



## A new method for the measurement of biogenic silica in suspended matter of coastal waters: using Si:Al ratios to correct for the mineral interference

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### Abstract

Measurement of biogenic silica (BSi) in the suspended matter of estuarine and coastal waters is not trivial because of the interference of lithogenic silica (LSi) that can represent up to 90% or more of the total particulate silica within the water column. Till date, no method has provided a satisfying way to correct this mineral interference when the most common wet-alkaline digestions are used. The most recently published method, Ragueneau and Tréguer (*Mar. Chem.*, 45 (1994) 43–51), presents the disadvantage to be site-specific. In this paper, we present a robust method to measure BSi in all estuarine and coastal waters and correction for LSi interference. It consists of a double wet-alkaline digestion where the filter sample is submitted to a first digestion (0.2 M NaOH, pH 13.3) at 100 °C for 40 min. At the end of this first leach, all the BSi and part of the LSi have been converted into Si(OH)<sub>4</sub>. Si and Al concentrations ([Si]<sub>1</sub> and [Al]<sub>1</sub>) in the supernatant are analyzed. After rinsing and drying, the filter is submitted to a second digestion, exactly identical to the first one, leading to the determination of the (Si:Al)<sub>2</sub> ratio that is characteristic of the silicate minerals present in the sample. The corrected biogenic silica concentration is thus given by  $[BSi] = [Si]_1 - [Al]_1(Si:Al)_2$ .

There are three basic assumptions behind the proposed method: (1) all the Al measured during the first leach is derived from silicate minerals, (2) all the biogenic silica has been digested during the first leach, so the second leach addresses the Si:Al ratio of silicate minerals only, and (3) silicate minerals dissolve during the second digestion exactly as during the first digestion, i.e. with a similar Si:Al ratio. Theoretical considerations and field experiments demonstrate that the above assumptions are accurate.

Blanks and repeatability tests indicate that the detection limit ranges from 0.6 to 1.5 nmol of BSi present on the filter and that the uncertainty is close to 10%. This method was successfully applied to more than 200 samples from five

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different coastal areas, demonstrating the importance of this correction, which can represent up to 92% of the apparent BSi concentration determined after the first digestion.

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## 1. Introduction

Biogenic silica (BSi) is produced in surface waters of the world's oceans mostly by diatoms, and also by radiolarian and silicoflagellate (Litszin, 1972; Nelson et al., 1995). Because of the role played by diatoms in carbon export, either towards higher trophic levels (Cushing, 1989) or the deep sea (Buesseler, 1998), there has been a major increase in the number of studies focusing on the Si cycle in the last two decades (review in Ragueneau et al., 2000). Accurate measurements of BSi standing stocks and fluxes, are a prerequisite for a good understanding of the aquatic Si cycle. The measurement of Si production and dissolution fluxes has undergone significant progress, owing to the development of the  $^{32}\text{Si}$  radioactive isotope technique (Tréguer et al., 1991), the  $^{30}\text{Si}$  stable isotope technique (Beucher et al., *in revision*), and the application of flow-through reactors to BSi (van Cappellen and Qiu, 1997). Surprisingly, the measurement of BSi standing stocks has remained very difficult in sediments and in the suspended matter of surface waters, especially those enriched with silicate minerals, i.e. estuarine and coastal waters.

Si plays an essential role in the functioning of coastal ecosystems, especially since N and P delivery have increased (Nixon et al., 1996; Howarth et al., 1996), and Si delivery has decreased because of eutrophication (Conley et al., 1993) and river manipulation (Humborg et al., 1997). These factors sometimes combine, leading to decreasing Si:N and Si:P ratios in the receiving coastal waters and Si limitation of diatom growth (Del Amo et al., 1997; Ragueneau et al., 2002a), with important implications in terms of coastal food webs (Officer and Ryther, 1980; Smayda, 1990; Turner et al., 1998). Recent studies have shown the poor ability of sole-nutrient mixing diagrams to describe the processes really

occurring along the land-ocean continuum (Ragueneau et al., 2002b). Measurements of BSi standing stocks, production and dissolution are required from the river basins to the coastal ocean to understand better the modifications of the Si cycle during its path from the watersheds to the ocean, and the consequences for: (1) the functioning of the coastal ecosystems, and (2) the global Si and C biogeochemical cycles. Unfortunately, it is in rivers and coastal waters that complications arise in the measurement of BSi standing stocks, because of the interference due to the presence of silicate minerals in high quantities (Ragueneau and Tréguer, 1994).

Before the early 1980s, numerous approaches attempted to distinguish between biogenic and lithogenic silica (LSi) in marine sediments (review in Eggimann et al., 1980). Today, most oceanographers use wet-chemical digestion methods for this purpose, as attested by a recent interlaboratory comparison of the measurement of BSi (Conley, 1998). Several studies have compared the efficiency of the extraction of BSi using different alkaline leaching solutions, be it in the sediments (Eggimann et al., 1980; Conley, 1998; Schlüter and Rickert, 1998) or in suspended matter (Krause et al., 1983). The difficulty of the BSi measurement resides not only in the extraction efficiency of BSi, which must be complete, but also in the correction for the dissolution of co-existing aluminosilicates (LSi). Several approaches have been attempted, including calibration curves constructed from mixtures of typical clay minerals and BSi (Hurd, 1973), single-step extraction with germanium analyses in the alkaline solution (Mortlock and Froelich, 1989), the use of  $\text{SiO}_2/\text{Al}_2\text{O}_3$  ratio of terrigenous silicates (Eggimann et al., 1980; Kamatani and Takano, 1984) or sequential extraction with back-extrapolation of the linear section of the extraction curve (DeMaster, 1981), following the idea that silicate minerals do dissolve

linearly with time (McKeys et al., 1974). For many years, the latter technique has been of wide use in marine sediments. Because the correction depends greatly upon the accurate determination of the slope at which silicate minerals dissolve (Conley, 1998), Müller and Schneider (1993) developed an automated method for continuous monitoring of the increasing  $\text{Si}(\text{OH})_4$  concentration in the leachate.

Recently, several articles have been published proposing different approaches, all based on the use of Si:Al ratios to correct the mineral interference in the measurement of BSi in marine sediments (Schlüter and Rickert, 1998; Kamatani and Oku, 2000; Köning et al., 2002). Silicate minerals do not necessarily dissolve linearly (Huertas et al., 1999; Kamatani and Oku, 2000), so the interpretation of sequential extractions based on Si measurements only can remain ambiguous (DeMaster, 1981), especially when the LSi:BSi ratio is high. To overcome this problem, Kamatani and Oku (2000) proposed to measure both Si and Al during a sequential extraction, with a low sampling resolution (20-min intervals). Köning et al. (2002) demonstrated the need for a better accuracy in the early steps of the digestion and used a much higher sampling resolution (1-s intervals), a technique that needs to be automated following the method proposed by Müller and Schneider (1993). Note that all these articles deal with the measurement of BSi in sediments.

The present article will address the use of Si:Al ratios for the determination of BSi concentrations in the suspended matter of estuarine and coastal waters, where the load of silicate minerals is high and the use of sequential extractions is difficult, since replicates are necessary. Ragueneau and Tréguer (1994) used a double extraction method described by Brzezinski and Nelson (1989) to separate BSi and LSi and proposed a method to correct for the mineral interference in these ecosystems. This correction makes use of a linear relationship found between apparent BSi and LSi concentrations (i.e. before correction), obtained during winter when BSi is minimum. This method presents several disadvantages, including the need to calibrate a relationship between winter LSi and BSi concentrations for a given site, and the

assumption that this curve is valid year-round for this site. Its advantage, however, is its simplicity, which must be a constant objective if we are to suggest that silicic acid and BSi concentrations become included in coastal monitoring programmes and that more Si budgets be determined for continental margins (Ragueneau et al., submitted). The method of Ragueneau and Tréguer (1994) has thus been modified to allow for a correction using Si:Al ratios, with the objective of gaining robustness and applicability without losing too much simplicity. For this purpose, we show that a double digestion with a single determination of Si and Al concentrations during each digestion is sufficient to provide BSi measurements with an uncertainty close to 10%.

## 2. Material and methods

### 2.1. Chemical extractions

The modification of the method described by Ragueneau and Tréguer (1994) consists in adding an intermediate leach, strictly identical to the first one, devoted to the determination of the Si:Al ratio characteristic of the silicate minerals dissolving under the experimental conditions proposed (Fig. 1).

The first part of the alkaline digestion is identical to the one described in Ragueneau and Tréguer (1994). A polycarbonate membrane, 0.6  $\mu\text{m}$  porosity, 47-mm diameter, is submitted to a first digestion with 4.0 ml of 0.2 M NaOH (pH 13.3) in a polymethylpentene centrifuge tube at 100 °C for 40 min. After cooling, 1.0 ml of 1.0 M HCl is added to stop the digestion by neutralizing the pH. Subsequent centrifugation for 10 min at 2500 rpm allows the separation of the supernatant from the remaining suspended material. Using a volumetric pipette, 0.5 ml of the supernatant is withdrawn and diluted to 10 ml with de-ionized water (DIW), for subsequent silicic acid analysis ( $[\text{Si}]_1$ ) corresponding to an apparent BSi concentration. Two milliliters is also withdrawn and diluted to 10 ml with DIW in another tube for subsequent determination of Al concentrations ( $[\text{Al}]_1$ ). Note that the extent of these dilutions

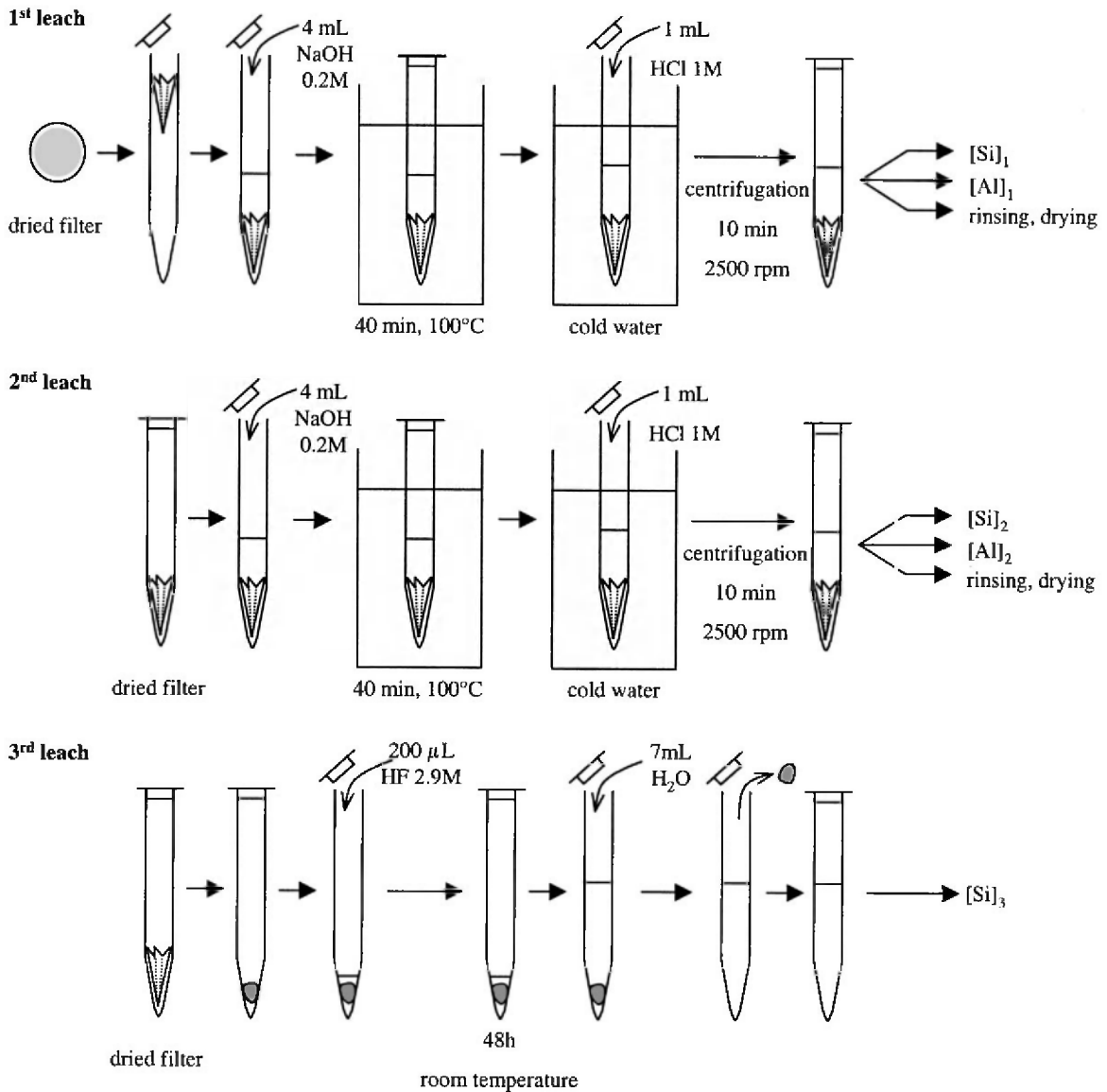


Fig. 1. Synopsis of the experimental protocol for the determination of biogenic and lithogenic silica in suspended matter of coastal ecosystems. Note that during the third leach, a saturated aqueous boric acid solution can be used instead of DIW to negate the interference of the HF with subsequent reactions (Brzezinski and Nelson, 1995), to increase the sensitivity of LSi measurements.

should be adapted to the samples studied and the range of standards in use.

At the end of this first leach, it is clear that all the BSi has been converted into  $\text{Si}(\text{OH})_4$  (Brzezinski and Nelson, 1989); the filter, suspended silicate minerals and 2.5 ml of the supernatant

remain in the polymethylpentene tube. Three rinsing operations are then performed. During each, DIW is added to 13 ml, the tube is shaken, centrifuged, and 10.5 ml are withdrawn. This stage is very important, because if a sample has a high BSi:LSi ratio, even a small amount of silicic acid

remaining in the tube will affect the determination of the Si:Al ratio of the silicate minerals present in the tube. After the rinsing stage, the 2.5 ml of filter and liquid are dried at 60 °C with the help of clean air circulation on top of the supernatant.

The second leach is identical to the first leach described above (4.0 ml of 0.2 M NaOH, 100 °C for 40 min).  $[\text{Si}]_2$  and  $[\text{Al}]_2$  concentrations in the supernatant are measured and the  $(\text{Si:Al})_2$  ratio is determined. This ratio is characteristic of the silicate minerals present in the sample, as all the BSi dissolved during the first leach. Multiplying this ratio and the Al concentration measured in the first leach ( $[\text{Al}]_1$ ) allows the quantification of  $\text{Si}(\text{OH})_4$  extracted from silicate minerals during the first leach. The corrected BSi concentration is thus given by

$$[\text{BSi}] = [\text{Si}]_1 - [\text{Al}]_1^* (\text{Si} : \text{Al})_2. \quad (1)$$

After the second leach, a third leach can be performed if the total particulate silica needs to be measured. This third leach allows the dissolution of all the remaining detrital silica. No Al measurement is needed during this digestion, which simply aims at providing a good sense of the total amount of silicate minerals present in the sample. This third leach is identical to the second leach in Ragueneau and Tréguer (1994): 0.2 ml of 2.9 M HF is added to the dried filter (Fig. 1). The filter is compressed until all air bubbles have been removed. The tube is then tightly covered and silicate minerals are digested for 48 h. At the end of this digestion, DIW is added so that the HF concentration in the final solution is lower than 0.002 M (Eggemann and Betzer, 1976), and silicic acid is measured ( $[\text{Si}]_3$ ). Note that the use of boric acid instead of DIW increases the sensitivity of LSi measurements (Brzezinski and Nelson, 1995), which becomes useful in open ocean waters. The corrected concentration of silicate minerals in the sample is given by the sum of the three contributions from the three leaches:

$$[\text{LSi}] = [\text{Si}]_3 + [\text{Si}]_2 + ([\text{Si}]_1 - [\text{BSi}]). \quad (2)$$

For the first two leaches,  $\text{Si}(\text{OH})_4$  is analysed by the automated acid/molybdate colorimetric method described by Gordon et al. (1993), using an

auto-analyser Alpkem RFA-300 (precision: 1%). For the last leach, because of the presence of HF,  $\text{Si}(\text{OH})_4$  is analysed manually according to Mullin and Riley (1965) modified by Tréguer and Le Corre (1975), with a precision of 2%.

Al concentration in this study was measured by means of ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy, ISA JOBIN-YVON JY 70 PLUS), at 396.152 nm, with a series of six standard solutions of known Al concentrations (0.2, 0.5, 1, 2, 5 and 10  $\mu\text{M}$ ), prepared in the same medium as the samples: the relative volumetric proportions of NaOH, HCl and  $\text{H}_2\text{O}$  were 4/1/20, respectively. The standard deviation (SD) was 1–2 ppb Al. Assumed to be 3 SD of low values, the detection limit of this method is 0.15  $\mu\text{mol Al l}^{-1}$ . The need for Al measurements on each sample using ICP-AES could be seen as an impediment for the adoption of the proposed method. Note, however, that there exist other means of measuring Al concentrations in the leachates which are sensitive enough for our purposes, such as graphite furnace atomic absorption or the colorimetric method used by Kamatani and Oku (2000), which should make this method available to most investigators.

## 2.2. Repetability, blanks and detection limit

Fifteen litres of seawater was sampled in the Bay of Brest (France) during March 1999, and filtered in 1-l aliquots. The 15 replicates of BSi were analysed as described above in order to evaluate the precision of the analysis. Results demonstrated the good reproducibility of the corrected BSi ( $1.09 \pm 0.12 \mu\text{mol l}^{-1}$ , i.e.  $\pm 11\%$ ) and LSi ( $4.83 \pm 0.33 \mu\text{mol l}^{-1}$ , i.e.  $\pm 7\%$ ) concentrations as well as Si:Al ratios ( $2.21 \pm 0.16$ ).

During each series of samples, including this repeatability experiment, three blank measurements were performed by applying the protocol described in Fig. 1 to three filters with no seawater filtered. Blank values usually ranged between 2 and 5 nmol, with standard deviations from 0.2 to 0.5 nmol, implying a range of detection limits between 0.6 and 1.5 nmol of BSi present on the filter.

### 3. Discussion

#### 3.1. Testing three underlying assumptions

There are three basic assumptions behind the proposed mode of correction of the mineral interference (Eqs. (1) and (2)). The first assumption is that all the Al measured during the first leach is derived from silicate minerals, with negligible influence from the BSi. The second assumption is that all the biogenic silica has been digested during the first leach, so the second leach really addresses the Si:Al ratio of silicate minerals only. The third assumption is that silicate minerals dissolve during the first and second digestions with a similar Si:Al ratio.

##### 3.1.1. Diatom-Al is negligible

The Al content of living diatoms generally is less than 1% (Lewin, 1961; Shemesh et al., 1988; van Bennekom et al., 1989). As a correction is needed when the BSi:LSi ratio is low (see below, Fig. 5), the interference from diatom-Al is really negligible in comparison to the Al extracted from silicate minerals (Schlüter and Rickert, 1998). This assumption may be violated when resuspended particles account for an important fraction of the suspended matter collected in the water column. Al can be passively incorporated in biogenic silica particles immediately after deposition at the sediment–water interface (van Cappellen and Qiu, 1997; Dixit et al., 2001), leading to an enrichment of BSi in Al. Typically, the Si:Al ratio of diatoms in sediments is close to 20 (van Bennekom et al., 1988) or 40 (Hurd, 1973), as observed in the Bering Sea and the Weber Deep in Indonesia, respectively. These ratios, however, are one order of magnitude higher than corresponding Si:Al ratios in silicate minerals, suggesting that most often, even in the presence of resuspended particles, the assumption that most of the Al comes from the minerals is reasonable. Note that for open ocean waters, diatom-derived Al may lead to overestimation of LSi concentrations when these are extremely low.

##### 3.1.2. Complete digestion of BSi

To ensure that the remains of BSi at the end of the first leach do not interfere in the determination

of the Si:Al ratio of the silicate minerals during the second leach, sequential extractions have been carried out over a wide range of apparent (i.e. before correction) BSi concentrations and BSi:LSi ratios. This has been achieved by filtering 250 ml of water collected along a salinity gradient in the Aulne estuary, a tributary of the Bay of Brest, France. At low salinity (0 and 5), where the apparent BSi concentration was supposed to be the highest, both 250 and 50 ml have been filtered for comparison of the digestion efficiency with variable amounts of BSi on the filters. At each sampling station, four samples have been collected in April 2000, allowing sequential extractions to be conducted with 4 digestion times of 12, 25, 40 and 90 min. The aim of this experiment was also to ensure that the length of 40 min (Ragueneau and Tréguer, 1994) is appropriate within the entire range of BSi quantities on the filters. The sequential extractions are shown in Fig. 2 and the (Si:Al)<sub>2</sub> ratios are reported on Fig. 3, as a function of the amount of BSi on the filters.

This experiment shows that the Si:Al ratio decreases with increasing digestion times from 12 to 25 and 40 min (Fig. 3). It then stabilises between 40 and 90 min, at a value close to 1.6. This value is close to the mean Si:Al ratios measured in the Bay of Brest between April and October 1993 (mean at 1.8, range from 1.4 to 2.1), where dominant silicate minerals are, in order of their relative importance, illite, chlorite and kaolinite (Bassoulet, 1979). Note

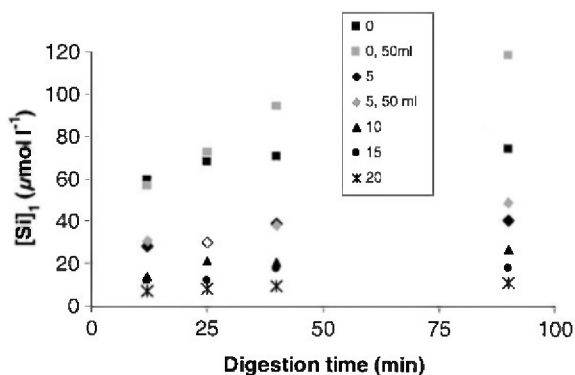


Fig. 2. Time course experiment carried out on samples collected at various salinities in the Aulne estuary (Bay of Brest, France). The legend indicates the salinity and the filtration volume when different from 250 ml.

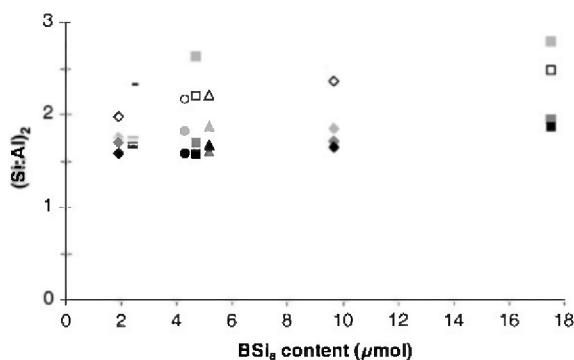


Fig. 3. Si:Al ratio measured during the second leach (Fig. 1), versus the apparent BSi ( $[Si]_1$ ) content on the filter for the Aulne estuary. Squares, diamonds, triangles, circles and dashes correspond to salinity 0, 5, 10, 15 and 20, respectively. Open symbols, light grey, dark grey and black symbols correspond to a digestion time increasing from 12 to 25, 40 and 90 min, respectively.

that the Si:Al molar ratios of these three minerals are comprised between 0.8 and 2.1 (Degens, 1965; Weaver and Pollard, 1973; Kamatani and Oku, 2000). The good correspondence between the theoretical and measured Si:Al ratios gives confidence to the protocol proposed and suggests that Si and Al are dissolved during the alkaline digestion with a ratio close to the crustal ratio.

For any given BSi concentration, the Si:Al ratio measured after 40 min appears to be stable and representative of silicate minerals. This suggests that over the range of BSi concentrations tested, a 40 min digestion is necessary and sufficient to dissolve all the BSi during the first leach and produce a reproducible Si:Al ratio during the second leach, characteristic of the silicate minerals present in the suspended matter, with one exception. The sample presenting the highest amount of BSi on the filter (17.5  $\mu\text{mol}$ ), encountered at salinity 0, had a  $(Si:Al)_2$  ratio which did not decrease down to 1.6, as at other salinities, even with a 90 min digestion (Fig. 3). Conversely, with the 50 ml 0 salinity sample, a 40 min digestion resulted in a  $(Si:Al)_2$  ratio similar to other samples in the salinity gradient. This comparison suggests that some BSi remained in the tube at the end of the first leach. The incomplete dissolution of the salinity 0 sample (250 ml filtered), is clearly seen in

Fig. 2. For this sample, there is a large departure in the apparent BSi concentration, depending on the amount of water filtered. For comparison, the apparent BSi concentration for samples at salinity 5 is similar, whether 50 or 250 ml has been filtered, which is expected in case of complete dissolution of the BSi. In the conditions of our experiment, 10  $\mu\text{mol}$  of BSi initially present on the filter (sample of salinity 5, 250 ml filtered) are completely dissolved whereas 17  $\mu\text{mol}$  (sample of salinity 0, 250 ml filtered) are not (Fig. 2). Because we have no data point between 10 and 17  $\mu\text{mol}$  of BSi present on the filter, we can conclude that 40 min are sufficient to completely digest 10  $\mu\text{mol}$  of BSi present on the filter.

It is difficult at this stage to state on the origin of the incomplete dissolution of BSi at 0 salinity. From a quantitative point of view, a problem may arise if the solubility limit of the BSi was reached during the first leach and further dissolution of the BSi would be prevented. However, the alkaline digestions are performed under pH and temperature conditions that preclude such a problem (Hurd, 1973; van Cappellen and Qiu, 1997). From a qualitative point of view, several problems may also arise. The first one is the way in which the water and solid phase have been separated. Paasche (1980) has shown that complete hydrolysis is possible, even with high amounts of BSi, if the sample is gently filtered and particles are well spread out on a filter. Experience has proven that digestion of biogenic material concentrated by centrifugation may not be complete, even after several hours (Gallinari, Personal Communication) and even if the amount of digestible BSi remains reasonable. Filtration is thus recommended for the initial separation of the particles and the water.

It is thus reasonable to conclude that all the BSi has been dissolved during the first leach of the proposed method (Fig. 1), provided that (1) separation of particulate and dissolved phases is performed using gentle filtration and not centrifugation, and (2) the amount of BSi present on the filter remains below a reasonable value of ca. 10  $\mu\text{mol}$ . This range, from 0 to 10  $\mu\text{mol}$  of BSi present on the filter, covers most of the regions of interest, including riverine environment (Conley,

1997), given that one can remain in this range by simply filtering appropriate volumes of water.

### 3.1.3. Similar mineral dissolution during digestions 1 and 2

Fig. 3 demonstrates that  $(\text{Si}:\text{Al})_2$  remains constant between 40 and 90 min, at a value close to the crustal ratio. This is important, because it shows that (a) the protocol proposed is accurate enough to measure such a ratio with a reasonable reproducibility, and (b) beyond 40 min, when all the BSi has been dissolved, the Si:Al ratio does not change any more. Unfortunately, it does not provide any information on the initial stages of the dissolution process during the first digestion. In fact, several mechanisms could lead to different dissolution ratios between the two digestions. The silicate mineral dissolution typically proceeds in a non-linear manner at the beginning of an experiment, followed by a linear dissolution process (Huertas et al., 1999; Kamatani and Oku, 2000; Köning et al., 2002). The high initial dissolution rate, which could occur during the first digestion and not during the second, would be the result of different processes such as dissolution of fine-grained materials, highly strained areas on large grains or defects (Chou and Wollast, 1984, 1985; Knauss and Wolery, 1988, 1989). Dissolution can be non-linear but still remain congruent, as observed for kaolinite at pH 12 by Huertas et al. (1999). In this case, it does not interfere in our experiment because the rapid initial dissolution would be accounted for by our measurement of Al concentrations during the first digestion. A problem would arise from non-stoichiometric dissolution of Si and Al from the minerals, which would be different during the two digestions.

Non-stoichiometry is typically explained by two different mechanisms. The first one is an incongruent dissolution resulting from the formation of a residual phase at the surface of the silicate minerals, which would have a different Si:Al ratio than the bulk mineral. The second one is a congruent dissolution with rapid precipitation of secondary phases such as aluminium hydroxyde. Aluminium hydroxydes may be formed at the surface of the silicate minerals, being either amorphous or poorly crystallized (Cozarelli

et al., 1987; Swoboda-Colberg and Drever, 1993). There is no way that in the experimental conditions proposed (pH 13.3, 100 °C), precipitation of aluminium hydroxydes occurs. Precipitation typically occurs at pH between 5 and 10 (Huertas et al., 1999) but not at pH 13.3. Even at the highest Al concentrations in our digestion tubes, we always remain far from saturation with respect to such phases, thereby preventing precipitation (Ganor et al., 1995; Devidal et al., 1997; Huertas et al., 1999).

A problem could arise if a residual phase was formed in the field (pH 7–8), prior to sampling, on silicate mineral surfaces, leading to incongruent dissolution (i.e. preferential dissolution of the residual phase during the first minutes of the first NaOH leach). However, for the dissolution to occur differently during the two digestions, this phase would have to be either very reactive or very thin, to have entirely disappeared during the first digestion. If very reactive, there are chances that such a phase could be gone in the field prior to sampling. If very thin, there are chances that our Si:Al measurements reflect more the bulk mineral than this surface residual phase, because the digestion proposed is very strong and attacks not only the mineral surfaces but also the bulk mineral (Wollast, personal communication).

To conclude, the hypothesis that the bulk of the silicate minerals dissolves similarly during both digestions appears reasonable under the experimental conditions proposed. Unfortunately, one must admit that it will never be proven with this method because (1) there is BSi dissolving during the first digestion, (2) commercial silicate minerals are not necessarily good analogues to minerals collected in the field and (3) performing successive digestions after all BSi is gone (such as between 40 and 90 min in Fig. 3), to study the dissolution of silicate minerals only, will not provide information on the very initial Si:Al dissolution ratio.

### 3.2. Validation of the protocol

The proposed protocol has been validated by performing a time course experiment on a sample collected during summer 1995 at the mouth of the river Danube, on the northwestern shelf of the

Black Sea. Eight filters of the same sample have been submitted to various digestion times, ranging from 2 to 40 min (Fig. 4). The apparent BSi concentration measured after 40 min was  $5.93 \mu\text{mol l}^{-1}$ . Applying a slope correction following DeMaster (1981) yields a corrected BSi

concentration of  $5.43 \mu\text{mol l}^{-1}$ . The proposed protocol, much easier to handle than a time-course experiment, yields a corrected BSi concentration of  $5.49 \mu\text{mol l}^{-1}$ , in excellent agreement with the time course correction ( $(\text{Si:Al})_2 = 2.78$ , see legend of Fig. 4).

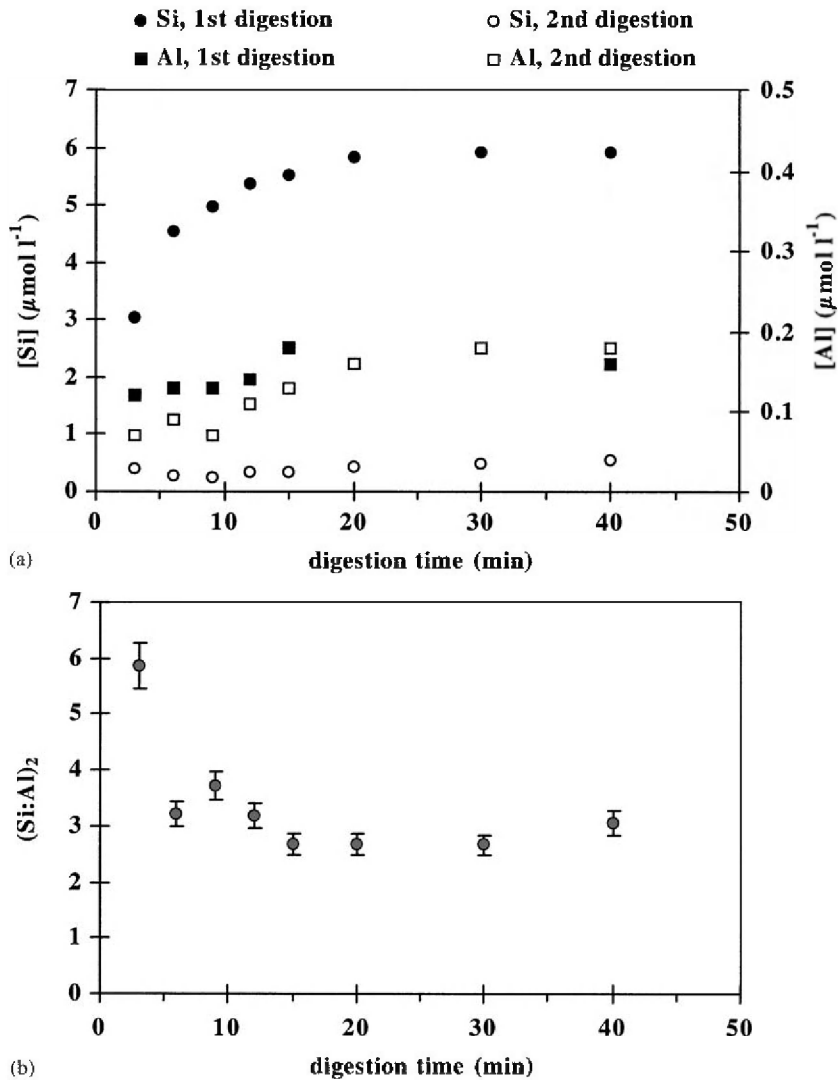


Fig. 4. Time course experiment conducted on a sample collected in the northwestern Black Sea during summer 1995. (a) Si (circles) and Al (squares) concentrations measured during the first (black symbols) and second (open symbols) digestions. (b) Si:Al molar ratio, as measured during the second digestion. Note that this ratio is constant ( $2.78 \pm 0.19$ ) after only 15 min, showing that for this sample, the digestion of BSi was completed within 15 min (see Fig. 3 for comparison). Error bars in (b) correspond to 7%, i.e. the repeatability on Si:Al ratios (see text).

### 3.3. Applicability of the method and extent of the correction

The method proposed herein has been tested in various coastal waters, namely the Bay of Brest, the Bay of Seine, the North sea, the Danube Delta, the Danube Plume in the Northwestern Black Sea and the Kerguelen Plateau in the Southern Ocean. Table 1 shows a representative sample of 52 data points among the 239 data of the complete data set collected in these areas. It is seen that  $(\text{Si}:\text{Al})_2$  varies between 1.4 and 5.4, with a mean value at  $2.97 \pm 1.10$ . This variability is not an experimental artefact due to incomplete digestion of BSi during the first leach, because the BSi amount on filters always remained largely below  $10 \mu\text{mol}$  (see the section on “complete digestion of BSi”). It implies that the Si:Al ratio has to be measured on each single sample. This clearly adds difficulty in the measurement, but this internal calibration also brings robustness. The corrected BSi concentrations can be much lower than the apparent BSi concentrations. Table 1 and Fig. 5 show that the extent of the correction for the six study sites varies between 3% and 92% of the apparent BSi and that the extent of the correction is strongly dependent upon the Lsi:BSi ratio, demonstrating the need to correct for the mineral interference in most coastal environments.

## 4. Conclusions and perspectives

In this paper, we have presented a robust method to correct for the mineral interference in the measurement of biogenic silica concentrations in coastal waters. It is based on the use of Si:Al ratios, as proposed for the determination of BSi in marine sediments by Eggiman et al. (1980), Schlüter and Rickert (1998), Kamatani and Oku (2000) or Köning et al. (2002). This method consists in introducing an intermediate leach between the two alkaline digestions of Ragueneau and Tréguer (1994). This leach is strictly identical to a first leach (NaOH 0.2 M,  $100^\circ\text{C}$ , 40 min) and is devoted to the determination of the Si:Al ratio of the dissolving silicate minerals. The interference arising from this dissolution is then corrected by

multiplying this Si:Al ratio by the Al concentration determined during the first leach (Fig. 1).

There are several assumptions implied in this method. Theoretical considerations have been used to demonstrate that the Al extracted from the diatoms is negligible and that the silicate minerals dissolve similarly during both digestions. Two time course experiments have been used to test the completeness of BSi dissolution during the first digestion, and to validate the protocol proposed. The first one, carried out in the Aulne estuary on filters with various amounts of BSi present on them, demonstrated that 40 min are both necessary and sufficient to ensure (1) that all the BSi has been dissolved during the first digestion, provided the amount of BSi present on the filter does not exceed  $10 \mu\text{mol}$ , and (2) that the Si:Al ratio can be determined accurately in the second digestion, provided important rinsing steps are performed between the two digestions (Figs. 2 and 3). The second time course experiment has been used to apply two different modes of correction on a sample collected in the Black Sea (Fig. 4). Corrected BSi concentrations using the proposed method or the DeMaster (1981) approach were not significantly different, thereby validating the proposed method.

The  $(\text{Si}:\text{Al})_2$  ratios measured in the field can be quite variable (see Table 1). This variability is natural and not an experimental artefact, as the reproducibility of this ratio is better than 0.2, all the samples presented in Table 1 have an Al concentration well above the detection limit of the ICP-AES and a BSi amount on the filter well below  $10 \mu\text{mol}$ . The method proposed will therefore be most robust if the Si:Al ratio is measured on each sample, to account for this natural variability.

The method proposed is of wide applicability. Indeed, contrarily to previous methods published, it is not site-specific and does not require assumptions on seasonal changes in the nature of the mineral particles present in the suspended matter, as does the method proposed by Ragueneau and Tréguer (1994). It does not require the use of a single Si:Al crustal ratio, as does the method proposed by Kamatani and Takano (1984). It avoids the use of sequential extractions, best

Table 1  
Application of the proposed method (Fig. 1)

Site	[Si] <sub>1</sub> ( $\mu\text{mol l}^{-1}$ )	[Al] <sub>1</sub> ( $\mu\text{mol l}^{-1}$ )	[Si] <sub>2</sub> ( $\mu\text{mol l}^{-1}$ )	[Al] <sub>2</sub> ( $\mu\text{mol l}^{-1}$ )	(Si/Al) <sub>2</sub>	[Si] <sub>3</sub> ( $\mu\text{mol l}^{-1}$ )	[BSi] ( $\mu\text{mol l}^{-1}$ )	[LSi] ( $\mu\text{mol l}^{-1}$ )	LSi:BSi	Correction (%)	Correction ( $\mu\text{mol l}^{-1}$ )
AE	20.7	4.1	8.1	5.0	1.6	89	14.1	103.4	7.4	32	6.6
	17.3	4.0	6.0	3.8	1.6	78	11.0	89.9	8.2	36	6.3
	38.8	9.2	13.3	7.8	1.7	196	23.1	225.0	9.7	40	15.7
	9.6	2.4	4.3	2.6	1.7	48	5.6	56.8	10.1	42	4.0
	38.2	9.9	12.6	7.4	1.7	206	21.3	235.2	11.0	44	16.9
	94.0	25.1	39.4	23.5	1.7	491	51.8	573.1	11.1	45	42.2
BoB	2.51	0.21	0.32	0.16	2.0	1.00	2.1	1.7	0.8	17	0.4
	2.00	0.16	0.36	0.19	1.9	1.16	1.7	1.8	1.1	15	0.3
	1.49	0.05	0.13	0.07	1.9	1.31	1.4	1.5	1.1	6	0.1
	1.10	0.08	0.22	0.11	1.9	1.23	0.9	1.6	1.7	14	0.2
	0.67	0.05	0.07	0.05	1.4	1.03	0.6	1.2	2.0	11	0.1
	1.03	0.16	0.32	0.20	1.6	2.21	0.8	2.8	3.6	25	0.3
	0.57	0.10	0.25	0.12	2.1	1.76	0.4	2.2	6.1	36	0.2
BoS	6.00	0.08	0.39	0.18	2.2	1.64	5.8	2.2	0.4	3	0.2
	1.66	0.02	0.18	0.04	5.2	0.43	1.6	0.7	0.5	6	0.1
	1.84	0.05	0.21	0.06	3.5	0.55	1.7	0.9	0.6	10	0.2
	1.27	0.05	0.20	0.07	2.7	0.45	1.1	0.8	0.7	10	0.1
	3.66	0.08	0.55	0.12	4.7	1.97	3.3	2.9	0.9	10	0.4
	1.96	0.18	0.40	0.11	3.6	1.09	1.3	2.1	1.6	32	0.6
	2.35	0.13	0.63	0.16	3.9	2.64	1.8	3.8	2.0	22	0.5
DD	2.57	0.12	0.55	0.12	4.6	1.84	2.0	2.9	1.4	21	0.5
	18.64	4.08	9.83	5.02	2.0	18.48	10.7	36.3	3.4	43	8.0
	11.23	2.88	6.51	2.88	2.3	17.96	4.7	31.0	6.6	58	6.5
	5.37	1.51	3.25	1.78	1.8	31.15	2.6	37.2	14.3	52	2.8
	2.86	0.86	2.69	1.11	2.4	12.76	0.8	17.5	22.4	73	2.1
	10.58	2.99	8.22	2.65	3.1	17.12	1.3	34.6	26.4	88	9.3
DP	1.14	0.02	0.13	0.03	4.3	0.06	1.1	0.3	0.3	7	0.1
	1.04	0.03	0.15	0.03	4.4	0.21	0.9	0.5	0.5	11	0.1
	3.81	0.08	0.35	0.14	2.5	1.60	3.6	2.1	0.6	5	0.2
	0.93	0.03	0.13	0.04	3.0	0.41	0.8	0.6	0.7	9	0.1
	0.37	0.01	0.07	0.01	5.4	0.23	0.3	0.3	1.1	13	0.0
	0.25	0.01	0.04	0.01	4.8	0.24	0.2	0.3	1.5	15	0.0
	0.35	0.01	0.09	0.05	2.0	0.36	0.3	0.5	1.5	9	0.0
	0.31	0.02	0.03	0.01	4.5	0.28	0.2	0.4	1.6	22	0.1
	0.27	0.01	0.04	0.01	3.0	0.44	0.2	0.5	2.3	14	0.0
	0.22	0.03	0.09	0.05	1.8	0.89	0.2	1.0	6.2	24	0.1
	8.42	2.57	5.64	2.66	2.1	19.83	3.0	30.9	10.4	65	5.4
	0.09	0.02	0.03	0.01	3.0	0.30	0.0	0.4	20.2	78	0.1
	0.07	0.02	0.05	0.02	2.5	0.59	0.0	0.7	26.0	64	0.0
NS	2.59	0.09	0.13	0.05	2.6	2.36	2.3	2.7	1.2	9	0.2
	1.72	0.09	0.26	0.07	3.6	2.81	1.4	3.4	2.4	18	0.3
	2.28	0.11	0.40	0.11	3.8	5.14	1.9	6.0	3.2	19	0.4
	0.79	0.08	0.16	0.06	2.8	2.15	0.6	2.5	4.5	29	0.2
	2.85	0.40	0.78	0.20	4.0	13.75	1.2	16.1	12.9	56	1.6
	1.43	0.27	0.64	0.18	3.6	17.83	0.5	19.4	41.4	67	1.0
	1.10	0.34	0.22	0.07	3.0	3.04	0.1	4.3	49.4	92	1.0
KP	0.60	0.03	0.05	0.01	4.2	0.16	0.5	0.3	0.7	22	0.1
	0.54	0.03	0.07	0.02	4.0	0.20	0.4	0.4	1.0	23	0.1

Table 1. (continued)

Site	[Si] <sub>1</sub> ( $\mu\text{mol l}^{-1}$ )	[Al] <sub>1</sub> ( $\mu\text{mol l}^{-1}$ )	[Si] <sub>2</sub> ( $\mu\text{mol l}^{-1}$ )	[Al] <sub>2</sub> ( $\mu\text{mol l}^{-1}$ )	(Si/Al) <sub>2</sub>	[Si] <sub>3</sub> ( $\mu\text{mol l}^{-1}$ )	[BSi] ( $\mu\text{mol l}^{-1}$ )	[LSi] ( $\mu\text{mol l}^{-1}$ )	LSi:BSi	Correction (%)	Correction ( $\mu\text{mol l}^{-1}$ )
	0.53	0.04	0.07	0.02	3.6	0.28	0.4	0.5	1.3	27	0.1
	0.45	0.06	0.09	0.02	4.1	0.23	0.2	0.6	2.5	51	0.2
	0.57	0.05	0.16	0.04	3.6	0.65	0.4	1.0	2.6	32	0.2
	0.52	0.12	0.19	0.06	3.4	0.70	0.1	1.3	12.1	80	0.4
Av.	6.37	1.41	2.48	1.33	2.97	25.01	3.74	30.12	6.98	31.77	2.63
SD	14.91	3.94	6.18	3.64	1.10	77.81	8.34	90.33	10.21	23.89	6.71
Min.	0.07	0.01	0.03	0.01	1.39	0.06	0.02	0.27	0.25	2.97	0.03
Max.	94.04	25.13	39.43	23.47	5.40	491.44	51.82	573.10	49.45	92.14	42.22

In different coastal areas including the Aulne estuary (AE), the Bay of Brest (BoB), the Bay of Seine (BoS), the Danube Delta (DD), the Danube Plume (DP), the North Sea (NS) and the Kerguelen Plateau in the Southern Ocean (KP). [BSi] and [LSi] are corrected BSi and LSi concentrations, as given by Eqs. (1) and (2). “Correction (%)” and “Correction ( $\mu\text{mol l}^{-1}$ )” represent the relative and absolute amounts of Si to be subtracted from the apparent BSi ([Si]<sub>1</sub>) concentration, respectively, to correct for the mineral interference. These 52 data constitute a representative sample of the complete data set that contains 239 data. Note that data samples where the Al concentration was below the detection limit at the ICP-AES have been removed from the original data set (305 samples). At the bottom of the table the average (Av.), the standard deviation (SD), and the minimum (Min.) and maximum (Max.) values of the parameters listed on top are reported. These average values and the corresponding ranges presented in this table for the 52 samples are very close to those calculated with the complete data set, which could not be entirely presented.

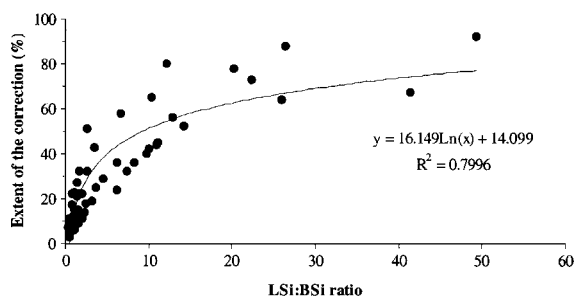


Fig. 5. Relationship between the extent of the correction (%) and the LSi:BSi ratio. See legend of Table 1.

adapted for sediments (DeMaster, 1981) but difficult to conduct on suspended matter, unless the method becomes automated (Köning et al., 2002). As the Al concentration can be measured even more simply by other means than ICP-AES, this method could be widely employed. Finally, the maximum amount of BSi present on filters (10  $\mu\text{mol}$ ) allows the measurement to be performed in any kind of aquatic environment, as one can always adapt the filtration volume so as to remain below this threshold.

The large potential for the error revealed in Table 1 and the combined robustness and wide

applicability of the proposed method justify its use despite the effort of measuring simultaneously Si and Al on each sample. It should be noted that the proposed protocol is less intensive than sequential extractions, which are best adapted for sediment analysis. It represents a first step towards the development of a routine method to measure BSi in the suspended matter of coastal waters. This is an important goal if we are to better understand processes affecting the Si cycle along the land-ocean continuum and their consequences for the functioning of coastal ecosystems and the global Si cycle (Ragueneau et al., submitted).

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and the Northwestern Black Sea were collected during the EU-EROS 21 program (ENV4-CT96-0286). All these institutions are thanked for their financial support. This manuscript strongly benefited from the comments by V. Franck, D.J. Conley, M. Schlueter and two anonymous reviewers, who are all greatly acknowledged. We are also very grateful to Robert Marc for his help during water sampling in the Aulne estuary.

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