

Persulfate chemical wet oxidation method for the determination of particulate phosphorus in comparison with a high-temperature dry combustion method

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Abstract

A persulfate chemical wet oxidation (CWO) method for the determination of particulate phosphorus (PP) was improved and then compared with a conventional high-temperature dry combustion (HTDC) method. In the improved CWO method, the concentration of the digestion reagent, potassium persulfate, was increased from 0.5% to 3% (w/v); the method exhibited high recoveries of P from various test materials under commonly used autoclave conditions (at 120°C for 30 min). The recoveries of P relative to those of the HTDC method were $102 \pm 6.7\%$ from standard organic and inorganic P compounds, and $100 \pm 3.8\%$ from natural particulate matter analogues including riverine suspended particulate matter (SPM), sediments, plankton, and geochemical reference materials of rock. However, low recoveries of 14% to 69% were observed for reference samples of clay minerals. Comparison using many samples of estuarine and pelagic SPM showed that the CWO method produced PP values consistent with those obtained by the HTDC method, except for some estuarine samples enriched in inorganic matter. The low recoveries were ascribed to the presence of an inorganic P fraction highly recalcitrant to chemical digestion. The procedural blank was lower for the CWO method than for the HTDC method. The filter blanks showed large variation among the filter materials tested; aluminum oxide membrane filters were shown to contain a considerable amount of P that could cause significant contamination in both methods. Analytical precision was equivalent between the two methods. Considering its simplicity and its less time-consuming nature, the CWO method presented here is suitable for PP determination in aquatic environments.

Introduction

Phosphorus (P) is an essential nutrient for sustaining marine productivity. Regeneration of bioavailable dissolved P from particulate matter is an important process maintaining P availability in the marine ecosystem (Karl and Björkman 2002;

Paytan et al. 2003; Benitez-Nelson et al. 2007). Phosphorus is a nutrient with high particle affinity (Benitez-Nelson and Bueseler 1999; Fang 2004); consequently, marine P cycling is largely regulated by the interaction between dissolved and particulate P (PP) pools. However, few studies have been conducted on the spatial and temporal variability of particulate P pools in pelagic environments (Loh and Bauer 2000; Suzumura and Ingall 2004; Yoshimura et al. 2007), and little is known about the chemical composition of P-containing species in suspended particulate matter (SPM) in these environments. Particulate organic carbon (POC) and nitrogen (PON) are frequently measured as a part of routine chemical analysis in marine observations, whereas PP determination is not often included. This is likely because PP determination requires an additional volume of sample water as well as additional analytical procedures and costs; POC and PON are generally determined simultaneously on a same filter using an elemental analyzer (CHN analyzer), whereas PP must be determined on a separate filter. Some protocols have been proposed for the simultaneous determination of PP with POC, with PON, or with both on the same filter (Pujo-Pay and Raimbault 1994;

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Raimbault et al. 1999; Lampman et al. 2001). These methods are expected to increase the frequency of PP determination, but at present these methods are not frequently used.

Chemical methods for the determination of PP are based on the liberation of orthophosphate as soluble reactive P (SRP) from organically bound forms and inorganic polyphosphates by oxidative treatment and acid hydrolysis. Following this treatment, the concentration of SRP is measured spectrophotometrically by standard phosphomolybdenum blue methods (e.g., Strickland and Parsons 1972; Hansen and Koroleff 1999). Chemical wet-oxidation (CWO) techniques have been used as digestion protocols for PP determination. Persulfate oxidation (Menzel and Corwin 1965) is a safer technique than other CWO protocols. Perchlorate digestion (Strickland and Parsons 1972), for example, is a more complicated procedure, requiring special facilities and safety precautions; the digestion involves a refluxing step that potentially causes the loss of P, and that necessitates the use of a specially equipped fume hood. The autoclave-promoted persulfate oxidation method is considerably simpler and, consequently, has often been used for marine PP determination (Koike et al. 1993; Vidal and Duarte 2000; Biddanda et al. 2001). Another useful option for oxidative treatments is high-temperature dry combustion (HTDC), which was originally developed for sedimentary P analyses (Anderson 1976; Aspila et al. 1976) and has been used for PP determination (Solózano and Sharp 1980). In HTDC methods, organically bound forms of P in particulate matter are digested at high temperature (~550°C) to form inorganic P compounds. Digestion is followed by an acid extraction to recover soluble orthophosphate measurable by spectrophotometry. In some cases, the addition of MgSO_4 or $\text{Mg}(\text{NO}_3)_2$ is recommended to promote oxidation during the combustion process (Nedashkovskiy et al. 1995; Ormaza-González and Statham 1996; Monaghan and Ruttenberg 1999). Various standard organic P compounds, including sugar phosphates, nucleotides, nucleic acids, and phospholipids, have been used to examine digestion efficiency by the HTDC and CWO methods. It was reported that the HTDC method recovered $100\% \pm 5\%$ of standard organic P compounds (Solózano and Sharp 1980; Kérouel and Aminot 1996; Ormaza-González and Statham 1996; Monaghan and Ruttenberg 1999). In contrast, the persulfate CWO method showed low P recoveries for some organic P compounds stable to chemical digestion, such as phosphonates (which contain C–P bonds) and phospholipids (Kérouel and Aminot 1996; Monaghan and Ruttenberg 1999).

There are some studies in which natural particulate samples are used to compare and evaluate HTDC and CWO methods for PP determination. Among these studies, the procedures for persulfate CWO differed in autoclave temperature (100–120°C), time (0.5–2 h), pH (acidic or slightly alkaline), and the concentration of persulfate (0.7–3.3%). The recovery of PP varied greatly among the CWO methods. Lampman et al. (2001) found no considerable difference between P recoveries in their CWO protocol and in the HTDC method of Anderson

(1976). They also showed, however, that another persulfate CWO protocol (Raimbault et al. 1999) had quite poor recovery, averaging 55% (and ranging from 28 to 72%) relative to that of the HTDC method. Langner and Hendrix (1982) reported that P recovery ranged from 93.6% to 101.4% for an alkali persulfate CWO method on epiphytic algal materials.

The CWO method is unquestionably simpler and less time consuming than the HTDC method. Weighing this advantage against the fact that CWO can produce low and variable P recoveries, this paper reports the results of a re-evaluation of the persulfate CWO method for the determination of PP in seawater. Because of the aforementioned concerns about the ability of the CWO method to digest various P forms, the digestion conditions were improved to obtain high recoveries. The HTDC method and the CWO method were then compared using standard P compounds, geochemical reference materials, and natural SPM analogues, including plankton, sediments, and riverine, estuarine, and seawater SPM samples. In addition, filters that are typically used for aquatic SPM separation were tested for blank values of P. In this paper, I discussed the applicability and limitations of the CWO method for PP determination in biogeochemical research of P cycling.

Materials and procedures

All reagent preparation, sample dilution, and rinsing were carried out using purified water (Q-water) prepared with a Milli-Q Gradient System (Millipore). All reagents were of analytical grade unless otherwise specified.

Procedures of the HTDC and CWO methods—The HTDC method used in this study was based on the protocols of Aspila et al. (1976) and Solózano and Sharp (1980). Samples were placed in 30-mL acid-cleaned borosilicate vials; 0.2 mL of 0.17 M $\text{Mg}(\text{NO}_3)_2$ was then added to each vial. The samples were dried, uncovered, on a 110°C hotplate. The vials were then covered with aluminum foil, placed in a muffle furnace, and combusted at 470°C for 90 min. After the vials cooled, phosphate was extracted by adding 20 mL of 1 M HCl to each vial, tightly capping the vials with Teflon-lined caps, and shaking them at room temperature for 14 h. Residues in the extract were removed using an acid-washed 0.45- μm syringe filter (Millex-HV, Millipore). Because the development of the molybdenum blue complex is inhibited in highly acidic conditions, the filtrate acidity was reduced to 0.1 M $[\text{H}^+]$ or less by dilution with Q-water or by addition of 1 M NaOH solution. It should be noted that there were some differences in these procedures from the original protocols. Aspila et al. (1976) used 50% (w/v) $\text{Mg}(\text{NO}_3)_2$, not 0.17 M, with ashing at 550°C for 2 h, whereas Solózano and Sharp (1980) used 0.17 M MgSO_4 with ashing at 450–500°C for 2 h.

As mentioned previously, various methods exist for the determination of PP and total dissolved P using persulfate CWO method. These methods are variations of Menzel and Corwin (1965), which originated from the protocol of Menzel and Vaccaro (1964) for CWO of dissolved organic C. Samples

were placed in 50-mL glass bottles (Duran). Twenty milliliters of a $K_2S_2O_8$ solution was dispensed into each bottle; various persulfate solutions were used, prepared from a low-P source (Wako Chemical, Japan), and ranging from 0.5% to 3% (w/v) $K_2S_2O_8$. The bottles were capped tightly with a Teflon-lined cap and heated at 120°C for 30 min using an autoclave (Model AS-2346, Ikeda Scientific). The bottles were vigorously shaken before and after autoclaving. After cooling, samples were filtered as above. It was found that color development in an orthophosphate standard was inhibited after digestion using > 2% $K_2S_2O_8$; consequently, filtrates were diluted to < 2% $K_2S_2O_8$ prior to measurement.

SRP concentrations in the HTDC extract and CWO solution were measured by means of the colorimetric method of Strickland and Parsons (1972) using a double-beam spectrophotometer (Shimadzu UV-1600) with a 5-cm pathlength.

Blank test—Because the PP concentrations in oligotrophic pelagic seawaters are relatively low (< 100 nM, Loh and Bauer 2000; Suzumura and Ingall 2004; Yoshimura et al. 2007), P contamination from analytical procedures and filter materials likely contributes considerable error to PP determination. Procedural blanks were analyzed and used to compare sources of contamination in the HTDC and CWO methods. Filter blanks were prepared from the filters of various materials that have most often been used in capturing particulate matter from seawater. The tested filters are listed in Table 1, including the glass fiber filters that are generally used for POC and PON analysis, mixed-cellulose membrane, polyvinylidene fluoride (PVF) membrane, polycarbonate (PC) membrane, nylon-mesh, and aluminum oxide membrane. Each filter (2.5 cm in diameter) was washed by passing 5 L of Q-water and 1 mL of Na_2SO_4 through it; filter samples were analyzed for total P (TP) by the HTDC and CWO methods. In all experiments using CWO for blank determination, a 3% $K_2S_2O_8$ digestion solution was used.

Standard P compounds—The HTDC and CWO methods were compared using the commercially available P compounds listed in Table 2, including five organic and four inorganic compounds. These compounds were dissolved in Q-water (except lecithin, which was dissolved in chloroform), and a portion of each solution containing approximately 200 nmol

of P was dispensed into digestion vials and bottles. The samples were gently heated to dryness on a hot plate, digested, and analyzed for SRP.

Natural analogues of aquatic particulate matter—Because the chemical composition of P in natural particulate matter and dissolved organic matter has not yet been fully characterized (Cade-Menun et al. 2005; Sannigrahi et al. 2006), tests using standard P compounds alone are insufficient to evaluate digestion procedures. Accordingly, various natural analogues of particulate matter were analyzed for TP by the HTDC and CWO methods. These natural analogues are listed in Table 3 and include riverine SPM, coastal and pelagic marine sediments, net-collected and cultured plankton, geochemical reference materials, and several samples of estuarine and pelagic SPM.

Riverine SPM. A riverine SPM sample that had been collected and used in Suzumura and Kamatani (1995) as an analogue of terrigenous particulate matter transported to coastal marine environments was used in this study. The sample was collected from the freshwater region of the Tamagawa River (Fig. 1) by decanting from several hundred liters of the river water. The sampling location and chemical analytical data of this sample are presented in Suzumura and Kamatani (1995).

Coastal and pelagic sediments. A coastal sediment sample was collected from the inner part of Tokyo Bay (35°36.5'N, 140°01.5'E, 10 m depth), a typical eutrophic basin in Japan, in August 1998, using an Eckman-Burge sampler. The top 1 cm of the surface sediment was recovered and dried in air. The pelagic sediment sample was a mixture of subsurface sediment collected from the western North Pacific station KNOT (44°N, 155°E, 4900 m depth; Tsurushima et al. 2002) in July 1998, using a multiple corer. The dried sediments were ground into powder with an agate mortar and pestle.

Plankton samples. A natural assemblage of plankton was collected during a cruise by R/V *Tansei-Maru* (KT-06-28) in the western North Pacific Ocean in November 2006 (Fig. 1). The seawater was collected about 4 m below the surface by ship's pump while the ship was steaming, and introduced to a laboratory sampling tap. Several tons of seawater were passed through a plankton net (mesh size 72 μ m, NNX17, RIGO Co.). A portion of the collected plankton sample was subjected to microscopic observation. The dominant phytoplankton were

Table 1. The procedure blanks and blanks of filters in TP analyses by the HTDC and CWO methods (mean \pm SD, $n = 3$)^{*}

	Nominal poresize (μ m)	TP _{HTDC} (nmol)	TP _{CWO} (nmol)
Procedure blank	Not applicable	0.33 \pm 0.08	0.08 \pm 0.07
Glass fiber (GF/F, Whatman)	0.7	2.22 \pm 0.34	0.76 \pm 0.06
Glass fiber (GF/C, Whatman)	1.0	1.75 \pm 0.13	1.75 \pm 0.19
Mixed-cellulose membrane (MF, Millipore)	0.8	0.84 \pm 0.13	0.57 \pm 0.13
PVF membrane (Durapore, Millipore)	0.1	5.89 \pm 0.18	1.10 \pm 0.21
PC membrane (Nuclepore, Whatman)	0.1	0.40 \pm 0.11	0.19 \pm 0.13
Nylon-mesh (Nylon net, Millipore)	11	2.86 \pm 0.46	3.73 \pm 0.02
Aluminum oxide membrane (Anodisc, Whatman)	0.2	16,300 \pm 380	18,300 \pm 1,480

^{*}The filter blanks are expressed as P contents (nmol) per 2.5 cm filter and include the procedure blank.

Table 2. List of the tested standard P compounds and the ratios of P concentrations determined by the HTDC and CWO methods (mean \pm SD, $n = 3$).

P compounds	Specification	Ratio of CWO/HTDC
<i>Organic P compounds</i>		
Deoxyribonucleic acid	Sigma, Sodium salt, Type III from salmon testes	1.02 \pm 0.02
Adenosine-5'-triphosphate	Sigma, Disodium salt, Grade I from bacterial source (99%)	1.01 \pm 0.01
Inositol hexaphosphate (phytic acid)	Sigma, Dodecasodium salt from rice (\geq 90%)	1.03 \pm 0.00
2-Aminoethyl phosphonic acid	Aldrich (99%)	1.01 \pm 0.01
Phosphatidyl choline (lecithin)	Sigma, Type III-B from bovine brain (approx. 99%)	0.97 \pm 0.01
<i>Inorganic P compounds</i>		
Phosphorus (V) oxide	Wako Chemicals (> 98%)	1.20 \pm 0.00
Pyrophosphate	Alfa Aesar, Sodium salt (98%)	1.00 \pm 0.00
Tripolyphosphate	Strem Chemicals, Sodium salt (> 85%)	1.00 \pm 0.00
Hexametaphosphate	Aldrich, Sodium salt (96%)	1.01 \pm 0.01

Table 3. List of the natural particulate matter analogues and the analytical results of PP determination by the HTDC and CWO methods (mean \pm SD, $n = 4$).

Samples	PP _{HTDC} ($\mu\text{mol g}^{-1}$)	PP _{CWO} ($\mu\text{mol g}^{-1}$)	Ratio of CWO/HTDC
<i>SPM and sediment samples</i>			
Riverine SPM	32.3 \pm 0.41	32.7 \pm 0.33	1.02
Coastal marine sediment	30.8 \pm 0.83	30.3 \pm 0.58	0.98
Pelagic marine sediment	9.62 \pm 0.08	8.80 \pm 0.12	0.92
<i>Plankton samples</i>			
Net-collected plankton (>72 μm)	174 \pm 2.59	175 \pm 3.82	1.01
Cultured <i>Skeletonema costatum</i>	10.0 \pm 0.13*	10.5 \pm 0.24*	1.05
Cultured <i>Chaetoceros pseudocurvisetus</i>	5.18 \pm 0.31*	5.37 \pm 0.31*	1.04
<i>Geochemical reference materials</i>			
Limestone (JLs-1)	4.15 \pm 0.05	4.12 \pm 0.04	0.99
Feldspar (JF-2)	0.16 \pm 0.01	0.16 \pm 0.01	1.00
Coral (JCp-1)	0.18 \pm 0.01	0.18 \pm 0.02	1.00
Montmorillonite (JCSS-3101)	1.44 \pm 0.03	1.00 \pm 0.02	0.69
Kaolinite (JCSS-1011)	22.0 \pm 0.96	3.04 \pm 0.04	0.14

*Values are in μM in culture media.

diatoms (particularly representatives of the genera *Pseudo-nitzschia* and *Chaetoceros*) and the dinoflagellate *Ceratium fusus*. Copepoda (nauplius) were the most abundant group among the zooplankton. The composite sample was freeze-dried and ground into powder with a stainless steel blender (Oster ST-1).

Two species of phytoplankton, Bacillariophyceae, were cultured at 20°C in 2-L polycarbonate bottles containing f/2 medium under a cycle of 12 h light/12 h dark. A strain of *Skeletonema costatum* (NIES-16) was obtained from the Microbial Culture Collection at the National Institute for Environmental Studies, Japan (MCC-NIES). The *Chaetoceros pseudocurvisetus* strain was the same as that used by Oku and Kamatani (1995). After 7 to 10 d of incubation, the cultured plankton was recovered on a GF/F filter and freeze-dried.

Geochemical reference materials. Three rock samples were obtained from GSJ Geochemical Reference Samples (National

Institute of Advanced Industrial Science and Technology, Japan), including limestone (JLs-1), feldspar (JF-2), and coral (JCp-1). Precise information about the samples is available at <http://riodb02.ibase.aist.go.jp/geostand/>. Reference samples of the clay minerals montmorillonite (JCSS-3101) and kaolinite (JCSS-1011) were obtained from the Clay Science Society of Japan.

Estuarine SPM. Estuarine SPM samples were taken from 9-10 stations located along the wide salinity gradient of the Arakawa River, which flows into Tokyo Bay (Fig. 1) between December 2000 and September 2003. Surface water samples were collected in a stainless steel bucket, stored in polyethylene bottles on ice, and analyzed within 6 h of sampling. At some sampling times, bottom-water samples (50 cm above the bottom) were also collected using a Van Dorn water sampler. A 20- to 150-mL aliquot of the water sample, with the volume depending on SPM concentration, was filtered through a glass fiber filter (GF/F, Whatman, 4.7 cm in diameter) that was

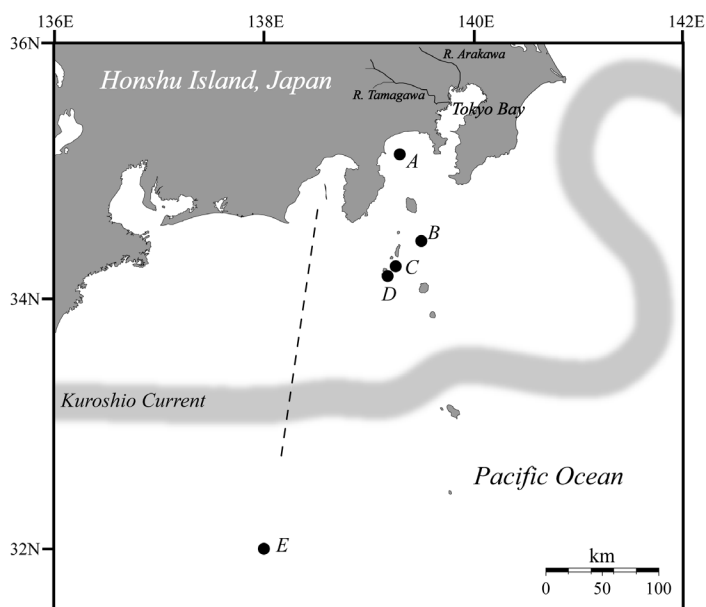


Fig. 1. Sampling locations of the pelagic SPM and net-collected plankton (dashed line).

prewashed by passing 5 mL of 1 M HCl and followed by a few milliliters of Q-water through it. After sample filtration, the filter was washed with 1 mL of 0.17 M Na_2SO_4 to flush away the remaining sample water containing dissolved P. Details of the sampling location, sample preparation, and chemical data for the samples collected between December 2000 and October 2001 are described elsewhere (Suzumura et al. 2004).

Pelagic SPM. During the cruise KT-06-28 mentioned above, regarding plankton sampling, epipelagic seawater samples were collected from 5 locations (Fig. 1). Seawater samples were collected by ship's pump as above. At stations A and E, the overall water depths were 1500 m and 3800 m, respectively. Water samples were collected at depth intervals of 10, 30, 50, 75, 100, 150, 150, 200, and 250 m using a CTD with a Rosette Multi-bottle Sampler. Before filtration, the samples were passed through a 100- μm mesh to remove large organisms, which might create considerable error between replicate filters from the same water sample. A 5-L aliquot of the sample was filtered through a GF/F filter (2.5 cm in diameter) using the same protocol as for the estuarine samples. Two replicate filters obtained from each seawater sample were freeze-dried and analyzed for PP by the HTDC and CWO methods.

Assessment

Complexity and time requirements of each method—When comparing the utility of chemical analytical methods, the procedures' complexity and potential time savings are essential considerations. The HTDC method involves 5 main steps before spectrophotometric analysis: 1) addition of $\text{Mg}(\text{NO}_3)_2$ reagent and drying (~1.5 h), 2) combustion in a muffle furnace

(~3 h), 3) acid extraction of orthophosphate from the combusted sample (~15 h), 4) refinement of the extracts with a syringe filter (0.5 h), and 5) neutralization and/or dilution (0.5 h). The CWO method requires fewer steps: 1) addition of persulfate solution and autoclaving (2 h), 2) purification of the digested solution using a syringe filter (0.5 h), and 3) dilution (0.5 h). The approximate total times, including cooling and reagent preparation times, were 20 h for the HTDC method and 3 h for the CWO method for 24 samples in a run.

Procedure and filter blanks—Table 1 shows the blank values derived from the procedures and various filters in the HTDC and CWO methods. The CWO method produced a lower procedural blank than did the HTDC method. This is likely because the CWO method has fewer steps, which reduces the chance of contamination. Monaghan and Ruttenberg (1999), however, found the higher procedural blank in their CWO method than in the HTDC method. They stated the necessity of reagent purification to reduce the blank. The use of the highly pure, low-P $\text{K}_2\text{S}_2\text{O}_8$ would contribute to minimize contamination in this study.

The filter blanks varied widely among the filter types tested. Blanks of GF/F filters obtained using CWO were one third as high in P content as those obtained using HTDC, and gave results fairly similar to those obtained in previous studies using CWO methods (Pujo-Pay and Raimbault 1994; Raimbault et al. 1999). GF/C filter blanks were equivalent for the two methods. PC membrane filters (Nucleopore) produced the lowest blanks using either method, and this result agrees with previous results (Raimbault et al. 1999). The PC membrane filter, therefore, appears to be advantageous for precise determination of low-PP samples. However, in the HTDC method, the use of resinous filters, including PC membrane filters, presented considerable inconveniences. During heating in the muffle furnace, resinous filters often burned eruptively, spattering the carbonized residue and causing the loss of P. Furthermore, the combustion of the resinous filters generated an odor that was possibly harmful, necessitating special ventilation equipment for the muffle furnace. Because of these drawbacks, it is more useful to process resinous filters for PP determination using the CWO method.

Aluminum oxide (alumina) membrane filters contained a surprisingly high amount of P (Table 1). Recently, alumina membranes have often been used for the separation of marine submicron particles, in particular for the epi-fluorescent microscopic enumeration of marine bacteria and viruses (e.g., Shibata et al. 2006). I learned that during manufacture of the alumina membrane, a phosphoric acid electrolyte is used in the anodic oxidation of aluminum to produce a porous film (Furneaux et al. 1989). The alumina filter blanks were four orders of magnitude higher in concentration of P than were the blanks of other filters; the high P concentration renders these filters unusable for the study of P in marine waters.

Hereafter, all PP concentration data presented are blank-subtracted.

Concentration of persulfate solution in the CWO digestion—In previous studies where the persulfate CWO digestion of DOP resulted in low P recovery from standard compounds and natural samples, relative to recoveries from HTDC methods, the concentration of persulfate ($K_2S_2O_8$) used was usually 0.5% or lower (Monaghan and Ruttenberg 1999 and cited therein). To achieve high recoveries using the CWO method for seawater DOP analysis, Ridal and Moore (1990) recommended autoclaving with more drastic conditions in both temperature and time; seawater samples containing 0.4% $K_2S_2O_8$ were digested at 125°C for 2.5 h.

For this study, the effect of increasing the persulfate concentration in the digestion solution was examined, instead of increasing time and temperature. This is because time savings are the major advantage of the CWO method, and because some low-end autoclaves have maximum operating temperatures as low as 120°C. Furthermore, unlike seawater samples, particulate and filter samples in which Cl^- is absent or low in concentration can be subjected to digestion by increased persulfate concentrations. High Cl^- content interferes with the analysis: the oxidation of Cl^- consumes persulfate and generates free chlorine that inhibits color development of the phosphomolybdenum blue complex. The author tested persulfate solutions ranging from 0.5% to 3% (w/v), using four analogues of natural particulate samples including riverine SPM, net-collected plankton, and coastal and pelagic marine sediments.

CWO digests using 0.5% persulfate exhibited low P concentration (PP_{CWO}) relative to P concentration determined by the HTDC method (PP_{HTDC}); PP_{CWO}/PP_{HTDC} ranged from 50.5% for pelagic sediment to 85.5% for plankton (Fig. 2). Increased persulfate concentration produced higher recoveries in all

samples tested; PP_{CWO} using the 3% solution was equivalent to PP_{HTDC} , excepting the pelagic marine sediment, for which PP_{CWO}/PP_{HTDC} reached 86.1%. Considering the solubility of $K_2S_2O_8$ in water and potential contamination from the reagent, further increases in persulfate concentration seem to be impractical, but the author carried out no test on higher-concentration persulfate solution. The 3% $K_2S_2O_8$ solution was thus employed in further assessments of the CWO method.

Effect of sample versus solution ratio—To determine optimal conditions, the author analyzed the samples of riverine SPM and net-collected plankton using various ratios of solid sample/solution (w/v) in the CWO digestion and the HTDC HCl extraction step. Using the HTDC method, P concentration values observed were invariable in both samples throughout the tested range (Fig. 3). However, using the CWO method, P concentrations decreased with increasing sample proportions of riverine SPM, particularly at ratios higher than ~1 mg/mL. Accordingly the author used solid/solution ratios no greater than 1 mg/mL, typically a 20 mg sample in 20 mL solution. Initially, it was thought that the low recovery at higher ratios might be due to insufficient oxidant, that is, that the increased amounts of organic matter might consume all the available persulfate. The CWO method, however, produced $PP_{CWO} \approx PP_{HTDC}$ for the plankton sample, which contained much larger amounts of organic C (see upper x axes, Fig. 3). Therefore, it was concluded that the low recoveries observed in the riverine SPM at high solid/solution ratios in the CWO method were not attributable to oxidant deficiency.

Comparisons for standard P compounds and particulate matter analogues—Various standard P compounds were tested using the CWO and HTDC methods, and PP_{CWO}/PP_{HTDC} results for

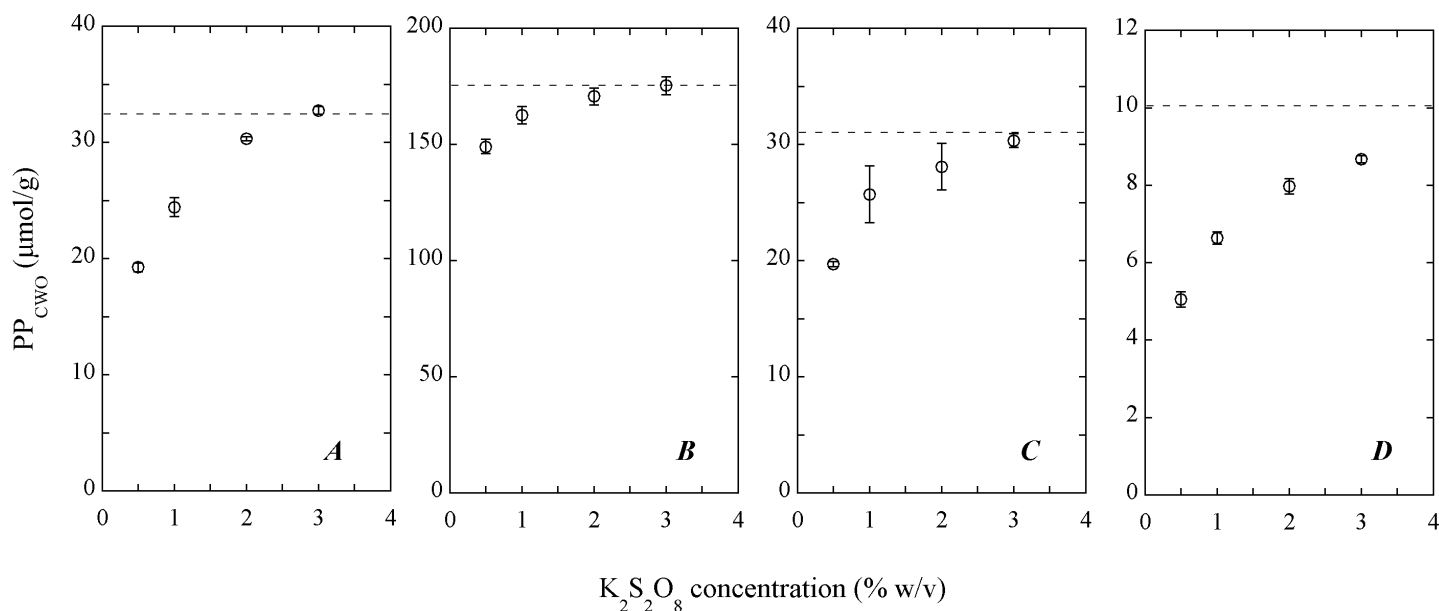


Fig. 2. PP_{CWO} in the samples of riverine SPM (A), net-collected plankton (B), coastal sediment (C), and pelagic sediment (D), using various concentrations (0.5% to 3%) of the $K_2S_2O_8$ solutions. The dashed lines in each graph represent the PP_{HTDC} .

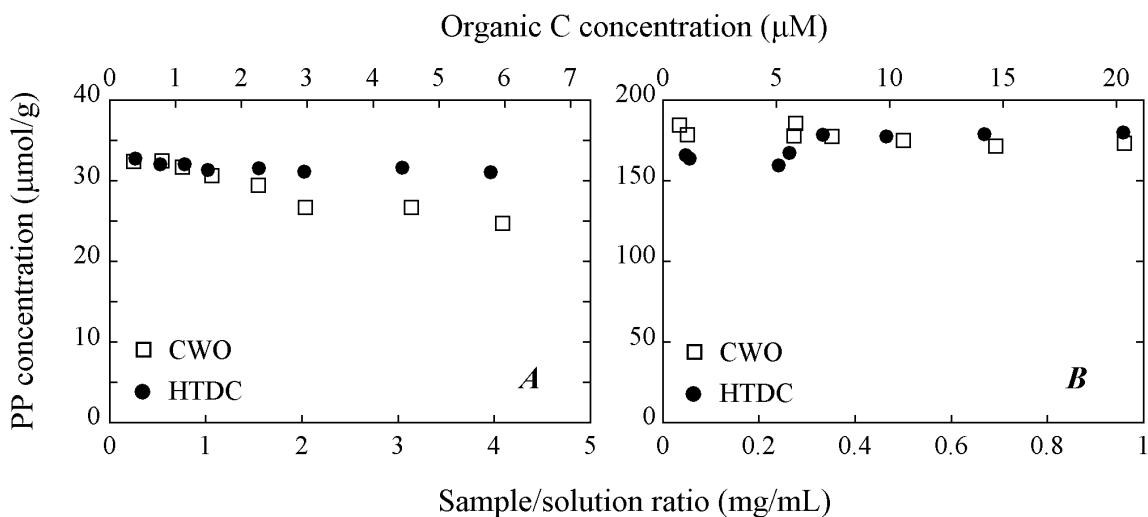


Fig. 3. PP concentrations in the samples of riverine SPM (A) and net-collected plankton (B) at various sample/solution ratios in the HTDC method and the CWO method. The upper x -axis represents organic C concentrations contained in the samples at the given sample/solution ratios.

these compounds are compiled in Table 2. In this study, recoveries could not be calculated accurately, because purity varied among the reagents; and consequently only approximate P recoveries could be obtained directly. Nevertheless, the HTDC methods provide almost complete recovery ($100 \pm 5\%$) of various P compounds, according to previous reports (e.g., Monaghan and Ruttenberg 1999). Thus, the digestion efficiency of the CWO method can be evaluated from the ratios of PP_{CWO}/PP_{HTDC} presented in Table 2.

Excepting phosphorus (V) oxide, for which PP_{CWO} exceeded PP_{HTDC} considerably, the two methods produced comparable results for the standard compounds tested (Table 2). The mean value of PP_{CWO}/PP_{HTDC} for five organic and four inorganic P compounds was 1.03 ± 0.07 . A concern regarding persulfate CWO methods has been their low recoveries of some organic P compounds stable to chemical digestion such as phosphonates (which contain C–P bonds) and phospholipids (K  rouel and Aminot 1996; Monaghan and Ruttenberg 1999). The CWO method used here demonstrated sufficient oxidation and hydrolysis capacity to liberate the P from these compounds, likely due to the increased concentration of the persulfate solution.

The results of PP determination for the analogues of naturally occurring particulate matter, and geochemical reference materials, are given in Table 3. Analysis of riverine SPM, coastal sediment, and net-collected and cultured plankton produced comparable results for the HTDC and CWO methods. The geochemical rock reference materials of limestone, feldspar, and coral produced comparable results as well. The PP_{CWO} in the pelagic marine sediment was slightly lower (92%) than PP_{HTDC} . Low recoveries were observed for CWO of montmorillonite and kaolinite, accounting for 69% and 14% of the PP_{HTDC} values, respectively.

Using the means and standard deviations calculated from 3 replicate measurements of each standard P compound, and 4

replicate measurements of each natural analogue sample, the averaged coefficients of variation were calculated as 2.1% for the HTDC and CWO methods. Thus, there was no significant difference (t test, $P < 0.05$) in analytical precision between the two methods.

Comparisons for estuarine and pelagic SPM—The HTDC and CWO methods were compared by analyzing 86 estuarine and 21 pelagic SPM samples for PP, using both methods. PP_{HTDC} concentrations in the estuary ranged from 0.23 to 15.2 μM , a few orders of magnitude higher than those in the pelagic seawaters (0.9–52.9 nM; Fig. 4). In the pelagic samples, PP_{HTDC} and PP_{CWO} were quite consistent; PP_{CWO}/PP_{HTDC} was 0.99 ± 0.10 (mean \pm SD) and ranged from 0.70 to 1.15. In estuarine samples, the methods were relatively comparable at low PP_{HTDC} concentrations ($< 3 \mu\text{M}$), with $PP_{CWO}/PP_{HTDC} = 1.05 \pm 0.12$ ($n = 60$), but the ratio decreased in the samples with high PP_{HTDC} ($> 3 \mu\text{M}$) to 0.85 ± 0.17 ($n = 26$). In the two samples of high PP_{HTDC} concentration ($> 10 \mu\text{M}$), PP_{CWO} was 53% and 70% of PP_{HTDC} .

Potential cause of the low recoveries of the CWO method—As mentioned, a major concern regarding persulfate CWO methods is their potential inefficiency in hydrolyzing and liberating P from chemically stable organic P compounds. Through the use of a high-concentration (3%) $\text{K}_2\text{S}_2\text{O}_8$ solution, the CWO method presented here recovered organic P compounds comparably with the HTDC method (Table 2). Inositol hexaphosphate has been identified as a major organic P compound among those tested in the riverine SPM sample used here (Suzumura and Kamatani 1995). The comparable values of PP_{CWO} and PP_{HTDC} for the riverine SPM suggest that even in a natural matrix containing various coexisting substances, the CWO method can efficiently recover chemically stable organic P.

During the comparison study, however, low recoveries of the CWO method relative to the HTDC method were observed

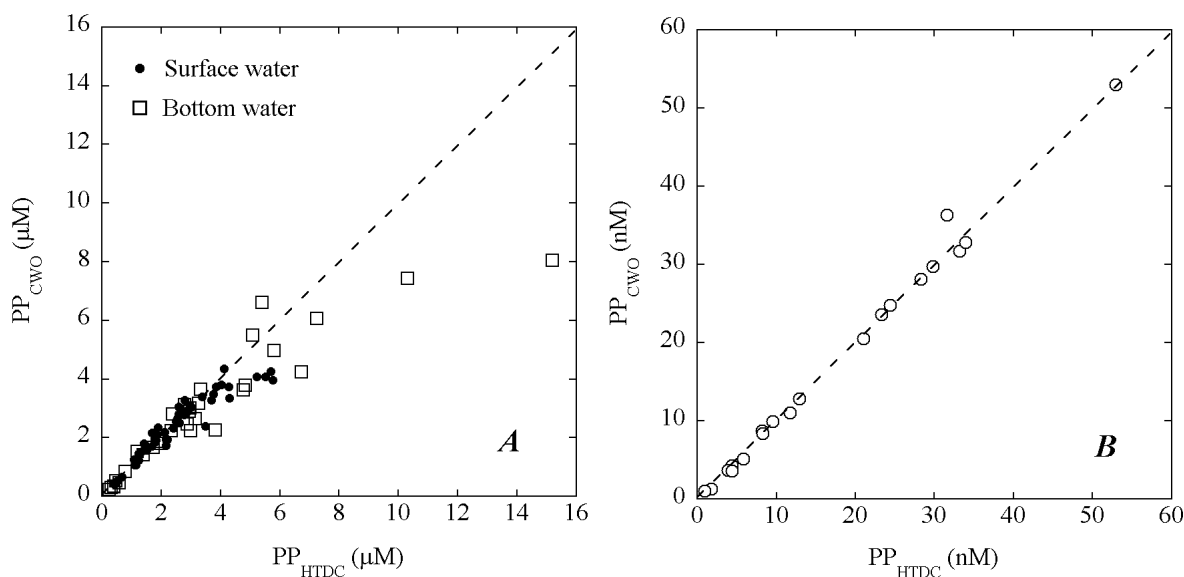


Fig. 4. Plots of PP_{HTDC} versus PP_{CWO} in the estuarine (A) and pelagic (B) samples. The dashed line represents $PP_{CWO}/PP_{HTDC} = 1$. Note different scales.

for pelagic marine sediment, some samples of estuarine SPM, and clay mineral reference materials. Particularly low recoveries were observed for clay minerals (Table 3). Montmorillonite and kaolinite have the chemical compositions $(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot H_2O$ and $Al_2Si_2O_5(OH)_4$, respectively, theoretically containing no P atom in their crystalline structures. But because the reference materials are not chemically pure, they may contain some unknown P components that cannot be easily liberated by CWO. The digestion solution's low acidity appeared to be a potential cause for the low CWO recoveries observed. Actually, the pH of the persulfate solution after digestion was about 1.1, approximately equivalent to a 0.08 M acid solution. In comparison, a higher concentration of acid solution (1 M HCl) was used in the HTDC method; this solution was expected to efficiently extract inorganic P from the clay minerals. To examine the effect of acidity on the efficiency of P extraction in the clay minerals, clay samples were then subjected to 1 M HCl extraction, on the basis of the protocol for particulate inorganic P (PIP) determination by Aspila et al. (1976).

A significant portion of P was extracted from montmorillonite with 1 M HCl, corresponding to 91.7% of PP_{HTDC} (Table 4). In contrast, acid-extracted P from kaolinite was only 12% of PP_{HTDC} . This result suggests that the low acidity of the

digestion solution was a minor factor in the low recovery observed in the CWO of kaolinite.

A further experiment was executed to examine the P forms and digestion mechanisms of kaolinite by a modified HTDC method in which the oxidant, $Mg(NO_3)_2$ was not added. This modified HTDC method recovered only 50.9% of the P in kaolinite determined by the original, $Mg(NO_3)_2$ -added, HTDC method (Table 4). The fraction of P that was not recovered from kaolinite by the CWO method, 1 M HCl extraction or the modified HTDC method was therefore in tightly bound forms and refractory structures that could be liberated efficiently only by the HTDC procedure with the addition of $Mg(NO_3)_2$.

The characteristics of the unrecovered fraction of P in kaolinite could not be fully identified in this study. However, the author does not believe that the lower CWO recoveries from the clay minerals were caused by insufficient oxidation efficiency of organic matter in the CWO method, because the organic C concentrations of these clay samples were quite low: approximately 0.1 mg/g. Thus, the unrecovered fraction was most likely composed of inorganic complexes, rather than organically bound forms. The SPM samples collected from the estuary are composites of particles of various origins: land-derived matter, fresh phytoplankton cells, and resuspended

Table 4. P concentrations in the reference samples of clay minerals determined by various procedures ($\mu\text{mol/g}$, mean \pm SD, $n = 4$)^a

Procedures	Montmorillonite	Kaolinite
HTDC with $Mg(NO_3)_2$ addition	1.44 \pm 0.03 (100%)	22.0 \pm 0.96 (100%)
CWO	1.00 \pm 0.02 (69.4%)	3.04 \pm 0.04 (13.8%)
HTDC without $Mg(NO_3)_2$ addition	1.42 \pm 0.04 (98.6%)	11.2 \pm 3.1 (50.9%)
1 M HCl extraction	1.32 \pm 0.02 (91.7%)	2.63 \pm 0.01 (12.0%)

^aThe values in parentheses are the P recoveries to the HTDC method with $Mg(NO_3)_2$ addition.

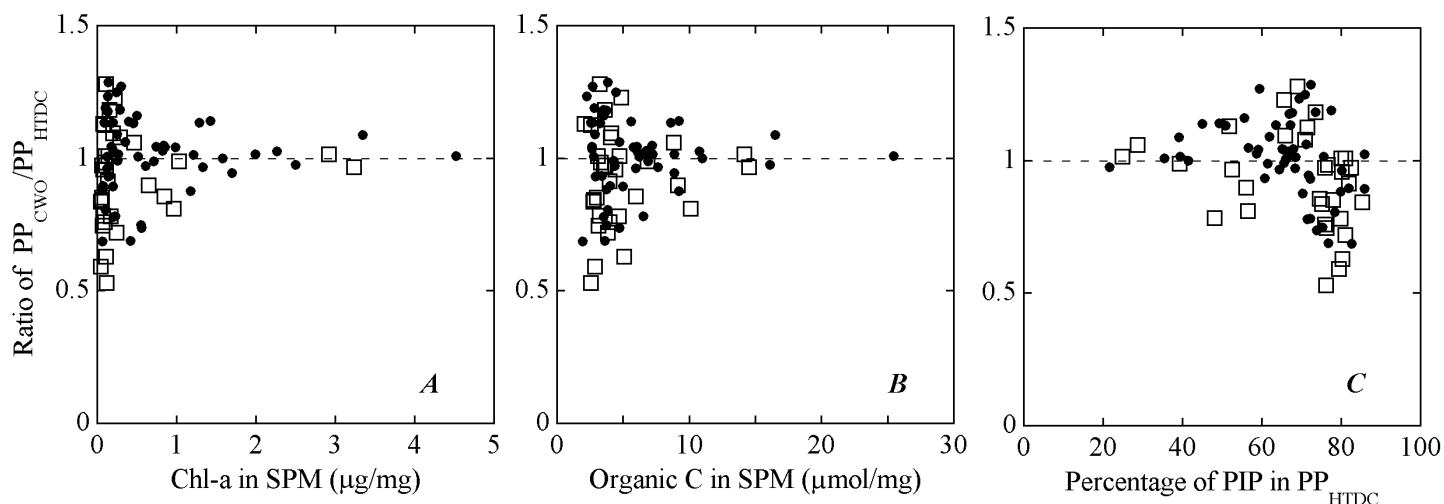


Fig. 5. Plots of the ratio of PP_{CWO}/PP_{HTDC} versus Chl *a* concentration in SPM (A), organic C concentration in SPM (B) and percentage of PIP in PP_{HTDC} (C). Symbols are same as Fig. 4A. The dashed line represents $PP_{CWO}/PP_{HTDC} = 1$. The concentrations of Chl *a*, organic C and PIP in SPM were calculated from the data of Suzumura et al. (2004).

sediments (Suzumura et al. 2004). The large variation observed in CWO recovery seems to be caused by the compositional variability of the estuarine SPM. Chlorophyll *a* (Chl *a*) concentration was employed as an index of SPM composition; Chl *a* concentration indicates the abundance of biological, phytoplankton-derived materials in the SPM. Relatively comparable results of PP_{CWO} with PP_{HTDC} were observed in Chl *a*-rich (> 2 mg/L) samples (Fig. 5A), whereas abiotic-particle-rich samples with low Chl *a* content exhibited high variability in PP_{CWO}/PP_{HTDC} , including some very low recoveries of PP_{CWO} (~53%). A similar trend was found in the relationship between the PP_{CWO}/PP_{HTDC} ratio and the organic C content in SPM (Fig. 5B). In addition, Fig. 5C shows the relationship between the PP_{CWO}/PP_{HTDC} ratio and the PIP concentration in SPM; the low recoveries of the CWO method was observed in PIP-rich samples. It is concluded that the low P recoveries from some estuarine samples by the CWO method can be ascribed to some inorganically bound structures that were recalcitrant to chemical digestion. The highly linear correlation found between PP_{HTDC} and PP_{CWO} in the pelagic samples (Fig. 4) implies that such a chemically stable fraction is negligible in the euphotic zone of the open ocean environments. The possibility remains that SPM and sinking particles in deep sea environments contain the recalcitrant inorganic P that is found in the estuarine environments.

The results presented here indicate that $Mg(NO_3)_2$ can efficiently destroy recalcitrant inorganic P structures as well as organic molecules. Information is limited in describing the structure of the recalcitrant inorganic P that is effectively destroyed in the $Mg(NO_3)_2$ added-HTDC method only. No proper compound of simple phosphate salts can be supposed. One possible explanation is that a portion of P might be tightly trapped within the interior of the crystalline structures of clay minerals.

Discussion

The clearest advantage of the CWO method over the HTDC method is its simplicity and its less time-consuming procedures. A concern regarding the CWO method—its presumed low efficiency in liberating some organic P compounds—was avoided, with almost complete recoveries from standard P compounds and organic-rich natural samples. The presence of a recalcitrant fraction of inorganic P was demonstrated in clay minerals and some estuarine samples; this leads to the underestimation of PP by the CWO method, relative to the HTDC method. Further investigation is needed to better understand the sources and characteristics of the recalcitrant fraction. The fraction of P that withstands drastic chemical oxidation, however, is probably inert to the biological and chemical processes of natural environments. Even though the recalcitrant fraction is quantitatively a major component of PP in some aquatic environments, including estuaries, its immobility and nonreactivity might hamper its use as a biologically available P source. The low recoveries of the CWO method can be then stated as an advantage of this method rather than a defect, by selectively excluding this unreactive and less available P fraction. This property of the CWO method is likely to be useful in improving the determination of particulate organic P (POP). POP concentration is usually estimated as the difference between PP and PIP concentrations (e.g., Aspila et al. 1976; Solózano and Sharp 1980). This experimental definition of POP is based on the assumption that all inorganic P can be recovered into the PIP fraction, which is usually measured as inorganic P extractable by dilute acid. In this study, however, the presence of some recalcitrant forms of inorganic P in natural particulate matter was strongly suggested; such inorganic P can be liberated by the HTDC method but not by the CWO method. It can be thus concluded that the HTDC method

leads to overestimation of POP by including such recalcitrant inorganic P as organic P.

Comments and recommendations

Except for the alumina membrane filter, the filter blanks contained less than 6 nmol P in each sample (each containing one filter 2.5 cm in diameter). The detection limit of PP by the CWO method presented here has not been fully discussed. The detection limit can theoretically be calculated from the standard deviation of the blank, and the volume of filtered sample water. For example, in the case where a GF/F filter (2.5 cm in diameter) is used to filter a 5-L aliquot of seawater, the detection limit of the CWO method is estimated as 0.04 nM, when the detection limit is defined as 3 standard deviations ($n = 4$, $P < 0.05$) of the measured filter blank (Table 1), divided by the filtered volume. Unfortunately, the author has no data on blank variation between lots of each filter type. In addition, the difference in pretreatment conditions of filtration, including concentration, time, and volume of acid washing of filters, probably causes deviation in the filter blank among investigations. To minimize the error derived from filter blank, adequate precautions must be taken, including frequent blank tests and the use of filters from the same lot during investigation, especially for the precise measurement of samples low in PP concentration.

This study demonstrated serious P contamination in alumina membrane filters. Since a significant fraction (> 95%) of P was removed from the alumina membrane using 1 M HCl, under the same conditions for PIP extraction reported by Aspila et al. (1976), acid prewashing might be effective in minimizing the contamination. Considering their extremely high P content, however, it is still difficult to recommend alumina membranes for use in PP determination. Furthermore, caution should be taken in the use of alumina membranes even for dissolved P analysis, because of the potential risk of contamination into filtrates.

Stoichiometric characterization of C, N, and P provides substantial information on the nature and origin of particulate matter, and important insights for additional understanding of the biogeochemical cycling of the bioelements. Considering its low blank (the lowest among the filters tested) and its sharply defined pore sizes alone, the PC membrane filter seems to be most suitable for PP determination. Nevertheless, because they are most frequently used in POC and PON determination, glass fiber filters are also recommended for PP determination.

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