

A method for the measurement of particulate C and P on the same filtered sample

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ABSTRACT: There is substantial interest in measuring C:P ratios in aquatic systems because these particulate ratios may influence food quality of phytoplankton to zooplankton grazers. In most analyses C is measured by dry combustion using a CN analyzer requiring that P be measured on a separate sample. We show for marine and freshwater phytoplankton cultures and naturally occurring seston that C can be measured by a variation of the same wet-persulfate digestion method frequently used for P analysis, allowing C and P to be measured on the same filter. Over a large range of C values (1 to 40 $\mu\text{mol C filter}^{-1}$), the persulfate method for measuring C gave the same C value for lake seston, estuarine seston, and marine and freshwater phytoplankton cultures as high temperature dry combustion on a Carlo-Erba CN analyzer. For C, the wet digestion method is much less expensive than the dry combustion method in terms of both equipment and per-sample materials costs. Further, the persulfate method is more precise (CV = 3%) than the dry combustion method (CV = 12%) for the measurement of C. This greater precision of C measurement, and the fact that C and P are measured on the same sample, means that small differences in C:P ratios can more readily be detected.

KEY WORDS: Particulate · Phosphorus · Carbon · Method · C:P ratio

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INTRODUCTION

Carbon to phosphorus ratios (C:P) in aquatic ecosystems are an important indicator of food quality (Sterner et al. 1997, Elser et al. 1998) and a critical determinant of oceanic export production (Sambrotto et al. 1993, Jingfeng et al. 2000). Most methods for particulate C analysis were developed over 30 yr ago (Maciolek 1962, Menzel & Vaccaro 1964, Wangersky & Gordon 1965, and others), and have since been altered and improved (e.g., Gordon & Sutcliffe 1974, Sharp 1974, Hilton et al. 1986, Nakajima 1986, McDowell et al. 1987, and others). All of these methods begin with chemical or physical oxidation to convert all forms of C to CO_2 , followed by its subsequent analysis using gas (GC) or liquid chromatography, or through the use of infrared CO_2 analyzers. In various versions, the oxidation step involves dry combustion in an ampoule or

CHN analyzer (American Public Health Association 1992), wet oxidation using persulfate or chromic acid (Strickland & Parsons 1968) or other oxidizing agents, or irradiation of samples with ultraviolet light (American Public Health Association 1992). Total P (TP) analysis has generally used a wet persulfate digestion since Menzel & Corwin (1965) introduced this method. Phosphorus is converted to PO_4 and analyzed colorimetrically on a spectrophotometer or autoanalyzer (American Public Health Association 1992).

Following the recommendation by Strickland & Parsons (1967) to analyze C using CH-analyzers when available, the C in seston has generally been measured using these instruments. When C:P ratios are to be evaluated, replicate seston samples for P undergo a separate, persulfate digestion and are analyzed by colorimetric techniques on separate filters (American Public Health Association 1992). This use of separate samples to determine C:P ratios increases the cost of analysis as well as the variability of the results because of slight differences in the amount of material on sepa-

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rate filters. Determined this way, the C:P ratio includes the variance resulting from sample collection on separate filters and the analytical variance of both methods.

In 1993, Malley et al. introduced a method that could determine C, N, and P on the same sample, but it required the use of a near-infrared spectroscope, expensive equipment not normally associated with aquatic research. Recently Raimbault et al. (1999) introduced a new method based on a basic persulfate digestion for the simultaneous determination of particulate C, N, and P on a single filter. While this method seems efficient at N and C digestion, we were concerned that it may not hydrolyze some forms of P. Polyphosphates may be especially difficult to hydrolyze under these conditions.

We present a wet digestion technique, which is a variation of the standard TP digestion of Menzel & Corwin (1965). We compare C measured by this technique with that on a Carlo-Erba C:N analyzer. Further, we compare P determination by this method with a recently proposed basic persulfate digestion method (Raimbault et al. 1999) and with a dry digestion method developed for sediments (Anderson 1976). Lastly, we show some initial results for particulate N determination in which we perform a redigestion under basic conditions of the original acid digest.

MATERIALS AND METHODS

The digestion is a variation of the persulfate method that Menzel & Corwin (1965) developed for TP. The procedure involves 4 steps: (1) collecting particulate matter on glass fiber filters; (2) digesting filters in sealed 20 ml serum vials containing 10 ml of 1% persulfate in an autoclave for 2.5 h at 120°C; (3) extracting the CO₂ in the head space and analyzing it using a GC equipped with a Thermal Conductivity Detector (TCD); and (4) analyzing the digested P as PO₄ in the aqueous portion by spectrophotometry.

Glass fiber filters (Whatman GF/F, 25 mm) were prepared for use with samples and standards by soaking for 1 h in 0.1 N H₂SO₄ and rinsing with nanopure water. These filters remained intact during the digestion, eliminating the need to filter the digestate for PO₄ analysis.

- Standards—Glucose, L-serine and tripolyphosphate mixed standards were prepared in concentrations that allowed 200 µl aliquots to be pipetted directly onto, and be absorbed by, prepared glass fiber filters.

- Seston—Surface water was collected from Chodikee Lake, Ulster County, New York, USA (dominated by Cyanobacteria), and at a number of sites along the freshwater tidal portion of the Hudson River Estuary in

1 l HDPE bottles. Aliquots of 100 to 200 ml were filtered through glass fiber filters that had been cleaned as above.

- Phytoplankton cultures—Three types of phytoplankton cultures were used. The marine diatom *Ditylum brightwellii* was purchased from Provasoli-Guillard National Center for Culture of Marine Phytoplankton (Bigelow Laboratory, ME, USA; CCMP #359) and grown in a 1/10 dilution of Stosch media (von Stosch & Drebes 1964). Cultures of a freshwater chrysophyte (*Ochromonas* spp.) and chlorophyte (*Selenastrum capricornutum*) were purchased from Carolina Biological Supply (Burlington, NC, USA) and cultured in lake water enriched with N, P, and bicarbonate in Redfield ratio (Redfield 1958) as described in Caraco & Miller (1998). Aliquots of 10 to 40 ml were filtered as for seston (above).

- Zooplankton samples—Samples were obtained by hauling a 120 µm plankton net through Upton Lake, Dutchess County, New York. Once collected, the zooplankton samples were not filtered. Rather, known numbers were picked individually using an Irwin loop and placed directly into the serum vials.

For the filtration of seston and phytoplankton, we used a vacuum of no greater than 0.65 atmospheres (61 kPa), and the filters were generally dried before running as required by the Carlo-Erba analyzer. Additionally, however, samples can be frozen for future analysis or run immediately because dried samples are not necessary in a wet digestion procedure. A filtered volume of 100 to 200 ml worked well for lake and river seston, while 10 to 40 ml was sufficient for phytoplankton cultures. If larger volumes of water need to be filtered, or samples have high DOC concentrations, tests should be run to evaluate DOC adsorption to the glass fiber filters (Abdel-Moati 1990).

Procedure. Samples and standards were digested by placing filters in 20 ml serum vials (Wheaton #223687) containing 10.0 ml of 1% low N potassium persulfate (Fisher Scientific ID #P282-500; 10.0 ml), sealed by crimping on aluminum rings (Kimble #73822A-20) containing butyl rubber septa (Tekmar #14-4385-043) and autoclaved for 2.5 h at 120°C. Using an autoclave with a slow pressure release is important since a rapid drop in pressure may cause the vials to leak, thus altering results.

Once samples reached room temperature the carbon headspace samples were extracted with a 20 ml syringe equipped with a 2-way stopcock and needle. With the stopcock open, the needle was inserted through the septa, the plunger drawn back to 15 ml, the stopcock closed, and the plunger allowed to draw back to neutral, approximately 11 ml. These headspace samples were then run on a Shimadzu GC-8A equipped with a thermal conductivity detector with the

injector/detector at 110°C and the column at 44°C. The remaining aqueous portion of the sample, containing the persulfate digested particulate P sample, was fully oxidized to PO_4 . After allowing the sample to settle, 3 ml of liquid was extracted. This subsample was then analyzed colorimetrically following the method of Murphy & Riley (1962) using a Shimadzu UV-160 spectrophotometer with a 1 cm spec cell and the wavelength set to measure at 880 nm.

For a limited number of samples, the possibility of total N determination was tested by redigesting the 1% persulfate digested sample in basic persulfate following the method of D'Elia et al. (1977). The digestion reagent was prepared by dissolving 2.0 g of low N potassium persulfate and 1.0 g of low N sodium hydroxide (Fisher Scientific ID #5318-100) in 50 ml of nanopure water. Screw top test tubes containing 3 ml of subsamples were then spiked with 0.45 ml of this digestion reagent, capped and autoclaved for 2.5 h at 120°C. The resulting nitrate was measured by the sulfanilamide method (American Public Health Association 1992) modified for an Alpkem Autoanalyzer (Alpkem Corporation 1992).

To compare the digestion efficiency of our carbon method, the peak areas derived from persulfate digested glucose/L-serine standards run using our method were compared with acidified bicarbonate standards in the same concentration range analyzed using the method of Stainton (1973). Both sets of stan-

dards were run on the same Shimadzu GC (GC-8A) equipped with a thermal conductivity detector and set up as described above. Similarly, replicate particulate C samples, as phytoplankton and seston, were run using our method and on a Carlo-Erba C-N-S elemental analyzer where C was oxidized at 1000°C, and resulting peaks were compared with a known mass of acetanilide standard to determine mass.

To evaluate TP digestion efficiency of our method, replicate seston samples from the Hudson River Estuary were run using our persulfate technique and compared with those run using a very rigorous TP ignition method designed for sediment and biogenic materials by Andersen (1976). In this method, samples are placed in a porcelain crucible and ignited in a muffle furnace (550°C for 1 h) followed by boiling in 1 N HCl for 15 min and colorimetric determination of P. Replicate Hudson River seston samples and tripolyphosphate (Sigma ID #T5883) standards analyzed using our technique were also compared with those analyzed using the basic persulfate digestion method designed by Raimbault et al. (1999), where samples are autoclaved at 120°C for 30 min in a basic solution of 0.75% persulfate.

RESULTS

Over a wide range of C (0.26 to 42 μmol per vial) and P (0.125 to 4.0 μmol per vial) masses, standards were linear for both analyses (Fig. 1). Most C analysis runs were done with standards ranging from 2.1 to 21.0 μmol . The variability in peak height of these standards, both within and between runs, was low and inversely correlated to C mass. For samples <2 μmol , more than 10 individual samples are needed so that the 95% CI does not exceed 10% of the mean value (Fig. 2). For samples >20 μmol , there is a 95% certainty that the mean of duplicates is within 10% of the true mean. Blank filters (no organic C added) produced a relative peak height (mean peak height = 296 000; coefficient of variation [CV] = 22.6) 29% lower than the lowest (2.1 μmol) standard (mean peak height = 419 000; CV = 12.1).

To evaluate the carbon digestion efficiency of our persulfate GC technique, the results of natural samples run using our GC technique were compared with those run on a high temperature (1000°C) dry combustion Carlo-Erba C-N-S elemental analyzer using an acetanilide standard. For sample filters containing from 1 to 40 μmol no significant difference was found between the 2 methods (Table 1, Fig. 3). In a linear regression between the 2 methods, the intercept (0.55 $\mu\text{mol} \pm 0.6$ [SE]) was not significantly different from 0 ($p > 0.35$) and the slope (0.983 ± 0.6 [SE]) of the regression

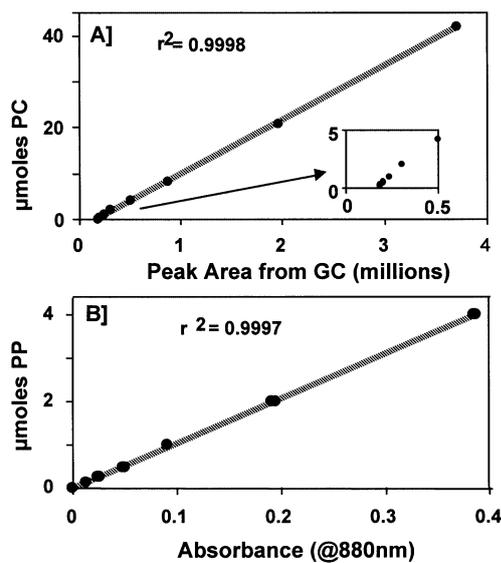


Fig. 1. Standard curves for the measurement of (A) particulate C (PC) and (B) particulate P (PP) using the 1% persulfate digestion technique. For (A), the equation for the line is $\mu\text{mol C} = 1.12 \times 10^{-5} \times \text{peak area} - 1.79$; $r^2 = 0.999$. The inset box shows the relation at very low amounts of particulate C (0 to 5 μmol C per filter). For (B), the equation for the line is $\mu\text{mol P} = 10.38 \times \text{absorbance} + 0.0068$; $r^2 = 0.999$. GC: gas chromatography

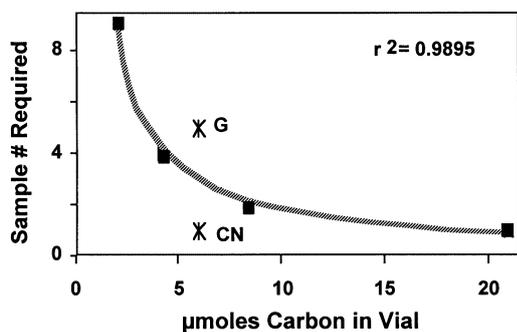


Fig. 2. Replication analysis for the 1% persulfate method to measure C. The heavy line with solid square points shows the number of samples required so that the 95% CI does not exceed 10% of the mean, as a function of the amount of C (as glucose/L-serine mixed stock standards). The equation for the line is $y = 16.74 \times x^{-0.95}$; $r^2 = 0.989$. Asterisks show the same calculation for *Ditylum brightwellii* cultures at one C level run on the Carlo-Erba CHN (CN) analyzer and on the gas chromatograph (GC) using the 1% persulfate method

was not significantly different from 1.0 (t -test, $p < 0.01$). This shows that in the 1 to 40 μmol range, digestion efficiency in persulfate is equivalent to that in the high temperature CN analyzer. Further, it shows that there is not a significant systematic bias between the 2 methods.

Any variation for individual samples from the 1:1 line could have been caused by analytical error or actual differences in the amount of material on replicate filters. Filter replicates may vary in the amount of material filtered, inclusion of rare particles on one filter, or the clumping of phytoplankton or suspended materials within the sample. This last possibility may be a particular problem for the seston filters where inclusion of a single zooplankton on one filter and not the other may alter the results.

The variability in carbon analysis using our persulfate digestion GC method was also compared with that using the Carlo-Erba CN analyzer. To do this, a culture of the marine diatom *Ditylum brightwellii* was used.

Table 1. Equations of the line for replicate samples run using our gas chromatograph technique (x-axis) and a combustion CN analyzer (y-axis)

	r^2	Slope	Slope (95% CI)	y-intercept
Chrysophytes	0.92	1.01	0.83–1.18	-1.62
Chlorophytes	0.88	0.97	0.76–1.18	1.34
Diatoms	0.97	0.97	0.88–1.07	-0.24
Lake seston	0.71	0.88	0.57–1.19	4.21
Estuarine seston	0.93	1.09	0.75–1.42	-0.53
All combined	0.90	0.98	0.91–1.02	0.55

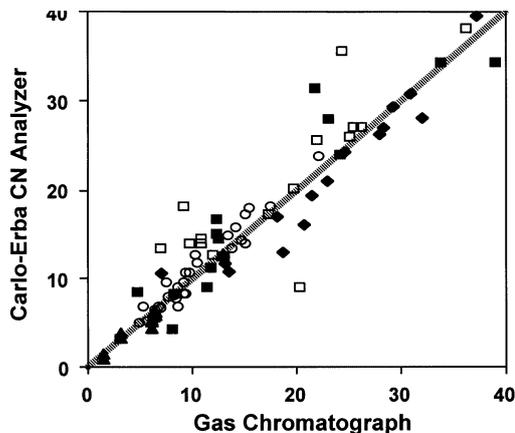


Fig. 3. Comparison of C analyzed by wet digestion and run on a gas chromatograph technique (our technique) with the traditional combustion CN analyzer. Replicate samples of phytoplankton and seston were compared: chrysophytes (\blacklozenge); chlorophytes (\blacksquare); diatoms (\blacktriangle); lake seston (\square); and estuarine seston (\circ). The line is the 1:1 ($y = x$) line. Actual regression equations are presented in Table 1. Results are plotted as total C measured in μmol

These cells do not form clumps; therefore, replicate filters should have similar amounts of material. The Carlo-Erba technique was found to be 4 times more variable than the GC technique in the sample size we tested (Table 2). The precision of the GC technique means that fewer replicates are necessary when looking for differences in samples with very similar carbon concentrations, thus saving time and expense.

To determine the efficiency of the persulfate digestion on particulate phosphorus, replicate samples of seston from the Hudson River Estuary were run using our 1% persulfate method and an ignition method for TP in lake sediments (Anderson 1976). No significant difference was found between the 2 methods. The intercept ($0.01 \mu\text{mol} \pm 0.03$ [SE]) was not significantly different from 0, nor was the slope (0.951 ± 0.14 [SE]) of the regression of micromoles P (ignition method) as a function of micromoles P (persulfate method) significantly different from 1.0 (t -test, $p < 0.0001$). This shows that in the 0.05 to 0.3 μmol range, there is complete oxidation of all forms of P in 10 ml of 1% persulfate.

The digestion efficiency of a new CNP method proposed by Raimbault et al. (1999) and our 1% persulfate method was also tested on polyphosphate standards and Hudson River Estuary seston. Our 1% persulfate method digested over 90% of the polyphosphate while the Raimbault et al. (1999) method digested under 20%

Table 2. Variability of data obtained by the Carlo-Erba combustion CN analyzer compared with the gas chromatograph (GC) method using 8 replicate diatom (*Ditylum brightwellii*) samples for each method. Phosphorus values were derived from the same sample run for C on the GC providing C:P ratios close to data obtained by Redfield (1958) with very low variation, while C:P ratios provided by separate C and P (where C is run on a CN analyzer) analysis yielded much larger variability. CV: coefficient of variation

	C (μmol)		P (μmol)	C:P ratio	
	CN analyzer	GC	1% persulfate	CN analyzer	GC
	6.30	6.58	0.065	96.6	100.9
	5.92	6.59	0.067	88.4	98.4
	4.99	6.11	0.063	79.3	97.1
	5.55	6.21	0.063	88.2	98.7
	5.33	6.10	0.064	83.5	95.6
	4.39	6.09	0.062	72.0	99.8
	6.31	6.24	0.062	102.6	101.5
	6.11	6.31	0.065	93.7	96.8
Mean	5.61	6.28	0.064	88.0	98.6
Range	4.39–6.31	6.09–6.59	0.061–0.067	72.0–102.6	95.6–101.5
CV	12.18	3.25	3.19	11.1	2.1

compared with sodium phosphate standards. Further, when replicate samples of Hudson River seston were run using both methods the results were significantly different (paired *t*-test; $p < 0.001$). The Raimbault et al. (1999) method returned particulate phosphorus values averaging 55% lower (range 28 to 72%) than those digested in 1% persulfate.

When 10 individual *Daphnia* spp. were digested in 1% persulfate, without filters, their resulting C:P ratios were lower, but similar to those found by Anderson & Hessen (1991), and the variability was small (mean C:P = 53.75; CV = 8.2) (Table 3). Attempts to digest

Table 3. Results from 9 replicate samples of 10 individual zooplankton (*Daphnia* spp.) digested in each vial using the 1% persulfate digestion technique with the resulting C and P masses. The resulting C:P ratios are similar to those of Anderson & Hessen (1991), although slightly lower, and show little variability. CV: coefficient of variability

C (μmol)	P (μmol)	C:P ratio
180	3.48	52
297	5.73	52
248	4.83	51
388	7.17	54
275	4.93	56
172	3.79	45
362	5.92	61
277	4.91	57
317	5.65	56
	Mean	54
	Range	45–61
	CV	8

individual *Daphnia* spp. failed because of low C mass of individuals.

In an effort to analyze individual samples for C, P, and N, a limited number of samples were taken and the persulfate digested portion was redigested in basic persulfate following the method of D'Elia et al. (1977) (Fig. 4). Although, in the 0.25 to 3.0 μmol range tested, the results were linear and the intercept of the regression of basic persulfate as a function of persulfate/basic persulfate was not significantly different from 0 (0.03 ± 0.07 [SE]), the slope of the line was greater than 1 (1.15 ± 0.05 [SE]). This indicates that 10 to 20% of the N is lost during the initial digestion, possibly through volatilization. Recovery of some of this may be possible during the C analysis step using a GC capable of detecting N_2 and N_2O .

DISCUSSION

Since C:P ratios are so important in understanding aquatic food webs, our simple, efficient, yet inexpensive technique that analyzes particulate P and C without the use of separate samples should be useful to many researchers. We have found only 2 other methods for measurement of particulate P and C on the same sample in the literature. The earlier technique by Malley et al. (1993) is limited by the need for a very expensive near-infrared reflectance spectrophotometer. The recent method by Raimbault et al. (1999)

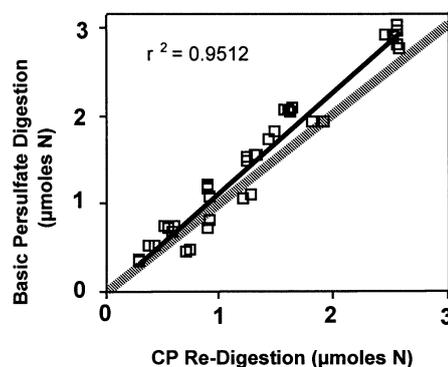


Fig. 4. Comparison of replicate filtered samples run using a standard basic persulfate digestion of D'Elia et al. (1977) with redigestion of previously digested 1% persulfate samples handled as for C:P analysis. The regression line is $y = 1.15x - 0.027$; $r^2 = 0.95$

uses a new wet oxidation procedure for the simultaneous digestion of C, N, and P. Although C and N may be completely oxidized by this method, we found this technique to inefficiently hydrolyze both polyphosphate standards and natural estuarine seston samples. Since some phytoplankton can contain polyphosphate (Kuhl 1962), an inefficient digestion may result in substantial errors in C:P ratios.

Errors in C:P ratios can also occur when replicate samples are run separately for C and P, which is true when C is run using dry combustion and P is analyzed separately using a wet digestion. The error resulting from nonmatched C and P samples are most severe when there is large variation in C and P content between samples. For zooplankton samples this error can be particularly large because, even with a matched number of zooplankton per sample, the size of zooplankton can cause substantial differences in C and P content per sample. For example, for 9 replicate samples of zooplankton with 10 zooplankton per sample (Table 3), the CV for C and P was 26 and 22%, respectively, but was only 8% for C:P ratios on matched samples. On the other hand, when we randomly matched C and P replicates, which would be true for separate analysis of C and P samples, the CV of C:P ratios increased to 37%. For well replicated samples this increase in variance should be far less. For example, for the well mixed diatom sample (Table 2), CV for C:P ration increased only from 2.1 to 3.9% when we randomly matched filter pairs. For seston samples with moderate between-sample variation the problem of errors generated from nonmatched samples are likely intermediate between these 2 extremes.

Another advantage of the 1% persulfate method over others is the cost associated. Equipment and materials costs as well as time can be reduced by using this method. For example, the cost of a Carlo-Erba CN analyzer exceeds \$30 000, and the per-sample analysis cost is approximately \$3.80. For the GC method, equipment costs are less than \$8000 and per-sample analysis costs just over \$0.50. The preparation and analysis rate for both methods is roughly 8 to 12 samples h⁻¹, but if phosphorus is also to be run, substantial time is saved by using the GC method since the samples have already been digested for that analysis.

The use of persulfate digestion to oxidize organic materials has been widely accepted in aquatic research. When we compare our adaptation of this with the other accepted methods the results are indistinguishable. On phytoplankton and seston samples in the range of 1 to 40 µmol and digested in 1% persulfate, both P and C are oxidized as completely as through the use of high temperature combustion. Further, C sample replication was found to be more precise with this procedure. The relatively low cost of the

equipment and sample analysis make this method useful for the numerous determinations necessary to characterize the C:P ratios in aquatic communities.

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