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# Method for analysing phosphate $^{18}\text{O}/^{16}\text{O}$ ratios for waters with high C:P ratios

Groundwater Science Programme

Open Report OR/14/067





BRITISH GEOLOGICAL SURVEY

GROUNDWATER PROGRAMME

OPEN REPORT OR/14/067

# Method for analysing phosphate $^{18}\text{O}/^{16}\text{O}$ ratios for waters with high C:P ratios

D J Lapworth, B Surridge, P J Williams, T H E Heaton & D C  
Gooddy

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### **BGS Central Enquiries Desk**

Tel 0115 936 3143

Fax 0115 936 3276

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email [bgs\\_london@bgs.ac.uk](mailto:bgs_london@bgs.ac.uk)

### **Columbus House, Greenmeadow Springs, Tongwynlais, Cardiff CF15 7NE**

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Fax 029 2052 1963

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Tel 01491 838800

Fax 01491 692345

### **Geological Survey of Northern Ireland, Colby House, Stranmillis Court, Belfast BT9 5BF**

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## Summary

In this report we outline a method to isolate phosphate as pure  $\text{Ag}_3\text{PO}_4$  for waters with high C:P ratios for  $\delta^{18}\text{O}$  analysis. This report details a method that can be used to minimise residual organic contamination of the final  $\text{Ag}_3\text{PO}_4$ , therefore significantly reducing uncertainties in the final interpretation of  $\delta^{18}\text{O}$  results.

This includes the use of column resins in series to (i) remove the majority of dissolved organic carbon and (ii) isolate/pre-concentrate  $\text{PO}_4$ . This is followed by the use of a modified McLaughlin et al (2004) method for  $\text{PO}_4$  precipitation, with an additional final hydrogen peroxide clean-up step to remove residual organic matter following precipitation of  $\text{Ag}_3\text{PO}_4$ . The reagents required for this method are first listed, and then a step-by-step account of the process is outlined. Importantly, it contains adequate detail to be used by other researchers in this field or modified to suit their particular research objectives.

# 1 Introduction

There has been growing interest in recent years in the application of phosphate  $\delta^{18}\text{O}$  for investigating sources and processing of P in the aquatic environment (e.g. McLaughlin et al., 2004; Elsbury et al., 2009; Chang and Blake 2014). It is now widely acknowledged that these early methods first used for ocean and estuary studies are not ideal for samples with high C:P ratios, such as groundwater, river water and treated sewerage waters, due to the potential for co-precipitation of organics with  $\text{Ag}_3\text{PO}_4$ . For such samples, the McLaughlin (2004) method has been found to not fully remove organics with resulting C contents  $>1\%$  possible in samples used for analysis. This can potentially lead to  $\delta^{18}\text{O}$  values that are not specific to  $\text{PO}_4$ , but rather are a mixture of organic oxygen and  $\text{PO}_4$  oxygen, leading to erroneous values if the two pools have different  $\delta^{18}\text{O}$  signatures. This report brings together work undertaken at the BGS Wallingford laboratory to develop and use a method for extracting phosphate ( $\text{PO}_4$ ) from waters, with high C:P ratios, for phosphate  $\delta^{18}\text{O}$  characterisation.

Anion exchange resins have been used for several decades (e.g. Hoering 1957; Qian et al., 1992) to isolate nutrients for elemental and isotope analysis including phosphate  $\delta^{18}\text{O}$  analysis of rocks (Crowson et al., 1991), and more recently for waters (e.g. Gross et al., 2013). DAX-8 resins have been used for removal of organics prior to phosphate  $\delta^{18}\text{O}$  analysis for soil extractable P (Tamburini et al., 2010) and waters (Gross et al., 2013). Li et al (2011) presented a modified McLaughlin et al (2004) method which involved recycling steps within the original method to remove organics and obtain a more pure  $\text{Ag}_3\text{PO}_4$  precipitate. However, this is a very time consuming alternative to that presented here, and independent tests carried out at the BGS laboratories suggest that it may not be a fully effective method and reduced P recoveries are also inevitable.

This method includes the use of a front-end two-step column system first employed by Dr Ben Surridge (co-author from Lancaster University) which combines the use of column resins in series to (i) remove the majority of dissolved organic carbon and (ii) isolate/pre-concentrate  $\text{PO}_4$ . This is followed by the use of a modified McLaughlin et al (2004) method for  $\text{PO}_4$  precipitation, with an additional final hydrogen peroxide clean-up step to remove residual organic matter following precipitation of  $\text{Ag}_3\text{PO}_4$ . The reagents required for this method are listed and then a step-by-step account of the process is outlined. Importantly, we hope that it contains adequate detail to be used by other researchers in this field or modified to suit their particular research objectives.

## 2 Reagent required for silver phosphate precipitation

### 2.1 INITIAL PREPARATION

- Dowex 1X8-200 anion exchange resin (Sigma Aldrich) Approx. 50 g per sample.
- Dax-8 resin (Supelco, Supelite) Approx. 50 g per sample.
- Methanol (Analar) For preparing and cleaning Dax-8 resin – volume needed can be estimated according to requirements.
- Hydrochloric acid (Aristar) For preparing and cleaning Dowex anion exchange resin – volume needed can be estimated according to requirements.
- Potassium Chloride (Analar) 44.73 g required to make up 1litre – Two litres needed ideally just to elute for PO<sub>4</sub>.
- Potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) used to make up a PO<sub>4</sub> standard.

### 2.2 MAGIC PRECIPITATION

- Magnesium Chloride (MgCl<sub>2</sub>.6H<sub>2</sub>O) (Analysis grade) 2 g per sample.
- Sodium Hydroxide (Analysis grade) 0.4 g per sample.

### 2.3 CERIUM PRECIPITATION

- Acetic acid, glacial (Analar) At least 2 ml per sample
- Nitric acid (Aristar) At least 1 ml per sample.
- Potassium acetate (Analar) Approx. 0.49 g needed to make up 0.5 M solution for 10 samples.
- Potassium hydroxide (may be necessary for adjusting pH)
- Cerium (III) nitrate hexahydrate (From Merck described as ‘Extra pure’) 400 mg per sample.

### 2.4 CATION RESIN

- Dowex 50W X-8 cation exchange resin (Sigma Aldrich) Approx. 4 g per sample.
- Nitric acid (Aristar) For preparing and cleaning Dowex cation exchange resin – volume needed can be estimated according to requirements.

### 2.5 SILVER PHOSPHATE PRECIPITATION

- Silver nitrate (AgNO<sub>3</sub>) (Analar) Approx. 2.54 g required to make up solution per 10 samples.
- Ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) (Analar) Approx. 4.3 g required to make up solution per 10 samples
- Ammonium hydroxide (NH<sub>4</sub>OH) (Analytical grade – From Fluka described as ‘for trace analysis’) Approx. 7-8ml required per 10 samples.

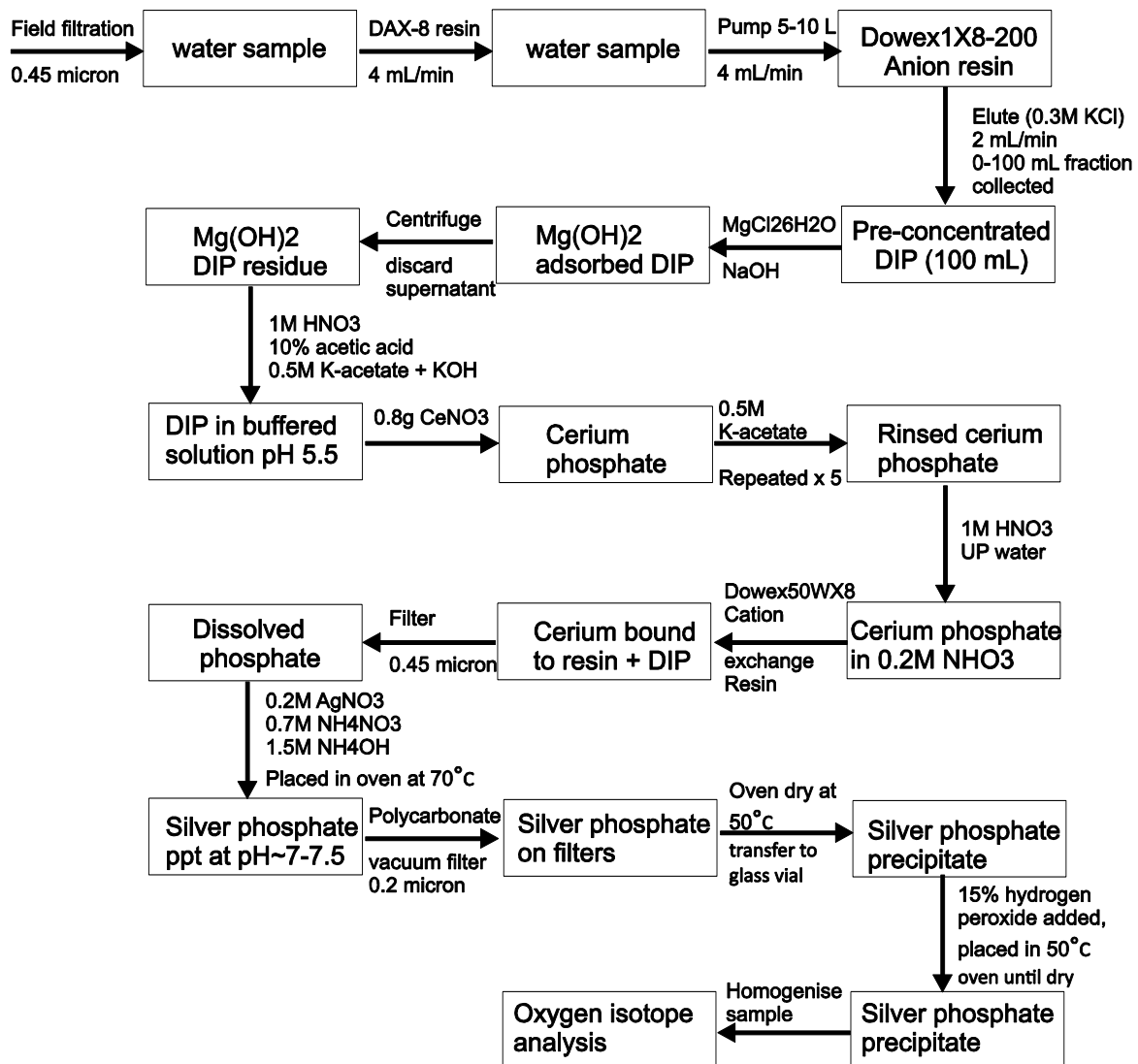
### 2.6 REMOVAL OF RESIDUAL ORGANICS FROM SILVER PHOSPHATE

- Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Analar) 0.25ml required per sample.



### 3 Overview of method

A schematic of the method described in this report and used to process water samples is shown in Figure 1.



**Figure 1** Schematic overview of the method described in this report to process water samples

### 4 Preparation of resins

The conditioning of resins can be time consuming so should be carried out prior to sample collection.

Dowex 1X8-200 anion exchange resin is conditioned with 1 M HCl (Aristar) solution in a large (1000ml or 2000ml) conical flask and placed on a shaker for 1 hour at approx. 90 motions per minute, then the 1 M HCl is poured off and the resin slurry rinsed repeatedly with Ultrapure water until a pH of 6 is achieved.

Dax-8 resin is conditioned with analytical grade methanol in a large conical flask on a shaker for 2 hours and then rinsed several at least 5 or 6 times with Ultrapure water to leave a clean resin slurry.

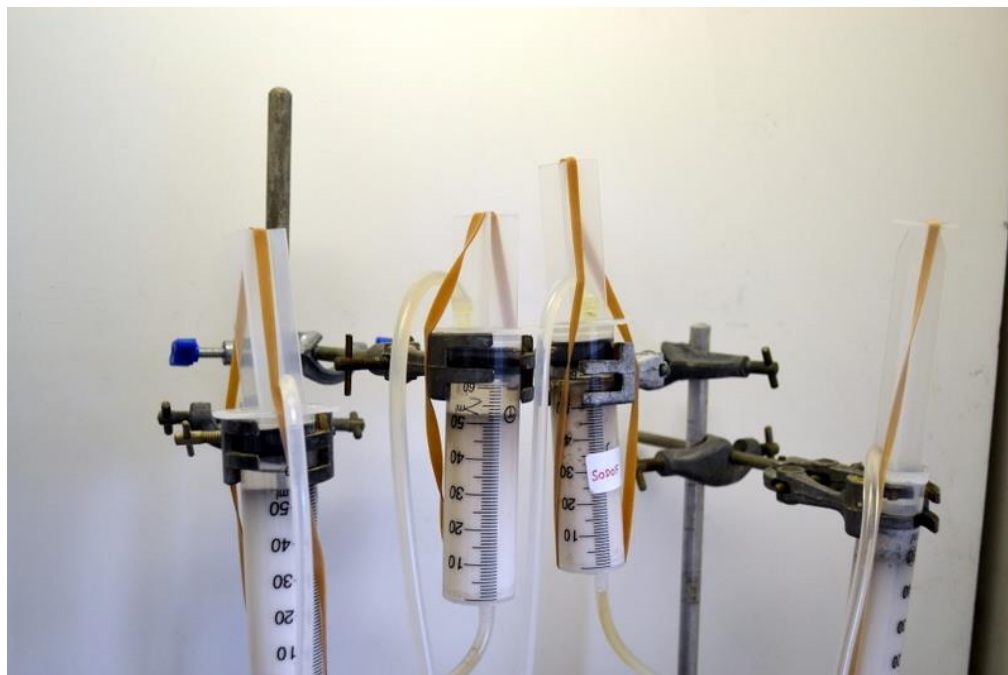
Dowex 50W X-8 cation exchange resin is conditioned with 1M HNO<sub>3</sub>. As less cation exchange resin is required (approx 4 g per sample) this can be carried out in a 500ml or 1000ml conical flask. The resin and acid is mixed overnight on a shaker at approx. 90 motions a minute. The 1M HNO<sub>3</sub> is then tipped away and the resin rinsed repeatedly with Ultrapure water. When the resin is fully rinsed, Ultrapure water added to the resin will retain a pH of 5.5-6, although the pH of the resin slurry itself remains at around 2.

## 5 Setting up resin column

For each sample, a column was prepared comprising two 60ml capacity syringes loaded with resin, the first syringe containing Dax-8 resin to remove organic compounds and the second containing Dowex anion exchange resin to collect the phosphate and other anions. The 60ml plastic syringes were prepared with a 'frit' made up of a triple layer of Whatman glass microfiber prefilters at the nozzle end of the syringe. The syringe plungers had a small hole drilled through them to allow tubing (1/4" external diameter, 1/8" internal diameter and flexible/soft) to be connected. Making sure that the nozzle of the syringe was sealed, resin slurry was then poured into the syringe and allowed to settle so that the resin comprised 50ml of the syringe volume with Ultrapure water the top 10ml. These proportions can be achieved exactly by adjusting with a small dropping pipette and any air voids in the resin removed at the same time. When all the syringes are filled, a second frit is placed at the top of the resin, and the plungers attached. The Ultrapure water at the top of the syringe backfills into the tubing attached to the plunger as they are inserted. Once assembled, a rubber band was stretched around each syringe to prevent any possibility of internal pressure disconnecting the plunger.



**Plate 1** Syringes containing amber coloured Dowex anion exchange resin



**Plate 2 Syringes containing white Dax-8 resin**

A Watson Marlow 205U peristaltic pump was used to pump the samples through the resin columns. Pump tubes (purple-white colour code) with an internal diameter of 2.79mm were used to allow adequate pumping speeds. The assembly from the sample to the pump and the resin column was connected throughout with Tygon flexible tubing (1/4" external diameter, 1/8" internal diameter). The column was drained to a sink.

The pump speed was set at 4ml/min which allowed 1 litre of sample to be pumped through the resin column every 4hours and 10 minutes.



**Plate 3 Samples being pumped through the resin column by a peristaltic pump**

## 6 Eluting phosphate from anion exchange resin

Elution of the phosphate (and minor quantities of other anions) from the resin was performed using 0.3 M potassium chloride solution and was carried out by passing the eluent through the anion exchange resin section of the column.

First the peristaltic pump was disconnected from the tubing at the top of the Dax-8 syringes and reconnected directly to the tubing leading into the anion exchange resin syringes.

0.3 M KCl was pumped through the anion exchange resin at 2 ml/min and the eluted phosphate sample collected in 125ml nalgene™ bottles. The first 100 ml of sample was collected for the phosphate sample. Tests were carried out to establish that this fraction isolates the phosphate peak from other anions that are subsequently eluted. This volume was carefully measured with a stopwatch and by checking premeasured volume markers on the sample bottles to account for any slight variations in flow rate between samples.



**Plate 4** Elution in progress, with the samples are being collected in 125 ml nalgene™ bottles



**Plate 5** Close up of the eluant being collected from the resin column

## 7 Phosphate co-precipitation with brucite ( $\text{Mg}(\text{OH})_2$ )

This step is commonly referred to in the literature as the Magic precipitation step. 100ml of each sample was collected in 125ml nalgene™ bottles. To each sample bottle the following reagents were added, both were dissolved in minimum quantities of ultra-pure water:

- 2 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$
- 0.4 g NaOH

When added, this formed a cloudy white precipitate ( $\text{Mg}(\text{OH})_2$ ). The sample was allowed to stand for at least 2 hours to allow maximum precipitation. If time permits, samples may be left overnight at this stage.

The samples were then transferred to 50ml centrifuge tubes and centrifuged for 10 minutes at 3000rpm. The supernatant was then poured off (into a separate nalgene™ bottle) leaving a gel pellet at the base of the centrifuge tube. The bottles of supernatant were labelled and stored for later testing of residual phosphate concentration.

## 8 Cerium phosphate precipitation

To each sample was added:

- 20 ml ultrapure water
- 4 ml 10% acetic acid
- 2 ml 1M  $\text{HNO}_3$
- 2 ml 0.5M potassium acetate solution

The sample was then shaken, to fully dissolve the gel. The pH should be approximately 5.0 to 5.5 when fully dissolved, but KOH was added to adjust the pH if necessary.

- Add 800 mg cerium nitrate in 5ml ultrapure water

The sample should go cloudy and must then be left at least overnight.

## 9 Isolation of cerium phosphate precipitate

The cerium precipitants were transferred to 50 ml centrifuge tubes and centrifuged at 3500 rpm for 15 minutes and the supernatant discarded. 20 ml of 0.5 M potassium acetate was then added, shaken vigorously with the sample to resuspend the precipitate and centrifuged at 3500 rpm for 15 minutes and the supernatant discarded. This procedure was repeated twice more to give a total of 5 potassium acetate washes.

- 4ml of 1 M HNO<sub>3</sub> was then added to each sample tube

This should dissolve all sample 'gel' pellets, but some required extra time and extra HNO<sub>3</sub> to fully dissolve.

- 18 ml of ultrapure water added to each sample.

## 10 Cation- exchange to remove cerium

Dowex 50w X-8 cation exchange resin was pre-prepared (see Preparation of Resins above) by shaking with 1M HNO<sub>3</sub> overnight and then rinsing with ultrapure water until a pH of approximately 6 was achieved.

- 4ml of cation exchange resin was added to each sample

The samples were then shaken and left overnight.

Samples were then filtered using 0.45µm Whatman puradisc filters.

## 11 Silver phosphate precipitation

The samples were transferred to small 75ml beakers.

To each sample was added:

- 7.5 ml of solution containing 0.2 M AgNO<sub>3</sub>; 0.7 M NH<sub>4</sub>NO<sub>3</sub>; 1.5 M NH<sub>4</sub>OH

The samples were placed in an oven at temperature 70-80°C. As the ammonia evaporates, crystals begin to form. After 3-4 hours crystals should be visible on the surface of the sample and after 12 hours the precipitation of the dark yellow silver phosphate crystals was considered to be complete. When precipitation is complete the pH of the sample should be between 7.0 and 7.5. If it is higher the samples should be left in the oven longer. If evaporation is drastically reducing the liquid volume of the sample, Ultrapure water may be carefully added. The beakers must not be allowed to dry out, and this step should be carried out in a vented chamber as the reaction releases ammonia.

## 12 Filtering and drying the silver phosphate precipitate

To recover the silver phosphate crystals from the beaker it is necessary to filter them through a polycarbonate filter. Polycarbonate filters are used as they are strong enough to allow the crystals to be carefully scraped from their surface without disintegrating. A 0.2  $\mu\text{m}$  polycarbonate filter was used, but 0.4  $\mu\text{m}$  is adequate.

The contents of each sample beaker were filtered through the polycarbonate filter using a 25mm narrow necked Buchner filtration funnel attached to a vacuum pump. The precipitate floating on the surface of the meniscus was easily recovered, but some crystals needed to be scraped from the sides and bottom of the beaker with a narrow spatula. These were then flushed from the beaker into the filtration funnel with Ultrapure water.

Once all the precipitate had been rinsed onto the polycarbonate filter, the precipitate caked polycarbonate filter was removed (with the utmost care) from the filtration assembly and placed on a watch glass before being put in the oven for drying. The oven was set at 50 °C.

When dry, the precipitate was scraped from the filter paper onto a folded glaucine weighing paper. This is important as the weighing paper has anti-static properties and prevents the precious crystals flying away. The silver phosphate crystals settle safely into the crease of the fold in the paper and can be transferred easily to a small glass vial. The vials were weighed before and after the addition of the silver phosphate crystals, thus allowing the weight of the crystals to be measured.



**Plate 6** Final silver phosphate crystals visible in the bottom of sample vials

## 13 Hydrogen peroxide ‘polishing’

The final step is to clean the silver phosphate crystals with hydrogen peroxide to remove any residual organic material.

0.5 ml of 15% hydrogen peroxide solution was added slowly (so as to avoid excessive bubbling) to all sample vials. The sample vials were then placed back in the oven at 50°C and left until all the hydrogen peroxide had evaporated. This step usually visibly improves the colour of the crystals, bleaching them from a dark brown to a lighter shade.

After the cleaning procedure, the vials are reweighed and a final sample weight determined. This sample weight was used to calculate the phosphate yield from the original sample.

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British Geological Survey holds most of the references listed below, and copies may be obtained via the library service subject to copyright legislation (contact [libuser@bgs.ac.uk](mailto:libuser@bgs.ac.uk) for details). The library catalogue is available at: <http://geolib.bgs.ac.uk>.

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