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# An online solid phase extraction method for the determination of ultratrace level phosphate in water with a high performance liquid chromatograph

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ABSTRACT

Phosphorous monitoring is important for eutrophication control in aquatic ecosystems, but ultratrace level concentrations may not be detected by conventional analytical methods. A method for measuring ultratrace level phosphate by online solid-phase extraction combined with HPLC was developed. A short column (50 mm) packed with octadecylsilane (ODS) was used for extraction of phosphoantimonylmolybdenum blue and dodecyltrimethylammonium hydrophobic ion-pair complexes. The ion-pair complexes entrapped on the ODS column were eluted with $CH_3CN/H_2O$ (35/65; flow rate, 1.0 ml $min^{-1}$) and monitored by an ultraviolet/visible spectrophotometer ($\lambda = 872$ nm). Phosphate concentration was determined from the peak area of the ion pair. The limit of detection for orthophosphate was 0.15 μg $PO_4$ $l^{-1}$ and the dynamic range was 0.15–100 μg $PO_4$ $l^{-1}$. Although our method was susceptible to silicate interference, it could be corrected by a proposed interference correction equation.



## 1. Introduction

Phosphorous is a limiting factor for primary production in aquatic ecosystems because it is an essential nutrient for photosynthetic organisms. Agricultural runoff and discharged wastewater contain excess phosphate ($PO_4$) that causes eutrophication in closed water bodies, leading to undesirable growth of algae and toxic microbes such as *Microcystis* (Lürling and Roessink, 2006), anaerobic conditions at the bottom of the water bodies, and progression of organic pollution. Monitoring of ultratrace level phosphate (<2 μg $PO_4$ $l^{-1}$) including its distribution, transport, and chemical and biological transformations, is an important aspect of eutrophication control and of aquatic ecosystem modeling (Zhang and Chi, 2002; Luff and Moll, 2004; Wei et al., 2004; Arhonditsis and Brett, 2005; Asaoka and Yamamoto, 2011; Tsiaras et al., 2014). Because of biological uptake, phosphate in surface water frequently goes undetected by conventional analytical methods even in eutrophic water bodies (Wei et al., 2004). In oligotrophic water bodies, phosphate is often present at concentrations less than 1 μg $l^{-1}$, below the limit of detection (LOD) of standard methods for measuring phosphate concentration (APHA, 1999).

Phosphate analysis is commonly performed with a phosphoantimonylmolybdenum blue (PAMB) method (APHA, 1999) originally described by Murphy and Riley (1962). In our proposed method, ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid, or phosphomolybdic acid. The phosphomolybdic acid is reduced to intensely colored phosphoantimonylmolybdenum blue (PAMB) by ascorbic acid (APHA, 1999). Because PAMB is an anionic species, it can form an ion pair with an organic cation such as quaternary ammonium ions (Kiso et al., 2002).

Other methods for detecting trace phosphate are solvent extraction of PAMB; complex formation of PAMB with cationic dyes such as rhodamine (Nasu and Minami, 1989; Taniai et al., 2003; Frank et al., 2006), crystal violet (Fogg et al., 1977), and malachite green (Susanto et al., 1995a,b); and flow-injection analysis (APHA, 1999; Dinz et al., 2004; Estela and Cerdà, 2005; Mesquita et al., 2011; Ribeiro et al., 2013). Ultratrace levels of phosphate have also been detected by flow-injection analysis systems combined with PAMB-complex formation with a cationic dye (Susanto et al., 1995a,b; Zhang and Chi, 2002; Yaqoob et al., 2004; Li et al., 2005; Motomizu and Li, 2005). These systems required specially designed sophisticated instruments, and the reagent solutions were not stable (Li et al., 2005).

PAMB paired with quaternary ammonium salts can be extracted using a solid phase extraction technique (Liang et al., 2007; Ma et al.,

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2008). We developed a simple method for the detection of ultratrace phosphate by solid-phase extraction with HPLC. This method incorporates PAMB, an ionic species that forms hydrophobic ion-pair complexes with organic cation. We previously used PAMB and quaternary ammonium ion-pair formation to develop a spot test based on color-band formation in a mini column to detect $PO_4$ at concentrations of 3–18 mg $l^{-1}$ (Kiso et al., 2002). In this study, an online solid-phase extraction unit equipped with an HPLC system was used to concentrate PAMB–quaternary ammonium ion pairs, which were detected by an ultraviolet/visible (UV/Vis) spectrophotometer after elution with an organic solvent.

In this study, the conditions for color development, ion-pair formation, extraction, and elution of the ion-pair were optimized to detect dissolved ultratrace level phosphate with a modified HPLC system.

## 2. Experimental

### 2.1. Reagents and solutions

All reagents were of analytical grade or the highest grade available. All reagent solutions were prepared with ultrapure water (18.2 MΩ $cm^{-1}$, Milli-Q Gradient A10; Millipore, Billerica, MA, USA) and stored in polypropylene or poly(tetrafluoroethylene) bottles.

The stock solution of phosphate (1000 mg $PO_4$ $l^{-1}$) was prepared with $KH_2PO_4$ (Sigma-Aldrich, St. Louis, MO, USA), and the standard solutions were prepared by dilution of the stock solution at concentrations of 0.1–100 μg $PO_4$ $l^{-1}$.

The mixed color reagent solution was prepared according to the US Standard Method (APHA, 1999) with reagents used as received from Nacalai Tesque (Kyoto, Japan): 5 ml of 2.743 g $l^{-1}$ $K(SbO)C_4H_4O_6 \cdot 1/2H_2O$ solution, 15 ml of 40 g $l^{-1}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ solution, and 30 ml of 0.1 mol $l^{-1}$ L-ascorbic acid solution were added to 50 ml of 2.5 mol $l^{-1}$ $H_2SO_4$ solution. Dodecyltrimethylammonium (DTMA) bromide was dissolved in methanol at 0.1% w/v. HPLC grade acetonitrile ($CH_3CN$) was used as the solvent for the solid-phase extraction.

Some researchers have reported the effects of interference ions such as Fe, Cu, Mn, Al, Zn, Pd and Ca on phosphate detection with using PAMB. $Cu^{2+}$ caused a positive interference on phosphate determination over 10 mg $l^{-1}$ (Galhardo and Masini, 2000). However, this concentration level is obviously low in natural and seawater samples. It was also reported that silica and arsenic reacted with ammonium molybdate (Murphy and Riley, 1962). Therefore, we tested the interference with co-ionic species for $PO_4^{3-}$ detection using the following solutions: arsenate (As) (III) solution prepared with $NaAsO_2$, As(V) solution with $Na_2HAsO_4 \cdot 7H_2O$, and Si solution with an analytical standard of Si for atomic absorbance.

### 2.2. Color development and ion-pair formation

To form PAMB, 2 ml of the mixed color reagent solution was added to 50 ml of the standard solution containing 10 μg $PO_4$ $l^{-1}$. The reaction was conducted for 10 min at room temperature (approximately 20 °C). Thereafter, 1.5 ml of DTMA solution was added to the PAMB solution to form the PAMB–DTMA ion-pair complexes. This reaction was allowed to proceed under ultrasonication in an ice bath for 15 min.

### 2.3. Online solid-phase extraction–HPLC system

We combined an online solid-phase extraction unit with an HPLC system consisting of two pumps (880-PU and PU-2080 plus, JASCO), a sample injector (Rheodyne Model 7725i) equipped with a sample loop of 5 ml, a 6-way valve (Rheodyne Model 7000) equipped with a short column (4.6 mm i.d. × 50 mm long) packed with octadecylsilane (ODS; ULTRON VX-ODS, Shinwa Chemical Industry, Kyoto, Japan), and a UV/Vis detector (UV-2070 plus, JASCO). Chromatogram data were processed with JASCO-BORWIN/HSS-2000 programs. The system diagram and the operational procedure are shown in Fig. 1.

### 2.4. Online solid-phase extraction protocol

The solid-phase extraction of the PAMB–DTMA ion pairs was influenced by sample-loading and column-washing conditions, mobile-phase composition, and the flow rate of the mobile phase. On the basis of our examination of these variables, the following procedure was employed.

The solution containing PAMB–DTMA ion-pair complexes was injected into the sample loop (5 ml), then the solution was introduced into the ODS column with water at a 2.0 ml $min^{-1}$ flow rate by pump 1 (880-PU), followed by washing with 7 ml of water at a 2.0 ml $min^{-1}$ flow rate. The flow channel of the 6-way valve was changed and the entrapped ion-pair was eluted with $CH_3CN/H_2O$ (35/65) at a 1.0 ml $min^{-1}$ flow rate by pump 2 (PU-2080 plus). Then the eluent was monitored by the UV/Vis detector (UV-2070 plus) at $\lambda = 872$ nm. The 6-way valve was returned, and the ODS column was washed with water for approximately 3 min to condition the column.

### 2.5. Interference with co-ionic species

The solutions containing As(III), As(V), and Si were added to the standard solution containing 5 μg $PO_4$ $l^{-1}$ at the following concentrations: 5 μg As(III) $l^{-1}$; 1, 3, and 5 μg As(V) $l^{-1}$, and 5, 100, 2000, and 5000 μg

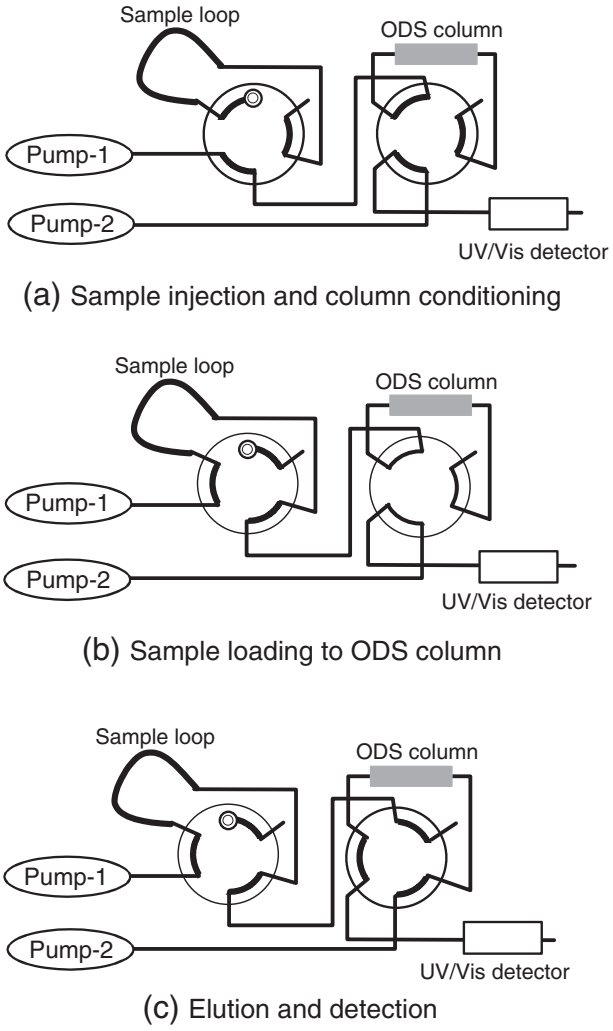


**Fig. 1.** Diagram of the online extraction system and operational procedure.