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Limnology and Oceanography, Vol. 43, No. 7 (Nov., 1998), 1565-1577.

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Thu Feb 24 13:04:56 2005

Limnol. Oceanogr., 43(7), 1998, 1565–1577
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Application of a novel method for phosphorus determinations in the oligotrophic North Pacific Ocean

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Abstract

Phosphorus (P) is an essential macronutrient and is present in seawater as both dissolved inorganic (primarily orthophosphate, P*i*) and organic compounds. Current analytical techniques measure soluble reactive phosphorus (SRP) as a surrogate for P*i* and soluble nonreactive phosphorus as a surrogate for dissolved organic phosphorus (DOP). A new method provides a measure of P*i* concentrations directly and eliminates interference from other reactive P compounds. The same method can also be used to make direct measurements of non-P*i* phosphorus (N-P*i*P) concentrations (i.e., [total dissolved P] − [P*i*]) with greater precision and accuracy limits than current methods provide. With this new method, P*i* concentrations in North Pacific surface waters were found to be lower, by up to 50% or more, than SRP concentrations measured with standard autoanalyzer techniques. Mean (±SD) N-P*i*P concentrations in the deep (>1,000 m) North Pacific were determined to be 16.2 ± 12.5 nM. These results have significant implications for turnover and residence times of dissolved P pools and for utilization of DOP in the deep sea. This new method for P*i* and N-P*i*P measurements has numerous potential applications in marine and freshwater research.

Life in aquatic environments depends on the presence of several necessary elements for cell survival and balanced growth. Among these are macronutrients such as carbon, nitrogen, phosphorus, and sulfur and trace elements such as iron, molybdenum, and zinc. Recent evidence suggests that the nutrient phosphorus (P) may limit production rates in selected oligotrophic marine habitats (Karl et al. 1995; Thingstad and Rassoulzadegan 1995; Michaels et al. 1996), which has stimulated a renewed interest in P dynamics in these ecosystems.

In the ocean, the total dissolved P (TDP) pool is partitioned into dissolved inorganic P (DIP) and dissolved organic P (DOP). The DIP pool is sometimes referred to as orthophosphate (HPO_4^{2-}, hereinafter referred to as P*i*), but this pool also includes pyrophosphate (P_2O_7) and inorganic polyphosphates. The DOP pool consists of a variety of compounds, including nucleotides, nucleic acids, P-containing derivatives of sugars and lipids, and phosphonate compounds. Dissolved P pools are operationally defined by current measurement techniques as soluble reactive P (SRP) and soluble nonreactive P (SNP), which are not necessarily congruent with DIP and DOP pools (*see* Burton 1973). The SRP pool is sometimes equated with P*i*, but it includes a variety of other P compounds such as pyrophosphate and certain DOP compounds that are analytically indistinguishable from P*i* (Jones and Spencer 1963; Rigler 1968). It is currently unclear to what extent the SRP pool is comparable to the concentration of P*i* and precisely which P compounds (organic and inorganic) contribute to SRP concentrations. Moreover, it is uncertain what relevance these analytically defined pools have to the biologically available P pool that is readily assimilated by marine microorganisms.

Accurate determination of P*i*, DIP, SNP, and DOP concentrations are essential for understanding and quantifying rates of P uptake and cycling. Currently, SRP determination is made by initial sample acidification, subsequent reduction to a colored molybdenum blue solution, and spectrophotometric analysis of the solution after a reaction time of 15 min (Fiske and Subbarow 1925; Murphy and Riley 1962; Strickland and Parsons 1972). Evidence from lake research indicates that the acidic conditions, necessary for color development, and the addition of the molybdate reagent may hydrolyze some organic P compounds and overestimate the concentration of P*i* (Chamberlain and Shapiro 1969; Tarapchak et al. 1982). For this reason, Strickland and Parsons (1972) introduced the term "soluble reactive phosphorus" to describe the material measured with current techniques.

Much of the research conducted to date concerning the concentration differences between SRP and P*i* has focused on identifying the presence and source of the discrepancy. Using experiments with anion exchange resins, Jones and Spencer (1963) found that the resin removed 98% of P*i* from prepared solutions, but only 30% of the P was removed from seawater containing natural SRP compounds. Similar experiments by Rigler (1968) using P*i* added to lake water showed that the resin removed 96% of the ^{32}P*i* but only 74% of SRP. Kuenzler and Ketchum (1962) measured P*i* and SRP using an algal bioassay system with known ^{32}P*i* tracer additions. Radiochemical equilibrium measurements on samples from a variety of freshwater habitats indicated that only a portion of the molybdate-reactive material was present as P*i*. In studies with hydrous zirconium oxide, a material with a high affinity for P*i*, Rigler (1968) observed a discrepancy between uptake of P*i* and the uptake of SRP and reached a similar conclusion regarding SRP bioavailability. Chamberlain and

Acknowledgments

We thank the HOT program scientists for their assistance in the field and K. Björkman and G. Tien for laboratory assistance. E. Ingall and T. Fisher provided constructive criticism during the review process. L. Lum assisted in the preparation of the text and figures, and L. Fujieki provided computer access to the HOT program data set. This research was supported in part by National Science Foundation grant OCE-9301368 (awarded to D.M.K.) and by an NSF Graduate Research Fellowship (awarded to A.T.-B.). This is SOEST contribution 4673 and U.S. JGOFS contribution 451.

Shapiro (1969) developed a method also based on the molybdenum blue reaction that allowed color development at low acidity for only 6 s, at which point additional acid was added to stop the reaction at approximately 75% color development and thereby preventing interference from subsequent hydrolysis of acid-labile DOP compounds. Their method yielded lower SRP concentrations than did standard analytical procedures and placed an upper limit on P*i* concentrations. Consistent results from each of these studies suggest that a significant fraction (25–70%) of the SRP in lake and seawater is not P*i*.

An alternative approach to the standard SRP analysis was developed by Karl and Tien (1992). Their magnesium-induced coprecipitation (MAGIC) technique employed the addition of NaOH to seawater (or to Mg^{2+}-amended freshwater) samples to facilitate a coprecipitation of P compounds with the mineral brucite ($Mg(OH)_2$) under slightly alkaline (pH 9–10) rather than acidic conditions. One advantage of this technique is that it can selectively isolate and concentrate inorganic P compounds in the sample before acidification, thereby reducing analytical interference by the hydrolysis of organic P compounds that may also be present in the water sample.

The method developed in this study was aimed at determining the concentration of P*i* by a modification of the MAGIC procedure and by making direct measurements of the nonphosphate phosphorus (N-P*i*P) pool in samples with high P*i*: N-P*i*P ratios (>3–5) where existing difference measurements do not perform well. By exploiting the adsorption characteristics of different P compounds onto $Mg(OH)_2$ and by tracking P*i* removal in the sample using a ^{32}P*i* radiotracer, it is possible to separate P*i* from N-P*i*P. This modified MAGIC method provides a more accurate estimate of P*i* concentrations in seawater and a more precise and accurate estimate of N-P*i*P by analyzing the nonprecipitated portion of the sample, known to contain the P*i*-free TDP pool.

Materials and methods

Sample collections and storage—Seawater was collected on selected Hawaii Ocean Time-series (HOT) cruises at Sta. ALOHA (22°45′N, 158°W). Samples were obtained from predetermined depths using polyvinylchloride sampling bottles with Teflon®-coated springs, mounted on a conductivity–temperature–depth rosette system (Karl and Lukas 1996). After recovery, subsamples were drawn into 500-ml high-density polyethylene bottles and stored frozen (−20°C) for subsequent analyses (Dore et al. 1996). Each bottle was washed with 1 M HCl, rinsed with deionized distilled water, and air dried prior to use. For these oligotrophic open-ocean waters, the subsamples were withdrawn without filtration to avoid contamination. Independent measurements of particulate P (Whatman GF/F filters) pools at Sta. ALOHA during the period of this investigation demonstrated that this fraction is small relative to the total dissolved P pool (e.g., the average 0–100-m particulate P pool is 17 μmol P m^{-3} compared with 300 μmol m^{-3} TDP, or approximately 6% of total P). For selected comparisons during the course of this investigation, water samples were also collected by hand from a variety of nearshore coastal marine habitats and from several freshwater ecosystems. All sample treatments were identical to the open ocean Sta. ALOHA samples except the coastal marine and freshwater samples were filtered (0.8-μm Nuclepore membranes) prior to freezing. To effect the authigenic formation of brucite in freshwater samples, Mg^{2+} was added as a $MgCl_2$ solution to achieve a final concentration of 55 mM.

SRP analyses—SRP was analyzed using several different methods: (1) the standard molybdenum-blue method of Murphy and Riley (1962) with modifications by Strickland and Parsons (1972) (the MR method), (2) the MAGIC method (Karl and Tien 1992), (3) automated flow analysis (the AA method; Karl and Tien 1997), and (4) a modification of the MAGIC method developed during this study. All determinations, with the exception of the AA method, were corrected for arsenate (AsO_4) interference using the method of Johnson (1971). The modified-MAGIC method differed from the MAGIC method only by the volume of NaOH added, the volume of HCl used to dissolve the pellets, and the use of a ^{32}P*i* radiotracer. Selected seawater samples previously collected at Sta. ALOHA using nutrient sampling protocols (Dore et al. 1996) and stored frozen as part of the HOT program archive were thawed and analyzed for P*i* to compare with paired measurements of SRP by AA and MAGIC methods.

TDP analyses—TDP was also analyzed using a variety of methods: (1) ultraviolet (UV) photooxidation followed by automated flow analysis (the UV-AA method), (2) persulfate oxidation, and (3) an alkaline-persulfate oxidation method.

For the UV-AA method, TDP measurements were made by treatment of a 30-ml sample contained in a quartz tube by UV light photooxidation according to Armstrong et al. (1967), which utilizes a 2-h UV irradiation period. Details of the UV irradiation unit were described by Walsh (1989). Typical standard deviations for replicate analyses in shallow (<100 m) and deep (>100 m) seawater samples are ±40 nM P and ±50 nM P, respectively (Smith et al. 1986).

TDP determinations using the persulfate oxidation method described by Menzel and Corwin (1965) have typically yielded low precision, at times approaching ±50% for TDP concentrations in the 100–300 nM range. During our research, significant effort was invested in refining the persulfate oxidation technique to yield a more precise measurement of TDP, including the use of 50-ml autoclavable glass centrifuge tubes with Teflon®-lined caps. Furthermore, a postoxidation MAGIC concentration step was employed to increase sample precision (Karl and Yanagi 1997). This concentration step, coupled with the use of tubes with Teflon®-lined caps, resulted in a TDP procedure with an approximately ±3% precision over a broad range of TDP concentrations (50 nM to 3 μM).

An experimental alkaline-persulfate oxidation method was attempted in hopes of improving the recovery and ease of analysis of TDP. This method was based on a procedure developed for particulate organic nitrogen and phosphorus (Raimbault and Slawyk 1991; Libby and Wheeler 1994; Pujo-Pay and Raimbault 1994) and modified for analysis of

dissolved organic carbon (Karl et al. 1998). Applied to TDP analyses, this procedure required addition of a NaOH-$K_2S_2O_8$ (100 g $K_2S_2O_8$ per liter of 1 M NaOH) solution in a ratio of 1 : 7 reagent : sample volume. The samples were sealed and autoclaved for 200 min at 126°C. After cooling, samples were centrifuged for 1 h at 1,000 × *g*. The supernatant was aspirated and discarded, and the $Mg(OH)_2$ pellet containing P*i* was treated as a standard MAGIC sample.

Pi and N-PiP measurements—A tracer amount of ^{32}P*i* (carrier free; ICN Radiochemicals) was added to each seawater sample to an activity of approximately 1,800 Bq ml^{-1} (total P*i* addition was approximately 1.78 fmol ml^{-1}). Samples for total ^{32}P*i* activity were taken, and the remaining water was distributed into 50-ml polypropylene centrifuge tubes. NaOH (1 M) was added to each sample (0.3%, vol : vol), and tubes were inverted, thoroughly mixed, and centrifuged for 1 h at 1,000 × *g*. After centrifugation, 1 ml of the supernatant was counted for total ^{32}P*i* activity and 40 ml of the remainder, primarily N-P*i*P compounds, was removed for TDP analyses. The remainder of the supernatant was aspirated and discarded, and the pellet was dissolved in 9 ml of trace metal grade HCl (0.1 M) and treated as a standard MAGIC sample from this step forward (Karl and Tien 1992).

Phosphorus standard curves were made for both P*i* and N-P*i*P analyses. A concentrated stock solution of analytical grade KH_2PO_4 solution (CSK nutrient element, Wako Chemicals) was diluted into seawater, with standard concentrations covering the expected range of seawater sample concentrations. All standards were made up in surface seawater and treated identically to the samples. These determinations were used to establish the slope of the standard curve. To determine a reagent blank, it was necessary to obtain P*i*-free and TDP-free seawater. For the former, supernatant from a standard MAGIC precipitation was used because it is P*i* free. TDP-free seawater was obtained using Nuclepore (0.2 μm)-filtered artificial seawater (Instant Ocean) and removing any phosphate contamination by standard MAGIC precipitation.

The percentage of the ^{32}P*i* removed following NaOH addition is equivalent to the percentage of ^{31}P*i* removed from the seawater sample. The measured concentration of P*i* from the dissolved pellet was corrected by the fraction of ^{32}P*i* remaining in solution to calculate the total P*i* concentration in the original sample. The P*i* that was not recovered in the precipitate was also subtracted from the N-P*i*P pool, because TDP would be overestimated by the minor contribution from the unrecovered P*i*. In practice, these corrections were relatively small because ^{32}P*i* removal was typically >90% with NaOH (1 M) additions of 0.3% (vol : vol) and >98% with additions of 0.5% (vol : vol).

Representative P compounds—A variety of organic and inorganic P compounds was selected to represent the broad range of pools expected to occur in the marine environment (Table 1). All compounds were obtained from Sigma Chemical Company, with the exception of DNA, which was obtained from Kodak, and were of analytical grade quality or better. Compound stock solutions were prepared gravimetrically, typically to concentrations of 2 mM P. The concentrated stocks were divided into 1-ml aliquots and stored frozen until needed. All standard solutions were discarded immediately after a single use.

Concentrations of stock nucleotide solutions were independently confirmed by absorbance spectrometry. Samples were diluted in deionized distilled water or trace metal grade HCl (0.1 M), and the concentrations were determined from spectrophotometric absorbance at a diagnostic wavelength for each compound and corresponding molar extinction coefficient (Fasman 1975).

P compound interference—Each compound was tested for $Mg(OH)_2$ coprecipitation and to determine if its presence in seawater affected the removal of P*i*. All P compounds were made up to 0.5 μM P equivalent concentrations in filtered (0.2-μm Nuclepore filters) surface seawater and were spiked with ^{32}P*i* (approximately 1,800 Bq ml^{-1}). The mixture was distributed into 10-ml aliquots, and NaOH (1 M) was added in eight different volume-to-volume (%) amounts ranging from 0% to 2.5%. Samples were centrifuged for 1 h at 1,000 × *g*. After centrifugation, 1 ml of the supernatant was removed and counted for ^{32}P*i* activity. Radioactivity was determined by adding 10 ml of Aquasol II liquid scintillation cocktail to samples and counting using a Packard Tri-Carb scintillation counter.

Selected P compound adsorption experiments—The adsorption of selected P compounds by $Mg(OH)_2$ was examined using a seawater or tap water solution containing one or more test compounds and ^{32}P*i*. Filtered surface seawater was used to prepare solutions containing one or more P compounds, at final concentrations of 1 μM P. A tracer addition of ^{32}P*i* (approximately 1,800 Bq ml^{-1}) was added to the compound mixtures to quantify the removal of P*i* from solution. For the tap water experiments, Mg^{2+} was added (as $MgCl_2$) to a final concentration of 55 mM to initiate $Mg(OH)_2$ precipitation following NaOH addition. Initial samples were taken for total ^{32}P*i* activity and SRP and TDP determinations. The remainder of the mixture was distributed into 50-ml polypropylene centrifuge tubes to which variable amounts of NaOH (1 M) were added. Triplicate subsamples were prepared and analyzed for each treatment. The samples with NaOH additions were mixed and then centrifuged for 1 h at 1,000 × *g*. Following the centrifugation step, 1 ml of the supernatant was counted for ^{32}P*i* activity. Of the remaining supernatant, 40 ml was removed to a 50-ml glass centrifuge tube for TDP analyses by the Menzel and Corwin (1965) method. Five milliliters of the supernatant was also reserved for SRP determinations with the MR method. Any remaining supernatant was aspirated, and pellets were dissolved in a small volume of acid (0.5 ml of 3 M HCl). The dissolved pellets were collected and counted for total ^{32}P*i* activity.

Phosphorus standard curves were prepared for both TDP and SRP analyses. A concentrated stock solution of analytical grade KH_2PO_4 solution (CSK nutrient element, Wako Chemicals) was diluted in seawater, with standard concentrations covering the range of sample concentrations. All standards were made up in surface seawater and were treated identically to samples. These standards were used to determine the slope of the standard curve.

Table 1. List of P-containing compounds used in this study.

Compound	Abbreviation	Chemical formula	Molecular weight (g)
Nucleotides			
Adenosine-5′-triphosphate	ATP	$C_{10}H_{16}N_5O_{13}P_3$	507.2
Cytidine-5′-triphosphate	CTP	$C_9H_{16}N_3O_{14}P_3$	483.2
Guanosine-5′-triphosphate	GTP	$C_{10}H_{16}N_5O_{14}P_3$	523.2
2-Deoxyadenosine-5′-triphosphate	dATP	$C_{10}H_{16}N_5O_{12}P_3$	491.2
Adenosine-5′-diphosphate	ADP	$C_{10}H_{15}N_5O_{10}P_2$	427.2
Thymidine-5′-diphosphate	TDP	$C_{10}H_{16}N_2O_{11}P_2$	402.2
Uridine-5′-diphosphate	UDP	$C_9H_{14}N_2O_{12}P_2$	404.2
2-Deoxyguanosine-5′-diphosphate	dGDP	$C_{10}H_{15}N_5O_{10}P_2$	427.2
Thymidine-5′-monophosphate	TMP	$C_{10}H_{15}N_2O_8P$	322.2
2-Deoxyadenosine-5′-monophosphate	dAMP	$C_{10}H_{14}N_5O_6P$	331.2
2-Deoxycytidine-5′-monophosphate	dCMP	$C_9H_{14}N_3O_7P$	307.2
2-Deoxyguanosine-5′-monophosphate	dGMP	$C_{10}H_{14}N_5O_7P$	347.2
2-Deoxyuridine-5′-monophosphate	dUMP	$C_9H_{13}N_2O_8P$	308.2
Sugar phosphates			
Glucose-1-phosphate	G-1-P	$C_6H_{13}O_9P$	260.1
Glucose-6-phosphate	G-6-P	$C_6H_{13}O_9P$	260.1
Fructose-6-phosphate	F-6-P	$C_6H_{13}O_9P$	260.1
Fructose-1,6-diphosphate	F-1,6-diP	$C_6H_{14}O_{12}P_2$	340.1
Galactose-6-phosphate	Gal-6-P	$C_6H_{13}O_9P$	260.1
Ribose-1-phosphate	R-1-P	$C_5H_{11}O_8P$	230.1
Ribose-5-phosphate	R-5-P	$C_5H_{11}O_8P$	230.1
Ribulose-1,5-biphosphate	RuBP	$C_5H_{12}O_{11}P_2$	310.1
Monophosphate esters			
Phosphoglyceric acid	PGA	$C_3H_7O_7P$	186.1
Phosphoenol pyruvate	PEP	$C_3H_5O_6P$	168.0
Inorganic polyphosphates			
Pyrophosphate	PP_i	$H_4O_7P_2$	178.0
Tripolyphosphate	PPP	$H_5O_{10}P_3$	258.0
Polyphosphate (n = 31)	POLY-31	$H_{33}O_{94}P_{31}$	2,497.3
Polyphosphate (n = 91)	POLY-91	$H_{93}O_{274}P_{91}$	7,296.0
Phosphonates			
1-Aminoethylphosphonic acid	1-AEP	$C_2H_8NO_3P$	125.0
2-Aminoethylphosphonic acid	2-AEP	$C_2H_8NO_3P$	125.0
Phosphonoformic acid	PFA	CH_3O_5P	126.0
Amino acid derivatives			
Phospho-L-tyrosine	P-Tyr	$C_9H_{12}NO_6P$	261.2
Phospho-L-serine	P-Ser	$C_3H_8NO_6P$	185.1
Nucleic acids			
Ribonucleic acid	RNA	*	
Deoxyribonucleic acid	DNA		
Phospholipids			
Glycerophosphate	GYP	$C_3H_9O_6P$	172.1
Lecithin	LEC		
Choline phosphate	COL	$C_5H_{15}NO_4P$	184.2
Other phosphorylated organics			
Phytic acid	PHY	$C_6H_{18}O_{24}P_6$	660.0
Inositol phosphate	INS	$C_6H_{13}O_9P$	260.1

* Variable.

Because these adsorption experiments were performed in surface seawater, any measurement of TDP or SRP would have some contribution from the background P in seawater in addition to the added P-containing compounds. Also, this contribution would differ in each sample as a function of the amount of NaOH that was added. As a result, a separate blank value was prepared for each of the NaOH concentration treatments. A series of samples was taken from the initial common seawater and treated identically to those samples in the adsorption curve for both SRP and TDP, with the exception that no exogenous P compounds or ^{32}Pi were added. The measured absorbance of these control seawater samples was subtracted from the absorbance of those samples amended with P compounds.

Results

^{32}Pi and ^{31}Pi comparisons—Before attempting any field experiments, it was essential to verify that the independent assessments of the percentage removal of ^{32}Pi and nonlabeled P*i* (i.e., ^{31}Pi) were identical. The concentration of adsorbed orthophosphate in the $Mg(OH)_2$ pellet was examined in a sample spiked with 1 μM P*i* and radiolabeled ^{32}Pi (Fig. 1, top). The results of this experiment demonstrated that the two adsorption curves are indistinguishable (mean percentage remaining in supernatant [^{31}Pi − ^{32}Pi] = −0.11% ± 0.60%). As a result, observations of the adsorption of ^{32}Pi can be interpreted as representing the adsorption of nonlabeled P*i*. An additional comparison was made between the removal of P*i* and removal of AsO_4 from seawater. These compounds behave identically with respect to adsorption onto authigenic brucite (Fig. 1, bottom), so all samples were treated to eliminate AsO_4 interference.

Phosphorus compound interference—In the presence of different P compounds added at concentrations of 0.5 μM P, ^{32}Pi was quantitatively removed from solution at 0.5% (vol : vol) NaOH (1 M) addition for all tested compounds (n = 37). Mean removal of ^{32}Pi in the presence of these various compounds was 99.42% ± 0.35%. Seven compounds were also tested at concentrations exceeding 20 μM, and with the exception of ATP, removal of ^{32}Pi was also quantitative (mean ± SD removal = 99.34% ± 0.23%). In the presence of 20 μM exogenous ATP, removal of ^{32}Pi was 97.53%. Concentrations of 20 μM P in the DOP pool exceed TDP in oligotrophic surface seawater by nearly two orders of magnitude and in deep sea water (>1,000 m) by approximately one order of magnitude. These results indicate that adsorption of P*i* onto $Mg(OH)_2$ from seawater samples is not influenced by the presence of DOP in solution.

Phosphorus compound contributions to SRP measurements—One aspect of this study was to determine the contribution of selected organic and inorganic P compounds to conventional SRP measurements. The SRP concentrations of seawater samples spiked with a known concentration of 39 separate organic and inorganic P compounds were measured by MR, AA, and MAGIC techniques. The measured SRP concentrations, corrected for background, were compared with the original concentrations (typically 1 μM) to estimate

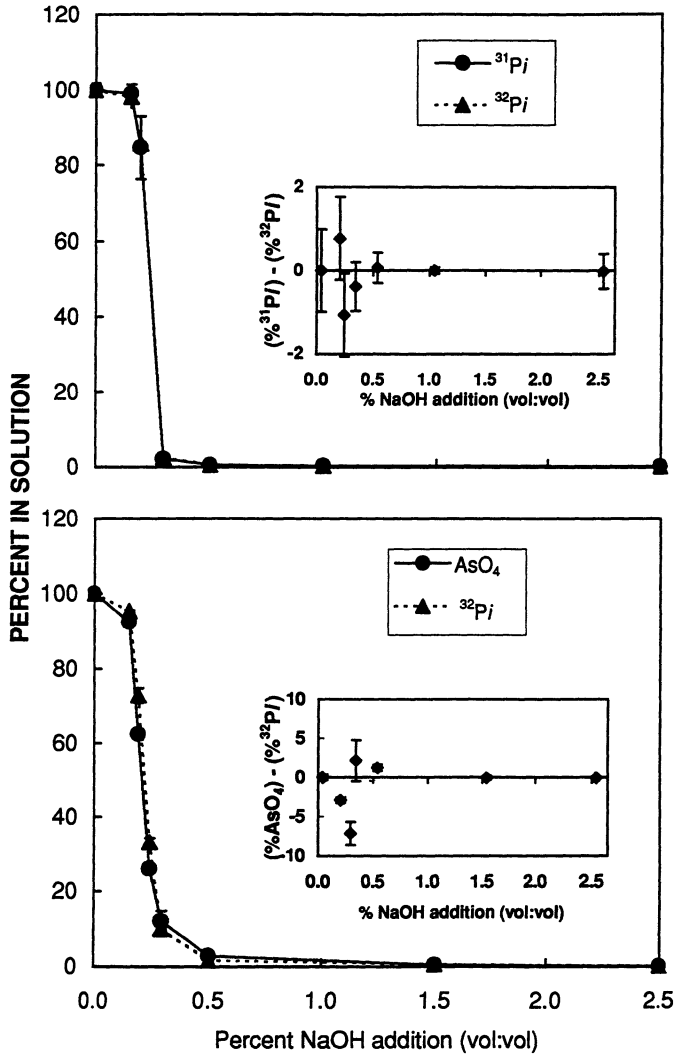


Fig. 1. Removal of ^{31}Pi and ^{32}Pi (top) and arsenate (AsO_4) and ^{32}Pi (bottom) by authigenic $Mg(OH)_2$ as a function of NaOH addition. Insets: Difference plots of percent remaining in solution.

the percent contribution from these P compounds to SRP measurements (Table 2). The tested compounds segregated into three groups. The first group, approximately half of the compounds tested, contributed about equally to SRP determinations with all three techniques. This group included nucleotide monophosphates, monophosphorylated sugars, nucleic acids, phosphorylated amino acids, phosphonates, and monophosphate esters. The second group contained compounds that contributed more to SRP determinations with the MAGIC procedure (an alkaline-based method). This group included most of the remaining compounds, particularly nucleotide di- and triphosphates, inorganic polyphosphates, and hexose diphosphate. The remaining compound, ribose-1-phosphate, contributed more to SRP determinations with the MR and AA acid-based methods.

SRP measurements—SRP measurements made with the modified MAGIC method developed in this study were done

Table 2. Mole percent contribution* of P-containing compounds by various soluble reactive P methods.

	Method		
Compound†	MAGIC	Murphy-Riley	Autoanalysis
dAMP	1.0±0.18	0.5±0.20	2.5±0.00
dCMP	0.7±0.15	2.1±0.07	0.5±0.74
dGMP	1.5±0.23	2.8±1.39	7.4±10.5
TMP	2.0±0.17	2.3±0.18	0.5±0.78
dUMP	0.5±0.16	0.5±0.08	1.7±0.78
ADP	13.1±0.08	8.8±0.65	3.9±0.80
dGDP	9.0±0.36	1.7±2.35	1.6±1.14
TDP	6.8±0.95	1.6±0.36	2.5±1.16
UDP	11.2±0.03	7.4±0.36	3.2±1.49
ATP	14.2±0.10	1.2±0.18	0.5±0.70
dATP	14.1±0.24	1.8±0.64	1.5±0.69
CTP	15.6±0.03	6.8±2.91	2.7±0.75
GTP	11.3±0.08	2.2±0.04	3.2±0.00
PP*i*	4.2±0.12	1.6±0.64	1.5±0.71
PPP	19.7±0.75	3.1±0.16	4.0±0.00
POLY-31	19.4±0.44	1.5±0.36	6.0±4.24
POLY-91	21.7±0.13	2.5±0.11	3.0±0.00
G-1-P	3.0±0.01	3.2±1.16	3.0‡
G-6-P	1.6±0.64	3.2±0.96	1.3±0.58
Gal-6-P	2.4±0.18	1.7±0.20	2.0±0.00
F-6-P	5.3±0.29	3.5±0.00	3.0‡
F-1,6-diP	12.2±0.35	0.5±0.44	2.0±0.00
RuBP	44.8±0.83	7.7±0.86	NA
R-1-P	40.0±2.35	85.6±1.57	83.4±1.12
R-5-P	6.3±0.81	3.4±0.84	1.7±0.58
DNA	2.8±0.12	0.6±0.32	3.0‡
RNA	1.3±0.45	1.3±1.27	0.0±1.00
GYP	1.0±0.17	1.4±0.47	1.0±0.00
PGA	3.2±0.80	3.0±0.16	4.0±0.00
PEP	11.9±0.19	2.0±0.26	0.5±0.72
P-Ser	6.7±0.09	5.0±0.68	7.5±0.71
P-Tyr	7.0±0.13	6.6±0.04	6.5±0.71
1-AEP	1.9±0.32	1.8±0.36	2.0±1.42
2-AEP	1.1±0.04	4.1±0.14	1.0±1.42
PFA	6.5±0.09	2.2±0.00	0.0±1.41
LEC	6.8±0.35	9.4±0.36	2.0±0.00
COL	0.9±0.61	3.8±0.32	0.5±0.71
INS	0.6±0.33	0.6±0.15	NA
PHY	0.0±0.59	0.4±0.16	1.5±0.71

* Values presented are mean percentages ± 1 SD (n = 3) for P compounds tested at 1 μM P concentrations. NA = sample not analyzed.
‡ Standard deviation not determined (i.e., n = 1).

to minimize the alkaline sample environment and, therefore, minimize the contribution of organic and inorganic P compounds to SRP concentrations. If samples analyzed by the MAGIC method, the AA method, and the modified MAGIC method all yield the same value, then the SRP concentration measured by all three techniques can be assumed to be close to the actual P*i* concentrations in seawater. Any discrepancy in results among the methods would indicate the additional contribution of N-P*i*P to SRP concentrations measured by the MAGIC and AA methods. Profiles of SRP concentrations analyzed by all three of these methods have been made at Sta. ALOHA (Fig. 2, Table 3). These results indicate that the P*i* measurements using the modified MAGIC method are consistently lower than those obtained with the standard AA method. Depth-integrated (0–100 m) SRP inventories for three separate months show that automated flow analysis can be >50% greater than the same profile analyzed with the modified MAGIC method (Table 3). However, the two MAGIC-based methods provide similar estimates of P*i*, indicating that the same P pools are probably measured by both. This finding suggests that those compounds showing variable contribution to SRP concentrations in an alkaline environment were not a significant fraction of P compounds in seawater during this 4-month period of investigation.

Retrospective comparisons of P*i* and SRP pool determinations at Sta. ALOHA over an 8-yr period document a variable offset among these three independent methods and indicate what we interpret to be time-varying changes in the chemical composition of the dissolved P pool (Fig. 3). For example, on HOT cruises 31, 41, 76, 79, and 80, the MAGIC and modified MAGIC methods yielded essentially identical estimates of P*i*, whereas paired SRP measurements using the AA method were significantly higher but by different amounts depending on the cruise. During other time periods (e.g., HOT cruises 45 and 85), estimates of SRP by both the MAGIC and AA methods were similar but higher than those obtained with the modified MAGIC method by up to fivefold. On still other cruises, all three estimates were different. Despite these variations, the estimate of P*i* using the modified MAGIC method was consistently <40 nM in these surface waters and, even with this partial data set, confirmed the trend of decreasing P*i* concentration with time that was recently reported for SRP measurements at this site by Karl and Tien (1997).

Comparison of TDP methods—Three TDP methods were evaluated to determine the recovery of selected P compounds. Results indicated that persulfate oxidation and alkaline-persulfate oxidation yielded greater recoveries of P compounds, whereas UV photooxidation was less effective at recovering nucleotide di- and triphosphates and inorganic polyphosphates (Table 4). These results suggest that samples analyzed with persulfate oxidation should yield as high or higher concentrations of TDP than those obtained with UV photooxidation, if the compounds poorly hydrolyzed by the UV photooxidation method are present in seawater.

Brucite adsorption experiments—A preliminary series of adsorption experiments was performed by measuring P compound removal at a single 2.5% (vol : vol) addition of NaOH (1 M). Results from these experiments indicate that some compound groups shared adsorption characteristics (Table 5). For seawater samples using the conditions recommended by the MAGIC procedure (Karl and Tien 1992), nucleotide triphosphates tested were almost completely removed from seawater (97.03% ± 1.30%; n = 4), as were nucleotide diphosphates (95.07% ± 2.50%; n = 4) and inorganic polyphosphates (97.05% ± 2.31%; n = 5). In contrast, a much lower percentage (29.43% ± 5.09%; n = 5) of the nucleotide monophosphates were coprecipitated with authigenic

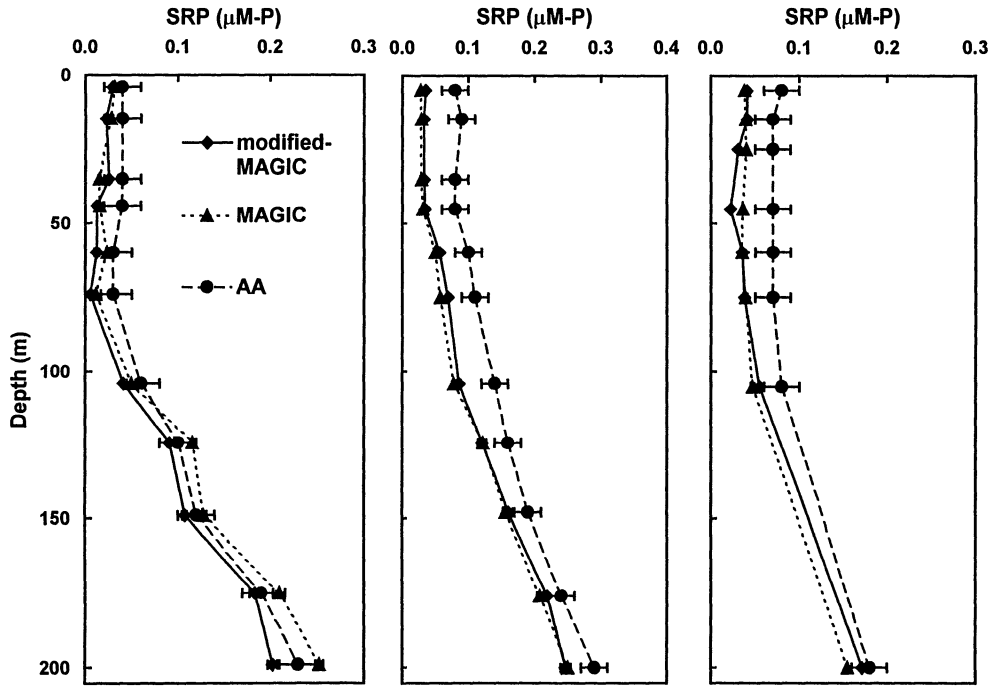


Fig. 2. Phosphorus concentration versus depth profiles measured with the modified MAGIC method, the SRP automated flow analysis (AA), and the MAGIC method at Sta. ALOHA: October 1996 (HOT-76) (left); January 1997 (HOT-79) (center); February 1997 (HOT-80) (right).

$Mg(OH)_2$. Five different P-containing compounds were also tested for removal from freshwater (Table 5). Results show that percentage removal from solution by brucite coprecipitation is similar for certain compounds (e.g., P*i* and ATP) but not for others (e.g., GYP, see Table 1). This suggests that further comparisons may be required before using the modified MAGIC method in freshwater habitats.

Complete adsorption curves were generated for a representative subset of marine P compounds listed in Table 1 (Figs. 4, 5). As expected, adsorption varied significantly among the different compounds. Although some compounds (e.g., dAMP; Fig. 4) were easily distinguished from $^{32}P_i$ by selective adsorption onto $Mg(OH)_2$, others were not (e.g., pyrophosphate [PP*i*]; Fig. 4). With this treatment, compounds with two or more phosphate groups per molecule were adsorbed to a greater degree than were those with only a single phosphate group. The positive surface charge of $Mg(OH)_2$ appears to attract negatively charged phosphate groups so that the greater the number of phosphate groups present, the stronger the interaction. If monophosphate organic derivatives dominate the dissolved P pool in seawater, the method developed here will be very effective in attaining a separation between P*i* and N-P*i*P. However, significant overestimation of P*i* could potentially occur from the presence of organic compounds with multiple phosphate groups.

Table 3. Depth-integrated (0–100 m) SRP concentrations at St. ALOHA.

	SRP (mmol P m^{-2})*		
Cruise and date	Automated flow analysis	MAGIC	Modified MAGIC
HOT-76, Oct 1996	4.28±1.40	2.54±0.09	2.08±0.01
HOT-79, Jan 1997	9.95±1.06	4.77±0.02	5.40±0.03
HOT-80, Feb 1997	7.50±1.14	4.14±0.03	3.90±0.08

* Values presented are integrated values ±1 SD.

A survey of selected coastal marine habitats around Oahu, Hawaii, revealed significant differences in the adsorption characteristics of TDP both among the various coastal sites and especially between the coastal sites and the open ocean waters collected at Sta. ALOHA (Table 6). For example, the removal of TDP by a 2.5% (vol:vol) addition of NaOH (1 M) ranged from 70% (±2.1%) to 92% (±0.3%) for a variety of coastal marine habitats. By comparison, surface seawater samples collected at Sta. ALOHA treated with the same NaOH additions removed only about 50% of TDP. This response was remarkably consistent over an approximately 8-yr period (mean = 52.3%, SD = 4.5%; range = 46.2–60.8%; 14 cruises from October 1989 to July 1997). These results provide strong evidence for differences in the chemical composition of the respective N-P*i*P pools from diverse ecosystems and for relative constancy in the chemical composition of the N-P*i*P pool at Sta. ALOHA.

N-PiP measurements—Estimates of deep water N-P*i*P concentrations were obtained for several profiles from Sta. ALOHA. By the addition of suboptimal concentrations of NaOH, the removal of P*i* was incomplete but the separation of P*i* and N-P*i*P pools was maximized. With the observation that P*i* is the most easily removed compound from seawater and that incomplete coprecipitation of P*i* leaves the vast ma-

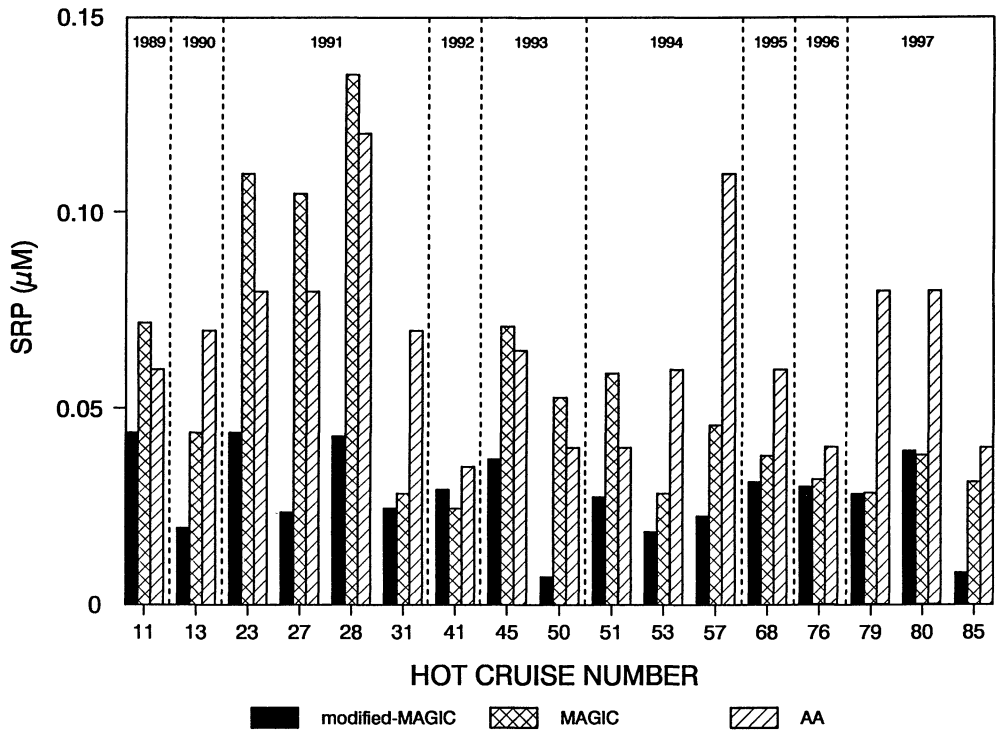


Fig. 3. Comparisons of SRP determinations, by MAGIC and AA protocols, and P*i* estimates, by the modified MAGIC method, for selected HOT cruises. The data presented are for surface seawater samples (5 m) collected at Sta. ALOHA. The HOT cruises were conducted in the following months of the years shown: January (cruises 13, 51, 79), February (23, 45, 80), March (53), June (27), July (28, 85), September (57), October (11, 31, 41, 50, 76), November (68).

jority of other P compounds in solution, the total P concentration of the nonprecipitated sample fraction was analyzed. After correcting for the small fraction of P*i* remaining in the supernatant (generally <10% of total) through the use of ^{32}P*i*, the N-P*i*P concentration of the seawater can be determined. The deep water profiles (>1,000 m) analyzed yielded N-P*i*P concentrations <50 nM, with some values indistinguishable from zero (0 ± 10 nM, Fig. 6).

"Missing" P pools—By estimating P*i* concentrations concurrently with N-P*i*P and TDP concentrations in surface water, this modified MAGIC method may make it possible to define a previously undescribed P pool. After correcting the P*i* and N-P*i*P concentrations for residual ^{32}P*i* in solution, the independent estimates of P*i* and N-P*i*P concentrations should sum to the initially measured TDP if all P pools are measured with this technique. In fact, a discrepancy beyond that of analytical error is sometimes observed. For example, the "missing" P pools for the 0–100 m depth-integrated inventories for HOT-76 and HOT-79 are 3.2 ± 0.1 mmol P m^{-2} and 3.3 ± 0.1 mmol P m^{-2}, respectively, values equivalent to approximately 13% of the TDP pool. This discrepancy must be due to the adsorption of compounds from seawater onto $Mg(OH)_2$ (therefore not part of the N-P*i*P concentration measurement) that do not react to form a phosphomolybdate complex (and not part of the P*i* concentration measurement). From observations of the adsorption characteristics of a wide variety of P-containing compounds that have been performed to date (Table 1), only inorganic polyphosphates, which adsorb similarly to P*i* (Fig. 4) but do not fully react to contribute to SRP concentrations (<20% contribution to SRP, see Table 2), have these necessary characteristics. The "missing" P may be an indication, albeit indirect, of the presence and concentration of these compounds in seawater.

Discussion

The modified MAGIC method developed in this study provides high-precision estimates of both P*i* and N-P*i*P concentrations and has ecological application for surface to deep waters in both oligotrophic and nutrient-rich regions of the world oceans and limnetic habitats. The method can also be employed for broad chemical characterizations of the dissolved P pools in seawater. Other researchers have attempted such classifications based on compound reactivity to different analytical methods (Solórzano and Strickland 1968; Strickland and Parsons 1972; Ridal and Moore 1992; Shan et al. 1994; Karl and Yanagi 1997). A synthesis of these different classification schemes on the same seawater sample may lead to a more comprehensive understanding of composition and dynamics of dissolved phosphorus pools in seawater. Two unique features of the modified MAGIC method are (1) the ability to separate the dissolved P pools into pellet and supernatant fractions by adsorption to $Mg(OH)_2$ and (2)

Table 4. Percentage recovery* of P compounds using a variety of total dissolved P methods.

Compound†	Method		
	Persulfate	NaOH–Persulfate	UV
dAMP	89±1.2†	106±10.7	91±20.6
dCMP	102±1.9	66±4.5	97±0.7
dGMP	96±0.9	136±23.5	84±21.0
TMP	110±2.3	74±2.1	96±8.5
dUMP	101±1.4	75±4.5	93±1.6
ADP	103±0.7	103±29.1	19±4.8
dGDP	100±1.5	47±30.2	11±4.9
TDP	112±1.0	108±5.4	15±2.3
UDP	107±2.7	93±24.0	14±1.5
ATP	108±1.3	91±8.9	35±13.3
dATP	101±1.0	95±5.7	35±6.9
CTP	96±0.6	67±9.3	26±4.5
GTP	96±1.3	86±9.5	30±15.0
PP*i*	102±1.6	92±0.2	3±2.8
PPP	96±1.3	97±2.6	18±0.0
POLY-31	94±1.0	79±11.0	19±8.5
POLY-91	88±0.5	72±13.8	26±6.4
G-1-P	98±0.7	88±1.1	96±0.0
G-6-P	101±2.0	116±44.3	97±1.0
Gal-6-P	87±0.3	103±6.0	89±0.7
F-6-P	98±1.7	98±5.2	94±0.0
F-1,6-diP	99±0.5	103±9.2	88±6.3
RuBP	97±0.4	NA	NA
R-1-P	85±1.0	78±12.9	84±6.8
R-5-P	97±4.2	103±30.1	93±8.7
DNA	75±0.2	74±1.4	30±0.0
RNA	78±1.1	64±8.8	56±3.8
GYP	105±2.2	66±18.2	86±14.1
PGA	99±0.6	94±2.5	89±3.5
PEP	97±1.1	71±15.4	93±11.6
P-Ser	101±0.7	84±7.2	99±2.1
P-Tyr	97±0.7	97±8.7	91±2.8
1-AEP	98±0.6	69±0.8	94±4.2
2-AEP	81±1.5	89±30.0	81±12.0
PFA	104±1.0	69±2.8	100±4.2
LEC	39±2.1	61±8.4	89±3.5
COL	73±0.4	66±26.6	88±14.1
INS	97±0.6	NA	NA
PHY	63±2.1	103±5.6	69±12.0
Overall mean ± SD	94±13.7	86±18.4	65±34.1

* Values presented are mean percentages ± 1 SD (n = 3) for P compounds tested at 1 μM P concentrations. NA = sample not analyzed.
† See Table 1 for compound names.

Table 5. Percentage removal from supernatant of selected P compounds by $Mg(OH)_2$ coprecipitation.

Compound*	Sample type†	Removal‡ (%)
dAMP	SW	21.4±8.4
dCMP	SW	29.7±11.8
dGMP	SW	24.6±17.2
TMP	SW	33.5±4.8
TMP	FW	51.3±0.3
dUMP	SW	29.6±4.7
ADP	SW	91.7±2.3
dGDP	SW	97.8±1.7
TDP	SW	95.5±1.4
UDP	SW	95.3±1.8
ATP	SW	97.1±1.3
ATP	FW	99.5±0.1
dATP	SW	96.7±0.7
CTP	SW	96.0±0.7
GTP	SW	98.9±0.6
P*i*	SW	99.9±0.9
P*i*	FW	99.8±0.1
PP*i*	SW	99.5±1.2
PPP	SW	98.1±0.5
POLY-31	SW	95.4±0.6
POLY-91	SW	94.4±0.7
F-6-P	SW	49.7±1.9
F-6-P	FW	73.0±0.5
F-1,6-diP	SW	99.9±1.1
RNA	SW	98.8±1.4
RuBP	SW	95.4±1.4
GYP	SW	12.0±1.8
GYP	FW	90.3±0.4
PEP	SW	40.1±2.2
P-Ser	SW	58.7±1.2
2-AEP	SW	26.9±1.7
INS	SW	20.5±1.3
PHY	SW	95.7±5.7

* See Table 1 for compound names.
† SW = seawater; FW = tap water, supplemented with $MgCl_2$.
‡ Following authigenic brucite ($Mg(OH)_2$) formation at 2.5% (vol:vol) NaOH (1 M) addition. Values presented are mean percentages ± 1 SD (n = 3).

the ability to recover the two isolated fractions for P analysis by a variety of methods.

SRP and Pi concentrations—The relative contributions of selected organic and inorganic P compounds to the traditional SRP analysis (Table 2) suggest that MAGIC-based SRP concentrations (both MAGIC and modified MAGIC) should be higher than those determined by the AA method. The fact that the three profiles analyzed in this study show that the two MAGIC-based methods yielded similar concentrations, both less than those obtained with the AA method, may give some indication of the nature of nonphosphate SRP compounds (e.g., acid-labile organic compounds) in seawater. The modified MAGIC method is designed to minimize the contribution of alkaline-labile compounds to SRP concentrations, and the fact that little difference was seen between the two separate MAGIC-based analyses suggests that alkaline-labile compounds were not present at significant concentration during this period of investigation.

Significant differences in SRP concentrations obtained with the MAGIC-based versus AA methods likely arise from two sources. First, both MAGIC and modified MAGIC samples are reduced to eliminate AsO_4 interference. AA methods

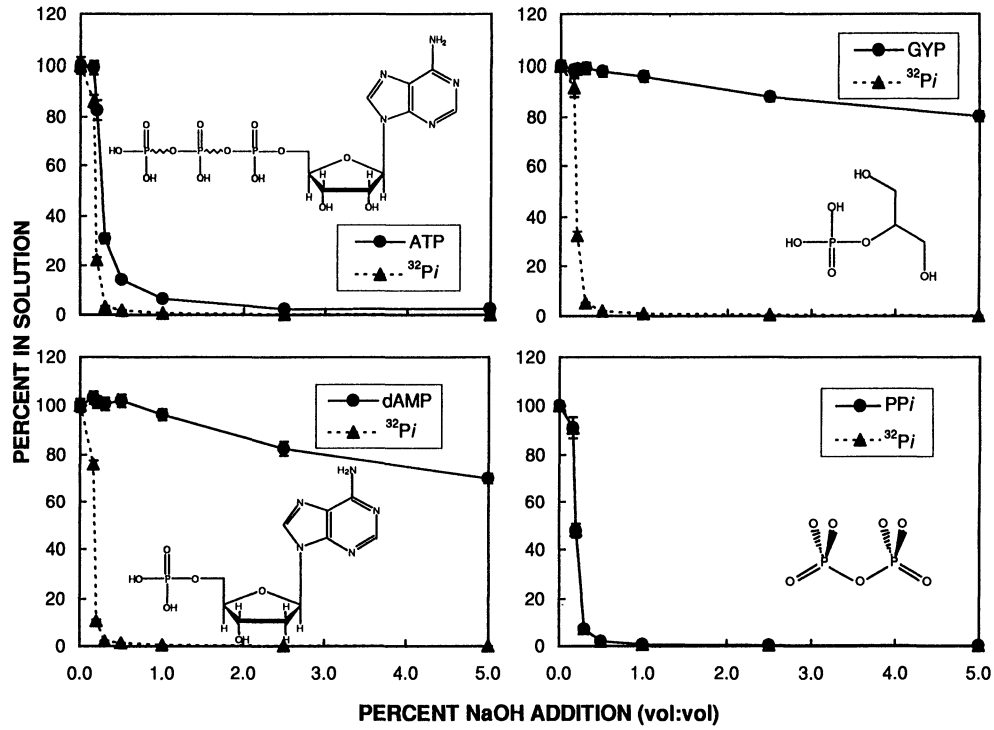


Fig. 4. $Mg(OH)_2$ adsorption experiments for selected P compounds, relative to ^{32}Pi adsorption, as a function of NaOH addition. Error bars indicate ± 1 SD of the mean values but may be within the symbol. Clockwise from upper left: ATP, GYP, PP*i*, dAMP.

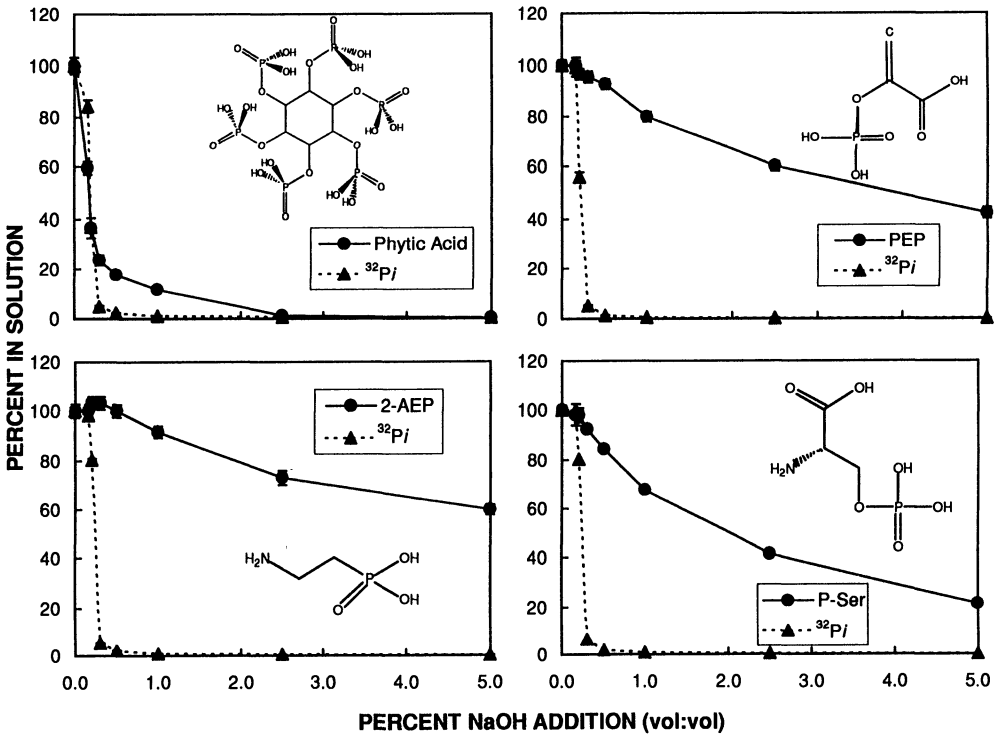


Fig. 5. Adsorption experiments, as in Fig. 4, except for PHY, PEP, P-Ser, 2-AEP.

Table 6. Phosphorus pool determinations and percentage of total dissolved P coprecipitation onto authigenic brucite for a variety of coastal marine and open ocean ecosystems around Oahu, Hawaii.

Location	Phosphorus pool determinations (μMP)*				TDP§ removed by $Mg(OH)_2$ (%)
	P*i*†	SRP‡	N-P*i*P†	TDP	
Kailua Beach, Oahu	0.034 (±0.002)	0.056 (±0.000)	0.103 (±0.004)	0.206 (±0.009)	70 (±2.1)
Ala Moana Beach, Oahu	0.063 (±0.002)	0.104 (±0.001)	0.123 (±0.005)	0.277 (±0.006)	74 (±2.6)
Ala Wai Canal, Oahu	0.196 (±0.010)	0.257 (±0.001)	0.258 (±0.009)	0.686 (±0.006)	81 (±0.7)
Ala Moana Boat Basin, Oahu	0.821 (±0.016)	0.769 (±0.003)	0.133 (±0.006)	0.966 (±0.011)	92 (±0.3)
ALOHA (HOT-85, Jul 1997)	0.008 (±0.001)	0.030) (±0.001)	0.138 (±0.004)	0.222 (±0.015)	52 (±4.1)

* Mean (±1 SD) (n = 3).
† Measured using modified MAGIC method.
‡ Measured using MAGIC method.
§ Calculated as difference between TDP in original sample and TDP remaining in solution following a standard (2.5% NaOH, vol:vol) MAGIC treatment.

do not routinely include this step, so these samples would overestimate SRP. Historical data from Sta. ALOHA for 1988–1996 indicate a depth-integrated (0–100 m) AsO_4 pool of 2.14 (±1.02) mmol m^{-2} (Karl and Tien 1997). If SRP concentrations obtained with AA methods are corrected downward by this amount, they would still exceed P*i* concentrations by almost 50% during 2 of the 3 months studied. It is also likely that this correction overestimates AsO_4 interference because the AA procedure does not allow samples to react long enough for full AsO_4 interference to develop (90 min; Johnson 1971). This excess SRP measured using the AA technique indicates the potential presence of a substantial pool of acid-labile compounds that do not adsorb to $Mg(OH)_2$. If a compound is acid labile but does not adsorb to $Mg(OH)_2$, it cannot contribute to SRP concentrations measured by MAGIC because it is separated from the analyzed portion of the sample prior to acidification. Conversely, if a compound adsorbs to $Mg(OH)_2$ and is acid labile, it should contribute equally in both SRP measurement methods. By comparing the compounds that are adsorbed to $Mg(OH)_2$ under standard MAGIC conditions (Table 5) with the compounds that contribute to SRP concentrations (Table 2), it is possible to determine which compounds or compound classes might be present in a given seawater sample.

Adsorption to $Mg(OH)_2$ does not necessarily mean that a compound hydrolyzes to contribute to the SRP concentration. For example, PP*i* is almost completely adsorbed to $Mg(OH)_2$, but a 1 μM P solution of PP*i* will only yield 0.04 μM SRP, a relative molar reactivity of 4%. This observation suggests that there are at least three P pools in seawater: (1) those that are alkaline labile and contribute to SRP concentrations when exposed to a basic environment, (2) those that are acid labile, and (3) those that are stable over a wide pH range. Within the group of acid-labile compounds are those that adsorb to $Mg(OH)_2$ and those that do not.

The chemical characteristics of these P pools can be used to infer the magnitude of their presence from differences between the three methods. For example, if SRP concentrations obtained with MAGIC methods exceed those obtained with AA, it would indicate the presence of a pool of alkaline-labile compounds. Similar measured concentrations could result from the absence of N-P*i*P compounds that contribute to SRP (i.e., P-containing compounds that are stable over a wide pH range) or the presence of acid-labile compounds that are fully adsorbed to $Mg(OH)_2$. These fully adsorbed compounds would contribute to SRP estimation with both AA and MAGIC methods. In this case, results from the modified MAGIC approach would be used to estimate the P*i* concentrations. If the three estimates are the same, SNP compounds are neither alkaline nor acid labile (i.e., nucleotide monophosphates). If modified MAGIC SRP concentrations are lower than the those obtained with the other two methods, the characteristics of the nonphosphate SRP measured by the AA and MAGIC methods could be determined.

N-PiP concentrations—Concentrations of N-P*i*P in deep seawater measured in this study were always <50 nM from 1,000 to 4,500 m depth. These concentrations varied from 41 nM to zero (±10 nM), indicating that at times, N-P*i*P is completely removed from deep seawater. Although other investigators have determined deep water SNP (also referred to as DOP) concentrations (Holm-Hansen et al. 1966; Williams et al. 1980; Ridal and Moore 1992), measurements have been plagued by relatively high error estimates (as much as ±90 nM; Smith et al. 1986). With our modified MAGIC method, N-P*i*P concentrations can be estimated with relatively high precision. The presence of low concentrations of N-P*i*P in deep waters suggests that combined P compounds produced in surface waters become available to organisms, and the availability is simply a function of time and mixing rates (Ridal and Moore 1992). The carbon:nitrogen:phosphorus (C:N:P) ratio of deep water dissolved organic matter (DOM) at the 1,000-m reference depth estimated from the 9-yr data set at Sta. ALOHA is 2,600:129:1,

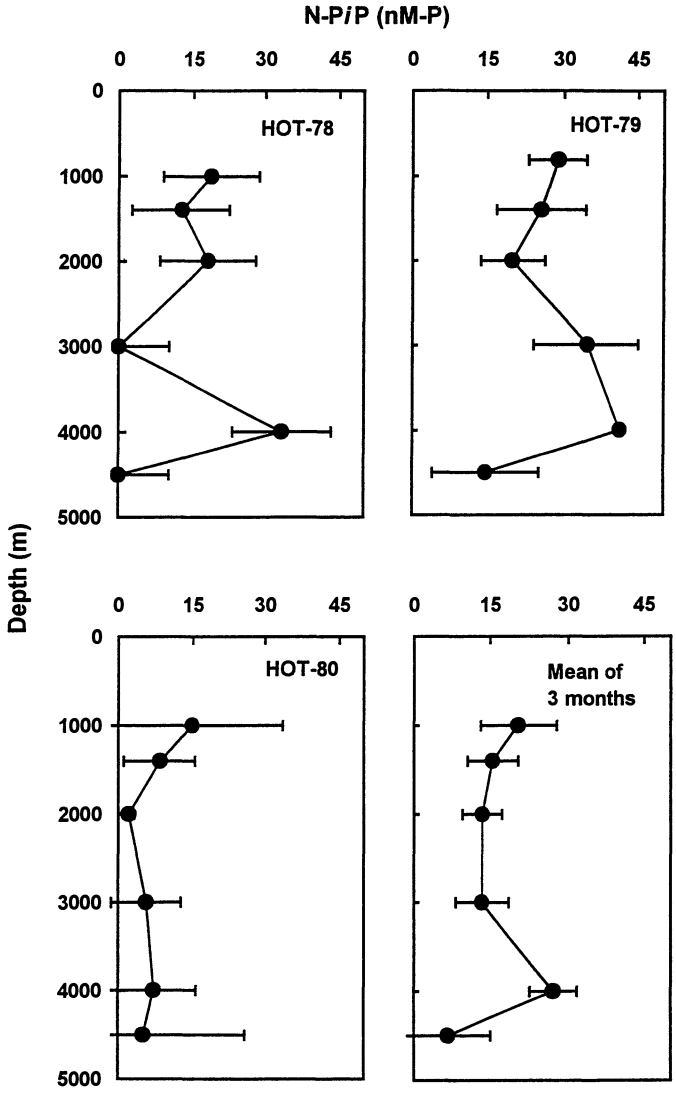


Fig. 6. Concentration versus depth profiles for deep water N-P*i*P at Sta. ALOHA during December 1996 (HOT-78), January 1997 (HOT-79), and February 1997 (HOT-80). Also shown is a mean N-P*i*P concentration versus depth profile for the 3-month period of observation.

using a mean N-P*i*P concentration of 16.2 (±12.5) nM and mean dissolved organic nitogen and dissolved organic carbon concentrations from the HOT program database (http://hahana.soest.hawaii.edu/). Compared with the measured 1,000-m particulate organic matter (POM) composition ratio of 190:26:1 (HOT program database), the dissolved pool is significantly depleted in N and P. This result suggests that as POM is degraded and cycles through the DOM pool, P-containing DOM is preferentially remineralized, resulting in the accumulation of less reactive DOM at depth with extremely high C:P and N:P ratios. These observations allow some speculation as to the types of DOM compounds that dominate the refractory pool in the deep ocean.

Other applications—The modified MAGIC method has several applications in P*i* uptake and regeneration experiments. First, a more accurate estimation of the P*i* pool leads to improved calculation of P*i* uptake and regeneration rates using ^{32}P*i* or ^{33}P*i* radiolabeled tracers. In addition, experiments to measure the production of DOP have been difficult, in part because of the problem of the separation of a generally small pool of ^{32}P-DOP from a generally large pool of ^{32}P*i* (Orrett and Karl 1987). The modified MAGIC method can be used to separate these two fractions, thereby providing direct estimations of ^{32}P-DOP production in seawater incubation experiments.

An additional application of this method is in the concentration and separation of specific P-containing compounds in the laboratory. For example, compounds that are present in seawater at very low concentrations could be concentrated 100-fold or more with the modified MAGIC technique. Alternatively, other compounds could be excluded from the concentration by carefully selecting a NaOH : sample volume ratio, something that may be useful when attempting to separate different compounds of similar reactivity (i.e., dissolved ATP from dissolved AMP). This concentration step could allow analysis of compounds previously hampered by values near detection limits and could increase the analytical precision of existing methods.

Summary

The characterization of the dissolved P pools in the ocean is still far from complete, but this new modified MAGIC method provides another tool for reaching this goal. The ease of analysis and potential for combination with other techniques makes the modified MAGIC method a valuable approach for the description of compound classes in the N-P*i*P pool. Combining this technique with other dissolved P pool characterization methods such as enzyme hydrolysis (Kobori and Taga 1979), partial UV photooxidation (Karl and Yanagi 1997), and preanalysis molecular weight separations would present a more complete picture of the N-P*i*P pool in seawater. Moreover, estimations of P*i* and deep water N-P*i*P concentrations are vital to our understanding of P turnover and residence times and of the relative utilization of DOM in the deep sea. If P is truly limiting over large regions of the world oceans, accurate and simultaneous measurements of P*i* and N-P*i*P concentrations are crucial for predicting primary and secondary production in the sea. Ultimately, these calculated rates of ocean productivity can lead to more accurate estimations of CO_2 uptake and sequestration into the ocean.

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Received: 8 December 1997
Accepted: 25 June 1998