A new technique to determine the phosphate oxygen isotope composition of freshwater samples at low ambient phosphate concentration

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ABSTRACT

The oxygen isotope composition of dissolved inorganic phosphate ($\delta^{18}O_P$) offers new opportunities to understand the sources and the fate of phosphorus (P) in freshwater ecosystems. However, current analytical protocols for determining $\delta^{18}O_P$ are unable to generate reliable data for samples in which ambient P concentrations are extremely low, precisely the systems in which $\delta^{18}O_P$ may provide new and important insights into the biogeochemistry of P. In this paper, we report the development, testing and initial application of a new technique that enables $\delta^{18}O_P$ analysis to be extended into such ecosystems. The Twist Spinning Mode (TSM) protocol described here enables >1000 L of sample with a P concentration <0.016 mg P L^{-1} to be initially processed within the field in approximately 24 hours. Combined with a new freeze-drying method to maximise the yield and minimise the contamination of silver phosphate generated for isotope ratio mass spectrometry, the TSM protocol is able to generate accurate and precise $\delta^{18}O_P$ data. We evaluated the TSM protocol using synthetic test solutions and subsequently applied the protocol to samples from locations around the Saint-Lawrence River in Montreal, Canada. We believe that the novel technique reported here offers the methodological basis for researchers to extend the application of $\delta^{18}O_P$ into a much wider range of freshwater ecosystems than has been possible to date.

INTRODUCTION

Phosphorus (P) is integral to the structure and function of many key biomolecules, including DNA, RNA, adenosine triphosphate and phospholipids, meaning that this element is essential to all life [1]. Human society has dramatically increased the input of reactive P to the biosphere[2], often resulting in undesirable ecosystem changes that lead to significant, adverse economic and social impacts [3-5]. In this context, understanding the sources and the biological impacts of P within ecosystems representkey challenges in many parts of the world.

Stable isotope analysis offers a potentially powerful tool through which to address these challenges. However, P has only one stable isotope (^{31}P), preventing the application of traditional stable isotope approaches that rely on an element possessing more than one isotope. Within natural environments, P is only strongly bound to oxygen (O) in the phosphate molecule. The P-O bonds within phosphate are resistant to inorganic hydrolysis under typical temperature and pressure conditions experienced within the Earth's surface environments [6]. Cleavage of P-O bonds relies on metabolic processes catalysed by the action of intracellular or extraceullar enzymes, which impart a range of equilibrium or kinetic isotope fractionations on the O atoms within resulting phosphate molecules [7-9]. In addition, initial evidence suggests that the biological uptake of phosphate molecules from solution can be associated with isotope effects under certain conditions [9]. Analysis of the O isotope composition of dissolved inorganic phosphate ($\delta^{18}O_P$), followed by interpretation of $\delta^{18}O_P$ data through the isotopic effects ascribed to a range of metabolic processes, offers the potential for new insights into the sources and the cycling of P within natural ecosystems [10-17].

Accurate determination of $\delta^{18}O_P$ relies on extraction of phosphate from an environmental sample, purification and finally measurement by isotope ratio mass spectrometry. Substantial methodological research has been undertaken within a range of environmental matrices to address these analytical challenges [18, 19]. Within aquatic ecosystems, initial work was undertaken within marine environments with large volumes of water usually requiring processing to generate reliable $\delta^{18}O_P$ data [20]. However, transfer of the methodologies developed for marine matrices to freshwaters can be problematic. For example, the presence of additional sources of O including dissolved organic carbon, nitrate and sulphate within freshwaters can bias the $\delta^{18}O_P$ values. Further, in our experience, freshwater matrices can interfere with certain stages of the protocols developed for $\delta^{18}O_P$ analysis within marine matrices, because they often present relatively low PO_4 / (NO_3, SO_4 or total organic carbon) abundance ratios. More recent work has developed protocols for analysis of $\delta^{18}O_P$ that seek to address the particular challenges of freshwater matrices [13]. However, when P concentration in a sample is particularly low and the volume of sample required for $\delta^{18}O_P$ analysis high, even the methodologies developed specifically for freshwaters may prove impracticable or may fail to generate reliable $\delta^{18}O_P$ data. This represents a significant barrier to application of $\delta^{18}O_P$ analyses within freshwater ecosystems at low ambient P concentrations, precisely the environments in which the importance of bioavailable P is likely to be maximised and in which $\delta^{18}O_P$ analyses are of particular interest.

In this paper, we report the development, testing and initial application of a new method designed to extract phosphate for $\delta^{18}O_P$ analysis from large volumes of freshwater (c.1000 L), over relatively short timescales (<24 hours) and using techniques that avoid potential biases in $\delta^{18}O_P$ values from additional sources of O. We believe that the novel approach we report provides researchers with the methodological basis on which to extend $\delta^{18}O_P$ analyses to a wide range of freshwater ecosystems in which this technique has proved impossible to date. Moreover, the new approach we present combined with a new freeze-drying method avoids the use of a MagIC precipitation in the field, thereby reducing the use of toxic reactants such as NaOH and HNO_3 in the field, lowers costs and minimizes the risk of incorporating organic phosphate and organic carbon componds, known to bias $\delta^{18}O_p$ values with additional sources of O.

MATERIALS AND METHODS

Previously, we have developed and reported a protocol based on the use of chloride-form anion exchange resin in column mode to separate phosphate from competing oxyanions within freshwater samples [13], hereafter termed the Modified McLaughlin Method (MMM). However, pumping large volumes of sample (> 20 L) through these columns can prove problematic, for example due to swelling of the resin bed. Pre-concentrating phosphate, for example using co-precipitation with brucite through the MagIC protocol [21], offers one potential solution. However, brucite precipitation requires the use of large masses of NaOH and $MgCl_2$, which incurs significant cost and which may bias $\delta^{18}O_p$ in samples containing low P concentration, due to the trace amounts of P within reagents. Further, our previous research has demonstrated that brucite is not specific to the inorganic phosphate ion, with additional organic P compounds and oxyanions including nitrate and sulphate being co-precipitated, which may confound accurate determination of $\delta^{18}O_p$ [8].

Here, we report a new approach that enables extraction of phosphate from large volumes of freshwater, termed the Twist Spinning Mode (TSM) protocol. The TSM protocol involves pumping a sample into a large (120 L) plastic barrel at a flow rate of 800 mL min^{-1} and subsequently through a column (96 cm tall, internal diameter of 10.5 cm) containing 50 mL of Dowex 1X8-200 chloride-form anion exchange resin with an exchange capacity of 1.2 meq/ml (Figure 1). The inlet to the column is angled at 30°, causing the sample to rotate during passage through the column. This allows the resin to be permanently suspended in a continuous flow of sample, rather than pumping a sample through a static resin bed in column mode. The maximum flow rate is limited to avoid resin spilling into the plastic barrel. However, if a small amount of resin is lost from the column it is collected from the plastic barrel and returned to the inlet of the column.

Phosphate and other oxyanions bind to the anion exchange resin as a sample passes through the column. After sampling is complete, the anion exchange resin is recovered from the base of the cylinder by decanting the overlying water, and then packed into a separate column. In the laboratory, the recovered resin column is eluted with 0.3 M KCl and eluent fractions containing phosphate are collected for further processing, following the protocol described in the MMM. The physical components that underpin the TSM protocol are designed to be inexpensive and simple, allowing for field deployment, ra ther than requiring the transport of large volumes of sample to a laboratory for processing. Using this new approach, 1000 L of sample can be processed within 24 h without having to attend to the sampling apparatus. Given this, it is assumed here that the $\delta^{18}O_p$ value obtained from the TSM protocol represents a daily mean of $\delta^{18}O_p$ within a sampling location such as a river or lake.

Laboratory Testing of the TSM protocol

The TSM protocol was initially tested using synthetic laboratory solutions. Three, 1000 L solutions containing varying but environmentally relevant concentrations of NO_3, SO_4 and humic acid as dissolved organic carbon (DOC) (Table 1) were prepared from KNO_3, K_2SO_4 and commercially-available humic acid (H16752-Humic acid sodium salt, technical grade, Sigma-Aldrich). Competing oxyanions and DOC were included in these solutions to evaluate the ability of the TSM protocol to generate accurate $\delta^{18}O_P$ data given the presence of contaminant sources of O within a sample. It is acknowledged that environmental DOC can include both humic and fulvic acids which may behave slightly differently to our test solutions. To each of these solutions, either KH_2PO_4 (to yield a final P concentration of 16.3 µg P L^{-1}) or a 20% P_2O_5 fertiliser (to yield a final P concentration of 19.5 µg P L^{-1}) was added, generating six synthetic test solutions in total. The two sources of phosphate were chosen to provide distinctly different $\delta^{18}O_P$ compositions: KH_2PO_4 = 9.0 (±0.3)‰ vs VSMOW (Vienna Standard Mean Ocean Water) and the 20% P_2O_5 fertiliser = 16.6 (±0.3)‰ vs VSMOW, as determined by the MMM following dissolution of KH_2PO_4 or fertiliser in milliQ water.

Field Testing of the TSM protocol

Further testing of the TSM protocol was subsequently undertaken in the field, based on a study area located in and around the Saint-Lawrence River in Montreal, Canada. Samples were collected in winter 2017 from two locations on tributaries of the Saint-Lawrence, the Assomption river (Pa) and Achigan river (Pe), situated in predominately agricultural catchments, as well as from the outflow of a waste water treatment works (Pw) downstream of the city of Montreal (Figure 2). Samples from the tributaries and from the waste water treatment works were processed using both the TSM protocol (1000 L of sample) and using the MMM (20 L of sample), in order to compare $\delta^{18}O_P$ generated using the two protocols.

Freeze-drying of silver phosphate precipitates

The protocols reported in both McLaughlin et al.[20] and Gooddy et al. [13] require a slow precipitation (SP) step in order to generate silver phosphate (Ag_3PO_4), which is subsequently analysed by EA-IRMS (Elemental Analysis Isotope Ratio Mass Spectrometry) to determine $\delta^{18}O_P$. This commonly involves the addition of an ammoniacal-silver nitrate solution to the purified phosphate solution (Equation 1) which is heated to 70°C. This solution is strongly basic and can promote the precipitation of silver oxide [5] (Equations 2 and 3), a potential source of contaminant-O in the determination of $\delta^{18}O_P$.

$$NH_4OH + NH_4NO_3 + 3AgNO_3 + H_3PO_4 \rightarrow Ag_3PO_4\downarrow + 2NH_3\uparrow + 4HNO_3 + H_2O \qquad (1)$$

$$AgNO_3 + HO^- \rightarrow AgOH + NO^{3-} \qquad (2)$$

$$2AgOH \rightarrow Ag_2O\downarrow + H_2O \qquad (3)$$

In an attempt to mitigate this, a freeze drying (FD) step in the generation of Ag_3PO_4 was tested in the research we report here (see Figure 3 for the full, revised protocol). This low-temperature process is performed at a near neutral pH, meaning that it is less favourable for silver oxide formation compared to SP.

A comparison of the performance of FD and SP approaches was undertaken. A 1 L synthetic test solution was prepared using KH_2PO_4 to achieve a final concentration of 19.5 mg P L^{-1}. Precipitation of brucite from the 1 L solution using the MagIC approach was followed by stages 6-12 inclusive in the protocol reported in Figure 3. The purified phosphate solution achieved after step 12 in Figure 3 was divided into 10 equal aliquots. Ammoniacal silver nitrate solution was added to each aliquot. Five aliquots were subjected to precipitation of Ag_3PO_4 by the FD method. By freeze drying under vacuum, water and ammonia in the sample were sublimated and silver phosphate precipitated. For a sample aliquot of 30 mL volume, this takes approximately 24 h. The process of formation of Ag_3PO_4 crystals occurs gradually. After precipitation of Ag_3PO_4, the crystals are washed with deionized water on a 0.45 μm acetate filter and placed in the freeze dryer again. Silver phosphate from the remaining five aliquots was generated using the SP approach described within the MMM. The Ag_3PO_4 obtained by both SP and FD methods was analyzed by Scanning Electron Microscopy (SEM) and XRD to assess the purity of the crystals.

Isotopic analysis of silver phosphate

A minimum of 200 μg of Ag_3PO_4 was encased in silver capsules with the same mass of carbon black, with special care taken to minimize the inclusion of air [22]. Samples were analyzed using an Elementar^{TM} Vario PyroCube^{TM} elemental analyzer via a heated autosampler (80ºC), where Ag_3PO_4 is carbothermally reduced in a glassy carbon reactor at 1450°C for complete oxygen sample conversion into CO. The sample gas is carried by a continuous flow of helium, passes through a water trap filled with hygroscopic magnesium perchlorate and is collected in a CO trap. The carbon monoxide is then released and carried through a fresh helium flow to the TCD for oxygen content analysis and then to the isotope ratio mass spectrometer (IRMS Isoprime VisION^{TM}) for isotopic measurement. The CO sample is measured after two monitoring CO pulses. Raw $\delta^{18}O_p$ values are normalised on the VSMOW scale by a two-point linear calibration using IAEA-601 (+24.1‰) & USGS-35 (+56.8‰) and verified with IAEA-C3 (+31.9).

RESULTS AND DISCUSSION

Evaluation of the TSM protocol

The initial mass of P contained in each 1000 L synthetic laboratory solution, alongside the mass of P eluted from the anion exchange resin using 0.3 M KCl, are reported in Table 2. For the synthetic solutions containing KH_2PO_4, the mass of P eluted from the resin decreased consistently as the mass of competing ions in solution increased (Solutions 1-3, Table 1). The P yields derived from the anion exchange resin ranged from 6% (Solution 1) to 1.5%

(Solution 3) of the total mass originally contained within each solution. For solutions that included the inorganic phosphate fertiliser, which contained a slightly higher initial mass of P, yields of P as a proportion of the original mass within the solutions were similar to those observed for solutions containing KH_2PO_4, ranging from a maximum of 5.7% in Solution 2 to a minimum of 2.7% in Solution 3.

In absolute terms, these P yields are clearly low. Excluding the humic acid added to the synthetic solutions, the anion content of Solution 1 equates to approximately 14 meq, which is substantially below the capacity of the anion exchange resin used with the TSM protocol (60 meq). Despite this, P yields for Solution 1 only reached a maximum of 6%. For Solutions 2 and 3, the anion content equates to approximately 640 and 1360 meq respectively, and considerably in excess of the capacity of the anion exchange resin. Whilst saturation of the capacity of the anion exchange resin and competition for binding sites between phosphate and other anions in solution may explain low yields of P in Solutions 2 and 3, this is not the case for Solution 1 where there was an excess of available binding sites on the anion exchange resin. Instead, we believe that the low yields of P are associated with the relatively high flow rate of the solution through the column containing the anion exchange resin in the TSM protocol. Processing c.1000 L of solution within 24 h requires a flow rate of approximately 800 mL min^{-1}. Our data suggest that this flow rate is too high for a significant proportion of the phosphate within the test solutions to be bound to the anion exchange resin. Increasing the mass of anion exchange resin within the column in the TSM protocol may enhance P yields. Alternatively, solutions could be recirculated through the column, although this would effectively increase the total volume of solution to be processed through a column and therefore the time required to complete sampling using the TSM protocol. However, because the TSM protocol allowed c.1000 L of solution volume to be processed, sufficient P was extracted from solution by the anion exchange resin to ultimately enable a sufficient mass of Ag_3PO_4 to be generated for $\delta^{18}O_P$ determination. Importantly, the protocol reported by Gooddy et al. (2015) would have been unlikely to generate a sufficient mass of Ag_3PO_4 for analysis, based on processing a maximum solution volume of 50 L which would have taken in excess of eight days to load through the anion resin column at 4 mL min^{-1}.

Beyond a limit on the mass of Ag_3PO_4 that can ultimately be generated, low recovery of P from solution using the TSM protocol would be problematic if isotopic fractionation was associated with low P recovery from a solution. However, our data do not indicate significant isotopic fractionation during sample processing using the TSM protocol. Table 3 reports $\delta^{18}O_P$ and O yield data for test Solutions 1-3 following the addition of either KH_2PO_4 or inorganic fertiliser as sole sources of P. Based on the MMM, Ag_3PO_4 synthesised from a pure KH_2PO_4 solution had a $\delta^{18}O_P$ value of 9.0‰ (±0.3), whilst from a pure solution of inorganic fertiliser $\delta^{18}O_P = 16.6$‰ (±0.3). For synthetic test solutions containing KH_2PO_4, $\delta^{18}O_P$ based on the TSM protocol ranged from 8.6‰ in Solution 2 to 9.5‰ in Solution 3 with a mean of 9.1‰. For solutions containing inorganic fertiliser, $\delta^{18}O_P$ ranged from 16.3‰ in Solution 1 to 16.4‰ in Solutions 2 and 3, with a mean of 16.4‰ based on the TSM protocol. Further, O yield data ranged from 15.6% to 16.2% with a mean of 15.9%. This compares to an expected O yield for stoichiometrially-pure Ag_3PO_4 of 15.3%, supporting the purity of the Ag_3PO_4

produced through the TSM protocol (also see below). Therefore, despite low P yields from the original solutions, the TSM protocol appears able to generate accurate $\delta^{18}O_p$ data from solutions in which low P concentrations would preclude analysis using alternative protocols.

Freeze Drying versus Slow Precipitation of silver phosphate

The results of the comparison between FD and SP approaches to the generation of Ag_3PO_4 are reported in Table 4. Both methods generated very similar $\delta^{18}O_p$ (averaging 8.7‰ (±0.2) and 8.6 (±0.3) for FD and SP respectively) and oxygen yield (averaging 15.0% (±0.3) and 14.7 (±0.4) for FD and SP respectively) data. Importantly, the yield of Ag_3PO_4 appears to be somewhat higher and less variable for the FD approach compared to the SP approach. Figure 4 reports example SEM spectra for Ag_3PO_4 precipitated with SP and FD methods. Both approaches appear to produce relatively pure Ag_3PO_4 crystals.

Application of TSM and MMM to samples from the Saint Lawrence River catchment

The MMM protocol was unable to produce sufficient Ag_3PO_4 for analysis from either the Assomption (Pa) or Achigan (Pe) river samples (Table 5). However, $\delta^{18}O_p$ data were successfully generated from the wastewater treatment work effluent (Pw) using the MMM approach, giving a value of 17.4‰ compared to 18.3‰ from the TSM protocol. Whilst sufficient for determination of $\delta^{18}O_p$, yields of Ag_3PO_4 for site Pw using the TSM protocol were low. We ascribe this observation to the fact that 1000 L of sample with high ionic concentration was passed through the resin column, likely leading to competition for binding sites on the resin and low P recoveries leading, ultimately, to low Ag_3PO_4 yields. Although the present dataset is limited, it is interesting to observe that the $\delta^{18}O_p$ values generated by both TSM and MMM protocols are broadly consistent with the global range of $\delta^{18}O_p$ reported previously for municipal wastewaters, although these values do appear to exceed the range reported specifically for other sites in North America by at least 3.8‰ [11, 14, 17]. Discussions on the actual sources of phosphate within the wastewater is beyond the scope of this paper and further data would be required in order to establish whether this observation reflects isotopically enriched P within the influent to sample location Pw, compared to other wastewater treatment works that have been analysed in North America, or isotopic enrichment associated with treatment processes within the specific works we sampled in the research reported here. In contrast to the MMM approach, the TSM protocol produced good yields of Ag_3PO_4 for both of the tributary river sites. Values of $\delta^{18}O_p$ for the Assomption and Achigan rivers were >5‰ enriched compared to expected equilibrium values. This observation may indicate the presence of an isotopically-enriched source of P in the catchments of these two tributaries, in excess of the $\delta^{18}O_p$ composition of the inorganic fertiliser used in the laboratory tests reported in this paper. Further tracking of in-river changes in $\delta^{18}O_p$ using the TSM protocol we report here would enable the importance of multiple P sources and in-river P cycling to be explored within these tributaries of the Saint Lawrence river. In Canadian ecosystems, nutrient fluxes are very low, human activities bring discrete changes to nutrient budgets that lead rapidly to drastic changes in ecosystem dynamics. We hope that, by applying this new technique more broadly to such ecosystems, that it will be possible to better understand P sources, assimilation and re-cycling.

Future Use of TSM to determine $\delta^{18}O_P$ in the Context of Samples with Low P Concentration

Despite relatively low recovery of P from synthetic test solutions, the TSM protocol we report offers new opportunities to determine $\delta^{18}O_P$ for freshwaters in which P concentrations are below the threshold suitable for processing using existing protocols. Given the mass of P retained on the anion exchange resin in proportion to that in the original test solutions, the final Ag_3PO_4 yields from the TSM protocol are relatively high and enable robust mass spectrometry to determine $\delta^{18}O_P$. Our SEM analyses coupled with quantification of O-yields indicate that the Ag_3PO_4 we produced was pure. Critically, no significant fractionation of $\delta^{18}O_P$ was revealed following the TSM protocol, despite the low P yields described above, meaning that the TSM protocol, in combination with FD, appears able to generate accurate $\delta^{18}O_P$ data in samples with low ambient P concentrations (<0.016 mg P L^{-1}). Further optimisation of the TSM protocol may be achieved through increases in the mass of anion exchange resin used to bind phosphate from large volumes of solution processed at relatively high flow rates. However, this will also necessitate changes to the subsequent elution of anion exchange resin using KCl, in order to maintain chromatographic separation of phosphate from competing anions, which is dependent on KCl molarity but also on resin bed depth. Despite opportunities for further development, we believe that the TSM protocol reported here provides new opportunities to apply $\delta^{18}O_P$ analyses to questions surrounding P biogeochemistry in freshwaters that have not been feasible sites for phosphate oxygen isotope analyses to date.

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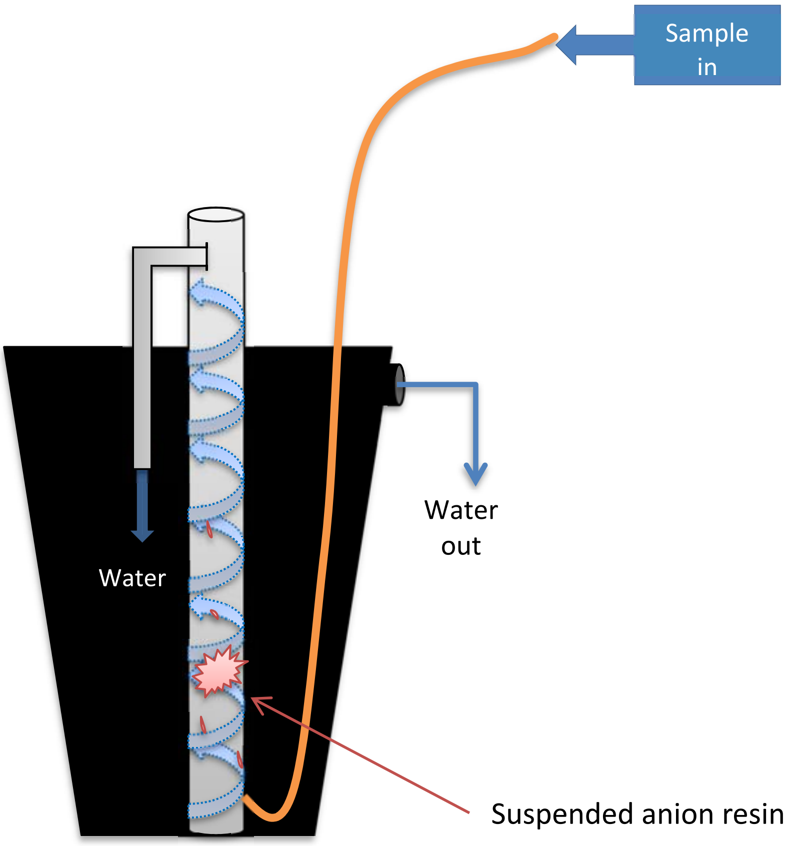
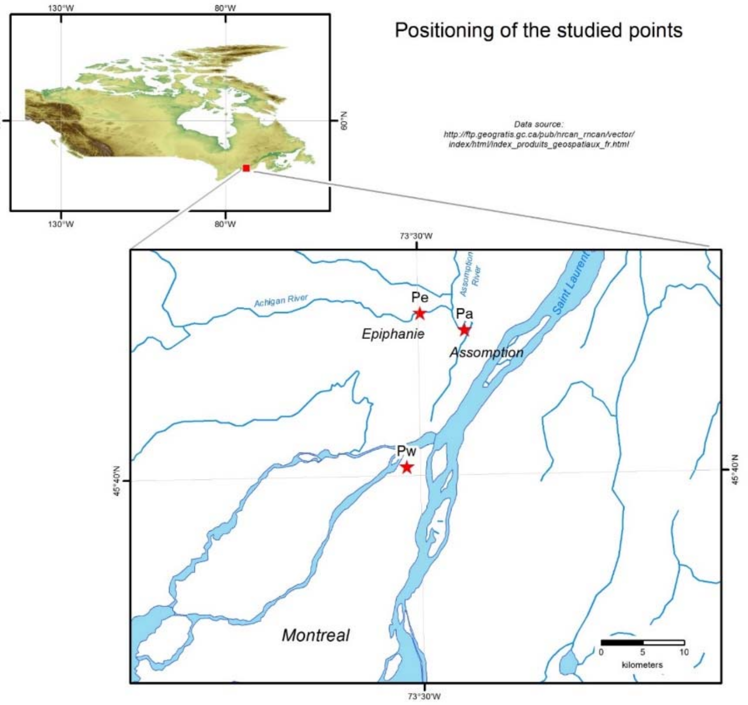


Figure 1. Schematic diagram of Twist Spin Method (TSM) experimental setup. The sample container is made of plastic and has a volume of 120 litres. The internal column containing the anion exchange resin is 96 cm high and has an internal diameter of 10.5cm. The water inlet to the column is fixed at an angle of 30° at the base to induce spinning of the water column as it twists through the anion resin. The flow is adjusted to maintain the resin suspended in the internal column but also to minimise overspillage in the larger container.


Positioning of the studied points

Data source:
http://ftp.geogratis.gc.ca/pub/nrcan_rncan/vector/
index/html/index_produits_geospatiaux_fr.html

Figure 2. Location of sample sites within the Montréal region used for initial application of TSM protocol.

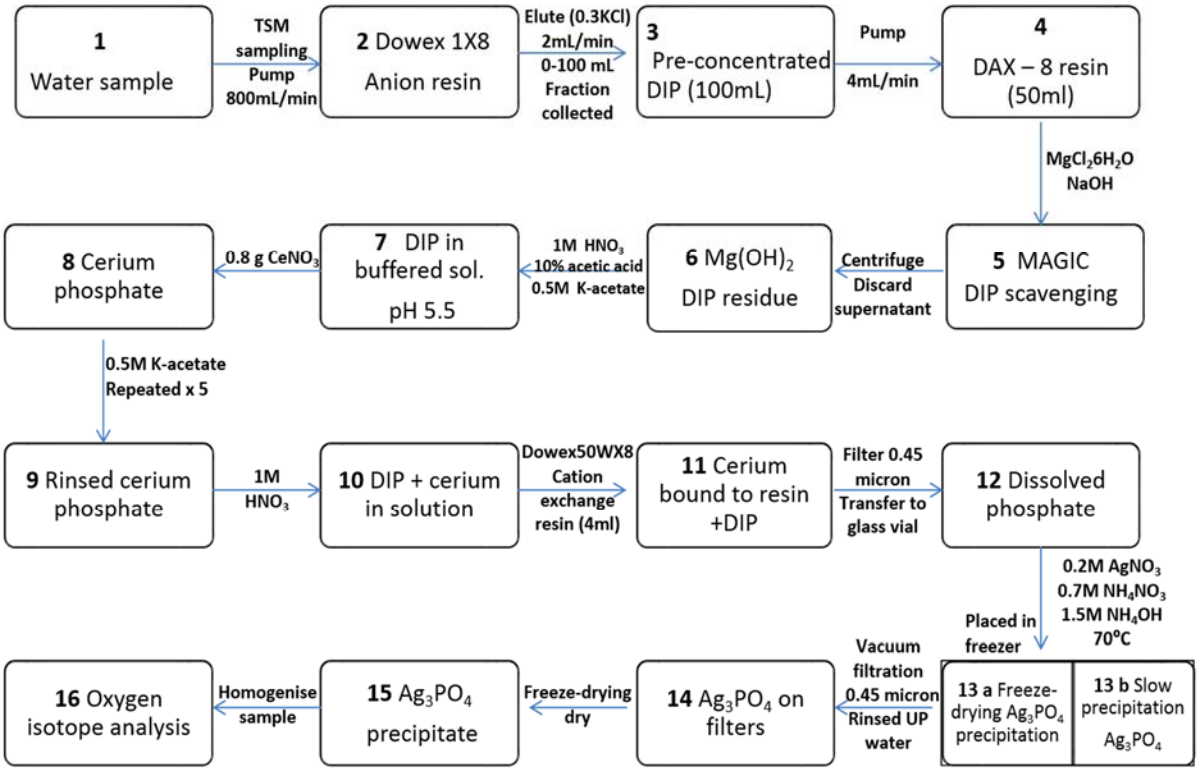


Figure 3. Summary of Twist Spinning Mode (TSM) protocol for determination of ^{18}Op

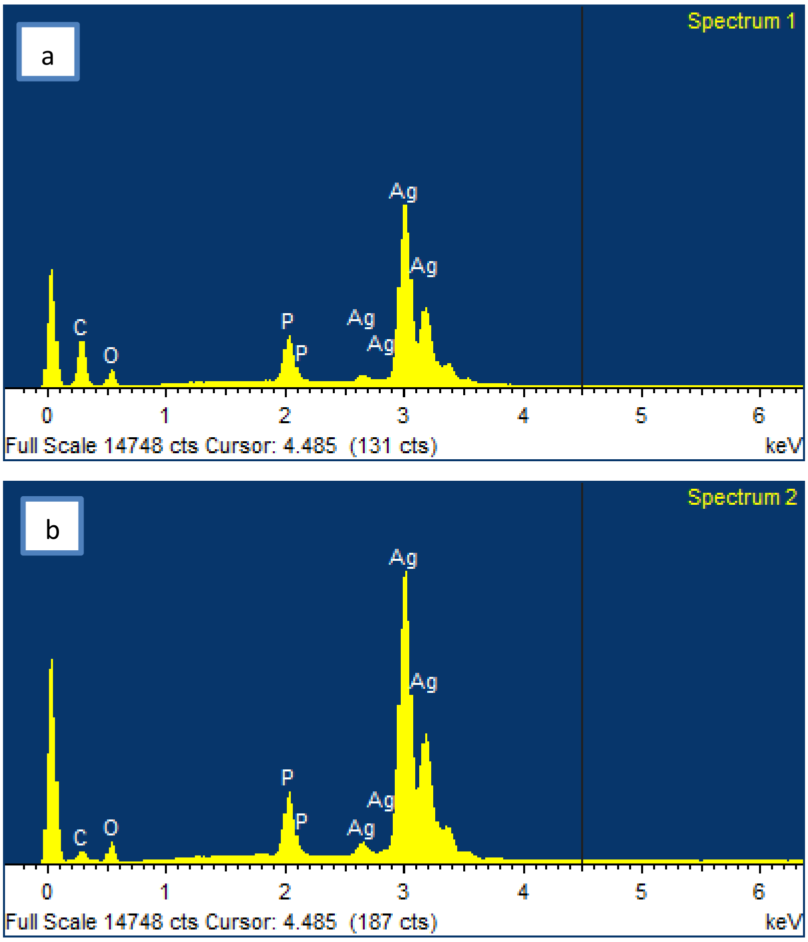


Figure 4. SEM spectra for Ag_3PO_4 synthetized from DIP and precipitated by a) the slow precipitation method and b) the Freeze-Drying method.

Table 1. Properties of synthetic solutions used in laboratory evaluations of TSM protocol. Pure humic acid was used as source of DOC for this synthetic solution.

	Solution 1	**Solution 2**	**Solution 3**
NO_3 (mg/L)	0.5	5	11
SO_4 (mg/L)	0.2	27	57
Humic Acid (mg/L)	0.5	5	10
Conductivity (µS/cm)	15	130	200

Table 2. Initial mass of P in synthetic solutions and final mass of P eluted from anion resins during during laboratory evaluations of TSM protocol.

	Solution 1	**Solution 2**	**Solution 3**
KH_2PO_4			
Total Mass P in (mg)	16.30	16.30	16.30
Mass P eluted (mg)	0.98	0.95	0.25
% Yield	6.0	5.8	1.5
Fertiliser 20%P_2O_5			
Total Mass P in solution (mg)	19.56	19.56	19.56
Mass P eluted mg	0.78	1.08	0.52
% Yield	4.0	5.7	2.7

Table 3. Measured $\delta^{18}O_p$ for each synthetic test solution containing KH_2PO_4 or a 20% inorganic fertiliser after the TSM extraction and precipitation protocol. The measured $\delta^{18}O_p$ for KH_2PO_4 synthesised from solution is 9.0‰ and for inorganic fertilizer is 16.6‰. The oxygen yield expected for stoichiometrically-pure Ag_3PO_4 is 15.3%. The P concentrations are those originally in 1000 L synthetic solutions used in these laboratory tests.

	Source Phosphate	**P (μgL^{-1})**	**$\delta^{18}O_{PO4}$ (±0.3 ‰ *vs* VSMOW)**	**O_{Ag3PO4} (% weight, ±0.2 %)**
Solution 1	KH_2PO_4	16.3	9.3	16.2
	Fertilisers 20%	19.5	16.3	15.8
Solution 2	KH_2PO_4	16.3	8.6	16.1
	Fertilisers 20%	19.5	16.4	16.0
Solution 3	KH_2PO_4	16.3	9.5	15.8
	Fertilisers 20%	19.5	16.4	15.6

Table 4. Silver phosphate yields, measured $\delta^{18}O_p$ and oxygen context of Ag_3PO_4 for samples prepared by freeze drying (FD) or slow precipitation (SP) techniques.

Precipitation method	**Sample**	**Yield (%)**	$\delta^{18}O_p$ **(‰)**	O_{Ag3PO4} **(%)**
FD	1	86.7	8.7	15.4
	2	89.3	8.9	14.7
	3	90.3	8.5	14.9
	4	93.0	8.6	14.9
	5	103.1	9.0	15.2
Mean		***92.5***	***8.7***	***15.0***
SP	1	88.4	8.5	14.7
	2	81.7	8.5	14.9
	3	74.6	8.6	14.1
	4	64.2	8.3	15.1
	5	104.2	9.0	14.9
Mean		***82.6***	***8.6***	***14.7***

Table 5. Data from application of TSM and MMM protocols to determine phosphate oxygen isotope composition of samples from the Saint Lawrence river system. Equlibrium value is calculated using the approach reported by Chang and Blake (2015).

Sample	**P** (µg L^{-1})	**SEC** (µs cm^{-1})	**T** °C	**$\delta^{18}O_{H2O}$** ‰	**Ag_3PO_4** (MMM) (mg)	**Ag_3PO_4** (TSM) (mg)	**$\delta^{18}O_P$** (MMM) (‰)	**$\delta^{18}O_P$** (TSM) (‰)	**$\delta^{18}O_P$** Equlibrium (‰)
Pw	500	1472	6.2	-9.34	21.0	0.2	17.4	18.3	15.9
Pa	9.1	196	0.4	-10.47	0.1	34.4	-	23.9	15.8
Pe	9.8	298	0.4	-9.99	0.2	30.9	-	21.4	16.3