An automated photo-oxidation method for the determination of dissolved organic phosphorus in marine and fresh water

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Abstract:

A segmented flow automated method with on-line photo-oxidation for the determination of dissolved organic phosphorus+soluble reactive phosphorus (DOP+SRP) in seawater and fresh water is described here. A low-power lamp was used for a compact, easy-to-handle and low-ozone-producing manifold. The influence of seawater matrix components was studied in detail using natural seawater and salt solutions spiked with DOP model compounds. Bromide was found to be the most inhibitory species in seawater. The work shows that most salt solutions referred to as artificial seawater are not satisfactory model matrices to test the actual seawater matrix effect. Since DOP recovered in undiluted seawater samples was about half that obtained in fresh water samples, the described method includes a 5- to 6-fold dilution of seawater samples. This simple procedure overcomes matrix effects and provides satisfactory DOP recovery. No pH effect was found in the 6–9 range corresponding to most natural waters. The standard deviation was 0.007 µmol l^{-1} and the limit of detection 0.02 µmol l^{-1}. Linearity was tested up to 5 µmol l^{-1} of DOP, i.e. far above naturally occurring values. A throughput of 20 samples per hour is easily achieved.

Keywords: Dissolved organic phosphorus; Total dissolved phosphorus; Seawater; Fresh water; Measurement

AN AUTOMATED PHOTO-OXIDATION METHOD FOR THE DETERMINATION OF DISSOLVED ORGANIC PHOSPHORUS IN MARINE AND FRESH WATER

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ABSTRACT

A segmented flow automated method with on-line photo-oxidation for the determination of dissolved organic phosphorus + soluble reactive phosphorus (DOP + SRP) in seawater and fresh water is described here. A low-power lamp was used for a compact, easy-to-handle and low-ozone-producing manifold. The influence of seawater matrix components was studied in detail using natural seawater and salt solutions spiked with DOP model compounds. Bromide was found to be the most inhibitory species in seawater. The work shows that most salt solutions referred to as artificial seawater are not satisfactory model matrices to test the actual seawater matrix effect. Since DOP recovered in undiluted seawater samples was about half that obtained in fresh water samples, the described method includes a 5 to 6-fold dilution of seawater samples. This simple procedure overcomes matrix effects and provides satisfactory DOP recovery. No pH effect was found in the 6-9 range corresponding to most natural waters. The standard deviation was 0.007 μmol l^{-1} and the limit of detection 0.02 μmol l^{-1}. Linearity was tested up to 5 μmol l^{-1} of DOP, *i.e.* far above naturally occurring values. A throughput of 20 samples per hour is easily achieved.

Keywords: Dissolved organic phosphorus, total dissolved phosphorus, seawater, fresh water, measurement.

1 INTRODUCTION

Phosphorus is well known as an essential micronutrient for microalgae. It is present in waters under various physico-chemical forms (Strickland and Parsons, 1972; McKelvie et al., 1995). Dissolved organic phosphorus (DOP) may provide a phosphorus reservoir for microalgae, but because of the lack of well-established methods, this DOP is rarely measured. With growing interest in the organic matter pool, several works have focused in the past decade on the accurate determination of DOP in marine waters (Ridal and Moore, 1990, 1992; Kérouel and Aminot, 1996; Ormaza-Gonzalez and Statham, 1996; Karl and Yanagi, 1997; Monaghan and Ruttenberg, 1999). Studies on C-N-P stoichiometry of dissolved organic matter (DOM) in conjunction with remineralization processes also rely on accurate determination of DOP (Clark et al., 1998). Presently, no direct DOP determination method is available and, despite long-standing interest in DOP, no simple routine method for its determination exists. Strictly speaking, DOP is the difference between the total dissolved phosphorus (TDP) and dissolved inorganic phosphorus (DIP). The DIP pool consists mainly of orthophosphate, but also includes pyrophosphate and inorganic polyphosphates (McKelvie et al., 1995; Thomson-Bulldis and Karl, 1998). However, DIP is often not distinguished from orthophosphate, or more exactly, from soluble reactive phosphorus (SRP). SRP represents orthophosphate and potentially some easily-hydrolyzable DOP and inorganic phosphorus compounds that would react under the phosphomolybdate-blue method's reaction conditions.

Nevertheless, most published DOP data have been obtained as the difference between TDP (measured by an oxidative conversion of all phosphorus forms into orthophosphate) and soluble reactive phosphorus (SRP). Therefore DOP may include some polyphosphates which occur naturally in waters (Solorzano and Strickland, 1968; Solorzano, 1978). However, when photo-oxidation with UV light is used as the digestion technique, DOP is converted into orthophosphate but DIP compounds are largely unaffected (Armstrong and Tibbits, 1968; Solorzano and Strickland, 1968).

Table 1. Technical characteristics of automated methods for the determination of total dissolved phosphorus or the sum of soluble reactive phosphate and dissolved organic phosphorus in natural waters. References: 1. Grasshoff (1967); 2. McKelvie et al. (1989); 3. Collos and Mornet (1993). SFA = segmented flow analysis; FIA = flow injection analysis.

| | Method reference | | |
1	2	3	This work
Flow type			
SFA	FIA	SFA	SFA
Digestion mode			
UV	persulfate-OH^--UV	H^+-UV + persulfate-OH^--UV	H_2O_2-UV
Hg-lamp: power consumption and λ max			
900 W (252 nm)	40 W (265 nm)	100 W (254 nm)	100 W (254 nm)
Digestion time			
15 min	5 s	8 min + 8 min	7 min
Medium for model compounds tested			
Seawater	Fresh water	Fresh water and 30 g l^{-1} NaCl	Sea and fresh water
Sample medium			
Seawater	Fresh water	Seawater	Sea and fresh water
Sample throughput (h^{-1})			
20	50-72	no data	20

The concentration of DOP is low in seawater (generally lower than a few tenths of a micromole) and good performance in both TDP and SRP are required in order to obtain accurate and precise DOP data. Intercomparison exercises have shown that the determination of TDP was a difficult task for many laboratories (Aminot et al., 1997). Only few automated methods have been described in the literature for the determination of TDP in natural waters although automation is a way of minimizing random errors, because the procedure is highly repetitive and sample handling (and hence potential contamination) is reduced. These

attempts included different flow techniques, as well as digestion and reaction conditions. Some methods have been developed for flow analysis of TDP in various mediums: soil leachate and runoff waters by Peat et al. (1997), wastewaters by Aoyagi et al. (1988), Hinkamp and Schwedt (1990) and Williams et al. (1993). These methods generally use drastic digestion conditions and their limits of detection are inappropriate for unpolluted natural waters, especially seawater. Table 1 summarizes the characteristics of methods designed for natural (sea and fresh) surface water analysis. Grasshoff (1967) used a high power UV digestor while McKelvie et al. (1989) and Collos and Mornet (1993) used lower power lamps. Low power systems are easier to handle and preferable for staff safety in laboratories, since ozone production is greatly reduced. To produce TDP results, Grasshoff (1967) pretreated his samples with acid for 1 hour at 90 °C in order to hydrolyze traces of polyphosphates, then the samples were neutralized and submitted to digestion without any additional reagent. In other methods, digestion of organic compounds is carried out either in an acidic persulfate medium (Peat et al., 1997) or an alkaline persulfate medium (McKelvie et al., 1989) or a combination (Collos and Mornet, 1993). Kérouel and Aminot (1996) have shown that chemical or UV digestion of organic phosphorus compounds was more difficult in seawater than in deionized water. Recovery rates of 100 % (within analytical accuracy) were achieved for all tested compounds in deionized water, whatever the digestion method, while only 50 % or less may be recorded for some molecules in seawater. This suggests that calibration with organic standards run in deionized water may lead to erroneous data for DOP in seawater. Grasshoff (1967) did not specify the medium for his DOP standards, while Collos and Mornet (1993) operated in 30 g l^{-1} NaCl, which cannot be strictly regarded as seawater (see below). The test medium used by McKelvie et al. (1989) was assumed to be demineralized water, since they worked in fresh water.

To determine DOP for DOM stoichiometry studies, an automated low-power photo-oxidation method has been used in our laboratory for many years . The method was chosen over other published methods since it combined several advantages: i) precision is improved by automation; ii) the throughput is significantly higher than in manual or semi-automated (batch digestion, then automated SRP measurement) methods; iii) photo-oxidation does not digest polyphosphates; iv) few reagents are required. In order to eliminate matrix effects, digestion was promoted by dilution of the sample while adding H_2O_2 (as suggested by Armstrong et al., 1966) to increase the medium's oxidizing strength. The SRP measured after digestion resulted from SRP + DOP initially present in the sample, therefore DOP was computed by subtracting SRP measured in parallel from that sample. Although empirical results had shown the reliability of this method, no attempt was previously made to determine specific factors influencing the process until the preparation of standard solutions of model compounds was accurately controlled (Kérouel and Aminot, 1996).

The paper presents criteria influencing the recovery of DOP by on-line photo-oxidation and describes an automated method for the determination of DOP in natural waters (from seawater to fresh water). The discussion deals with the composition of the phosphorus pool in waters with respect to the described method's performance for DOP determination.

2   MATERIAL AND METHOD

All materials used are commercially available. The manifold combines dilution, on-line UV photo-oxidation and phosphate determination (Fig. 1). It will be referred to as the automated dilution photo-oxidation (AD-UV) method below. The digestion unit is composed of a quartz coil from Technicon (ref. 188-B09702; 48 turns, int. diam. 2 mm, 15 ml) and a 100 W low-pressure Hg lamp (Pen-Ray, model 3SC9 from U-V Products). The digestion unit can be inserted upstream of any phosphate manifold provided that the ascorbic acid

concentration is adjusted (see below). Because of the high dilution of the sample, the Technicon SCIC colorimeter was set at maximum sensitivity (std cal = 10.0). Despite this, the background noise and drift remained quite acceptable (see section 3.2.).
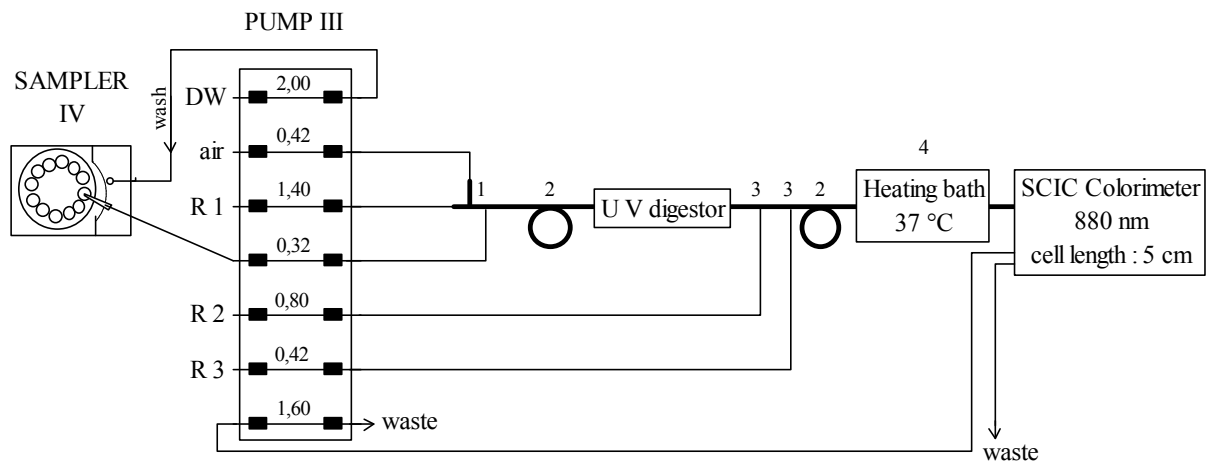
Figure 1. The automated dilution photo-oxidation method for the determination of SRP+DOP in sea and fresh waters. The manifold is based on Technicon Autoanalyzer II material. Part # 1: injector 116-0489-01; part # 2: mixing coils 10 turns 157-0226-01; part # 3: injectors 116-B034-01; part # 4: heating bath 157-B273-03. R1: digestion/dilution reagent; R2: ascorbic acid reagent; R3: heptamolybdate reagent; (see Material and method section). Flows are in millilitre per minute.

Irradiation takes place in the close-to-neutral or slightly alkaline medium of natural samples, which prevents the chlorine formation observed under acidic conditions (Grasshoff, 1967). To measure phosphate following digestion, reagent concentrations were adjusted according to Murphy and Riley (1962), but the concentration of ascorbic acid was multiplied by about 5, to remove residual hydrogen peroxide which interferes in the determination of PO_4. Tests have shown that, provided other reaction conditions are optimal (Pai et al., 1990), a large excess of ascorbic acid does not alter phosphate determination. The refractive index blank (RIB) was corrected systematically. For RIB determination, samples were re-run without ammonium heptamolybdate added to reagent 3 (see below) to prevent color development (Tréguer and Le Corre, 1975; Loder and Glibert, 1977). The reagents are prepared with high purity Milli-Q demineralized water (DW).

-   Reagent 1 (digestion/dilution reagent): 12.4 mmol l^{-1} H_2O_2 prepared by diluting 30 % H_2O_2 Baker 7047 (1.4 ml l^{-1}). The Baker reagent was selected for its low P contamination. In the reaction medium the H_2O_2 concentration is 10 mmol l^{-1}. At higher concentrations, O_2 resulting from H_2O_2 decomposition disrupts flow segmentation. Since 30 % H_2O_2 decomposes slowly, its concentration should be checked about twice a year (10 µl of 30 % H_2O_2 discolor 2.07 ml of 0.02 mol l^{-1} $KMnO_4$ in H_2SO_4 0.5 mol l^{-1}) and working solution prepared accordingly.
-   Reagent 2: 18 g l^{-1} ascorbic acid (Merck 127) and 0.5 ml Aerosol 22 (Sigma A-9753) as wetting agent.
-   Reagent 3: 150 ml of ammonium heptamolybdate solution (40 g l^{-1}, Merck 1182) were mixed with 800 ml of 1.5 mol l^{-1} H_2SO_4 (Merck 731), then 50 ml of potassium antimonyl tartrate solution (3 g l^{-1}, Merck 8092) were added.
-   RIB reagent (replaced reagent 3 for RIB determination): 200 ml of DW were mixed with 800 ml of 1.5 mol l^{-1} H_2SO_4.

The P compounds used to test the method are shown in Table 2. In order to check photo-oxidation recovery, the model compounds were dissolved, either in natural phosphate-depleted coastal seawater (low-P water: SRP < 0.005 μmol l^{-1}) obtained by aging in light conditions in the laboratory, or in the artificial saline matrices shown in Table 3.

In all tests, model compounds were used at a concentration of 1 μmol l^{-1}. Most of the corresponding data result from duplicate determinations, generally carried out in the same run, but not successively. Since the standard deviation computed from these duplicates was within 0.01 μmol l^{-1} (1 %), the precision was not indicated in graphs and tables, for simplification.

Table 2. Model compounds tested in this study for phosphorus recovery as phosphate. Sodium salts were used, except when otherwise mentioned. nP = number of P atoms per molecule.

Class (P-bond type) and compounds	Abbreviation	nP
Nucleotides (C-O-P)		
Adénosine 5' monophosphate	AMP	1
Guanosine 5' monophosphate	GMP	1
Adénosine 5' diphosphate	ADP	2
Inosine 5' diphosphate	IDP	2
Adénosine 5' triphosphate	ATP	3
Cytidine 5' triphosphate	CTP	3
Uridine 5' triphosphate	UTP	3
Phosphosugars (C-O-P)		
Glucose 6' phosphate	GluP	1
Ribose 5 phosphate	RP	1
β-glycerophosphate	GP	1
Other organics (C-O-P)		
Phosphoenolpyruvic acid**	PEPA	1
Riboflavine 5' monophosphate	RMP	1
Phosphoryl choline chloride*	PCC	1
Phytic acid	PA	6
Phosphonate (C-P)		
2-Aminoethylphosphonic acid	AEPA	1
Inorganic poly-P (P-O-P)		
Pyrophosphate ($H_4P_2O_7$)	PP	2
Tripolyphosphate ($H_5P_3O_{10}$)	TPP	3

*calcium salt
**tri(cyclohexylamine) salt

SRP determination was always run in parallel, from the same sample bottle, using the concentrations recommended by Murphy and Riley (1962) for all the reagents.

Sampling of seawater was performed using classical Niskin-type bottles. A subsample was taken in a 0.5-1 litre glass bottle, then filtered without delay through a precombusted Whatman GF/F disk hold on a glass filter-holder fitted on a separatory funnel (this enables sample bottles to be filled without pouring). The filtered aliquot (~ 100 ml) was placed in a precombusted 125 ml borosilicate vial which was closed with a linerless polyethylene screw-cap and immediately placed in a freezer (- 25 °C). Samples were thawed within a few hours before analysis and sample bottles were placed directly on the Autosampler, without any

transfer, in order to prevent potential contamination (Kérouel and Aminot, 1987). Gloves were always worn during drawing off and handling of samples.

3   RESULTS

3.1   Examination of photo-oxidation conditions for DOP recovery

Photo-oxidation conditions used for developing the AD-UV method were adjusted using a testing manifold without automated sample dilution. When necessary, samples were diluted manually before analysis.

3.1.1   *Effect of salinity on the recovery of DOP in natural seawater samples*

Natural seawater samples from the Bay of Seine (French coast of the English Channel) were analyzed for DOP concentration (range 0.06-0.2 µmol l^{-1}) with and without dilution.
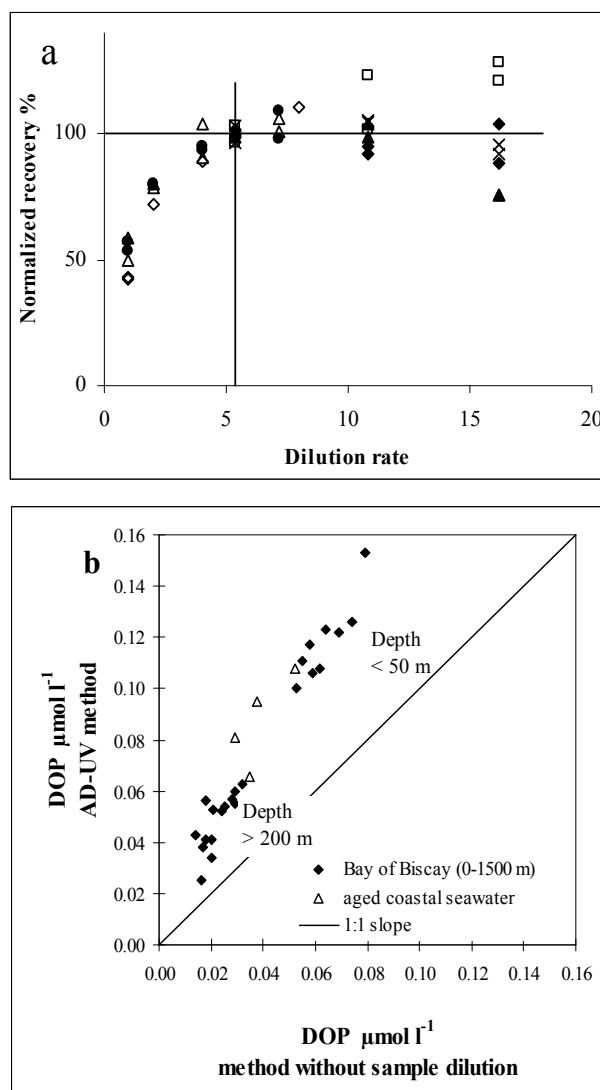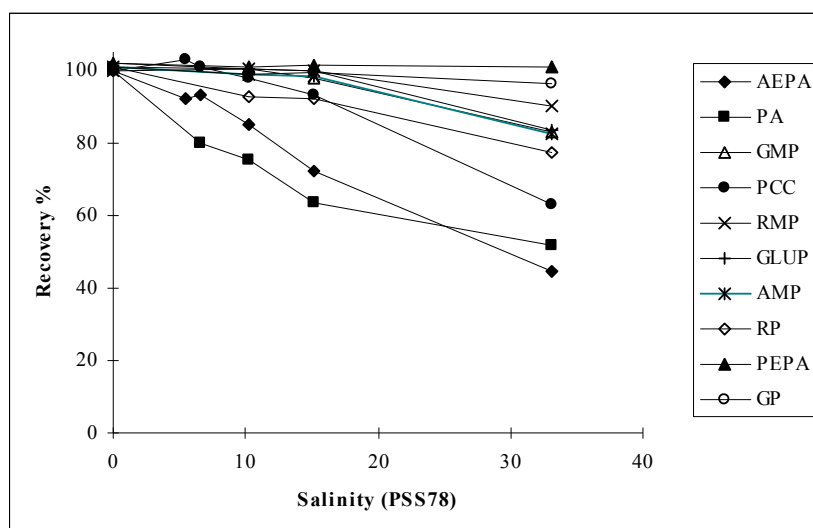

Figure 2. Effect of dilution on the recovery of DOP in natural seawater samples. a) Recovery as a function of dilution; values are standardized with respect to the dilution rate of 5.4; the symbols identify the various samples (most were duplicated). b) Comparison of DOP results found in seawaters using photo-oxidation without dilution and with a dilution of 5.4 (the AD-UV method).

Figure 2a illustrates the non linear effect of dilution (hence of salinity) on DOP results, the recovery of DOP increasing as a function of the dilution rate. In order to make the comparison independent of sample concentration, data were standardized with respect to the value found at a dilution rate of ~ 5-6. Indeed, above this dilution rate DOP recovery reached a threshold, so that the average recovery remained virtually constant (dispersion of data at high dilution rates results from increased relative standard deviation with decreased measured signals). This suggested a partial inhibition of photo-oxidation due to some salt effect. Consequently, the method presented here was essentially based on the sample's dilution, with a dilution rate empirically set at 5.4, a compromise which provided good recovery and satisfactory signal magnitude. This corresponds to a salinity lower than 7 when analyzing oceanic seawater.

The importance of dilution for DOP recovery has been confirmed for oceanic water (two 0-1500 m profiles from the Bay of Biscay) and aged waters (four coastal waters stored for about a year). Figure 2b shows that, whatever the nature and age of the water, dilution by 5.4 enhanced DOP recovery by a factor of about 2.

3.1.2   Effect of salinity on the recovery of DOP model compounds

The model compound standards were prepared in low-P water with a salinity of about 33, then dilutions of about one half (S ~ 15) and one third (S ~ 10) were tested. Figure 3 shows that the recovery of refractory compounds is greatly enhanced when salinity decreases below about 15 and that around a salinity of 10 virtually 100 % recovery is achieved for the majority of compounds. Additional dilutions, down to salinities of about 6.5 and 5.5, for the two refractory compounds 2-aminoethylphosphonic acid (AEPA; containing a C-P bond) and phosphoryl choline chloride (PCC; containing a C-O-P bond) increased their recovery rates to 93 and 100 % (± 2 %), respectively. Phytic acid (PA; containing 6 C-O-P bonds) was the most difficult to recover, with ~ 80 % at a salinity of 6.5. These results confirmed that the selected dilution rate for the automated method was able to decrease the matrix effect of natural seawater to very low levels if mixtures of DOP compounds were considered.

Figure 3. Effect of salinity on the recovery of DOP test compounds in saline water by on-line photo-oxidation. Abbreviations of compounds are listed in Table 2.

3.1.3   Recovery of DOP model compounds in artificial solutions

In order to determine whether the main seawater ions or minor UV-absorbing species (bromide and nitrate) may potentially alter DOP recovery, standards of highly refractory DOP

compounds were prepared in artificial salt solutions (see Table 3). AEPA and PCC, were systematically tested and PA was tested in bromide and nitrate solutions. Reference solutions containing phosphate standards were run simultaneously for internal calibration.

Table 3. Artificial matrices prepared to check the recovery of model compounds by photo-oxidation. Only Merck Reagent Grade products and Milli-Q demineralized water were used.

Matrix	Abbreviation
artificial seawater (from Grasshoff, 1976) containing the major salts: sodium, potassium, magnesium, calcium, chloride and sulfate	ASW
ASW + NaHCO$_3$ at seawater concentration: 200 mg l^{-1} (2.38 mmol l^{-1})	ASW+HCO$_3$
ASW + KBr at seawater concentration:100 mg l^{-1} (0.84 mmol l^{-1})	ASW+Br
ASW + NaHCO$_3$ 200 mg l^{-1}+ KBr 100 mg l^{-1}	ASW+HCO$_3$+Br
demineralized water	DW
demineralized water + NaCl 30 g l^{-1}	DW+NaCl
demineralized water + KBr 85 mg l^{-1} (0.71 mmol l^{-1})	DW+Br (1)
demineralized water + KBr 170 mg l^{-1} (1.43 mmol l^{-1})	DW+Br (2)
demineralized water + KNO$_3$ 8.6 mg l^{-1} (85 μmol l^{-1})	DW+NO$_3$ (1)
demineralized water + KNO$_3$ 42.5 mg l^{-1} (420 μmol l^{-1})	DW+NO$_3$ (2)

The results confirmed the complete recovery previously found in deionized water (Kérouel and Aminot, 1996). They showed that DOP recovery of AEPA and PCC was also complete in sodium chloride, and only slightly depleted in artificial seawaters, whether buffered with bicarbonate or not, but in the absence of bromide (Fig. 4). However, a poor yield was measured when bromide was present. This effect was slightly attenuated by bicarbonate. The bromide inhibition on photo-oxidation was confirmed by pure solutions of potassium bromide. Nitrate solutions also produced a measurable drop in yield at concentrations that can be encountered in coastal waters or estuaries (up to 7 % for AEPA at 420 μmol l^{-1} of NO$_3$). Bromide is, therefore, one of the main interfering factors in photo-oxidation of DOP in seawater. Since bromide and nitrate strongly absorb UV in the 210-220 nm range, the results suggested that this absorption may be the main cause of reduced DOP mineralization. Since bicarbonate increases yields, despite a role in radical scavenging agent (Peat et al., 1997), it will not interfere in natural waters. Significant reduction of H$_2$O$_2$ by bromide is unlikely since H$_2$O$_2$ is introduced in great excess in the medium (6 times that of bromide in seawater), but resulting products may alter recovery. Removal of bromide was considered, but ruled out since it would not be applicable in routine work.

3.1.4 *Influence of H$_2$O$_2$ concentration on DOP recovery*

Tests with refractory model compounds in undiluted seawater showed that addition of hydrogen peroxide improved the recovery of AEPA and PCC by factors of 1.5 and 3.3, respectively. Because the medium is still slightly saline in the dilution photo-oxidation method, it was considered preferable to add hydrogen peroxide in order to ensure the highest possible oxidizing conditions.
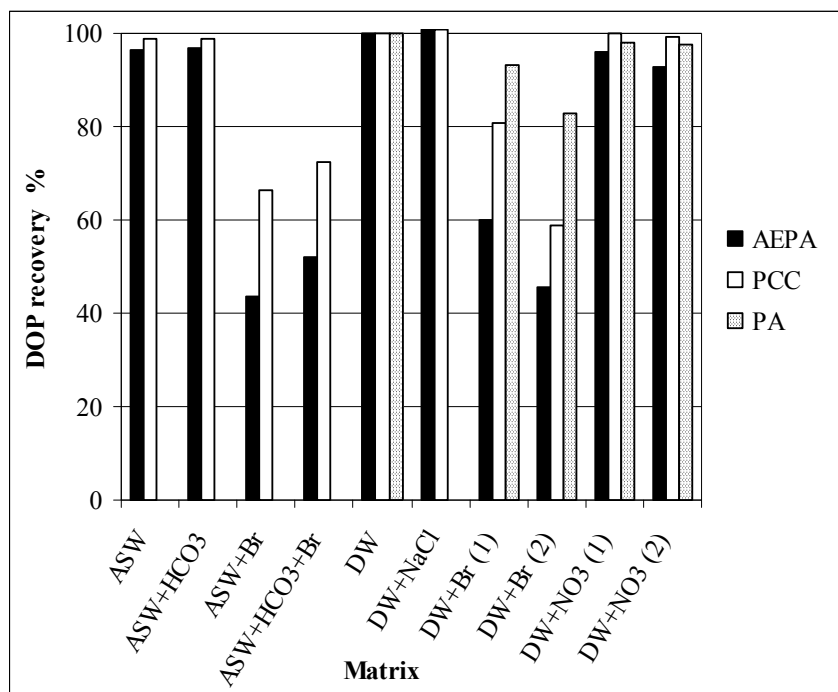
Figure 4. Effect of matrix composition on the recovery of refractory DOP model compounds in undiluted samples. AEPA = 2-Aminoethylphosphonic acid; PCC = Phosphoryl choline chloride; PA = phytic acid. Matrix types are detailed in Table 3.

3.1.5 Influence of pH on DOP recovery

AEPA, PCC and PA were photo-oxidized in seawater at pH 8.2 (no treatment), then in slightly alkaline conditions, *i.e.,* pH 8.8 (seawater + borate) and pH 9.2 (seawater + carbonate), and slightly acidic conditions, *i.e.,* pH 6.9 and 6.1 (seawater + HCl). All the corresponding recovery rates from undiluted samples remained within a ± 1 % range. This showed that DOP recovery by photo-oxidation was unaffected by pH in the range of most natural surface waters (pH 6 to 9). The PA peak shape was degraded at the highest pH value. It was concluded that no improvement could be expected from photo-oxidizing the samples in slightly alkaline or acidic conditions.

3.1.6 Influence of lamp aging on DOP recovery

One frequent criticism of photo-oxidation is the risk of insidious decay of the lamp leading to an underestimation of concentrations. A test was performed using DOP model compounds to compare a used lamp (after about a thousand hours in use) and a new lamp of the same model described above. The samples were not diluted in order to generate the maximum matrix effect and strengthen the test. Overall recovery data with the new lamp (figure 5a) were quite similar to those found previously (Kérouel and Aminot, 1996). The results showed that the used lamp produced recovery rates only a few percent lower than the new lamp on all tested compounds. The difference was relatively greater for the most refractory compounds, which indicated that refractory model compounds are useful and sensitive tools for assessing the method's DOP recovery. The test indicated that it is important to check and replace the lamp periodically to guarantee good recovery.
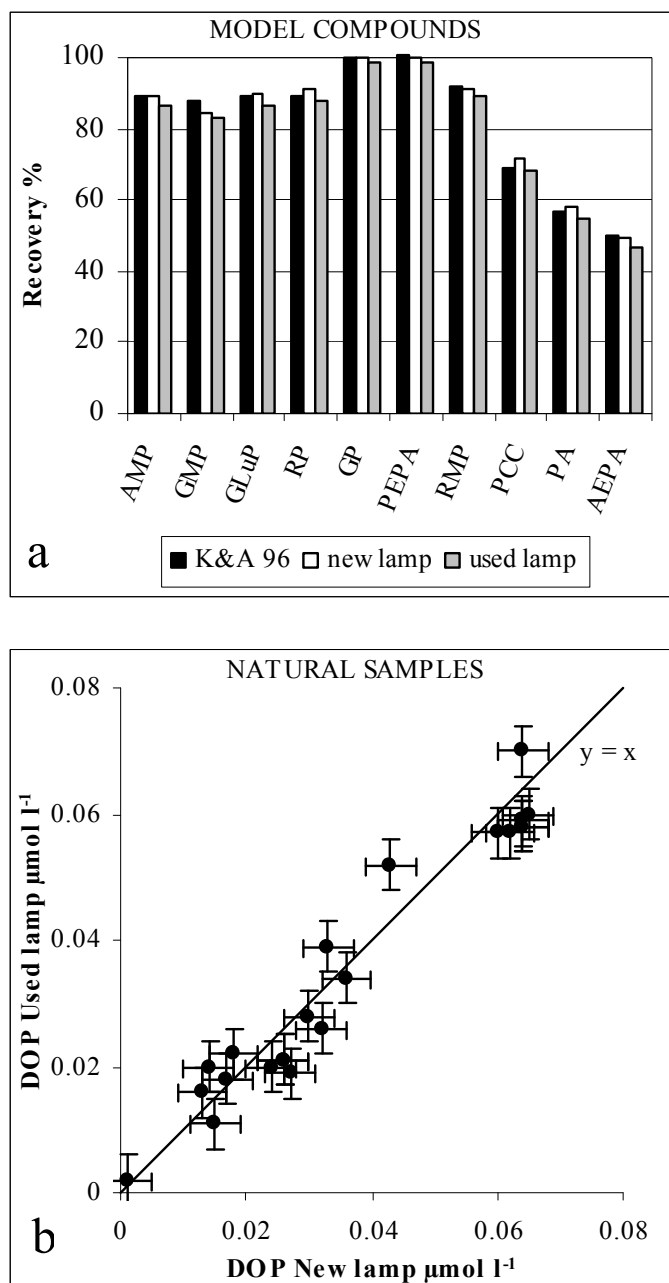
Figure 5. Comparison of recovery rates of DOP in undiluted seawater with a new lamp and used lamp (about one thousand hours in use). a) Recovery of model compounds compared with data previously found (Kérouel and Aminot, 1996). b) Recovery of naturally occurring DOP in various aged and fresh seawaters. Abbreviations of compounds are listed in Table 2.

The comparison was then broadened to twenty-four samples of fresh coastal and aged offshore seawater which were analyzed successively using the two lamps (Fig. 5b). No significant difference appeared between the two sets of data which fell within the precision expected with undiluted samples ($s = 0.004$ µmol l^{-1}). Although relative precision at these low concentrations (< 0.08 µmol l^{-1}) was lower than in model compound measurements, the test indicated that there was no major drop in recovery of naturally occurring DOP with the used lamp.

3.2 The dilution photo-oxidation method

3.2.1 Sample signal and throughput

A typical record of SRP+DOP peaks obtained with the dilution photo-oxidation (AD-UV) method is shown in Fig. 6. The wide plateaus obtained provide precise determination of signal values despite a slight noise due to the colorimeter being set at maximum sensitivity. The usual sample throughput is 20 h^{-1}, but this rate can be slightly increased without creating major problems. The limiting factor is inherent to the determination of phosphate by the molybdenum blue method with antimony since sorption onto the glass wall progressively generates peak tailing and sharpening if rinsing is not sufficient (Hansen and Grasshoff, 1983).
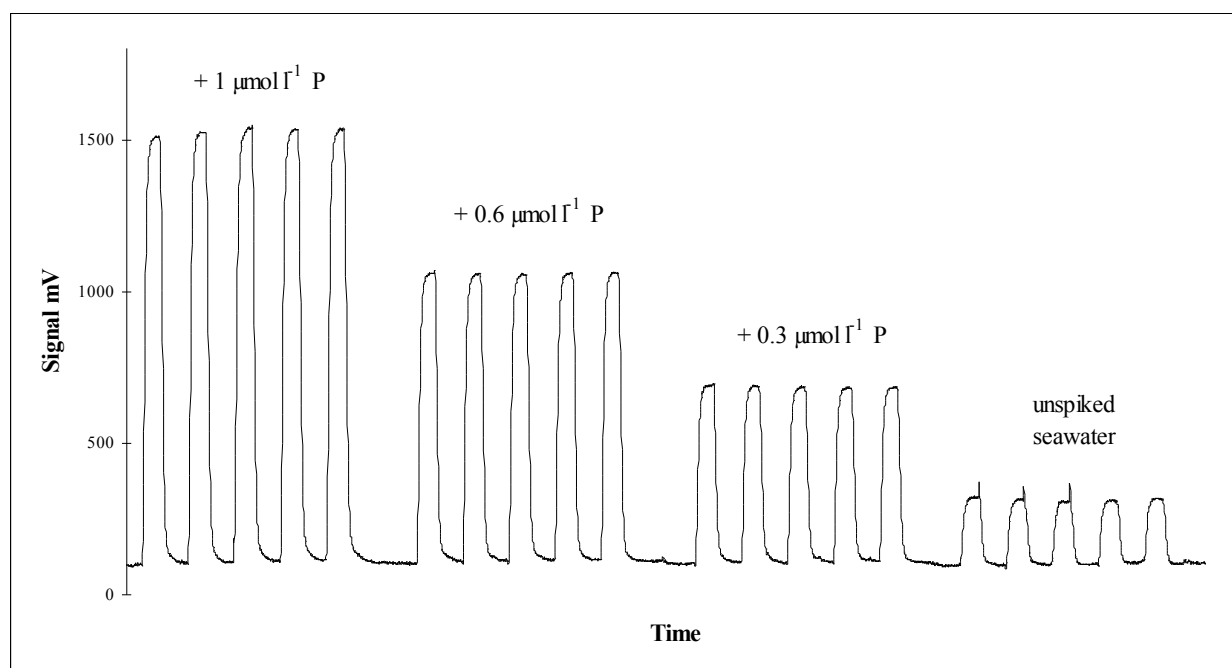
Figure 6. Typical record of SRP+DOP peaks obtained with the described automated dilution photo-oxidation method (figure 1); sample throughput is 20 h^{-1}.

3.2.2 Calibration and precision

The calibration curve was made using phosphate standards and checked with refractory DOP model compounds, usually AEPA (Fig. 7). Linearity was verified up to 5 µmol l^{-1} of AEPA, which greatly exceeds expected concentrations of naturally occurring DOP concentrations in waters. The slope of the AEPA standard curve is 94 % that of phosphate (as expected from previous experiments; section 3.1.2.) with R^2 = 0.9996 and standard error = 0.5 % for both compounds.

Since DOP is calculated as a difference (TDP minus SRP), its precision is a function of several variables. Due to the highly variable proportion of DOP in TDP (from almost 0 % in deep waters to almost 100 % in phosphate-depleted surface waters), standard deviations of TDP and SRP (and hence, DOP) can theoretically cover a wide range of values. Since variances add up ($s^2_{DOP} = s^2_{TDP} + s^2_{SRP}$), there is no simple rule for predicting DOP precision from those of SRP and TDP as a function of concentrations. Therefore, reproducibility was estimated from replicate analyses of samples of various origins separated by variable time intervals. We found that the standard deviation of TDP (0.004-0.010 µmol l^{-1}) was slightly

greater than that of SRP (0.001-0.004 µmol l^{-1}), as expected from signal amplification. However, no relationship was found between standard deviations and concentrations of SRP and TDP in the range encountered in most oceanic, coastal and estuarine waters (< 6 µmol l^{-1}). Data on DOP, summarized in Table 4, showed that a standard deviation of 0.007 µmol l^{-1} can be retained as an overall maximum value for DOP throughout the 0-6 µmol l^{-1} TDP range.

The limit of detection of the dilution photo-oxidation method for DOP, estimated at three times the standard deviation of a small concentration (Taylor, 1990), is necessarily based on values obtained at a high TDP, *i.e.* $3 \times 0.007 \sim 0.02$ µmol l^{-1}.
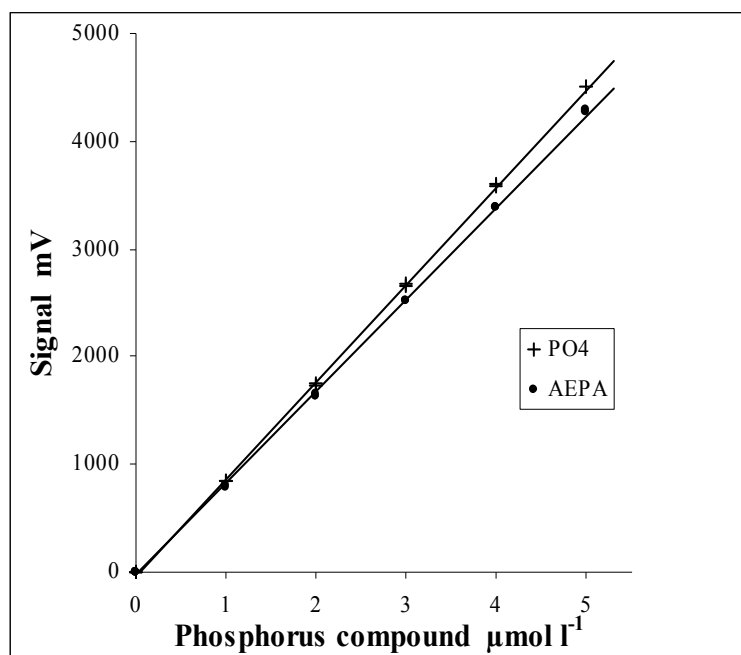
Figure 7. Calibration curve made in seawater with the described automated dilution photo-oxidation method using phosphate and control curve with the refractory DOP compound 2-aminoethylphosphonic acid (AEPA: recovery: 94 %, as expected).

Table 4. Standard deviations of DOP measured by the automated dilution photo-oxidation method in various conditions from series of duplicate samples.

Sample type (sample number)	Phosphate concentr. µmol/l	Time between measures min	Standard deviation µmol/l
Aged seawaters (3)	< 0.005	9	0.002
Coastal waters, any season (9)	0.02 to 1.1	30-60	0.006
Estuarine waters, any season (4)	3 to 6.5	30-60	0.007
Coastal waters, undiluted and diluted (12)	0.15 to 0.7	9	0.004

3.2.3 *Interference from polyphosphates*

Standard solutions (1 µmol l^{-1}) of pyrophosphate, tripolyphosphate and several di- and triphosphate nucleotides (see Table 2) in seawater were analyzed with the SRP and AD-UV methods. The SRP response was in the range 0.5-2.5 % of the standard concentration. With the dilution photo-oxidation method, inorganic phosphates were recovered within 0.5-1.5 %,

organic diphosphates within 1-3.5 % and organic triphosphates within 10-14 %. This agrees with previous findings that P-O-P bonds are strongly resistant to UV depolymerization in neutral pH conditions (Armstrong et al., 1966; Ormaza-Gonzalez and Statham, 1996).

3.2.4   Comparison with the manual alkaline persulfate digestion method

A series of seawater samples from various French coastal and offshore areas (Bay of Seine, Bay of Brest and Bay of Biscay) was analyzed with the dilution photo-oxidation method and with the alkaline-persulfate digestion method (Koroleff, 1983). The widely used alkaline persulfate method was selected according to previous studies (Kérouel and Aminot, 1996) which showed it was highly efficient in digesting refractory DOP compounds. The HTC (high temperature combustion then hydrolysis) method of Solorzano and Sharp (1980) was previously taken as a reference method for TDP (Kérouel and Aminot, 1996; Monaghan and Ruttenberg, 1999). But, according to Monaghan and Ruttenberg (1999), its results are comparable to those obtained by the acid-persulfate method (though less efficient than the alkaline version; Kérouel and Aminot, 1996).

The relationship between the two determinations (Fig. 8) is characterized by the equation: $DOP_{Chem} = 0.99\ DOP_{UV} + 0.003$ ($R^2 = 0.91$; n = 40). Standard errors on the slope and intercept are respectively 0.05 and 0.006 μmol l^{-1}. At the 95 % confidence level, there is no difference between the results of the two methods. Actually, photo-oxidation should provide slightly lower yields than chemical digestion since some kinds of inorganic condensed phosphate present in the phosphorus pool are not measured by photo-oxidation but are by chemical digestion. Previous studies (Ridal and Moore, 1992) obtained significantly lower (~ 30 %) DOP values using photo-oxidation than when it is used in combination with persulfate digestion. However, the matrix effects shown in this study may also alter DOP data obtained using manual batch photo-oxidation or chemical digestion. This makes it difficult to compare methods accurately.
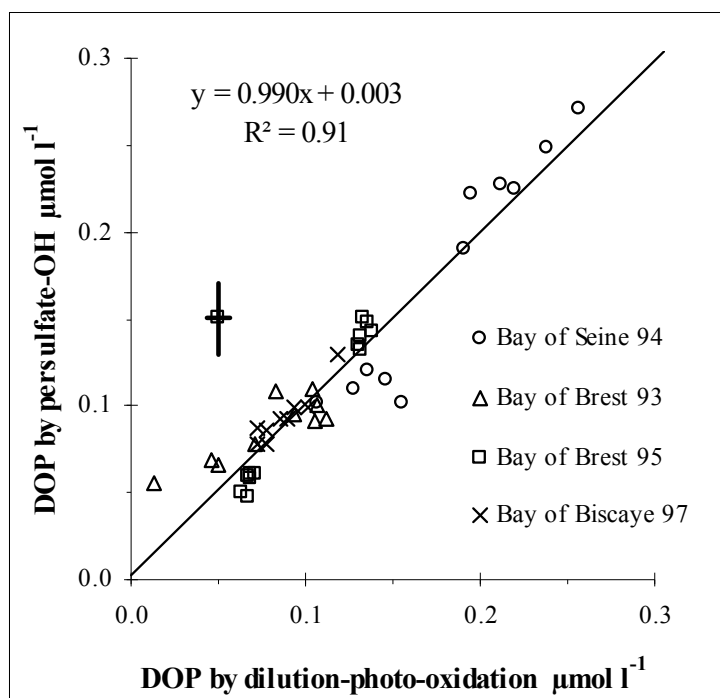
Figure 8. Comparison of DOP measurements in seawater samples by the automated dilution photo-oxidation method and the manual alkaline persulfate method. The cross represents plus and minus one standard deviation for each of the corresponding methods.

4   DISCUSSION

The automated method described for DOP determination in seawater uses a low-power lamp for a compact, easy-to-handle and low ozone producing manifold. The segmented flow analysis (SFA) technique used provides much lower sample throughput than flow injection analysis (FIA), but FIA cannot achieve the precision provided by SFA (Zhang, 2000). This is an essential criterion since DOP is obtained as the difference (often relatively small) between two determinations. The high sensitivity required (by the necessary dilution of the sample) is also more easily obtained using SFA, which enables higher values of absorbance to be achieved than with FIA.

Since matrix effects have been found to be a major problem for DOP digestion in marine samples, experiments showed that a 5 to 6-fold dilution of seawater samples was the simplest way to achieve satisfactory DOP recovery. The dilution rate applied is a necessary compromise between reducing the matrix effects and maintaining a satisfactory signal-to-noise ratio. Indeed, the signal must be amplified to compensate for the decrease in concentration, but modern high-performance optical equipment is expected to overcome this problem. In spite of this, the sensitivity and reproducibility obtained (using the Technicon SCIC colorimeter in widespread use) match or exceed those previously published using automated systems.

Until now, no automated method had been described for the specific determination of DOP in waters and only two had been described for the determination of TDP in seawater. The high-power UV version by Grasshoff (1967) is not very easy to use in the field, in particular on board ships. The low-power UV version by Collos and Mornet (1993) is more complicated than the present method and its actual recovery of DOP in seawater has not been documented. For the automated determination of dissolved organic carbon and nitrogen, it had been argued that coupling acidic and alkaline oxidative conditions enhanced recovery (Aminot and Kérouel, 1990; Collos and Mornet, 1993). The present study shows that DOP does not need drastic conditions for complete or almost complete recovery by photo-oxidation, provided that adequate dilution and close-to-neutral pH are met. In addition, this soft digestion approach enables the subsequent phosphate determination to be performed without complex reagent adjustment.

The efficiency of the lamp over a long period and the availability of refractory DOP model compounds, which provides an easy way to check recovery, may encourage the use of on-line photo-oxidation for automated methods.

The results also point out that tests using any so-called artificial seawater, assumed to represent seawater, but which contains only NaCl (or even some other main salts), may lead to erroneous conclusions about the actual recovery of DOP in seawater samples.

The accurate determination of DOP depends, on the one hand, on the specificity of both SRP and SRP+DOP determinations, and on the other hand, on the recovery of P-compounds with respect to the relative composition of the dissolved P pool.

SRP should not include significant contributions from either DOP or inorganic poly-P compounds. The average contributions of about fifty of these compounds, determined in this study or by Kérouel and Aminot (1996), Thomson-Bulldis and Karl (1998), Monaghan and Ruttenberg (1997), amount to about 1-3 percent of their concentrations. A few molecules produced slightly higher responses (5-10 %) and one gave 85 % (ribose-1-phosphate). Because it is difficult to quantify impurities, mainly orthophosphate, in the test compounds (Kérouel and Aminot, 1996), the lower responses cannot be attributed with certainty to interference from the compounds. Consequently, the average contribution of non-

orthophosphate compounds to SRP can be assumed to be negligible in comparison with the precision of DOP measurements.

Regarding the specificity of the method for the digestion of organic phosphorus, the experiments showed that polyphosphates do not interfere in our dilution photo-oxidation method, as was previously described for photo-oxidation methods with short exposure times (Armstrong et al., 1966; Armstrong and Tibbits, 1968; Solorzano and Strickland, 1968; Ormaza-Gonzalez and Statham, 1996). This may be important in coastal and fresh waters or in productive areas, as shown by the significant concentrations attributed to inorganic polyphosphates (up to 0.20 μmol l^{-1}) found in such areas at certain periods (Solorzano and Strickland, 1968; Solorzano, 1978). It may be objected that dissolved organic polyphosphates are also missed by the method. Their concentrations in solution have not been documented, but in phytoplankton cells adenosine triphosphate (ATP) is known to amount to 0.2-0.5 % of the carbon content (Strickland et al., 1969; Hunter and Laws, 1981; Laws et al. 1983). This roughly corresponds to an average P/chlorophyll *a* ratio of 1 nmol μg^{-1}. As turnover of polyphosphated nucleotides is fast and production stops when cells die, their concentrations in solution are expected not to contribute significantly to DOP.

Owing to the great number of phosphorus compounds in biological systems, and lack of information on DOP composition in natural waters, the recovery of organic phosphorus compounds must rely on model compounds that are assumed to be representative of the expected DOP pool. Investigations on DOP class sizes from lakes and a green alga culture by Minear (1972) showed that the DOP pool was dominated by two classes of 2500 daltons or less (peak at ~ 1400) and 25 000 daltons or greater (peak at ~ 50 000). Most of the DOP was generally found in the low-molecular-weight fraction, while the high-molecular-weight fraction contained 20-45 % deoxyribonucleic acid. In seawater, recent studies on the class size fraction > 1000 daltons (Clark Kolowith et al., 2001) reported 25 % of phosphonates (C-P bonds) and 75 % of mono- and diester phosphates (C-O-P bonds), which is in good agreement with Minear's data. Tests of model compounds have shown that digestion of C-O-P bonds by the AD-UV method is complete, with the exception of phytic acid, a cyclic molecule with 6 C-O-P bonds. Phytic acid is produced by higher plants and it has been identified in sediments of fresh water lakes and marsh or of brackish ponds (Groot and Golterman, 1993). In addition, it was found to precipitate with all polyvalent cations and to be strongly adsorbed on iron oxyhydroxides (Groot and Golterman, 1993). The presence of phytic acid was suspected in waters polluted by livestock waste, so studies were carried out which showed that marine microalgae can use it as a P source (Balazsi and Wikfors, 2000). In the AD-UV method, phytic acid is digested with a yield ranging from 80 % in seawater to 100 % in fresh water. Because phytic acid can not be found in natural waters, except potentially in areas polluted with livestock waste, this compound will not alter the overall recovery of DOP from natural samples. Initially selected as a potentially good model compound for recovery tests, phytic acid was not chosen for automated analysis because poor signals are generated under some conditions of pH and/or divalent cations concentrations.

As for the digestion of phosphonates, the AD-UV method was shown to achieve a yield of 94 % for 2-aminoethylphosphonic acid (AEPA) in seawater, which is identical to results obtained by Kérouel and Aminot (1996) and Monaghan and Ruttenberg (1999) with the ash-hydrolysis method of Solorzano and Sharp (1980), giving respectively 94 % and 95 %. Wet oxidation methods generally produce lower recovery rates for this compound in seawater (Cembella et al., 1986; Ormaza-Gonzalez and Statham, 1996; Kérouel and Aminot, 1996; Monaghan and Ruttenberg, 1999). It may be noted, from method comparisons of Cembella et al. (1986) and Monaghan and Ruttenberg (1999), that AEPA is the most refractory among the phosphonates tested. According to Cembella et al. (1986) a 1 hour UV digestion achieved only 37 % recovery for AEPA (and 96 % in 18 h), compared to 100 % for the other

phosphonates tested (chemical digestions were respectively < 92 % and < 97 %). Therefore, for phosphonate compound digestion, the AD-UV method is equivalent or superior to any other. Regarding the recent findings that phosphonates amount to 25 % of the high-molecular-weight fraction of DOM in seawater (Clark Kolowith et al., 2001), a 94 % recovery for these compounds would not miss more than 1.5 % of DOP. It has been concluded that AEPA is a good model compound to test method efficiency.

Finally, quite comparable results were obtained by the automated dilution photo-oxidation method and the alkaline persulfate oxidation. This allows us to assume that i) the described method does not miss major organic compounds in seawater, ii) concentrations of polyphosphates were negligible in the samples. Ridal and Moore (1992) found that TDP values obtained after photo-oxidation are generally lower (by ~ 30 % of the DOP concentration) than those resulting from chemical oxidation or a combination of photo- and chemical oxidations. Our results suggest that the difference may be attributed to a matrix effect.

The present method was designed for DOP determinations, but TDP and non-SRP phosphorus can be obtained after acid hydrolysis of the samples (Grasshoff, 1967; see Introduction).

5   ACKNOWLEDGMENTS

6   REFERENCES

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