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Determination of total phosphorus in natural waters with a simple neutral digestion method using sodium persulfate

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Abstract

The determination of total phosphorus (TP) in an aqueous sample is based on digestion of the sample to convert phosphorus compounds into orthophosphate, which can then be determined based on spectrophotometry. The widely used oxidant, potassium persulfate, has poor aqueous solubility and requires careful handling with furnace, autoclave, or oven. Here, a very simple method for the determination of TP has been developed, using an inexpensive portable water boiler for neutral digestion and highly water soluble sodium persulfate instead of potassium persulfate. The key experimental factors have been evaluated and optimized. The optimal conditions for efficient TP determination were found to be 3 h of digestion time at sub-boiling temperature, followed by addition of ascorbic acid to eliminate free chlorine generated from the saline sample, and then addition of the ammonium molybdate reagent for detection by UV-Vis spectrophotometry. The detection limit is 0.02 μM using 5-cm cuvette. Under these optimized conditions, both artificial solutions containing model phosphorus compounds ($n = 19$) and natural water samples ($n = 14$) were analyzed using both sodium persulfate and potassium persulfate. There is no significant difference between the digestion performances of these two oxidants. This new method was used to analyze natural water samples ($n = 28$) in comparison with the standard autoclaving method using potassium persulfate. The analytical results using this method agreed very well with the standard method. Samples of different matrix were analyzed, showing the successful application of this method in both environmental monitoring (time series and spatial survey) and marine science (spatial and vertical distribution).

Phosphorus (P) is an essential macronutrient for life on earth (Paytan and McLaughlin 2007). P is a limiting nutrient for algal growth in oligotrophic aquatic ecosystems, but is also known as a pollutant in coastal and estuarine waters (Chen et al. 2015). An excess of P can cause eutrophication of natural waters and the development of blooms, which has become a worldwide environmental problem (Correll 1998; Statham 2012). Total phosphorus (TP) is the sum of the contents of all P-containing compounds present in a water sample, including orthophosphate, phosphorus monoester (C-O-P bond), phosphorus diester (C-P bond), phosphonate (C-P bond), polyphosphate monoester (C-O-P-O-P-O-P bond), polyphosphate (P-O-P-O-P bond), and pyrophosphate (P-O-P bond) (Kolowith et al. 2001; Karl and Björkman 2014).

Although orthophosphate is the most accessible form of P for organisms, TP represents the maximum potential amount of bioavailable P (Karl and Björkman 2014). Therefore, TP is an important environmental variable that can be used as an indicator of water quality and as a control parameter in waste water treatment processes. Numerous criteria have been developed based on TP background concentration in a given environment (Zhang 2012). As a consequence, the accurate determination of TP is extremely important for aquatic studies of phosphorus cycling, ecosystem management, and restoration (Maher and Woo 1998).

Various TP determination methods, including fusion, dry ashing, boiling samples in perchloric, sulfuric or nitric acid on a hot plate, high-temperature combustion, UV irradiation, persulfate oxidation, and nitrate oxidation have been reported and applied for environmental analysis (Maher and Woo 1998; Worsfold et al. 2008, 2016). These methods are generally based on the transformation of all the P-containing compounds into orthophosphate by hydrolysis or oxidation, and the orthophosphate is then determined using the phosphomolybdenum blue (PMB) method (Murphy and Riley 1962; Nagul et al. 2015). Different digestion methods have been compared (Ridal and Moore 1990; Kerouel and Aminot 1996; Ormaza-Gonzalez and Statham

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Additional Supporting Information may be found in the online version of this article.

1996). Every reported method has shown some advantages along with some drawbacks in terms of the different aspects, such as digestion efficiency, applicability, sample throughput, economy, complexity of the instruments and procedures, possibility for automation, etc. Therefore, TP is often a problematic parameter in routine water quality monitoring programs. For example, for total dissolved phosphorus (TDP) determination, more than half of the laboratories that participated in international laboratory performance studies (nutrient section) could not produce consistent results (Aminot et al. 1997).

One of the most popular techniques for TP sample digestion is persulfate oxidation under acidic (Ridal and Moore 1990; Ormaza-Gonzalez and Statham 1996; Monaghan and Ruttenberg 1999) or alkaline (Lambert and Maher 1995; Maher et al. 2002; Dafner and Szmant 2014) conditions. This technique was recommended as the standard method for TP determination by US Environmental Protection Agency (USEPA, Method 365.1 1993) and the American Public Health Association (APHA, Method 4500-P 2012). The effects of the post-persulfate digestion procedures have been evaluated to attempt improving the efficiency of TP determination (Zhou and Struve 2004). Various problems were found related to the measurement of the released orthophosphate using the PMB method. It was revealed that the intermediate products of persulfate oxidation, and not the slight change in pH, caused the slowdown of color formation (Huang and Zhang 2008). Subsequently, Huang and Zhang (2009) reported a neutral persulfate method for sample digestion at 90°C in an oven overnight. The procedure is simple and easy to perform as it eliminates the need for addition of acid or base before digestion, and also the subsequent tedious process of pH adjustment after digestion. Moreover, it is more convenient and safer to substitute the high pressure autoclave with an oven, but a more portable digestion instrument will be beneficial for field applications (e.g., shipboard analysis).

There have been many studies on the digestion mode using persulfate; however, very few studies evaluating the effect of the persulfate compound have been reported to date. Both potassium persulfate ($K_2S_2O_8$) and ammonium persulfate ($(NH_4)_2S_2O_8$) have been widely used as the oxidant. The former is more popular in marine science and the latter is mostly used in environmental monitoring, depending on the standard methods released by different agencies. However, the acid/ammonium persulfate solution is not stable and has to be prepared fresh daily (Shen and Dasgupta 1991). Moreover, there is an increasing requirement for a single digestion procedure for both total nitrogen (TN) and phosphorus measurement (Valderrama 1981; Raimbault et al. 1999; Dafner and Szmant 2014; Gibson et al. 2015), and for such applications, the use of ammonium persulfate would be unsuitable as it introduces extra nitrogen into the sample. However, the water solubility of $K_2S_2O_8$ is low ($\sim$50 g L^{-1} at room temperature) and can be much lower when the solution is stored in refrigerator ($\sim$ 20 g L^{-1} at 0°C), which makes it relatively difficult and time consuming to prepare and use the oxidant solution, especially in cold areas. Sodium persulfate ($Na_2S_2O_8$) is more soluble than $K_2S_2O_8$ and has been widely used for in situ chemical oxidation of contaminated soil and groundwater (Tsitonaki et al. 2010). The solubility comparison of $Na_2S_2O_8$ and $K_2S_2O_8$ solutions with different concentrations is shown in Supporting Information Fig. S1. To the best of our knowledge, compared with the commonly used $K_2S_2O_8$, the more soluble and cheaper $Na_2S_2O_8$ has not yet been applied for the determination of TP in aqueous samples.

The objective of this study is to optimize a simple and inexpensive neutral digestion method using $Na_2S_2O_8$ as oxidant. The digestion is performed in a portable domestic water boiler under moderate sub-boiling conditions. The digestion performance of two common oxidants, $K_2S_2O_8$ and $Na_2S_2O_8$, are also compared. As there is no certified reference material for dissolved organic phosphorus (DOP) currently available (Ormaza-Gonzalez and Statham, 1996; Yoshimura 2013), it is common practice to use model P-containing compounds for testing the digestion efficiency of TP determination method (Kerouel and Aminot 1996). Both refractory (e.g., aminoethylphosphonic acid, phytic acid (PA), or phosphorylcholine chloride) and labile compounds (e.g., phosphoenolpyruvate, glycerophosphate, or riboflavin-5-phosphate) should be used to evaluate the reliability as well as the weak points of the method (Kerouel and Aminot 1996). Thus, this study uses model P-containing compounds and real natural water samples to evaluate the method. Finally, the established method was applied to natural water samples collected from rivers, estuarine, coastal sea, and open oceans to test its potential for actual environmental monitoring and marine science applications.

Materials and methods

Reagents and standards

All the reagents were of analytical grade or better quality, and purchased from Sinopharm Chemical Reagent, China, unless stated elsewhere. Ultra-pure water used for preparing standards and reagents was collected from a Millipore water purification system (Millipore, U.S.A., 18.2 MΩ). All the reagents were stored in polypropylene bottles, which were soaked in 2 M HCl solution overnight, followed by thorough rinsing with pure water before use.

The $K_2S_2O_8$ solution of 50 g L^{-1} was prepared by dissolving 5 g potassium persulfate in 100 mL pure water, and then stored at room temperature away from light exposure. The $Na_2S_2O_8$ solution of 250 g L^{-1} was prepared by dissolving 25 g sodium persulfate in 100 mL pure water, and then stored at 4°C in a refrigerator. Ascorbic acid (AA) solution of 100 g L^{-1} was prepared fresh daily. The mixed reagent (MR)

solution of ammonium molybdate was prepared by mixing 100 mL of 130 g L^{-1} $(NH_4)Mo_7O_{24}{\cdot}4H_2O$ solution, 100 mL of 3.5 g L^{-1} potassium antimony tartrate, and 300 mL of diluted H_2SO_4 (1 : 1, H_2O : H_2SO_4). It was kept stored at 4°C, and is stable under these conditions for several months. Phosphate stock solution of 8.00 mM was prepared by dissolving pre-dried potassium dihydrogen phosphate (KH_2PO_4) in pure water and stored at 4°C. Sixteen organic phosphorus and three inorganic phosphorus compounds including phosphate esters, phosphonates, organic condensed phosphates, and inorganic polyphosphates were purchased from different vendors and stored as recommended by manufacturers. Details about these chemicals are listed in Supporting Information Table S1. Stock solutions of these P-containing chemicals (mostly 2 mM) were prepared by dissolving the specific amounts of the solid chemicals in pure water and then kept stored in a refrigerator at 4°C. Artificial seawater (ASW) was prepared for testing the matrix effect by dissolving 31 g NaCl and 10 g $MgSO_4{\cdot}7H_2O$ in 1 L pure water.

Instruments

A UV-Visible spectrophotometer equipped with a 5-cm cuvette was used to measure the absorbance of PMB complex at 700 nm (723-PC, Shanghai Spectrum Instruments, China). The kinetic studies were performed using a UV-Vis 1800 spectrophotometer (Shanghai Meipuda Instrument, China) with a computer for data collection. A domestic water boiler was used to maintain the digestion temperature at ~ 95°C. Compared with the autoclave or oven, the boiler is cheaper (~ 30 US dollar) and more portable (~ 1 kg). The potassium persulfate autoclaving method was chosen for method comparison because it is recommended as the standard method for TP determination in water and wastewater by the Water Environment Federation (Method 4500-P). An autoclave (G154DWS, Zealway, U.S.A.) was used for method comparison.

Procedure

In a typical procedure for neutral digestion, 70 μL of the 250 g L^{-1} $Na_2S_2O_8$ solution was added to a brown glass bottle containing 25 mL of the aqueous sample (P-containing model compounds at ~ 2 μM or real samples), and mixed. Then these bottles were placed in the water boiler at ~ 95°C for 3 h. After digestion, 0.5 mL of the AA and MR solutions were added sequentially to the cooled digested solution, and mixed thoroughly. The characteristic blue color fully developed within 5 min at room temperature. The absorbance of the formed PMB compound was measured at 700 nm with a spectrophotometer using a 5-cm cuvette.

The procedures for acidic and alkaline digestions are the same as that for the neutral condition except that the $Na_2S_2O_8$ solution was prepared with acid or base accordingly. Both acidic and alkaline digestion processes were strictly according to the standard oxidation methods (Hansen and Koroleff 1999).

Sampling

Water samples were collected from the Jiulong River, coastal areas near Xiamen City and the South China Sea (SCS) for studying the application of this method. River water samples were collected every day for 7 d at two fixed sites in reservoirs of the West Jiulong River and the North Jiulong River in January 2016. Surface coastal waters were collected from different locations near Xiamen City in February 2016. The TDP samples were filtered using PES 0.45 μm filter prior digestion and measurement. The sample locations and basic physical parameters of the samples (measured with YSI professional plus in situ sensor) are shown in Supporting Information Table S2. During a cruise in August 2015 in the SCS, seawater samples were collected using Niskin bottles attached to the Rosette sampler, and were gravity filtered using a GF/F filter (0.7 μm, Whatman^{TM}), then were immediately frozen at −20°C. Before analysis, these samples were completely thawed and mixed thoroughly. Shipboard analysis of the temperature and salinity of the samples was conducted using a SBE 911 plus CTD recorder (Sea-Bird, U.S.A).

Assessment and discussion

Optimization of the digestion conditions

The effect of digestion time

Based on the recommendations in the previous publications (Kerouel and Aminot 1996; Maher et al. 2002, Huang and Zhang 2009), four organic phosphorus compounds containing C-P bond or C-O-P bond and two inorganic phosphorus compounds containing P-O-P-O-P bond were chosen as the model compounds to evaluate the effect of digestion time. The detailed information of these compounds are shown in Supporting Information Table S1. As shown in Fig. 1, the maximum digestion percentages of organic phosphorus compounds were reached at 5 min, and no significant increase in the digestion percentage was observed with further increase in the digestion time from 5 min up to 7 h. It should be noted that some compounds cannot be completely oxidized even with longer digestion time. The amount of phosphate released from polyphosphates increased rapidly within the first 2 h and then reached the maximum value. Therefore, a 3 h digestion time was considered sufficient for all the samples, and used as the standard procedure for sample analysis. Under this condition, organic phosphorus compounds containing C-P bond and C-O-P bond can decompose immediately, while the hydrolysis of P-O-P-O-P bond is more difficult. The polyphosphates required much longer time to decompose than the organic phosphorus compounds (Maher and Woo 1998). The different digestion kinetics could potentially be used as the basis to distinguish between the different P-containing compounds, which need further investigation with more model compounds.

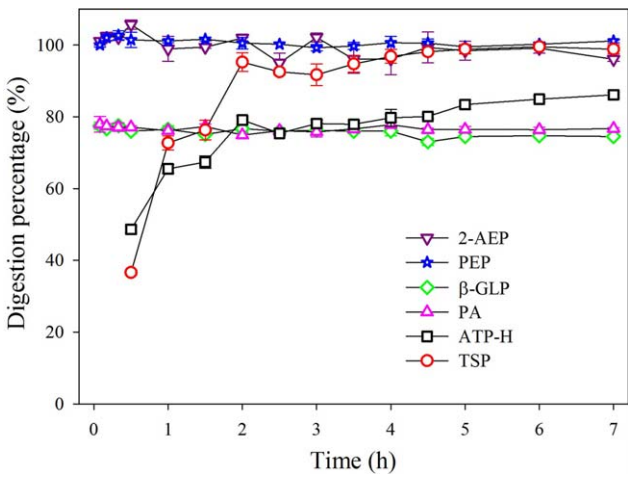

Fig. 1. Effect of digestion time on the digestion percentages of model phosphorus compounds. 2-AEP, 2-aminoethylphosphonic acid; PEP, phospho(enol)pyruvic acid monopotassium salt; β-GLP, glycerol phosphate disodium salt hydrate; PA, phytic acid sodium salt hydrate; TSP, sodium pyrophosphate; ATP-H, adenosin 5′-triphosphate. 2-AEP and PA are refractory compound according to Kerouel and Aminot (1996).

The effect of persulfate concentration

To evaluate the influence of the persulfate concentration on the digestion and compare the performances of $Na_2S_2O_8$ and $K_2S_2O_8$, the final concentration of persulfate in the sample was varied from 0.35 mg mL^{-1} to 2.8 mg mL^{-1}. PA was chosen as the model compound because it gets digested only up to 80% generally (Fig. 1), and it was assumed that adding more persulfate might increase its oxidation efficiency. However, it was found that the digestion percent of PA did not show any significant improvement with various concentrations of added persulfate, while the pH of the digested solution decreased with increased persulfate concentration (detailed data shown in Supporting Information Table S3). Since the persulfate oxidation is often accompanied by release of protons (H^+), the pH of digested sample is lowered with increase in the amount of added persulfate. There was no significant difference between the two persulfate compounds, $Na_2S_2O_8$ and $K_2S_2O_8$, in terms of the digestion percent and pH in digested samples, indicating that both chemicals have comparable performance. Huang and Zhang (2009) found the higher persulfate concentration could decrease formation rate of the PMB complex and the reaction would require several hours for completion when the sample pH is lower than 1.3. Therefore, 0.7 mg mL^{-1} was chosen as the final concentration of $Na_2S_2O_8$ in the sample, which requires adding 70 μL of the 250 g L^{-1} $Na_2S_2O_8$ solution to 25 mL of the digestion sample. It should be noted this concentration was lower compared with other references (e.g., Huang and Zhang 2009, APHA, Method 4500-P). We have tested the concentration of digestion reagent (0.7 mg mL^{-1} vs. 10 mg mL^{-1}) on the digestion efficiency of real samples. It was found there was no significant difference between the absorbance of digested samples after PMB reaction (absorbance of 0.093 ± 0.001 vs. 0.092 ± 0.001 and absorbance of 0.138 ± 0.000 vs. 0.136 ± 0.001). This concentration can be increased for sample containing more phosphorus.

The effect of acidic, neutral, and alkaline conditions

Three pH conditions for persulfate digestion have been reported in the previous studies. Neutral digestion is the simplest as no acid or base is added into the sample, eliminating the potential contamination due to extra chemicals. Acidic condition is required for hydrolyzing polyphosphates (Armstrong et al. 1966), but the full color formation of PMB is known to be affected by the final pH of digested sample (Zhang et al. 1999). Under alkaline conditions, the H^+ released from persulfate would be neutralized by OH^- provided by sodium hydroxide or other bases, and result in higher digestion percent due to the complete release of persulfate. Thus, the alkaline condition is suitable for the single digestion determination of TP and TN (Gibson et al. 2015). One of the refractory compounds, 2-aminoethylphosphonic acid, was used as model compound during this experiment (Kerouel and Aminot 1996). The kinetic curves of PMB formation of samples after digestion under different conditions are shown in Fig. 2a. The absorbance of digested samples in acidic conditions reached the normal level after more than 10 min. For samples digested under alkaline and neutral conditions, a reaction time of 2 min was sufficient. Thus, it is considered that all the three digestion conditions have the same capacity to break down the organic phosphorus compounds and polyphosphates into orthophosphate. However, slow formation of the PMB complex in acidic persulfate digested samples might give lower values than actual in the determination of TP. The final pH of the three different digested samples after adding the AA and MR reagents were measured and found to be about 1.0 (alkaline), 0.9 (neutral), and 0.6 (acidic). Obviously, the alkaline and neutral conditions are more suitable for digestion as the recommended pH for the PMB reaction is about 1 (Zhang et al. 1999). Neutral digestion was used for the subsequent experiments due to its operational simplicity.

Effect of the sequence of reagent addition to digested samples

It has been reported that the color formation of PMB complex was greatly influenced by the residual chlorine in digested saline samples. The excess free chlorine could be removed by placing digested samples in a hot water bath for 2 h (Yoshimura 2013). The autoclave persulfate oxidation technique reported by Menzel and Corwin (1965) requires a well ventilated autoclave to release the generated free chlorine gas. These sample treatments are too complicated for routine analysis in both land and shipboard laboratories. Thus, we evaluated whether the residual chlorine could be removed by adding AA, and whether the AA should be added

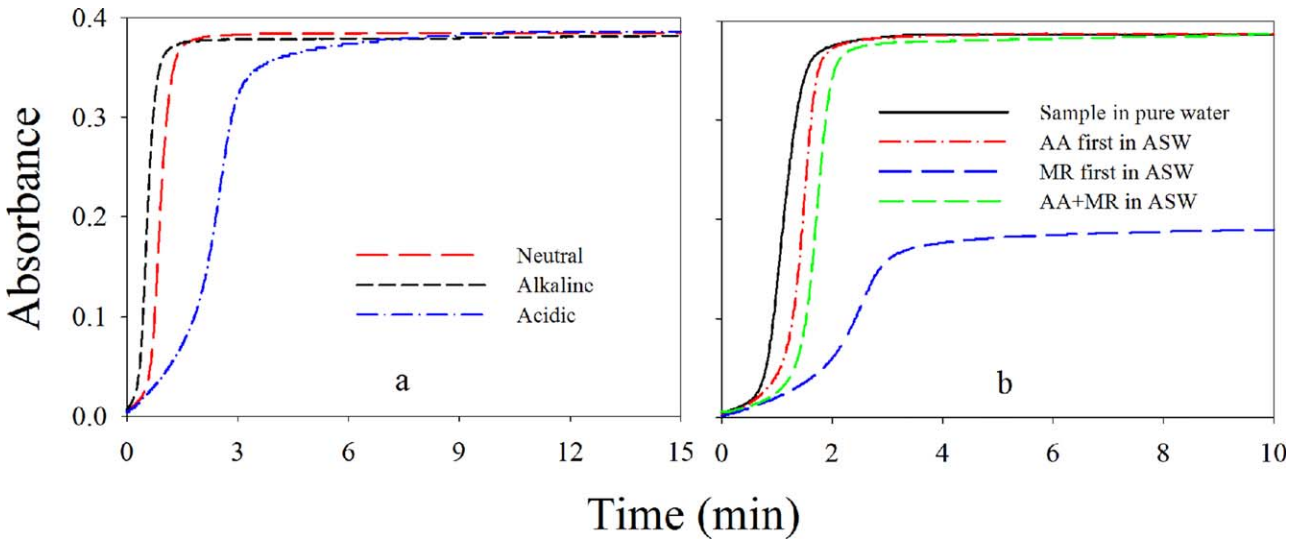

Fig. 2. Effect of digestion condition (**a**) and sequence of reagents addition (**b**) on the formation of PMB compound in digested samples. The model compound during this experiment was 2-aminoethylphosphonic acid.

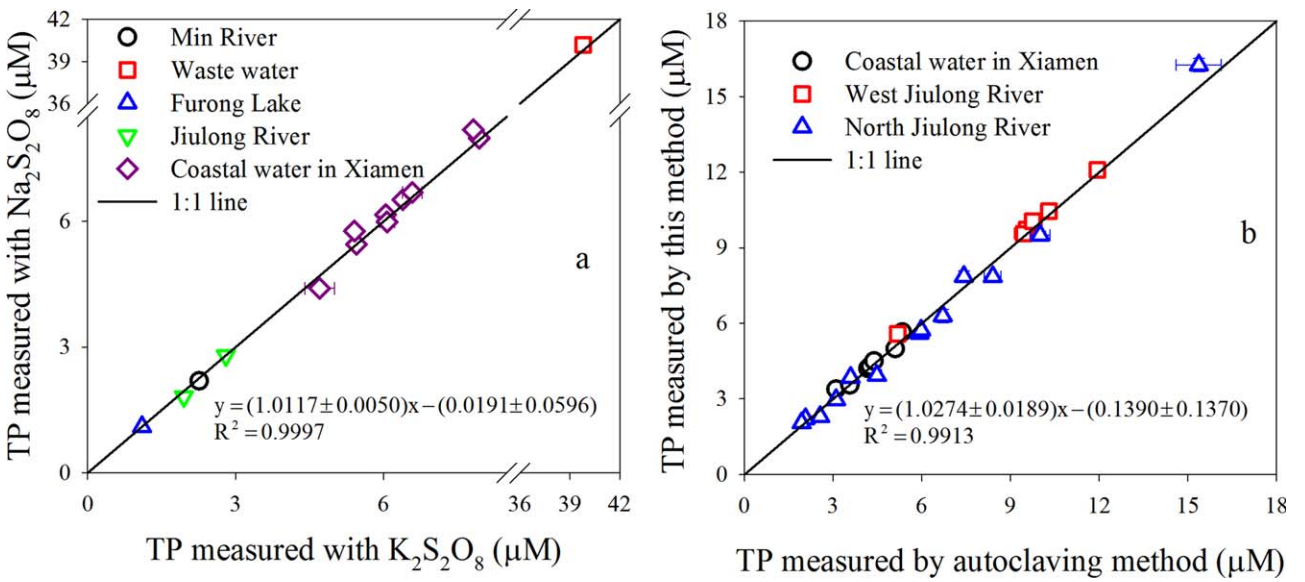

Fig. 3. Comparison of analytical results obtained using two oxidants in same digestion mode (**a**) and this method and standard method (**b**).

before or after the MR for best results. The PMB formation curves for different orders of reagent addition to digested samples are shown in Fig. 2b. For digested seawater samples, AA should be added prior to MR in order to reduce the generated chlorine, otherwise the signal can be seriously reduced.

Free residual chlorine was detected using the N,N-diethyl-p-phenylenediamine colorimetric method. The concentration of residual chlorine in the digested sample (salinity of 35) was less than 6 mg L^{-1}. This amount of residual chlorine can be reduced with 3.72 μL AA (100 g L^{-1}), which is much less than the 0.5 mL solution added into sample for color formation. Therefore, it is highly recommended to add AA first for digested saline sample, which is in accordance with the previous study (Hansen and Koroleff 1999).

Comparison of the digestion performance of $Na_2S_2O_8$ and $K_2S_2O_8$

The digestion performance of $Na_2S_2O_8$ and $K_2S_2O_8$ has been demonstrated to be comparable for digesting PA (data shown in Supporting Information Table S3). In order to further validate this conclusion, 19 model phosphorus compounds prepared in both pure water and ASW and different natural samples ($n = 14$) of varied matrices were analyzed using both $Na_2S_2O_8$ and $K_2S_2O_8$. All the model samples and

Table 1. Determination of DOP recoveries in Jioulog River (AMP, ddenosin-5′-monophosphoric acid; PE, O-phosphorylethanolamine; PFA, sodium phosphonoformate hexahydrate; RMP, riboflavin 5monophosphate sodium salt).

DOP	Sample concentration (μM)	Added concentration (μM)	Found concentration (μM)	Recovery (%)	Added concentration (μM)	Found concentration (μM)	Recovery (%)
RMP	2.19	2	4.01 ± 0.04	91.0	6	7.50 ± 0.15	88.5
PFA	1.21	2.04	3.36 ± 0.03	98.1	6.12	7.43 ± 0	97.5
PE	1.21	2.03	3.37 ± 0.04	99	6.09	7.40 ± 0.09	100.8
AMP	1.21	1.96	3.18 ± 0.04	96.9	5.88	7.01 ± 0.03	89.7

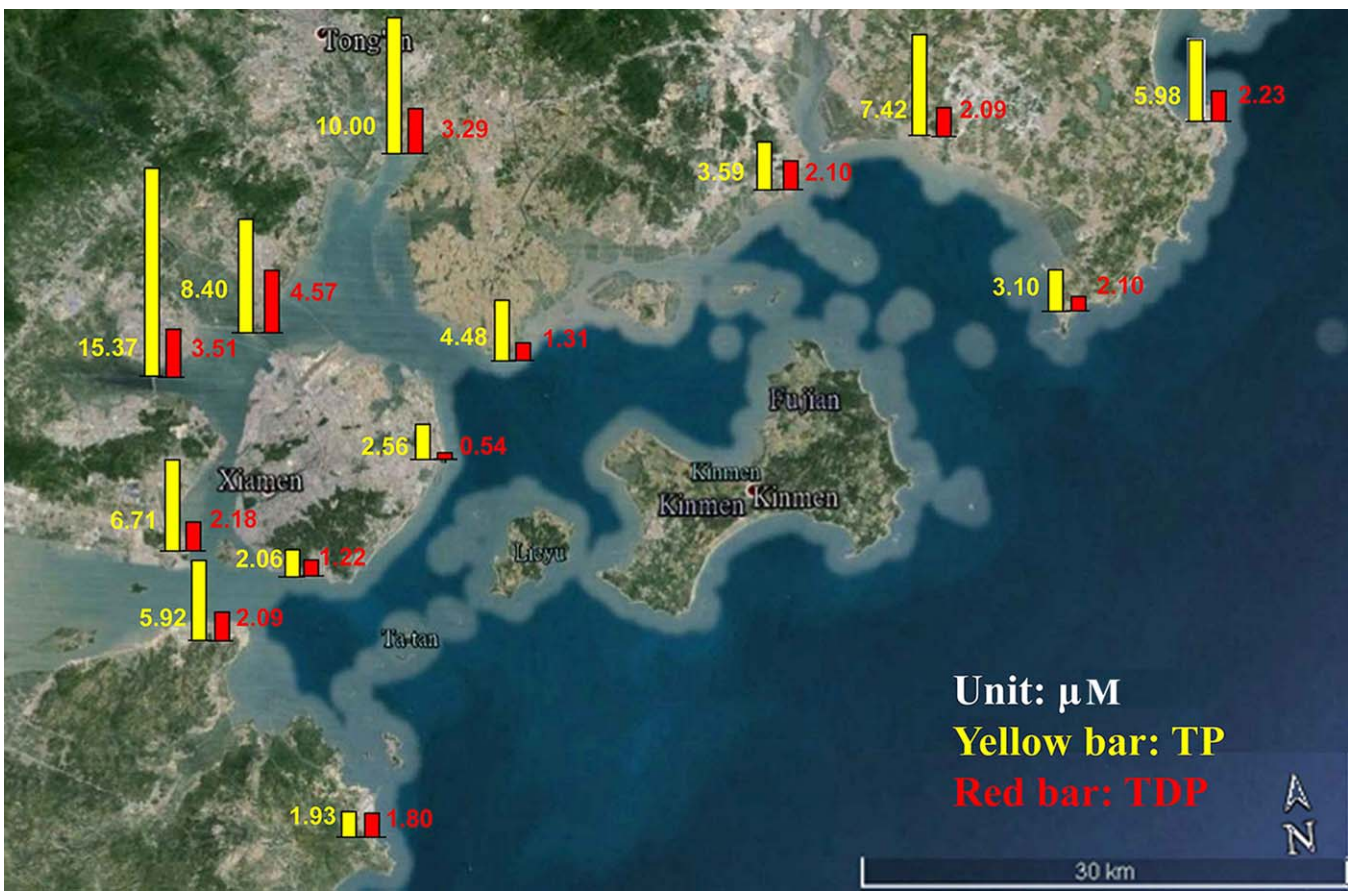

Fig. 4. The spatial distribution of TP and TDP in the surface coastal waters of Xiamen.

natural samples were determined three times. As shown in Supporting Information Table S4, the digestion percentages of 15 P-containing compounds are within a range of 86.1–109.3% and four compounds have digestion percentages from 53.7% to 76.7%, which are similar to the values reported for other digestion methods (Kerouel and Aminot 1996; Ormaza-Gonzalez and Statham 1996; Maher and Woo 1998; Huang and Zhang 2009). It should be noted that almost all the model compound solutions have similar digestion efficiencies in both pure water and ASW, showing the insignificant interference from chloride.

The analytical results using $Na_2S_2O_8$ and $K_2S_2O_8$, as oxidants for environmental samples are shown in Fig. 3a. There was no significant difference (at 95% confidence level) between the measured TP concentrations using these two oxidants in the different sample matrices.

Evaluation of the method

The limit of detection for this simple digestion method was found to be 0.02 μM, which was estimated as three times the standard deviation of the measured blanks ($n = 10$)

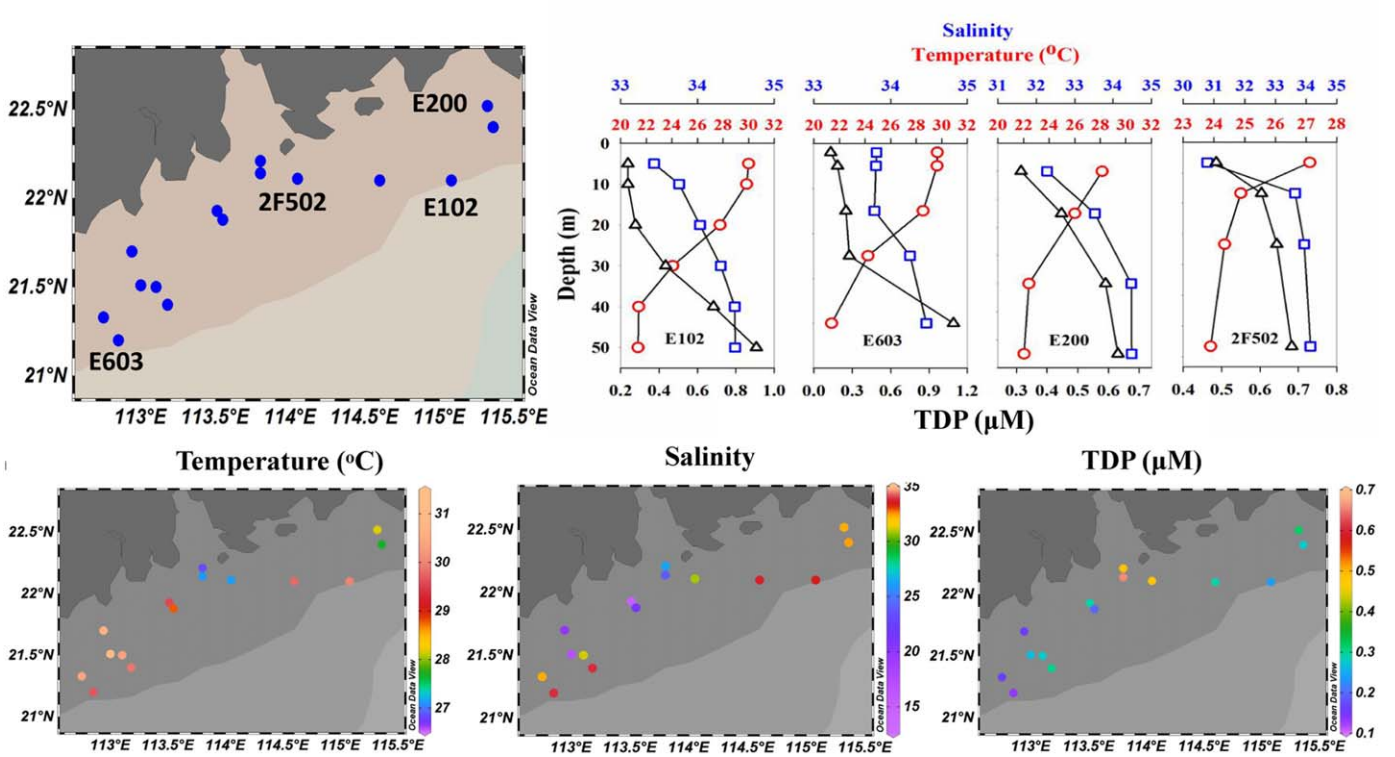

Fig. 5. The sampling stations (**a**), typical vertical profiles of temperature, salinity, and TDP in 4 stations in the SCS (**b**), and spatial distribution of temperature (**c**), salinity, (**d**) and TDP (**e**) in the surface waters.

divided by the slope of calibration curve ((0.0761 ± 0.0004)/μM with 5-cm cuvette).

Four typical DOP compounds were spiked in samples collected from Jiulong River to evaluate the recoveries. The recovery data varied from 88.5% to 100.8% as shown in Table 1, indicating little matrix interference on the determination of TP in natural samples.

The digestion performances of $Na_2S_2O_8$ and $K_2S_2O_8$ have been comprehensively compared under the same digestion protocol. However, it is also necessary to compare this new neutral $Na_2S_2O_8$ digestion method using a portable boiler with a standard method (APHA, Method 4500-P). As shown in Fig. 3b, the analytical results obtained using these two methods for TP determination in river and coastal water samples agree very well, illustrating that this simple method has high accuracy and is comparable with the standard methods in current use.

Application to natural water samples analysis

In order to evaluate the wide applicability of this established method, three different types of samples were analyzed for different purposes. These include the time-series monitoring of TP concentration in Jiulong River waters (Supporting Information Fig. S2), environmental survey of different coastal waters near Xiamen (Fig. 4), and spatial and vertical distribution study of TDP in the northern SCS (Fig. 5).

As shown in Fig. 4, the spatial distribution of TP in the surface coastal water of Xiamen ranged from 1.93 μM to 15.37 μM, whereas the TDP ranged from 0.54 μM to 4.57 μM. The spatial pattern generally indicates where and how the phosphorus enters the marine environment, and in this case, shows that continental discharge is the primary source of phosphorus delivered to the marine environment. The concentration of TP is mainly regulated by the particulate form of phosphorus in this area.

Sixty samples were collected from 15 stations in the Pearl River estuary and northern SCS shelf in August 2015. The salinity, temperature, and TDP concentration of the samples were measured and the results are shown in Fig. 5. The TDP concentration at the surface was higher (0.49 μM) near the Pearl River estuary than at the offshore stations (< 0.30 μM), indicating riverine input was transporting the TDP to the SCS. As the biogeochemical cycle of P is not the focus of this research, further explanations will be discussed in our future communications.

Comments and recommendations

A simple neutral digestion method was developed for the determination of TP in natural waters. Highly water soluble sodium persulfate was comprehensively evaluated as an alternative oxidant for persulfate potassium for the digestion of aqueous samples. Due to its higher solubility, less volume of oxidant solution can be added to the sample, thus

reducing the matrix change and other potential risks because of sample dilution. Compared with the standard autoclave method, digestion at sub-boiling temperature in a boiler is safer, and a large number of samples can be heated simultaneously without any constant monitoring. We have tested the possibility of batch digestion of samples and performed the P determination with a commercial continuous flow nutrient analyzer (AutoAnalyzer III). The results showed no significant difference between the automated determination and the manual determination at 95% confidence level.

As DOP (e.g., 2-AEP, PEP, β-GLP, and PA, as shown in Fig. 1) can be oxidized in less than 5 min, this simple procedure can be combined with flow techniques for the automatic determination of phosphate and DOP at normal or trace levels (Ma et al. 2008, 2014, 2017). Furthermore, the digestion procedure can be further optimized for single digestion of TN and maybe total organic carbon measurement in the future (Gibson et al. 2015).

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Conflict of Interest

None declared.

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