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Rapid Determination of Dissolved Phosphorus in Environmental Waters Using Inductively Coupled Plasma Tandem Mass Spectrometry

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Abstract: Dissolved phosphorus (P), an important bioavailable nutrient element, is one of the key indicators of eutrophication levels, and has been routinely measured for decades in aquatic environments. The current EPA recommended colorimetric method has been criticized for its time-consuming step of sample preparation and generation of toxic waste through the use of heavy metal catalysts. In this study, a fast, accurate, and interference-free method for the determination of dissolved P in environmental waters based on inductively coupled plasma tandem mass spectrometry (ICP-MS/MS) was developed. This ICP-MS/MS was operated in the MS/MS mode to provide an interference-free measurement. The developed method exhibited excellent linearity ($R^2 = 0.9999$) over a concentration range of 0.5–500 μg L^{-1}, with a limit of detection of 8.1 ng L^{-1} in 5% nitric acid solutions. The recoveries (90%–105% in water samples) and the concentrations determined by this method were in good agreement with those determined using the recommended colorimetric method. Comparing with the colorimetric method, the proposed method for determination of dissolved P exhibited great advantages such as shorter experiment time, lower limit of detection and higher sample throughput.

Key Words: Dissolved phosphorus; Rapid determination; Inductively coupled plasma tandem mass spectrometry; Environmental waters

1 Introduction

Phosphorus (P) is considered to be a limiting nutrient[1,2] in many freshwater[3,4] and marine environment[5]. Excessive anthropogenic P inputs can result in eutrophication[6], which would cause a rapid growth of algae, phytoplankton and bacterioplankton[7,8]. In aquatic systems, P is distributed in various chemical fractions such as dissolved P (defined as that P which passes through a filter of 0.45-μm pore size) and colloidal particulate P[9]. These different fractions can vary greatly in terms of bioavailability. As it is believed that the dissolved P is the most bioavailable form of P for algae[10] and thus plays a critical role in eutrophication, it is of great importance to measure dissolved concentration of P in environmental waters. Several methods have been developed and applied for this purpose, including fluorescence[11] and spectrophotometric methods[12,13]. Among them, the EPA recommended colorimetric, phosphomolybdenum (PMB) method is the most widely used technique for the measurement of dissolved P. In this approach, filtered water samples are firstly digested (heating and acidifying) to convert all forms of P (inorganic and organic phosphate) to

orthophosphate, followed by reaction with molybdate to form a blue compound. Antimonyl tartrate is used to catalyze the reaction and to inhibit the formation of silicomolybdic acid, which is a common interference in this assay. Besides the well-known advantages of this technique (e.g., simplicity and availability of small and affordable apparatus), they also have disadvantages related to, for instance, time-consuming sample preparation step. In addition, the sample preparation involves the use of heavy metal catalysts, which create a toxic waste cleanup problem. Last but not the least, the frustrating detection limit for this method is approximately 20 $\mu g\ L^{-1}$. Because imperceptible change of dissolved P concentrations in environmental waters could significantly influence the equilibrium of aquatic ecosystems, less sensitive methods should be utilized to identify serious eutrophication problems[14]. The measurement of low concentration (less than 10 $\mu g\ L^{-1}$) of dissolved P in natural water makes the monitoring of P still challenging.

A more preferred method for the determination of dissolved P is direct analysis using ICP-MS, which either simplifies the sample treatment process or enhances the selectivity, sensitivity, precision and sample throughput. However, the determination of dissolved P using traditional quadrupole-based ICP-MS has been a challenge due to its high ionization potential. In addition, severe polyatomic interferences such as $^{15}N^{16}O$, $^{14}N^{16}O^{1}H$ and $^{12}C^{18}O^{1}H$ on the ^{31}P must be overcome to achieve accurate and sensitive determination. The recent application of the tandem ICP-MS allows for ICP-MS operation in the tandem-MS (MS/MS) mode that is not prone to polyatomic interferences. To date, this technique has been successfully applied to the measurement of phosphopeptides[15].

The present work aims to develop a highly rapid, sensitive and accurate method for the determination of dissolved P in environmental waters using ICP-MS/MS. The analysis of the dissolved P was achieved without time-consuming and complex digestion (heating an acidifying) process, and completed within a few minutes compared to traditional colorimetric method. This method has a wide linear range (0.5–500 $\mu g\ L^{-1}$) and a limit of detection of 8.1 ng L^{-1}, and can be successfully applied to water sample detection.

2 Experimental

2.1 Instrumentation

An 8800 ICP-MS/MS instrument (Agilent, Japan) equipped with two quadrupoles (Q1 and Q2), and a third generation octopole reaction system (ORS^3) positioned between Q1 and Q2 was used throughout the work. The ORS^3 was pressurized with pure oxygen to convert P to PO in MS/MS mode. The first quadrupole (Q1) was set to 31 to allow only the ions of interest (i.e., ^{31}P, $^{15}N^{16}O$, $^{14}N^{16}O^{1}H$ and $^{12}C^{18}O^{1}H$) to enter the O$_2$-pressurized reaction cell. It is expected that ^{31}P would form $^{31}P^{16}O$ and that this higher mass species could be separated from the interfering ions ($^{15}N^{16}O$, $^{14}N^{16}O^{1}H$ and $^{12}C^{18}O^{1}H$). Finally, only $^{31}P^{16}O$ passed through the second quadrupole (Q2, set to 47) and detected by the MS detector. The instrument operation conditions are listed in Table 1.

2.2 Reagents and materials

Ultrapure water (resistivity of 18.2 MΩ cm) obtained from Milli-Q system (Advantage A10, Millipore, USA) was used for all analytical purposes. Single element solution of P (1000 mg L^{-1}, National Analysis Center for Iron & Steel, China) was used for external calibration and spiking purposes. Aladdin humic acid as a dissolved organic matter (DOM) model (Lot No. E1329065) from Aladdin Chemistry Co. Ltd. (Shanghai, China) was used to assess carbon-matrics interference on dissolved P determination. A total of 100 mg of the humics solid was dissolved in 1 L of ultrapure water, filtered, and DOC concentration, analyzed with a TOC analyzer (TOC L-CN200, Shimadzu, Japan), was (50.5 ± 0.3) mg L^{-1}.

2.3 Water samples collection

Rain water samples were collected from laboratory roof during storm time. Lake water and river water samples were respectively collected from Liuhuahu Lake and Donglangchong River (Guangzhou, China). Reservoir water samples were collected from Longdong Reservoir (Guangzhou, China). Seawater samples were collected from Macau (China). All samples were filtered through a 0.45-µm membrane syringe filter before the analysis. Seawater samples were diluted 50 times by ultrapure water so as to prevent high dissolved solids from clogging the cone orifices and giving rise to signal loss and instability, and other filtered water samples were analyzed directly. External calibration of aqueous P standards was employed.

3 Results and discussion

Table 1 Optimum ICP-MS/MS settings for P determination

Agilent 8800	
Plasma	
RF power (W)	1600
Sampling depth (mm)	8.0
Carrier gas (L min^{-1})	1.1
Lenses	
Extract 1 (V)	0
Extract 2 (V)	−180
Deflect (V)	-80
Reaction Cell	
Oxygen flow rate (mL min^{-1})	0.25
Octpole Bias (V)	−5.0
Octpole RF (V)	190
Energy discrimination (V)	−7.0

3.1 Selection of ICP-MS/MS operation modes

As discussed in the introduction, interference-free determination of trace dissolved P by traditional quadrupole-based ICP-MS is remaining as a challenging task. ICP-MS/MS has demonstrated to be a powerful tool to overcome polyatomic interferences. As ICP-MS/MS can be operated in either single quadrupole (SQ) mode or tandem mass (MS/MS) mode, analytical performances of these two modes, in terms of sensitivities (the slope of analytical curve) and background equivalent concentrations (BECs) (calculated as the average intensity for 10 consecutive measurements of procedure blank containing 5% (*V/V*) nitric acid divided by the slope of analytical curve), were compared. For the SQ mode, ^{31}P (without O_2 in reaction cell) and $^{31}P^{16}O$ (with O_2 introduction in reaction cell) were monitored, respectively. For the MS/MS mode, O_2 was bled into the ORS^3 and $^{31}P^{16}O$ (Q2) was monitored. Analytical curves using 5% (*w/V*) nitric acid standards in the 1.0–200 μg L^{-1} range were obtained. As shown in Table 2, although the linearity for both the SQ and MS/MS mode was very good, the difference of BECs between two modes was significant. Higher BECs observed in SQ mode with or without O_2, compared to dramatically reduced BECs of 0.2 μg L^{-1} in MS/MS mode, indicated that there were still substantial polyatomic interferences, despite better sensitivity achieved using SQ mode. Hence, the use of SQ mode was considered less suitable owing to the occurrence of polyatomic interferences. As a conclusion, MS/MS mode seemed to be the choice for the determination of dissolved P and was chosen for further development of ICP-MS/MS method.

3.2 ICP-MS/MS optimization

Due to the high ionization energy of P atoms (10.43 eV), plasma conditions were crucial for the efficient production of P ions. Hence, RF power was changed and subsequently optimized to obtain the maximum signal intensity by adjusting sampling depth and carrier gas flow rate.

Figure 1 shows the influence of RF power and sampling depth on PO intensity for 10 μg L^{-1} P solution. As shown in Fig.1, the maximum signal intensity was obtained under high RF power owing to the increased plasma temperature promoting the increase of ionization of P. Based on these results all further experiments were carried out under plasma RF power conditions of 1600 W. Sampling position was independently optimized by measuring PO intensity. The resulting values indicated a maximum intensity at 8.0 mm sampling depth. For further analysis, a sampling depth of 8.0 mm was used.

As shown in Fig.2, carrier gas flow rate (0.4–1.5 L min^{-1}) also strongly affected the signal intensity. The highest signal intensity was found to be approximately 1.1 L min^{-1}. Signal intensity decreased dramatically as the flow rate continued to increase, which indicated that on one hand lower flow velocity "punch" through the plasma less readily, resulting in deteriorated signal intensities and sensitivity; on the other hand, the plasma cooling effect and reduced residence time of aerosols in the plasma owing to much higher carrier gas flow rate prevented the efficient production of P ions.

Table 2　Calibration data and background equivalent concentrations (BECs) for P determination using ICP-MS/MS operated in different modes. BECs was calculated as the average intensity for 10 consecutive measurements of 5% (*V/V*) nitric acid solution, divided by the slope of calibration curve

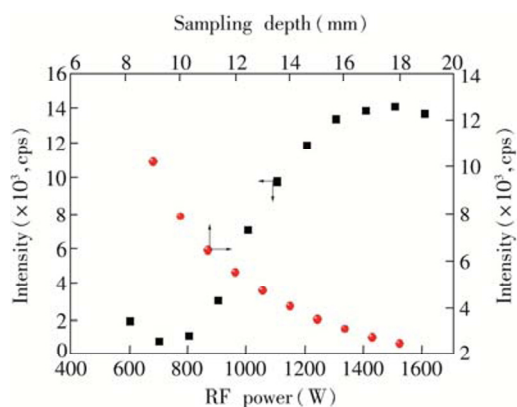| Gas	Mode	Q1 (amu)	Q2 (amu)	Sensitivity (L cps/μg)	Intercept (cps)	R^2	BEC (μg L^{-1})
No gas	SQ	-	31	3409	15894	0.9997	4.7
O_2	SQ	-	47	2676	2155	0.9996	1.2
O_2	MS/MS	31	47	1562	383	0.9998	0.2

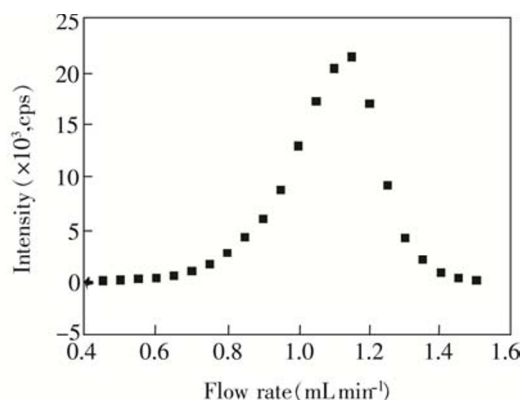Fig.1　Influence of RF power and sampling depth on PO intensity for 10 μg L^{-1} P solution

Fig.2　Effect of carrier gas flow rate on PO intensity for 10 μg L^{-1} P solution

To convert the P ions to PO species as much as possible, oxygen flow rates were optimized as illustrated in Fig.3. It was obviously to note that PO intensity increased with the O_2 flow rate, reaching the maximum value at an O_2 flow rate of 0.25 mL min^{-1}. After reaching the maximum signal intensity, the signal decreased gradually with the continuous increase of O_2 flow rate. These results could be attributed to the fact that the increase of the reaction gas increased collision opportunities and the loss due to scattering of parent and product ions with the relatively heavy reaction gas molecules. Thus, a cell gas flow rate of 0.25 mL min^{-1} was selected.

3.3 Influence of dissolved organic matter on P determination

It was reported that high carbon-content matrics could enhance signal intensity of some hard-to-ionize elements[16] when using ICP-MS technique, which would result in positive errors. Therefore, ubiquitous dissolved organic matter (DOM) in an environmentally-relevant concentration range (0–50 mg L^{-1} dissolved organic carbon, DOC) was chosen to study its potential influence on P determination. Figure 4 shows the PO intensity of solutions containing different concentrations of humic acid (HA) as a model of DOM. When P was determined in the solution containing different concentrations of DOC (0–50 mg L^{-1} DOC), the signal intensity kept constant, which implied that DOM had no significant influence on P determination.

3.4 Analytical performance

The method performances, such as linearity of calibration curve, detection limit and the limit of quantification under optimized conditions, were evaluated. Eleven-point external calibrations were performed with solutions of P (0 [5% (*V/V* nitric acid)], 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100, 200, 500 μg L^{-1}). Excellent linearity with correlation coefficients of 1.0000 over the studied range of 0.5–500 μg L^{-1} was realized. The detection limit (DL) was calculated as 3 times the standard deviation for 10 consecutive measurements of the blank divided by the calibration curve slope. The limit of quantification (LOQ) was calculated similar to DL with only difference that 10 times the standard deviation of 10 repetitive measurements of the blank. The DL and LOQ values obtained by this method were 8.1 and 27.1 ng L^{-1}, respectively. It should be noted that the DL presented in our work was instrumental DL as all measurements were based on non-matrix-matched 5% (*V/V*) nitric acid blank. Thus, any dilution factor should be taken into account. Even so, the obtained DL value was sufficiently low to measure P in real water samples.

3.5 Analysis of real water samples

Five real environmental waters (rain water, river water, lake water, sea water and reservoir water) were analyzed with this method. As shown in Table 3, the concentrations of DOC in five tested water samples were 1.8–54.7 mg L^{-1}, and the P content was 1.2–187.7 μg L^{-1}. The spiking recoveries for these samples were 90%–105%, suggesting that the proposed method was suitable to accurately determine dissolved P in environmental water. Additionally, the detection result of this method was consistent with that of colorimetric method, showing that this proposed method could be a promising alternative to traditional analytical protocol.

4 Conclusions

A rapid and accurate method for P determination of dissolve in real samples (river water, lake water, seawater, rain water and reservoir water) using ICP-MS/MS was developed. The proposed method exhibited a lower detection limit (8.1 ng L^{-1}) and shorter analysis time compared to EPA recommended colorimetric method. This method provides an attractive alternative of routine monitoring to the existing EPA recommended method and allows for its potential contribution in further study related to P biogeochemical cycle.
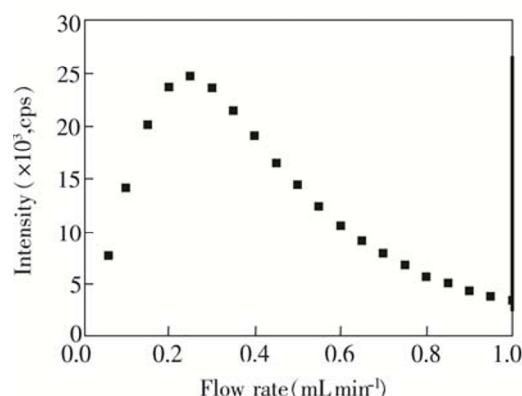
Fig.3　Optimization of reaction cell gas (O_2) flow rate on PO intensity for 10 μg L^{-1} P solution
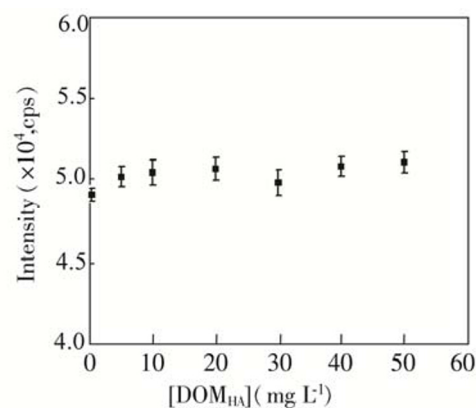
Fig.4　Effect of DOM concentration on PO intensity for 20 μg L^{-1} P solution. Error bars indicate standard deviations of three replicates

Table 3 Dissolved P in typical environmental water samples using ICP-MS/MS method and EPA recommended colorimetric approach (Average ± SD, *n* = 5)

Sample	DOC (mg L^{-1})	P addition (µg L^{-1})	P found (µg L^{-1})	Recovery (%)	Colorimetric method (µg L^{-1})
Donglangchong River water	54.7	0	187.7 ± 1.7	-	182.4 ± 3.8
		50	240.2 ± 0.9	105	-
		100	283.2 ± 1.3	95	-
		200	394.0 ± 1.8	103	-
Liuhuahu Lake water	5.8	0	36.1 ± 1.1	-	35.5 ± 2.7
		20	54.4 ± 0.9	91	-
		50	85.7 ± 1.4	99	-
		100	133.2 ± 1.6	97	
Longdong Reservoir water	5.3	0	3.0 ± 0.8	-	n.d^b.
		2	4.8 ± 1.5	90	
		5	7.6 ± 1.4	92	
		10	12.4 ± 1.4	94	
Rain water	2.1	0	1.2 ± 5.8	-	n.d.
		2	3.2 ± 2.1	100	-
		5	6.2 ± 2.4	100	-
		10	11.3 ± 1.4	101	-
Seawater	1.8	0	-	-	77.4 ± 0.5
Seawater^a	-	0	1.5 ± 1.2	-	-
		2	3.3 ± 2.9	90	-
		5	6.2 ± 2.9	94	-
		10	10.9 ± 2.8	94	-

^{a}With 50-fold dilution.^{b}Not detected (< method detection limits of 10 µg L^{-1}).

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