RESEARCH ARTICLE

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A new method for phosphate purification for oxygen isotope ratio analysis in freshwater and soil extracts using solid-phase extraction with zirconium-loaded resin

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Rationale: Phosphate (PO_4) oxygen isotope ($\delta^{18}O_{PO4}$) analysis is increasingly applied to elucidate phosphorus cycling. Due to its usefulness, analytical methods continue to be developed and improved to increase processing efficiency and applicability to various sample types. A new pretreatment procedure to obtain clean Ag_3PO_4 using solid-phase extraction (SPE) with zirconium-loaded resin (ZrME), which can selectively adsorb PO_4, is presented and evaluated here.

Methods: Our method comprises (1) PO_4 concentration, (2) PO_4 separation by SPE, (3) cation removal, (4) Cl^- removal, and (5) formation of Ag_3PO_4. The method was tested by comparing the resulting $\delta^{18}O_{PO4}$ of KH_2PO_4 reagent, soil extracts ($NaHCO_3$, $NaOH$, and HCl), freshwater, and seawater with data obtained using a conventional pretreatment method.

Results: PO_4 recovery of our method ranged from 79.2% to 97.8% for KH_2PO_4, soil extracts, and freshwater. Although the recovery rate indicated incomplete desorption of PO_4 from the ZrME columns, our method produced high-purity Ag_3PO_4 and accurate $\delta^{18}O_{PO4}$ values (i.e., consistent with those obtained using conventional pretreatment methods). However, for seawater, the PO_4 recovery was low (1.1%), probably due to the high concentrations of F^- and SO_4^{2-} which interfere with PO_4 adsorption on the columns. Experiments indicate that the ZrME columns could be regenerated and used repeatedly at least three times.

Conclusions: We demonstrated the utility of ZrME for purification of PO_4 from freshwater and soil extracts for $\delta^{18}O_{PO4}$ analysis. Multiple samples could be processed in three days using this method, increasing sample throughput and potentially facilitating more widespread use of $\delta^{18}O_{PO4}$ analysis to deepen our understanding of phosphorus cycling in natural environments.

1 | INTRODUCTION

Phosphorus (P) is an essential element for all living organisms and a limiting nutrient for primary production in several systems. Anthropogenic P loads have increased in numerous watersheds

because of industrialization and urbanization, changing P cycling and nutrient balances in the environment.[1] These human-induced alterations may promote severe eutrophication and can negatively affect biodiversity and food security.[1–3] To address these issues, it is necessary to understand the processes influencing P transformations

between inorganic and organic forms[4] in the environment and to identify the source of P reaching water bodies.[5,6]

Phosphate oxygen (O) isotope ($\delta^{18}O_{PO4}$) analysis is an increasingly applied tool to elucidate P sources and cycling in aquatic and terrestrial systems.[7–9] Although physicochemical reactions do not cleave the P&bond;O bonds in the phosphate ion (PO_4) at Earth's typical surface temperatures and pressures, enzyme-mediated reactions can cleave these bonds and incorporate new O atoms from water into PO_4.[8] Accordingly, $\delta^{18}O_{PO4}$ can be used to estimate input of P from sources with unique isotopic signatures to soil or water bodies and the degree of biological enzymatic processes that modulate $\delta^{18}O_{PO4}$. Previous studies using $\delta^{18}O_{PO4}$ analysis have successfully highlighted the source of P inputs to rivers,[6,10,11] internal P loads from sediment in lakes,[12] and biologically driven P cycling in soil.[13,14]

The procedure of $\delta^{18}O_{PO4}$ analysis involves extracting PO_4 from environmental samples (solid or water samples), concentrating PO_4 using magnesium-induced co-precipitation (MagIC)[15] or co-precipitation with iron (Fe) hydroxide,[16] removing other constituents in solution, precipitating the PO_4 as pure Ag_3PO_4, and measuring the $\delta^{18}O_{PO4}$ value of Ag_3PO_4 using an isotope ratio mass spectrometry (IRMS) instrument. Conventional sample preparation methods typically comprise (1) formation and dissolution of phosphate compounds (e.g., ammonium phosphomolybdate, magnesium ammonium phosphate, or cerium phosphate),[17,18] (2) resin treatments to eliminate other interfering ions,[17–19] and (3) formation of pure Ag_3PO_4. These processes require control of pH and chemical composition in the sample and efficient precipitation of PO_4 under a suitable temperature. The ultimate goal is to eliminate impurities, such as dissolved organic matter (DOM), metal ions, and chloride ions (Cl),[17,18] and produce pure Ag_3PO_4, which ideally contains most of the original PO_4 from the sample and does not involve any O exchange in the process. Because each sample may differ from another in terms of the type and concentration of impurities, some sample type-specific modifications (e.g., organic matter removal with activated carbon or reagents)[20–22] may be needed to obtain the desired outcome. Therefore, analytical methods continue to be developed and improved to increase processing efficiency and applicability to various samples. A new method, which can be applied to various samples with high processing efficiency, could facilitate more studies using $\delta^{18}O_{PO4}$.

In the study reported here, a new purification method for PO_4 using solid-phase extraction (SPE) with zirconium (Zr)-loaded resin was tested. SPE is a reliable technique for acquiring targeted chemicals and/or removing impurities by passing a solution through a column packed with resin.[23] The Zr-loaded resin and Zr oxide selectively adsorb PO_4 from solution without interference from Cl and nitrate ions,[24,25] because of the high affinity of the surface hydroxyl groups on Zr oxide to PO_4.[26,27] The adsorption of PO_4 on Zr materials occurs under acidic to neutral conditions, and desorption occurs under basic conditions.[24,25,28] Therefore, a Zr-loaded resin, under controlled pH, would effectively separate PO_4 from some impurities in solution.

The purification procedure suggested in the study comprises (1) sample concentration by MagIC, (2) PO_4 concentration by SPE, (3) cation removal, (4) Cl removal, and (5) precipitation of Ag_3PO_4. Multiple samples (e.g., 24 samples) can be processed in three days using this method. The method was tested by comparing the resulting $\delta^{18}O_{PO4}$ data obtained from paired samples that were processed using the method proposed by Tamburini et al,[18] using samples of KH_2PO_4 reagent, soil extracts, freshwater, and seawater. The adsorption/desorption features and potential reuse of the Zr-loaded resin were also evaluated.

2 | MATERIALS AND METHODS

2.1 | Materials

An InertSep ME-1 column (GL Science, Japan) with 250 mg of resin in a 6 mL reservoir was used as a solid-phase adsorbent column for PO_4. The resin has iminodiacetic acid functional groups on a polymer base, effectively adsorbing Zr ions without reacting with sodium and potassium ions. The manufacturer's certification shows that the cartridge has an adsorption capacity of 0.3 mmol/g (copper ions). An InertSep HLB column with 250 mg of resin in a 6 mL reservoir or 60 mg of resin in a 3 mL reservoir (GL Science) was used to remove DOM. The smaller column size was used for the HCl extract of soil samples which has low DOM. A BioRad AG50W X8 resin with 100–200 mesh and hydrogen form (BioRad, USA) was used to remove cations. All reagents used in this study were of analytical grade.

2.2 | Resin preparation

The conditioning method for the Zr-loaded InertSep ME-1 column (ZrME) followed that of Okumura et al,[25] with some modifications (Figure 1). The solutions were passed through the column using


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┌─────────────────┐
│ InertSep ME-1   │
└─────────────────┘
  ├─ Methanol, 5 ml, 3 ml/min
  ├─ UPW, 30 ml, 3 ml/min
  ├─ 0.1 M ZrO(NO₃)₂, 10 ml, 1 ml/min
  ├─ UPW, 30 ml, 3 ml/min
  ├─ 1 M KOH, 10 ml, 3 ml/min
  ├─ UPW, 30 ml, 3 ml/min
  ├─ 0.1 M HNO₃, 5 ml, 3 ml/min
┌──────┐
│ ZrME │
└──────┘
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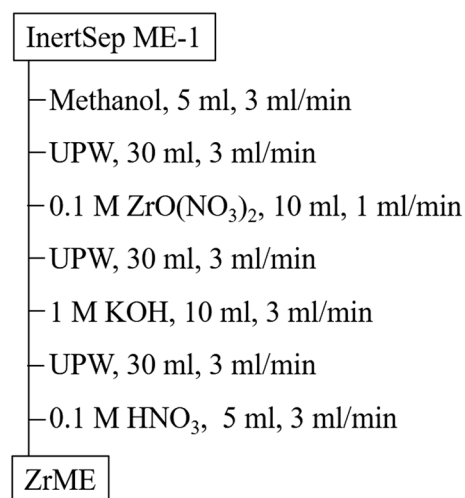
FIGURE 1 Conditioning protocol for ZrME column. The solution type, volume, and flow rate are listed

gravity-driven elution (Figure S1). To control the flow rate (mL/min), the average volume of one drop (mL/drop) was determined using a graduated cylinder. Then, the drop rate (drop/min) was calculated by dividing the target flow rate by the drop volume. Instead of gravity-driven elution, a multi-channel peristaltic pump can be an option to control flow rates. ZrME was sealed using Parafilm to avoid contamination and stored in a refrigerator until analysis. InertSep HLB columns (DOM column) were conditioned by passing 5 mL of methanol and 10 mL of ultrapure water (UPW) at a rate of less than 3 mL/min. A Biorad AG50X8 resin (cation resin) was prepared by adding 3 M HNO_3 (resin slurry:solution = 1:2) and shaking overnight, followed by washing using UPW. The HNO_3 addition and UPW wash were done twice. The cation exchange column (cation column) was prepared by adding 6 mL of resin slurry into a 12 mL empty reservoir with a frit (GL Science). UPW was allowed to flow through the column until the air was eliminated from the inside of the column. The DOM column and cation column were prepared just before use.

2.3 | Adsorption/desorption characteristics of PO_4 on ZrME

An adsorption and desorption experiment was conducted to determine PO_4 adsorption capacity and its desorption from the ZrME columns. A mixed solution made of 1mM KH_2PO_4, 55mM $MgCl_2$, and 100mM HNO_3 was prepared by dissolving KH_2PO_4, $MgCl_2 \cdot 6H_2O$, and concentrated HNO_3 in UPW (referred to as KH_2-P) to reproduce a solution close in chemical composition to that obtained after the MagIC step. To examine the adsorption capacity, the KH_2-P solution was passed through the ZrME column at 0.5 mL/min, and PO_4 concentration was measured in the eluent to calculate the adsorption efficiency. To determine PO_4 desorption, 0.1, 0.25, and 0.5 M KOH solutions were passed through the ZrME column that had previously absorbed 20 μmol of P from the KH_2-P solution. The desorption rate was calculated by measuring the PO_4 concentration in the eluent.

2.4 | Sample preparation (day 1)

Phosphate extracted from the KH_2-P solution, soil, fresh groundwater (GW), and seawater (SW) was used to test the ZrME method (Table S1). The soil sample was obtained from a rice field in central Japan (34°945′–34°921′ N, 136°200′–136°241′ E). Detailed information on the site and soil can be found in Ishida et al[29] (sample K5-WF). GW (20 L) was obtained from a monitoring well, at a depth of 30 m, at the University of Shiga Prefecture, central Japan (35.2589′ N, 136.2156′ E). SW (20 L) was obtained from the coastal area of Ikuchi Island, western Japan (34.2560′ N, 133.0826′ E).

2.4.1 | Soil sample

The soil sample was sequentially extracted using the Hedley procedure.[30] Water extraction was not conducted due to the low P amount extracted with water. Additionally, a single HCl extraction was conducted, which is commonly used for $\delta^{18}O_{PO_4}$ analysis in total inorganic P.[18] The extraction solutions were treated following the procedure of Zohar et al,[31] with some modifications as described below.

2.4.2 | Soil sample sequential extraction $NaHCO_3$ fraction ($NaHCO_3$-P)

The dried soil sample was shaken in 0.5 M $NaHCO_3$ for 16 h to extract weakly absorbed P. The solution was centrifuged and filtered through glass filter paper (GA-55, Advantec, Japan). To eliminate carbonate from the solution, 3 M HNO_3 was added to pH < 1. To concentrate the PO_4, MagIC was performed by adding 3 M $MgCl_2$ and adjusting the pH to about 10.5 by adding 5 M NaOH. The brucite precipitate was obtained by centrifugation and dissolution in 3 M HNO_3. It should be noted that lower concentrations of HNO_3 or NaOH (1 M) are better for pH adjustment to avoid unexpected hydrolysis of organic P in DOM-rich samples.[32] MagIC was repeated until an adequate PO_4 amount (e.g., 10 μmol) was contained in 50 mL (1–3 times). MagIC is not required for samples that contain more than 10 μmol of P in 50 mL of solution. The final solution was filtered through a 0.45 μm membrane filter (mixed cellulose ester, Advantec).

2.4.3 | Soil sample sequential extraction NaOH fraction (NaOH-P)

After the $NaHCO_3$ extraction, 0.1 M NaOH was added to the soil sample, and the mixture was shaken for 16 h to extract aluminum (Al) and Fe-bound P. The solution was separated from the soil in the same manner as described above. Because the resulting NaOH extraction solution has a high pH, $MgCl_2$ and HNO_3 were added to the solution to perform the MagIC step. The MagIC step was repeated until 10 μmol of PO_4 was contained in 50 mL, and the final solution was filtered through a 0.45 μm membrane filter.

2.4.4 | Soil sample sequential extraction HCl fraction (HCl-P)

Following the same procedure as described above, 1 M HCl was added to the soil sample to extract calcium-bound P, and the mixture was shaken for 16 h. The solution was then separated from the soil in the manner described above. Because the HCl extraction solution contains high concentrations of dissolved Al and Fe, which would interfere with PO_4 adsorption on ZrME,[25] 10 mL of conditioned

cation resin (see Section 2.2) was added, and the solution was shaken for 1 h to adsorb the Al and Fe. After separating the solution from the resin by filtration, MagIC was repeated until adequate PO_4 was contained in 50 mL, similar to the $NaHCO_3$ extraction.

2.4.5 | Soil sample solely extracted with HCl (In-P)

The dried soil sample was shaken in 1 M HCl for 16 h. The solution was then separated from the soil in the manner described above. Then, MagIC was repeated until adequate PO_4 was contained in 50 mL, in the same manner as for the $NaHCO_3$ extraction. Cation exchange resin was not added to the extract to assess the effect of metal ion removal on PO_4 adsorption on the ZrME column.

2.4.6 | Samples of GW and SW

The water samples were filtered through a 0.5 μm cartridge filter (TCW-05N-PPS, Advantec) within 24 h of the collection time. MagIC was used to concentrate PO_4 by adding $MgCl_2$ and adjusting the pH by adding NaOH for GW or just adjusting the pH for SW. The process was repeated until an amount of 10 μmol of PO_4 was contained in 50 mL.

2.5 | ZrME method

2.5.1 | DOM removal/PO_4 adsorption (day 2)

The pretreatment protocol using ZrME columns (ZrME method) is depicted in Figure 2. An empty reservoir (*upper*), DOM column (*middle*), and ZrME column (*lower*) were combined using adapters (GL Science) (Figure S1). The ZrME column and DOM column were

filled with 0.1 M HNO_3 before being combined to prevent air intrusion into the column. The filtered sample solution was passed through the combined columns at a rate of less than 0.5 mL/min. When gravity-driven elution did not work at the start of sample flow, the system was pressurized manually using a syringe attached to the adapter until steady flow occurred. The sample volume was kept below 50 mL and the amount of P below 16.4 μmol, to ensure complete and quick adsorption on ZrME. The adsorption efficiency of PO_4 on ZrME was calculated from the change in PO_4 concentration in the solutions before and after the sample was processed.

2.5.2 | Washing (day 2)

After removing the DOM column from the ZrME column, 20 mL of 1 M HNO_3 and 30 mL of UPW at 3 mL/min were sequentially passed through the ZrME column to remove any residual sample solution from the column. To calculate PO_4 loss during washing, the PO_4 concentration in the washing solution was measured.

2.5.3 | PO_4 elution (day 2)

To elute PO_4 from the column, 15 mL of 0.25 M KOH was passed through ZrME at a rate of less than 0.5 mL/min. To calculate the desorption efficiency from ZrME, the PO_4 concentration in the desorption solution was measured.

2.5.4 | Cation removal (day 2)

To remove any residual metal ions from the solution after the ZrME step, the sample solution was passed through a cation exchange column at a rate of less than 0.5 mL/min. To prevent air intrusion into
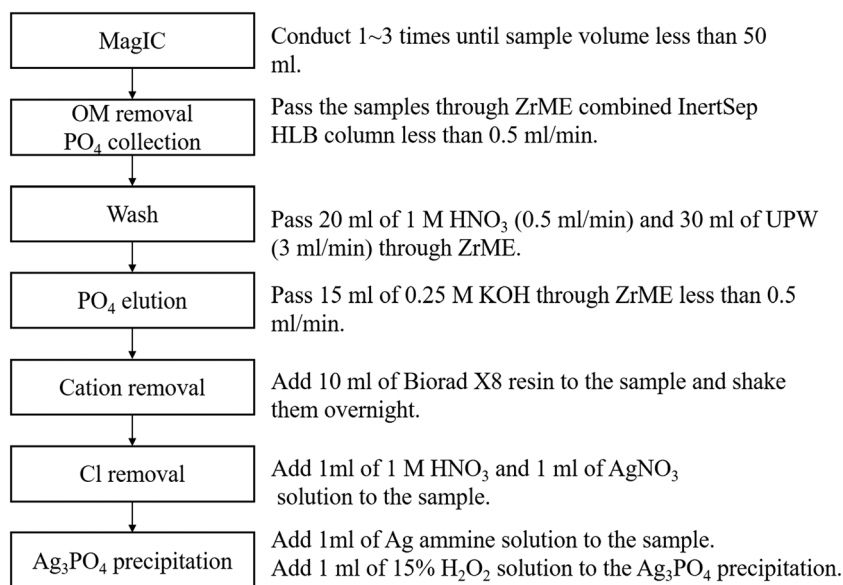
MagIC	Conduct 1~3 times until sample volume less than 50 ml.
OM removal PO_4 collection	Pass the samples through ZrME combined InertSep HLB column less than 0.5 ml/min.
Wash	Pass 20 ml of 1 M HNO_3 (0.5 ml/min) and 30 ml of UPW (3 ml/min) through ZrME.
PO_4 elution	Pass 15 ml of 0.25 M KOH through ZrME less than 0.5 ml/min.
Cation removal	Add 10 ml of Biorad X8 resin to the sample and shake them overnight.
Cl removal	Add 1ml of 1 M HNO_3 and 1 ml of $AgNO_3$ solution to the sample.
Ag_3PO_4 precipitation	Add 1ml of Ag ammine solution to the sample. Add 1 ml of 15% H_2O_2 solution to the Ag_3PO_4 precipitation.

FIGURE 2 Protocol of ZrME method to purify Ag_3PO_4 from soil extracts and freshwater

the column, the cation column was filled with UPW before the sample was added. The column was washed with 5 mL of UPW, and the washing solution was added to the sample solution. This operation can be performed simultaneously with the PO_4 desorption from ZrME step by connecting the ZrME column to the cation column with the adapter. To calculate the PO_4 loss during cation removal, the PO_4 concentration in the sample solution was measured.

2.5.5 | Cl removal (days 2–3)

Although most of the Cl was removed during the washing step, a small amount may remain in the sample after cation removal, especially in the case of the HCl extraction. To remove Cl, 1 mL of 1 M HNO_3 and 2 mL of 1 M $AgNO_3$ were added to the sample solution, and the solution was left overnight. The solution should be acidic due to the cation exchange reaction and the addition of HNO_3. Under acidic conditions, only AgCl is formed. If a white precipitate (AgCl) was observed, it was eliminated using a 0.2 μm membrane filter.

2.5.6 | Precipitation of Ag_3PO_4 (day 3)

A volume of 2 mL of Ag amine solution (10.2 g of $AgNO_3$, 9.6 g of NH_4NO_3, 18.5 mL of concentrated NH_4OH, and 81.5 mL of UPW) was added to the sample.[18] To promote Ag_3PO_4 precipitation, the sample was placed in an oven at 50°C for 3 h. The precipitate was collected by filtration with a 0.2 μm polycarbonate filter (Advantec) and washed with UPW five times. The filter was transferred to a 50 mL centrifuge tube to remove residual organic matter by adding 1 mL of 15% H_2O_2 neutralized with NH_4OH. The tube was left in the dark for 30 min, after which 1 mL of neutralized 15% H_2O_2 was added again. H_2O_2 addition was repeated until the vigorous foaming following the addition of H_2O_2 stopped (ca three times). After removing the filter paper using tweezers, the precipitate was washed using 5 mL of UPW five times under centrifugation. Lastly, purified Ag_3PO_4 was transferred, using a disposable plastic dropper, to a 1.5 mL microtube with about 1 mL of UPW. The Ag_3PO_4 was dried at 50°C. When rapid drying was necessary, the microtubes were centrifuged, and the supernatant was removed using a dropper before the samples were transferred to the oven for drying. The dried samples were kept in a desiccator until analysis.

2.6 | Establishing the most efficient ZrME method

To determine the most effective order of procedures for the ZrME method, the process was tested with some modifications. (1) In the DOM removal/PO_4 adsorption step, the combined columns (DOM and ZrME columns) were not used to calculate the adsorption rate of the ZrME column alone. The samples were first passed through the DOM column, and the solution, after passing through the DOM column, was split into several subsamples. The subsamples were then passed through the ZrME column. The PO_4 adsorption efficiency on the ZrME column was calculated from the change in PO_4 concentration in the solutions before and after the sample was processed by the ZrME column. (2) In the cation removal step, cation removal was performed in a batch mode for the $NaHCO_3$-P, NaOH-P, and HCl-P extracts. A volume of 10 mL of cation resin was added to the sample solution and shaken overnight. The sample solution was separated from the resin using filtration. The resin was washed using a small volume of UPW three times, and the washing solution was added to the sample solution. The other steps followed the procedure described above.

2.7 | Conventional protocol

To test for any isotopic fractionation during the ZrME method, Ag_3PO_4 was purified from the same samples using the methods of Tamburini et al[18] and $\delta^{18}O_{PO4}$ values were compared. The method comprised four steps: (1) formation and dissolution of ammonium phosphomolybdate, (2) formation and dissolution of magnesium ammonium phosphate, (3) cation removal by cation exchange resin, and (4) formation of Ag_3PO_4. For KH_2PO_4, Ag_3PO_4 was obtained by adding 1 M $AgNO_3$ solution to the KH_2PO_4 solution and then neutralizing it with NH_4OH.

2.8 | Regeneration of ZrME columns

To test the reuse of the ZrME column, cleaning and reuse experiments were performed. An amount of 15 mL (Lo-P) or 50 mL (Hi-P) of the KH_2-P solution was passed through ZrME columns, followed by washing step and PO_4 elution step. To clean the columns, 10 mL of 1 M KOH, 30 mL of UPW, 10 mL of 1 M HNO_3, and 30 mL of UPW were sequentially passed through the columns (Figure 3). To determine the residual PO_4 amount in the regenerated columns, 15 mL of 0.25 M KOH solution was passed through the columns at 0.5 mL/min. This series of operations was repeated three times. The adsorption/desorption rate of the column and the amount of residual PO_4 were calculated by measuring the PO_4 concentration of each elution.

Used ZrME
— 1 M KOH, 10mL, 0.5 mL/min
— UPW, 30 mL, 3 mL/min
— 1 M HNO_3 10 mL, 3 mL/min
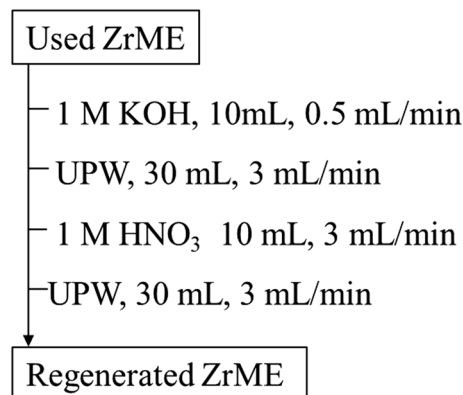— UPW, 30 mL, 3 mL/min
Regenerated ZrME

FIGURE 3   Protocol to regenerate used ZrME column

2.9 | Sample analysis

The PO_4 concentration of each sample was measured using the molybdenum blue method[33] with a microplate spectrophotometer (Multiskan GO, Thermo Fisher Scientific, USA). The $\delta^{18}O_{PO4}$ values are reported as conventional δ notation: $\delta^{18}O = R_{sample}/R_{standard} - 1$, where R_{sample} represents the $^{18}O/^{16}O$ ratio of the sample and $R_{standard}$ represents the $^{18}O/^{16}O$ ratio of Vienna Standard Mean Ocean Water (VSMOW). Ag_3PO_4 samples were measured using a thermal conversion elemental analyzer connected to an isotope ratio mass spectrometer, a Delta plus XP via ConFlo III or a Delta-V advantage via ConFlo IV (Thermo Fisher Scientific), at the Research Institute for Humanity and Nature in Kyoto, Japan. Two internal standards (Ag_3PO_4), with values of $10.7 \pm 0.35‰$ and $23.1 \pm 0.27‰$, were used for calibration and normalization. The values for these internal standards were determined using two independently calibrated standards (STDL: $11.3 \pm 0.15‰$; STDH: $20.0 \pm 0.25‰$) reported by McLaughlin et al.[17] These Ag_3PO_4 standards were made from KH_2PO_4 (Mallinckrodt, USA) and NaH_2PO_4 (Fisher, USA) and were calibrated against Ag_3PO_4 standards of TU1 ($21.09 \pm 0.15‰$) and TU2 ($5.51 \pm 0.15‰$), which were prepared using the BrF_5 fluorination method and calibrated to VSMOW (samples provided by Torsten Vennemann, University of Lausanne). The analytical precision ($\pm SD$) was $\pm 0.4‰$ for the samples of KH_2-P, In-P, and GW, and $\pm 0.7‰$ for those of $NaHCO_3$-P, NaOH-P, and HCl-P.

3 | ASSESSMENT

3.1 | Adsorption/desorption characteristics of ZrME method

For PO_4 purification, it is vital that the ZrME column has sufficient adsorption capacity and reversible adsorption/desorption properties. The adsorption curve indicates that the ZrME column with 250 mg of resin can completely adsorb up to 50 μmol of PO_4 under the tested conditions (Figure 4). 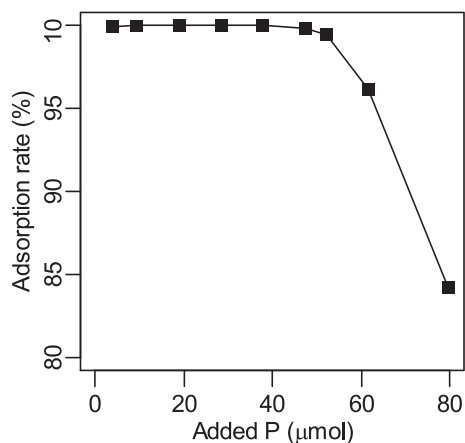Because the amount of PO_4 needed for $\delta^{18}O_{PO4}$ analysis is about 10 μmol, the adsorption capacity of the ZrME columns is sufficient.

The desorption curves for 0.25 M KOH and 0.5 M KOH were almost identical (Figure 5), and 93% of the adsorbed PO_4 was recovered in 10 mL. These results indicate that a 15 mL volume of 0.25 M KOH is sufficient for the desorption step as used in this protocol.

3.2 | Phosphate recovery using ZrME method

High PO_4 recovery during the purification procedure ensures sufficient Ag_3PO_4 is available for $\delta^{18}O_{PO4}$ analysis.[21] Using the ZrME columns the PO_4 in all but the SW samples was fully adsorbed on the ZrME column during the adsorption step (Table 1). During the washing step, most of the adsorbed PO_4 was retained in the column, while about 10% of PO_4 was lost for In-P (HCl total extraction). Desorption yield by 0.25 M KOH ranged from 82.7% to 98.4%, which means that a small fraction of the PO_4 remained on the resin. Previous studies have shown that the adsorption of PO_4 on Zr-loaded resin or on Zr oxide is not entirely reversible,[24,34] and this seems to be also true for the ZrME column. In addition, unexpected air bubbles inside the column may have prevented complete PO_4 desorption. Care must be taken in adding solution to the column to avoid the entry of bubbles. Overall, the total recovery by the ZrME method was 79.2–97.8%, which primarily reflects the desorption rate (Table 1). The overall PO_4 recovery rates in this study are in the range of those of previous reports (61–97%),[20,21] showing that the recovery rate is acceptable.

In contrast, the ZrME method was unsuitable for SW samples, given that little adsorption occurred (Table 1). PO_4 adsorption on Zr-loaded resin was hindered by the presence of fluoride (F; >1 mmol/L) and sulfate (SO_4; >10 mmol/L) ions in SW.[25] The concentration of these ions increased as they were concentrated in the MagIC step, causing poor PO_4 adsorption for the SW samples. Therefore, samples with high concentrations of F and SO_4 should be avoided when using the ZrME method. Since sufficient PO_4 could not be obtained from
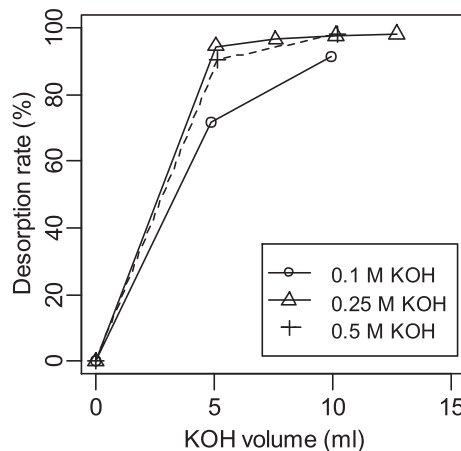

FIGURE 4 PO_4 adsorption rate on ZrME column (250 mg of resin) by passing KH_2-P solution

FIGURE 5 PO_4 desorption rate from ZrME column using KOH solutions

TABLE 1 PO_4 recovery in key steps of the ZrME method. Parameter n indicates the number of subsamples made after MagIC and DOM removal steps and applied to the ZrME column independently. The volume and PO_4 amount refer to those of subsamples. The numbers in parentheses show the standard deviation

ID	n	Sample Volume mL	PO_4 amount μmol/L	PO_4 amount μmol	PO4 recovery Adsorption (%)	Wash (%)	Desorption (%)	Cation removal (%)	Total (%)
KH_2-P	5	15	1000	15.0	99.9 (0.1)	100.0 (0.0)	93.0 (4.4)	99.9 (2.1)	92.6 (3.8)
$NaHCO_3$-P	1	41	116	4.7	100.0	100.0	82.7	96.0	79.2
NaOH-P	3	15	887	13.3	100.0 (0.0)	99.5 (0.3)	88.7 (1.7)	95.1 (3.8)	84.1 (3.3)
HCl-P	3	45	156	7.0	98.7 (1.0)	99.5 (0.9)	91.3 (2.5)	98.0 (0.9)	88.1 (2.1)
In-P	5	6	1760	10.6	98.7 (0.1)	90.8 (0.8)	98.0 (2.2)	93.6 (2.6)	82.2 (2.1)
GW	3	8	1602	12.8	99.8 (0.0)	99.6 (0.4)	98.4 (3.0)	−	97.8 (3.2)
SW	1	40.3	185	7.4	12.3	94.3	8.4	116.4	1.1

TABLE 2 The $\delta^{18}O_{PO4}$ values and Ag_3PO_4 purities from KH_2PO_4, soil extracts, and fresh GW treated with the ZrME and conventional methods. Parameter n indicates the number of subsamples made after MagIC and DOM removal steps and applied to the ZrME column independently. The yield shows the purity of Ag_3PO_4 samples, calculated by deviation of O yield of samples from theoretical values expected for Ag_3PO_4 in IRMS analysis. The numbers in parentheses show the standard deviation

ID	ZrME method n	$\delta^{18}O_{PO4}$ (‰)	Yield (%)	Conventional method n	$\delta^{18}O_{PO4}$ (‰)	Yield (%)	Method
KH_2-P	5	11.0 (0.42)	98–102	5	11.3 (0.23)	94–99	$AgNO_3$ addition
$NaHCO_3$-P	1	18.5	108	−			
NaOH-P	3	20.5 (0.39)	90–105	3	20.4 (0.23)	101–108	Tamburini et al[18]
HCl-P	3	19.1 (0.15)	94–100	1	19.6	100	Tamburini et al[18]
In-P	5	19.5 (0.20)	103–105	5	20.3 (0.29)	98–105	Tamburini et al[18]
GW	3	16.5 (0.16)	104–109	3	16.3 (0.32)	105–111	Tamburini et al[18]

SW using the ZrME method, $\delta^{18}O_{PO4}$ measurement was not performed.

Similarly, high concentrations of Fe (>50 mmol/L) and Al (>100 mmol/L) inhibit PO_4 adsorption on the Zr-loaded resin. Treatment of soil with HCl solution can release a large amount of these metals from the soil, reducing the adsorption efficiency. Comparing HCl-P (HCl extraction following cation removal) and In-P (HCl extraction without cation removal), In-P resulted in lower yield. The high concentration of metal ions of In-P may reduce PO_4 adsorption on the ZrME resin. The result illustrates however that cation removal before the MagIC or ZrME steps is effective in improving the PO_4 recovery of samples with high metal ion concentrations.

3.3 | Validating the ZrME method by comparing it to an established method

For accurate $\delta^{18}O_{PO4}$ analysis, it is necessary to obtain high-purity Ag_3PO_4 without isotopic fractionation. The purity of Ag_3PO_4 samples can be assessed by assessing the deviation of O yield of samples from the theoretical values expected for Ag_3PO_4 in IRMS analysis.[18] In the most stringent criterion, a deviation within 10% of the expected values (90–110% in yield) is acceptable for $\delta^{18}O_{PO4}$ analysis.[18] All of

the Ag_3PO_4 samples formed using the ZrME method met this criterion (Table 2). Thus, using the ZrME method, high-purity Ag_3PO_4 can be precipitated from soil extractions and freshwater samples.

Additionally, the ZrME method did not cause any significant isotope fractionations, as evidenced by the almost identical $\delta^{18}O_{PO4}$ values when comparing the data to results obtained using the established method of Tamburini et al[17] (Table 2). The differences in $\delta^{18}O_{PO4}$ values between the two methods for HCl-P and In-P (0.5–0.8‰) were larger than those for the other samples (0.1–0.3‰). However, the differences were less than 2 SD of the usual analytical precision of the $\delta^{18}O_{PO4}$ measurement by IRMS (e.g., 0.4‰). Although the cause of the differences is unclear, it would be an insignificant obstacle to $\delta^{18}O_{PO4}$ analysis. In conclusion, the ZrME method can be used to purify soil extractions ($NaHCO_3$, NaOH, and HCl) and freshwater samples before precipitation as Ag_3PO_4.

3.4 | Reuse of ZrME column

It is crucial to consider the reuse of ZrME columns to decrease experimental cost. Although the adsorption rate of Hi-P, which was introduced with 50 μmol of PO_4, gradually reduced with the number of reuses, the ZrME column retained sufficient adsorption capacity in the

TABLE 3 PO_4 recovery and residual PO_4 amount in regeneration experiment. Parameter n indicates the number of repetitions. The numbers in parentheses show the standard deviation

ID	Sample				Regeneration cycle	PO_4 recovery				Residual PO_4	
		Volume	PO$_4$ amount								
			µmol/								
	n	mL	L	µmol		Adsorption (%)	Wash (%)	Desorption (%)	Total (%)	Amount (µmol)	Rate (%)
Lo-P	3	15	1000	15	1	99.8 (0.0)	99.5 (0.1)	92.4 (4.4)	91.7 (4.4)	0.1 (0.0)	1.0 (0.0)
					2	100 (0.0)	99.6 (0.2)	88.9 (3.3)	88.5 (3.1)	0.1 (0.0)	0.9 (0.1)
					3	100 (0.0)	99.3 (0.2)	91.8 (1.7)	91.2 (1.6)	0.1 (0.0)	1.0 (0.1)
Hi-P	3	50	1000	50	1	99.9 (0.0)	99.2 (0.3)	96.7 (4.1)	95.8 (4.0)	0.4 (0.1)	0.8 (0.1)
					2	98.1 (0.4)	98.7 (0.3)	90.4 (0.7)	87.5 (0.4)	0.5 (0.0)	1.1 (0.1)
					3	95.2 (1.9)	97.6 (0.3)	94.2 (2.4)	87.5 (1.3)	0.4 (0.0)	0.9 (0.0)

range of normal sample amount (e.g., 15 µmol) (Table 3). After the cleaning step (Figure 3), the ZrME column contained only 1% PO_4 (0.1–0.5 µmol) relative to the amount introduced. These results show that ZrME columns can be reused at least three times with a minor effect of residual P. For processing samples with low P content, however, it would be better to use new resin because the impact of residual P is greater. Since the resin is physicochemically stable, the number of reuses could be increased. Further verification of the regeneration procedure to clean the column and to recover its adsorption capacity is required.

4 | CONCLUSIONS

The use of ZrME columns for the cleanup and purification of PO_4 from freshwater and soil extracts before Ag_3PO_4 precipitation for $\delta^{18}O_{PO4}$ analysis was successfully demonstrated. Multiple samples (e.g., 24 samples) could be processed using this method in three days, a much shorter time than that of current methods (e.g., five days). Materials of the ZrME column cost about 1500 yen/unit in Japan. The cost of analysis with column regeneration is comparable to that of the conventional method. The ZrME method was tested with a limited number of sample types. In future studies, the method should be tested using several different samples and extracts, for example, SEDEX extracts, commonly used sequential extractions for marine sediments.[35] The ZrME method could promote the use of $\delta^{18}O_{PO4}$ analysis due to its simplicity, resulting in a deeper understanding of P cycling in the environment and human impacts on the P cycle.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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