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High-temperature carbon reduction of silica: A novel approach for oxygen isotope analysis of biogenic opal

Andreas Lücke,[1] Robert Moschen,[1,2] and Gerhard H. Schleser[1,*]

[1]Institute of Chemistry and Dynamics of the Geosphere V: Sedimentary Systems, Forschungszentrum Jülich, D-52425 Jülich, Germany
[2]Geographical Institute, University of Cologne, D-50674 Cologne, Germany

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Abstract—A new technique has been developed for the determination of the oxygen isotope composition from biogenic silica. The iHTR method (inductive high temperature carbon reduction) is based on the reduction of silica by carbon with temperatures of up to 1830°C (maximum T 2200°C) to produce carbon monoxide for isotope analysis. Basically, samples of silica are mixed with graphite and filled into a sample holder made of a glassy carbon rod. The rod is introduced into a glassy carbon cylinder liner closed at the top which itself is enclosed by a double-walled glass vessel. The glassy carbon rod is inductively heated under vacuum to the temperature needed for quantitative conversion of the particular silica material to CO. The most critical process of dehydration (in the case of opal) and reduction to CO is routinely achieved in the iHTR device. Weakly bound oxygen and oxygen-containing contaminants, like hydroxyl groups, as well as remaining minor organic constituents are volatilized stepwise under high vacuum at temperatures of 850°C and 1050°C without isotopic exchange before the reduction of the silica. After completion of dehydration, the temperature is raised to the value needed for silica reduction. For both biogenic silica and quartz a temperature of 1550°C was found to be adequate. The technical design with a standard preparation routine and various test experiments is presented proving the reliability and capability of the new iHTR method, especially with respect to fresh diatom materials and diatom opal. The amount of sample material necessary at present is ~1.5 mg of silica and the reproducibility achieved for natural samples is better than ±0.15‰. Replicate analysis of the quartz standard NBS28 resulted in a $\delta^{18}O$ value of 9.62‰ ± 0.11‰ (n = 17). *Copyright © 2005 Elsevier Ltd*

1. INTRODUCTION

Opal from biogenic sources is a constituent of a wide variety of deposits such as lacustrine sediments, marine sediments and soils (Stoermer and Smol, 1999; Webb and Longstaffe, 2000). For paleoclimatic investigations in terrestrial regions lacustrine sediments are of particular importance since they often provide continuous records allowing the formation of good chronologies and high time resolution, especially in the case of a varved microstructure. In many lakes, however, the most commonly used biogenic carbonate isotope paleothermometer is of no assistance due to a lack of calcareous microfossils. In these cases a suitable alternative might be based on the oxygen isotopes of biogenic opal derived from diatoms. These microalgae are almost ubiquitous in freshwater lakes, their siliceous frustules are of proven autochthonous origin and are often well preserved in sediments.

Since the early works of Mopper and Garlick (1971) and Labeyrie (1974) in the 1970s, and of Juillet-Leclerc and Labeyrie (1987) and Matheney and Knauth (1989) in the 1980s, the subject of biogenic silica oxygen isotope composition has attracted increasing interest. In more recent years the isotopic composition of oxygen from diatoms deposited in marine or lacustrine sediments has been used to infer paleotemperature conditions (e.g., Mikkelsen et al., 1978; Wang and Yeh, 1984; Shemesh et al., 1992; Shemesh et al., 1995; Rosqvist et al., 1999; Leng et al., 2001; Shemesh et al., 2001; Hu and Shemesh, 2003; Jones et al., 2004).

Although the potential for the use of oxygen isotopes from biogenic silica is very promising, an even broader application as a paleoclimatic proxy has been limited for two main reasons: Firstly, there is considerable uncertainty with respect to the temperature dependence of the oxygen isotope fractionation during the formation of biogenic silica in water (Juillet-Leclerc and Labeyrie, 1987; Shemesh et al., 1992; Clayton, 1992; Shemesh, 1992; Brandriss et al., 1998) and, secondly, substantial analytical problems are encountered with the preparation of the silica frustules (Mopper and Garlick, 1971; Labeyrie and Juillet, 1982; Haimson and Knauth, 1983; Wang and Yeh, 1985; Schmidt et al., 1997, 2001; Brandriss et al., 1998). The main problem is separating the contaminating and exchanging oxygen containing compounds or molecular groups attached to or absorbed by the biogenic silica. This is decisive, since biogenic silica may have a water content of up to 7%–12% (H_2O and OH-groups) (Knauth, 1973). The water trapped in pores of the opaline structure is partially exchangeable and different in oxygen isotope composition when compared to the silica oxygen.

In the past, different approaches were chosen to overcome this problem. One alternative consisted in a separate dehydration of the samples under vacuum at temperatures close to 1000°C to remove exchangeable hydroxyl groups and molecular water (Labeyrie, 1974; Wang and Yeh, 1984; Brandriss et al., 1998). Another approach is to use a stepwise fluorination to remove loosely bound oxygen in a series of steps (Thorleifson and Knauth, 1984; Matheney and Knauth, 1989). A third ap-

* Author to whom correspondence should be addressed (g.schleser@fz-juelich.de).

Table 1. Materials of different origin and type used for test and calibration purposes during investigation of the performance of the iHTR method.

Name	Material/Origin	Type
Carbon monoxide (CO)	Cylinder gas, purity grade 4.7 (Messer-Griesheim)	—
Opal-1 (laboratory standard Jülich)	Diatomaceous earth, technical grade, purified and annealed (Merck)	Biogenic opal
NBS28 (reference standard)	Quartz standard, National Bureau of Standards (USA); IAEA Vienna	Quartz sand
Ethmodiscus rex (reference material)	Sample from a marine sediment core[a] (Weddell Sea; age ~4 Ma)	Fossil diatom opal
Fragilaria crotonensis	Sample from a continuous diatom culture[b]	Fresh diatom opal
Cyclotella meneghiniana	Sample from a continuous diatom culture[b]	Fresh diatom opal

[a] Sample kindly provided by Dr. R. Botz, Institute for Geology and Palaeontology, University of Kiel.
[b] Experiments done at ICG-V: Sedimentary Systems (Research Centre Jülich) by K. Kowalzcyk and B. Kammer.

proach is to perform controlled isotope exchange (CIE) of oxygen to approach the assumed original oxygen isotope compositions of loosely bound oxygen (Labeyrie and Juillet, 1982; Juillet-Leclerc and Labeyrie, 1987).

These approaches are not simple and they are relatively time-consuming, and hence less suitable for routine measurements. Therefore, the aim was to develop a method which is better suited for broader use, especially with regard to paleotemperature investigations. Though the preparation of silicates and oxides with the carbon reduction method for oxygen isotope investigations is not new (e.g., Baertschi and Schwander, 1952; Schwander, 1953; Clayton and Epstein, 1958; Sharp and O'Neil, 1989), our new technical approach avoids a number of draw-backs which prevented the reduction method from becoming a routine method and gives special emphasis to the preparation of fresh diatom samples.

2. SAMPLES AND METHODS

2.1. Samples

For the experiments siliceous materials of inorganic (quartz) and biogenic (opal) origin were used together with a commercial cylinder gas carbon monoxide (Table 1). The Jülich laboratory standard called Opal-1 was purchased from Merck (diatomaceous earth, LAB). The standard reference material NBS28 was purchased from the IAEA, Vienna and the given oxygen isotope value of 9.6‰ ± 0.1‰ vs. V-SMOW was adopted. Material of the fossil *Ethmodiscus rex* was provided by Dr. Botz in the form of pure marine diatom opal. Two freshwater diatom species, *Fragilaria crotonensis* and *Cyclotella meneghiniana* were cultured under steady-state conditions in a chemostat using continuous light conditions. The $\delta^{18}O$ value of the growth medium was −7.6‰. For each species one litre of sample suspension containing ~400 mg of diatom matter was filtered through a 0.45 μm filter and subsequently freeze-dried. To remove organic matter from the diatom frustules, samples from laboratory diatom cultures were digested three times in hydrogen peroxide (15%) at 60°C for 8 h at a time. Treated samples were repeatedly washed with distilled water and again freeze-dried.

2.2. iHTR Device

Based on experience with the determination of the oxygen isotope composition of biogenic silica a new preparation system should unite the following features. (i) It should enable the removal of any attached or absorbed water and hydroxyl groups, and loosely bound oxygen, (ii) the dehydration and the quantitative silica decomposition should take place in a single chamber forming part of a continuous routine, and (iii) it should help to minimize the amount of sample required and also reduce the prevailing analytical uncertainties.

To meet these criteria inductive heating was chosen as the method to accomplish fast and stepwise heating of a reaction cell up to temperatures of more than 2000°C if necessary. Dehydration can thus be achieved at lower temperatures while the silica-bound oxygen remains unaffected (<1100°C). Once this process is completed the temperature can be further increased to quantitatively decompose the silica (~1500 to 1700°C). The oxygen will instantly react with graphite to form carbon monoxide according to the equation

$$SiO_2 + 3C \rightarrow SiC + 2CO. \quad (1)$$

It was decided to use CO as the analyte rather than to convert it to carbon dioxide. Carbon monoxide can nowadays be used routinely for oxygen isotope analysis in an isotope ratio mass spectrometer (IRMS), offering twice the sensitivity as compared to carbon dioxide. In addition, this increases the throughput of samples, since the conversion of carbon monoxide to carbon dioxide is avoided. Problems reported in earlier studies in which gaseous interferences strongly distorted the oxygen isotope composition of samples while using CO as the analyte were not observed (e. g. Adams, 1949–1950; Schwander, 1953). In principle, this set-up would also enable the preparation of silicate minerals and rocks, for which temperatures of up to 2200°C might be necessary to achieve complete decomposition.

The technical design of the inductive high temperature carbon reduction device (iHTR) developed for the preparation of biogenic silica samples is illustrated in Figure 1. It primarily consists of three units: In the thermal reaction module (1) dehydration is achieved under high vacuum and, subsequently, silica materials are reduced while the liberated oxygen is converted to CO. The gas distribution module (2) enables flushing of the reaction chamber with helium and directs the generated CO either to a sample vial for condensation or, not yet realized, directly into a mass spectrometer using helium as carrier gas. The seal cap module (3) enables the introduction of samples into the thermal reaction module and seals the system. Not shown is the vacuum system, which consists of a rotary pump coupled to a turbo molecular pump (200 L/min), and the configuration of valves and pressure gauges necessary to control the gas flows. It should be noted that a special cryo-trap with a micro vial can be developed and attached to the iHTR device to freeze the water generated during dehydration for subsequent oxygen isotope analysis parallel to that of silica.

Samples to be decomposed are introduced into the sample chamber at the top of a rod (sample holder) (see Fig. 1). A lid is placed on top of the sample chamber to prevent ejection of sample material during vacuum pumping or rapid thermal reaction. The rod is housed in a cylinder liner closed at the top, which itself is enclosed by a double-walled Vycor glass vessel connected to a cryostat by flexible tubes. Thus, heating of the vessel's inner glass surface during the reduction process is prevented. The glass vessel is connected to the gas distribution module by a vacuum tight quick-flange fitting to enable quick replacement of the glass vessel if necessary. The longer cylinder liner extends into the gas distribution module where it is mounted with a Viton gasket vacuum tight against the glass vessel. Thus the emerging closed space between the interior glass wall and the exterior of the cylinder liner is constantly kept under high vacuum. This design was chosen for two reasons: (1) contaminations of the glass surface during sample loading are completely avoided and, more importantly, (2) any contact of the generated CO with the glass vessel, which might lead to nonquantifiable oxygen isotope exchange reactions with the glass surface in the heating zone, is prevented. The generated CO can never-

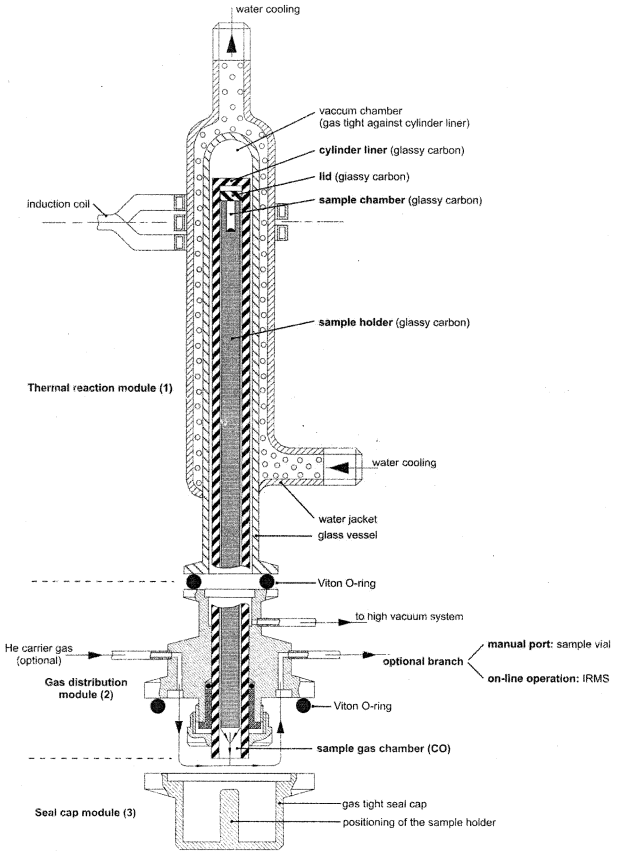

Fig. 1. Schematic view of the inductive high temperature reduction device (iHTR). It consists of three units: thermal reaction module (1), gas distribution module (2) and seal cap module (3) (dimensions are not to scale). Dehydration, decomposition of biogenic silica and generation of CO for oxygen isotope analysis takes place within the thermal reaction module. The dimensions of the various components are presently as follows: dimensions of the sample chamber: Ø = 3 mm, depth = 10 mm; volume of the sample chamber: ~71 μL, sample holder Ø external = 6.8 mm, total length = ~200 mm; cylinder liner: Ø external = 12 mm, Ø internal = 7 mm, total length = ~200 mm; free available sample gas volume: ~13 mL; double-walled quartz vessel: Ø internal = 16 mm, length = 150 mm.

theless easily expand towards the seal cap module where it is stored temporarily until the reaction is completed as the sample holder has vertical lane grooves directing the gas downwards. After complete reduction and cooling the generated gas can be distributed for further processing. At the manual port (not shown), a sample vial with molecular sieve beads is connected using an Ultra-Torr fitting (Cajon), which is sealed after complete condensation of the sample gas for offline isotope determination.

Most important, the central parts of the iHTR device, i.e., the sample holder (rod), the lid and the cylinder liner, are made of glassy carbon. In the first test runs a high purity graphite rod (spectroscopic grade graphite) was used, which can be heated inductively and can also supply the carbon needed for silica reduction. However, this did not lead to satisfactory oxygen isotope results. The standard deviation of repeatedly measured samples was high with partly erratic deviations from the mean. Moreover, the graphite rod continuously released a fine dust which gradually covered the inner surface of the quartz vessel. Therefore, it was decided to use glassy carbon as it is more or less inert due to the special molecular configuration of its structure (Dübgen and Popp, 1984). However, with glassy carbon as the sample holder it is necessary to mix the silica samples with high-purity graphite powder as the carbon source for their reduction.

The glassy carbon rod is heated inductively by a medium-frequency generator (type TIG10/300, Hüttinger Elektronik, Freiburg; Germany) under vacuum. With the present configuration of generator, heating coil and glassy carbon rod we have reached temperatures of up to 2200°C. Temperatures of the glassy carbon are measured by a quotient pyrometer calibrated by the Physikalisch-Technische Bundesanstalt (PTB), Germany, which is the National Metrology Institute. Heating can be performed fast in smaller or larger steps by increasing the power of the generator and can be controlled automatically with a programmable controller.

2.3. Standard iHTR Procedure

Before preparation, a sample vial is filled with seven beads of molecular sieve (5 Å, Ø ~2 mm; Merck), which is sufficient to trap the amount of CO generated and immediately connected to the iHTR manual port for evacuation. The molecular sieve beads are routinely preconditioned in a muffle furnace at 400°C for at least several hours to achieve initial degassing before use. Complete degassing of the beads is reached by heating the sample vial to 270 to 280°C in a heating block. A pressure of less than 10^{-3} mbar is reached within a very short time and is kept constant until preparation of the sample is finished (~45 min). This is achieved by the use of a vacuum bypass, which disconnects the vial from the gas distribution module by a valve.

~1.5 mg of a sample is accurately mixed with high-purity graphite powder. A mixture of 1:1 (mass ratio) guarantees the stoichiometric excess of carbon with respect to silica-bound oxygen. The amount of sample necessary is determined by the dual-inlet system IRMS (Optima, Micromass Ltd, UK). In the case of an online coupling to the IRMS in continuous flow mode this amount will be significantly reduced.

The sample is loaded into the sample chamber and the lid is closed. The sample holder is introduced and positioned into the cylinder liner. Subsequently, the seal cap module is fixed and the system is placed under vacuum. To avoid atmospheric contaminations while opening the iHTR device the cylinder liner is constantly flushed with helium. Preparation of a sample starts by evacuating the system immediately down to pressures of $<10^{-4}$ mbar. The whole procedure presently takes roughly 45 min and is run automatically and pressure-controlled. The pressure development is recorded to monitor the behavior of the sample investigated. Figure 2 presents a typical example of such pressure monitoring.

Since biogenic silica normally contains high and variable amounts of adsorbed water molecules, hydroxyl groups and weakly bound oxygen atoms, these components are removed by a thermal dehydration process before reduction of the silica structure. The water attached and trapped by the porous surface of biogenic silica is in principle volatilized by

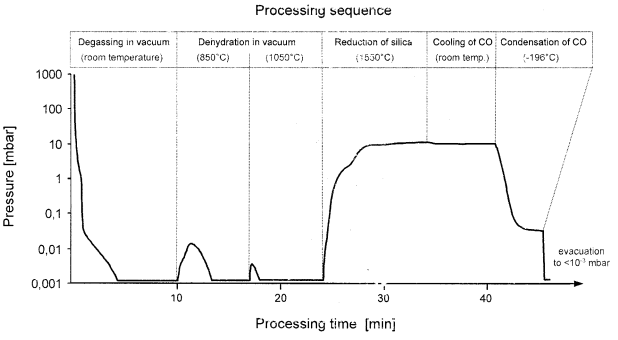

Fig. 2. Typical pressure recording of gas generation in the iHTR device during the dehydration and decomposition process of 1.4 mg of Opal-1. Dehydration starts at room temperature (22°C) and proceeds to 850°C and 1050°C while volatilized gases are evacuated. At the beginning of the reduction, the sample gas chamber is separated from the vacuum system and temperature is increased to 1550°C. The pressure drop at the end indicates absorption of the generated CO by the molecular sieve (5 Å) at liquid nitrogen temperature. Note logarithmic scale on the y-axis.

heating to ~100°C without any isotopic exchange with silica-bound oxygen (Labeyrie, 1974). A minor percentage of the water is chemically bound as hydroxyl to the silicon atoms and requires ~1000°C for its removal (Fripiat and Uytterhoeven, 1962). Therefore, the dehydration technique consists of a three step procedure whereby the volatilized oxygen-containing components are immediately removed by the high vacuum system to avoid isotopic exchange with the silica (Fig. 2). The duration of evacuation at room temperature (1) is extended to 10 min to achieve a thorough degassing of the sample. Subsequently, the temperature is raised to 850°C (2) and maintained for ~4 min to completely remove even small amounts of released gases. In the final step, the temperature is further raised to 1050°C (3) and kept constant for another 4 min. The release of substantial amounts of gas components during this dehydration procedure is shown by Figure 2. Since volatilized gas components are directly pumped off the respective peak areas do not quantitatively represent the amounts of released gases. The complete process is pressure-controlled to account for variable amounts of volatile components in different sample types. Only if a pressure of $<10^{-3}$ mbar is reached after a respective temperature increase and held for more than 2 min does the dehydration process proceed.

After dehydration is finished the connection to the vacuum line is closed and silica reduction starts. The temperature is raised stepwise according to the programmed controller entries until a temperature of 1550°C is reached when, in the case of biogenic silica and pure quartz, silica decomposition and conversion to CO is completed. The generated CO is cooled down to room temperature and the connection to the sample vial is opened. At liquid nitrogen temperature the CO is trapped in the molecular sieve of the sample vial until condensation is completed at pressures $<5 \times 10^{-2}$ mbar. The remaining gases are pumped off and the sample vial is sealed with a hand torch and is, thus, ready for offline oxygen isotope analysis.

Using this routine presently enables the preparation of 8 to 12 materials per day depending, for example, on working routine. Currently, a daily preparation program consists of four to five replicates of a standard material and, depending on sample inhomogeneities, of up to four replicates of one to two samples.

3. RESULTS AND DISCUSSION

Performance and reliability of the new iHTR technique with respect to the determination of the oxygen isotopic composition of biogenic opal were investigated in various experiments. The tests reported here are intended to demonstrate the quantitative extraction of silica bound oxygen, the efficiency of dehydration, the absence of a memory effect, the reproducibility of the oxygen isotope composition of diverse materials, the quality of the analysis of opaline and quartz reference materials and the capability to analyze fresh diatom samples. If not stated otherwise all experiments were performed by the standard preparation procedure specified in chapter 2.3.

3.1. Trapping of Carbon Monoxide

Preliminary tests with carbon monoxide as analyte proved the suitability of this approach for our purposes. Compared to the alternative conversion to carbon dioxide the utilization of CO has the advantage of doubling the sensitivity of a given amount of material. When analyzing biogenic silica, e.g., from sediment cores, this is of particular interest since the sample amount normally is very limited after the clean-up procedure.

As long as isotope determinations have to be performed offline the generated CO has to be trapped in a sample vial using a molecular sieve at liquid nitrogen temperature. To ensure that the adsorption and desorption of the sample gas at the IRMS manual port do not cause a significant shift in the original isotope composition, a reliable standard procedure was developed and tested. Best results were achieved by routinely preconditioning the molecular sieve beads (5 Å, Ø ~2 mm; Merck) in a muffle furnace at 400°C for at least several hours. This induces a first degassing of trapped gas molecules and material impurities before use in the iHTR. Before preparation of a sample, the molecular sieve beads have to be filled into a sample vial which is then connected to the iHTR and immediately placed under vacuum. Complete degassing of the beads in the sample vial is achieved by heating the vial to 270 to 280°C by a heating block. A constant pressure of less than 10^{-3} mbar is maintained during the whole time of sample preparation (~45 min) until immediately before condensation of CO at liquid nitrogen temperature. Desorption of CO from the molecular sieve is reached by again heating the closed sample vial to 270 to 280°C while attached to the manual port of the IRMS before intake of CO into the sample gas bellow.

Table 2. Oxygen isotope composition of cylinder CO after standard trapping at the iHTR device with molecular sieve beads (5 Å) at liquid nitrogen temperature and variable durations of temperature controlled desorption (heating time).[a]

Heating time (min)	10	30	45	60
$\delta^{18}O$ value (‰)	−9.00	−8.83	−8.73	−8.63
Offset (‰)[b]	−0.57	−0.40	−0.30	−0.20
SD (‰)	±0.18	±0.13	±0.08	±0.06

[a] CO desorption at the manual port of the IRMS was achieved using a hot air blower inducing a temperature of 260–270°C to the sealed sample vial with the molecular sieve (for details, see text). (SD = standard deviation; for each time step, n = 7).

[b] Offset describes deviation from the reference $\delta^{18}O$ value of CO gas (−8.43‰ vs. V-SMOW).

To investigate the reliability of the described procedure and the duration of heating required for desorption at the IRMS, cylinder CO of constant isotopic composition was introduced into the iHTR up to a pressure normally reached when processing a sample. The CO was processed according to the above procedure, however, with varying durations of heating at the IRMS. The respective oxygen isotope compositions were determined and are given in Table 2. They document that the described procedure for the CO treatment is adequate for minimizing the isotopic offset. The heating time needed for sufficient desorption is found to be relatively long possibly due to an unknown equilibrium reaction. Since with increasing time of heating also the reproducibility of analysis increases, a duration of 45 min for desorption of CO was accepted. A correction of 0.3‰ is applied to all samples.

3.2. Silica Reduction and Carbon Monoxide Generation with iHTR

Several experiments were performed to ensure the quantitative release of silica bound oxygen and its conversion to CO with graphite when processing biogenic opal (Opal-1) as well as quartz (NBS28) with the standard iHTR procedure (1550°C). Before this, a first test of quantitative silica reduction was done by spectroscopic investigation of the solid reaction residue remaining in the sample chamber after treatment and by mass spectrometry of the respective sample gas generated. For both materials infrared spectroscopy proved the absence of oxygen in the reaction residue (SiC) whereas IRMS mass scans

revealed only a negligible carbon dioxide peak but no other equivocal mass peaks in the respective spectra.

3.2.1. Decomposition Temperature

Theoretically, it can be expected that a higher temperature is necessary to achieve quantitative decomposition for crystalline quartz compared to biogenic opal. For reasons of laboratory routine, however, it is preferable to treat different materials identically especially in cases where calibration standards and samples are concerned. It thus had to be tested whether a temperature of 1550°C was also sufficient for the quantitative decomposition of quartz. In paired comparisons of Opal-1 and NBS28 the final temperature of reduction was further increased to 1700°C and 1830°C, respectively, for the two materials. In all cases, the sample amount was kept constant and Opal-1 was used as the reference. Compared to the standard treatment (1550°C), no difference in the isotopic composition of the quartz was observed at a temperature of 1700°C while an enrichment of ~0.35‰ was found for the 1830°C treatment (Table 3). The cause of this deviation between 1550°C and 1830°C is not clear and needs further verification. However, it was observed that the treatment with 1830°C caused anomalously depleted oxygen isotope raw values (~5‰) for NBS28 and Opal-1 alike. This was not accompanied by a detectable change in the oxygen yield. Obviously, increasing the temperature up to 1830°C affected the isotopic composition of both materials similarly and is unfavorable because of effects as yet unknown. It might be argued that despite its inert nature oxygen-containing contaminants are released from the glassy car-

Table 3. The relation between the oxygen isotope composition of quartz (reference material NBS28) and the reduction temperature used in the iHTR treatment. NBS28 values are against V-SMOW.

Temperature (°C)	NBS28 mean (‰)	SD	n
1550	9.63	0.12	4
1700	9.66	0.09	4
1830	9.98	0.09	3

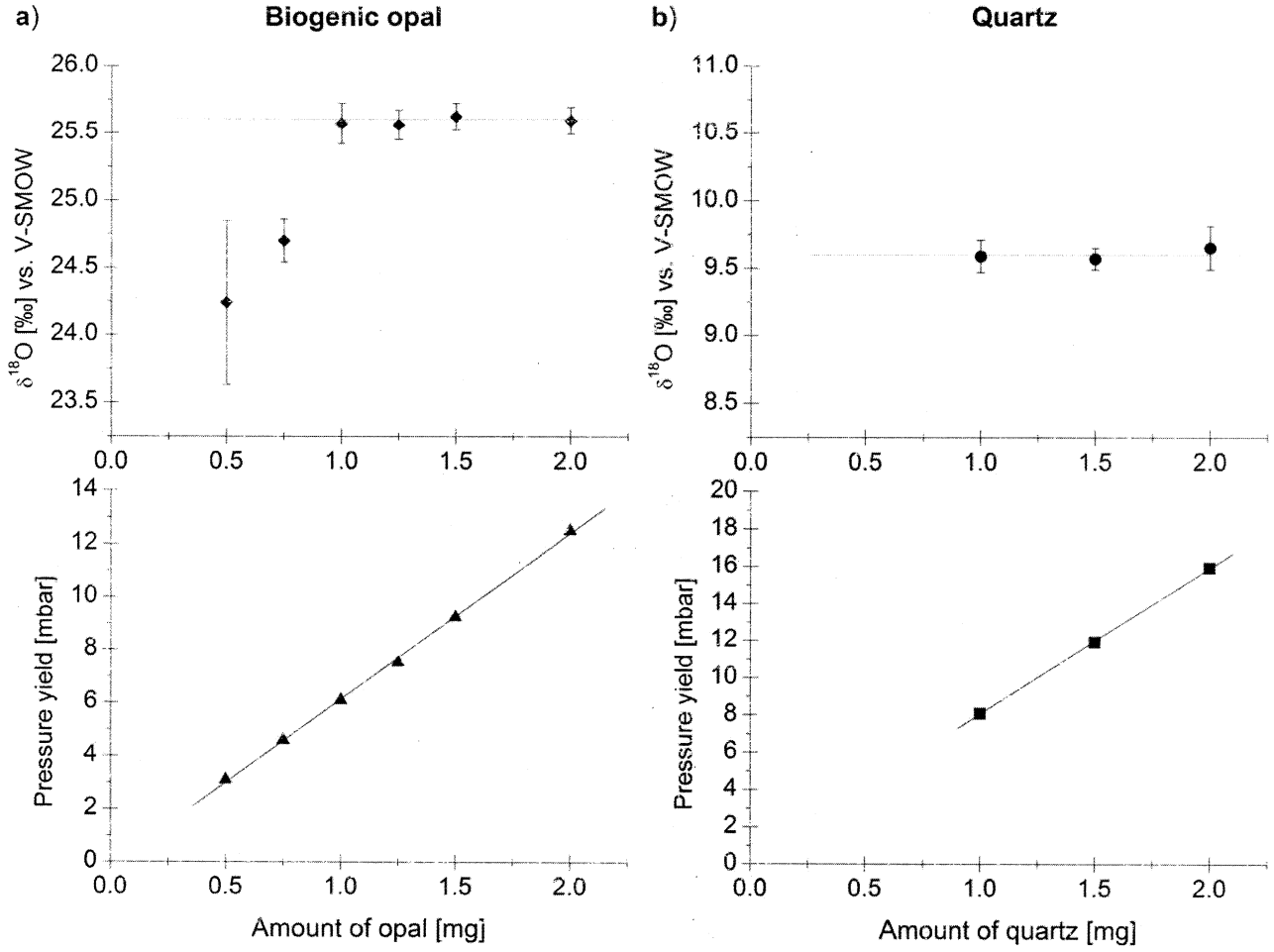

Fig. 3. Relationship between the amount of Opal-1 and NBS28 quartz, and CO generated in the iHTR device (lower panels). Note that the relations (black line) are strictly linear ($r^2 = 0.999$) and pass through the origin. In the upper panels the respective oxygen isotopic composition of the generated CO gas is given together with the standard deviation (bars) and the expected mean value (dotted line). The temperature used for disintegration was 1550°C, five replicates each were used for Opal-1 and four replicates each for quartz.

bon under these high temperature/low pressure conditions. This seems likely since at the inner wall of the glass vessel condensation in the form of a black coating was observed for the 1830°C treatment. Nevertheless, identical isotope values for the standard treatment and the 1700°C treatment proved that under vacuum a temperature of 1550°C is sufficient for the quantitative decomposition of biogenic silica and quartz alike.

3.2.2. Sample Size and Oxygen Yield

The quantitative yield of oxygen and a constant oxygen isotope composition have to be ensured irrespective of the amount of material used for the analysis. The results of experiments performed with iHTR using different amounts of Opal-1 and NBS28 are documented in Figure 3. For both materials a doubling of the sample amount resulted in a doubling of released CO (lower panels). The relations between sample amounts and pressure readings are strictly linear ($R^2 = 0.999$) and pass through the origin. Thus, a loss of sample from the sample chamber due to violent thermal decomposition is effectively prevented by the lid and the quantitative reaction takes place independent of sample amount and sample structure.

A similar result is achieved with respect to the oxygen isotope composition of both materials (Figs. 3a, b, top). They are constant within the uncertainties of analysis and, therefore, independent of the sample amount. Very depleted oxygen isotope values and increased analytical uncertainties emerge when using less than 1 mg of Opal-1 and quartz. This cannot be ascribed to the iHTR preparation, but is an artefact of strongly reduced signal strength arising when feeding a dual-inlet system with respectively less sample CO. The amount of biogenic opal routinely used for offline preparations with iHTR is, therefore, set to 1.4 to 1.5 mg.

Comparison of the amount of generated CO with the stoichiometrically expected oxygen yield gives further proof for the quantitative reduction of silica. As opal contains unknown amounts of water, OH-groups etc., this quantitative investigation could only be performed reliably for quartz. The determination of the oxygen yield for sample amounts of 1.0 and 2.0 mg of quartz (NBS28), respectively, prepared with iHTR resulted in an average value of 98.8%, indeed documenting a quantitative reduction of silica.

3.3. Reproducibility and Memory effect

3.3.1. Reproducibility

Several replicates of Opal-1 were prepared on individual days within a period of 2 months to investigate the reproducibility and long-term stability of the iHTR method using the standard procedure. The glassy carbon components were not changed during this time. The reproducibility of Opal-1 was found to be less than 0.1‰ in all cases and mean isotope values reported against the internal machine standard remained stable (Table 4). However, it was observed that the isotopic value of the first preparation of a day was generally enriched by up to 0.35‰. Since the system is kept under constant vacuum in stand-by conditions and flushed with helium during sample loading, atmospheric contaminations are very unlikely. Only the sample holder is exposed to the atmosphere while the sample is loaded. The phenomenon might be provoked by the instantaneous absorption of very small amounts of newly generated CO, i.e., 'preconditioning', of the glassy carbon surfaces after extended periods under vacuum. It might thus be seen as a 'preconditioning effect' also common to many elemental analyser systems. To avoid this possible bias, it was decided to routinely discard the initial preparation of any daily series run.

Table 4. Reproducibility of the isotopic composition of Opal-1 on five different days within 2 months. Note that the first value of each analytical series (day) deviates due to initialization conditions of the iHTR device.

Opal-1 $\delta^{18}O_{raw}$ (‰)		
Mean	SD	Individual analysis
16.28	0.06	(16.58[a], 16.36, 16.31, 16.19, 16.27)
16.22	0.04	(16.61[a], 16.28, 16.23, 16.16, 16.21)
16.22	0.04	(16.51[a], 16.19, 16.17, 16.24, 16.26)
16.16	0.07	(16.56[a], 16.21, 16.23, 16.12, 16.06)
16.61	0.08	(16.81[a], 16.55, 16.57, 16.75, 16.58)

[a] Value not included in mean and SD of samples.

Replicate analysis of the quartz standard NBS28 using the standard preparation routine resulted in a $\delta^{18}O$ value of 9.62‰ ± 0.11‰ vs. V-SMOW (n = 17). The respective reproducibility of fresh and sedimentary samples of diatom silica is better than ±0.15‰ and mostly in the order of 0.10‰.

3.3.2. Memory effect

Any memory effect produced by the preparation technique would significantly deteriorate the reliability of oxygen isotope determinations by this method. Two experiments with materials of large isotopic differences (>20‰) were performed to ensure that no memory effect occurred in the iHTR device. In a first experiment, cylinder CO was used alternately with Opal-1. CO was introduced into the sample gas chamber until pressures were reached that were commonly generated from samples of silica. Subsequently, the standard iHTR treatment without the degassing and dehydration steps was performed with the gas to guarantee comparability with results obtained by standard treatment of Opal-1. In the second experiment, a comparison between different biogenic opal materials was performed and Opal-1 and *Ethmodiscus rex* were alternately prepared by the standard procedure.

The results of the comparison between CO and Opal-1 (Fig. 4a), on the one hand, and between Opal-1 and *Ethmodiscus rex* (Fig. 4b), on the other hand, are such that a memory effect can be excluded. Each first analysis of a series after a switch between materials does not show any detectable deviation from the respective mean value. The reliable range for oxygen isotope determinations of different materials referenced with our laboratory standard Opal-1 is thus almost 50‰ (-2.5‰ to + 46.8‰) on the V-SMOW scale.

3.4. Calibration and Accuracy

3.4.1. Calibration of iHTR to V-SMOW Scale

Calibration of the new iHTR technique to the V-SMOW scale was achieved by using NBS28 (quartz), National Bureau

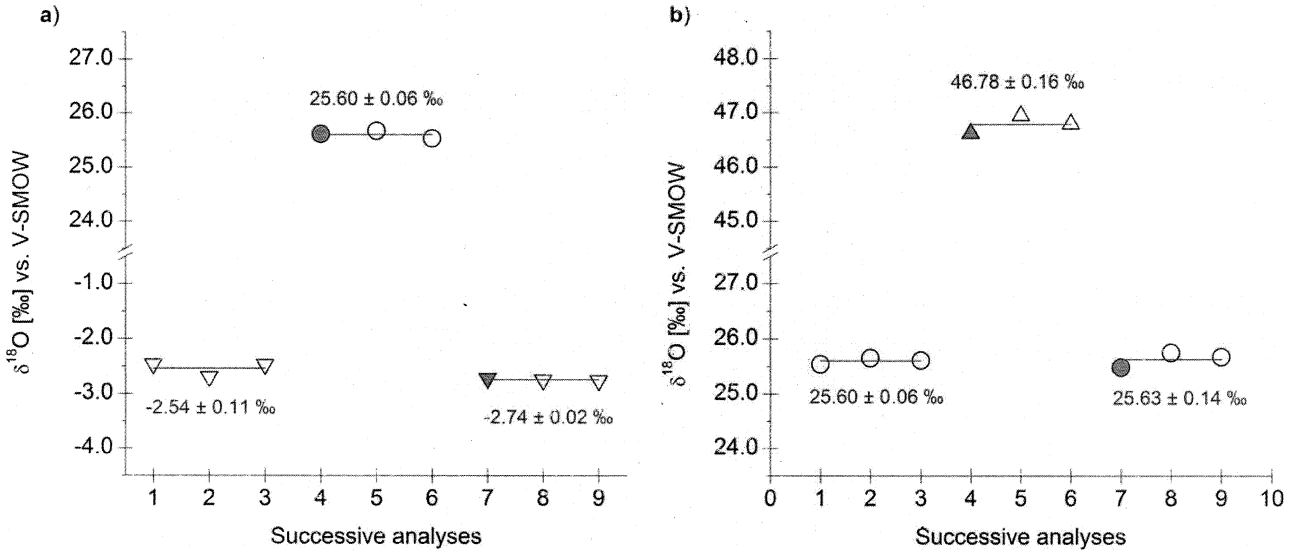

Fig. 4. Test of memory effects with two different experimental settings. Consecutive preparation/analysis of (a) cylinder CO alternating with Opal-1 and (b) Opal-1 alternating with *Ethmodiscus rex*. Cylinder CO was treated like a sample (for further details, see text). Each first analysis of a respective series of replicates is highlighted in gray. Mean values are indicated (gray lines) and given together with the standard deviation for each series of replicates.

of Standards (USA), with an accepted $\delta^{18}O$ value of 9.6‰ ± 0.1‰. The laboratory standard Opal-1 was alternately prepared with NBS28 as standard material on six different days within several weeks. Between 1.4 to 1.5 mg of each material was used for preparations and treated according to the standard routine.

The respective raw results (vs. machine standard) and the calculated means of both materials are presented in the first columns of Table 5. Based on the known value of NBS28 and the respective mean values, calibration of the Opal-1 was performed for single days (Table 5, last columns). Calculating the overall mean oxygen isotope value of the laboratory standard Opal-1 resulted in a value of +25.60‰ ± 0.19‰ against V-SMOW. Discarding the single deviating value of 25.94‰, which occurred without obvious explanation, would result in a significantly improved standard deviation of 0.11‰ without substantial change of the overall mean.

3.4.2. *Comparison with stepwise fluorination*

Based on this calibration, an experiment was undertaken to further verify the capability of the iHTR method by direct comparison with the stepwise fluorination method (SWF), which has been used to determine the oxygen isotope composition from biogenic opal. Schmidt et al. (1997, 2001) investigated a 4-million-year-old sample of frustules from the marine diatom *Ethmodiscus rex* from a sediment core of the Weddell Sea (Antarctic South Atlantic) using the SWF approach and determined its $\delta^{18}O$-value as 46.8‰ ± 0.29‰ against V-SMOW. In a set of measurements several samples of Opal-1 and *Ethmodiscus rex* were consecutively prepared with iHTR on different days according to the standard routine. The iHTR results for *Ethmodiscus rex* referenced to the V-SMOW scale with the calibrated laboratory standard Opal-1 are given in Table 6.

Table 5. Calibration of the laboratory standard Opal-1 to the V-SMOW scale using NBS28 quartz as reference material. Measurements were performed on six different days (single rows) over a period of several weeks (overall mean: n = 6).

NBS28 $\delta^{18}O_{raw}$ (‰)		Opal-1 $\delta^{18}O_{raw}$ (‰)		Opal-1 $\delta^{18}O$ V-SMOW (‰)	
Mean	Individual analysis	Mean	Individual analysis	Mean	SD
1.12	(0.96, 1.42, 1.05, 1.04)	17.12	(17.04, 16.97, 17.20, 17.26)	25.60	0.14
0.70	(0.69, 0.43, 0.99, −0.44[a])	16.70	(16.49, 16.79, 16.83, 15.96[a])	25.60	0.19
0.75	(1.19, 0.89, 0.44, 0.49)	17.09	(17.13, 17.11, 17.10, 17.01)	25.94	0.05
0.34	(0.31, 0.19, 0.40, 0.44)	16.36	(16.42, 16.22, 16.36, 16.42)	25.62	0.09
1.99	(2.12, 2.17, 1.71, 1.97)	17.83	(17.97, 17.82, 17.82, 17.72)	25.44	0.10
1.54	(1.94, 1.83, 1.25, 1.15)	17.32	(17.29, 17.31, 17.34, 17.34)	25.38	0.03
				Overall mean: 25.60 ± 0.19‰	

[a] Value not included in mean and SD of single days.

Table 6. Oxygen isotopic composition of Tertiary diatom frustules of *Ethmodiscus rex* prepared with the iHTR device using Opal-1 as reference standard.[a]

Ethmodiscus rex $\delta^{18}O$ (‰) vs. V-SMOW		
Average	SD	Individual analysis
46.71	0.03	(46.69, 46.70, 46.22[b], 46.74)
46.81	0.15	(46.70, 46.68, 47.00, 46.85)
46.69	0.02	(45.70[b], 46.67, 46.69, 46.71)
46.57	0.15	(46.45, 46.45, 46.77, 46.61)
Overall mean: 46.70 ± 0.10 ‰		

[a] Measurements were performed on four different days (single rows) within different weeks. The $\delta^{18}O$ value of *Ethmodiscus rex* using the stepwise fluorination technique is reported as 46.8 ± 0.29‰ versus V-SMOW (Schmidt et al., 1997, 2001).
[b] Value not included in mean and SD of samples.

The average value for *Ethmodiscus rex* is determined as 46.70‰ ± 0.10‰ (n = 4), which is in excellent agreement with the SWF result. This result emphasizes that iHTR is a reliable and highly precise technique for the analysis of the oxygen isotope composition of both opal and quartz materials.

3.5. Fresh Diatom Opal

3.5.1. Dehydration procedure

Determination of the oxygen isotope composition of biogenic silica requires the complete removal of loosely adsorbed water molecules, hydroxyl groups and weakly bound oxygen atoms from the 'stable' silica shell before decomposition of the sample starts. Any isotope exchange between the oxygen-bearing contaminants and silica has to be avoided during dehydration (Labeyrie and Juillet, 1982). This is of special importance, for example, for samples of fresh diatom frustules (Juillet, 1980; Brandriss et al., 1998).

To determine the effect of the dehydration procedure on the oxygen isotope composition of fresh biogenic silica, different dehydration treatments with increasing maximum temperatures in vacuum were tested for Opal-1 and *Fragilaria crotonensis*. In all cases the process was pressure-controlled and the end vacuum for each dehydration step was below 10^{-3} mbar. When the respective maximum dehydration temperature of 22°C (room temperature), 500°C, 800°C, 980°C and 1050°C was reached, the reduction of the sample started and the generated CO was trapped and analyzed.

The results of these experiments are presented in Table 7. In general, isotope values of both materials converge asymptotically towards the control run (1050°C) with increasing duration and temperature of dehydration. A similar effect is observed for the amounts of generated gas (Table 7) due to incomplete dehydration, i.e., contamination of the opaline oxygen at low temperatures. Most notably, the isotopic compositions of both materials deviated significantly below 800°C and were characterized by a high standard deviation. As the dehydration temperature is increased to 980°C a plateau is reached and a further increase to 1050°C does not lead to a change in the isotopic composition of the respective sample. The standard deviation is significantly improved at these high dehydration temperatures. A further increase in temperature immediately led to strong gas development, which is seen as the first decomposition reaction of silica.

Particularly $\delta^{18}O$ values of *Fragilaria crotonensis* show a rather strange composition for the low temperature treatments, which can only be explained by strong contaminations. Nevertheless, mass spectrometric scans did not show any anomalous mass peaks or increased carbon dioxide contents of the sample gas when compared to the mass scan of a standard treatment. It should be stressed here that thermal dehydration will also volatilize organic remains which are not destroyed by the hydrogen peroxide treatment and are possibly entrapped within the frustule structure. This seems to be at least one possibility (probably N_2 or NO components are formed) of explaining the large differences between the investigated materials at low dehydration temperatures.

Table 7. Dependence of the oxygen isotopic composition of Opal-1 and *Fragilaria crotonensis* on different dehydration treatments and the corresponding amount of generated CO. Isotope values are against V-SMOW.

Temperature treatment	Mean	SD	Individual analysis	mbar[a]
Dehydration experiments on Opal-1				
10 min. at 22°C	28.15	0.52	(28.18, 27.62, 28.65)	7.44
10 min. at 22°C; 5 min at 500°C	26.49	0.82	(26.77, 27.13, 25.57)	7.12
10 min. at 22°C; 4 min at 500°C; 5 min at 800°C	26.01	0.08	(26.03, 26.08, 25.92)	6.64
10 min. at 22°C; 4 min at 850°C; 5 min at 980°C	25.57	0.16	(25.69, 25.39, 25.64)	6.62
10 min. at 22°C; 4 min at 850°C; 5 min at 1050°C	25.60	0.04	(25.57, 25.59, 25.64)	6.60
Dehydration experiments on cultured *Fragilaria crotonensis*[b]				
10 min. at 22°C	90.67	0.89	(90.14, 91.70, 90.16)	10.68
10 min at 22°C; 5 min at 500°C	50.24	1.43	(49.47, 49.35, 51.89)	8.05
10 min at 22°C; 4 min at 500°C; 5 min at 800°C	26.95	0.19	(27.16, 26.80, 26.90)	5.37
10 min at 22°C; 4 min at 850°C; 5 min at 980°C	25.26	0.14	(25.10, 25.35, 25.32)	4.75
10 min at 22°C; 4 min at 850°C; 5 min at 1050°C	25.30	0.13	(25.33, 25.41, 25.15)	4.72

[a] Average pressure yield standardized to 1 mg of sample.
[b] Removal of organic matter by digestion in hydrogen peroxide (≈15%) at 60°C in three treatments for 8 h each. $\delta^{18}O_{H2O}$ value of culture medium: −7.6‰ vs. V-SMOW.

Table 8. Oxygen isotopic composition of *Cyclotella meneghiniana* (laboratory culture) after different periods of storage in deionized water (pH = 5.5) at room temperature (22°C). The $\delta^{18}O$ value of the deionized water was −7.2‰ vs. V-SMOW.

	Cyclotella meneghiniana[a] $\delta^{18}O$ (‰) vs. V-SMOW		
Storage time	Mean	SD	Individual analysis
Control	26.34	0.09	(26.37, 26.25, 26.29, 26.44)
7 d	26.26	0.14	(26.21, 26.42, 26.16, 25.97[b])
14 d	26.33	0.10	(26.26, 26.45, 26.28)
28 d	26.13	0.09	(25.89[b], 26.07, 26.19)
62 d	26.33	0.07	(26.25, 26.39, 26.35)
2 d pH 9.0	33.15	0.12	(32.02[b], 33.01, 33.23, 33.20)

[a] Removal of organic matter by digestion in hydrogen peroxide (≈15%) at 60°C.
[b] Value not included in mean and SD of samples.

These experimental results confirm investigations by Labeyrie (1979) and Brandriss et al. (1998), who stressed that especially when dealing with fresh diatom frustules dehydration was only exhaustive when the samples were treated at temperatures higher than 800°C under high vacuum. The iHTR device, additionally, prevents any further contamination with atmospheric water vapor since no transfer of the sample is needed after dehydration.

Based on these findings a three-step dehydration technique was adopted for routine iHTR preparations (see chapter 2.3. and Fig. 2). This standard dehydration procedure can, however, easily be varied according to the necessities of different silica materials. The water vapor generated during that dehydration process might be trapped and used for isotopic analysis parallel to that of the silica as soon as an adequate cryo-trap has been built.

3.5.2. *Storage effects and pH*

The question of oxygen isotope exchange reactions between silica, i.e., an 'exchanging surface silica layer', and loosely bound oxygen attached to the opal surface probably occurring during laboratory treatments is widely discussed (Lewin, 1961; Juillet, 1980; Labeyrie and Juillet, 1982). Therefore, an additional experiment was performed with fresh diatom frustules. After removal of organic matter, frustules of the cultured freshwater species *Cyclotella meneghiniana* were stored in closed bottles for periods of up to 62 d (7, 14, 28 and 62 d) at room temperature in deionized water with a $\delta^{18}O$ value of −7.2‰ (pH ≈ 5.5) and analyzed using the standard iHTR preparation. A sample not exposed to water was used as the control.

Despite potential oxygen exchange of the delicate organic-free fresh frustules and a slight solution of silica by this treatment (up to 2 mg/L of dissolved silicon occurred) no difference between the oxygen isotope composition of the control and the exposed samples was observed within the analytical uncertainties (Table 8). If this result can be generalized, the iHTR dehydration process is very effective in completely removing loosely bound oxygen. This is encouraging with regard to the cleaning steps necessary for sediment samples (Rings et al., 2004) and to possible applications since the time of settling of diatoms in small freshwater lakes is much shorter than 2 months.

A different situation arises, however, if the frustules are stored in water of pH 9.0 for 48 h (Table 8). Since a considerable amount of dissolved silicon was found in the water after exposure (~10 mg/L), the substantial enrichment of 6.8‰ in the oxygen isotope composition of the frustules might not be ascribed to isotopic exchange reactions but to strongly increased silica dissolution occurring at this pH on the valve surfaces. The extent of this isotopic shift is comparable with results from Juillet (1980), who, after a treatment with hot acid (60°C) for up to 6 h, determined isotope values enriched by up to 5.2‰ for diatom samples from lacustrine surface sediments.

This result may be of importance with respect to the long-term stability of biogenic silica and its $\delta^{18}O$ values because situations of increased pH may arise in the epilimnion of freshwater lakes for shorter periods or in closed lakes of endorheic regions for longer periods. Nevertheless, more experiments investigating the preservation of the juvenile oxygen isotope signature of sedimentary diatom frustules over longer periods are still necessary.

4. CONCLUSION AND IMPLICATIONS

Despite available and widely accepted standard methods for the preparation and determination of the oxygen isotope composition of biogenic opal a broader application as a proxy parameter for paleothermometry is clearly still hampered by analytical uncertainties. In this contribution a novel technical approach for the preparation of biogenic silica samples and subsequent oxygen isotope determination is described. The technique is based on inductive heating and high temperature carbon reduction of silica and is called iHTR. A temperature of 1550°C in vacuum turned out to be adequate for the quantitative reduction of biogenic opal as well as pure quartz. Dehydration of samples, including fresh diatom materials, is achieved in a three-step heating process immediately before disintegration of the silica within the iHTR device. Various experiments with respect to dehydration efficiency, quantitative conversion of silica, reproducibility and memory effects demonstrated the quality and reliability of the technique developed. Calibration of iHTR to the V-SMOW scale was achieved by using NBS28 as the reference standard. Further comparison of the iHTR method with the stepwise fluorination method clearly demonstrated the reliability and capability of the new method for the determination of biogenic opal oxygen isotopes.

The new iHTR method overcomes common problems of present standard methods as it enables complete dehydration and decomposition in a single continuous process. Complete dehydration without isotopic exchange is of especial importance with respect to fresh diatom samples from sediment traps and laboratory cultures. Problems with the preparation of living diatom samples from sediment traps reported by Schmidt et al. (1997) might be caused by an inadequate dehydration routine for those samples. iHTR also improves the reproducibility of natural opal samples to better than ±0.15‰ (1 SD), which is most important with respect to calibration experiments regarding the temperature dependence of the oxygen isotope signature recorded in diatom frustules. It also considerably reduces the amount of sample necessary for analyses to ~1.5 mg of silica

by the use of CO as the analyte. As soon as the ongoing online coupling to an IRMS is implemented the necessary sample amount will be reduced and a further reduction of analytical uncertainties can be expected. More experience seems to be necessary with respect to the lifetime of the glassy carbon components in routine application.

The new iHTR technique should help to further reduce uncertainties of temperature reconstructions based on biogenic silica oxygen isotopes and to clarify methodologic differences expressed in the differences of fractionations determined by different techniques. For example, the fractionation ($10^3 \ln\alpha$) between water and diatom silica of ~33.6‰ found for cultured diatoms with iHTR is in the range of values reported by Brandriss et al. (1998) for similar materials. However, more experiments are still needed with respect to the long-term conservation of the primary oxygen isotope composition in biogenic silica (see Schmidt et al., 2001). The new iHTR technique may help to promote the application of oxygen isotope compositions from marine and freshwater diatoms, from sponge spicules, crysophyte cysts as well as from phytoliths.

In its present configuration the iHTR device is already able to reach temperatures of up to 2200°C. Given that the cooling of the inner glass surface of the glass vessel can be ensured for a sufficient duration this would enable the technique to be applied to other silicates and oxides. First attempts in this direction using biotite as the test material encountered problems possibly due to the relatively high amount of other elements besides silicon and oxygen in this material.

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REFERENCES

Adams L. H. (1949–1950) Ratio of oxygen isotopes in quartz of contrasted origin. *Carnegie Inst. Wash. Yrbk.* **49,** 36–37.

Baertschi P. and Schwander H. (1952) Ein neues Verfahren zur Messung der Unterschiede im ^{18}O-Gehalt von Silikatgesteinen. *Helv. Chim. Acta* **35,** 1748–1751.

Brandriss M. E., O'Neil J. R., Edlund M. B., and Stoermer E. F. (1998) Oxygen isotope fractionation between diatomaceous silica and water. *Geochim. Cosmochim. Acta* **62,** 1119–1125.

Clayton R. N. (1992) Silica-carbonate isotopic temperature calibration. *Science* **258,** 1162–1163.

Clayton R. N. and Epstein S. (1958) The relationship between O^{18}/O^{16} ratios in co-existing quartz, carbonate and iron oxides from various geological deposits. *J. Geol.* **66,** 352–373.

Dübgen R. and Popp G. (1984) Glasartiger Kohlenstoff. Sigradur—Ein Werkstoff für Chemie und Technik. *Werkstofftechnik* **15,** 331–338.

Fripiat J.J. and Vytterhoeven J. (1962) Hydroxyl content in silica gel "aerosic." *J Phys. Chem.* **66,** 800–805.

Haimson M. and Knauth L. P. (1983) Stepwise fluorination—A useful approach for the isotopic analysis of hydrous minerals. *Geochim. Cosmochim. Acta* **47,** 1589–1595.

Hu F. S. and Shemesh A. (2003) A biogenic–silica δ^{18}O record of climatic change during the last glacial-interglacial transition in southwestern Alaska. *Quat. Res.* **59,** 379–385.

Jones V. J., Leng M. J., Solovieva N., Sloane H. J., and Tarasov P. (2004) Holocene climate of the Kola Peninsula: Evidence from the oxygen isotope record of diatom silica. *Quat. Sci. Rev.* **23,** 833–839.

Juillet A. (1980) Structure de la Silice biogénique: Nouvelles données apporteés par l'analyse isotopique de l'oxygène. *C. R. Acad. Sci. Paris* **290 D**, 1237–1239.

Juillet-Leclerc A. and Labeyrie L. D. (1987) Temperature dependence of the oxygen isotopic fractionation between diatom silica and water. *Earth Planet. Sci. Lett.* **84,** 69–74.

Knauth L. P. (1973) Oxygen and hydrogen isotope ratios in cherts and related rocks. Ph.D. thesis. California Institute of Technology.

Labeyrie L. D. (1974) New approach to surface seawater palaeotemperatures using $^{18}O/^{16}O$ ratios in silica of diatom frustules. *Nature* **248,** 40–42.

Labeyrie L. D. (1979) La composition isotopique de l'oxygène de la silice des valves de diatomées. Mise au point d'une nouvelle méthode de paléo-climatologie. Thèse d'État, Université de Paris XI, Note CEA n° 2086.

Labeyrie L. D. and Juillet A. (1982) Oxygen isotopic exchangeability of diatom valve silica; Interpretation and consequences for paleoclimatic studies. *Geochim. Cosmochim. Acta* **46,** 967–975.

Leng M., Barker P., Greenwood P., Roberts N., and Reed J. (2001) Oxygen isotope analysis of diatom silica and authigenic calcite from Lake Pinarbasi, Turkey. *J. Paleolimnol.* **25,** 343–349.

Lewin J. C. (1961) The dissolution of silica from diatom walls. *Geochim. Cosmochim. Acta* **21,** 182–198.

Matheney R. K. and Knauth L. P. (1989) Oxygen isotope fractionation between marine biogenic silica and seawater. *Geochim. Cosmochim. Acta* **53,** 3207–3214.

Mikkelsen N., Labeyrie L. D., and Berger W. H. (1978) Silica oxygen isotopes in diatoms: A 20,000 yr record in deep-sea sediments. *Nature* **271,** 536–538.

Mopper K. and Garlick G. D. (1971) Oxygen isotope fractionation between biogenic silica and ocean water. *Geochim. Cosmochim. Acta* **35,** 1185–1187.

Rings A., Lücke A., and Schleser G. H. (2004) A new method for the quantitative separation of diatom frustules from lake sediments. *Limnol. Oceanogr. Methods* **2,** 25–34.

Rosqvist G. C., Rietti-Shati M., and Shemesh A. (1999) Late glacial to middle Holocene climatic record of lacustrine biogenic silica oxygen isotopes from a Southern Ocean island. *Geology* **27,** 967–970.

Schmidt M., Botz R., Stoffers P., Anders T., and Bohrmann G. (1997) Oxygen isotopes in marine diatoms: A comparative study of analytical techniques and new results on the isotope composition of recent marine diatoms. *Geochim. Cosmochim. Acta* **61,** 2275–2280.

Schmidt M., Botz R., Rickert D., Bohrmann G., Hall S. R., and Mann S. (2001) Oxygen isotopes of marine diatoms and relations to opal-A maturation. *Geochim. Cosmochim. Acta* **65,** 201–211.

Schwander H. (1953) Bestimmung des relativen Sauerstoffisotopen-Verhältnisses in Silikatgesteinen und -Mineralien. *Geochim. Cosmochim. Acta* **4,** 261–291.

Sharp Z. and O'Neil J. (1989) A laser-based carbon reduction technique for oxygen isotope analysis of silicates and oxides. *Carnegie Inst. Wash. Yrbk.* **21,** 136–138.

Shemesh A. (1992) "Response to R. N. Clayton (Silica-carbonate isotopic temperature calibration, *Science* 258, 1162–1163)". *Science* **258,** 1163.

Shemesh A., Charles C. D., and Fairbanks R. G. (1992) Oxygen isotopes in biogenic silica: Global changes in ocean temperature and isotopic composition. *Science* **256,** 1434–1436.

Shemesh A., Burckle L. H., and Hays J. D. (1995) Late Pleistocene oxygen isotope records of biogenic silica from the Atlantic sector of the Southern Ocean. *Paleoceanography* **10,** 179–196.

Shemesh A., Rietti-Shati M., Rioual P., Battarbee R., de Beaulieu J.-L., Reille M., Andrieu V., and Svobodova H. (2001) An oxygen isotope record of lacustrine opal from a European Maar indicates climatic stability during the last interglacial. *Geophys. Res. Lett.* **28,** 2305–2308.

Stoermer E. F. and Smol J. P. (1999) *The Diatoms: Applications for the Environmental and Earth Sciences*. Cambridge University Press, Cambridge.

Thorleifson J. T. and Knauth L. P. (1984) An improved stepwise fluorination procedure for the oxygen isotopic analysis of hydrous silica (abstract). *Geol. Soc. Am. Abstr. Progr.* **16,** 675–676.

Wang C.-H. and Yeh H.-W. (1984) Oxygen isotopic analysis of marine diatoms. *Bull. Inst. Earth Sci. Acad. Sinica* **4,** 131–140.

Wang C.-H. and Yeh H.-W. (1985) Oxygen isotopic composition of DSDP site 480 diatoms: Implications and applications. *Geochim. Cosmochim. Acta* **49,** 1469–1478.

Webb E. A. and Longstaffe F. J. (2000) The oxygen isotopic compositions of silica phytoliths and plant water in grasses: Implications for the study of paleoclimate. *Geochim. Cosmochim. Acta* **64,** 767–780.