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MAGIC: A sensitive and precise method for measuring dissolved phosphorus in aquatic environments

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Abstract

A simple and straightforward method has been developed for the precise determination of nanomolar concentrations of soluble reactive phosphorus (SRP) and total dissolved phosphorus (TDP) in marine and freshwater environments. The method relies on the quantitative removal of P from solution by in vitro formation of brucite [$Mg(OH)_2$], initiated by the addition of NaOH. The precipitate is collected by centrifugation and either dissolved in HCl for SRP determinations or hydrolyzed (8 M HCl, 80°C, 1 h), dried, and ashed (450°C, 3 h) for TDP determinations. Both procedures are completed by reaction with the standard Murphy-Riley molybdenum blue reagents. This *mag*nesium-*i*nduced *co*precipitation (MAGIC) procedure can be used to effect a 100-fold concentration, thereby providing a method which can reliably detect 1 nmol SRP liter^{-1} of seawater. MAGIC can also be used to determine P in Mg^{2+}-amended freshwater samples. The method is highly reproducible; typical estimates of precision for triplicate SRP determinations in the 10–100 nM range are from 1 to 3%. Field trials of the MAGIC procedure in the North Pacific Ocean reveal mixed-layer SRP concentrations from 30 to 100 nM, with substantial temporal variability. The method is versatile and has many other potential applications.

Phosphorus is an essential nutrient for the growth of all organisms. In pelagic marine ecosystems, the supply rates of both N and P exert primary controls on ecosystem productivity (Smith et al. 1986). Over long time scales, P rather than N is probably the biomass- and production rate-limiting nutrient in the global ocean (Redfield 1958; Codispoti 1989), due to P exhaustion especially in shallow-water, carbonate-dominated ecosystems and to the potential role of bacterial N_2 fixation as a mechanism for relieving the ecosystem of N limitation. Consequently, field studies of P cycling in the epipelagic zone are of fundamental importance for our understanding of microbial processes in oligotrophic oceanic habitats. Nevertheless, investigations conducted beyond the continental shelf are rare (Perry and Eppley 1981; Herbland 1984; Sorokin 1985; Harrison and Harris 1986; Orrett and Karl 1987), especially in comparison to the large body of scientific literature on N cycling in oceanic habitats. This situation is due, in part, to technical limitations in the ability to obtain precise and accurate determinations of low concentrations of dissolved P.

Quantitative analyses of P in seawater have traditionally relied on the formation of a 12-molybdophosphoric acid (12-MPA) complex and its subsequent reduction to yield a highly colored blue solution, the extinction of which is measured by absorption spectrophotometry (Fiske and Subbarow 1925; Murphy and Riley 1962). Over the years, numerous improvements have been introduced to the basic method so that substantial variability now exists in the conditions used for color development, final reduction of the 12-MPA complex, and the treatment of potentially interferring compounds.

Although the stepwise chemical procedures for P determination are straightforward and fully amenable to automated analysis (Armstrong et al. 1967), there are many complexities, both analytical and conceptual, inherent in measuring and interpretating P concentrations in seawater (Tarap-

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chak 1983). For example, the soluble reactive P (SRP) pool measured by the standard Murphy and Riley procedure is not necessarily the concentration of (ortho)phosphoric acid but may also include dissolved organic P (DOP) compounds that are hydrolyzed under the acidic reaction conditions. The potential contribution to SRP concentration from P-ester hydrolysis is minimized by reducing [H^+], shortening the acidic contact period, or using citrate to consume the unused Mo reagent following a brief contact period sufficient for P reaction (Baginski et al. 1967).

Strickland and Parsons (1972) have defined eight classes of P compounds which are based on reactivity with the acidic molybdate reagents, ease of hydrolysis, and particle size. Most investigators measure both SRP and total dissolved P (TDP); TDP is also quantified by the molybdenum blue reaction following either UV photo-oxidation (Armstrong et al. 1966), high-temperature acid persulfate digestion (Menzel and Corwin 1965), or both (Ridal and Moore 1990). Depending on the hydrolysis method used, the difference between TDP and SRP is sometimes defined as DOP. However, it is not known whether these pragmatic distinctions have any relevance to the biologically available P (BAP) pool which is of primary interest in field studies of microbiological P uptake and P regeneration. The few studies which have compared SRP, TDP, and BAP pools indicate that differences do exist among these pools and that these differences are habitat-dependent.

In oligotrophic oceanic environments, dissolved-P determinations are unreliable because the ambient-P concentrations approach the limit of analytical detection (0.02–0.04 μM) with the standard Murphy and Riley method. At these low concentrations, the variability of replicate determinations can be $\pm 25\%$ or greater. Consequently, several modifications and new analytical procedures have been introduced to improve the detection limit and precision of environmental P analyses (e.g. long pathlength capillary cell absorption spectrometry, use of organic solvents to extract and concentrate the dye from aqueous solutions, use of malachite green oxalate which has a higher molar absorptivity than 12-MPA, X-ray fluorescence spectrometry, laser-induced thermal lensing colorimetry, and conversion of P to phosphine gas with subsequent detection by either gas chromatography or chemiluminescence).

In this report we describe a novel *mag*nesium-*i*nduced *co*precipitation (MAGIC) procedure which concentrates SRP by pH-induced, in vitro brucite [$Mg(OH)_2$] formation followed by dissolution and addition of the molybdenum blue reagents. The method is rapid, precise, and ideal for samples formerly at or below the lower limit of detection. SRP concentrations as low as 1 nmol P liter^{-1} can be measured. Experiments with various DOP compounds and inorganic polymers of P suggest that this procedure may also be used to determine TDP. Field trials conducted at the US JGOFS Hawaii Ocean Time-Series (HOT) Station ALOHA provide evidence for its successful application to oligotrophic seawaters.

Materials and methods

Reagents—All reagents were of at least analytical grade. Our intention was to devise a method for nanomolar-level determinations of P, so we were especially concerned about potential sources of contamination. Accordingly, we recommend the use of Fluka NaOH (Fluka BioChemika No. 71689) and Baker Instra-Analyzed HCl (Baker No. 9530-33) for $Mg(OH)_2$ precipitation and dissolution, respectively. All reagents required for the molybdenum blue reaction (sulfuric acid, ammonium molybdate, potassium antimonyl tartrate, and ascorbic acid) were also tested for P contamination. Of these, ammonium molybdate and sulfuric acid contained the highest concentrations of P. We compared ammonium molybdate distributed through Fisher, Baker, Mallinckrodt, and MCB for reactivity to a standard P concentration and for degree of P contamination. There were no significant differences among reagents from these commercial sources.

Standard brucite precipitation procedure—During the course of this study our standard procedure for P analysis in oligotrophic seawaters near Hawaii evolved considerably toward optimal sensitivity, repro-

ducibility, and ease of analysis. Seawater samples were collected with 12-liter Niskin or Go-Flo bottles (General Oceanics Co.) attached to a CTD-rosette system. Immediately upon retrieval, 500-ml subsamples were collected into clean, HCl-washed (1 M) linear polyethylene bottles that were rinsed three times with seawater before final filling. If total particulate P (PP) is $\leq 10\%$ of the expected TDP concentration, filtration is not necessary and is even undesirable because of potential contamination. For the oligotrophic seawaters at Station ALOHA (22°45′N, 158°W), PP was ≤ 20 nM so filtration was not routinely performed. If required, however, samples should be filtered through combusted (450°C, 4 h), acid-washed glass-fiber filters (Whatman GF/F-type) into acid-washed plastic filter units or their equivalent (Millipore Corp.). The samples are immediately frozen (−20°C), or immediately treated with NaOH to induce $Mg(OH)_2$ formation (*see below*). The number of sample replicates required for P determination by the MAGIC procedure will vary depending on the experimental objectives and sample source. For precise P determinations, we recommend triplicate measurements of both SRP and TDP.

Frozen samples are thawed, mixed thoroughly, and triplicate 50-ml aliquots placed into clean disposable polypropylene centrifuge tubes or acid-washed borosilicate glass test tubes (the latter are required for TDP analyses; *see below*). NaOH (1 M) is added at a 1 : 40 vol/vol ratio (e.g. 1.25 ml per 50 ml of seawater). At this point, a milky-white precipitate which we have identified as brucite (*see below*) forms. The tubes are capped, mixed thoroughly, incubated for 5 min at room temperature, then mixed and incubated for another 5 min before centrifugation (1,000 × *g* for 60 min at room temperature). The clear supernatant is carefully aspirated with a Pasteur pipette attached to a vacuum line and then discarded. Without further treatment, 8.5 ml of 0.1 M HCl is added, followed by intermittent agitation with a vortex mixer until the pellet is completely dissolved. Sample volumes are then brought to 10.0 ml with 0.1 M HCl. This standard procedure effects a quantitative 5-fold concentration of SRP and TDP. If desired, the original seawater volume can be increased or the volume of HCl used to dissolve the pellet can be decreased (or both) in order to concentrate P by a factor of 100-fold or more before analysis.

At this point, the concentrated samples are treated exactly as untreated seawater samples in the Murphy and Riley reaction either manually or with automated or flow injection analyses. For manual determinations, 1 ml of the molybdenum blue mixed-reagent (Murphy and Riley 1962; Strickland and Parsons 1972) with a $[H^+]$: [Mo] ratio of 71 is added, and the color is allowed to develop for ≥ 20 min before absorbance (880 nm) is read in a double-beam spectrophotometer (Varian model DMS 100S) with a 10-cm pathlength. Corrections for As^{5+} interference are made with thiosulfate reduction according to the method of Johnson (1971).

During the course of our investigation, we evaluated several and now recommend two separate procedures for TDP analysis: dry combustion-persulfate oxidation and acid hydrolysis-dry combustion-persulfate oxidation. The primary difference between the two methods is the initial hot HCl hydrolysis and potassium iodide treatment prior to dry ashing at 450°C. Other procedures are also possible (*see results and discussion section*) and the choice is up to the individual investigator.

For TDP analysis, replicate subsamples are treated by the standard MAGIC procedure, as described above, with glass test tubes. For the initial acid hydrolysis step, the $Mg(OH)_2$ pellets are dissolved in 1 ml of 8 M HCl and heated (80°C) for 1 h, followed by addition of 100 μl of potassium iodide solution (5% wt/vol in distilled water) and removal of the HCl by evaporation in vacuo (Savant Speed-Vac model HC-250). Arsenic present in the sample is reduced to the trivalent form by iodine in acid solutions and is subsequently lost by evaporation as chloride (Strickland and Parsons 1972). We have confirmed the quantitative loss of both As^{5+} and As^{3+} in the MAGIC procedure by this treatment. From this point, the two hydrolysis methods are identical. The tubes are placed into a furnace and dry ashed at 450°C for 3 h. After ad-

dition of 1.6 ml of $K_2S_2O_8$ (5% wt/vol in distilled water), the samples are placed in an autoclave and hydrolyzed at 120°C for 30 min. The samples are allowed to cool before bringing the total volume to 10 ml with distilled water. Finally, the samples are treated exactly as SRP samples (*see above*) in the Murphy and Riley reaction by either manual or automated analysis.

Preparation of standard curve—A P standard curve is routinely prepared in distilled water from a stock solution of reagent-grade KH_2PO_4. A commercial P standard (CSK-nutrient element, Wako Chemicals No. 27874099) is used as the primary standard. Internal standard additions of PO_4^{3-} were performed on selected samples as further verification of overall accuracy. The presence of Mg^{2+} in the concentrated seawater samples has no effect on the quantitative determination of SRP as assessed by direct comparison of PO_4^{3-} standard curves taken through the entire MAGIC procedure with control distilled water or seawater-based PO_4^{3-} standards. The additional PO_4^{3-} contributed by NaOH and HCl will vary depending on reagent source and concentrations used (*see results and discussion*) and must also be accounted for in the blank determination. In this regard, greater accuracy will be achieved when an entire standard curve, covering the full range of expected sample concentrations, is carried through the MAGIC procedure. For TDP determinations, the standard curve must also include $K_2S_2O_8$ and any other reagents used in the hydrolysis step. If P-free seawater or freshwater is required to provide a low-level (e.g. 1–30 nM) P standard curve, one can use the supernatant from a NaOH-treated sample as the diluent.

Results and discussion

Chemistry of P coprecipitation—During a series of experiments designed to evaluate the removal of PO_4^{3-} from seawater by coprecipitation with iron hydroxide, we discovered that adding NaOH to seawater, without added Fe (our negative control) initiated rapid formation of a milky-white precipitate which quantitatively removed $^{32}PO_4^{3-}$ from solution. The isolated precipitate was readily soluble in HCl.

When the precipitate was collected by low-speed centrifugation, washed with 0.01 M NaOH to remove seawater salts, dried at 60°C, and analyzed by X-ray diffraction analysis, $Mg(OH)_2$ was found to be the dominant species (Fig. 1A, C). An identical precipitate which is capable of scavenging $^{32}PO_4^{3-}$ also formed when NaOH was added to Mg^{2+}-amended (10 mM) distilled water (Fig. 1B). The absence of potentially co-occurring $MgCO_3$ (magnesite) and $CaCO_3$ (calcite/aragonite) in the X-ray pattern was confirmed by comparison of the weight percent of CO_3-C by CO_2-coulometry (Johnson et al. 1985). The inorganic C contents of our oven-dried seawater and freshwater-derived $Mg(OH)_2$ precipitates were 0.98 and 0.58%, respectively, compared to a value of 0.64% for the commercially available $Mg(OH)_2$ reagent (Fluka BioChemika No. 63081). Furthermore, when commercially available $Mg(OH)_2$ was added to a sample of either seawater or freshwater containing tracer amounts of $^{32}PO_4^{3-}$ [100 mg $Mg(OH)_2$ per 10-ml sample], the $^{32}PO^{3-}$ was immediately and quantitatively removed from solution. From these results, we conclude that pH-induced $Mg(OH)_2$ formation is responsible for the scavenging of dissolved P from solution.

Optimization of PO_4^{3-} removal—We conducted several experiments to investigate the conditions necessary for quantitative P recovery during $Mg(OH)_2$ formation. Variables tested include: NaOH concentration, centrifugation force and time, pellet rinsing, pellet dissolution, and application of MAGIC to freshwater samples.

The stepwise titration of surface seawater with 1 M NaOH results in rapid formation of $Mg(OH)_2$, which increases in mass with increasing NaOH concentration and, therefore, pH (Fig. 2A). $Mg(OH)_2$ formation appears to be directly responsible for the scavenging of dissolved PO_4^{3-} (Fig. 2B and C). However, it is important to note that removal of PO_4^{3-} from solution and recovery in the pellet are both quantitative following addition of a threshold concentration of 1 M NaOH equivalent to ~0.3% on a vol/vol basis (Fig. 2). At this NaOH concentration, the total mass of $Mg(OH)_2$ which is produced from a 10-ml sample of seawater is

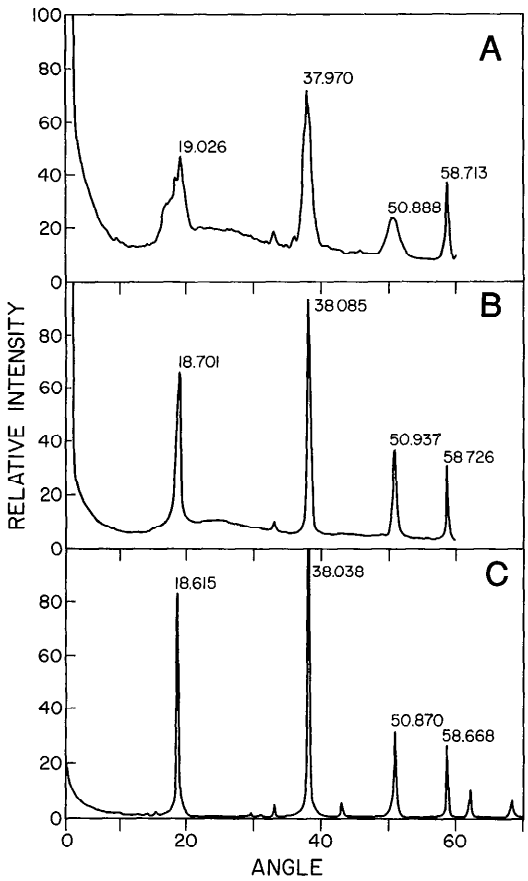


Fig. 1. Comparison of X-ray diffraction (angle expressed as °2θ) patterns. A. The precipitate formed after adding NaOH to a sample of filtered seawater. B. The precipitate formed after adding NaOH to a sample of Mg^{2+}-amended (10 mM) distilled water. C. Commercially available $Mg(OH)_2$ (Fluka). The halite (NaCl) contamination of the seawater precipitate is the result of residual seawater in the pellet volume.

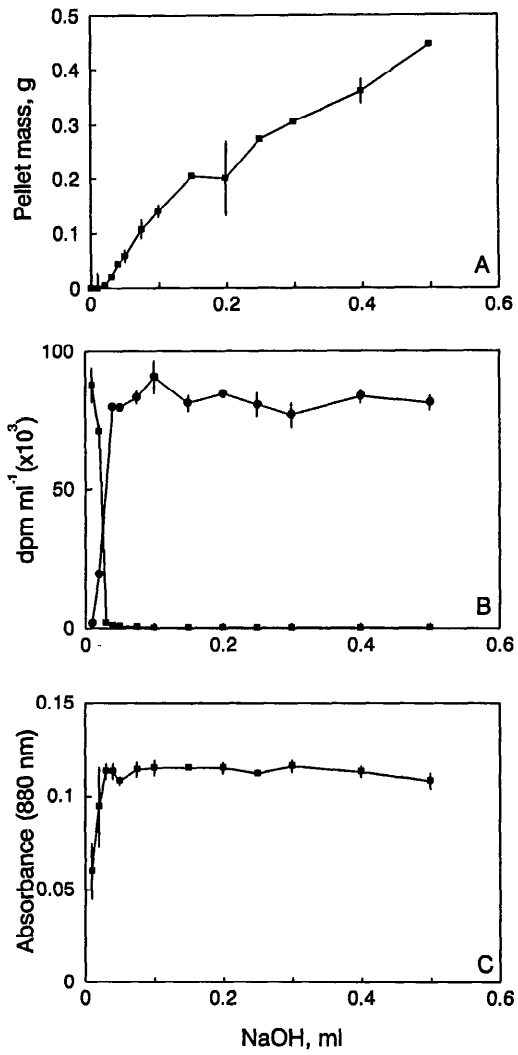


Fig. 2. Effects of adding NaOH (1 M) to a seawater sample (10 ml) containing PO_4^{3-} (0.5 μM) and $^{32}PO_4^{3-}$ (82,845±3,991 dpm ml^{-1}) on production of $Mg(OH)_2$ and recovery of PO_4^{3-}. A. Production of $Mg(OH)_2$ as a function of NaOH (ml) added. $Mg(OH)_2$ is expressed as the wet weight of the pellet after 60 min of centrifugation (*see text*). B. Removal of $^{32}PO_4^{3-}$ as a function of NaOH (ml) added. Data presented as (■) dpm ml^{-1} remaining in supernatant following 60 min of centrifugation (as above) and (●) dpm ml^{-1} recovered following $Mg(OH)_2$ solubilization by HCl treatment (concn factor = 1; *see text*). C. Recovery of PO_4^{3-} as determined by the molybdenum blue reaction following $Mg(OH)_2$ solubilization by HCl treatment (as in panel B) with a 1-cm pathlength. Data for all three graphs are presented as mean ±1 SD (n = 3).

~20±1 mg (wet wt basis). Addition of excess NaOH above this threshold does not improve P removal, but does yield a larger mass of $Mg(OH)_2$ precipitate which must be dissolved in a subsequent step of the procedure.

For our routine P determinations (i.e. 5-fold concn; *see materials and methods*), we recommend addition of 1 M NaOH at a ratio of 1 : 40 (i.e. 2.5% vol/vol). This procedure is effective for the quantitative removal of PO_4^{3-} from samples as large as 0.5 liter (Table 1), and there is no reason why it cannot be scaled up to larger volumes.

Table 1. Removal of SRP and $^{32}PO_4^{3-}$ from seawater by the MAGIC procedure as a function of initial seawater volume. The concentration factor (5-fold) was kept constant in the design of this experiment by varying the volume of acid used to dissolve the $Mg(OH)_2$ pellet.

Seawater vol.* (ml)	NaOH (1 M) vol.† (ml)	$^{32}PO_4^{3-}$ in supernatant (dpm ml^{-1})	$^{32}PO_4^{3-}$ removed (%)	HCl (0.1 M) vol.‡ (ml)	Absorbance at 880 nm§
10	0.25	249	99.7	2	0.545±0.015
25	0.625	218	99.7	5	0.537±0.002
50	1.25	230	99.7	10	0.548±0.006
100	2.5	362	99.5	20	0.500±0.004
250	6.25	751	99.0	50	0.560±0.009
500	12.5	189	99.8	100	0.522±0.018

* Seawater was collected from Kaneohe Bay and filtered (0.2 μm) before use. P was added to a final concentration of 10 μM. All treatments were run in triplicate with $^{32}PO_4^{3-}$ (7.67×10^4 dpm ml^{-1}) added to one of each set of triplicates.
† Used to form $Mg(OH)_2$.
‡ Used to dissolve $Mg(OH)_2$.
§ Mean ±1 SD of the mean ($n = 3$); 1-cm pathlength.

However, if concentration factors >5-fold are required, it is advisable to decrease the NaOH-to-sample volume ratio in order to minimize the final mass of $Mg(OH)_2$ formed. Furthermore, because even the highest purity grade NaOH that we tested (Fluka BioChemika No. 71689) has some contaminating P (according to manufacturer's specifications, <0.0002% P) the use of reduced NaOH volumes will also minimize the reagent blank and maximize the signal-to-noise ratio. Alternatively, one could use any other strong base provided it is free of contaminating P.

Once formed, the $Mg(OH)_2$ precipitate with adsorbed P is quantitatively recovered by low-speed centrifugation (Table 2). Centrifugation forces ≥12,000 × *g* are not recommended because they do not improve P recovery (Table 2) and the resultant pellets are difficult to dissolve. Unless it is absolutely essential to wash (i.e. resuspend and centrifuge) the pellet, we recommend against this practice. If washing is required, for instance to remove salts, use weak NaOH (0.01 M). Even under these conditions, however, we observed losses of PO_4^{3-} from the pellet (~2–3% per wash cycle) which may be attributed to dissolution of $Mg(OH)_2$, desorption of P, or both.

It is also possible to use the $Mg(OH)_2$ formed in vitro to remove P from repetitive additions of seawater added to a given sample tube. Following removal of the P-free supernatant liquid, a second equal or lesser volume of sample can be added followed by resuspension of the pellet (without further addition of NaOH), collection by centrifugation, and removal of supernatant liquid. This entire process can be repeated for several cycles to concentrate TDP from larger sample volumes than one might be able to accommodate in a given centrifuge.

Once collected by centrifugation, the $Mg(OH)_2$ pellet is readily dissolved in HCl. We recommend the use of Baker Instra-Analyzed HCl (or equivalent trace-metal grade) because of the relatively low concentration of contaminating PO_4^{3-}. The $[H^+]$ or volume required for complete dissolution of the concentrated $Mg(OH)_2$ is directly proportional to the pellet mass; the greater the pellet mass the greater the required $[H^+]$. For our standard 5-fold concentration procedure, 0.1 M HCl to a final volume of 10 ml is used to dissolve the pellet collected from 50 ml of seawater. Alternatively, 1 ml of 1 M HCl could be used to effect a 50-fold concentration.

The precise reaction conditions (volume and concentration of acid) will be dictated by the experimental design and objectives. For example, to measure SRP, the strength of the acid should be kept low (≤0.5 M) to minimize phosphate ester hydrolysis. Moreover, because color development in the molybdenum blue reaction is inhibited at high $[H^+]$, it is generally important to minimize $[H^+]$. If, on the other hand, only TDP determinations or measuring $^{32}PO_4^{3-}$ removal are of interest then final acidity is of lesser concern and concentration factors in excess of 100-fold can be easily achieved by increasing the molarity of the HCl used.

Table 2. Effects of centrifugation parameters on the concentration of P from seawater.

Exp. and sample	Incubation period (min)	Centrifugation parameters: Force (×g)	Centrifugation parameters: Time (min)	$^{32}PO_4^{3-}$ removed* (%)
Exp. 1 (10 ml of seawater per sample)				
1–3	10	1,000	10	96.1±1.1
4–6	10	1,000	30	97.4±0.3
7–9	10	1,000	60	98.1±0.1
10–12	120	1,000	10	95.7±0.3
13–15	360	1,000	10	95.0±0.2
Exp. 2 (40 ml of seawater per sample)				
1–3	10	1,000	60	99.8±0.1
4–6	10	12,000	10	99.4±0.1
7–9	10	48,400	10	99.3±0.1
10–12	10	12,000	60	99.5±0.1
13–15	10	48,400	60	99.9±0.1

* Mean ±1 SD of the mean (n = 3).

Table 3. The removal of ^{32}P and ^{31}P from Mg^{2+}-amended freshwater samples.

Sample	Mg^{2+} added* (mM)	$^{32}PO_4^{3-}$ removed† (%)	$^{31}PO_4^{3-}$ recovered‡ (abs. at 880 nm)
Tapwater	0	71.5±0.32	0.155±0.006
+ 10 μM	10	99.8±0.04	0.227±0.005
PO_4^{3-}	25	99.8±0.11	0.226±0.003
	55	99.8±0.02	0.228±0.004
	100	99.6±0.02	0.226±0
	200	99.3±0.04	0.224±0.002
Krauss Pond	0	92.7±4.03	0.210±0.008
+ 10 μM	10	99.8±0.01	0.230±0.004
PO_4^{3-}	25	99.5±0.09	0.230±0.002
	55	98.5±0.66	0.234±0.004
	100	98.2±0.61	0.235±0.005
	200	97.5±0.93	0.237±0.005

* As $MgSO_4 \cdot 7H_2O$.
† $^{32}PO_4^{3-}$ removed from solution after adding 1 M NaOH 2.5% (vol/vol) and centrifuging. Mean ±1 SD of the mean (n = 3); 1-cm pathlength.
‡ SRP measured in pellet after treatment with 10 ml of 0.1 M HCl and reaction with molybdenum blue reagents.

Although the MAGIC procedure was initially developed for seawater applications, we evaluated the efficacy of P removal from Mg^{2+}-amended freshwater samples (Table 3). The addition of $MgSO_4 \cdot 7\ H_2O$ to solutions of PO_4^{3-} made up in either tapwater or Krauss Pond (University of Hawaii campus) water resulted in the quantitative removal of PO_4^{3-} from solution. A concentration of 10–25 mM seems to be sufficient for ≥99% removal of $^{32}PO_4^{3-}$ from solution (Table 3). Subsequent experiments indicated that commercially available $MgCl_2$ had a much lower blank (less contaminating P) than $MgSO_4 \cdot 7H_2O$. If necessary, the reagents can be purified before use (by the MAGIC procedure, as described above), but it was not attempted in the present study. Although we did not extensively investigate the use of the MAGIC procedure for freshwaters, our initial results indicate its applicability.

Recovery of DOP, pyrophosphate, and polyphosphates—We also investigated the response of a variety of DOP and several polyphosphate compounds to the MAGIC preconcentration procedure. Of the compounds tested, including adenosine-5′-triphosphate (ATP), adenosine-5′-monophosphate (AMP), glycerol phosphate, acetyl phosphate, RNA, DNA, phosphonoformic acid, pyrophosphate, and tripolyphosphate, we observed a quantitative coprecipitation of each with the in vitro formation of $Mg(OH)_2$ as determined by the analysis of total P before and after NaOH addition and centrifugation. Furthermore, our results indicate that the MAGIC process does not significantly hydrolyze P-esters and polyphosphates. However, in this regard, it is advantageous to minimize the $Mg(OH)_2$ pellet mass and thereby minimize the $[H^+]$ required for pellet dissolution (*see above*).

We evaluated the efficacy of several previously reported procedures for the measurement of TDP using several representative compounds (Fig. 3). The individual treatments included: dry ashing, dry ashing followed by acid hydrolysis (Solórzano and Sharp 1980), and dry ashing followed by either persulfate or acid persulfate hydrolysis (Menzel and Corwin 1965). We did not evaluate the acid hydrolysis-dry combustion-persulfate oxidation procedure in this experiment but, on theoretical grounds, we would expect it to perform at or beyond the capability of the methods that were evaluated.

Our results indicate that the dry ashing-acid persulfate procedure consistently yielded a maximum amount of P from the compounds tested. Because the TDP in our procedure is transferred from a soluble to a particulate form during $Mg(OH)_2$ coprecipitation, the dried pellet could theoretically be exposed to even more extreme hydrolysis conditions (e.g. nitrate or carbonate fusion

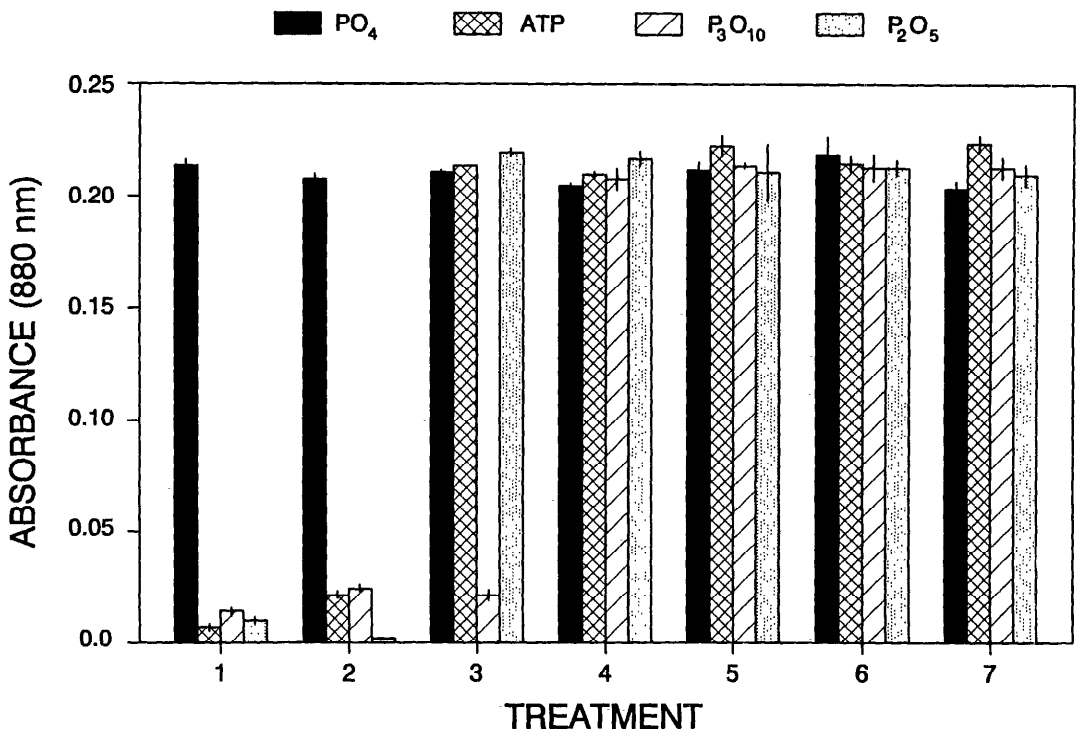


Fig. 3. Analysis of TDP derived from four different P-containing compounds by the molybdenum blue assay following a variety of treatments including: 1—control, no treatment; 2—$Mg(OH)_2$ precipitation and HCl dissolution (concn factor = 1); 3—$Mg(OH)_2$ precipitation, dry ash (450°C, 3 h), HCl dissolution; 4—$Mg(OH)_2$ precipitation, $MgSO_4$-dry ash (450°C, 3 h), HCl dissolution and hydrolysis (80°C, 20 min) (after Solórzano and Sharp 1980); 5—$Mg(OH)_2$ precipitation, dry ash (450°C, 3 h), $K_2S_2O_8$ dissolution and hydrolysis (120°C, 30 min); 6—$Mg(OH)_2$ precipitation, dry ash (450°C, 3 h), HCl/$K_2S_2O_8$ dissolution and hydrolysis (120°C, 30 min) (a variation of Menzel and Corwin 1965); 7—as 6, except for extension of hydrolysis period (60 min.).

techniques), if desired, to recover P from highly refractory compounds (e.g. aminoethylphosphonates). We are currently using these methods to evaluate the hypothesis that refractory dissolved P compounds analogous to the refractory dissolved organic N and C compounds recently described in natural seawater samples might also exist (*see* Ridal and Moore 1990).

During our TDP investigations, we observed what we interpret to be a volatilization loss of acetyl P during the dry-ashing procedure. This potential problem can be alleviated by the preashing HCl-hydrolysis step. Although this step was identified as a potential problem for selected substrates, we have not observed this effect with natural samples. Nevertheless, if the acid hydrolysis-dry combustion-persulfate oxidation TDP method is used, it will ensure against potential losses. In any case, TDP hydrolysis conditions should be tailored to the specific goals of the investigator.

For practical interest, our recovery and stability experiments on selected dissolved-P compounds are dependent upon whether they are truly representative of the P-containing compounds in natural aquatic environments. Perhaps the best way to evaluate the efficacy of total P recovery by the MAGIC procedure is to compare TDP concentrations measured for various natural habitats before and after $Mg(OH)_2$ precipitation (Table 4). From the results obtained in these comparative studies, we conclude that the MAGIC precipitation removes an average of 95% (range 88–100%) of TDP for the environments evaluated.

Effects of sample storage—Because of the relative ease of MAGIC, the entire proce-

Table 4. Efficacy of the MAGIC procedure for removing TDP from various freshwater and marine samples.

Sample source	Absorbance at 880 nm		Removal‡ (%)
	Initial TDP*	Final TDP†	
Freshwater§			
Krauss Pond	0.145±0.010	0.018 (n = 2)	88
Quarry Pond	0.293±0.004	0.007±0.010	98
MRTC aquaculture pond	1.056±0.006	0.051±0.004	95
tapwater	0.352±0.006	0±0.002	100
Marine			
Ala Moana Beach	0.766 (n = 2)	0.033±0.007	96
Mamala Bay	0.179±0.070	0.016±0.013	91
Ala Wai Canal	0.344±0.008	0.003±0.003	99
Sta. ALOHA + 3 μM PO_4^{3-}	0.352±0.006	0.030±0.010	91
			mean = 95

* Initial TDP (absorbance mean ±1 SD, n = 3, except where noted) measured on GF/F filtered samples with the $K_2S_2O_8$ oxidation procedure of Menzel and Corwin 1965, corrected for reagent blank.
† Measured on the sample supernatant following in vitro $Mg(OH)_2$ formation and centrifugation with methods as above.
‡ [Mean final TDP ÷ mean initial TDP] × 100%.
§ Samples were supplemented with 10 mM Mg^{2+} (as $MgCl_2$) before TDP determinations.

dure can be performed at sea. If real time shipboard analyses are not necessary, either the water samples or the $Mg(OH)_2$ precipitates can be stored for subsequent shore-based laboratory analysis. We evaluated the effects of pellet storage (Table 5). Our results indicate that concentrated samples can be stored at room temperature (~24°C) for up to 4 d without detectable change in SRP concentration. Subsequent experiments, not reported here, indicate that $Mg(OH)_2$-precipitated P samples are stable for several months when stored at 4°C.

Potential interferences—The MAGIC procedure was designed for a quantitative concentration of dissolved inorganic and organic P compounds from marine and freshwater samples. Once collected, SRP or TDP can be measured by any available spectrophotometric, fluorometric, or other analytical procedures. The influence of potentially interfering elements such as As^{5+}, As^{3+}, and $Si(OH)_4$ will depend entirely on the method used for to detect P. As most aquatic chemists use the molybdenum blue reaction, we focus our attention on that detection system.

Both As^{5+} and $Si(OH)_4$ form heteropoly acids with Mo (Levine et al. 1955). However, selectivity for P can be maintained by control of the reaction conditions, including $[H^+]$, time, temperature, and molarity of reactants. For example, $Si(OH)_4$ interference in the standard Murphy and Riley (1962) modified single-reagent method for P determinations in seawater is eliminated by control of solution acidity (Pai et al. 1990), but As^{5+} interference remains. Under the conditions typically used for automated P analyses, As^{5+} molar reactivity can be reduced (by up to 70%), relative to that of P, simply by adjusting temperature and reaction time. Other methods involve the selective reduction of arsenate (As^{5+}) to nonreactive arsenite (As^{3+}) before adding the molybdenum blue reagents (Johnson 1971), selective solvent extraction of the 12-MPA complex before spectrophotometric determination (Paul 1966), or selective volatilization of As^{5+} with strong acids. There are, however, conflicting reports about both the necessity to correct for As^{5+} interference and the effectiveness of the various treatments.

We believe that it is essential to evaluate

Table 5. Effects of sample (pellet) storage on the measurement of SRP by the MAGIC procedure. P-amended (2 μM PO_4^{3-}) seawater was concentrated by $Mg(OH)_2$ precipitation at t = 0 d, and the pellets were stored at room temperature for the periods indicated.

Storage time (d)	Optical density at 880 nm*
0	0.193±0.002
1	0.195±0.002
2	0.197±0.003
3	0.192±0.003
4	0.192±0.001

* Mean ±1 SD of the mean (n = 3); 1-cm pathlength.

Table 6. Selected depth profiles of SRP and As^{5+} concentrations for water samples collected at Station ALOHA.

	Depth (m)	SRP* (nM)	As^{5+}* (nM)	[SRP]/ [As^{5+}]
Sep 89	5	105±4	28±9	3.8
	25	108±1	21±7	5.1
	45	101±5	23±7	4.4
	75	105±4	24±1	4.4
	100	108±8	37±9	2.9
	150	262±6	17±5	15
Jan 90	0	76±4	26±1	2.9
	5	52±5	27±3	1.9
	15	56±7	22±2	2.5
	25	58±1	44±2	1.3
	45	47±4	34±1	1.4
	60	47±3	22±1	2.1
	75	40±5	20±2	2.0
	85	65±2	24±2	2.7
	95	75±5	26±2	2.9
	115	100±4	26±1	3.8
	125	139±1	64±2	2.2
	135	177±4	37±1	4.8
Feb 90	5	126±8	19±1	6.6
	26	131±8	23±2	5.7
	46	108±4	34±1	3.2
	59	94±2	40±5	2.4
	75	101±10	24±1	4.2
	84	109±4	27±3	4.0
	105	87±5	41±6	2.1
	114	85±7	35±1	2.4
	125	85±9	25±1	3.4
	135	156±2	39±3	4.0
	148	185±7	38±1	4.9
	176	256±1	35±1	7.3
Apr 90	14	107±1	18±1	5.9
	36	91±1	16±1	5.7
	76	97±4	23±1	4.2
	105	96±3	12±1	8.0
	116	102±6	18±2	5.7
	134	111±4	7±1	15
	149	116±13	10±1	12
	176	199±7	28±3	4.3
	203	219±6	11±1	20

* SRP and As^{5+} values are given as mean values ±1 SD of the mean (n = 3).

and correct for As^{5+} interference if one desires accurate estimates of dissolved-P concentrations in oligotrophic oceanic environments. Previous measurements of As^{5+} in seawater indicate concentrations ranging from 18 to 23 nM in Cape Basin (Statham et al. 1987), 30 to 110 nM in the Caribbean Sea (Johnson 1971), and 10 to 60 nM in the Gulf of Mexico (Johnson 1971) with [As^{5+}]: [PO_4^{3-}] ratios ranging from >2 in surface waters to <0.01 in deep waters (*see above*). As^{5+} is quantitatively coprecipitated with PO_4^{3-} in the MAGIC procedure and, in our hands, the [As^{5+}]: [PO_4^{3-}] reactivity ratio is >0.9. Consequently we used the method of Johnson (1971) to measure and correct for As^{5+} interference on the quantitative determination of SRP.

For accurate TDP determinations, it is also necessary to correct not only for As^{5+} but also for other As compounds which might form As^{5+} upon oxidation or hydrolysis. For example, the MAGIC procedure also concentrates As^{3+}, which is normally nonreactive to the molybdenum blue reagents but which is oxidized to the reactive As^{5+} form during the TDP procedure. Consequently, it is inappropriate to use the [As^{5+}] determined during the SRP analysis to correct for total As interference in TDP analyses. Furthermore, it is conceivable that other pools of As compounds may exist, analogous to those of P (Andreae 1979), which become reactive only after the initial hot HCl treatment. The addition of potassium iodide to the acidified sample before evaporation (Strickland and Parsons 1972) appears to be a simple solution to an otherwise difficult analytical problem. In our hands, both As^{5+} and As^{3+} were quantitatively removed from solution during the sample evaporation step. Consequently, we recommend that the acid hydrolysis-dry combustion-persulfate oxidation method be used for the most accurate estimates of TDP.

Comparison of MAGIC to other high-sensitivity methods—High-sensitivity P determinations in aquatic environments have traditionally relied on the use of either solvent extraction or capillary cell absorption spectrometry. The MAGIC procedure offers several advantages to these methods. First, the precision of P determinations with MAGIC is greater than that of other methods because the concentration step occurs before the addition of the molybdenum blue reagents, thereby increasing the signal-to-reagent blank ratio. In contrast, other methods do not concentrate the sample or concentrate it after reagent addition, thereby amplifying both signal and reagent blank.

In our hands, the precision of P determinations in the concentration range of 10–100 nM is typically 1–3%. The detection

limit is also lower with MAGIC than with the other methods, because of concentration of the material analyzed. If one uses a concentration factor of 20-fold, the absorbance (10-cm pathlength at 880 nm) of 5 nM PO_4^{3-} is 0.0577 (±0.0032; $n = 3$) compared to a procedure blank value of 0.0387 (±0.0032; $n = 3$). Even lower detection limits are theoretically possible, but are currently limited by P contamination in the reagents used in our analysis. For nanomolar and lower detection, reagent purification is necessary. Furthermore, the MAGIC procedure is relatively rapid and straightforward and does not require the use of any specialized equipment, except for a low-speed centrifuge and spectrophotometer. Finally, for applications such as P uptake and regeneration where $^{32}PO_4^{3-}$ and [^{32}P]DOP must be quantitatively collected for analysis, MAGIC presents a unique methodology.

Station ALOHA profiles—The Hawaii Ocean Time-Series (HOT) program has established a deep-water hydrostation ~100 km north of Oahu (22°45′N, 158°W) at a site referred to as Station ALOHA (a long-term oligotrophic habitat assessment). Since October 1988, hydrographic and biogeochemical measurements have been made at Station ALOHA on an approximately monthly sampling schedule (Chiswell et al. 1990). It has provided an opportunity to evaluate the MAGIC procedure under oligotrophic oceanic field conditions and to obtain a time-series database on SRP and As^{5+} concentrations at this site. Selected data are presented in Table 6.

SRP concentrations at Station ALOHA vary temporally with mean surface layer (0–50 m) values ranging from 50 to 130 nM (Table 6). On several cruises, we observed a positive slope in SRP concentration from a depth of ~60–120 m to the surface. We did not observe corresponding increases for nitrate or silicate which suggests that this distribution structure may be unique for SRP. We hypothesize that the near-surface enrichment in SRP may be caused by either atmosphere-derived P or a complex redistribution of nutrients in the euphotic zone, perhaps as a result of P scavenging by rising particles and migrating organisms or bubble scavenging (Karl et al. in press). The depth distributions of As^{5+} at Station ALOHA were less variable with time. The mean surface ocean concentration (0–100 m) of As^{5+}, 26.2±7.1 nM (range, 16–44 nM), was nearly identical to that reported by Statham et al. (1987) for water off Cape Basin.

Other applications of MAGIC—We have focused on the application of MAGIC to SRP and TDP analyses in seawater. There are, however, other potential applications in marine chemistry and biogeochemistry. For example, MAGIC can be used as an alternative to the charcoal-adsorption technique (Hodson et al. 1976) for measurements of dissolved nucleotides in seawater samples or cell extracts. Another potential application is measurement of total P flux from in situ sediment trap experiments. It is well known that a variable fraction of the particulate P that enters a sediment trap partitions into the solute phase which, unless measured and corrected for, results in a potentially large error in particle flux determinations (Karl and Knauer 1989). MAGIC can be used to recover this lost P even at very low concentrations. Finally, it is likely that other metal and nonmetal ions are coprecipitated by in vitro $Mg(OH)_2$ formation, providing a potential noncontaminating concentrating method.

Conclusion

We have devised and field tested a novel and flexible method for precise and accurate determinations of SRP and TDP in the range of 1–100 nM P. With attention to, and alleviation of, P contamination of the colorimetric reagents, the method can measure subnanomolar concentrations of P. The method relies on in vitro formation of $Mg(OH)_2$ via the addition of NaOH to seawater or Mg^{2+}-amended freshwater samples. Coprecipitation of SRP and of most dissolved inorganic and organic P compounds with $Mg(OH)_2$ is rapid and quantitative. Concentration of P compounds from natural waters can also be used for studies of P uptake and regeneration with PO_4^{3-} radiotracers and can be used to isolate and detect specific P compounds such as

nucleotides, sugar phosphates, and nucleic acids.

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