

Oxygen isotope analysis of phosphates: a comparison of techniques for analysis of Ag_3PO_4

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Abstract

A comparison has been made of oxygen isotope analyses of natural and synthetic phosphates using three methods in current use and Ag_3PO_4 as the analyte. Of these methods, conventional fluorination using BrF_5 provides the most precise and accurate measurements and these analyses serve as the basis for comparison. Fluorination liberates 100% of the oxygen in Ag_3PO_4 and the isotopic composition of this oxygen can be readily normalized to accepted oxygen isotope ratios of international reference standards. The widely used method of high-temperature reaction with graphite in isolated silica tubes is also precise but requires calibration for scale compression resulting from a combination of factors including incomplete extraction of oxygen, reaction temperature, possible oxygen exchange with the silica tube and/or differences in the grain size of the graphite used. The recently developed method based on high-temperature carbon reduction and continuous flow mass spectrometric analysis of CO is relatively fast, requires little sample and provides 100% yields for oxygen. At the present time, this method is less precise than the other methods examined and requires calibration against standards on a run to run basis. Five phosphate reference standards with $\delta^{18}\text{O}$ values ranging from -5.2‰ to 34.0‰ were prepared and packaged for distribution to active workers in the field. Analyses of these standards will allow normalization and calibration of results obtained using any available method of oxygen isotope analysis of phosphate. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Early in the development of oxygen isotope paleothermometry, measurements of oxygen isotope frac-

tionations between cogenetic phosphate and carbonate in fossil marine shells held high promise of providing ocean paleotemperatures that were independent of the isotopic composition of the water inhabited by the organism. With this goal in mind, Tudge (1960) devised a reliable but tedious method of analysis of oxygen isotope compositions of biogenic phosphate. Temperature coefficients of the phosphate–water and

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carbonate–water fractionations proved to be so similar, however, that no useful temperature information could be gleaned from parallel measurements of the oxygen isotope composition of carbonate and phosphate (Longinelli, 1966; Longinelli and Nuti, 1973a). Longinelli (1965, 1966) made a comprehensive study of orthophosphate from marine organisms and subsequently on phosphorites, fish teeth and bones (Longinelli and Nuti 1968, 1973a,b). The field lay relatively dormant for a decade until the publication of landmark papers by Kolodny et al. (1983) and Longinelli (1984). These studies showed that oxygen isotope analyses of phosphate from bones and teeth of mammals and fish, as well as from phosphorites can be effective tools in paleohydrological and paleoclimatological research. Oxygen isotope compositions of biogenic phosphate can provide powerful paleoenvironmental proxies because biogenic apatite appears to form in isotopic equilibrium with environmental fluids, including body fluids, and because the strong P–O bonds in apatite are highly resistant to low-temperature alteration processes. During the 1990s, analytical techniques were refined and diverse applications of the tool were made (e.g., Ayliffe et al., 1992; Lécuyer et al., 1993; Barrick and Showers, 1994; Bryant et al., 1994; Fricke and O’Neil, 1996; Lécuyer et al., 1996; Blake et al., 1997; Fricke et al., 1998; Vennemann and Hegner, 1998; Fricke and Rogers, 2000; Vennemann et al., 2001) and the field blossomed.

Biogenic apatite can be represented by the general formula $\text{Ca}_5(\text{PO}_4, \text{CO}_3, \text{F})_3(\text{OH}, \text{F}, \text{Cl}, \text{CO}_3)$. Because oxygen is present in three different sites in the mineral, the PO_4^{3-} radical is normally isolated for isotopic analysis. Tudge (1960) isolated PO_4^{3-} as BiPO_4 and, despite lengthy chemical procedures and the hygroscopic nature of its most common form, BiPO_4 was used in all early studies of oxygen isotope variations of biogenic phosphate. Firsching (1961) developed a more simple method of isolating PO_4^{3-} from natural substances as Ag_3PO_4 and, many years later, this method was resurrected and refined for oxygen isotope work by Crowson et al. (1991). Ag_3PO_4 soon became the analyte of choice because it is easier to prepare than BiPO_4 and, very importantly, because it is not hygroscopic.

Oxygen isotope analyses of Ag_3PO_4 are made by a variety of methods including fluorination (using BrF_5 ,

ClF_3 or F_2) specifically refined for phosphates by Crowson et al. (1991) and Lécuyer et al. (1996), resistance heating Ag_3PO_4 –graphite mixtures in isolated silica tubes (O’Neil et al., 1994), reaction with bromine (Stuart-Williams and Schwarcz, 1995) and laser heating Ag_3PO_4 –graphite mixtures (Wenzel et al., 2000). In all these methods, the O_2 generated is converted to CO_2 for mass spectrometric analyses, but only the fluorination reaction produces O_2 in 100% yield. O_2 can be released for isotopic analysis from tiny amounts of Ag_3PO_4 placed directly on the filament of a mass spectrometer configured for negative ion analysis (Holmden et al., 1997), but this method is time-consuming, relatively expensive and its accuracy and precision are not comparable to those of other techniques. A very promising analytical technique that has recently been developed for analysis of small amounts of Ag_3PO_4 , is on-line high-temperature reduction with graphite in a glassy carbon reactor (e.g., Kornexl et al., 1999). In this method, oxygen in Ag_3PO_4 is converted quantitatively to CO and analysed by continuous flow mass spectrometry.

Considerable effort has been directed toward minimizing the amount of sample required, improving the spatial resolution of analysis and eliminating chemical isolation of the PO_4^{3-} . Laser ablation (Cerling and Sharp, 1996) and laser fluorination (Jones et al., 1999) work well for tooth enamel and, with future refinements, these techniques may be applicable to analyses of other substances as well. Interference by oxygen from hydroxyl and carbonate ions, both of which may occur in substantial amounts in biogenic apatite, require correction factors, though. Such corrections are not necessary when PO_4^{3-} is isolated for isotopic analysis.

Because the field has advanced very quickly, analytical techniques employed vary considerably and complications arise in comparing results obtained in different laboratories. The aim of this contribution is to make a critical comparison of oxygen isotope analyses of both natural and synthetic phosphates of widely different oxygen isotope composition using three of the available methods of analysis of Ag_3PO_4 . The methods chosen are: (1) conventional fluorination using BrF_5 as reagent, (2) high-temperature reaction with graphite in isolated silica tubes and (3) high-temperature reduction and continuous flow mass spectrometric analysis of CO. The graphite method

described by O'Neil et al. (1994) is currently one of the most widely used methods of oxygen isotope analysis of phosphates because it is relatively simple, precise, and easy to establish in any functioning stable isotope laboratory. The new method of high-temperature reduction of phosphate is also simple, very rapid and has the advantage that only small amounts of sample are required for analysis. Conventional fluorination is a well-known and reliable procedure in which 100% of the oxygen in phosphate is extracted and converted to CO₂ for isotopic analysis. For these reasons, analyses by the fluorination method will be taken here as the measure for comparison with analyses made by the other techniques.

2. Analytical techniques

2.1. Sample preparation

Isolation of PO₄³⁻ from natural samples at the University of Tübingen (UT) (Table 1) followed the

techniques described in detail by Stephan (2000), adapted after Crowson et al. (1991) and O'Neil et al. (1994). About 20 mg of crushed sample was soaked for 12 h in 2.5% NaOCl to remove soluble organic matter, washed several times in distilled water and then soaked for 48 h in 0.1 M NaOH to remove humic acids. After several rinse cycles with distilled water, the phosphate was dissolved in concentrated HF and the CaF₂ residue discarded. The solution was neutralized with 2 M KOH, added to a solution of ammoniacal silver nitrate heated to 60 °C. Over a period of several hours, the phosphate slowly precipitated as Ag₃PO₄. Filtered crystals of Ag₃PO₄ were washed several times in distilled water and dried overnight at 70 °C.

Synthetic standards TU-1, TU-2, 130-9 and 130-1, were prepared by dissolving commercial reagent grade di-potassium hydrogen phosphate (K₂HPO₄; δ¹⁸O = 12.8 ‰) in two different samples of distilled water (δ¹⁸O values of -10.9 ‰ and +16.8 ‰, relative to VSMOW) and allowing the phosphate and water to exchange at 150 °C for several days.

Table 1
Summary of samples used, methods of preparation and type of analyses

Sample	Type of sample	Ag ₃ PO ₄ preparation	Reference
GW-1	Natural; mixture of teeth from one <i>Carcharodon carcharias</i>	UT	A
NBS-120c-UT	Natural; phosphorite rock	UT	A
SM2-9	Natural; mixture of teeth from several <i>Somniosus microcephalus</i>	UT	A
TU-1	Synthetic; from K ₂ HPO ₄	UT	A
TU-2	Synthetic; from K ₂ HPO ₄	UT	A
130-1	Synthetic; from K ₂ HPO ₄	UT	A
130-9	Synthetic; from K ₂ HPO ₄	UT	A
KH ₂ PO ₄	Synthetic	No preparation	
NBS-120c-GL	Natural; phosphorite rock		
Kodak	Synthetic	GL	B
Hoering 4-18	Synthetic; from K ₂ HPO ₄	GL	B
BAP 5-2	Natural; igneous apatite	GL	B
Mod Ele	Natural; tooth enamel apatite	GL	B
Ambo Ele	Natural; tooth enamel apatite	GL	B
ID Std	Natural; tooth enamel apatite	GL	B
SP Std	Natural; tooth enamel apatite	GL	B
JR Turt	Natural; turtle shell	GL	B
YR-1	Synthetic; from KH ₂ PO ₄	Y	B
YR-2	Synthetic; from KH ₂ PO ₄	Y	B
YR-3.1	Synthetic; from KH ₂ PO ₄	Y	B

Abbreviations: UT—University of Tübingen; GL—Geophysical Laboratory; Y—Yale. References: A—Stephan (2000); B—O'Neil et al. (1994).

Table 2

Summary of the results of the oxygen isotope analyses of Ag_3PO_4 , KH_2PO_4 and quartz obtained at the University of Tübingen

Sample	Fluorination with BrF_5^a			Reaction with graphite in sealed silica tubes ^b			High-temperature reduction ^c				
	$\delta^{18}\text{O}$ (‰, VSMOW)	1σ	n	$\delta^{18}\text{O}$ raw (‰)	1σ	n	$\delta^{18}\text{O}$ raw ^d (‰)	1σ	$\delta^{18}\text{O}$ normalized ^e (‰, VSMOW)	1σ	n
GW-1	22.65	0.18	13	21.30	0.12	5	20.97	0.61	22.66	0.14	52
NBS120c ^f	22.58	0.09	3	21.27	0.02	2	20.58	0.57	22.09	0.51	18
TU-1	21.11	0.19	7	21.38		1	19.56	0.71	21.11	0.57	22
TU-2	5.45	0.10	7	6.23	0.17	2	3.51	0.83	5.35	0.62	14
130-9	8.43	0.10	3	9.10	0.20	2	6.55	0.65	8.42	0.29	35
130-1	-1.14	0.06	3	1.08	0.02	2	-3.10	0.66	-1.13	0.17	32
SM2-9	26.17	0.22	2	24.67	0.11	8	24.14	0.23	26.13	0.25	12
YR-1	-5.19	0.15	3	-0.87	0.16	2	-7.20	0.54	-5.77	0.33	18
YR-2	13.06	0.21	4	12.78	0.12	2	11.72	0.49	13.05	0.31	19
YR-3.1	34.03	0.22	3	29.88	0.20	3	32.74	0.60	33.54	0.58	6
KH_2PO_4	12.21	0.06	2				10.86	0.43	12.70	0.36	17
Kodak	18.06	0.21	2				16.29	0.46	17.94	0.46	9
Hoering 4-18	11.53		1				9.93	0.08	11.57	0.08	3
BAP 5-2	11.07		1				9.20	0.10	10.83	0.10	3
Mod Ele	15.71		1				13.74	0.29	15.39	0.29	3
Ambo Ele	21.44		1				19.21	0.27	20.87	0.27	3
ID Std	10.98		1				9.10	0.18	10.73	0.18	3
ELE Cory 6	7.22		1				-	-	-	-	-
HVAP	10.43		1				-	-	-	-	-
SP Std	19.60		1				15.90	0.22	17.56	0.22	3
JR Turt	13.02		1				12.55	0.53	14.19	0.54	3
Mean of 1σ		0.13			0.12			0.56		0.33	
NBS-28 quartz ^g	9.56	0.07	12								
NCSU quartz ^h	11.62	0.17	20								

$g = \delta^{18}\text{O}$ values with a different batch of silica glass used earlier: GW-1: $\delta^{18}\text{O} = 22.11\text{‰}$ ($1\sigma = 0.25$; $n = 16$); NBS120c: $\delta^{18}\text{O} = 21.70\text{‰}$ ($1\sigma = 0.38$; $n = 4$); SM2-9: $\delta^{18}\text{O} = 25.26\text{‰}$.

^a Values are averages from eight different runs between July 1999 and October 2000.

^b Values for GW-1 are averages from four different runs and chemical extractions. All others are single extractions.

^c Values are averages of 10 different runs between May and November 2000.

^d $\delta^{18}\text{O}$ values using reference gas calibration with water reduced at 1350 °C.

^e $\delta^{18}\text{O}$ values of samples normalized to GW-1, 130-9 and 130-1 analyzed with each run and using $\delta^{18}\text{O}$ values obtained by fluorination of these samples.

^f Average from one NBS120c-GL and two NBS120c-UT.

^g Values are averages from one run in October 2000. Accepted value is 9.64‰.

^h Values are averages from a number of runs between July 1999 and October 2000, analyzed in parallel with the phosphates. Accepted value is 11.67‰.

After the exchange period, the dissolved phosphate was precipitated as Ag_3PO_4 by addition of ammoniacal silver nitrate solution, filtered and dried at 70 °C overnight.

Samples YR-1, YR-2, and YR-3 were prepared at Yale University by enzyme-catalyzed oxygen isotope exchange between dissolved phosphate and three waters with $\delta^{18}\text{O}$ values ranging from -19.1‰ to

+44.1‰ relative to VSMOW. For standards YR-1 and YR-2, $\text{PO}_4\text{-H}_2\text{O}$ oxygen isotope exchange was catalyzed by alkaline phosphatase (APase) from calf intestine (SIGMA) using β -glycerophosphate as a substrate and PO_4 source. APase catalyzes the hydrolytic cleavage of phosphomonoester bonds. The APase exchange was carried out at 35–37 °C in 50 mM glycine buffer (pH 9) with 0.5 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$,

Table 3

Comparison of oxygen isotope analyses of Ag_3PO_4 obtained using the ST method in different laboratories (UT—University of Tübingen; GL—Geophysical Laboratory; Y—Yale)

Sample	UT			GL			Y		
	$\delta^{18}\text{O}$ raw (‰)	1σ	n	$\delta^{18}\text{O}$ raw (‰)	1σ	n	$\delta^{18}\text{O}$ raw (‰)	1σ	n
GW-1	21.30	0.12	5						
NBS120c	21.27	0.02	2	21.8		1			
TU-1	21.38		1				21.7	0.11	2
TU-2	6.23	0.17	2				7.5	0.07	2
130-9	9.10	0.20	2						
130-1	1.08	0.02	2						
SM2-9	24.67	0.11	8						
YR-1	−0.87	0.16	2	0.0	0.3	3	−1.38		1
YR-2	12.78	0.12	2	14.3	0.2	3	14.31	0.07	2
YR-3.1	29.88	0.20	3				33.51	0.02	2
Kodak				18.8	0.1	3			
Hoering 4-18				13.8	0.2	2			
BAP 5-2				12.6	0.2	42			
Mod Ele				16.5		1			
Ambo Ele				21.0		1			
ID Std				12.8		1			
ELE-6				8.5	0.1	2			
HVAP				11.9	0.1	5			
SP3-3 Std				10.3	0.3	4			
JR Turt				15.1		1			

and β -glycerophosphate. The YR-3 standard was prepared using orthophosphate from reagent grade KH_2PO_4 as a substrate and inorganic pyrophosphatase, (pPiase) from yeast (Sigma) which catalyzes the reversible hydrolysis of pyrophosphate into two orthophosphates. The pPiase reaction was carried out at 22.4 °C in 50 mM HEPES (*N*-[2-hydroxyethyl] piperazine-*N'*-[2-ethane-sulfonic acid]) buffer (pH 7.4) with 15 mM KH_2PO_4 and 15 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

Ag_3PO_4 for samples NBS-120c-GL, BAP 5-2, Mod Ele, Ambo Ele, ID Std, SP Std and JR Turt (Table 1) was prepared at the University of Michigan and the Geophysical Laboratory in a manner similar to that described in O'Neil et al. (1994), adapted after Crowson et al. (1991). About 50 mg of apatite was dissolved in concentrated HF, the solution neutralized with 2 M KOH and subsequently precipitated as Ag_3PO_4 by addition of an ammoniacal silver nitrate solution. Filtered crystals of Ag_3PO_4 were washed several times in distilled water and dried overnight. A synthetic GL standard, Hoering 4-18, was prepared by dissolving KH_2PO_4 in solution and precipitating it as

Ag_3PO_4 . In addition, analyses were made of a synthetic Ag_3PO_4 standard, purchased from Kodak Chemicals.

2.2. Fluorination (FL)

Between 15 and 25 mg Ag_3PO_4 and about 14 mg KH_2PO_4 were loaded into nickel reaction vessels while flushing the vessels with dry nitrogen. Sample sizes (on an oxygen basis) and vessels used were the same as those routinely used for silicates and oxides. The samples were evacuated for a minimum of 3 h at 150 °C down to a vacuum of better than 10^{-5} mbar and reacted with doubly distilled BrF_5 at 500 °C for 15 h on a conventional extraction line modified after Clayton and Mayeda (1963). The released O_2 was quantitatively converted to CO_2 by passing the extracted O_2 over a carbon rod heated by a platinum coil. Gas yields were measured in a calibrated volume using a pressure transducer with a precision of $\pm 2\%$ for sample sizes of between 10 and 200 μmol of CO_2 . Yields for Ag_3PO_4 and KH_2PO_4 were $100 \pm 2\%$. The oxygen isotope composition of CO_2 was measured on

a Finnigan MAT 252 dual-inlet mass spectrometer. Oxygen isotope compositions are expressed in the conventional δ -notation relative to Standard Mean Ocean Water (VSMOW) in parts per thousand (‰). Replicate analyses of the quartz NBS-28 standard analyzed as a single run during the same period as the phosphates had an average $\delta^{18}\text{O}$ value of $9.56\text{‰} \pm 0.07\text{‰}$ ($n=12$), while our own in-house quartz standard (NCSU) analyzed in parallel with the phosphate samples averaged $11.62\text{‰} \pm 0.17\text{‰}$ ($n=20$; expected value is 11.67‰). Two samples each of TU-1, TU-2 and GW-1 were also heated in a vacuum at 500 °C prior to analysis.

2.3. Reaction with graphite in sealed silica tubes (ST)

The techniques used in the three different laboratories are similar and are based on that described by O'Neil et al. (1994). The method used at the University of Tübingen (UT) is described in detail by Stephan (2000). About $20 (\pm 0.05)$ mg of Ag_3PO_4 are carefully mixed with $0.75 (\pm 0.02)$ mg of graphite in a small Al-boat and then transferred from this boat into 6 mm o.d. (1 mm wall) silica tubes that have previously been roasted in air at 600 °C for 1 h. The sample mixture is then evacuated to better than 10^{-3} mbar and heated with a small resistance furnace to about 500 °C for 5 min to remove any adsorbed water and organic contaminants. The tubes are sealed under vacuum and reacted for 3 min in a large muffle furnace held at 1200 °C to produce CO_2 . During this reaction about 25% of oxygen from the Ag_3PO_4 is released and fixed as CO_2 . However, small amounts of CO are also produced, necessitating fairly exact proportions of Ag_3PO_4 to graphite. Using higher amounts of graphite increases the amount of CO (the presence of which can clearly be recognized by the ion-source pressure in the mass spectrometer), while smaller amounts of graphite will yield less than 25% of oxygen from the phosphate. The CO_2 produced from the phosphate is then expanded into the inlet system of a Finnigan MAT 252 mass spectrometer and analyzed for its isotopic composition relative to the same reference gas as that for the CO_2 produced by fluorination.

The methods used at the Geophysical Laboratory (GL) and at Yale (Y) differ from that used at Tübingen in the amount of graphite used (0.3 mg) for 20 mg of

Ag_3PO_4 , the reaction temperature (1400 °C instead of 1200 °C), and the methods of cooling from high temperature. The reason that different graphite/ Ag_3PO_4 ratios are required to obtain precise results in the different laboratories is not clear, but it may be related to the grain size of the graphite (Stephan, 2000). The oxygen isotope compositions at GL and Yale were measured on Finnigan MAT 251 mass spectrometers.

2.4. High-temperature reduction (HTR)

The methods used at UT and GL are similar and are based on continuous flow techniques and high temperature reduction of Ag_3PO_4 , forming CO as the analyte gas (e.g., Kornexl et al., 1999). It utilizes the TC-EA (high-temperature conversion-elemental analyzer) from Finnigan MAT, which includes a reactor consisting of a glassy carbon tube filled with glassy carbon chips and a small graphite reaction cup, all encased within a ceramic tube (Koziet, 1997). About 0.2–1.0 mg of Ag_3PO_4 are weighed into silver cups that are 4 mm in diameter and 6 mm high. The cups are tightly folded to minimize the amount of trapped air and loaded into an autosampler (Carlo Erba AS128) that is attached to the reaction tube. At UT, 3×5 mm Sn-cups were used for one run (Run 8, Table 4). This run had a higher blank (up to 50 mV on mass 28 collector compared to < 10 mV for silver cups), but gave a similar result to that obtained using the silver cups. After a brief He purge within the loading port of the autosampler, the folded sample cup is allowed to fall into the reactor held at 1450 °C and continuously flushed with He (purity 99.999%; flow about 90 ml/min). Reaction gases produced are carried in the He-stream through a gas chromatograph (molecular sieve 5A) held at 70 °C , and admitted to a Delta Plus XL mass spectrometer via a ConFlo interface. This interface also allows for the generation of reference CO pulses such that individual CO peaks can be measured against those of the reference gas.

The reference gas used at UT has a $\delta^{18}\text{O}$ value of about 10.5‰ , that at GL has a value of about -190‰ . The former was estimated by analyzing VSMOW and SLAP using the TC-EA, while the latter was estimated by the analysis of Ag_3PO_4 standards with known oxygen isotope composition. Daily drifts

in the $\delta^{18}\text{O}$ values measured for samples can occur when using the TC-EA, presumably depending on the exact settings and operating conditions of the TC-EA for the given day. Corrections for these drifts in the $\delta^{18}\text{O}$ values measured for the samples at UT and GL were made by analyzing standards of different $\delta^{18}\text{O}$ values (e.g., GW-1, 130-1 and 130-9 at UT) with each run of phosphates, commonly about 30 samples per run. The slope and intercept of the relation between measured and true values were, hence, adjusted on a per run basis. The $\delta^{18}\text{O}$ values of these standards were determined by fluorination techniques outlined above. Samples and correction standards were run in triplicate or more.

Yields of CO were determined via calibrations of peak area versus oxygen yield, deduced from the weight of benzoic acid ($\text{C}_7\text{H}_6\text{O}_2$) and its theoretical oxygen content (26.23 wt.% oxygen). The benzoic acid was reacted at the same temperature as the phosphates and the calibration has a typical standard error of 2.5% for a calibration based on 10 samples. Yields for Ag_3PO_4 and KH_2PO_4 normally average 100% but can decrease to about 90% when the graphite reaction cup becomes filled with residual Ag (after running about 100 or more samples), or when reaction temperatures are decreased to 1400 °C. This decrease in yield does not appear to influence the reproducibility of the analyses. Standard reproducibility within one run for phosphates and benzoic acid are typically better than 0.3‰.

After each run of 30 samples, the glass tubes of the Conflo interface are disassembled and cleaned in an ultrasonic bath with distilled water to remove yellow-brown deposits that we believe to be deposits of elemental phosphorus. Elemental phosphorus is known to volatilize from phosphorous compounds in a reducing atmosphere at high temperatures. These deposits readily hydrate to produce a viscous liquid when exposed to air (such as at the outlet of the Conflo glass tube as well as on the outside wall of the glassy carbon reactor tube) and when dissolved in water produce an acidic solution. Buildup of these deposits has adverse effects on the analyses, including poor reproducibility for repeated measurements of the reference gas only (worse than $\pm 0.15\text{‰}$), as some of this material also accumulates on the outside of the capillary tubing used to introduce the reference gas into the Conflo and mass spectrometer. The capillaries

are cleaned by wiping them with a clean, dry cloth. After washing in distilled water, the glass tubes comprising the Conflo unit are dried briefly at 110 °C and reassembled. The system is ready for use within minutes of reassembly. No deposits of the yellowish material are formed when samples of water or benzoic acid are reacted.

Similar yellowish deposits of Al- and Si-carbide and oxide compounds (+ phosphorous?) are found on the outside of the glassy carbon tube of the reactor itself, which also necessitates cleaning the reactor, particularly when it has been vented to air and/or has not been used for extended periods. The glassy carbon tube and chips are cleaned with distilled water and dried in an oven before reassembly.

3. Results

Oxygen isotope analyses of Ag_3PO_4 and KH_2PO_4 measured at the University of Tübingen (UT), the Geophysical Laboratory (GL) and Yale (Y) using the FL, ST and HTR techniques are summarized in Tables 2–5 and Figs. 1–3. The results of the FL and ST techniques in Tables 2 and 3 are reported as measured, corrected only for ^{17}O and ^{13}C interferences on mass 45 and 46 (Craig, 1957; Santrock et al., 1985). For both the FL and ST methods at UT, the CO_2 samples were analyzed against the same CO_2 reference gas.

3.1. Fluorination

$\delta^{18}\text{O}$ values of GW-1, NBS 120c, TU-1, 130-9, 130-1, YR-1 and YR-2 determined using the FL method represent averages of a number of analyses performed over the course of about 2 years (February 1999 to September 2000). The average for NBS 120c given for the FL method includes analyses of one sample of Ag_3PO_4 prepared at the Geophysical Laboratory and two separate preparations made at the University of Tübingen. $\delta^{18}\text{O}$ values for samples of GW-1, SM2-9, YR-1, YR-2, and YR-3.1 are also averages of analyses of more than one preparation of Ag_3PO_4 . The reproducibility of $\delta^{18}\text{O}$ values obtained for analyses of different batches of the standards ($\pm 0.2\text{‰}$) was similar to the reproducibility of individual preparations of each and to analyses of quartz standards made during the same period.

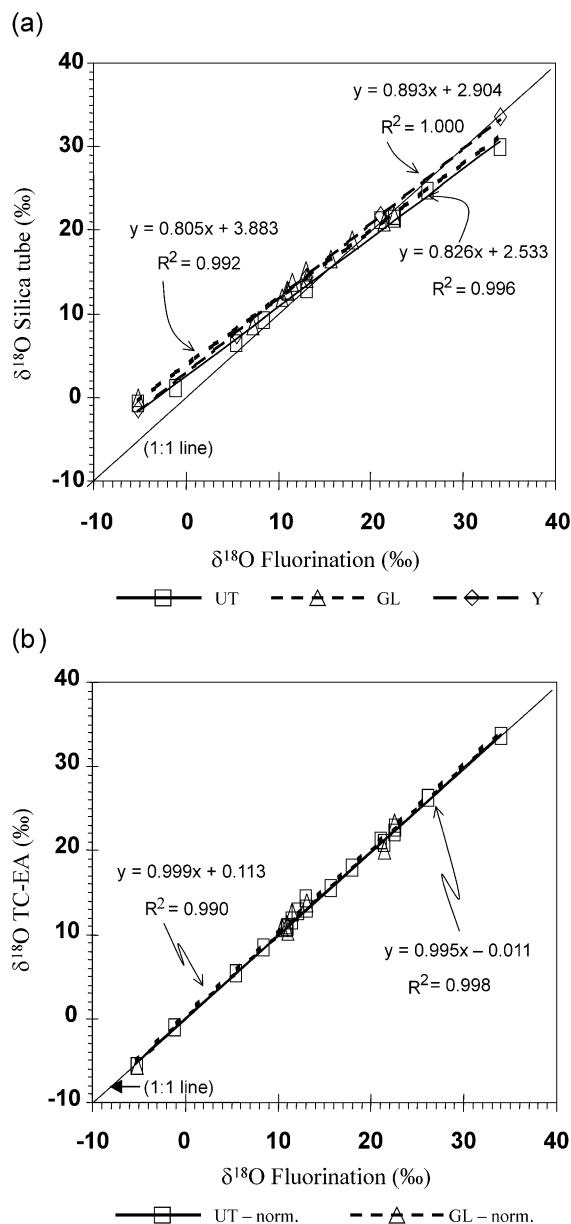


Fig. 1. (a) Comparison of $\delta^{18}\text{O}$ values obtained from reaction of Ag_3PO_4 with graphite in sealed silica tubes to those obtained by conventional fluorination. (b) Comparison of $\delta^{18}\text{O}$ values obtained from reaction of Ag_3PO_4 with graphite in a continuous flow TC-EA to those obtained by conventional fluorination.

Three samples each of TU-1, TU-2 and GW-1 were heated in a vacuum at 500°C , in order to test for any effects of heating on the isotopic composition of the

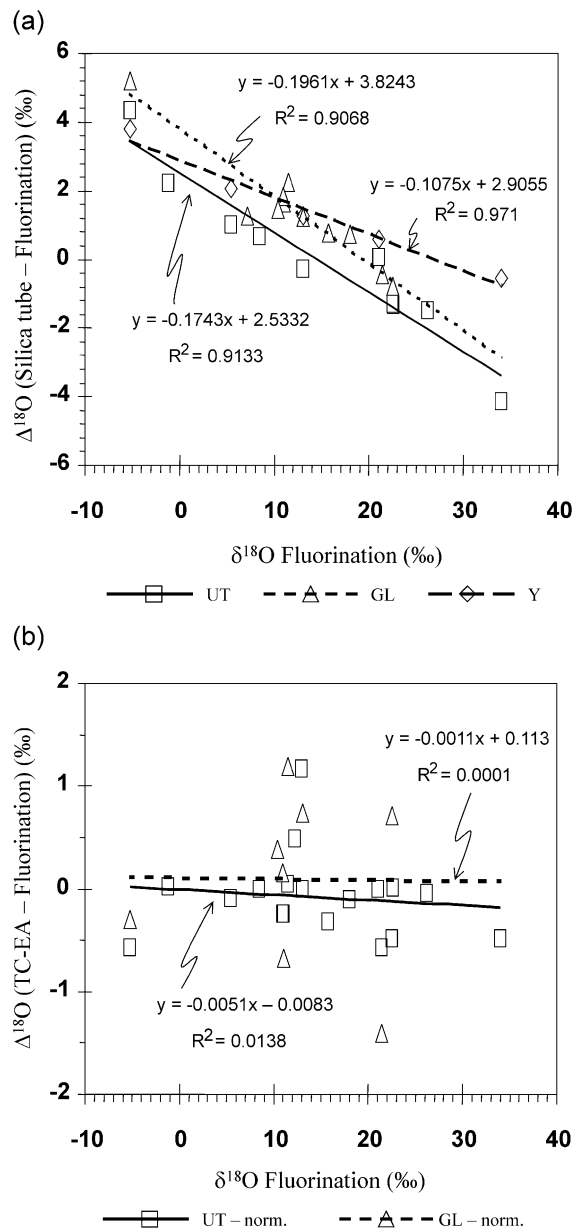


Fig. 2. (a) Plot of the differences in the $\delta^{18}\text{O}$ values between the silica tube (ST) and the fluorination method [$\Delta^{18}\text{O}$ (silica tube—fluorination)] as a function of the $\delta^{18}\text{O}$ value obtained by fluorination. Note the significant scale compression of the ST method. (b) Plot of the differences in the $\delta^{18}\text{O}$ values between the high-temperature reduction (HTR) and fluorination method [$\Delta^{18}\text{O}$ (TC-EA—fluorination)] as a function of the $\delta^{18}\text{O}$ value obtained by fluorination. Note that there is no significant scale compression for the HTR method.

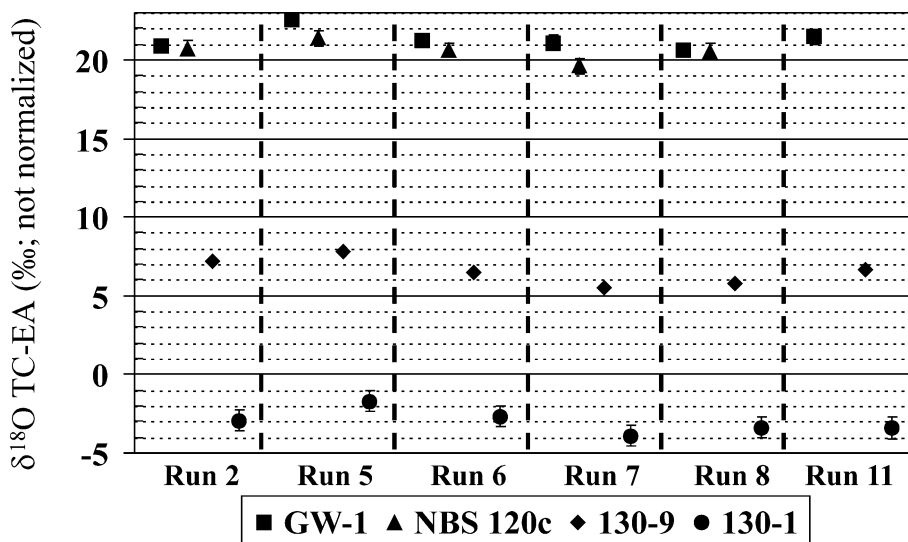


Fig. 3. Variation in the uncorrected $\delta^{18}\text{O}$ values obtained from six different analytical runs over the course of 1 year for four samples.

Ag_3PO_4 . Heating in vacuo had no effect on the analyses of TU-1 and TU-2 which are samples of Ag_3PO_4 precipitated from solutions of KH_2PO_4 . These analyses were included in the average value given in Table 2. The same is true for GW-1, a sample of Ag_3PO_4 prepared from phosphate in Recent shark teeth.

3.2. Reaction with graphite in sealed silica tubes

Measurements using the ST method at UT and Y were all performed over the course of 2 months and over the course of 2 years at GL. Previous measurements of different preparations of Ag_3PO_4 from GW-1, NBS 120c and SM2-9 using a different batch of silica tubing had $\delta^{18}\text{O}$ values that were about 0.6‰ higher than those given in Table 2.

In Table 3, the raw oxygen isotope compositions of the same samples of Ag_3PO_4 measured using the ST method in three different laboratories (UT, GL and Y) are compared. In Fig. 1a, the results of these methods are compared to the oxygen isotope composition of the same Ag_3PO_4 measured using the FL method.

3.3. High-temperature reduction

All $\delta^{18}\text{O}$ values of the HTR method presented in Table 2 for TU and Table 4 for TU and GL are averages of a number of analyses made of different preparations

of Ag_3PO_4 over the course of about a year. Detailed analyses with average values for each run are given in Table 5. For this method, the raw $\delta^{18}\text{O}$ values of the samples were determined assuming an oxygen isotope composition of the reference gas CO of about 10.5‰. The normalized UT values are calibrated against GW-1, 130-9 and 130-1, which were analyzed with each run. A similar approach has been taken for the samples analyzed at GL (Table 4), where the raw values are reported relative to a CO reference gas of about -190 ‰, while normalized GL values are calibrated against a variety of standards which were analyzed with each run. $\delta^{18}\text{O}$ values obtained by fluorination for all these standards at TU were used for the normalization.

4. Discussion

4.1. Fluorination

The good reproducibility of $\delta^{18}\text{O}$ values of different preparations of Ag_3PO_4 in the same or even in different laboratories analyzed over the course of 1 1/2 years, indicates that preparation of Ag_3PO_4 from apatite or other phosphate source using the techniques described above provides accurate oxygen isotope compositions of the phosphate. However, the $\delta^{18}\text{O}$ values measured for NBS 120c, averaging 22.6‰

Table 4

Comparison of oxygen isotope analyses of Ag_3PO_4 obtained using the HTR method at the University of Tübingen (UT) and the Geophysical Laboratory (GL)

Sample	UT			GL		
	$\delta^{18}\text{O}$ norm (‰)	1σ	n	$\delta^{18}\text{O}$ norm (‰)	1σ	n
GW-1	22.66	0.14	52			
NBS120c	22.09	0.51	18	23.29	0.47	8
TU-1	21.11	0.57	22			
TU-2	5.35	0.62	14			
130-9	8.42	0.29	35			
130-1	-1.13	0.17	32			
SM2-9	26.13	0.25	12			
YR-1	-5.77	0.33	18	-5.49	0.42	11
YR-2	13.05	0.31	19	13.8	0.78	4
YR-3.1	33.54	0.58	6			
KH_2PO_4	12.70	0.36	17			
Kodak	17.94	0.46	9			
Hoering 4-18	11.57	0.08	3	12.72	0.22	7
BAP 5-2	10.83	0.10	3	10.40	0.34	11
Mod Ele	15.39	0.29	3			
Ambo Ele	20.87	0.27	3	20.03	0.14	7
ID Std	10.73	0.18	3	11.14	0.18	7
ELE-6	–	–	–			
HVAP	–	–	–	10.81	0.19	2
SP Std	17.56	0.22	3			
JR Turt	14.19	0.54	3			
Mean of 1σ		0.33				

(Table 2), are about 1.3–0.9‰ higher than those previously reported for Ag_3PO_4 prepared from NBS 120c using conventional fluorination with BrF_5 reagent (Crowson et al., 1991; Lécuyer et al., 1993, 1996). As reaction temperature, time and the gas yields are similar to those of Crowson et al. and Lécuyer et al., and the $\delta^{18}\text{O}$ values for NBS-28 quartz are also identical within the analytical errors cited to those measured here, the question arises whether these differences in $\delta^{18}\text{O}$ for NBS 120c are related to differences in the chemical treatment used during isolation of the phosphate radical and subsequent precipitation as Ag_3PO_4 . Both Crowson et al. (1991) and Lécuyer et al. (1993, 1996) used an organic ion exchange resin (Amberlite) to isolate the phosphate ion prior to precipitation as Ag_3PO_4 . Crowson et al. (1991) also found that heating the Ag_3PO_4 prior to oxygen isotope analyses by fluorination is required for good reproducibility. For samples TU-1, TU-2 and

GW-1, no differences in $\delta^{18}\text{O}$ values were observed for samples heated and those not heated to 500 °C in a vacuum. This observation is in contrast to those of Crowson et al. (1991). It was suggested by these authors that the heating step removes or stabilizes the organic material that may be caught up in the Ag_3PO_4 during crystallization. The fact that we did not observe a change in gas yield or $\delta^{18}\text{O}$ value between heated and unheated samples suggests that at least part of the organic contaminants may have been derived from the ion-exchange resin used by Crowson et al. (1991), and that our cleaning procedure for natural phosphate samples effectively eliminates the organic matter.

4.2. Variability using graphite reduction

The total range of $\delta^{18}\text{O}$ values obtained by ST methods is less than that obtained by fluorination of the same samples of Ag_3PO_4 (Figs. 1a and 2a). Linear regression of the $\delta^{18}\text{O}$ values obtained by fluorination (at UT only) and those for the ST methods employed at UT, GL and Y yield slightly different slopes and distinctly different intercepts (Table 3; Fig. 2a):

$$\text{UT: } \delta^{18}\text{O}(\text{ST}) = 0.826(\pm 0.019) \times \delta^{18}\text{O}(\text{FL}) + 2.533(\pm 0.361),$$

$$\text{GL: } \delta^{18}\text{O}(\text{ST}) = 0.805(\pm 0.022) \times \delta^{18}\text{O}(\text{FL}) + 3.884(\pm 0.319),$$

$$\text{Y: } \delta^{18}\text{O}(\text{ST}) = 0.893(\pm 0.011) \times \delta^{18}\text{O}(\text{FL}) + 2.904(\pm 0.207).$$

This observation indicates a $\delta^{18}\text{O}$ scale compression that is likely to be related to exchange between the CO_2 produced and the glass and/or, more probably, the residual Ag–P–O compounds. Such exchange processes explain the shift in $\delta^{18}\text{O}$ values observed for the three Ag_3PO_4 preparations of GW-1, NBS 120c and SM2-9 reacted in different batches of silica glass at UT (Table 2). While the possibility of such isotopic exchange was noted by O’Neil et al. (1994), the magnitude of this scale compression is likely to change as a function of the glass tubing used, the size and surface area of the tubing, and the amount of Ag_3PO_4 and

Table 5

Run summaries for the TC-EA analyses obtained at the University of Tübingen (UT; Ag-cups, except Run 8 in Sn-cups) and the Geophysical Laboratory (GL; Ag-cups)

Sample	UT					GL					
	$\delta^{18}\text{O}$ (‰) raw	1σ	n	$\delta^{18}\text{O}$ (‰) norm.	Run	$\delta^{18}\text{O}$ (‰) raw	1σ	n	$\delta^{18}\text{O}$ (‰) norm.	Run	
GW-1	20.41	0.42	7	22.43	1						
	20.70	0.32	8	22.72	1						
	20.49	0.21	6	22.51	1						
	20.87	0.07	4	22.54	2						
	22.55	0.27	4	22.76	5						
	21.20	0.37	4	22.77	6						
	21.10	0.38	2	22.84	7						
	20.58	0.45	3	22.81	8						
	20.38	0.31	5	22.46	9						
	20.39	0.00	2	22.73	10						
	21.46	0.30	7	22.70	11						
NBS 120c	20.74	0.24	3	22.41	2	30.5	0.55	5	22.93	A	
	21.37	0.46	5	21.60	5	30.6	0.34	3	23.9	B	
	20.62	0.21	4	22.20	6						
	19.61	0.41	3	21.43	7						
TU-1	20.56	0.17	3	22.80	8						
	20.33	0.14	3	22.00	3						
	20.02	0.17	4	21.69	4						
	20.25	0.11	5	20.50	5						
	19.51	0.74	4	21.10	6						
TU-2	18.58	0.35	3	20.45	7						
	18.69	0.38	3	20.94	8						
	4.39	0.38	5	4.94	5						
	3.42	0.17	3	5.16	6						
130-9	2.20	0.36	3	4.90	7						
	4.03	0.32	3	6.42	8						
	6.28	0.25	5	8.55	1						
	7.16	0.23	4	8.79	2						
	7.78	0.49	5	8.26	5						
	6.51	0.05	4	8.22	6						
	5.49	0.13	3	8.03	7						
	5.75	0.34	3	8.12	8						
130-1	6.81	0.39	5	8.96	9						
	6.52	0.13	3	8.34	10						
	6.68	0.36	3	8.51	11						
	−3.66	0.28	5	−1.21	1						
	−2.93	0.20	4	−1.35	2						
	−1.70	0.67	4	−1.04	5						
	−2.67	0.16	3	−1.01	6						
SM2-9	−3.90	0.16	3	−0.89	7						
	−3.40	0.47	3	−0.95	8						
	−3.64	0.27	5	−1.42	9						
	−2.57	0.18	3	−1.08	10						
	−3.41	0.51	2	−1.18	11						
	23.81	0.09	4	25.78	1						
	24.31	0.37	4	26.26	1						
	24.29	0.37	4	26.35	9						
	YR-1	−7.69	0.28	6	−6.12	2	3.99	0.38	6	−5.47	A

(continued on next page)

Table 5 (continued)

Sample	UT					GL				
	$\delta^{18}\text{O}$ (‰) raw	1σ	n	$\delta^{18}\text{O}$ (‰) norm.	Run	$\delta^{18}\text{O}$ (‰) raw	1σ	n	$\delta^{18}\text{O}$ (‰) norm.	Run
YR-2	-6.82	0.10	4	-5.24	4	2.40	0.46	5	-5.52	B
	-6.54	0.79	5	-5.79	5					
	-7.77	0.60	3	-5.93	6					
	11.17	0.35	6	12.81	2	20.92	0.78	4	13.8	B
	11.36	0.18	4	13.00	4					
	12.43	0.63	5	12.83	5					
KH ₂ PO ₄	11.92	0.45	4	13.58	6					
	10.99	0.31	4	12.40	4					
	11.10	0.28	3	12.77	6					
	10.96	0.06	2	12.63	6					
Kodak	10.01	0.35	3	12.34	8					
	11.23	0.35	5	13.35	9					
	15.82	0.16	6	17.48	2					
Hoering 4	16.75	0.25	3	18.41	3					
	9.93	0.08	3	11.57	2	20.81	0.22	5	12.55	A
BAP 5-2						20.28	0.23	2	13.13	B
	9.20	0.10	3	10.83	3	19.04	0.30	4	10.65	A
						17.51	0.40	7	10.25	B
Mod Ele	13.74	0.29	3	15.39	3					
SP Std 5-16	15.90	0.22	4	17.56	3					
JR Turts	12.55	0.53	4	14.19	4	19.19	0.19	2	10.81	A
Ambo Ele	19.21	0.27	4	20.87	4	28.18	0.07	3	20.44	A
						26.60	0.19	4	19.73	B
ID Std	9.10	0.18	4	10.73	4	19.47	0.20	3	11.11	A
						18.39	0.17	4	11.17	B

graphite used. Thus, while the results of the ST method may be as precise as those of the FL technique (Table 2), the accuracy may vary in different laboratories, requiring individual calibration for the silica tubing used and the amount of Ag₃PO₄. This method must be calibrated separately in each laboratory, requiring Ag₃PO₄ standards of known isotopic composition, preferably with a large range of $\delta^{18}\text{O}$ values.

4.3. Variability using HTR

A comparison of normalized $\delta^{18}\text{O}$ values of the HTR method to those obtained on the same Ag₃PO₄ sample by fluorination (Fig. 1b), shows that the two data sets are linearly correlated with a slope close to 1 and insignificant $\delta^{18}\text{O}$ scale compression (Figs. 1b and 2b):

$$\text{UT: } \delta^{18}\text{O}(\text{HTR}) = 0.995(\pm 0.011) \times \delta^{18}\text{O}(\text{FL}) - 0.007(\pm 0.184),$$

$$\text{GL: } \delta^{18}\text{O}(\text{HTR}) = 0.999(\pm 0.042) \times \delta^{18}\text{O}(\text{FL}) + 0.113(\pm 0.590).$$

This is also true for the uncorrected data for which the following regression can be obtained:

$$\text{UT: } \delta^{18}\text{O}(\text{HTR}) = 1.006(\pm 0.011) \times \delta^{18}\text{O}(\text{FL}) - 1.802(\pm 0.182).$$

However, the average standard deviation about the uncorrected mean $\delta^{18}\text{O}$ values are higher than those for $\delta^{18}\text{O}$ values that were normalized for each run (Table 2). This difference in standard deviation indicates that much of the variation in uncorrected $\delta^{18}\text{O}$ values measured for a given sample is related to slight variations in operating conditions.

This effect is illustrated in Fig. 3, where uncorrected $\delta^{18}\text{O}$ values for four samples from six separate runs are observed to increase or decrease in

parallel. However, closer inspection of Fig. 3 reveals short term drifts that are more difficult to explain. For example, for runs 2 and 8, no significant difference in $\delta^{18}\text{O}$ values is observed between GW-1 and NBS 120c (Fig. 3 and Table 4), that is, both are the same within analytical error. In contrast, for runs 5 and 7, the difference between average $\delta^{18}\text{O}$ values of GW-1 and NBS 120c is 1.2‰ and 1.5‰, respectively. Several factors that may account for these differences include memory effects, sample heterogeneity and/or short term fluctuations in operating conditions (reaction temperature, variability in the reference gas, sample position for reaction). Memory effects for these two samples can be eliminated, however, as NBS 120c was always analyzed directly after GW-1. Furthermore, even in cases where samples with widely different $\delta^{18}\text{O}$ values (such as 130-9 and 130-1) were analyzed in sequence, memory effects were not observed for reaction temperatures of 1450 °C and sequences of 50–100 samples. If the reactor is not cleaned after about 100 samples, CO yields decrease, peaks widen and slight memory effects may become apparent such that the first sample of a set may be influenced by previous samples. Variation in $\delta^{18}\text{O}$ values of different splits of a single precipitate has been noted before, particularly for precipitates that comprise different grain sizes of Ag_3PO_4 (e.g., O'Neil et al., 1994). While internal reproducibility for GW-1 and NBS 120c in runs 5 and 7 is somewhat worse than that in other runs, the $\delta^{18}\text{O}$ values obtained are, nonetheless, significantly different. Hence, sample inhomogeneity can not account for this difference. This leaves short term fluctuations in the operating conditions, perhaps in combination with some variation due to sample heterogeneity, as the most likely source of such variations.

Fortunately the impact of fluctuating operating conditions on measured $\delta^{18}\text{O}$ values can be easily corrected for by running a suite of standard Ag_3PO_4 materials with known oxygen isotope composition. For each run, uncorrected HTR values of these standards are regressed with known values determined by fluorination. The result is a correction equation that can then be applied to the raw data (e.g., Fig. 3). The corrected $\delta^{18}\text{O}$ values are as accurate, but slightly less precise, than those determined using fluorination (Tables 2 and 4).

4.4. Comparison of FL, ST and HTR methods

Of the three methods compared in this study, the fluorination method is the method of choice for both accurate and precise measurements of the oxygen isotope composition of Ag_3PO_4 without correcting data. Furthermore, it produces oxygen in 100% yield and allows a direct comparison to be made between $\delta^{18}\text{O}$ values of phosphates and those of silicates and carbonates. However, it requires the use of dangerous chemicals, a complex extraction line and fairly large samples, usually about 4–5 mg or more of Ag_3PO_4 , depending on the construction of the extraction line.

The ST method is simple, inexpensive, rapid and has an accuracy similar to that for fluorination. However, it also requires fairly large samples of Ag_3PO_4 (a minimum of about 8 mg), gives reproducible but incomplete oxygen yields and must be calibrated for the amounts and type of graphite and the type and size of silica tubing using well calibrated standards of Ag_3PO_4 . Such standards are best calibrated using the fluorination method.

The HTR method is similar to the fluorination method in that it provides 100% oxygen extraction. It is fast and simple to use, but its main advantage is that only small amounts of sample are required for analysis (2 mg of Ag_3PO_4 will suffice for three to four single measurements). To achieve a precision and accuracy of 0.3‰ or better, however, analyses must be made along with analyses of Ag_3PO_4 reference standards, as is true for the ST method.

4.5. Comparison to other techniques

Laser-based analysis of phosphate have been made by reacting BrF_5 or F_2 with portions of tooth enamel or bone heated directly with a CO_2 laser (Kohn et al., 1996), by direct heating enamel with a CO_2 laser to produce CO_2 in a helium-stream with isotopic analysis by continuous flow mass spectrometry (Sharp and Cerling, 1996), and by ablating samples with a UV laser to produce a plasma in the presence of F_2 (Jones et al., 1999). All these methods have the advantage of providing rapid analyses, spatial resolutions of about 100 μm and a precision of about $\pm 0.4\text{‰}$. However, they suffer from inconsistent, variable offsets in $\delta^{18}\text{O}$ values, possibly as a result of variable amounts of carbonate and hydroxyl in the biogenic phosphate. In

an attempt to eliminate such offsets, Wenzel et al. (2000) opted to react small amounts of Ag_3PO_4 with graphite using a CO_2 laser. These authors also noted an offset from the expected $\delta^{18}\text{O}$ values, in this case perhaps related to the production of both CO and CO_2 . However, the offset was constant for a range of $\delta^{18}\text{O}$ values between 2‰ and 22‰ and, thus, a reliable correction to the data can be made. In principle, this method is similar to the HTR method described above.

All laser-based methods must ultimately be calibrated using phosphates of known isotopic composition. The accuracy of analyses made directly on natural carbonate apatite will always be subject to the uncertainty that arises from variable or inconsistent contents of carbonate and hydroxyl groups. Owing to the complex nature and chemical variability of natural biogenic phosphates, chemical isolation of the PO_4^{3-} ion is the only way to avoid this uncertainty. It is possible to fluorinate Ag_3PO_4 using a laser-based extraction system. Initial results at the University of Tübingen, however, indicate that Ag_3PO_4 as well as biogenic phosphate from bones (i.e. dentine) can partially react with the reagent gas (F_2) while other samples in the same reaction chamber are being reacted. Partial reaction does not occur with pure fluorapatite (e.g., the enameloid of shark teeth), for which good agreement in absolute $\delta^{18}\text{O}$ values has been obtained with conventional fluorination. Young (1993) and Jones et al. (1999) reported, however, that at least some fluorapatite may not react completely.

Two published methods of analysis of Ag_3PO_4 were not included in this comparison study but are discussed here for the sake of completeness. Stuart-Williams and Schwarcz (1995) reacted Ag_3PO_4 with Br_2 to produce O_2 for oxygen isotope analysis. While this method is somewhat more convenient to use than conventional fluorination and produces results of similar precision, it requires the use and disposal of hazardous chemicals, construction of a fairly complex furnace and extraction line specifically for phosphates, and a relatively large amount of sample (about 20 mg). In addition, not all oxygen is released during the reaction and, hence, isotopic fractionation may occur between residue and gas produced, necessitating a strict control on the reaction temperature.

A method employing negative thermal ion mass spectrometry was developed by Holmden et al. (1997). This method is promising for the analysis of relatively small samples, but currently has a low precision of about $\pm 1\%$. While samples as small as <21 nmol of equivalent CO_2 gas can be analysed by the mass spectrometer, the phosphate must still be isolated from the natural sample and converted to Ag_3PO_4 , which may limit sample size to about 1 mg because of sample handling constraints.

4.6. Need for standards

The discussion of ST and HTR methods makes it clear that Ag_3PO_4 standards are needed in order to produce accurate oxygen isotope data that can be compared rigorously among laboratories. Ideally, these standards should cover a wide range in $\delta^{18}\text{O}$ values. Ag_3PO_4 standards can be prepared directly from solutions of commercial, reagent grade K_2HPO_4 . The standards TU-1 and TU-2 and YR-1, YR-2 and YR-3, prepared at the University of Tübingen and Yale University, respectively, are of this type, and aliquots of these materials can be requested from T.W.V. or R.E.B.

The use of Ag_3PO_4 standards for calibration purposes does not address variation that may be introduced during the extraction and isolation of PO_4^{3-} from natural materials. For example, NBS 120c has commonly been used as an interlaboratory standard. However, NBS 120c is a sedimentary phosphate with significant concentrations of hydroxyl and carbonate ions as well as organic and silicate matter, all of which may interfere differently depending on extraction methods (e.g., digestion in HF or HNO_3 , ion-exchange resin to isolate the phosphate ion versus pipetted removal of dissolved phosphate from CaF_2 residues). The result could be isotopic offsets between laboratories.

To provide a detailed calibration of the extraction methods used for isolating the phosphate ion it will be necessary to provide natural apatite standards with known oxygen isotope composition. It is very important that these apatites be the same type of material that is commonly analyzed. For example, tooth enamel apatite provides an ideal standard for the case where biogenic apatite samples are the focus of study, whereas sedimentary phosphate does not.

5. Conclusions

A number of techniques are available for oxygen isotope analysis of phosphates, many of which are ideally suited for specific needs and capabilities of individual laboratories or research groups. For most methods, the nature of the biogenic phosphate will require chemical isolation of the PO_4^{3-} ion in order to avoid interference from nonphosphatic oxygen. Both carbonate and hydroxyl groups in apatite have isotopic compositions that are vastly different from that of the phosphate. The preferred method for isolating phosphate is precipitation as Ag_3PO_4 because this compound is not hygroscopic and is ideally suited to oxygen isotope analysis. During isolation of phosphate, it is important to eliminate any organic matter present in the apatite. Results of this study suggest that this is best achieved by pretreatment of finely powdered biogenic phosphate with NaOCl followed by NaOH .

Of the presently available analytical techniques, conventional fluorination is the method of choice for precise and accurate measurements of the oxygen isotope composition of Ag_3PO_4 . It is ideally suited for the calibration of standards, allowing direct comparisons to be made to values of international oxygen isotope reference standards like the NBS-28 quartz standard. Other techniques suitable for measurements of the oxygen isotope composition of biogenic phosphate include methods based on the partial or complete reduction of the Ag_3PO_4 using graphite. Of these, the method of O'Neil et al. (1994) is simple and relatively easy to use, and has a precision similar to that achieved by conventional fluorination. Other graphite reduction techniques, such as the new TC-EA methods (e.g., Kornexl et al., 1999) and the laser-based graphite reduction techniques (Wenzel et al., 2000), are fast and require little sample (1–2 mg) and are also fairly precise (presently about $\pm 0.3\text{‰}$). For comparison, an average analytical error of 0.1‰ for the $\delta^{18}\text{O}$ value of phosphate will lead to an error in calculated temperature of $\pm 0.4\text{ °C}$, on the basis of the phosphate–water fractionation of Longinelli and Nuti (1973a) and excluding errors inherent in the temperature calibration and the uncertainty in the $\delta^{18}\text{O}$ value of water. An error of 0.3‰ , in contrast, will lead to an error in the calculated temperature of $\pm 1.3\text{ °C}$.

Techniques based on direct extraction of oxygen from biogenic phosphate, such as the new laser-based extraction methods (e.g., Kohn et al., 1996; Sharp and Cerling, 1996; Jones et al., 1999), are fast and relatively precise, but may produce offsets in $\delta^{18}\text{O}$ values that are critically dependant on the chemical composition of the biogenic phosphate. They may, therefore, be of more limited use.

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References

- Ayliffe, L.K., Lister, A.M., Chivas, A.R., 1992. The preservation of glacial–interglacial climatic signatures in the oxygen isotopes of elephant skeletal phosphate. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 99, 179–191.
- Barrick, R.E., Showers, W.J., 1994. Thermophysiology of *Tyrannosaurus rex*: evidence from oxygen isotopes. *Science* 265, 222–224.
- Blake, R.E., O'Neil, J.R., Garcia, G.A., 1997. Oxygen isotope systematics of biologically mediated reactions of phosphate: I. Microbial degradation of organophosphorus compounds. *Geochim. Cosmochim. Acta* 61, 4411–4422.
- Bryant, J.D., Luz, B., Frölich, P.N., 1994. Oxygen isotopic composition of fossil horse tooth phosphate as a record of continental paleoclimate. In: McFadden, B., Bryant, J.D. (Eds.), *Interpreting Ancient Diets and Climates: The Geochemical Records of Fossil Vertebrates*. *Palaeogeogr., Palaeoclimatol., Palaeoecol.*, 107, 303–316.
- Cerling, T.E., Sharp, Z.D., 1996. Stable carbon and oxygen isotope analysis of fossil tooth enamel using laser ablation. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 126, 173–186.
- Clayton, R.N., Mayeda, T.K., 1963. The use of bromine pentafluoride in the extraction of oxygen from oxides and silicates for isotopic analysis. *Geochim. Cosmochim. Acta* 27, 43–52.
- Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* 12, 133–149.
- Crowson, R.A., Showers, W.J., Wright, E.K., Hoering, T.C., 1991. Preparation of phosphate samples for oxygen isotope analysis. *Anal. Chem.* 63, 2397–2400.
- Firsching, F.H., 1961. Precipitation of silver phosphate from homogeneous solution. *Anal. Chem.* 33, 873–874.

- Fricke, H.C., O'Neil, J.R., 1996. Inter- and intra-tooth variation in the oxygen isotope composition of mammalian tooth enamel phosphate: implications for palaeoclimatological and palaeobiological research. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 126, 91–99.
- Fricke, H.C., Rogers, R.R., 2000. Multiple taxon-multiple locality approach to providing oxygen isotope evidence for warm-blooded theropod dinosaurs. *Geology* 28, 799–802.
- Fricke, H.C., Clyde, W.C., O'Neil, J.R., Gingerich, P.D., 1998. Evidence for rapid climate change in North America during the latest Paleocene thermal maximum: oxygen isotope compositions of biogenic phosphate from the Bighorn Basin (Wyoming). *Earth Planet. Sci. Lett.* 160, 193–208.
- Holmden, C., Papanastassiou, D.A., Wasserburg, G.J., 1997. Negative thermal ion mass spectrometry of oxygen in phosphates. *Geochim. Cosmochim. Acta* 61, 2253–2263.
- Jones, A.M., Iacumin, P., Young, E.D., 1999. High-resolution $\delta^{18}\text{O}$ analysis of tooth enamel phosphate by isotope ratio monitoring gas chromatography mass spectrometry and ultraviolet laser fluorination. *Chem. Geol.* 153, 241–248.
- Kohn, M.J., Schoeninger, M.J., Valley, J.W., 1996. Herbivore tooth oxygen isotope compositions: effects of diet and physiology. *Geochim. Cosmochim. Acta* 60, 3889–3896.
- Kolodny, Y., Luz, B., Navon, O., 1983. Oxygen isotope variations in phosphate of biogenic apatites: I. Fish bone apatite—re-checking the rules of the game. *Earth Planet. Sci. Lett.* 64, 398–404.
- Kornel, B.E., Gehre, M., Höfling, R., Werner, R.A., 1999. On-line $\delta^{18}\text{O}$ measurement of organic and inorganic substances. *Rapid Commun. Mass Spectrom.* 13, 1685–1693.
- Koziet, J., 1997. Isotope ratio mass spectrometric method for the on-line determination of oxygen-18 in organic matter. *J. Mass Spectrom.* 32, 103–108.
- Lécuyer, C., Grandjean, P., O'Neil, J.R., Capetta, H., Martineau, F., 1993. Thermal excursions in the ocean at the Cretaceous–Tertiary boundary (northern Morocco): $\delta^{18}\text{O}$ record of phosphatic fish debris. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 105, 235–243.
- Lécuyer, C., Grandjean, P., Emig, C.C., 1996. Determination of oxygen isotope fractionation between water and phosphate from living linguids: potential application to palaeoenvironmental studies. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 126, 101–108.
- Longinelli, A., 1965. Oxygen isotopic composition of orthophosphate from shells of living marine organisms. *Nature* 207, 716–718.
- Longinelli, A., 1966. Ratios of oxygen-18: oxygen-16 in phosphate and carbonate from living and fossil marine organisms. *Nature* 211, 923–926.
- Longinelli, A., 1984. Oxygen isotopes in mammal bone phosphate: a new tool for paleohydrological and paleoclimatological research? *Geochim. Cosmochim. Acta* 48, 385–390.
- Longinelli, A., Nuti, S., 1968. Oxygen isotopic composition of phosphorites from marine formations. *Earth Planet. Sci. Lett.* 5, 13–17.
- Longinelli, A., Nuti, S., 1973a. Revised phosphate–water isotopic temperature scale. *Earth Planet. Sci. Lett.* 19, 373–376.
- Longinelli, A., Nuti, S., 1973b. Oxygen isotope measurements of phosphate from fish teeth and bones. *Earth Planet. Sci. Lett.* 20, 337–340.
- O'Neil, J.R., Roe, L.J., Reinhard, E., Blake, R.E., 1994. A rapid and precise method of oxygen isotope analysis of biogenic phosphate. *Isr. J. Earth Sci.* 43, 203–212.
- Sanrock, J., Studley, S.A., Hayes, J.M., 1985. Isotopic analyses based on the mass spectrum of carbon dioxide. *Anal. Chem.* 57, 1444–1448.
- Sharp, Z.D., Cerling, T.E., 1996. A laser GC-IRMS method for in-situ carbon and oxygen isotope analysis of carbonates and phosphates. *Geochim. Cosmochim. Acta* 60, 2909–2916.
- Stephan, E., 2000. Oxygen isotope analysis of animal bone phosphate: method refinement, influence of consolidants, and reconstruction of paleotemperatures for Holocene sites. *J. Arch. Sci.* 27, 523–535.
- Stuart-Williams, H.L.Q., Schwarcz, H.P., 1995. Oxygen isotopic analysis of silver orthophosphate using a reaction with bromine. *Geochim. Cosmochim. Acta* 59, 3837–3841.
- Tudge, A.P., 1960. A method of analysis of oxygen isotopes in orthophosphate and its use in measurements of paleotemperatures. *Geochim. Cosmochim. Acta* 18, 81–93.
- Young, E.D., 1993. Oxygen isotope analysis of phosphate in biogenic and non-biogenic apatite by laser-heating in F_2 gas: a spectroscopic study of the fluorination process. *Geol. Soc. Am., Abstr. Program* 25, A205.
- Vennemann, T.W., Hegner, E., 1998. Oxygen, strontium, and neodymium isotope composition of fossil shark teeth as a proxy for the palaeoceanography and palaeoclimatology of the Miocene northern Alpine Paratethys. *Palaeogeogr., Palaeoclimatol., Palaeoecol.* 142, 107–121.
- Vennemann, T.W., Hegner, E., Cliff, G., Benz, G.W., 2001. Isotopic composition of recent shark teeth as a proxy for environmental conditions. *Geochim. Cosmochim. Acta* 65, 1583–1599.
- Wenzel, B., Lécuyer, C., Joachimski, M.M., 2000. Comparing oxygen isotope records of Silurian calcite and phosphate- $\delta^{18}\text{O}$ compositions of brachiopods and conodonts. *Geochim. Cosmochim. Acta* 64, 1859–1872.