Impact of sample pretreatment on the measured element concentrations in the bivalve *Arctica islandica*

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[1] Correlating metal to calcium (Me/Ca) ratios of marine biogenic carbonates, such as bivalve shells, to environmental parameters has led to contradictory results. Biogenic carbonates represent complex composites of organic and inorganic phases. Some elements are incorporated preferentially into organic phases, and others are incorporated into inorganic phases. Chemical sample pretreatment to remove the organic matrix prior to trace element analysis may increase the applicability of the investigated proxy relationship, though its efficiency and side effects remain questionable. We treated inorganic calcium carbonate and bivalve shell powder (*Arctica islandica*) with eight different chemical treatments including H_2O_2, NaOH, NaOCl, and acetone and analyzed the effects on (1) Me/Ca ratios (Sr/Ca, Mg/Ca, Ba/Ca, and Mn/Ca), (2) organic matter (≈N) content, and (3) mineralogical composition of the calcium carbonate. The different treatments (1) cause element and treatment specific changes of Me/Ca ratios, (2) vary in their efficiency to remove organic matter, and (3) can even alter the phase composition of the calcium carbonate (e.g., formation of $Ca(OH)_2$ during NaOH treatment). Among all examined treatments there were none without any side effects. In addition, certain Me/Ca changes we observed upon chemical treatment contradict our expectations that lattice-bound elements (Sr and Ba) should not be affected, whereas non-lattice-bound elements (Mg and Mn) should decrease upon removal of the organic matrix. For instance, we observe that NaOCl treatment did not alter Sr/Ca ratios but caused unexpected changes of the Mg/Ca ratios. The latter demonstrates that the buildup of complex biogenic composites like the shell of *Arctica islandica* are still poorly understood.

1. Introduction

[2] Since the 1940s when *Urey* [1948] and *McCrea* [1950] showed that information about past environmental conditions is preserved in the elemental/isotopic signature of biogenic marine carbonates the field of paleoreconstruction has grown rapidly. Since then Me/Ca (Me stands for divalent metal ions like Mg, Mn, Sr, and Ba which can be substituted for Ca in the calcium carbonate) ratios of biogenic calcium carbonates (e.g., bivalves [*Schöne et al.*, 2011; *Vander Putten et al.*, 2000], corals [*Mitsuguchi et al.*, 1996; *Watanabe et al.*, 2001], ostracods [*Holmes*, 1996; *Keatings et al.*, 2006], and foraminifera [*Barker et al.*, 2003; *Bryan and Marchitto*, 2010]) are widely used to reconstruct past environmental conditions [*Foster et al.*, 2008a]. Sample preparation for Me/Ca analysis often includes chemical treatment to remove organic matter from the biogenic calcium carbonate [*Gaffey and Bronnimann*, 1993]. However, the efficiency of chemical removal of organic matter remains questionable and a variety of side effects may alter the outcome of the analysis.

[3] Biogenic calcium carbonates consist of mineral and organic phases [*Lowenstam and Weiner*, 1989]. The organic matrix occurs both within and between $CaCO_3$ crystals [*Bourgoin*, 1987; *Schöne et al.*, 2010] and plays an important role in the biomineralization process by controlling crystal growth and structural organization [*Addadi et al.*, 2006; *Krampitz et al.*, 1983; *Levi-Kalisman et al.*, 2001; *Nudelman et al.*, 2006; *Takeuchi et al.*, 2008]. The organic matrix of mollusk shells is composed of water-insoluble chitin and the soluble organic matrix [*Bourgoin*, 1987; *Schöne et al.*, 2010; *Takesue et al.*, 2008]. The average content of organic matter varies among species, and was found to range from 0.3 to 4.0 wt % in mollusk shells [*Bourgoin*, 1987]. According to *Schöne et al.* [2010] shells of the species *Arctica islandica* contain on average 99.54 wt % calcium carbonate and water-soluble organic matrix, and only 0.46 wt % water-insoluble organic matrix.

[4] Trace elements can be incorporated into biogenic calcium carbonates (e.g., mollusk shells) in various ways: (1) as lattice-bound elements that substitute for calcium in the calcite or aragonite crystal lattice and (2) as non-lattice-bound elements, such as surface adsorbed elements or constituents of organic compounds [*Dodd*, 1967; *Takesue et al.*, 2008]. For Me/Ca analyses it has to be taken into account that the insoluble organic matrix of aragonitic bivalve shells is enriched in certain elements (e.g., Mg) and depleted in others (e.g., Sr and Ca) in comparison to the entire biomineral [*Schöne et al.*, 2010]. Today's high-resolution techniques for Me/Ca analysis, such as laser ablation inductively coupled mass spectrometry (LA-ICP-MS), however, do not distinguish between mineral and organic phases, but analyze the ablated sample as bulk. The Ca concentration, which is commonly used as internal standard, is difficult to determine for the exact volume ablated by the laser. Furthermore, the organic matter is unevenly distributed across shells. As a consequence, LA-ICP-MS analyses may overestimate, e.g., the Mg content locally in shell regions with very high organic matter content where Ca concentrations are lower than on average [*Schöne et al.*, 2010]. Thus, the latter authors strongly recommended to either remove of the organic matrix prior to LA-ICP-MS analyses or to mathematically correct for the Me content of the insoluble organic matrix.

[5] Previous studies question the effectiveness of different treatment agents and point out that pretreatment itself may alter the trace element composition of the calcium carbonate sample [*Keatings et al.*, 2006; *Love and Woronow*, 1991; *Watanabe et al.*, 2001]. For example, *Gaffey and Bronnimann* [1993] studied the effectiveness of sample treatment to remove organic matter from green algae and echinoid skeletons. They concluded that NaOCl was the most and H_2O_2 the least effective agent, while NaOH treatment failed to remove the organic material. In addition, they observed that H_2O_2 treatment caused dissolution of the calcium carbonate. The authors did not evaluate changes in the element composition of the samples. *Mitsuguchi et al.* [2001] and *Watanabe et al.* [2001] examined changes in the trace element content of coral carbonate as a consequence of chemical treatment. Both studies observed that Mg/Ca ratios of coral carbonate were significantly altered by chemical treatment, while Sr/Ca ratios showed little or no variation. However, they detected opposite Mg/Ca changes upon H_2O_2 treatment. *Mitsuguchi et al.*

Inorganic calcium carbonate powder (HB01)	Calcium carbonate shell powder (*A. islandica*)

6 sub-samples	9 sub-samples

Treatment 1 to 5 + control (c)	Treatment 1 to 8 + control (c)

ICP-MS, N analyzer, XRD

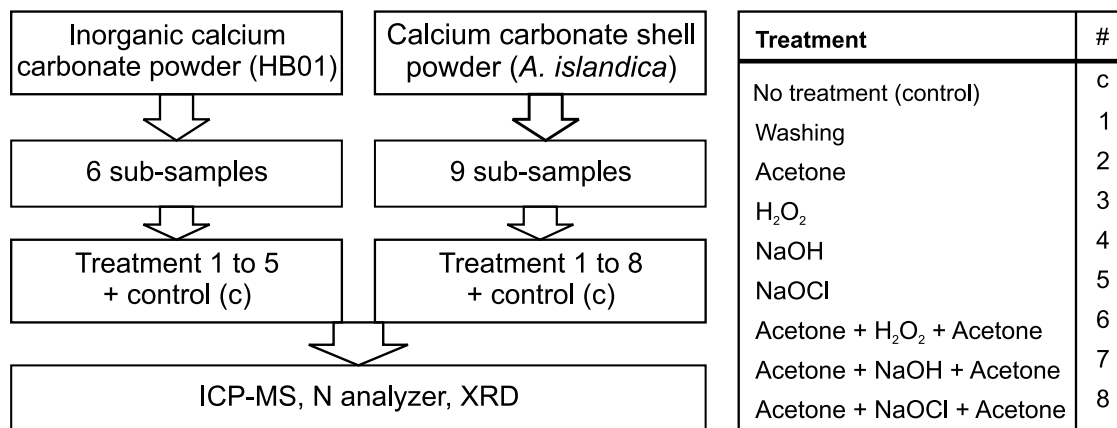| Treatment	#
No treatment (control)	c
Washing	1
Acetone	2
H_2O_2	3
NaOH	4
NaOCl	5
Acetone + H_2O_2 + Acetone	6
Acetone + NaOH + Acetone	7
Acetone + NaOCl + Acetone	8

Figure 1. We divided inorganic (HB01) and organic (*A. islandica*) calcium carbonate powder into six and nine subsamples, treated them with different chemicals/cocktails (control "c"; treatments 1–8), and analyzed the effects on (1) Me/Ca ratios (Sr/Ca, Mg/Ca, Ba/Ca, and Mn/Ca; ICP-MS), (2) organic matter (≈N) content (N analyzer), and (3) calcium carbonate structure and composition (XRD).

[2001] also observed dissolution of the calcium carbonate as a result of H_2O_2 treatment. Very few studies focus on bivalve shells when analyzing the effects of chemical treatment on biogenic calcium carbonates. *Takesue et al.* [2008], for example, found that oxidative removal of the organic matter decreased Mg/Ca and Mn/Ca, but affected neither Sr/Ca nor Ba/Ca ratios in three powdered shells of the estuarine bivalve *Corbula amurensis*.

[6] Here, we carried out a systematic investigation on inorganic calcium carbonate and bivalve shell powder (*Arctica islandica*) to examine the efficiency of 8 chemical treatments and their impact on the carbonate composition. We combine different analytical techniques (inductively coupled plasma mass spectrometry (ICP-MS), nitrogen (N) analyzer, X-ray diffractometry (XRD)) to analyze the effect of each treatment on Me/Ca ratios (Mg/Ca, Sr/Ca, Ba/Ca, Mn/Ca), organic matter content using N as a proxy, and the composition of the carbonate and of newly formed phases.

2. Material and Methods

2.1. Preparation of the Powder Samples

[7] We used inorganic calcitic carbonate powder (HB01; heavy calcium carbonate, p.a. quality, by Janssen Chimica) and the right valves of two aragonitic *Arctica islandica* specimens (A and B) to examine the effect of sample pretreatment in biogenic calcium carbonates. Pure calcitic inorganic carbonate, which is at normal conditions the stable among the three calcium carbonate polymorphs,

was chosen as an end-member for the experiments to separate the effect of chemical treatment on inorganic and organic calcium carbonate.

[8] We prepared and weighed six HB01 subsamples (170.9 ± 2.2 mg, Figure 1) that were all kept and treated in 2 ml Eppendorf tubes. The use of a sample divider was not necessary, because HB01 did not contain any organic matter to be unevenly distributed.

[9] The shells had been live collected in the North Sea at 40 m water depth in 2005. Upon collection the shells were dried in the lab. The organic periostracum was ground from the outside of the valves on a two-speed grinder polisher (Buehler Alpha wheels) with P600 sandpaper and by using a hand dremel (PROXXON Minimot 40/E). Afterward, we sonicated the valves for 30 s and dried them in closed petri dishes for 1 h at 54°C. We then pestled each valve to a grain size of less than 30 μm using an agate sphere mortar. We did not differentiate between the inner and outer shell layer, but analyzed both valves as bulk samples, because according to *Zhang* [2009] the trace element composition (Mg, Sr, Ba, and Mn) differs only slightly between both shell layers. A grain size of less than 30 μm was considered necessary to ensure that the chemical agents would completely penetrate into the grains and access all organic material embedded within the calcium carbonate. We divided the shell powder of each valve into nine subsamples (172.0 ± 58.6 mg, Figure 1) by means of a sample divider to ensure identical grain size distribution in all subsamples.

Mg/Ca	Sr/Ca	Ba/Ca	Mn/Ca	c	% calcite	other constituents after sample treatment
					100	
NS	NS	NS	NS	1	100	
NS	NS	NS	NS	2	100	
-	NS	NS	+	3	100	
NS	NS	-	+	4	0	$Ca(OH)_2$, Na_2CO_3, $Na_2Ca(CO_3)_2*5H_2O$, NaOH
NS	NS	NS	+	5	75	NaCl

Figure 2. Effects of treatments (control "c"; treatments 1–5) (left) on the Me/Ca ratios and (right) on the carbonate composition of the HB01 powder samples (see also Figure 1). NS indicates no significant difference between the treated sample and the control. Plus indicates a significant increase; minus indicates a significant decrease.

[10] In the following text we will refer to the shell powder samples of both valves (A and B) as "shell powder," due to the fact that both sets of samples were treated exactly the same way.

2.2. Treatment of Powder Samples

[11] We chose eight different treatments and applied treatment 1 to 5 to the HB01 samples and treatment 1 to 8 to the shell powder samples (Figure 1). One sample of each powder remained untreated and served as a control (Figure 1). We used highly purified chemicals (suprapure 30% NaOH by Merck, ultrapure 31% H_2O_2 by Roth, ultrapure Acetone by AppliChem, and ultrapure 60% HNO_3 by Merck), except for NaOCl (13% NaOCl by AppliChem) that was available in technical quality only. After adding 1300 μl of each solution to the corresponding powders, we mixed each sample using a vortex shaker and incubated for 2 h at 60°C, except for H_2O_2 treatments, which had to be incubated at room temperature. To prevent the sample tubes from bursting in the heat, we placed them into larger tubes filled with water. Over the course of 2 h, samples were mixed on the vortex shaker every 15 min to ensure that the powder did not agglomerate and that the reagent penetrated the entire sample. After 2 h, we centrifuged each sample at 22°C for five minutes and transferred the supernatants into new plastic tubes. The remaining solid was washed six times with reverse osmosis water (ROW, conductivity <0.067 μS); between combined treatments we washed each shell powder sample three times with ROW. To wash the samples, we filled the tubes with ROW, mixed them on a vortex shaker, and centrifuged each sample. HB01 samples were not washed after chemical treatment in order to examine the effect of washing and of chemical treatment separately. At the end,

we dried all samples over night at 60°C. Samples were now ready for analyses.

2.3. ICP-MS Analyses

2.3.1. Sample Preparation for ICP-MS Measurements

[12] The treated and untreated HB01 and shell powder samples were analyzed for their element concentrations (Mg, Sr, Ba, Mn, Ca) using a ThermoFinnigan Element2 ICP-MS at the University of Bremen, Germany. We transferred 10 to 20 mg of each sample into 13 ml tubes and noted the exact weight. The HB01 samples were dissolved in 10 ml of 2% ultrapure HNO_3. These solutions were diluted 1:100 with HNO_3 and spiked with 2.5 ng/ml indium as internal standard. Each shell powder sample was dissolved in 100 μl of 60% HNO_3 and dried for 3 h at 100°C. Next, we added ROW water to a total volume of 10 ml. These solutions were again diluted 1:100 and spiked for analysis.

[13] In addition, we determined the trace element content of the two most effective agents, NaOH and NaOCl. For this 10 μl of the reagent was diluted 1:100 with ultrapure HNO_3 and spiked with 2.5 ng/ml indium as internal standard. In the same way we prepared the NaOH and NaOCl supernatants of the HB01 samples for ICP-MS analyses.

2.3.2. Statistical Analyses of the ICP-MS Data

[14] From the ICP-MS data we calculated the Mg/Ca, Sr/Ca, Ba/Ca, and Mn/Ca ratios for each sample, as well as the standard deviations (σ) of these ratios by using the respective analytical precision and the Gaussian propagation of uncer-

Table 1. Me/Ca Ratios in Untreated (c) and Treated HB01 Samples

Treatment	Mg/Ca (mg/g)	Sr/Ca (mg/g)	Ba/Ca (μg/g)	Mn/Ca (μg/g)
c	1.42	1.01	114.0	4.43
1	1.37	0.99	109.9	4.69
2	1.43	1.01	111.0	4.68
3	1.33	1.00	109.9	19.68
4	1.41	0.99	78.6	14.37
5	1.39	1.00	110.7	6.67

Table 2. The σ Values of the Me/Ca Ratios of Untreated (c) and Treated HB01 Samples

Treatment	Mg/Ca (mg/g)	Sr/Ca (mg/g)	Ba/Ca (μg/g)	Mn/Ca (μg/g)
c	0.024	0.016	2.503	0.301
1	0.023	0.017	2.017	0.206
2	0.026	0.016	1.884	0.204
3	0.017	0.014	1.803	0.604
4	0.020	0.011	1.869	0.589
5	0.021	0.018	1.843	0.228

tainty. Next, we tested for a significant difference in Me/Ca ratios before (control) and after treatment (sample). We considered a difference in Me/Ca ratios between sample and control as significant if it was larger than three standard deviations (equation (1)):

$$\left(\text{Ratio}_{\text{sample}} - \text{Ratio}_{\text{control}}\right) > 3 * \text{sqrt}\left[\left(\sigma^2_{\text{Ratio (sample)}} + \sigma^2_{\text{Ratio (control)}}\right)\right] \tag{1}$$

Finally, the treatments were classified into three groups using the following criteria: (1) "No significance" means that there was no significant change in Me/Ca ratios observed for specimen A and B, or both specimens showed significant but opposite changes after the same treatment. (2) "Significant increase" means that sample treatment caused increasing Me/Ca ratios for both specimens A and B, with at least one increase being significant. (3) "Significant decrease" means that sample treatment caused decreasing Me/Ca ratios for both specimens A and B, with at least one decrease being significant.

2.4. Nitrogen Analyses

2.4.1. Nitrogen Measurements

[15] We analyzed all shell powder samples of specimen A und B for their nitrogen concentration using an element analyzer (Euro EA by HEKAtech, Germany). Nitrogen rather than carbon was used as an indicator for the organic content of the shell powder, as the EA cannot distinguish between organic and inorganic carbon. We analyzed as many 20 mg aliquots of each sample as possible and calculated the average N concentration and standard deviations of all measurements (specimen A and B) for each shell powder. To monitor the accuracy of our measurements, we measured a soil reference sample with a known N concentration of

0.214 ± 0.050 μg/mg N. In total, we measured 24 soil samples along with 138 shell powder samples. Acetanilid was used as calibration standard.

2.4.2. Statistical Analyses of the Nitrogen Data

[16] The N data were Box-Cox transformed to achieve normality and homogeneity of variances. Next, we applied a fully factorial two-way analysis of variance (ANOVA) followed by a post hoc Tukey HSD test on differences between means to test on effects of treatment and of specimen on the N content of the shell powder.

2.5. Sample Preparation for XRD Analyses

[17] Semiquantitative X-ray diffraction (XRD) analyses were conducted at the University of Bremen, Germany, using a Siemens D500 diffractometer to characterize the composition of polycrystalline phases. We measured all HB01 powder samples as well as two shell powder samples of specimen B. The first shell sample (200 mg of untreated shell powder) was measured to examine the possible effect of grinding on the calcite-aragonite ratio of the calcium carbonate. The second shell sample (200 mg of untreated shell powder incubated over night at 60°C) was

Table 3. RSD Values (%) of ICP-MS Analyses of Untreated (c) and Treated HB01 Samples and of the Supernatants of Treatments 4 and 5

Treatment	Mg	Sr	Ba	Mn	Ca
c	1.3	1.1	1.9	6.7	1.1
1	1.4	1.4	1.6	4.3	0.9
2	1.4	1.1	1.2	4.2	1.2
3	0.8	1.0	1.3	2.9	1.0
4	1.1	0.7	2.2	4.0	0.9
5	1.2	1.6	1.4	3.3	0.9
Supernatant_4	18.7	1.1	1.0	17.8	0.8
Supernatant_5	3.2	5.1	4.1	9.9	1.2

Table 4. Element Concentrations of the Reagents and HB01 Supernatants of Treatments 4 and 5[a]

	Mg (ng/mL)	Ca (ng/mL)	Mn (ng/mL)	Sr (ng/mL)	Ba (ng/mL)
Supernatant of treatment 4	0.09	0.00	0.01	5.90	8.66
NaOH	0.17	0.00	0.00	0.01	0.00
Supernatant of treatment 4 minus NaOH	*−0.08*	*0.00*	*0.01*	*5.89*	*8.66*
Supernatant of treatment 5	0.00	0.00	0.01	0.00	0.13
NaOCl	7.80	25.57	1.24	17.66	6.42
Supernatant of treatment 5 minus NaOCl	*−7.80*	*−25.57*	*−1.23*	*−17.66*	*−6.29*

[a]Italic rows show differences between reagent and supernatant.

measured to examine the effect of heating on the calcite-aragonite ratio.

3. Results

3.1. Inorganic Carbonate Powder (HB01) Samples

[18] XRD analyses of the treated HB01 samples reveal the formation of new compounds during treatment 4 (NaOH) and 5 (NaOCl) (Figure 2). After treatment 4 the sample consists of $Ca(OH)_2$, Na_2CO_3, $Na_2Ca(CO_3)_2 * 5H_2O$, and NaOH; calcium carbonate was not detected. After treatment 5 the sample consists of circa 75% calcite and 25% NaCl.

[19] The Me/Ca ratios of the HB01 samples before and after the different treatments, their standard deviations (σ values), as well as the respective analytical precisions (RSD values) of the ICP-MS analyses are shown in Tables 1–3. (For element concentrations in untreated (c) and treated HB01 samples, see auxiliary material.)[1] ICP-MS analyses of the treated and untreated HB01 samples (Figure 2) show that there is no significant difference in Mg/Ca ratio between control and treated samples except for a significant decrease of Mg/Ca after treatment 3 (H_2O_2). Sr/Ca ratios are not significantly affected by any treatment. For Ba/Ca there is no significant dif-

ference between the control and the treated samples except for a significant decrease after treatment 4 (NaOH). In contrast, all treatments cause a significant increase in Mn/Ca with the exception of treatment 1 (washing) and 2 (acetone). We note that due to the newly formed Na compounds, treatment 4 reduced the Ca concentration in the solid by 19.3 wt % and treatment 5 by 9.8 wt %, compared to the untreated control (39.9 wt %).

3.2. NaOH and NaOCl Supernatants of the HB01 Samples

[20] The concentrations of Ca, Ba, and Mn in the suprapure NaOH solution were below their detection limits (Ca < 0.0966 ng/mL; Ba < 0.0058 ng/mL; Mn < 0.0067 ng/mL); the Mg concentration was 0.17 ng/mL, the Sr concentration 0.01 ng/mL. In the NaOCl solution all analyzed elements had concentrations between 1.24 ng/mL (Mn) and 25.57 ng/mL (Ca) (Table 4). For the respective analytical precisions (RSD values) of the ICP-MS analyses of the supernatants see Table 3.

[21] The element concentrations of the HB01 samples before the treatments (control) and after treatment 4 (NaOH) and 5 (NaOCl), as well as the respective differences, are shown in Table 5. The data show that, except for Mn, all concentrations in the sample decreased during both sample treatments, which is due to the addition of Na compounds to the solid sample.

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[1]Auxiliary materials are available in the HTML. doi:10.1029/2011GC003630.

Table 5. Element Concentrations of HB01 Solid Products After Treatments 4 and 5 and of the Control[a]

	Mg (μg/g)	Ca (wt %)	Mn (μg/g)	Sr (μg/g)	Ba (μg/g)
HB01 control (untreated)	566	39.9	1.77	403	45.50
HB01 sample treatment 4	291	20.6	2.96	204	16.18
HB01 sample treatment 5	419	30.1	2.01	302	33.31
HB01 sample treatment 4 minus HB01 control (untreated)	*−275*	*−19.3*	*1.19*	*−199*	*−29.32*
HB01 sample treatment 5 minus HB01 control (untreated)	*−147*	*−9.8*	*0.24*	*−101*	*−12.19*

[a]Italic rows show differences between control and treatment.

Table 6. Summary of the Changes in Element Concentrations of the Solid Products (Relative to the Untreated Sample) and in the Supernatants After Treatments 4 (NaOH) and 5 (NaOCl) (Relative to the Reagent)

Treatment	Sample	Mg	Ca	Mn	Sr	Ba
		Increase, Decrease, or No Change of the Element Concentration After Sample Treatment[a]				
4 (NaOH)	supernatant	↓	NC	NC	↑	↑
	solid	↓	↓	↑	↓	↓
5 (NaOCl)	supernatant	↓	↓	↓	↓	↓
	solid	↓	↓	↑	↓	↓

[a]Increase (upward arrows), decrease (downward arrows), or no change (NC).

[22] The element concentrations of the reagent and supernatants of the treatments 4 and 5 are shown in Table 4. Despite the decrease of the calcium concentration in the solid products after treatment 4 and 5 (Table 5), there is no corresponding increase in the supernatants of both treatments. During treatment 4 the concentration of Sr and Ba in the solution increased and Mg decreased, whereas during treatment 5 these concentrations all decreased.

[23] Table 6 summarizes the changes of element concentrations in the solids and solutions during treatment 4 and 5. We notice opposite changes of concentrations in solutions and solids for Ba and Sr during treatment 4, and for Mn during treatment 5.

Table 8. The σ Values of the Me/Ca Ratios of Untreated (c) and Treated Shell Powders

Specimen	Treatment	Mg/Ca (mg/g)	Sr/Ca (mg/g)	Ba/Ca (μg/g)	Mn/Ca (μg/g)
A	c	0.006	0.036	0.428	0.416
A	1	0.005	0.039	0.433	0.394
A	2	0.008	0.049	0.613	0.563
A	3	0.006	0.033	0.457	0.600
A	4	0.006	0.041	0.481	0.379
A	5	0.008	0.032	0.521	0.447
A	6	0.008	0.038	0.630	0.520
A	7	0.009	0.053	0.584	0.624
A	8	0.005	0.031	0.465	0.485
B	c	0.006	0.037	0.605	0.342
B	1	0.005	0.034	0.901	0.363
B	2	0.006	0.036	0.746	0.340
B	3	0.009	0.038	0.794	0.518
B	4	0.005	0.025	0.595	0.369
B	5	0.006	0.042	0.819	0.428
B	6	0.005	0.033	0.911	0.588
B	7	0.004	0.031	0.792	0.354
B	8	0.005	0.027	0.663	0.630

In all other cases changes in element concentrations of the solutions are not complementary to those of the powder samples.

3.3. Shell Powder (*A. islandica*) Samples

[24] XRD analyses of the untreated and of the heated shell powder samples indicate that both grinding and heating do not cause aragonite-calcite conversion. Both samples were found to contain less than circa 0.5% calcite.

Table 7. Me/Ca Ratios in Untreated (c) and Treated Shell Powders

Specimen	Treatment	Mg/Ca (mg/g)	Sr/Ca (mg/g)	Ba/Ca (μg/g)	Mn/Ca (μg/g)
A	c	0.31	2.71	15.88	8.49
A	1	0.29	2.71	17.12	13.98
A	2	0.35	2.73	20.36	11.22
A	3	0.47	2.70	24.35	15.07
A	4	0.34	2.62	17.62	10.60
A	5	0.37	2.72	22.39	9.11
A	6	0.31	2.70	18.80	11.28
A	7	0.38	2.40	13.37	11.14
A	8	0.37	2.71	21.96	11.40
B	c	0.37	2.60	25.46	9.21
B	1	0.36	2.57	29.26	10.76
B	2	0.34	2.55	24.57	8.92
B	3	0.53	2.56	31.65	15.44
B	4	0.32	2.46	16.14	7.91
B	5	0.36	2.59	28.09	10.08
B	6	0.29	2.57	24.85	8.99
B	7	0.31	2.54	31.29	8.29
B	8	0.41	2.56	29.94	14.13

Table 9. RSD Values (%) of the ICP-MS Measurements of Untreated (c) and Treated Shell Powders

Specimen	Treatment	Mg	Sr	Ba	Mn	Ca
A	c	1.6	0.9	2.5	4.8	1.0
A	1	1.4	1.2	2.4	2.7	0.8
A	2	1.9	1.4	2.8	4.9	1.1
A	3	1.0	0.9	1.7	3.9	0.8
A	4	1.2	1.1	2.5	3.4	1.1
A	5	1.8	0.6	2.1	4.8	1.0
A	6	2.3	1.0	3.2	4.5	1.0
A	7	2.0	1.6	4.1	5.4	1.5
A	8	1.2	0.9	2.0	4.2	0.7
B	c	1.4	1.1	2.2	3.6	0.9
B	1	1.2	1.1	3.0	3.3	0.7
B	2	1.4	1.1	2.9	3.7	0.9
B	3	1.3	1.1	2.3	3.2	1.0
B	4	1.3	0.6	3.6	4.6	0.8
B	5	1.2	1.2	2.7	4.1	1.1
B	6	1.5	1.1	3.6	6.5	0.7
B	7	1.2	0.9	2.4	4.2	0.8
B	8	1.0	0.8	2.1	4.4	0.7

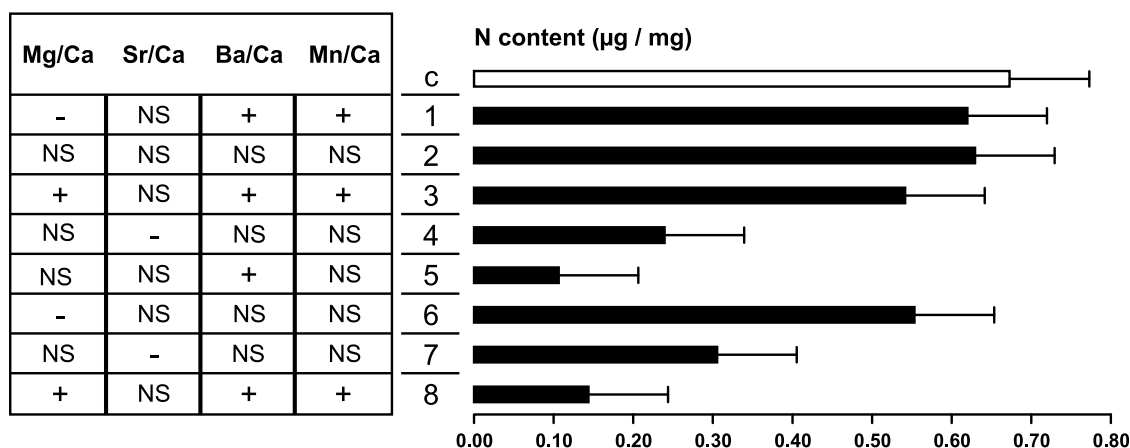| Mg/Ca	Sr/Ca	Ba/Ca	Mn/Ca
-	NS	+	+
NS	NS	NS	NS
+	NS	+	+
NS	-	NS	NS
NS	NS	+	NS
-	NS	NS	NS
NS	-	NS	NS
+	NS	+	+

Figure 3. Treatment effects and organic content in *A. islandica* shells powder. (middle) Treatment number (control "c"; treatments 1–8). (left) Summary of treatment effects on the Me/Ca ratios (NS indicates no significant difference between the treated sample and the control, plus indicates a significant increase, and minus indicates a significant decrease). (right) Nitrogen content (μg/mg, mean and one SD) of control (white bar) and posttreatment samples.

[25] The Me/Ca ratios of the shell powder samples before and after the different treatments, their standard deviations (σ values), as well as the respective analytical precisions (RSD values) of the ICP-MS analyses are shown in Tables 7–9. An overview of the changes of the Me/Ca ratios in shell samples after the different treatments is shown in Figure 3. In the shell powder samples there is a significant increase in Mg/Ca after treatment 3 (H_2O_2) and 8 (NaOCl + Acetone), and a significant decrease after treatment 1 (washing) and 6 (H_2O_2 + Acetone). Sr/Ca decreased significantly during both NaOH treatments (4 and 7), but was not affected by the other treatments. Ba/Ca and Mn/Ca showed similar behavior and increased significantly during treatment 1 (washing), 3 (H_2O_2), and 8 (NaOCl + Acetone). In addition, Ba/Ca increased significantly during treatment 5 (NaOCl).

[26] In comparison to the untreated control samples the Ca concentrations of both shell powder samples did not decrease during treatment 4 and 5 (NaOH and NaOCl) as observed in the HB01 samples. Instead, the Ca concentration increased on average by 5.6 wt % during treatment 4 and decreased by only 2.7 wt % during treatment 5.

3.4. Nitrogen Analyses

[27] The average N concentration of the 24 soil reference measurements is 0.213 ± 0.024 μg/mg N.

[28] The N content of the shell powder is significantly affected by treatment ($P < 0.0001$; F value = 1021.83; DF = 8); by specimen ($P < 0.0001$;

F value = 126.70; DF = 1) and by the treatment x specimen interaction ($P < 0.0001$; F value = 10.94; DF = 8). The N content differs significantly between all treatment samples except between washing and Acetone and between both H_2O_2 treatments (Post Hoc Tukey HSD Test).

[29] N concentration of the untreated control sample (0.67 μg/mg = 100% N) was the highest among all samples (Figure 3 and Table 10). NaOCl treatment removed 84.2% (treatment 5) and 78.6% (treatment 8) of the N contained in untreated sample powder. The second most efficient treatment agent was NaOH, which removed 64.4% (treatment 4) and 54.6% (treatment 7) of the original N concentration. Both H_2O_2 treatments (3 and 6)

Table 10. Average N Concentrations and Decrease of the N Content of Each Shell Powder Sample After Treatment (1 to 8) in Comparison to the Control

Treatment	Average N Concentration (μg/mg)	Removed N (%)
c	0.67	0.00
1	0.62	7.87
2	0.63	6.46
3	0.54	19.51
4	0.24	64.42
5	0.11	84.20
6	0.55	17.75
7	0.31	54.60
8	0.14	78.62

removed less than 20%, washing and acetone treatment removed less than 10%.

4. Discussion

[30] The treatments we examined vary in their efficiency in removing the organic matrix. Moreover, each treatment altered the Me/Ca ratios differently, although the changes we observe in pure HB01 carbonate after chemical treatments do not always coincide with those we observe in the shell powder samples. These inconsistencies indicate effects of the organic matrix, which are investigated and discussed below.

[31] Analyses of the NaOH and NaOCl supernatants were carried out for the HB01 samples. Theoretically, an increase of the element concentration in the treated sample powder should be accompanied by a decrease of the element concentration in the supernatant (and vice versa). However, this is only the case as long as no new reaction products form during chemical treatment and as long as no other treatment (e.g., washing) is applied prior to Me/Ca analysis of the sample. With few exceptions (see section 3.2.) the changes of the element concentrations in the supernatants do not match those we observe in the HB01 powder samples (Table 6). This may be due to the formation of new Na compounds and sample loss during several handling steps per treatment. Analysis of the element concentrations of the supernatants was not carried out for the shell powder samples. Unlike the HB01 samples, the shell powder samples were washed multiple times between and after treatments. As a consequence, analysis of the element concentrations of the supernatants of the shell powder samples cannot yield proper mass balances and was not considered a helpful tool.

4.1. Effect of Treatments on Organic Matter

[32] According to our quantitative N analyses, the most efficient agent in removing the organic matter from biogenic calcium carbonate powder is NaOCl followed by NaOH, while H_2O_2 is the least effective agent (Figure 3 and Table 10). However, none of the examined treatments removed all organic material contained in the bivalve shell powder.

[33] As previously observed by *Takesue et al.* [2008] changes in Me/Ca ratios due to chemical sample treatment can vary among different specimen of the same species. Statistical analyses of our N

measurements reveal an effect of specimen on the N content of the shell powder, but this effect is rather small (F value = 126.70) in comparison to the effect of chemical treatment (F value = 1021.83) and is of no concern when analyzing Me/Ca ratios. Thus, the focus of our N analyses remains on the impact of chemical treatment on the shell powder samples.

[34] The main constituents of the organic matrix are water-insoluble structural proteins (β chitin), water-soluble polyanionic proteins, and silk-like proteins [*Levi-Kalisman et al.*, 2001; *Schöne et al.*, 2010]. Chitin, a cellulose derivate, belongs to the polysaccharides and often occurs associated with proteins, in the case of the organic matrix as a chitin-protein complex [*Beyer et al.*, 1998]. Our N concentrations integrate all N-containing organic substances, such as proteins, chitin, and their degradation products, thus, the most important constituents of the organic matrix. It neglects, however, the part of the organic matrix referring to carbohydrates and lipids.

[35] NaOH is a leach that cracks peptide and ester bonds. That way, it removes proteins, including those of the chitin-protein complex, and lipids, but no carbohydrates [*Hänsel and Sticher*, 2009]. NaOCl, an oxidant and leach, oxidizes organic partial structures and causes alkaline hydrolyzes. Like NaOH, it removes proteins and lipids, but no carbohydrates [*Endres and Siebert-Raths*, 2009]. In addition, alkaline agents such as NaOH and NaOCl split off acetyl groups from chitin and that way convert water-insoluble chitin into water-soluble chitinosan [*Nachtigall*, 2002]. Multiple washing at the end of NaOH and NaOCl treatments completes the removal of the chitin-protein complex from the shell powder. In comparison, H_2O_2, an oxidant and acid, oxidizes organic partial structures and causes acid hydrolyzes. It dissolves proteins, lipids, as well as carbohydrates. The glycoside bounds of polysaccharides, however, are harder to crack, though oxidative breakdown of polysaccharides into smaller components should be possible [*Klemm et al.*, 2005; *Domininghaus*, 2004].

[36] As H_2O_2 loses its reactivity over time by exothermic decomposition, probably more N will be removed if the treatment agent is replaced over the course of incubation. Harsher H_2O_2 treatment (e.g., elevated temperature) may succeed in removing the same amount of N containing organic matter as NaOCl or NaOH treatment, but it presumably increases the amount of carbonate dissolution. In addition, *Gaffey et al.* [1991] point out

that elevated temperatures may cause transition from aragonite to calcite and alter the chemical composition of the calcium carbonate.

[37] In agreement with previous findings we conclude that none of the examined chemical treatments can effectively remove all organic material contained in the sample powder [*Love and Woronow*, 1991; *Weber et al.*, 1976].

4.2. Effect of Treatments on Calcium Carbonate

[38] Aragonite is a metastable polymorph of calcium carbonate at ambient conditions and may readily convert to calcite at heat or stress exposure. *Foster et al.* [2008b] detected a significant conversion of aragonite to calcite in otolith samples as an effect of micromilling. In our experiments we detected no calcite conversion in the shell powder after grinding or incubation for 2 h at 60°C. *Love and Woronow* [1991] achieved the same result after heating abiogenic aragonite for 2 h at 400°C. From our observations we conclude that the formation of new phases and the changes we observe in the chemical composition of the carbonate were not influenced by sample preparation (grinding or heating), but solely result from chemical treatment.

[39] Due to the decrease of the Ca concentration in HB01 samples after NaOH and NaOCl treatment, the Me/Ca ratios cannot be directly compared among treatments. Opposite to our results, *Love and Woronow* [1991] observed no changes of the Ca concentration after NaOCl, NaOH, and H_2O_2 treatment of abiogenic aragonite. However, they chose different incubation parameters (temperatures and duration), which may alter the effect of treatment on the carbonate chemistry and formation of reaction products. In comparison to the pure HB01 carbonate, we do not observe the same decrease of the Ca concentration after NaOH and NaOCl treatment in the shell powder samples. This result may be ascribed to the effect of the organic matrix on the structure and chemistry of the carbonate, e.g., due to its calcium binding properties [*Bourgoin*, 1987]. The polypeptide building of the organic matrix could form Ca complexes that are stable enough to withstand the conditions of leaching with NaOH and NaOCl.
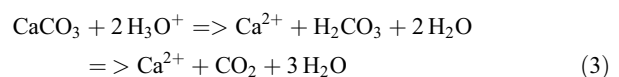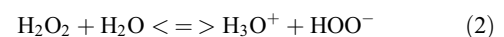
4.2.1. Washing and Acetone Treatment

[40] Neither washing nor acetone treatment resulted in the formation of new solid phases in the HB01 samples (Figure 2).

[41] Washing and acetone treatments do not significantly alter any of the Me/Ca ratios of the HB01 samples (Figure 2). For the shell powders, however, washing causes a significant decrease of the Mg/Ca ratio (Figure 3). The results are consistent with the observation that Mg seldom substitutes for Ca in abiogenic calcium carbonates but occurs, for example, as surface adsorbed or interstitial ions [*Love and Woronow*, 1991]. According to *Foster et al.* [2008a], Mg in *Arctica islandica* shells occurs as a non-lattice-bound ion, e.g., hosted in the organic matrix. Thus, washing the sample can remove non-lattice-bound Mg ions and consequently decrease the Mg/Ca ratio of the shell powder. It remains unclear why this outcome is limited to washing of the shell powder sample.

[42] The observation that washing resulted in a significant Ba/Ca and Mn/Ca increase in the shell powder samples is surprising. ROW, however, with a conductivity of <0.067 μS, and thus, an approximate pH of 7 may partially dissolve the carbonate and cause similar Me/Ca changes as described for H_2O_2 treatment (see section 4.2.2., last paragraph).

4.2.2. H_2O_2 Treatment

[43] H_2O_2 treatment of pure HB01 calcite did not produce new solid phases in significant amounts (Figure 2), but partial dissolution of the sample may have occurred. H_2O_2 has been shown to dissolve abiogenic and biogenic calcium carbonates, even when buffered with NaOH [*Boiseau and Juillet-Leclerc*, 1997; *Gaffey and Bronnimann*, 1993; *Mitsuguchi et al.*, 2001; *Pingitore et al.*, 1993; *Keatings et al.*, 2006], because H_2O_2 is a strong enough acid (pK_a 11.6) [*CRC Press*, 2010–2011] to corrode $CaCO_3$ (equations (2) and (3)):

$$H_2O_2 + H_2O <=> H_3O^+ + HOO^- \qquad (2)$$

$$CaCO_3 + 2\,H_3O^+ => Ca^{2+} + H_2CO_3 + 2\,H_2O$$
$$=> Ca^{2+} + CO_2 + 3\,H_2O \qquad (3)$$

A 31% H_2O_2 solution as used in this study has a pH of approximately 5.3 (calculated from pK_a value and molar concentration). Under such circumstances calcium carbonate dissolves and forms an equilibrium mixture of circa 85 mol % CO_2 and 15 mol % HCO_3^- (calculated from Hägg diagram). In addition, H_2O_2 can cause oxidative dissolution of proteins, which further induces the formation of organic acids (amino acids or derivates thereof). Those organic acids are even stronger acids (pK_a 2

to 6) [*CRC Press*, 2010–2011] than H_2O_2 itself and can in turn contribute to carbonate dissolution.

[44] We found that Mg/Ca decreases significantly after H_2O_2 treatment of HB01 and after combined H_2O_2/Acetone treatment of shell powder (Figures 2 and 3). This observation agrees with previous research which examined the effect of H_2O_2 treatment on Mg/Ca ratios of inorganic [*Love and Woronow*, 1991] and organic calcium carbonate (corals [*Watanabe et al.*, 2001], bivalves [*Takesue et al.*, 2008], and foraminifera [*Barker et al.*, 2003]). According to *Love and Woronow* [1991] the Mg/Ca decrease in pure HB01 carbonate may result from the occurrence of Mg as surface adsorbed or interstitial ions, which are removed more easily than lattice-bound ions during chemical treatment. As discussed above, the Mg/Ca decrease in the shell powder is due to the preferred location of Mg within the organic matrix rather than in biogenic calcium carbonate, and the removal of the organic matrix by H_2O_2. In contrast, the observed significant Mg/Ca increase in the shell powder samples after pure H_2O_2 treatment is difficult to explain. The magnitude of this change (51.6% and 43.2% in specimens A and B, respectively) and consistency of both values render a contamination scenario as unlikely. It is possible that Mg deliberated from organic matrix dissolution became incorporated in residual matrix material and was not removed during subsequent washing.

[45] In contrast to Mg/Ca, none of our H_2O_2 treatments caused significant changes in the Sr/Ca ratio of inorganic or organic calcium carbonate. In bivalve shells, opposite to Mg, Sr occurs dominantly in the carbonate phase rather than in the matrix because Sr substitutes easily for Ca in the aragonite lattice [*Foster et al.*, 2009; *Takesue et al.*, 2008]. Thus, chemical treatment removing the organic matrix does not alter the Sr/Ca ratio of the sample. This result of our study corroborates results on the chemical treatment of corals [*Watanabe et al.*, 2001] and foraminifera [*Barker et al.*, 2003] samples, but we discuss below that NaOH treatment is an exception (Figures 2 and 3).

[46] Regarding Ba/Ca and Mn/Ca, the (partial) removal of the organic matrix does not explain the changes in the ratios we observe throughout this study (Figures 2 and 3). Ba, similar to Sr, is a lattice-bound cation preferably located within aragonite [*Takesue et al.*, 2008], thus, the removal of organic matrix by chemical treatment should not alter the Ba/Ca ratios. Mn, similar to Mg, is a small cation preferably associated with the organic matrix

[*Carriker et al.*, 1980; *Takesue et al.*, 2008] so that Mn/Ca ratios are expected to decrease after removal of the organic matrix. Accordingly, *Takesue et al.* [2008] detected a Mn/Ca decrease and no Ba/Ca change after removal of the organic matrix from clam shells, whereas *Love and Woronow* [1991] observed no Mn/Ca changes after chemical treatment of abiogenic calcium carbonate. Contrary to these findings, we observe significant Mn/Ca and Ba/Ca increases for the shell powder samples, and a significant Mn/Ca increase for HB01, after H_2O_2 treatment. The Me/Ca increases we observe here during H_2O_2 treatment may be ascribed to the fact that acid treatment had a stronger impact on the calcium carbonate than on the organic phase. Dissolution of the carbonate results in increased Me/Ca ratios for elements (e.g., Mn) preferentially located in the organic matrix. In comparison, Ba occurs in the carbonate lattice as $BaCO_3$, which is slightly less soluble than $CaCO_3$. As a consequence, Ba is removed less easily than Ca during carbonate dissolution and Ba/Ca ratios increase. Thus, not all Me/Ca changes we observe can be ascribed to the removal of the organic matrix, but may be results of complex reactions between the acid treatment agent and the shell powder.

4.2.3. NaOH Treatment

[47] In the case of NaOH treatments we distinguish between two main processes that can be coupled to each other: (1) chemical reactions between calcite and hydroxide resulting in the formation of new solid phases and (2) chemical reactions causing dissolution of the organic matrix. The main inorganic reaction is the conversion of $CaCO_3$ (40 wt % Ca) to $Ca(OH)_2$ (portlandite; 54 wt % Ca) and Na_2CO_3 (equation (4)):

$$CaCO_3 + 2\,NaOH <=> Ca(OH)_2 + Na_2CO_3 \quad (4)$$
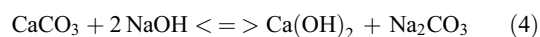
The same formation of portlandite was reported by *Pingitore et al.* [1993]. Our XRD data show that in addition $Na_2Ca(CO_3)_2 * 5H_2O$ and NaOH formed in the solid residue during drying of the sample. The composition of the resulting solid clearly depends on the amount of reagents; in our HB01 experiments the entire calcium carbonate had reacted. Remains of NaOH are easily removed by washing the samples at the end of chemical treatment.

[48] The consequences for element concentrations and Me/Ca ratios in the treated sample are twofold. First, element concentrations in the sample change simply because the mass and type of solid compounds changes. The same mass of Ca has a higher

concentration in a portlandite-dominated sample than a calcite-dominated one. Second, the ability of the newly formed phases to incorporate trace elements such as Sr strongly differs from that of calcite or aragonite because of the different crystal structures. Portlandite, for example, has a layered structure that strongly contrasts with the calcite or aragonite structure.

[49] The Mg/Ca ratios of both the HB01 and shell powder samples are not significantly affected by the NaOH treatments (Figures 2 and 3). For the HB01 samples this observation suggests that both elements remained quantitatively in the solid phases during the chemical reactions. For the shell powder samples, however, a decrease in Mg/Ca due to removal of the organic matrix would be expected. We suggest that the released Mg was incorporated into the newly formed solids, possibly in the form of $Mg(OH)_2$ (brucite), the amounts of which would be too small as to be recognized by the XRD investigations.

[50] Sr/Ca in the HB01 samples did not significantly decrease during the NaOH treatments (Figure 2). This shows that most of the Sr released during calcite decomposition may have been fixed in the newly formed solid phases, and possibly in minor amounts of $SrCO_3$ (strontianite) that cannot be revealed by the XRD analyses. For the shell powder samples there is a significant decrease in Sr/Ca during NaOH treatments (with and without acetone; Figure 3). In contrast to the HB01 samples, the shell powder samples were washed multiple times after chemical treatment. This procedure obviously resulted in repeated solvation of Sr cations and their subsequent removal, which explains the significant decrease in Sr/Ca compared to the HB01 experiments. This shows that washing after chemical treatments can result in reequilibration between solid phases and solution and can significantly affect Me/Ca ratios, even though simple washing does not.

[51] The strong Ba/Ca decrease in the HB01 samples compared to Sr/Ca is expected for NaOH treatment. Both elements show similar chemical behavior but owing to its better solubility (lower ionic potential) more Ba becomes solvated and removed during centrifuging. The fact that we do not observe a similar Ba/Ca decrease in the shell powder samples is puzzling and presumably due to the impact of the organic matrix. The Mn/Ca increase in the HB01 samples after NaOH treatment is associated with the simultaneous increase of the Mn concentration and decrease of the Ca

concentration (Table 5). The fact that the Ca concentration did not decrease in the shell powder sample may explain why Mn/Ca ratios of the shell powder samples remain unchanged.

4.2.4. NaOCl Treatment

[52] NaOCl treatment does not cause reaction of calcium carbonate to other Ca phases as is shown by XRD analyses of treated HB01 samples (Figure 2). However, drying of the centrifuged sample resulted in the precipitation of NaCl that makes up circa 25 wt % of the remaining solid. For the shell powder samples, multiple washing after NaOCl treatment most likely removed all of the NaCl owing to its high solubility in H_2O.

[53] As expected, the Sr/Ca ratios of the HB01 and shell powder samples are not significantly affected by NaOCl treatment because the carbonate-bound Sr remains fixed in the crystal lattice (Figures 2 and 3). Also, the Mg/Ca ratio of the HB01 powder did not change significantly during NaOCl treatment. For the shell powders, however, a significant decrease of Mg/Ca due to organic matrix removal would be expected but is not shown by our data; to the contrary, one of the treatments yielded an increase (Figure 3). Mg contamination by the NaOCl reagent, despite its limited purity, does not seem a viable explanation for ambiguous Mg behavior because the unwashed HB01 sample was not affected. Instead, interactions between the reagent and the organic matrix during treatment and subsequent washing may be reason for these ambiguous results. Washing in particular may have a subtle control on the final Mg/Ca of the dried sample. The same effect of washing may apply to the Ba/Ca increase, which we observe in the shell powder, but not the HB01 samples.

[54] The Mn/Ca increase in the HB01 samples may again be associated with the combined increase of the Mn and decrease of the Ca concentration after NaOCl treatment. The same explanation may apply to the Mn/Ca increase we observe in the shell powder samples, though the decrease of the Ca concentration after NaOCl treatment is rather small.

5. Summary

[55] We used inorganic calcite and bivalve shell powder (*Arctica islandica*) to examine the efficiency of 8 chemical treatments and their impact on the chemical and phase composition of the residual carbonate. The treatments vary in their efficiency in

removing organic matter with NaOCl being the most efficient treatment agent followed by NaOH. The latter treatment, however, removes significant portions of the carbonate and produces new compounds including $Ca(OH)_2$, and is thus not suitable for chemical treatment of calcium carbonates. Such reactions do not occur during NaOCl treatments. H_2O_2 is the least efficient agent in removing organic matter and causes partial dissolution of the calcium carbonate. For these reasons, we do not recommend H_2O_2 treatment to remove the organic matrix from biogenic carbonates. Regarding the effect of treatment on the Me/Ca ratios, every treatment has some impact on the chemical composition of the calcium carbonate, although certain Me/Ca changes we observed do not match the expectations. Lattice-bound elements (Sr and Ba) should not be affected, while non-lattice-bound elements (Mg and Mn) should decrease upon removal of the organic matrix. In agreement with these assumptions, we detected, for instance, that NaOCl treatment did not alter Sr/Ca ratios. However, it caused unexpected changes of the Mg/Ca ratios. For a summary of all Me/Ca changes we observed see Figures 2 and 3.

[56] To predict the outcome of chemical reactions chemical equilibrium conditions are generally assumed. Bivalve shells, however, are complex structures or inorganic and organic compounds. As a consequence, we often cannot predict the results of local reactions at the boundary layer between the inorganic and organic phase. Our results demonstrate that the composition of complex biogenic composites like the shell of *Arctica islandica* are still poorly understood and sample pretreatment prior to Me/Ca analyses has to be conducted with extreme caution.

[57] As previously suggested by *Keatings et al.* [2006], we recommend to avoid sample treatment prior to Me/Ca analyses when possible. The necessity to remove the organic matrix also depends on the amount of organic matter in the biogenic carbonate [*Lingard et al.*, 1992] and may be less crucial for samples with low organic content. If pretreatment is essential, NaOCl treatment can be applied prior to Sr/Ca analyses.

6. Outlook

[58] In addition to the chemical side effects discussed above, further complications arise when applying chemical treatment to cross sections of bivalve shells which are used for LA-ICP-MS

analysis. Some bivalves, such as *Arctica islandica*, have a distinctive homogeneous shell structure composed of small, irregular aragonite granules. These granules are each surrounded by an organic membrane and arranged in regular sheets and columns [*Kennedy et al.*, 1969]. Due to the dense structure of the shell and fine distribution of the organic matrix, it is difficult to achieve deep penetration of the treatment agent and subsequently extract the dissolved organics from the sample. In conclusion, this technique fails to remove all organic matter from bivalve shell powder and is consequently even less applicable for cross sections of bivalve shells.

[59] Instead, the use of a mathematical correction to account for the trace element content of the insoluble organic matrix as proposed by *Schöne et al.* [2010] may be an alternative approach for future studies.

Acknowledgments

References

Addadi, L., D. Joester, F. Nudelman, and S. Weiner (2006), Mollusk shell formation: A source of new concepts for understanding biomineralization processes, *Chemistry*, *12*(4), 980–987, doi:10.1002/chem.200500980.

Barker, S., M. Greaves, and H. Elderfield (2003), A study of cleaning procedures used for foraminiferal Mg/Ca paleothermometry, *Geochem. Geophys. Geosyst.*, *4*(9), 8407, doi:10.1029/2003GC000559.

Beyer, H., W. Walter, and W. Francke (1998), *Lehrbuch der Organischen Chemie*, 23rd ed., Hirzel, Stuttgart, Germany.

Boiseau, M., and A. Juillet-Leclerc (1997), H_2O_2 treatment of recent coral aragonite: Oxygen and carbon isotopic implications, *Chem. Geol.*, *143*(3–4), 171–180, doi:10.1016/S0009-2541(97)00112-5.

Bourgoin, B. P. (1987), *Mytilus edulis* shells as environmental recorders for lead contamination, Ph.D. thesis, McMaster Univ., Hamilton, Ont., Canada.

Bryan, S. P., and T. M. Marchitto (2010), Testing the utility of paleonutrient proxies Cd/Ca and Zn/Ca in benthic foraminifera from thermocline waters, *Geochem. Geophys. Geosyst.*, *11*, Q01005, doi:10.1029/2009GC002780.

Carriker, M. R., R. E. Palmer, L. V. Sick, and C. C. Johnson (1980), Interaction of mineral elements in sea water and shell of oysters (*Crassostrea virginica* (Gmelin)) cultured in controlled and natural systems, *J. Exp. Mar. Biol. Ecol.*, *46*(2–3), 279–296.

CRC Press (2010–2011), *CRC Handbook of Chemistry and Physics*, 91st ed., Boca Raton, Fla.

Dodd, J. R. (1967), Magnesium and strontium in calcareous skeletons: a review, *J. Paleontol.*, *41*(6), 1313–1329.

Domininghaus, H. (2004), *Die Kunststoffe und ihre Eigenschaften*, Springer, Heidelberg, Germany.

Endres, H.-J., and A. Siebert-Raths (2009), *Technische Biopolymere*, Hanser, München, Germany.

Foster, L. C., A. A. Finch, N. Allison, C. Andersson, and L. J. Clarke (2008a), Mg in aragonitic bivalve shells: Seasonal variations and mode of incorporation in *Arctica islandica*, *Chem. Geol.*, *254*, 113–119, doi:10.1016/j.chemgeo.2008.06.007.

Foster, L. C., C. Andersson, H. Høie, N. Allison, A. A. Finch, and T. Johansen (2008b), Effects of micromilling on d^{18}O in biogenic aragonite, *Geochem. Geophys. Geosyst.*, *9*, Q04013, doi:10.1029/2007GC001911.

Foster, L. C., N. Allison, A. A. Finch, and C. Andersson (2009), Strontium distribution in the shell of the aragonite bivalve *Arctica islandica*, *Geochem. Geophys. Geosyst.*, *10*, Q03003, doi:10.1029/2007GC001915.

Gaffey, S. J., and C. E. Bronnimann (1993), Effects of bleaching on organic and mineral phases in biogenic carbonates, *J. Sediment. Res.*, *63*(4), 752–754.

Gaffey, S. J., J. J. Kolak, and C. E. Bronnimann (1991), Effects of drying, heating, annealing, and roasting on carbonate skeletal material, with geochemical and diagenetic implications, *Geochim. Cosmochim. Acta*, *55*(6), 1627–1640, doi:10.1016/0016-7037(91)90134-Q.

Hänsel, R., and O. Sticher (2009), *Pharmakognosie-Phytopharmazie*, Springer, Heidelberg, Germany.

Holmes, J. A. (1996), Trace-element and stable-isotope geochemistry of non-marine ostracod shells in Quaternary palaeoenvironmental reconstruction, *J. Paleolimnol.*, *15*(3), 223–235, doi:10.1007/BF00213042.

Keatings, K. W., J. A. Holmes, and T. H. E. Heaton (2006), Effects of pre-treatment on ostracod valve chemistry, *Chem. Geol.*, *235*(3–4), 250–261, doi:10.1016/j.chemgeo.2006.07.003.

Kennedy, W. J., J. D. Taylor, and A. Hall (1969), Environmental and biological controls on bivalve shell mineralogy, *Biol. Rev. Cambridge Philos. Soc.*, *44*(4), 499–530, doi:10.1111/j.1469-185X.1969.tb00610.x.

Klemm, D., B. Heublein, H.-P. Fink, and A. Bohn (2005), Cellulose: Faszinierendes Biopolymer und nachhaltiger Rohstoff, *Angew. Chem.*, *117*(22), 3422–3458, doi:10.1002/ange.200460587.

Krampitz, G., H. Drolshagen, J. Hausle, and K. Hof-Irmscher (1983), Organic matrices of mollusk shells, in *Biomineralization and Biological Metal Accumulation*, edited by P. Westbroek and F. W. de Jong, pp. 231–247, D. Reidel, Dordrecht, Netherlands.

Levi-Kalisman, Y., G. Falini, L. Addadi, and S. Weiner (2001), Structure of the nacreous organic matrix of a bivalve mollusk shell examined in the hydrated state using Cryo-TEM, *J. Struct. Biol.*, *135*(1), 8–17, doi:10.1006/jsbi.2001.4372.

Lingard, S. M., R. D. Evans, and B. P. Bourgoin (1992), Method for the estimation of organic-bound and crystal-bound metal concentrations in bivalve shells, *Bull. Environ.*

Contam. Toxicol., *48*(2), 178–184, doi:10.1007/BF00194369.

Love, K. M., and A. Woronow (1991), Chemical changes induced in aragonite using treatments for the destruction of organic material, *Chem. Geol.*, *93*(3–4), 291–301, doi:10.1016/0009-2541(91)90119-C.

Lowenstam, H. A., and S. Weiner (1989), *On Biomineralization*, Oxford Univ. Press, New York.

McCrea, J. M. (1950), On the isotopic chemistry of carbonates and a paleotemperature scale, *J. Chem. Phys.*, *18*(6), 849–857, doi:10.1063/1.1747785.

Mitsuguchi, T., E. Matsumoto, O. Abe, T. Uchida, and P. J. Isdale (1996), Mg/Ca thermometry in coral skeletons, *Science*, *274*(5289), 961–963, doi:10.1126/science.274.5289.961.

Mitsuguchi, T., T. Uchida, E. Matsumoto, P. J. Isdale, and T. Kawana (2001), Variations in Mg/Ca, Na/Ca, and Sr/Ca ratios of coral skeletons with chemical treatments: Implications for carbonate geochemistry, *Geochim. Cosmochim. Acta*, *65*(17), 2865–2874, doi:10.1016/S0016-7037(01)00626-3.

Nachtigall, W. (2002), *Bionik: Grundlagen und Beispiele für Ingenieure und Naturwissenschaftler*, Springer, Heidelberg, Germany.

Nudelman, F., B. A. Gotliv, L. Addadi, and S. Weiner (2006), Mollusk shell formation: Mapping the distribution of organic matrix components underlying a single aragonitic tablet in nacre, *J. Struct. Biol.*, *153*(2), 176–187, doi:10.1016/j.jsb.2005.09.009.

Pingitore, N. E., S. B. Fretzdorff, B. P. Seitz, L. Y. Estrada, P. M. Borrego, G. M. Crawford, and K. M. Love (1993), Dissolution kinetics of CaCO$_3$ in common laboratory solvents, *J. Sediment. Petrol.*, *63*(4), 641–645, doi:10.1306/D4267B9A-2B26-11D7-8648000102C1865D.

Schöne, B. R., Z. Zhang, D. E. Jacob, D. P. Gillikin, T. Tütken, D. Garbe-Schönberg, T. McConnaughey, and A. L. Soldati (2010), Effect of organic matrices on the determination of the trace element chemistry (Mg, Sr, Mg/Ca, Sr/Ca) of aragonitic bivalve shells (*Arctica islandica*)—Comparison of ICP-OES and LA-ICP-MS data, *Geochem. J.*, *44*(1), 23–37.

Schöne, B. R., Z. Zhang, P. Radermacher, J. Thébault, D. E. Jacob, E. V. Nunn, and A.-F. Maurer (2011), Sr/Ca and Mg/Ca ratios of ontogenetically old, long-lived bivalve shells (*Arctica islandica*) and their function as paleotemperature proxies, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *302*(1–2), 52–64, doi:10.1016/j.palaeo.2010.03.016.

Takesue, R. K., C. R. Bacon, and J. K. Thompson (2008), Influences of organic matter and calcification rate on trace elements in aragonitic estuarine bivalve shells, *Geochim. Cosmochim. Acta*, *72*(22), 5431–5445, doi:10.1016/j.gca.2008.09.003.

Takeuchi, T., I. Sarashina, M. Iijima, and K. Endo (2008), In vitro regulation of CaCO$_3$ crystal polymorphism by the highly acidic molluscan shell protein Aspein, *FEBS Lett.*, *582*(5), 591–596, doi:10.1016/j.febslet.2008.01.026.

Urey, H. C. (1948), Oxygen isotopes in nature and in the laboratory, *Science*, *108*, 489–496, doi:10.1126/science.108.2810.489.

Vander Putten, E., F. Dehairs, E. Keppens, and W. Baeyens (2000), High resolution distribution of trace elements in the calcite shell layer of modern *mytilus edulis*: Environmental and biological controls, *Geochim. Cosmochim. Acta*, *64*(6), 997–1011, doi:10.1016/S0016-7037(99)00380-4.

Watanabe, T., M. Minagawa, T. Oba, and A. Winter (2001), Pretreatment of coral aragonite for Mg and Sr analysis:

Implications for coral thermometers, *Geochem. J.*, *35*(4), 265–269.

Weber, J. N., P. Deines, P. H. Weber, and P. A. Baker (1976), Depth related changes in the $^{13}C/^{12}C$ ratio of skeletal carbonate deposited by the Caribbean reef-frame building coral *Montastrea annularis*: Further implications of a model for stable isotope fractionation by scleractinian corals, *Geochim.*

Cosmochim. Acta, *40*(1), 31–39, doi:10.1016/0016-7037(76)90191-5.

Zhang, Z. (2009), Geochemical properties of shells of *Arctica islandica* (Bivalvia)—Implications for environmental and climatic change, Dr. rer. nat. thesis, Goethe-Univ. in Frankfurt am Main, Frankfurt, Germany.