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A precise method for the analysis of $\delta^{18}O$ of dissolved inorganic phosphate in seawater

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Abstract

A method for preparation and analysis of the oxygen isotope composition ($\delta^{18}O$) of dissolved inorganic phosphate (DIP) has been developed and preliminary results for water samples from various locations are reported. Phosphate is extracted from seawater samples by coprecipitation with magnesium hydroxide. Phosphate is further purified through a series of precipitations and resin separation and is ultimately converted to silver phosphate. Silver phosphate samples are pyrolitically decomposed to carbon monoxide and analyzed for $\delta^{18}O$. Silver phosphate samples weighing 0.7 mg (3.5 μmol oxygen) can be analyzed routinely with an average standard deviation of about 0.3‰. There is no isotope fractionation during extraction and blanks are negligible within analytical error. Reproducibility was determined for both laboratory standards and natural samples by multiple analyses. A comparison between filtered and unfiltered natural seawater samples was also conducted and no appreciable difference was observed for the samples tested. The $\delta^{18}O$ values of DIP in seawater determined using this method range from 18.6‰ to 22.3‰, suggesting small but detectable natural variability in seawater. For the San Francisco Bay estuary DIP $\delta^{18}O$ is more variable, ranging from 11.4‰ near the San Joaquin River to 20.1‰ near the Golden Gate Bridge, and was well correlated with salinity, phosphate concentration, and $\delta^{18}O$ of water.

Phosphorus (P) is a limiting macro-nutrient in many aquatic ecosystems and therefore has a major influence on primary production (Delaney and Filippelli 1994; Karl and Tien 1997; Benitez-Nelson 2000; Wu et al. 2000). Over geologic time scales, P may be the ultimate limiting nutrient and may affect the carbon cycle through its role in regulating productivity (Filippelli 1997; Tyrrell and Law 1997; Delaney 1998; Tyrrell 1999). Dissolved phosphate depth profiles in the oceans show low concentrations in surface waters due to intense biological uptake in the euphotic zone and increasing concentrations at depth as organic phosphorus is remineralized (Benitez-Nelson 2000). P is primarily lost from surface waters in the form of sinking particulate matter and almost all P is remineralized in the water column (Broecker and Peng 1982). Studies of phosphorus turnover in the coastal ocean indicate that P recycling rates vary seasonally and are very rapid (less than a day to 2 weeks), indicating that low phosphorus concentrations can support relatively high primary production (Lal et al. 1988; Lal and Lee 1988; Lee et al. 1991; Benitez-Nelson and Buesseler 1999). There is also evidence that dissolved organic P is preferentially remineralized from dissolved organic matter in the water column (Clark et al. 1998). Similarly, in sinking particulate matter, certain dissolved organic phosphorus compounds are preferentially remineralized and hydrolysis of organic P occurs throughout the water column, though more prevalently in shallow depths (Paytan et al. 2003).

Because P has only one stable isotope, it cannot be used as a stable isotopic tracer. However, most of the P found in nature is strongly bound to oxygen, which has three stable isotopes; hence, phosphate can be analyzed for $\delta^{18}O$. Because the P-O bond in phosphate is resistant to inorganic hydrolysis and does not exchange oxygen with water without biological mediation (Longinelli et al. 1976; Blake 1997; Blake et al. 1997), systematic isotopic variability of the oxygen isotopic composition of phosphate may provide information about temperature and the $\delta^{18}O$ of water, and can potentially determine P sources and the extent of P cycling in the ocean.

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Oxygen isotopic analysis of phosphatic mineral samples (apatite) and of dissolved phosphate in water samples has long been realized as an important tool for paleoclimate reconstruction (Longinelli and Nuti 1973; Lécuyer et al. 1993; Filippelli and Delaney 1994; Follmi 1995), ecology (Blake et al. 1997; Blake et al. 1998), and biogeochemical cycling research (Longinelli and Nuti 1968; Longinelli et al. 1976; Paytan et al. 2002). These analyses however have not been widely applied to dissolved inorganic phosphate (DIP) because traditional phosphate preparation techniques using $BiPO_4$ fluorination for isotope analyses require large samples, are labor intensive, time consuming, and involve handling dangerous substances such as BrF_5 (Tudge 1960; Longinelli 1966; Longinelli et al. 1976; Kolodny et al. 1983; Vennemann et al. 2002). To overcome some of these issues, Firsching (1961) introduced a method to precipitate the phosphate as silver phosphate, a pure, stable, nonhygroscopic compound (Baxter and Jones 1910). Subsequently, O'Neil et al. (1994) developed a method that reacted silver phosphate with graphite in sealed silica tubes at high temperatures to produce CO_2 for isotope analysis as an alternative to the fluorination procedure (Vennemann et al. 2002). Although these methods made analyses more efficient, these modifications still require a relatively large sample size (10 to 20 mg silver phosphate), which is not always practical or available, in particular for soluble reactive phosphate (SRP) in open ocean waters where phosphate concentrations could drop to less than 0.2 μM (GEOSECS). In addition, Révész and Böhlke (2002) have found that combustion of nitrates in sealed silica tubes results in isotopic exchange between the sample and the glass combustion tube. Because phosphate, like nitrate, is an oxygen-bearing compound, it is possible that a similar fractionation may occur during decomposition of phosphates for isotopic analysis in silica tubes. Kornexl et al. (1999) have developed a method for online oxygen isotope ratio determination by pyrolysis of silver phosphate (and other compounds) at 1400°C in an elemental analyzer, and analysis of the CO produced by a continuous flow gas chromatograph coupled with an isotope ratio mass spectrometer (EA-IRMS), which greatly increased the ease and throughput of isotopic analyses of phosphates.

Karl and Tien (1992) developed a method to precisely determine nanomolar concentrations of SRP and total dissolved phosphorus from marine and freshwater environments, which was subsequently improved by Thomson-Bulldis and Karl (1998). This approach, magnesium-induced coprecipitation (MagIC), removes P from solution via coprecipitation with $Mg(OH)_2$ and results in the quantitative concentration of phosphate from large volumes of water into more manageable volumes for analyses. Many methods have been developed for the purification of phosphate from apatite for isotopic analysis (Firsching 1961; Crowson et al. 1991; O'Neil et al. 1994), each of which involves dissolution of the solid sample into homogenous solution for precipitation of silver phosphate. However, they are not easily applied to DIP purification and conversion to silver phosphate from a salt-water matrix due to the abundance of chloride ions, which interfere with the silver phosphate precipitation. Here we propose a new method for purifying phosphate by precipitation as cerium phosphate (cerium does not form an insoluble salt with chloride and thus provides a means to separate phosphate from seawater). This unpublished technique was previously used by HSW for processing biogenic phosphates. We chose cerium phosphate as an intermediate step because of this experience, although other light lanthanides may work as well (Byrne et al. 1996; Liu and Byrne 1997). Using selective parts of each of these methods, we have been able to quantitatively remove phosphate from large volumes of seawater and purify it as cerium phosphate before final precipitation as silver phosphate for isotopic analysis.

This progress in chemical extractions and analytical techniques opens new avenues for application of $\delta^{18}O$ measurements to natural samples with low phosphate concentration. Here we describe a method for the extraction and analysis of $\delta^{18}O$ in SRP in natural water samples. Our method only requires 3.5 μmol of oxygen, corresponding to 0.7 mg of silver phosphate. This is the typical yield from approximately 8 L of coastal water and 30 L of oligotrophic water, thus making routine analysis of DIP in seawater much more manageable.

Materials and procedures

Laboratory supplies—We used 8-L High Density Poly-Ethylene (HDPE) Nalgene bottles, 250-mL HDPE Nalgene bottles, 50-mL HDPE depressed cap Fisher Scientific centrifuge tubes, Kimball 15 mL glass columns, Millipore 13-mm glass frit, Millipore 15-mL glass reservoir, and Millipore 125-mL vacuum filtration flask. All laboratory supplies were acid-washed in 10% nitric acid and rinsed in deionized water prior to usage.

Chemical supplies—Chemical supplies included 1 M American Chemical Society (ACS)-grade sodium hydroxide (Fisher Scientific), concentrated ACS-grade acetic acid (Fisher Scientific), 10 M ACS-grade nitric acid (Fisher Scientific), cerium nitrate hexahydrate (Sigma-Aldrich), 0.5 M ACS-grade potassium acetate (Fisher Scientific), 1 M ACS-grade nitric acid (Fisher Scientific), BIORAD AG 50 × 8 cation exchange resin, 7 M ACS-grade nitric acid (Fisher Scientific), 2 M ACS-grade ammonium nitrate (Fisher Scientific), concentrated ammonium hydroxide (Fisher Scientific), silver nitrate (Fisher Scientific), Milli-Q deionized water, Universal pH Indicator Strips, bromothymol blue indicator (Sigma-Aldrich), and Millipore Polycarbonate filters (filter code: HTTP).

Equipment—We used a centrifuge capable of 3000 to 4000 rpm (Beckman-Coulter), a laboratory oven set at 50°C, shaker table (New Brunswick Scientific C1 Platform Shaker), a Costech Zero Blank Autosampler (serial number 41020032), a Eurovector Elemental Analyzer, and a Micromass IsoPrime mass spectrometer.

Concentration of DIP from seawater—DIP was stripped from seawater via MagIC (Karl and Tien 1992; Thomson-Bulldis and Karl 1998). We used 150 mL of 1 M sodium hydroxide solu-

tion for 8 L of seawater with phosphate concentration greater than 0.8 μM (larger volumes of seawater are required for lower concentrations). The $Mg(OH)_2$ (brucite) floc was allowed to settle and the overlying seawater siphoned off (leaving typically 2 L in the bottle). This seawater/$Mg(OH)_2$ mixture was gradually transferred to a 250-mL bottle where each sample was centrifuged at 3500 rpm for 10 min, and the supernatant discarded. The end result was approximately 100 mL of wet $Mg(OH)_2$ in the 250-mL bottle.

The magnesium hydroxide precipitate was then dissolved in 5 mL of concentrated acetic acid and the minimum volume of 10 M nitric acid required (~5 mL) to dissolve the pellet. The solution was then buffered at pH 5.5 (as indicated with universal pH indicator paper) with 10 mL of 1 M potassium acetate. Potassium acetate was selected as a buffer because it is inexpensive, nontoxic, and has a low P blank. Four hundred milligrams of cerium nitrate dissolved in 3 mL of water (prepared fresh) was added to the solution to precipitate cerium phosphate, which was white to cream in color. Samples stood at room temperature for 1 to 3 h for precipitation to go to completion. It is possible that fractionation of the oxygen isotopes may occur if this reaction is incomplete; therefore the amount of cerium nitrate added should be sufficient to ensure that cerium ions are present in large excess. Additionally, the acetate buffering at a slightly acidic pH is necessary for three reasons. First, it will prevent hydrolysis of both cerium and of other interfering ions such as iron and magnesium. Second, it will maintain the flocculation of cerium phosphate, whose crystals are so small that they barely have an XRD signature and cannot be centrifuged or easily filtered. Finally, cerium phosphate is negligibly soluble in weak acidic solutions and therefore buffering at slightly acidic pH will prevent dissolution of the precipitate during rinsing.

The cerium phosphate with associated solution was then gradually transferred into a 50-mL centrifuge tube, and the solids were separated by centrifugation at 3500 rpm for 15 min. The 250-mL bottle was rinsed 3 times with potassium acetate solution to ensure complete transfer of cerium phosphate into the centrifuge tubes. The cerium phosphate was then repeatedly rinsed with 20 mL of 0.5 M potassium acetate and centrifuged until all the Cl^- ions are removed from the supernatant. This was indicated by adding silver nitrate to the supernatant and testing for silver chloride precipitation. Complete removal of chloride ions was usually achieved in 3 rinses. The cerium phosphate was shaken to resuspend the precipitate into the rinse solution between each centrifugation. Note that cerium phosphate should not be rinsed in deionized water because, without the potassium acetate, the cerium phosphate will deflocculate and become impossible to centrifuge. The retained precipitate was subsequently dissolved in the minimum amount of 1 M nitric acid (approximately 2 to 4 mL) and the amount of water required to reduce the molarity of the acid to less than 0.2.

The solution was then mixed with 4 mL of cation exchange resin (BioRad AG-50×8) and shaken on a shaker table overnight to remove cerium ions from solution. Alternatively, manually shaking the sample every half hour for the first 3 h and then leaving the sample overnight may be sufficient. Samples were eluted from the resin using Kimball glass columns, and the solution was collected in a second set of 50-mL centrifuge tubes. Two drops of bromothymol blue indicator was added to the solution. The solution should be acidic, and the color of the indicator solution should be yellow. The pH of the solution was raised with 1 mL ammonium hydroxide and 1 mL 3 M ammonium nitrate was added to the sample (solution should be blue). The pH was brought to 7 with 3 M nitric acid (the color of the indicator solution should be green).

Silver phosphate was then precipitated rapidly by adding 0.5 g silver nitrate in 2 mL deionized water (prepared fresh). Addition of silver nitrate will reduce the pH and the color of the indicator solution will return to yellow. Bright yellow precipitate forms immediately, and may turn black over time, possibly due to hydrogen reduction of the silver (Baxter and Jones 1910). Dettman et al. (2001) found that the fast precipitation of silver phosphate gives the same isotopic composition as the slow precipitation method used in O'Neil et al. (1994). At this stage, pH of the sample solution can be checked with universal indicator pH strips to ensure that the pH was approximately 7 (±0.5). pH values much greater or less than 7 will result in incomplete precipitation of silver phosphate. The silver phosphate was then vacuum filtered onto polycarbonate filters and rinsed several times with deionized water. The filter was left in the oven overnight at 50°C to remove excess water (Fig. 1).

Sample analysis by mass spectrometry—Samples of silver phosphate are pyrolyzed (thermally decomposed without added oxygen) and analyzed by continuous-flow isotope ratio mass spectrometry (CF-IRMS). Silver phosphate samples between 0.6 and 0.9 mg are weighed out into silver capsules along with approximately 1 mg of 50% nickelized carbon (Elemental Microanalysis LTD, part nr B1182) and placed into a zero-blank autosampler (Costech Analytical Technologies). To release any contaminant oxygen the nickelized carbon was crushed to a powder in an agate mortar and baked overnight in a stream of He at 1270°C before its use. The apparatus used for $\delta^{18}O$ analyses was a Eurovector Elemental Analyzer coupled to a Micromass (now GVI) IsoPrime mass spectrometer (Fig. 2). A continuous flow of He at 80 mL min^{-1} passes through the entire system and carries the sample gas to the mass spectrometer. The pyrolysis unit was similar to the one described by Kornexl et al. (1999), in which the reaction vessel was a glassy carbon tube within a ceramic tube. However, the unit used in these analyses also includes a tube containing pure copper granules at 650°C, which removes trace oxygen from the helium carrier gas. Samples were dropped from an autosampler into the pyrolysis tube that was held at 1270°C. He was used to flush the resulting CO from the pyrolysis tube through a packed molecular sieve GC column (1-m long, 6-mm outer diameter, and 4-mm

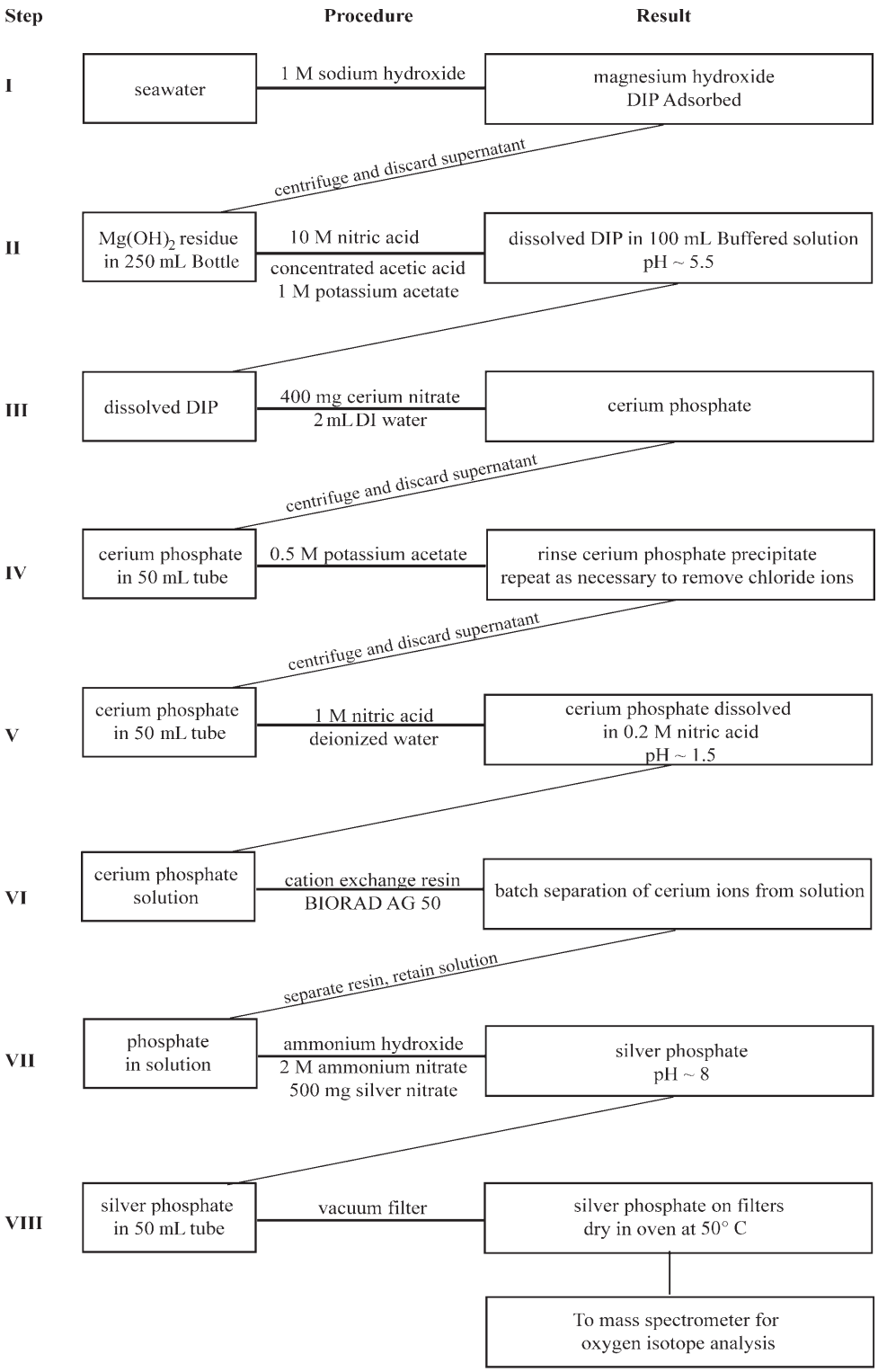

Fig. 1. Procedure for extraction of dissolved inorganic phosphate from seawater

inner diameter) at 650°C. This removes trace contaminants before the sample enters the mass spectrometer. A "raw" (uncorrected) $\delta^{18}O$ value was calculated from the 30:28 mass ratio of the sample gas with respect to the 30:28 ratio of a pulse of CO from a tank analyzed just prior to each sample. The delta value was calculated as follows:

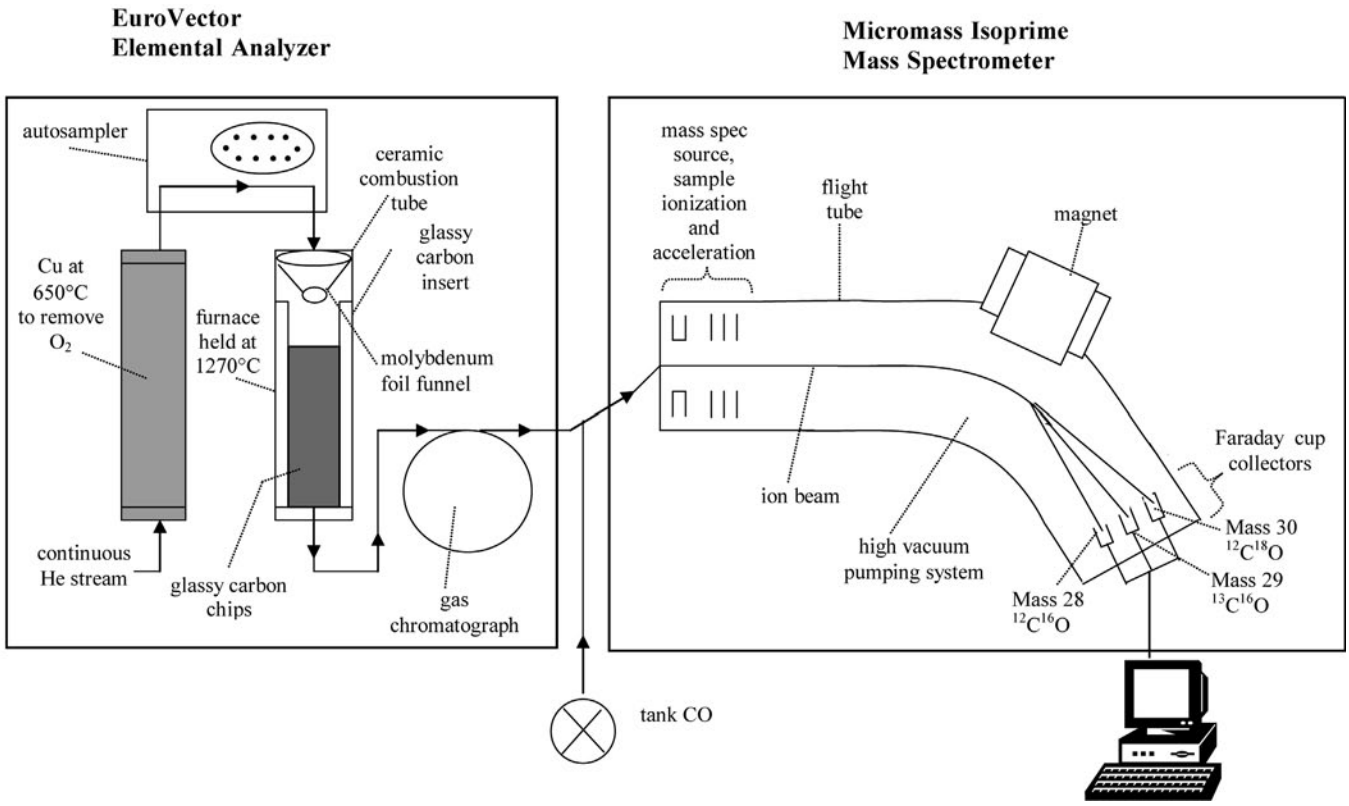

Fig. 2. Schematic diagram of the coupled elemental analyzer and mass spectrometer used for oxygen isotope analyses of silver phosphate. Sample oxygen is converted to CO in the pyrolysis tube at a temperature of 1270°C in the presence of carbon to form carbon monoxide, which is purified in a gas chromatograph and injected into a mass spectrometer for isotopic analyses.

$$\delta^{18}O = \left[\frac{(30/28)\ \text{sample}}{(30/28)\ \text{reference}} - 1\right] \times 1000 \quad (1)$$

The raw values are corrected by normalizing to internal working standards, which have been calibrated to Vienna standard mean ocean water (VSMOW) and are run in duplicate at 10 sample intervals.

Standards—Internationally recognized standards for phosphate oxygen isotopic composition, other than apatite, are not widely available. Therefore we prepared two silver phosphate internal standards from potassium phosphate (STDL) and sodium phosphate (STDH). The new standards were calibrated against silver phosphates TU1 (21.09 ± 0.15‰) and TU2 (5.51 ± 0.15‰), which were prepared using the BrF_5 fluorination method and calibrated to VSMOW (samples provided by Torsten Vennemann, Universität Tübingen). STDL was made from dibasic potassium phosphate (Mallinckrodt lot: 7080 KCSR-R) and has a $\delta^{18}O$ value of 11.30 ± 0.02‰ and STDH was made from dibasic sodium phosphate (Fisher lot: 996161) and has a $\delta^{18}O$ of 20.00 ± 0.09‰. These working standards were used to assess instrumental drift and linearity and to correct raw $\delta^{18}O$ values to VSMOW. The average 1 σ precision on 65 duplicate analyses of STDH and STDL was ± 0.3‰.

Assessment

Evaluation of extraction and precipitation method—During chemical extractions, there is a possibility of isotopic fractionation of the phosphate oxygen resulting from incomplete recovery of phosphate at each precipitation step whereby some isotopically distinct fraction is not recovered. To determine if this was occurring in our method, we conducted several steps to check for complete recovery. A 1-µM and 3-µM potassium phosphate spike with a known isotopic composition (STDL) was added to 8 L of a solution of magnesium chloride in deionized water (DI), and solutions were processed as seawater samples to measure any isotopic fractionation involved in the purification process. There was no significant difference between the $\delta^{18}O$ of the potassium phosphate standard and the spiked solution samples (Table 1). To more closely simulate the extraction procedure, STDL was added to seawater that had been stripped of phosphate (using the MagIC technique). These solutions with 1-µM, 2-µM, and 3-µM phosphate concentrations were processed as above to silver phosphate. No significant fractionation was observed and the $\delta^{18}O$ of these spiked samples were identical, within analytical error, to the standard. Similarly, we evaluated the

Table 1. Summary of results for method evaluation experiments

Sample	$\delta^{18}O$ (‰, VSMOW)	1σ	*n*
1 µm KH_2PO_4 (STDL)-spiked DI water	11.0	n/a	1
3 µm KH_2PO_4 (STDL)-spiked DI water	10.8	±0.3	2
1 µm KH_2PO_4 (STDL)-spiked seawater	11.0	±0.4	2
2 µm KH_2PO_4 (STDL)-spiked seawater	11.0	±0.6	4
3 µm KH_2PO_4 (STDL)-spiked seawater	11.0	±0.6	2
Unfiltered Monterey Bay seawater	19.9	±0.4	7
Filtered Monterey Bay seawater	19.6	±0.6	4

blank associated with this procedure by processing a sample of distilled water amended with magnesium chloride and were not able to precipitate any silver phosphate, implying no significant blank.

The yield of silver phosphate was difficult to assess for this procedure due to the precipitation of trace amounts of elemental silver with the silver phosphate (as observed by scanning electron microscope); however, weights of silver phosphate sample were equal to or greater than expected, suggesting ~100% yield. In addition, we measured the SRP after the MagIC separation and after the cerium phosphate precipitation to ensure that 100% of the phosphate was stripped from the seawater samples and processed. Soluble reactive phosphate concentrations in the supernatant after precipitations were undetectable.

To determine if particulate matter in the samples has an effect on the isotopic composition of the dissolved phosphate, 8-L samples of filtered and unfiltered water obtained from the Monterey Bay were processed. For the Monterey Bay samples, there was no significant difference between the filtered and unfiltered samples (Table 1).

Table 2. Summary of the reproducibility of this study compared with other studies of $\delta^{18}O$ analysis by high temperature reduction

Sample	$\delta^{18}O$ (‰, VSMOW)	1σ	*n*	Study
STDL	11.3	0.4	24	This study
STDH	19.8	0.4	44	This study
TU1	21.1	0.5	6	This study
TU2	5.4	0.4	5	This study
KP65	26.2	n/a	1	This study
UMS1	10.9	0.2	4	This study
KP65	25.1			Blake unpubl. data
UMS1	12.0			Blake unpubl. data
Ag_3PO_4	10.9	0.2	5	Kornexl et al. 1999
GW-1	22.7	0.1	52	Vennemann et al. 2002
NBS120c	22.1	0.5	18	Vennemann et al. 2002
TU1	21.1	0.6	22	Vennemann et al. 2002
TU2	5.4	0.6	14	Vennemann et al. 2002
130-9	8.4	0.3	35	Vennemann et al. 2002

Table 3. Duplicates of similar weight often have increased precision

Sample	Ag_3PO_4 weight (mg)	$\delta^{18}O$ (‰, VSMOW)	Average $\delta^{18}O$ (‰, VSMOW) for duplicates	1σ
Monterey Bay 5	0.805	20.2		
Monterey Bay 5	0.875	19.7		
Monterey Bay 5	0.86	19.6		
Monterey Bay 5	0.783	19.7		
Monterey Bay 5	0.815	19.9	19.8	0.3
SFBAY1	0.992	20.1		
SFBAY1	0.999	20.1	20.1	0.0
SFBAY3	0.962	18.6		
SFBAY3	0.95	18.7	18.6	0.1
SFBAY4	0.861	15.4		
SFBAY4	0.967	15.7	15.6	0.2
Monterey Bay 3	0.95	19.9		
Monterey Bay 3	0.89	19.8	19.9	0.1

Instrument function—The memory, precision, and drift of the instrument were determined using our internal standards (STDH and STDL) and the instrument blank was established using silver capsules containing 1 mg of nickelized carbon. No observable CO peak was detected when a silver capsule blank was dropped into the pyrolysis column, nor was there any detectable memory effect when samples of varying weights and isotopic compositions are added in sequence. Instrument drift is corrected using the STDH and STDL analyzed throughout each run (two standards every 10 samples). Variation between runs is corrected using the true values for the STDH and STDL standards as determined by repeated analyses (Table 2). The instrument precision for the standards over time ranged from 0.02‰ up to 0.6‰, and is typically around 0.4‰ of expected values. This is similar to values reported by Kornexl et al. (1999) and Vennemann et al. (2002) for high temperature reduction of phosphates (Table 2). Duplicates of similar weight have better precision (0.3‰ or better) than splits of the sample processed separately or of duplicates with weights varying more than 0.2 mg (Table 3).

Standards from two other laboratories were used to assess the consistency of our data with other laboratories (Table 2). In each case the measured value for the standard is the same as the reported value within analytical error and all studies report similar standard deviations. Results from Vennemann et al. (2002) indicate that different laboratories running the same standards often report slightly different values, therefore, it is important to have a universal silver phosphate standard made and run in all laboratories so results can be directly compared.

Discussion

This new method for the extraction and measurement of low concentrations of DIP from relatively small quantities of

Table 4. San Francisco Bay transect

Sta. nr	SRP (μM)	Temp (°C)	Equilibrium Temp* (°C)	pH	Salinity (psu)	$\delta^{18}O_w$ (‰) (VSMOW)	$\delta^{18}O_p$ (‰) (VSMOW)
1	1.15	16.0	22.2	7.80	32.3	–0.6	20.1
3	1.66	16.9	23.1	7.81	29.6	–1.9	18.6
4	0.91	17.2	33.2	7.81	23.6	–2.6	15.5
6	0.70	17.4	26.9	7.80	15.0	–5.2	14.5
7	1.36	17.2	17.2	7.85	9.5	–7.0	14.9
9	1.83	17.5	8.5	7.80	0.4	–10.5	13.4
14	1.73	19.0	9.5	7.51	0.3	–10.4	13.3
16	1.66	19.0	12.6	7.32	0.3	–10.2	12.8
20	1.53	16.9	16.4	7.92	0.4	–10.7	11.4

*Equilibrium temperature calculation from Eq. 2.

seawater has proven to be both accurate and precise, involving no blank. Samples can be processed without isotopic fractionation. Silver phosphate can be analyzed on a high temperature elemental analyzer configured for pyrolysis and containing a GC column coupled to a mass spectrometer. Such methodologies may provide insight into the sources and cycling of phosphate in aquatic ecosystems. This method is an advancement over previous methods, which require large volumes of seawater for analyses and time-intensive chemical separations. The current precision of the high temperature reduction of silver phosphate (1 σ ~ 0.3‰) is not as good as reaction with bromine (1 σ ~ 0.07‰) (Stuart-Williams and Schwarcz 1995), fluorination techniques (1 σ ~ 0.22‰), or reaction with graphite in sealed silica tubes (1 σ ~ 0.20‰) (Vennemann et al. 2002), although the reaction in silica tubes may result in exchange of oxygen between the sample and tube (Révész and Böhlke 2002). However, the sample size required for our approach is significantly smaller than for each of these techniques (0.6 mg compared to 20 mg for fluorination, reaction with bromine, and reaction with graphite) making it more desirable for analysis of seawater samples where phosphate concentrations are low (8 L of seawater containing micromolar concentrations of phosphate may be collected as opposed to 230 L required for other methods). The current precision of this technique is sufficient for evaluation of trends in many aquatic systems and may be particularly useful in coastal and estuarine systems where variations in phosphate cycling and sources are likely to be more pronounced.

There are several possible reasons for naturally occurring variations in $\delta^{18}O$ of dissolved inorganic phosphate: inorganic hydrolysis and polymerization, organic (enzyme-mediated) hydrolysis and polymerization, and mixing of phosphate of different isotopic composition (source phosphate). The first of these possibilities is unlikely because at low temperatures and moderate pH, nonbiological isotope exchange between phosphate and water is known to be negligible (Lécuyer et al. 1999; O'Neil et al. 2003). Eliminating inorganic hydrolysis as a potential cause of isotopic exchange leaves enzyme-mediated hydrolysis and polymerization and/or mixing of different sources of isotopically distinct phosphate as the likeliest possibilities for observed natural variability. Below we present preliminary data of $\delta^{18}O$ of DIP in an estuary and in seawater to demonstrate the potential of using $\delta^{18}O$ of phosphate as a tracer for both source phosphate as well as phosphate recycling.

San Francisco Bay—A preliminary study was conducted along a surface water transect in the San Francisco Bay (~70 km) on 17 and 18 October 2002. Seawater was collected from 9 stations from the mouth of the bay up into the San Joaquin River. The oxygen isotopic composition of dissolved inorganic phosphate decreases along the transect from the open ocean value of 20.1‰ to 12.8‰ in the San Joaquin River and 11.4‰ in the Sacramento River. The $\delta^{18}O$ of phosphate was well correlated with both salinity and $\delta^{18}O$ of the water (determined on Finnegan MAT 251) (Table 4 and Fig. 3b,3c) but was not correlated with pH (Table 4). We corrected for the change in the isotopic composition of phosphate as a result of the isotopic composition of water ($\delta^{18}O_{phosphate} - \delta^{18}O_{water}$) and found a correlation with the concentration of phosphorus but no correlation with water temperature (Table 4 and Fig. 3d,e).

Using the equilibrium equation for isotopic fractionation of oxygen in phosphate as a function of temperature (Longinelli 1965; Kolodny et al. 1983; Kolodny and Raab 1988):

$$T(°C) = 111.4 - 4.3\,(\delta^{18}O_p - \delta^{18}O_w) \quad (2)$$

where T is the average equilibration temperature (for apatite), $\delta^{18}O_p$ is the isotopic composition of the phosphate, and $\delta^{18}O_w$ is the isotopic composition of the water with which the phosphate has equilibrated. We calculated the expected temperature for each station if equilibrium had been reached. The data indicate that for all but one station, the $\delta^{18}O_p$ is not concordant with the attainment of oxygen isotope equilibrium between phosphate and water at this temperature. Because nonbiological isotopic exchange between phosphate and water is negligible at these low temperatures and pH values (O'Neil et al. 2003), we did not necessarily expect to see concordance with isotopic equilibrium. However, if there were rapid biological turnover of phosphate within the system, we would expect the

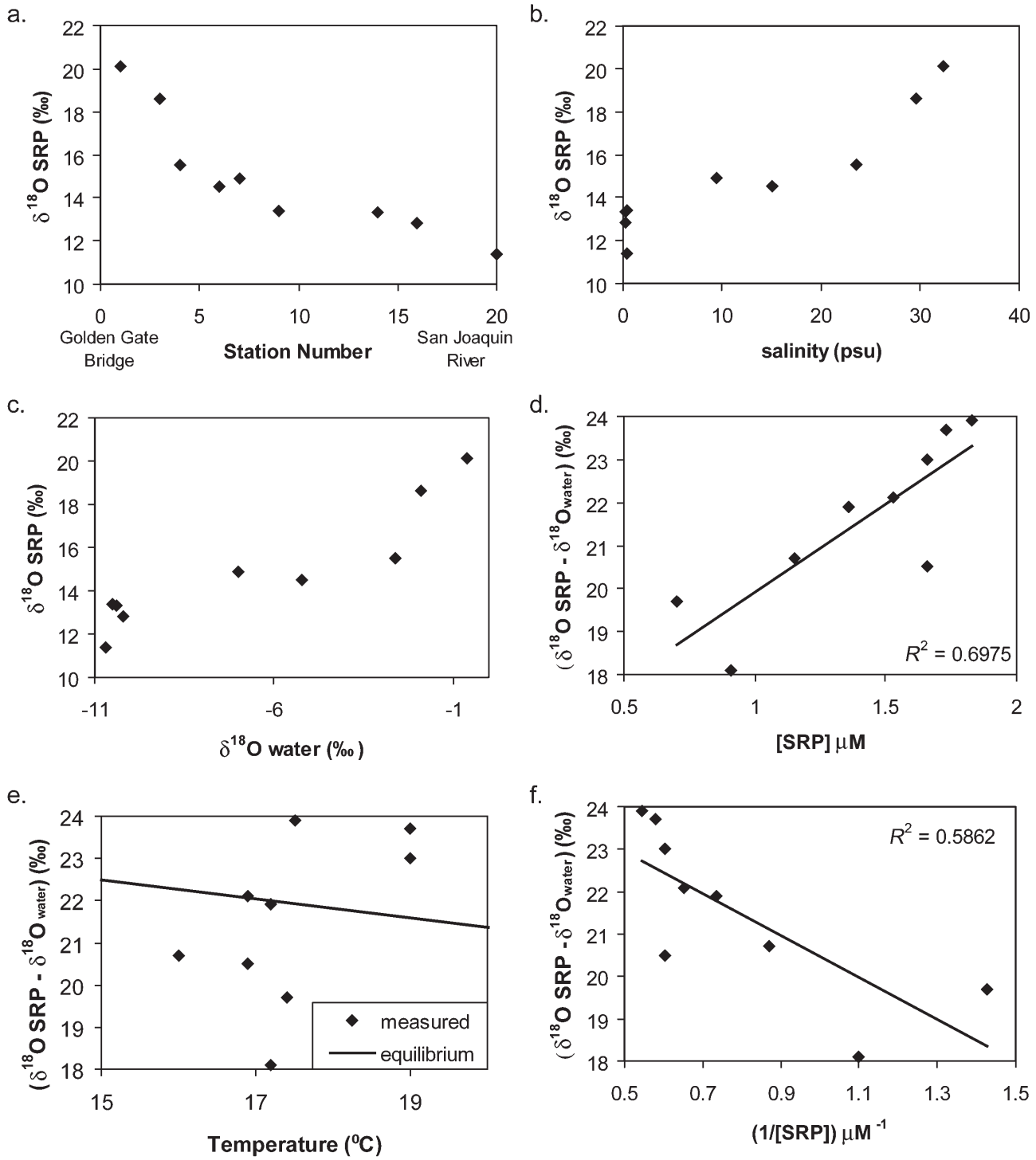

Fig. 3. (a) $\delta^{18}O$ phosphate along a transect in the San Francisco Bay taken in October 2002. $\delta^{18}O$ phosphate correlates well with salinity (b), $\delta^{18}O$ water (c), and SRP (d), but not with temperature (e) or pH (data not shown), and is not in equilibrium with water according to Eq. 2 (f). The $\delta^{18}O$ of phosphate corrected for $\delta^{18}O$ of water plotted against the reciprocal of the SRP concentration indicates a two end member mixing between phosphate sources of different isotopic composition.

$\delta^{18}O_p$ to approach the theoretical equilibrium value because a higher phosphorus demand would stimulate enzyme-mediated reactions to cleave phosphate from organic molecules, which is the primary exchange mechanism for $\delta^{18}O_p$ at these temperatures (Blake et al. 1997). Our data indicate that full biologically mediated $\delta^{18}O_p$ exchange of oxygen isotope between

Table 5. Open ocean $\delta^{18}O$ of inorganic phosphate

Station	Depth (m)	$[PO_4]$ (μM)	Temperature (°C)	$\delta^{18}O_{phosphate}$ (‰)	Equilibrium temperature (°C)
California Current Sta. 3	665	3.27	4.9	20.2	24.5
California Current Sta. 13	40	0.64	11.3	21.8	17.7
California Current Sta. 14	309	2.44	6.6	18.6	31.4
California Current Sta. 15	400	2.27	6.3	21.5	19.0
Hawaii Transect Sta. 18	1000	3.33	3.9	20.3	24.1
Monterey Bay M1 27 January 2003	100	1.40	10.4	19.9	25.8
Monterey Bay M1 27 January 2003	200	1.98	8.8	21.0	21.1
Monterey Bay M1 29 April 2003	40	1.98	9.7	22.3	15.5
Monterey Bay M1 29 April 2003	100	2.06	9.2	21.9	17.2
Monterey Bay M1 29 April 2003	200	2.26	8.2	22.0	16.8

phosphate and water has not occurred in this system, suggesting that the phosphate is not heavily used and recycled by organisms. This was not unexpected due to the relatively high concentrations of phosphate along the transect.

An interesting result of this study was the correlation between $\delta^{18}O_p$ and phosphate concentration. The $\delta^{18}O_p$ of our samples increased as phosphate concentration increased, which is opposite what one would expect for recycling of phosphate (one would expect phosphate concentrations to decrease as $\delta^{18}O_p$ increased toward the theoretical equilibrium value as mentioned above). The observed trend in the San Francisco Bay is potentially a mixing between isotopically distinct sources of phosphate. San Francisco Bay has two riverine inputs mixing with open ocean water at the mouth of the bay. Therefore it is highly likely that some of the variation of the $\delta^{18}O_p$ is due to mixing of different sources of phosphate to the system, one input from the San Joaquin and Sacramento Rivers and another oceanic phosphate source. The high correlation with salinity and $\delta^{18}O_w$ strengthen this argument, and it may be that the correlation we observe with phosphate concentrations is the result of mixing of agricultural phosphate sources with seawater sources of different isotopic composition and concentrations. This is further evidenced by plotting the $\delta^{18}O$ of phosphate corrected for the $\delta^{18}O$ of water versus the reciprocal of the SRP concentration (Fig. 3f), which implies a two-end member mixing line. These data suggest that $\delta^{18}O_p$ can be used to identify different sources of phosphate in natural systems.

Ocean variability—Seawater oxygen isotope values for SRP from a variety of different stations and depths also show significant variation from 18.6‰ to 22.3‰, suggesting small but detectable natural variability in seawater (Table 5). As in the San Francisco Bay study, we calculated the expected temperature based on the oxygen isotopic composition of the DIP measured using Eq. 2. We observed that these limited data do not correlate well with temperature and are all isotopically lighter than that predicted for equilibrium fractionation (Fig. 4a). This disequilibrium suggests that dissolved inorganic phosphate was incompletely recycled within the ecosystem. Phosphate concentration accounts for 12% of the variance in $\delta^{18}O$ of phosphate (Fig. 4b). This low correlation with phosphate concentration may be a function of greater dependence of $\delta^{18}O$ of phosphate on factors such as productivity. Further

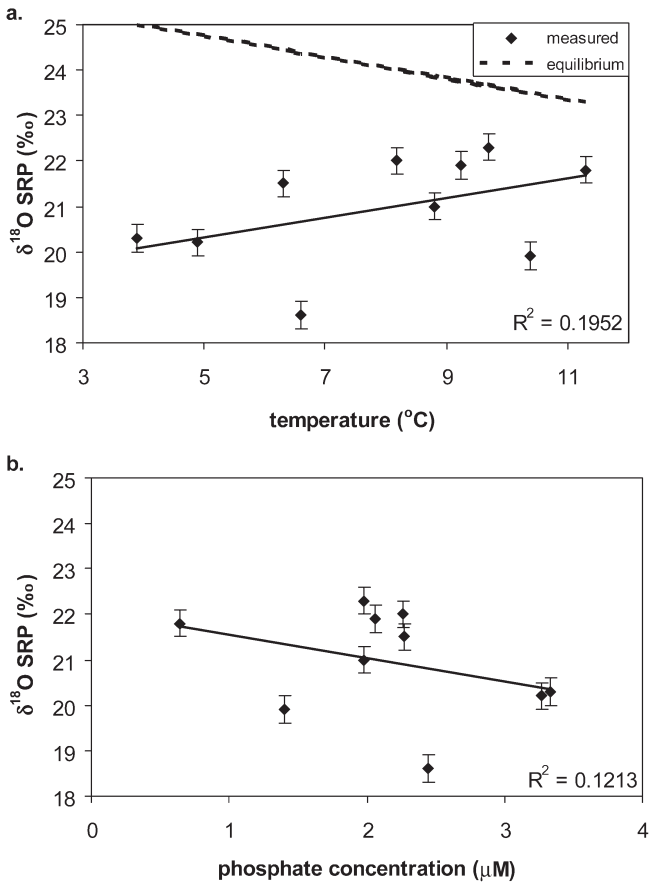

Fig. 4. (a) Oxygen isotopic composition of phosphate for various sites in the open ocean are not in equilibrium with seawater, and all samples are isotopically lighter than predicted from Eq. 2. (b) The oxygen isotopic composition of phosphate is not well correlated with phosphate concentration, which only explains 12% of the variance.

investigation is being conducted to determine the possible causes for the observed variability.

One interesting finding regarding the Monterey Bay data is that during January 2003 the samples are isotopically lighter than those sampled during April 2003. Monterey Bay is characterized by an upwelling season from March through late August where the combination of increased nutrients, sunlight, and some degree of water-column stratification lead to high primary production and chlorophyll values, as well as a change in phytoplankton flora to dominance by diatoms (Pennington and Chavez 2000). In September and throughout the winter, upwelling favorable winds relax, resulting in a marked decrease in primary production and a species composition shift among the phytoplankton flora to dominance by oceanic picoplankton (Pennington and Chavez 2000). The increase in $\delta^{18}O_p$ during the spring bloom coincides with the increase in productivity at the onset of upwelling and the species composition shift. It is interesting that $\delta^{18}O_p$ approaches the theoretical value calculated using Eq. 2 during the spring bloom and not during the winter months when a lack of nutrients produces an annual minima in the phytoplankton populations. It may be that during the period of higher primary productivity and subsequent grazing would stimulate the rapid recycling of phosphate in the surface waters, whereas during the winter months deep mixing and reduced sunlight availability limit productivity levels such that recycling rates are incomplete. Further investigation is being conducted within the Monterey Bay to trace these changes through time; however, we believe these data demonstrate the utility of using the oxygen isotopic composition of phosphate as a tracer for phosphate cycling.

Comments and recommendations

The extraction of phosphate from seawater is largely pH dependent; hence, maintaining the appropriate pH at each step in the procedure is important for 100% yield of phosphate. We have found that it is sufficient to test the pH at each stage with a pH indicator strip. Special attention should be given to the removal of chloride ions during the cerium phosphate-rinsing step; otherwise chloride will react with silver in the final precipitation and interfere with the silver phosphate precipitation. During both the cerium phosphate and silver phosphate precipitation steps, cerium and silver should be present in excess to ensure complete precipitation of phosphate. Incomplete precipitation may result in fractionation of the phosphate oxygen and inaccurate measurement of the isotopic composition.

For samples high in dissolved organic matter, a resin purification step may need to be incorporated following the MagIC dissolution step to ensure complete removal of DOM. In some estuarine samples, humic acids remained in the sample solutions at each step and persisted in the samples even until the silver phosphate step. We did not yet test to see if the DOM interferes with this precipitation but a resin step would ensure exclusion of DOM prior to precipitation of silver phosphate.

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