of reagent. One milliliter of 0.5 mM KH₂PO₄ (0.06805 g KH₂PO₄ in 1 L DW) was diluted to 100 mL to prepare the daily 5 mM working solution. Different volumes of the working solution were directly added in the centrifuge pre-gauged polycarbonate bottles to prepare nine standards (from 0.125 to 10 mL for 2.5 to 200 nM standards). The vol-

ume was adjusted to 250 mL by adding pre-filtered (0.6 μm) seawater. Seawater with a phosphate concentration close to zero was used in order to perform a calibration curve in the same range of concentration as for the field samples. The standards were then treated as previously described (see Section 2.4).

The S&P calibration was conducted with seven standards prepared by adding different volumes of the 5 μM phosphate solution in 40 mL borosilicated glass bottles (Schott®) (from 0.4 to 2.4 mL for 50 to 300 nM standards). A pre-filtered seawater was used to fit volume to 40 mL. The standards were then treated as previously described (see Section 2.4).

2.6. Optimization experiments

2.6.1. Matrix effect

Matrix effect was examined through a Student’s t-test comparison between averages of calibration coefficients obtained firstly with 17 MAGIC25 calibration curves (concentration range = 2.5–200 nM) and secondly with 16 S&P calibration curves (concentration range = 100–600 nM). A third type of curve, referred to “extra” MAGIC25 calibration curve, was undertaken in triplicates with seven standard solutions, ranging from 60 to 1500 nM phosphate concentration. They were prepared by direct additions of phosphate into 21 MAGIC concentrates obtained from one seawater sample.

2.6.2. Rate of reaction

The time-course of the blue complex formation in seawater solutions containing 500, 1000, 4000 nM phosphate using S&P and MAGIC25 procedures was followed, respectively. Low phosphate seawater was used for standard preparation. The chamber of measurement was open as frequently as possible to prevent warming and to maintain cell temperature ≤25 °C.

2.6.3. Blanks: particles and reagent contamination

Interference of particles was examined through several experiments conducted with nearshore and offshore seawater samples.

MAGIC25 SRP concentration (without turbidity correction) was determined on samples before and after 0.2 μm polycarbonate filtration.

Polycarbonate filters (0.2 and 0.6 μm) as well as GF/F filter were processed in order to decrease particles interference with a minimum duration of filtration. Such filters are largely used in macronutrient studies (GF/F filter shows a porosity of 0.7 μm which is generally enough in classical nutrient analysis [9]; 0.6 μm filters are used to remove the main fraction of pico plankton and bacteria). Two liters of two nearshore and one offshore marine water were sampled. Turbidity blanks (see Section 2.3) were determined in 250 mL, triplicate sub-samples filtered through 0.2 μm and through 0.6 μm filters.

The range value of the turbidity blank was determined by considering all data obtained through MAGIC25 analysis conducted during the present study with marine offshore and nearshore waters filtered on 0.6 μm polycarbonate membranes (n = 35).

Phosphate retention on 0.6 μm polycarbonate membrane was measured after filtration of 100 mL samples of 5, 20, 50 and 100 nM phosphate standards labelled with 122 kBq 32PO₄⁻ (Amersham BF1003). The retention was <0.04%.

Although GF/F filtration was very rapid, such filters were not used since the turbidity blank measured after filtration reached a 16 nM value (mean value = 10; S.D. = 3 nM; n = 25).
Absence of phosphate contamination after 0.6 μm polycarbonate membrane filtration was verified after filtration of a 5 mM phosphate standard, prepared with pre-filtered seawater. No significant difference of absorbance was observed before and after filtration (data not shown).

The reliability of the synthetic MAGIC25 blank was studied by comparing eight replicates of procedural blank, as proposed by Thompson-Bulldis and Karl [31]. Supernatant from a MAGIC25 precipitation was used to have a solution without phosphate. After sub sampling, a second MAGIC25 procedure was proceeded in order to measure a realistic reagent contamination.

Average and standard deviation (S.D.) of the reagent blank were calculated by considering all the data obtained. Specific contamination of the different reagents was studied. Reagent 2 contamination was measured by proceeding a 2.5 times diluted solution of HCl 0.25 M to obtain a [H⁺]/[Mo] ratio = 107. The contamination of reagent 3 was measured by comparing synthetic MAGIC25 blanks prepared with and without it.

2.6.4. Interfering ions
Arsenate and silicate are considered as the main interfering ions in the phosphomolybdic method because they form arseno- and silico-molybdic complexes that absorb at 880 nm. While arsenate is ubiquitous in marine waters with a concentration ranging between 10 and 40 nM [32,33], silicates concentrations are usually lower than 150 μM in seawater [34]. The silicate and arsenate interferences were measured by conducting the MAGIC25 procedure on a first sample treated with and without addition of 40 nM arsenate, and on a second sample treated with and without 160 μM silicate. Enrichment were undertaken by addition of 2 mL of a 5 mM arsenic acid solution (Na₂H₂AsO₄·7H₂O, SIGMA) and by addition of 16 mL of a 2.5 mM silicate solution (Na₂SiO₃·5H₂O, PROLABO, ref. 28.092.290) in 250 mL of pre-filtered seawater.

2.6.5. Storage
Three possibilities of sample storage were tested: poisoning with mercuric chloride (HgCl₂), freezing and precipitate refrigeration. Phosphate traces in HgCl₂ were determined by comparing MAGIC25-SRP of three samples poisoned or not at 5 mg-HgCl₂/L per sample (ref. 1.04417.0100). Freezing and refrigeration were tested by comparing MAGIC25-SRP determination after 0, 7 and 15 days of storage. Each analysis was undertaken in triplicate.

2.6.6. Comparison of methods
Two data sets were used to compare the MAGIC25 and the S&P method. The first one corresponded to 18 paired data obtained with standards concentration ranging from 2.5 to 200 nM of phosphate prepared in 1.3 L of filtered seawater. Three replicates of 250 and 40 mL were sub-sampled and treated by the MAGIC25 and the S&P method, respectively. The second set of data corresponded to a depth-profile (0–60 m) obtained in April 2004, at the Dyfamed station (43°25'N, 7°52'E, France—JGOFS). This station showed seasonally trophic pattern shifts from nitrogen in winter to phosphate limitation in summer [35]. Samples were analysed within the half a day after sampling. For both standards and field-samples, 1.3 L was filtered and stirred before sub sampled into different bottles used for chemical measurements.

2.6.7. Statistics
All statistics were performed according to Skoog et al. [36]. The reproducibility of the method was determined by standard deviation (S.D.) calculation and accuracy of the method was the 95% confidence interval of the mean concentration (IC₉₅%). Comparison of average concentration or calibration coefficient was performed through a Student’s t-test (IC₉₅%) and the detection limit of the method was determined as:

Minimum detectable absorbance = ε × S.D._reagent+blank 
× \sqrt{\frac{\text{Blank turbidity} + \text{Reagent blank}}{\text{Blank turbidity} \times \text{Reagent blank}}} 

S.D._reagent+blank = standard deviation of the blanks (turbidity + reagent) absorbance and blank turbidity blank and n_reagent are the number of replicates for turbidity and reagent blank measurement.

3. Results and discussion

3.1. Matrix effect
Calibration curves were established routinely by running procedural standards. The MAGIC25 and the S&P calibration slopes averaged 0.00440 (S.D. = 0.00032; n = 17) and 0.00020 (S.D. = 0.00001; n = 16), respectively (Fig. 1). Contrary to the five-fold concentration MAGIC method reported Fig. 1. Typical calibration curves of S&P and MAGIC25 method.
by Karl and Tien [5], the mean calibration slopes of the S&P method times the concentration factor was statistically different from the MAGIC25 method one (IC95%). This could be explained by the MAGIC matrix effect that produces a 12% decrease of the phosphomolybdic complex absorbance. Actually, there are important physical and chemical discrepancies between the seawater and the MAGIC25 concentrate, especially conductivity is higher in the MAGIC concentrate (conductivity at 20°C = 635 cm⁻¹ against 39 in seawater). An absorbance decrease >10% was reported by Aminot and Chaussepied [34] with increasing salinity from 0 to 35 g/L and Tue-Ngeun et al. observed a decrease of 20% of the slope after a chloride addition of 20 g/L [37]. The [H⁺]/[Mo] ratio of 91 of the MAGIC25 concentrate (after all reagent addition) maintain optimum conditions for a complete coloration [12]. The known acidity inhibition of phosphomolybic reaction cannot explain the signal underestimation. The extra calibration curve showed the same factor of 0.0044. The agreement between procedural- and extra calibration coefficients confirmed the matrix effect in the MAGIC concentrate. It also showed a 100% recovery of added phosphate by the MAGIC precipitate.

3.2. Aspects of reaction rate

In the S&P method, colour development was fast and slows down with decreasing phosphate concentration: absorbance peaks were achieved for 500, 1000 and 4000 nM concentrations after 3.75, 3.25 and 2.5 min respectively (Fig. 2a). This agrees with Pai et al. [12] who gave a <5 min time reaction for a 5000 nM concentration at room temperature with the same method. In the MAGIC25 concentrate, only 37, 87 and 98% of the peak values were achieved for 500, 1000 and 4000 nM phosphate concentration, after 4 min (Fig. 2b). In accordance with the S&P method in seawater, the colour development slows down with decreasing phosphate concentration. However all three peak values were achieved after 25 min. The slow down of the phosphomolybic complex formation in the MAGIC25 concentrate might be explained by the higher acidity [12,30]. A minimum reaction time of 30 min was recommended for the MAGIC25 method.

3.3. Blank determinations

In contrast to the S&P method or to the five-fold pre-concentration MAGIC method [5], the MAGIC25 method is subject to turbidity interference. MAGIC25 SRP showed an overestimation when samples were not pre-filtered. The average differences between not pre-filtered and pre-filtered samples were equivalent to 6 nM (S.D. = 3; n = 7), in offshore water and to 15 nM (S.D. = 10; n = 7) in nearshore waters (Fig. 3). One nanomolar is equivalent to 0.0044 absorbance unit. Minimum and maximum values were 1–8 and 2–31 nM, respectively. These values were higher than the 0.5–1 nM obtained by Wu et al. in the Sargasso Sea, after a 0.4 μm pre-filtration [7].

Turbidity blanks determined in three samples showed average values equivalent to 0.8 nM (S.D. = 0.2), 1.4 nM
Fig. 3. Suspended matter interference on MAGIC25-SRP measurement.

Fig. 4. Turbidity blank measured after 0.2- and 0.6 μm filtration of two nearshore and one offshore seawater samples.

(S.D. = 0.3) and 2.4 nM (S.D. = 0.3) (n = 3) after 0.2 μm filtration and equivalent to 0.7 nM (S.D. = 0.0), 1.5 nM (S.D. = 0.5) and 2.7 nM (S.D. = 0.8) after 0.6 μm filtration, respectively (Fig. 4). They were under 3 nM and showed no significant difference between 0.2 and 0.6 μm filtration. This allowed using 0.6 μm filtration in order to reduce considerably duration of filtration and to minimize risks of membrane filling-up and cell breakage. 0.2 μm filtration would be recommended in areas where the <0.6 μm size fraction is particularly abundant.

Turbidity blank after 0.6 μm filtration ranged from 0.2 to 5 nM (n = 35) with an average of 3 nM and a coefficient of variation of 30% (Table 1). The omission of turbidity blank would induce an overestimation rising up to 5 nM on the MAGIC25-SRP determination even in oligotrophic offshore water.

Thus, it was necessary to remove the particles by filtration and to take into account turbidity blank after pre-filtration, in order to achieve nanomolar precision with the MAGIC25 method. With the exception of Wu et al. [7] in the Sargasso Sea, no pre-filtration step was previously considered in the MAGIC treatment of offshore waters [5,8,24,26,31]. Turbidity blank was never considered in these previous studies.

Measurements of eight replicates of a synthetic MAGIC25 blank and of a procedural blank gave average concentration equivalent to 0.8 nM (S.D = 0.7) and 1.0 nM (S.D = 0.5), respectively. A t-test comparison of average values showed no significant difference (IC95%). This result demonstrates the reliability of the synthetic MAGIC25 blank that simplifies the method in comparison to the Thompson-Bulldis and Karl procedure [31]. By considering all the data obtained, the synthetic MAGIC25 blank averaged 0.5 nM (S.D. = 0.7) (n = 35) (Table 1). It was six times lower than the value of 3.3 nM reported by Karl and Tien [5].

Same reagent blank values for the S&P method were observed in DW and in 0.1 M HCl (0.1 nM; Table 1). This confirmed the negligible turbidity of reagents 4 and 5 and the undetectable phosphate contamination of HCl solution. Similarly, the addition of reducing reagent in the synthetic MAGIC blank did not enhance the signal (Table 1). Thus, the only source of phosphate contamination was sodium hydroxide.

The synthetic MAGIC25 blank seemed lower and less variable (mean = 0.5 nM; S.D. = 0.7) than the turbidity blank (mean = 3 nM; S.D = 1) (Table 1). In the nanomolar range of concentration, turbidity can be considered as the first controlling factor of the MAGIC25 precision. Optimal conditions are expected in oligotrophic open ocean areas where suspended matter level is low.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Average and standard deviation of blank values of the MAGIC25 and the S&amp;P method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance average</td>
<td>Equivalent concentration (nM)</td>
</tr>
<tr>
<td>Synthetic MAGIC25 blank (reagents 1, 2, 3, 4 and 5)</td>
<td>0.0021</td>
</tr>
<tr>
<td>Turbidity blank (reagents 1, 2 and 5)</td>
<td>0.0113</td>
</tr>
<tr>
<td>S&amp;P method reagent blank (reagents 4 and 5)</td>
<td>0.0002</td>
</tr>
<tr>
<td>S&amp;P method reagent blank in HCl 0.1 M (reagents 2, 4 and 5)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Procedural MAGIC25 blank (reagents 1, 2, 3 and 5)</td>
<td>0.0083</td>
</tr>
<tr>
<td>MAGIC25 reagent blank without reducing reagent (reagents 1, 2, 4 and 5)</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

a S.D. is the standard deviation.
b n is the number of measurements.
3.4. Performance of the MAGIC25 method

3.4.1. Efficiency

Efficiency of the MAGIC treatment varied depending on the ratio of 1 M NaOH addition to the sample. The MAGIC25 procedure used a volume ratio of 0.7% (v/v), which was close to the lower limit recommended by Karl and Tien (from 0.3 to 2.5% addition) [5]. A lower addition would create a thin layer of precipitate, which could be damaged during deceleration of centrifugation or during the supernatant elimination. The value of 0.7% (v/v) is over the threshold of 0.3% under which phosphate recovery does not match 100% with 60 min centrifugation [5]. No significant differences between SRP measurement after 10 and 60 min of centrifugation were shown (Fig. 5).

This underlined that a 0.7% (v/v) NaOH addition might minimize the interference of organic compounds as reported by Thompson-Bulldis and Karl [31]. Their work showed that the precipitation separated orthophosphate before acidification from several organic compounds. Even if other organic phosphate compounds would be trapped in the precipitate, their hydrolysis would be reduced by the low molarity of hydrochloride solution used [5].

A low NaOH addition gave a low quantity of precipitate, which allowed an increase of the concentration factor by decreasing the volume for dissolution. These advantages improve the accuracy and the sensitivity of the MAGIC25 method.

3.4.2. Linearity and sensitivity

Recorded absorbance versus concentration over the 2.5–200 nM phosphate range showed a highly significant linear relationship. The MAGIC25 curves exhibited an average slope (mean = 0.0044; S.D. = 0.0003; n = 17) 22-fold higher than the S&P one (mean = 0.00025; S.D. = 0.00003; n = 16). The difference between 22 and the expected value of 25 was attributed to the matrix effect. The coefficient of variation of the calibration coefficient amounted to 6.8% and involved a systematic error on the concentration calculation.

In optimal conditions (no suspended matter), 5 nM concentration gives a signal 10 times higher than the synthetic MAGIC25 blank (12 nM) and matched the S&P method precision (30 nM) for concentrations over 200 nM. Therefore, accuracy of the MAGIC25 method is optimal for low nanomolar concentrations.

3.4.3. Reproducibility, precision and detection limit

Reproducibility was carried out through eight replicates of a coastal sample with a high level of suspended matter. The SRP absorbance was 0.021 nM (S.D. = 0.007; n = 8), the turbidity blank absorbance was 0.016 nM (S.D. = 0.002; n = 8) and the synthetic MAGIC25 blank absorbance was 0.001 nM (S.D. = 0.001; n = 8). This corresponded to a phosphate concentration after turbidity and blank correction of 0.9 nM with a S.D. of 1.6 nM (Table 2). This demonstrated the reproducibility of the MAGIC25 method at low concentration, even in the presence of suspended matter.

Table 2

<table>
<thead>
<tr>
<th>Reproducibility (with high turbidity)</th>
<th>Average</th>
<th>S.D.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy 95% confidence interval</td>
<td>0.9</td>
<td>1.6</td>
<td>8</td>
</tr>
<tr>
<td>Accuracy 95% confidence interval</td>
<td>2</td>
<td>2</td>
<td>3</td>
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<td>Accuracy 95% confidence interval</td>
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<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Accuracy 95% confidence interval</td>
<td>2</td>
<td>0.5</td>
<td>3</td>
</tr>
<tr>
<td>Accuracy 95% confidence interval</td>
<td>200</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>Detection limit</td>
<td>1.8</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Detection limit without turbidity</td>
<td>0.8</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

* S.D. is the standard deviation.

* n is the number of measurements.

* Number of replicate considered for the calculation.

The precision is defined by reproducibility of the analytical process. An S.D. < 0.004 was observed between triplicates of SRP absorbance measured in the range 2–200 nM and a S.D. of 0.008 (equivalent to 1.7 nM) was measured for the reagent + turbidity blanks absorbance (Table 1). By considering an error of 6.8% on the calibration coefficient, we calculated also a relative S.D. of 102% for a concentration of 2 nM and a concentration of 200 nM (Table 2).

Because of the variability of the calibration coefficient, the error increased with concentration and matched the S&P method precision (30 nM) for concentrations over 200 nM. Therefore, accuracy of the MAGIC25 method is optimal for low nanomolar concentrations.
agreed with the accuracy of 0.2 nM obtained with the 5- and 100-fold concentration MAGIC procedures for a 2 nM concentration without turbidity consideration [5,7].

The detection limit was calculated with and without taking into account the S.D. of the turbidity blank measurement. It was 1.8 and 0.8 nM, respectively (Table 2).

3.4.4. Interfering ions
Arsenate and silicate are the principal interfering ions in the phosphomolybdic method [6,12,25]. The different modifiation of phosphomolybdic methodologies induced different interferences [38]. Thus, each modified recipes must be tested. In agreement with Johnson [27], no interference was observed after the addition of 40 nM arsenate and the reducing reagent. Regarding silicate, a 160 μM enrichment showed no interference in our studies. Actually, silicate interference varies with the H+/Mo molar ratio and with temperature [12,39]. The acid conditions used in the MAGIC25 concentrate ([H⁺]/[Mo] molar ratio, ranging from 91 to 95) guaranteed at the prevailing temperature (20–25 °C) inhibition of the silicomolybdic complex formation [12,28,38].

3.4.5. Storage
The current storage of sample for nutrient analysis is by mercuric chloride poisoning or freezing. Nevertheless, a 9 nM contamination was observed in three samples poisoned at a level of 5 mg mercuric chloride per liter. Such a blank value did not allow nanomolar detection.

Freezing after filtration (Table 3) showed no significant differences of SRP measurement after a 15 days storage (<2 nM) as already shown by Dore et al. [29] using the five-fold concentration MAGIC procedure.

Maintaining the precipitate at 4 °C is another possibility to store sample before analysis (Table 3). A sample of 7.5 nM phosphate (S.D. = 0.9 nM; n = 3) showed no significant difference in concentration after 7 and 15 days of precipitate refrigeration before analysis (Table 3). This feature of the present MAGIC25 method allowed the colorimetry to be performed several weeks after the centrifugation, with the same precision as already reported in the Karl and Tien procedure. Such a delay allows the analysis of sample in optimal laboratory condition when on board conditions are not convenient.

3.5. Validation: cross methodologies comparison
A linear and significant correlation (y = 0.95x – 0.3, r² = 0.99) was established in the 25–200 nM range value between the MAGIC25- and the S&P-SRP measurements obtained with orthophosphate standard solutions. Values under 25 nM were not included in the correlation because they were under the detection limit of the S&P method. The S&P SRP concentrations were close to 0 for standard solution up to 10 nM. The slope of 0.95 and the residual value of 0.3 nM were not significantly different from 1 and 0, respectively. The mean SRP determination by the S&P and the MAGIC25 methods were similar in the range 25–200 nM orthophosphate. Comparison of the S&P and MAGIC25 methods on field samples showed similar SRP profiles (Fig. 6). Average concentration of SRP in the first 60 m of the water column ranged from 3 to 157 nM and from 8 to 161 nM for the S&P and the MAGIC25 methods, respectively. However the average MAGIC25 SRP concentrations were seven times more precise than the S&P, since average S.D. amounted for an equivalent of 1 and 7 nM, respectively. In the first 30 m of the water column, average SRP concentrations were below 15 nM and the weak SRP gradient with depth (from 7 to 13 nM) was only assessed by the MAGIC25 method. Turbidity blank was also determined for each depth. It was around 4 nM in the first 30 m and reached 50% of the corrected MAGIC25 SRP signal. The differences between averages of MAGIC25-SRP and S&P-SRP concentrations reached 12 nM, and was much lower than the 60 nM values observed between the MAGIC25- and the S&P-SRP measurements.

### Table 3

<table>
<thead>
<tr>
<th>Storage period (days)</th>
<th>Freezing</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Refrigeration</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean concentration (nM)</td>
<td>S.D.</td>
<td>n</td>
<td></td>
<td></td>
<td>Mean concentration (nM)</td>
<td>S.D.</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>14.2</td>
<td>1.1</td>
<td>3</td>
<td></td>
<td></td>
<td>7.5</td>
<td>0.9</td>
<td>3</td>
<td></td>
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<tr>
<td>7</td>
<td>12.9</td>
<td>0.9</td>
<td>3</td>
<td></td>
<td></td>
<td>7.0</td>
<td>0.9</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>13.6</td>
<td>1.0</td>
<td>3</td>
<td></td>
<td></td>
<td>7.0</td>
<td>1.1</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

* S.D. is the standard deviation.  
  b n is the number of measurements.
by Karl and Tien [25] by comparing MAGIC5-SRP with the automated S&P-SRP. Karl and Tien [25] reported over- and under-estimations while we only observed an underestimation with the MAGIC25 method. The difference of SRP value can be explained by the interference by arsenic, and hydrolysable inorganic and organic phosphate. The latter would particularly vary with phosphorus composition across season and habitats [25,30,31,40].

This reveals that SRP-MAGIC25 concentration is more selective for orthophosphate concentration determination than the S&P method or the 5- to 20-fold concentration MAGIC procedure of Karl and Tien [5,25].

4. Conclusion

The MAGIC method to preconcentrate seawater orthophosphate before analysis was revisited. The new protocol giving a 25 times preconcentration allows to improve spectrophotometric signal 5 and 22 times in comparison to the MAGIC5 and S&P methods, respectively. Duration of centrifugation was considerably decreased from 60 to 10 min. A previously unknown matrix effect was demonstrated, which needs to be taken into account to convert absorbance into concentration. A pre-filtration step, as well as a turbidity blank measurement, seemed necessary to achieve nanomolar precision, at least in Mediterranean seawater. The new synthetic blank simplifies also procedure to achieve nanomolar precision, at least in Mediterranean as well as a turbidity blank measurement, seemed necessary.

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The MAGIC25 procedure showed a calculated detection limit of 0.8 nM with a precision of 0.5 nM level for seawater in comparison to the procedural blank of previous MAGIC method.

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