

Determination of the rate of production and dissolution of biosilica in marine waters by thermal ionisation mass spectrometry

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Abstract

A new method is described for a precise and simultaneous determination of the rate of production and dissolution of biosilica in marine waters, using isotopic dilution technique. No HF or F₂ is required for chemical preparations as the change in isotopic composition is measured on silica producing SiO₂⁻ ions. The seawater sample flask is spiked with ³⁰Si(OH)₄ (<10% of increase in situ concentration) and incubated in situ conditions. At the end of incubation, changes of the ³⁰Si:²⁸Si ratios in particulate and liquid phases are measured by using a thermal ionisation mass spectrometer Finnigan THQ. The relative analytical precision of the isotopic ratio measurements is <0.5%. The limit of detection of the change in isotopic ratio during incubation is 0.02 atom%. The overall repeatability determined on eight subsamples (average production: 0.23 μM day⁻¹; average dissolution: 0.07 μM day⁻¹) is ±0.02 and ±0.01 μM day⁻¹ for production and dissolution, respectively. Using mass and isotopic balances of the particulate and dissolved phases in the incubation flask, the best estimates for production and dissolution rates are calculated iteratively. This method was applied to 112 samples of marine waters (production, range: 0.00–2.38 μM day⁻¹; dissolution, range: 0.00–1.18 μM day⁻¹).

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1. Introduction

Diatoms which contribute to about 50% of the total primary production and export production of the world ocean, are key players in the biological pump of CO₂ [1,2]. The growth of diatoms relies on the availability of silicic acid in the surface ocean. By uptaking silicic acid, diatoms build up their frustules composed of amorphous silica. Amorphous silica is unstable in the conditions prevailing in the surface layers of the modern world ocean. Although, silica dissolution is not excluded for living diatoms, it mostly occurs at the death of diatoms, when frustules are no longer protected by organic coatings. The uptake of silicic acid by diatoms corresponds to a transfer of Si from the dissolved phase to the

particulate phase; this process is herein called “production” of biosilica, the reverse process is called “dissolution”.

Since 1973 and later, the measurement of the production and the dissolution rate of biosilica have been performed by using an isotopic dilution technique with ²⁹Si or ³⁰Si [3,4], and applied to different marine environments. After incubation of a seawater sample enriched in ²⁹Si or ³⁰Si the changes in isotopic composition of silicon of the particulate and dissolved phase were determined with a gas source mass spectrometer as SiF₃⁺ ions. This went through the transformation of SiO₂ in BaSiF₆ by HF attack, which remains harmful. In 1991, Tréguer et al. [5] introduced the use of ³²Si for the determination of the production rate of biosilica, this radio-tracer being measured by scintillation counting. This method is much more sensitive and practical, although not less expensive, than mass spectrometry. Tréguer et al.'s ³²Si original method and/or modified by Brzezinski and Philips [6]

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represented a tremendous improvement for the study of the silica cycle in marine waters and those methods have been extensively used to measure the production of biosilica in various marine environments [7]. Up today however, the use of ^{32}Si for the determination of biosilica dissolution rate in marine waters has been unsuccessful, and mass spectrometry using isotope dilution technique remains an obligatory method. For most marine chemists, measuring the rate of dissolution of biosilica in the surface ocean using an isotopic dilution technique remains a challenge. Up to 2003, only 56 profiles have been performed [8] worldwide. In fact, two major problems make this mass spectrometry determination difficult. First, this is because the absolute rate of biosilica dissolution is usually slow in seawater leading to a small variation in the isotopic ratios of the liquid phase during the time of incubation (usually 24 h); this means that a high precision is required. Secondly, because our planetary environment is silicate-minerals rich, contamination of samples by lithogenic silica through atmospheric transport in the laboratory easily occurs. This especially happens during chemical preparations, preliminary to mass spectrometry determinations. This explains why such preparations must be conducted in a clean room or under a laminar flow hood.

Here we describe a new method for the simultaneous determination of the rate of production and dissolution of biosilica in marine environments. It is based on new developments of the sample preparation protocol and precise measurements of the isotopic composition of SiO_2^- ions in the particulate and dissolved phase of seawater samples, by using a thermal ionisation mass spectrometer Finnigan THQ. A new method for calculating the production and the dissolution rates has also been developed. Thereafter it is briefly described and discussed. The calculations and comparison with previous method [3,4] are detailed elsewhere [9].

2. Experimental

2.1. Outline of the method (Fig. 1)

The isotopic dilution technique with ^{30}Si aims to simultaneously determine the rate of production of biosilica by diatoms and the rate of dissolution of biosilica in the same seawater sample (Fig. 1). In seawater, silicon isotopes have natural abundances of 92.23%, 4.67%, and 3.10% for ^{28}Si , ^{29}Si , and ^{30}Si , respectively [10]. It is assumed that, neither the process of uptake of silicic acid by diatoms, nor that of dissolution of biosilica significantly changes these abundances [11]. Samples from euphotic layer (which generally contains biogenic silica in the form of diatoms and radiolarians) are spiked by $^{30}\text{Si}(\text{OH})_4$ and incubated in situ conditions during a given time (usually 24 h). Then the isotopic dilution of the enriched seawater from the dissolving silica is used to estimate the dissolution of biosilica. Likewise the change in the isotopic composition of the silica itself is used to estimate the production of biosilica. Both these liquid and solid phases

undergo a chemical treatment to finally obtain pure silica for mass spectrometric measurements. Details of the sampling, incubation, chemical purification and mass spectrometry follow in Sections 2.4–2.9.

2.2. Instrumentation

A quadrupole thermal ionisation mass spectrometer (THQ-MS, Finnigan MAT, Bremen, Germany) is used for silica isotopic measurements. Ionic currents are measured using a secondary electron multiplier SEM 217 (Balzers-Pfeiffer GmbH, Asslar, Germany) mounted 90° off-axis, coupled to an ion counter PM6665 (Fluke & Philips, Eindhoven, The Netherlands). High vacuum (about 7×10^{-7} Pa) is obtained using a turbomolecular pump TPH240 (Balzers-Pfeiffer GmbH, Asslar, Germany).

An auto-analyser Technicon AAII (Bran + Luebbe, Norderstedt/Hamburg, Germany) is used for quantification of silica or orthosilicic acid concentrations, with a colorimetric method according to Tréguer and Le Corre [12].

2.3. Reagents and standard solutions

All the plastic ware used (flasks, filtration material, etc.) is acid pre-cleaned in HCl (Merck, p.a.) 10%. All solutions are prepared with 18 M Ω deionised water (Milli-Q Plus system, Millipore, Molsheim, France).

Spike solutions of sodium silicate are prepared with Milli-Q water after the fusion of ^{30}Si enriched silica powder (Oak Ridge National Laboratory product, higher than 96.00%) with anhydrous sodium carbonate (Merck, suprapur) [3]. 0.2 M sodium hydroxide (Merck, p.a.) is used for alkaline digestion of biosilica. 2.9 M fluorhydric acid (Merck, p.a.) is used for the second digestion, for the determination of lithogenic silica. The co-induced brucite precipitation adapted from MAGIC (MAGnesium Induced Coprecipitation [13]) used for the preconcentration of silicic acid is performed with sodium hydroxide (Merck, p.a.) 1 M. The brucite precipitate is re-dissolved in 2 M nitric acid (Merck, extra pure). Orthosilicic acid is precipitated with triethylamine molybdate (TEA-Moly): ammonium heptamolybdate tetrahydrated (Merck, p.a.), triethylamine chlorhydrate (Merck for synthesis), and acidification by hydrochloric acid (Merck, p.a.), according to De La Rocha et al. [14]. A solution of 73 mM octahydrated barium hydroxide (Prolabo, RP Normapur) is used for the activation of ionisation for mass spectrometry measurement. Sodium silicate solution (Merck, extra pure) diluted to 0.5 M is used as standard solution for mass spectrometer calibrations.

2.4. Sample collection, spiking and incubation (Fig. 1A)

Seawater is collected in the euphotic layer with a Niskin bottle, usually fitted on a CTD rosette, and a 5 l subsample is taken in a polyethylene flask and well stirred for homogenisation. A few millilitres of this subsample are withdrawn and

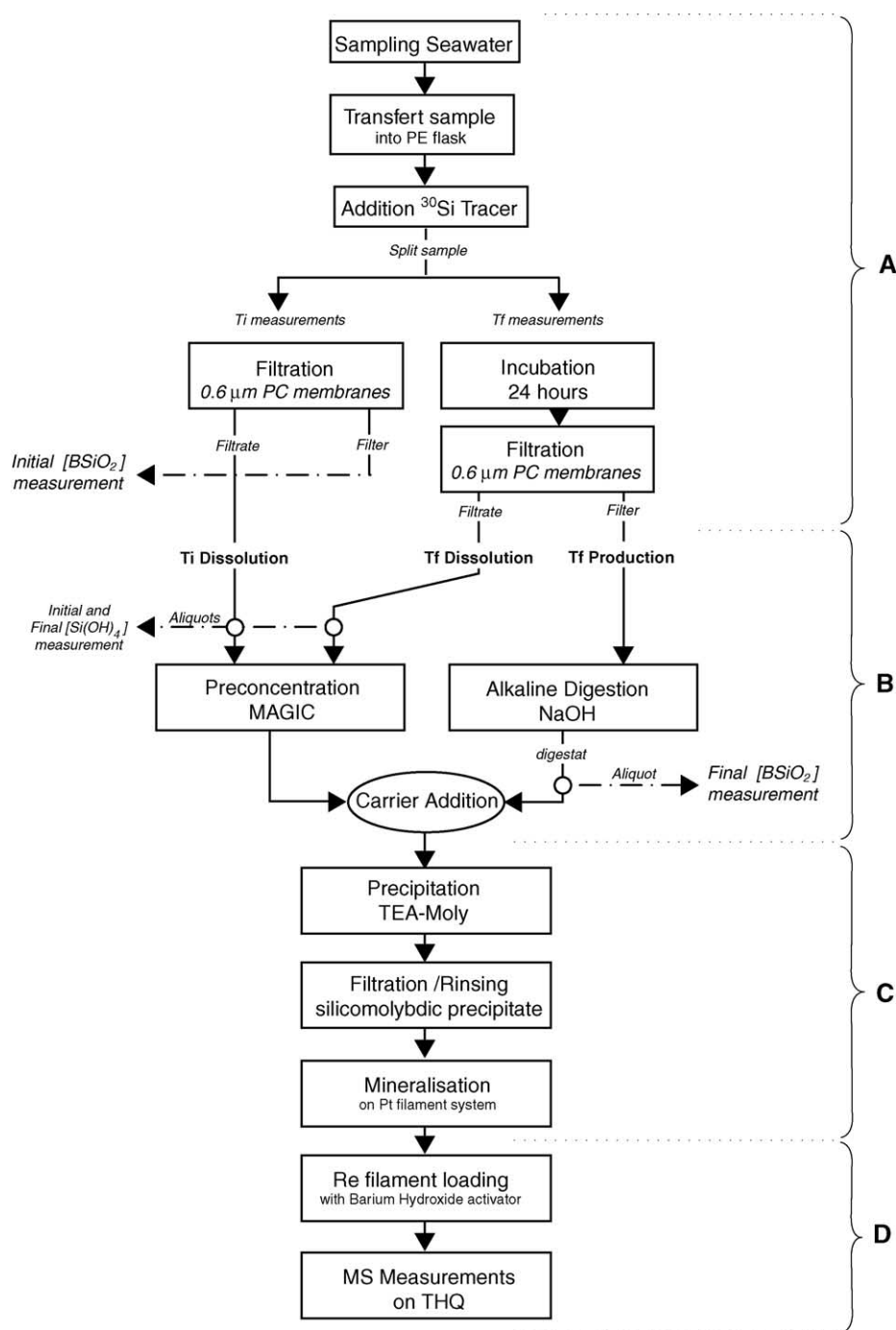


Fig. 1. Description of the whole procedure for the determination of the production and dissolution rates of biosilica in marine waters.

filtered on $0.6\ \mu\text{m}$ Nuclepore polycarbonate membrane for determination of the initial silicic acid concentration. Four litres are spiked with $^{30}\text{Si}(\text{OH})_4$ in the form of sodium silicate solution, and in amount that increase the in situ concentrations of silicic acid less than 10%.

Immediately after the spike addition, half of the 4 l subsample is filtered ($0.6\ \mu\text{m}$, PC membrane) for the determination of initial time variables. The membrane is dried at room temperature under a laminar flow hood and stored for fur-

ther determination of the initial biosilica concentration. The filtrate is stored in the dark at $+4\ ^\circ\text{C}$ for further determination of the silicic acid concentration and of the initial isotopic abundances.

The second half of the 4 l subsample, is poured into a polycarbonate flask and incubated in light conditions simulating those prevailing in situ. Usually the flask is put in a plexiglas incubator exposed to natural sunlight. The flask is fitted, when necessary, with nickel screen or neutral optical

filter to simulate the light attenuation of the sampling depth. The incubator temperature is regulated by circulating surface seawater. At the end of the incubation period (usually 24 h), the subsample is filtered and the filtrate and the filter stored as described above to determine the final time variables.

2.5. Digestion of biosilica (Fig. 1B)

The material collected on the polycarbonate membrane at initial time is used to determine the initial BSiO_2 concentration. It is digested with 0.2 M NaOH during 40 min at 100 °C according to Ragueneau and Tréguer [15]. When necessary (coastal waters) the apparent biosilica concentration in the alkaline digestat is corrected from the lithogenic silica contribution according to Ragueneau and Tréguer [15] in a second digestion with HF 2.9 M.

The particulate matter collected after the incubation undergoes the same protocol. An aliquot of the alkaline digestat is used to determine the final concentration of BSiO_2 . The remaining digestat is used to determine the Si isotopic composition of the biosilica after the incubation.

2.6. Pre-concentration of orthosilicic acid (Fig. 1B)

When Si(OH)_4 concentration in seawater is $<7 \mu\text{M}$ the filtrate needs to be pre-concentrated for allowing a quantitative precipitation with TEA-Moly reagent. This pre-concentration is achieved with a method adapted from MAGIC [13]: a quantitative scavenging of Si(OH)_4 by the brucite (Mg(OH)_2) precipitate is obtained by adding 1 volume of 1 M NaOH in 20 volumes of seawater. The precipitate is recovered by successive centrifugations, after removing the supernatant. The precipitate is then dissolved in pure 2 M HNO_3 (preferred to HCl because Cl^- ions perturb the SiO_2^- signal during mass spectrometry procedure).

2.7. Recovering and purification of silicon from orthosilicic acid by precipitation (Fig. 1C)

As pure silica is required for a suitable Si isotopes measurement, both Si(OH)_4 from seawater and Si(OH)_4 from the alkaline digestion of BSiO_2 (2.5) must be converted to pure silica. The conversion stands into two steps: first, the silicic acid is complexed and second, the precipitate is mineralised into pure silica (2.8). The first step is performed by adding 6 volumes of TEA-Moly reagent [16,14] to 10 volumes of this seawater or MAGIC Si acidified solution. When the total amount of Si(OH)_4 in the sample is smaller than the 1 μmol required for reaching optimal MS measurement conditions, an ad hoc amount of $^{29}\text{Si(OH)}_4$ carrier solution (solution prepared the same way as the $^{30}\text{Si(OH)}_4$ solution) can be added before precipitation. Nevertheless, increasing the volume of the seawater sample to obtain the minimal quantity of SiO_2 required, remains the most appropriate solution.

The silicomolybdc precipitate formed is filtered on a Nucleopore polycarbonate membrane (22 mm, 0.4 μm) and

rinsed with a diluted TEA-Moly reagent (37.5% with Milli-Q water) to remove salts. Filters are dried and stored at room temperature under a laminar flow hood.

2.8. Sample mineralisation (Fig. 1C)

As all previous steps, it is critical to perform the mineralisation of the silica in a clean atmosphere (under laminar flow hood). The filter containing the silico-molybdc precipitate is deposited on the centre of a HF cleaned platinum ribbon. The ribbon is progressively heated with a high intensity current, until reaching about 1100 °C. All the organic matter is eliminated by combustion and then molybdenum oxide is eliminated by sublimation. Thus a white residue of pure silica is obtained.

2.9. Mass spectrometer measurements (Fig. 1D)

Each pure silica sample is deposited on a Re filament. Beforehand, Re filaments (Rhenium Alloys Inc., USA; thickness 0.0381 mm, width 0.7112 mm) are outgassed at 4.5 A for 40 min, and left for about 1 week at ambient atmosphere under a laminar flow hood for re-oxidation. This process allows a better grip of the silica deposit. A suspension of the pure silica mineralised sample, in a few microlitres of MilliQ water is deposited and dried on the rhenium filament over a thin layer of Ba(OH)_2 (1 μl of 73 nM). As SiO_2 is difficult to ionise because of its very high ionisation potential (11.7 eV) [17], a Ba(OH)_2 activator is necessary to enhance and stabilise the SiO_2^- ions emission.

Two standards are measured for each set of samples. Standards (Na_2SiO_3 solutions), are loaded on the Re filament: 1 μl of 0.5 M Na_2SiO_3 solution over 1 μl of a 73 mM Ba(OH)_2 solution.

To get strong and long duration emission signals both for samples and standards, the amount of silica deposited on the Re filament has to be about 1 μmol , and the Ba/Si ratios between 0.6 and 1.5.

The Re filament is progressively heated through the following steps: 400 mA mn^{-1} until 2200 mA, 200 mA mn^{-1} until 2500 mA, 100 mA mn^{-1} until 2700 mA and finally 50 mA mn^{-1} until reaching ionic flux of 2×10^5 to 3×10^5 ions s^{-1} on the major isotope as recorded by the SEM. This final step corresponds to a filament temperature of about 1300 °C. The Si isotopes are measured as SiO_2^- ions at masses 60, 61, 62, thus giving 61/60 and 62/60 ratios. For accurate determinations of these isotopic ratios a total of 50 measurements gathered in five blocks is needed.

3. Results and discussion

3.1. Analytical precision

The analytical precision was determined for 30 successive deposits of 1 μmol of the Na_2SiO_3 standard solution.

Table 1
Isotopic ratios: standard deviation for 30 Na₂SiO₃ standard measurements

Ratio	Mean	1 S.D.	R.S.D. (%)
61/60	0.0513	0.0001	0.23
62/60	0.0372	0.0002	0.49

Measured ratios are converted to abundances as following:

$${}^{60}\text{A} = \frac{1}{1 + R_{61/60} + R_{62/60}}, \quad {}^{61}\text{A} = \frac{R_{61/60}}{1 + R_{61/60} + R_{62/60}},$$

$${}^{62}\text{A} = \frac{R_{62/60}}{1 + R_{61/60} + R_{62/60}} \quad (1)$$

The mean, absolute and relative standard deviations of the isotopic ratios ($R_{61/60}$ and $R_{62/60}$ being ${}^{61}(\text{SiO}_2)/{}^{60}(\text{SiO}_2)$ and ${}^{62}(\text{SiO}_2)/{}^{60}(\text{SiO}_2)$, respectively) are given in Table 1.

The corresponding abundance of the different Si isotopes (as SiO₂) are given in Table 2.

3.2. Blank determination

Because the mass spectrometer measures ionic fluxes of SiO₂⁻, our method allows the determination of the contamination of a given sample by lithogenic silica surreptitiously introduced during the different steps of the protocol. This determination is carried out using a two-step procedure. First step: 1 μm of the Na₂³⁰SiO₃ spike solution is directly deposited on Re filaments and run through the mass spectrometer for direct determination of the reference isotopic abundances A_{blank} . The contamination is assumed to be natural, for example coming from lithogenic aerosol (with natural isotopic abundances). The amount of contamination (hereafter called n_{natural}) represents the overall blank of the method. This blank is:

$$n_{\text{natural}} = n_{\text{spike}} \frac{A_{\text{spike}} - A_{\text{blank}}}{A_{\text{blank}} - A_{\text{natural}}} \quad (2)$$

where n_{spike} is the amount of spike solution (μmol), A_{spike} the isotopic abundance of the spike solution (atom%), n_{natural} the amount of contamination (μmol), A_{natural} the natural isotopic abundance of silica (atom%), A_{blank} the isotopic abundance of the blank (atom%).

The contamination of the blanks is always <20 nmol Si. The mean, determined on 30 blanks, is 9 nmol Si ± 11. Whatever the Si isotope introduced in the above equation, the amount of contaminant remained invariant, which confirms that the contamination was brought by natural lithogenic silica (and not by remnant of enriched material used for the isotopic dilution).

Table 2
Natural abundance of the three isotopes (Na₂SiO₃ standard solution)

Mass	Mean (atom%)	1 S.D. (atom%)	R.S.D. (%)
60	91.87	0.02	0.02
61	4.72	0.01	0.2
62	3.42	0.02	0.47

Previous experiments showed that the main source of contamination came from the mineralisation step. So, different combustion methods have been tested, going from combustion in platinum crucibles in classical furnaces to a sophisticated CO₂ laser system. The lowest contamination level was obtained for a combustion performed on a platinum ribbon (cf. 2.8).

3.3. Repeatability of the rates of production and dissolution

The overall repeatability of the rate of production was estimated for a sample of seawater collected in the bay of Brest in April 2002, i.e. during a period when the production usually overwhelmed the dissolution rate and the dissolution rate is usually at average level in this coastal ecosystem [18]. Forty litres of surface seawater (Si(OH)₄ = 3.45 μM; BSiO₂ = 1.17 μM), collected using a 51 Niskin bottle, were poured in a plastic carboy. After addition of the tracer and homogenisation these 40 l were split in eight subsamples. These eight subsamples underwent exactly the same treatment, from incubation to isotopic measurements. For uptake rate, the mean is 0.23 ± 0.02 μM day⁻¹. For the dissolution rate, the mean is 0.07 ± 0.01 μM day⁻¹. These standard deviations are reasonable, given the usual temporal and/or spatial variability of the production and dissolution rates in coastal and/or open ocean ecosystems [18].

3.4. Weighted least squares estimation of production and dissolution rates

In contrast to the previous approach based on two simplified equations from Nelson and Goering [19,4], the biosilica production and dissolution rates can be obtained with a two-compartment model (Fig. 2). This is a class of models for which the governing law is conservation of mass. Compartmental models are also lumped parameter models because the system is described by a finite number of changing variables, and thus can be described by ordinary differential equations:

$$\begin{aligned} \frac{d\text{Si(OH)}_4}{dt} &= \rho_D - \rho_P, & \frac{dA_d}{dt} &= \frac{A_{d(f)}\rho_D}{\text{Si(OH)}_4}, \\ \frac{d\text{BSiO}_2}{dt} &= \rho_P - \rho_D, & \frac{dA_p}{dt} &= \frac{(A_d - A_p)\rho_P + A_p\rho_D}{\text{BSiO}_2} \end{aligned} \quad (3)$$

where the variables Si(OH)₄, BSiO₂, A_d , and A_p are the concentrations and isotopic enrichments in the dissolved and particulate phase, respectively, and where (ρ_P , ρ_D) denote the

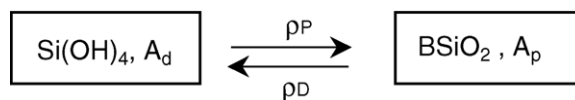


Fig. 2. Compartmental model used for the estimation of the biosilica production and dissolution rates.

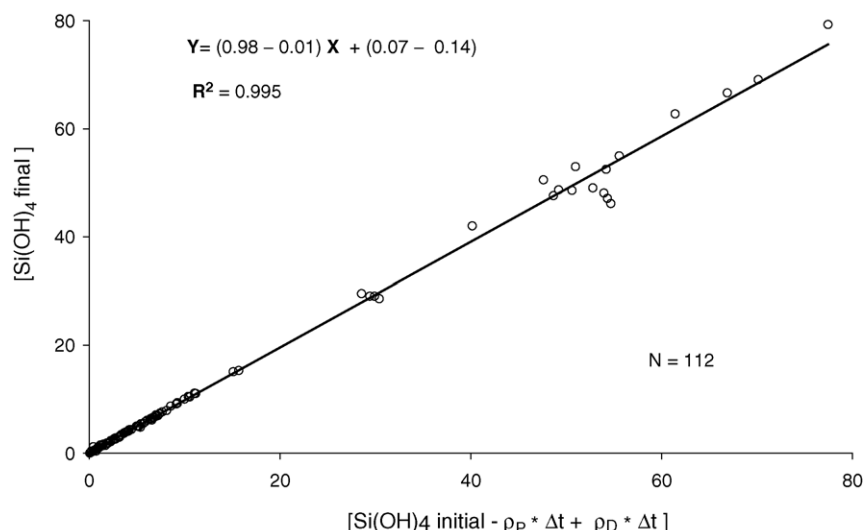


Fig. 3. Constraining the determination of the rate of production and dissolution of biosilica by mass and isotopic balances. Slope and ordinate (\pm S.E.) of the linear regression are shown in the figure.

model parameters to be optimized, i.e. the production and dissolution rates. Important features of Eq. (3) are that all Si that leaves the dissolved phase is assumed to appear as particulate biosilica (production), and that Si recycling (dissolution) is regarded as process that transfers Si from the particulate to the dissolved pool.

Straightforward integration of the differential equations corresponding to Eq. (3) yields the following balances for concentrations and isotopic enrichments:

$$\text{Si(OH)}_{4(f)} = \text{Si(OH)}_{4(i)} + (\rho_D - \rho_P)\Delta t,$$

$$A_{d(f)} = A_{d(i)} \left(1 + \frac{\rho_D - \rho_P}{\text{Si(OH)}_{4(i)}} \Delta t \right)^{\rho_D/(\rho_P - \rho_D)},$$

$$\text{BSiO}_{2(f)} = \text{BSiO}_{2(i)} + (\rho_P - \rho_D)\Delta t,$$

$$A_{P(f)} = \frac{\text{Si(OH)}_{4(i)} A_{d(i)}}{\text{BSiO}_{2(i)} + (\rho_P - \rho_D)\Delta t} \times \left(1 - \left(1 + \frac{\rho_D - \rho_P}{\text{Si(OH)}_{4(i)}} \Delta t \right)^{\rho_D/(\rho_P - \rho_D)} \right) \quad (4)$$

where subscript ‘i’ and ‘f’ refer to the initial and final times of the incubation.

Production and dissolution rates (ρ_P , ρ_D) are estimated with a weighted least-squares technique, and the uncertainty analysis of the least squares estimation was performed with Monte-Carlo simulations [20]. Because there are random and systematic variations, which are responsible for differences between the observations and the model counterparts, the best estimates for ρ_P and ρ_D are those values that minimize all four equations simultaneously, instead of those solving one or two equations analytically as proposed by Nelson and Goering. The advantage of using a weighted least squares cost function for such optimisation problems and the comparison with the previous method will be further discussed [9].

3.5. Applying this method to coastal and open ocean waters

This method has been used for the determination of the rates of production and dissolution in coastal waters (Bay of Brest) and in open ocean waters (Southern Ocean). The total number of samples was 112. The production rate ranged between 0.00 and 2.38 $\mu\text{M day}^{-1}$ and the dissolution rate between 0.00 and 1.18 $\mu\text{M day}^{-1}$ [18,8].

In order to validate the calculations of ρ_P and ρ_D (cf. 3.5) we calculated the mass balance on silicic acid in each incubated polycarbonate flask using the data extracted from the model described above, and the concentrations of silicic acid measured on three replicates (precision 0.04 μM). Δt being the time of incubation, the values of $[\text{Si(OH)}_4 \text{ final}]$ are plotted versus $[\text{Si(OH)}_4 \text{ initial} - \rho_P \Delta t + \rho_D \Delta t]$ in Fig. 3. Measured $[\text{Si(OH)}_4 \text{ final}]$ is linearly correlated to the calculated value ($R^2 = 0.995$, $n = 112$). The slope is 0.98, i.e., very close to 1 as expected.

4. Conclusion

This method is suitable for a precise determination of the production and the dissolution rates of biosilica in marine systems. The analytical precision of the isotopic ratios measurements given by the TIMS is better than 1%. The standard deviation for the whole method (seawater sampling + analytical procedure + mass spectrometry) is 0.02 $\mu\text{M day}^{-1}$ for the production and 0.01 $\mu\text{M day}^{-1}$ for the dissolution rate.

This methods contrast to previous ones [3,4]. First, because by measuring the SiO_2^- ions the TIMS method allows the determination of potential contamination of samples during the whole protocol: the blank is 9 nmol Si. Secondly, because we provide a better constraint of the biosilica production and dissolution rates as the rates are estimated by

the requirement to fit mass and isotopic balances, i.e. four equations and only two unknowns.

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