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Kiso, Yoshiaki
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Okamura, Hideo

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A Membrane Extraction Method for Trace Level Phosphate Analysis

Satoshi Asaoka[1*], Yoshiaki Kiso[2], Masahiro Nagai[3], Hideo Okamura[1]

1) Research Center for Inland Seas, Kobe University
   5-1-1 Fukaeminami, Higashinada, Kobe, 658-0022, Japan
2) Department of Environmental and Life Sciences, Toyohashi University of Technology, Tempaku-cho, Toyohashi 441-8580, Japan
3) Division of Human Environment, University of Human Environments, Motojuku-cho, Okazaki 444-3505, Japan

Correspondence to
Satoshi ASAOKA
Research Center for Inland Seas, Kobe University
5-1-1 Fukaeminami, Higashinada, Kobe, 658-0022 Japan
Tel & Fax: +81-78-431-6357
e-mail: s-asaoka@maritime.kobe-u.ac.jp

Abstract

The concentration of phosphate in surface and oligotrophic waters is sometimes below the detection limit of conventional analytical methods. In this work, a membrane extraction method using a hydrophilic PTFE (H-PTFE) membrane filter was developed to detect trace level phosphate. Phosphoantimonyl-molybdenum blue (PAMB) was prepared under conventional conditions. The PAMB was made to react with hexadecyltrimethyl ammonium (HTMA) ion by unltrasonication in an ice bath to form an ion pair of PAMB and HTMA. The ion pair was entrapped on a H-PTFE filter by filtration, and the ion pair on the filter was extracted with CH_3CN. When 10 mL of CH_3CN was used as the extraction solvent for 100 mL of water sample (10 times of concentration factor), 0.80 μg PO_4 L^{-1} (0.26 μg P L^{-1}) was successfully detected using a conventional spectrophotometer equipped with a 5 cm cell. Total phosphorus could also be detected after oxidation with potassium peroxosulfate.

Keywords:

ion pair; membrane extraction ; oligotrophic waters; phosphate, trace level analysis

1. Introduction

Phosphorous is an important chemical species useful for investigating geochemical behavior in order to control eutrophication. Excessive loadings of phosphate from agricultural fields and via wastewater discharge trigger eutrophication in estuarine, coastal waters, and lakes. Unfortunately, phosphate in surface water frequently is not detectable by conventional analytical methods [1]. The biological uptake of phosphate depletes its concentrations in surface waters to nano molar levels [2, 3]. In oligotrophic lakes, mountain streams and the open ocean, phosphate concentration is often at trace levels [4-6] , which is below the limit of detection (LOD) of standard methods for measuring phosphate concentration (10 μg-P L^{-1}; [7]). The investigations of phosphate behavior in natural water systems and the development of phosphate removal technologies in wastewater treatment processes require a simple detection technology for trace level phosphate. The analysis of trace level phosphate is commonly conducted by solvent extraction method or flow injection analysis [7]. However, the solvent extraction method involves troublesome handling and requires large volume of water sample for trace level phosphate detection. Flow analysis equipped with a liquid waveguide capillary flow cell (LWCFC) provided detection limits of around 15 ng-P L^{-1} , and this flow method requires a small sample volume (2 mL) [8, 9] . Unfortunately, the LWCFC method requires some special instruments and which are very expensive to use those in a wide variety of laboratories, especially in many developing countries. Magnesium-induced co-precipitation (MAGIC) method to concentrate dissolved phosphorus is currently commonly used for nanomolar level phosphate determination in oligotrophic waters [10,11]. The MAGIC method takes over 60 min to precipitate and collect the co-precipitation although this method requires a small volume of water sample and no special instrument.

We developed a simple method to detect trace level phosphate by membrane extraction with common laboratory ware. The phosphate analysis method was designed on the basis of formation of phosphoantimonylmolybdenum blue (PAMB) reported originally by Murphy and Riley [12]. It is well known that PAMB and phosphomolybdate are anionic species that form an ion pair with an organic cation such as a cationic dye and quaternary ammonium ions [13-15]. In our previous work, ion pair formation was employed for the spot test for phosphate analysis, where PAMB was entrapped on the packing material in a mini column with a blue color band formed in the column. The packing materials were silica gel or poly vinyl chloride (PVC) particles coated with a quaternary ammonium ion. The color band was formed by formation of an ion pair of PAMB and the quaternary ammonium ion, and the phosphate concentration was measured by the color band length [16-19].

The ion pair can also be entrapped by a C-18 (ODS) column and can be extracted with polar solvent such as methanol and CH_3CN. On the basis on these properties, an online solid phase extraction using high performance liquid chromatography (HPLC) as a sensitive phosphate analysis has been developed, where an LOD of 0.15 μg PO_4 L^{-1} was obtained [6].

The ion pair can be also trapped effectively on a membrane filter by suction filtration [20, 21]. The membrane filter entrapped the colored ion pair, and rough phosphate concentration of a water sample can be detected visually by the color intensity of the membrane. A solvent extraction is necessary for accurate analysis, and previous researchers dissolved both the ion pair and membrane for absorption photometry. In this case, the membrane polymer may affect background absorbance. Moreover, the cleaning of the optical cell filled with the solution dissolved membrane polymer may be troublesome. Therefore, it is necessary to dissolve only the ion pair entrapped on the membrane filter.

In this work, hydrophilic PTFE (H-PTFE) membrane filter was used for the membrane entrapment and only the ion pair was extracted from the membrane using acetonitrile (CH_3CN). The conditions for the ion pair formation, filtration, and extraction were examined for trace level analysis of phosphate. In addition, the method was applied for T-P analysis.

2. Materials and methods

2.1 Reagents

Mixed color reagent solution: the solution was prepared based on the US Standard Method[7] as follows: 50 mL of 2.5 mol L^{-1}-H_2SO_4, 5 mL of potassium antimonyl tartrate solution ($K(SbO)C_4H_4O_6 \cdot 1/2H_2O$: 2.743 g L^{-1}), 15 mL of ammonium molybdeate solution ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 40 g L^{-1}), and 30 mL of ascorbic acid solution (0.1 mol L^{-1}) were mixed in this particular order just before use. Phosphate standard solutions: the solutions of 0.1-100 μg-PO_4 L^{-1} were prepared with KH_2PO_4. Hexadecyl trimethyl ammonium bromide (HTMABr) solution: HTMABr was used as a quaternary ammonium salt and 0.1 w/v% of methanol solution was prepared. Potassium peroxodisulfate solution: 4 g of potassium peroxodisulfate was dissolved in water and diluted to 100 mL. All reagents used in this work were analytical grade (Nakalai Tesque, Inc., Japan) as well as distilled deionized water.

2.2 Color formation

The reagent solution of 2 mL or 4mL was added to the standard phosphate solution of 50 or 100 mL, and the reaction was carried out for 10 min at room temperature (ca. 20°C).

For total phosphorous (T-P) analysis, 100 mL of the phosphate standard solution and 20 mL of the potassium peroxodisulfate solution were contained in a digestion bottle (200 mL) with a screw cap and the mixture was heated in an autoclave for 30 min at 120 °C. The solution was filtered using the H-PTFE filter (0.2 μm) after cooling and the color formation was conducted by the procedure mentioned above.

2.3 Effects of HTMABr dosage

The HTMABr solution of 0.8-2.0 mL was added to 50 mL of the reaction mixture of the blank sample. The solution was made to react by ultrasonication in an ice bath for 15 min and filtrated with hydrophilic PTFE (H-PTFE) membrane filter (0.2 μm of pore size, 47 mm diameter: ADVANTEC, Japan). The membrane filter was washed twice by filtration with ca. 5 mL of ultra pure water.

The membrane filter was submerged in 5 mL of CH_3CN and the ion pair was extracted under ultrasonication in an ice bath for 5 min. The absorbance of the CH_3CN solution was measured by a spectrophotometer (V-530: JASCO, Japan) at 690 nm after filtration with H-PTFE of 0.2 μm.

2.4 Conditions of ion pair formation and extraction

The HTMABr solution of 1.0 mL was added to the 50 mL of PAMB solution containing 100 μg-PO_4 L^{-1} and the ion pair formation was conducted for 5-30 min under the following conditions: stirring, shaking at room temperature, ultrasonication at ambient temperature and ultrasonication in an ice bath. The filtration and extraction were conducted by same procedure described above. In addition, methanol, ethanol, and 4-methyl-2-pentanone were examined as an extraction solvent.

The ion pair solutions prepared with 50 mL of 10-100 μg-PO_4 L^{-1} solutions were filtered using H-PTFE membrane filters of 0.1, 0.2, and 0.5 μm. The filters were extracted by the same procedure described above.

2.5 Interference with co-ionic species

Silicate and arsenate reacted with ammonium molybdate [12]. Therefore, we tested the interference with co-ionic species for PO_4^{3-} detection using the following solutions: arsenate solution with $Na_2HAsO_4 \cdot 7H_2O$, and silicate solution with an analytical standard of silicate for atomic absorbance.

The solutions containing arsenate and silicate were added to the standard solution containing 10 μg PO_4 L^{-1} at the following concentrations: 5-50 μg L^{-1}. These solutions were also employed for the analytical procedure described above. The absorbance was measured by a 5 cm optical cell at concentration factors of 10.

3. Results and discussion

3.1 Optimum dosage of HTMABr

An excess amount of HTMABr is necessary to form an ion pair, but the residual HTMABr may disturb absorbance due to its low solubility in water. The effect of the HTMABr dosage on the absorbance was examined with a blank sample, because the blank sample may consume the smallest HTMABr. The absorbance was kept at a low level in the range of 0.8-1.2 mL of the HTMABr solution as shown in **Fig 1**. The absorbance increased rapidly due to HTMABr precipitation over 1.2 mL of the HTMABr solution. From the results, 1.0 mL of the HTMABr solution was added to 50 mL of the solution formed with PAMB, where the mole ratio of HTMABr/PAMB was more than 6 even for 100 μg-PO_4 L^{-1} solution.

3.2 Reaction conditions for ion pair formation

The formation of the ion pair of HTMA-PAMB was examined by stirring, shaking at room temperature, ultrasonication at ambient temperature and untrasoniction in an ice bath. The plots of the absorbance of the extracted solutions against the reaction time under various conditions to form the ion pair are shown in **Fig. 2**. In the case of the ultrasonication in an ice bath, the highest and maximum absorbance was obtained within 15 min and became stable within 30 min. In the following experiments, the ion pair formation was conducted by ultrasonication for 15 min in an ice bath.

3.3 Extraction conditions

Methanol, ethanol, acetonitrile, and 4-methyl-2-pentanone were used as extraction solvents. In the cases using methanol, ethanol, and 4-methyl-2-pentanone, a blue precipitate was observed in the solutions and the residual color was observed on the filter. Therefore, these solvents were not appropriate for use as extraction solvents. Acetonitrile extracted effectively the ion pair. The ion pair extracted by acetonitrile showed the maximum absorbance at 690 nm (**Fig. 3**).

The H-PTFE membrane filters of 0.1, 0.2 and 0.5 μm were tested to examine the effect of the pore size on the entrapment of the ion pair. The standard solutions of 10-100 μg-PO_4 L^{-1} were used and the relationship between the phosphate concentration and the absorbance was shown in **Fig. 4**, where the absorbance was measured by a 10 mm cell. Although the effects of the pore size were not observed to be significant (**Fig.4).** The flow rate of sample solutions through the H-PTFE filter was not controlled. However, the average flow rate was approximately 20 mL min^{-1}.

It was also concluded that the absorbance of the ion pair increased linearly with increasing phosphate concentration up to 100 μg-PO_4 L^{-1}. Therefore, at least, the proposed membrane extraction method can be applied for concentrations up to 100 μg-PO_4 L^{-1}.

3.4 Calibration curve for trace level phosphate

The membrane extraction method was applied to the lower level phosphate standards. The calibration curve for phosphate (0-50 μg PO_4 L^{-1}) is shown in **Fig. 5**. A 10 mm optical cell was employed to obtain the calibration curve at concentration factors of 10. The calibration curve showed clear linearity and its correlation coefficient is 1.000. Based on the standard deviation of the blank sample, the LOD (S/N ratio = 3) was evaluated to be 1.7 μg PO_4 L^{-1} (0.55 μg P L^{-1}).

The proposed membrane extraction method was compared with n-butanol extraction method, which extracts and concentrates PAMB itself at 10 times. As shown in **Fig. 6**, the membrane extraction method gave a high absorbance. Although the membrane extraction method includes the ion pair formation process, the extraction was conducted more easily than in the solvent extraction procedure.

To improve the LOD, a 5 cm optical cell was employed. Since the 5 cm optical cell was necessary for 10 mL of extracted sample solution, 100 or 200 mL of the sample solutions were used and the extraction was conducted with 10 mL of CH_3CN: the concentration factors are 10 and 20 times. The calibration curves are shown in **Fig. 7.** In each case, the calibration curves showed high linearity and their correlation coefficients were 1.000. The LODs were 0.80 μg PO_4 L^{-1} (0.26 μg P L^{-1}) and 2.0 μg PO_4 L^{-1} (0.65 μg P L^{-1}) at concentration factors of 10 and 20, respectively. As shown in **Fig. 7**, in the case of the concentration factors of 20 times, the standard deviations were higher than the case of 10 times due to precipitation of the ion pair of HTMA-PAMB. In the case of the concentration factors of 10 times, the LOD is lower than the case of 20 times. Therefore, a 100 mL of sample volume was required at concentration factors of 10 by using a 5 cm optical cell, indicating that 1 μg-PO_4 L^{-1} level of phosphate can be analyzed accurately using a conventional spectrophotometer.

Based on the results indicated above, the protocol shown in **Fig. 8** is proposed for trace level phosphate analysis. The membrane extraction was conducted more easily than solvent extraction and the proposed analytical method requires no special apparatus, and therefore the method can be used by a wide range of researchers.

3.5 Interference with co-ionic species

The interfering effects of arsenate and silicate for the standard solution of 10 μg PO_4 L^{-1} using a 5 cm optical cell at concentration factors of 10 were investigated because the molybdenumblue method is susceptible to interference from arsenate and silicate. Arsenate caused positive error for determining phosphate (**Fig. 9**). The filled circle in the

Fig. 9 shows absorbance of 10 μg PO_4 L^{-1} and 0-50 μg L^{-1} of arsenate solution. The open circle is absorbance of arsenate calculated by subtracting absorbance of 10 μg PO_4 L^{-1} from absorbance of 10 μg PO_4 L^{-1} and arsenate solution. The positive error increased quantitatively with increasing concentration of arsenate, indicating that the positive error was attributable to the formation of arsenoantimonylmolybdenum blue-HTMA ion pairs. Therefore, interference with arsenate is unavoidable. Interference from silicate gave error rates of +14.6% at 5 μg-Si L^{-1}, +19.0% at 10 μg L^{-1} and +24.1% at 50 μg-Si L^{-1}. The error could be alleviated to change the final concentration of H_2SO_4 in a sample solution more than 0.35 g L^{-1} [6].

3.6 Total phosphorous analysis

Total phosphorous was converted to orthophosphate by oxidation with potassium peroxosultae and orthophosphate was analyzed by the membrane extraction method described above. The calibration curve for T-P and PO_4 are shown in **Fig. 10.** The slope of the calibration curve for T-P was lower at ca. 20% than that for PO_4, and this corresponded to the dilution effect in the sample preparation procedure, where the standard solution was diluted at 1.2 times.

Natural water samples collected from a pond were analyzed by the methods, where the pond water was treated by filtration with fibrous filter media in order to remove suspended matter such as algae. The results for the raw water and the treated water are summarized in **Table 1**. The low level phosphorous was analyzed and the effects of the treatment were evaluated successfully.

4. Conclusions

In this work, a simple method for trace level phosphate analysis was developed using a membrane extraction technique. This method may have the following advantages:

(1) Phosphoantimonylmolybdenum (PAMB) was prepared by conventional procedure.

(2) The ion pair of PAMB and HTMA formed by ultrasonication in an ice bath was entrapped by the H-PTFE filter (0.2 μm) and extracted effectively with CH_3CN. This proposed simple protocol for entrapping the ion pair can be employed on site.

(3) In the case where 100 mL of a sample was used, phosphate of 0.8 $\mu g\text{-}PO_4\ L^{-1}$ was detected by this proposed method.

(4) Since oxidation procedures with potassium peroxosulfate exhibited no significant influence, this method is applicable to phosphate analysis for T-P determination as well.

Since this proposed method requires no special apparatus, it may be a useful technique for a wide range of researchers.

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Figure captions

Fig. 1 The effect of the HTMABr dosage on the blank absorbance

Fig. 2 The change of the absorbance over time under various conditions to form the ion pair of HTMA-PAMB

Fig. 3 Absorption spectra of the HTMA-PAMB ion pair

Fig. 4 The effect of the pore size on the entrapment of the HTMA-PAMB ion pair

Fig. 5 Calibration curve for phosphate obtained by the proposed membrane extraction method using a 10 mm of optical cell
Concentration factor is 10 times. Error bars indicates standard deviation (n=4).

Fig. 6 Comparison of the calibration curves for phosphate obtained by the two extraction methods, n-butanol extraction method and the proposed membrane extraction method

Fig. 7 Calibration curves for phosphate obtained by the proposed membrane extraction method using a 50 mm of optical cell
Concentration factors are 10 or 20 times. Error bars indicates standard deviation (n=4).

Fig. 8 The protocol of the proposed membrane extraction method for trace level phosphate analysis

Fig. 9 Absorbance of phosphate with different arsenate concentration

Fig. 10 The calibration curve for total phosphorus and phosphate obtained by the proposed membrane extraction method
Concentration factors are 10 times using a 50 mm of optical cell.

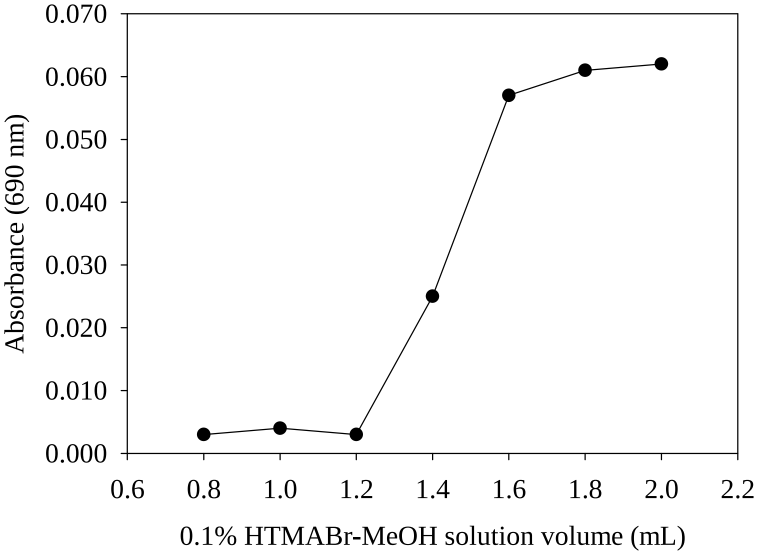
0.070
0.060
0.050
0.040
0.030
0.020
0.010
0.000
Absorbance (690 nm)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
2.2
0.1% HTMABr-MeOH solution volume (mL)

Fig.1

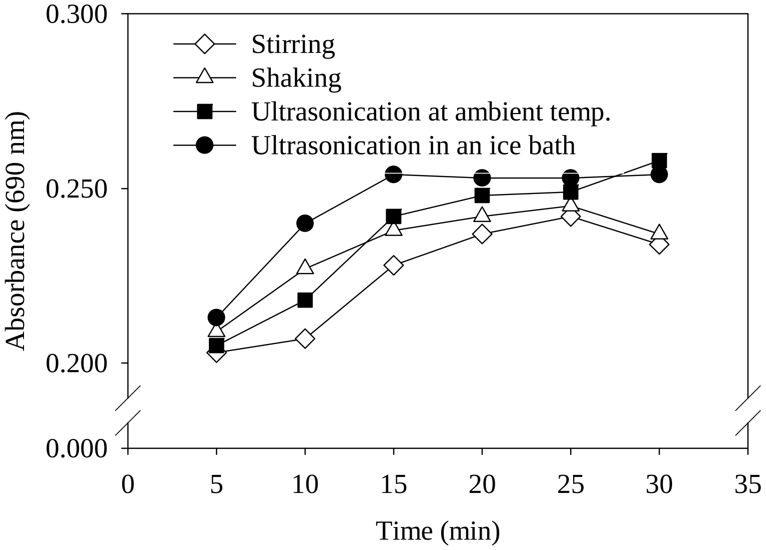
0.300
0.250
0.200
0.000
Absorbance (690 nm)
Stirring
Shaking
Ultrasonication at ambient temp.
Ultrasonication in an ice bath
0
5
10
15
20
25
30
35
Time (min)

Fig.2

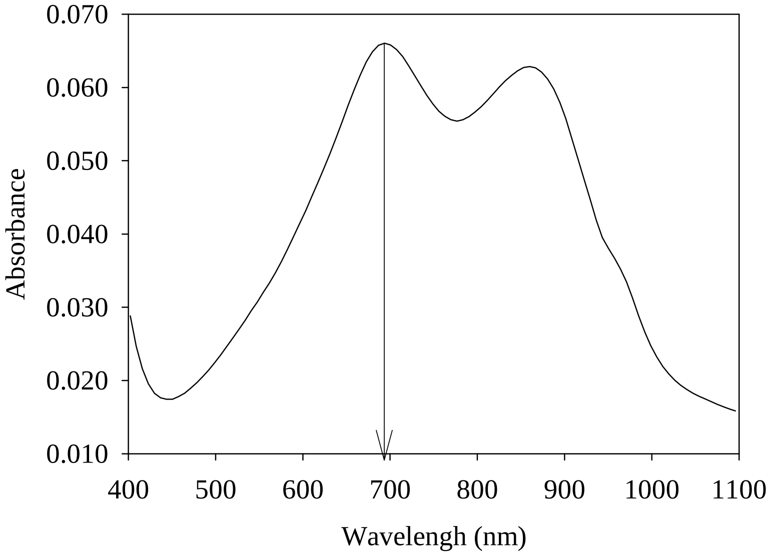
0.070
0.060
0.050
0.040
0.030
0.020
0.010
Absorbance
400
500
600
700
800
900
1000
1100
Wavelengh (nm)

Fig.3

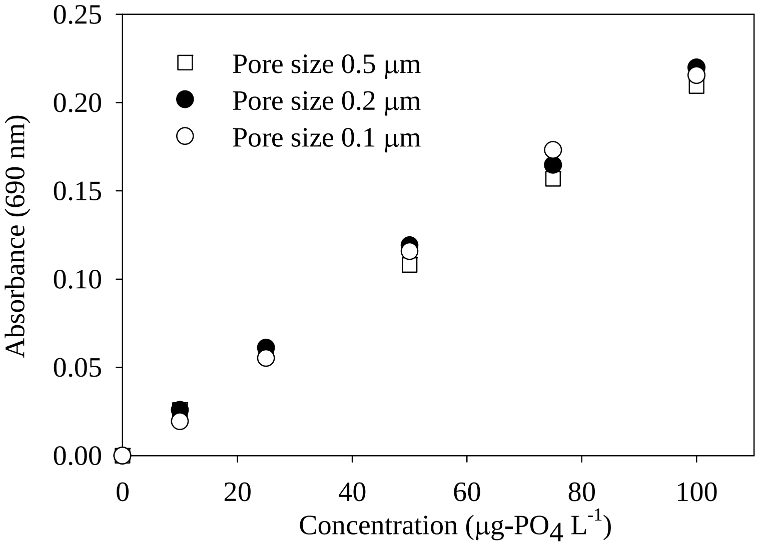
0.25
0.20
0.15
0.10
0.05
0.00
Absorbance (690 nm)
□ Pore size 0.5 μm
● Pore size 0.2 μm
○ Pore size 0.1 μm
0
20
40
60
80
100
Concentration (μg-PO4 L-1)

Fig.4

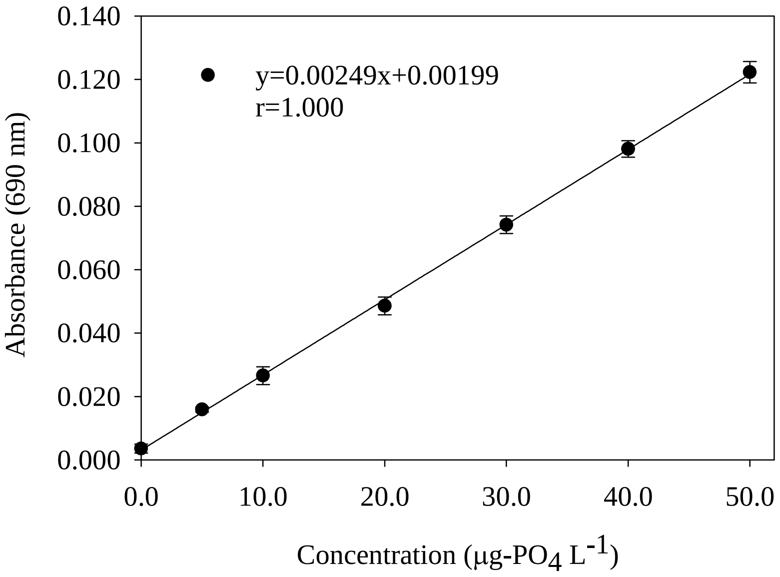
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
Absorbance (690 nm)
y=0.00249x+0.00199
r=1.000
0.0
10.0
20.0
30.0
40.0
50.0
Concentration (µg-PO4 L-1)

Fig.5

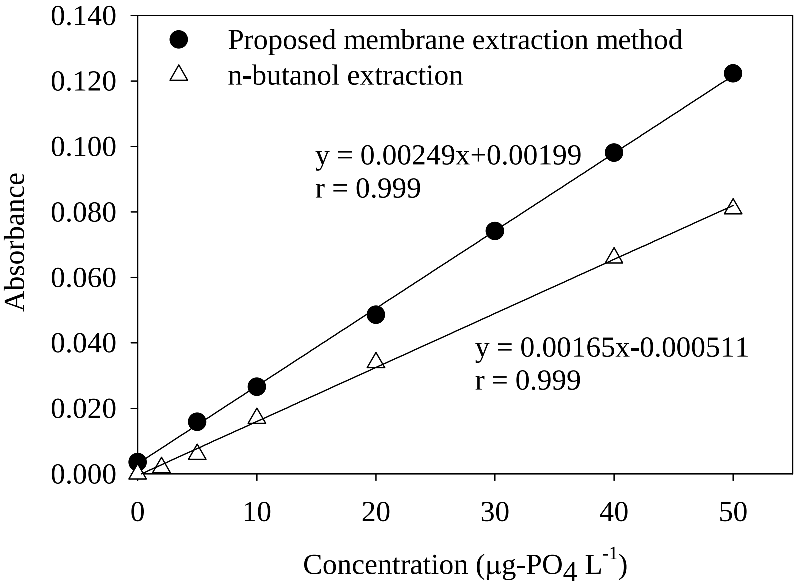
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
Absorbance
● Proposed membrane extraction method
△ n-butanol extraction
y = 0.00249x+0.00199
r = 0.999
y = 0.00165x-0.000511
r = 0.999
0
10
20
30
40
50
Concentration (µg-PO4 L-1)

Fig.6

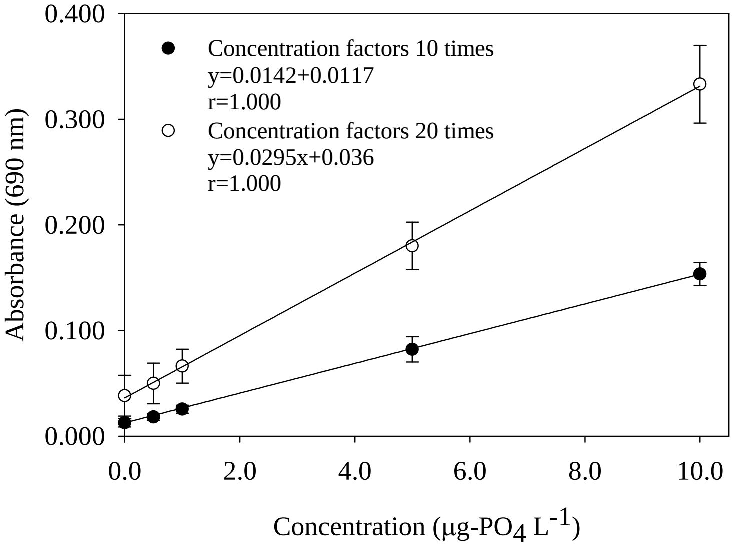
0.400
0.300
0.200
0.100
0.000
Absorbance (690 nm)
● Concentration factors 10 times
y=0.0142+0.0117
r=1.000
○ Concentration factors 20 times
y=0.0295x+0.036
r=1.000
0.0
2.0
4.0
6.0
8.0
10.0
Concentration (μg-PO4 L-1)

Fig.7

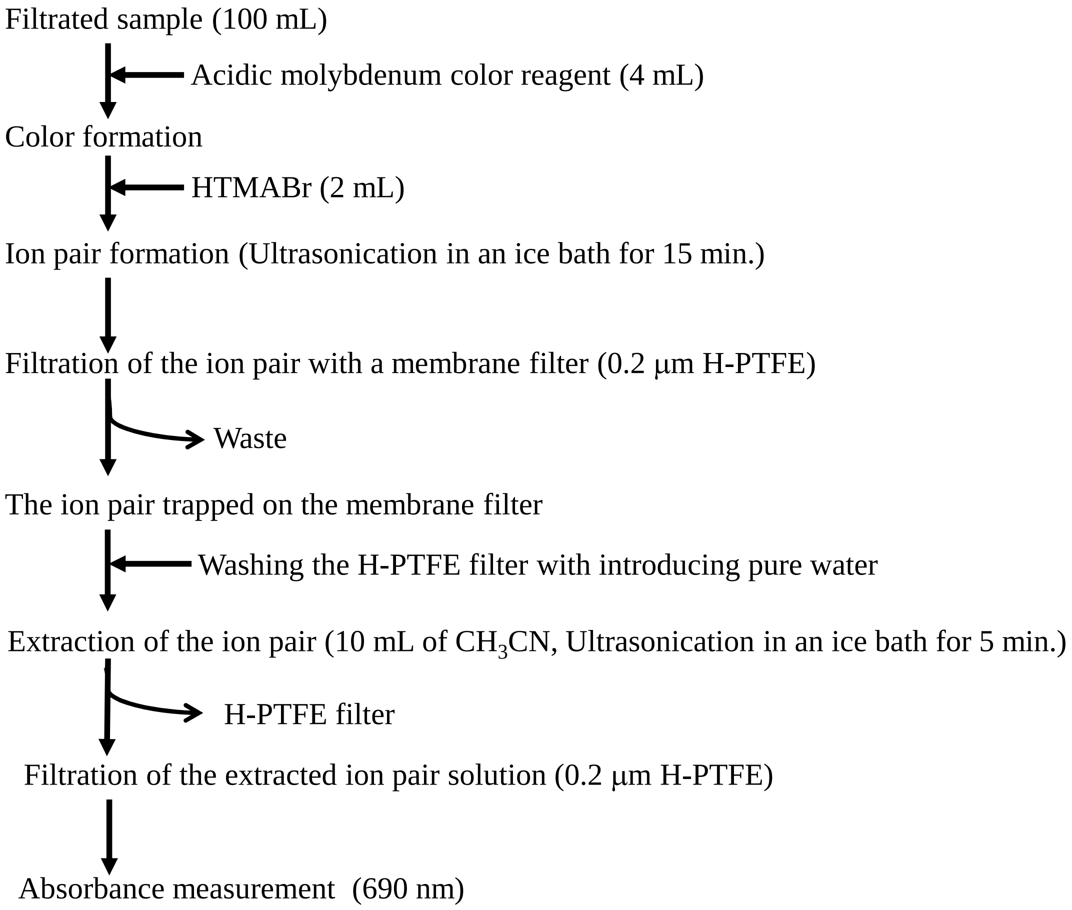
Filtrated sample (100 mL)
Acidic molybdenum color reagent (4 mL)
Color formation
HTMABr (2 mL)
Ion pair formation (Ultrasonication in an ice bath for 15 min.)
Filtration of the ion pair with a membrane filter (0.2 μm H-PTFE)
Waste
The ion pair trapped on the membrane filter
Washing the H-PTFE filter with introducing pure water
Extraction of the ion pair (10 mL of CH3CN, Ultrasonication in an ice bath for 5 min.)
H-PTFE filter
Filtration of the extracted ion pair solution (0.2 μm H-PTFE)
Absorbance measurement (690 nm)

Fig. 8

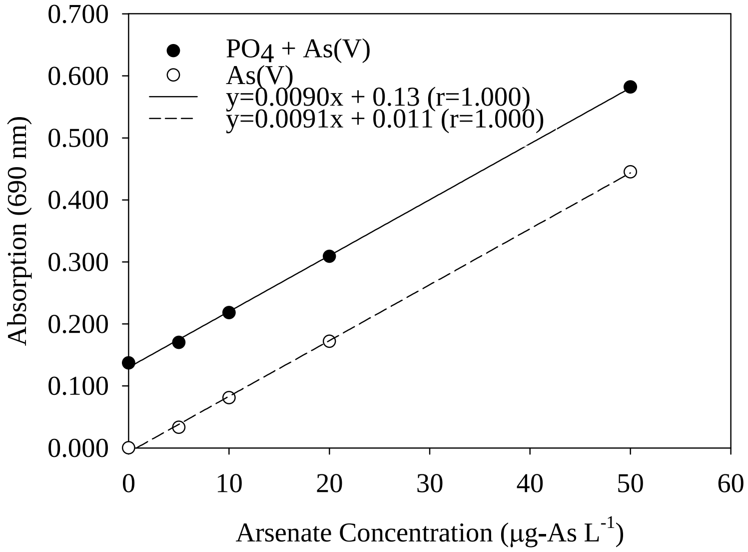

Fig. 9

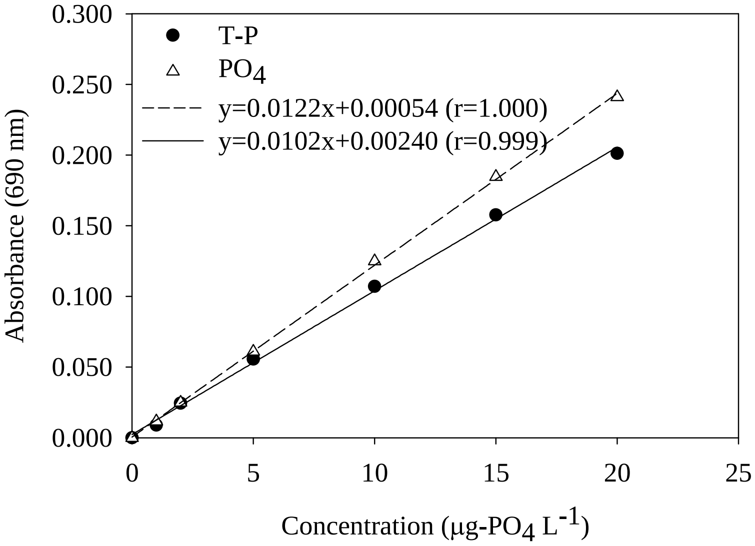

Fig.10

Table

Table1 Total phosphorus and phosphate concentrations in raw water sample measured by the proposed membrane extraction method

Sample	T-P Concentration		PO_4 Concentration	
	(μg-PO_4 L^{-1})	(μg-P L^{-1})	(μg-PO_4 L^{-1})	(μg-P L^{-1})
Pond raw water	20.4	6.6	6.5	2.1
Fibrous filter media treatment water	8.5	2.8	6.3	2.1