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Conference paper

Bruno Thien, Agathe Martignier, Jean-Michel Jaquet and Montserrat Filella*

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Abstract: Intracellular inclusions of amorphous Ba- and Sr-rich calcium carbonates – referred to as “micropearls” – have recently been detected in Lake Geneva. These micropearls are formed under conditions of pronounced Ba and Sr undersaturation in the lake waters. Their formation can be explained by the ability of certain microorganisms to preconcentrate these trace elements in tandem with a non-equilibrium solid-solution growing mechanism.

Keywords: amorphous carbonate; barium; biomineralisation; calcium; growth entrapment; ISSP-17; lake water; solid solution; strontium; uptake kinetic effect.

Introduction

Intracellular inclusions of amorphous Ba- and Sr-rich calcium carbonates – referred to as “micropearls” – have recently been detected in Lake Geneva [1]. Initially, they were thought to exist as agglomerates inside an organic matrix but it was subsequently discovered that they are in fact intracellular mineral inclusions in unicellular phytoplankters. Scanning electron microscope (SEM) images and energy dispersive X-ray spectroscopy (EDXS) analyses have revealed two distinct types of micropearls, distinguished mainly by their chemical composition: Sr-rich and Ba-rich ones with the Ba-rich type also containing minor amounts of Sr. Micropearls always appear in clusters, embedded in the organism’s cell (Fig. 1a and b). Clusters can contain ~10–200 micropearls (diameter: 0.2–3 μm). The unicellular alga Tetraselmis cf. cordiformis, the organism where Sr-micropearls was detected, has a diameter of 15 μm and the micropearls contain up to 25 mol% SrCO₃. The organisms which contain Ba-micropearls are, as yet, unidentified biflagellate eukaryotes. The associated micropearls can contain up to 97 mol% BaCO₃ with a maximum of 10 mol% SrCO₃ (Fig. 1 in Sup Info file). They range from 7 to 20 μm in size. The micropearls in the smaller organisms (7–10 μm) contain more Ba than those in the larger ones (20 μm).

Considering the low concentration of Ba and Sr in Lake Geneva waters, the formation of micropearls requires strong preconcentration inside the cells. This might be complemented by a solid solution formation-linked mechanism. A solid solution is a mixture of two crystalline solids that coexist as a new crystalline solid. Its solubility is usually lower than that of the two pure phases. Thermodynamic concepts have already
been successfully applied to account for equilibrium of trace elements between microorganisms and water [2, 3]. In this study, we test whether solid solution-based modeling provides an insight on the formation of the observed micropearls.

Some theoretical background

The distribution of a trace element Tr (relative to the host component Hc) between a mineral and an aqueous solution can be described by a fractionation coefficient, $\Delta_{\text{Tr/Hc}}$:

$$\Delta_{\text{Tr/Hc}} = \frac{\{\text{Tr/Hc}\}_{\text{mineral}}}{\{\text{Tr/Hc}\}_{\text{solution}}}$$

where $\{\text{Tr/Hc}\}$ are molar concentration or activity ratios. $\Delta_{\text{Tr/Hc}}$ can be experimentally measured (e.g. [4]) or derived from theoretical calculations (e.g. [5]). It has been shown that the higher the host mineral growth rate is, the more $\Delta_{\text{Tr/Hc}}$ deviates from the equilibrium value (e.g. [6]). This effect has been theoretically explained by Watson [7] with the “growth entrapment model” that postulates that a growing mineral has a tendency to take the composition of its surface layer. It implies that the measured $\Delta_{\text{Tr/Hc}}$ can be higher or lower than the value measured at equilibrium and that the measurement of $\Delta_{\text{Tr/Hc}}$ at equilibrium requires a very low growth rate of the host mineral. An alternative model has been proposed by DePaolo [8]. This model gives quantitatively similar results but the mechanisms are different: precipitation and re-dissolution kinetics. The deviation of $\Delta_{\text{Tr/Hc}}$ from the equilibrium value has been called the uptake kinetic effect and can be implemented in geochemical modeling codes [9]. This is the approach considered in this study. Heberling and co-workers offer a different concept called “thermodynamic adsorption/entrapment” [10] where the deviation from the equilibrium is due to different thermodynamic properties between the bulk mineral and the surface layers.

Methods

The incorporation of a trace element in a host mineral can be described by an aqueous – solid solution with two end-members, for instance HcCO$_3$ and TrCO$_3$ in the case of carbonates, with the composition of the mineral varying between these two limits. In this study, we considered aqueous – solid solutions with carbonates having a rhombohedral structure (RH) like calcite and an orthorhombic structure (OR) like aragonite.
The geochemical modeling package GEM-Selektor v.3 [11, 12] makes it possible to calculate the mineral composition as a function of the aqueous solution composition. To do so, the thermodynamic properties of the two end-members and the interaction coefficient of the aqueous-solid solution \( W_g \), which accounts for its non-ideality [13], must both be known. The non-ideality implies that \( \Delta_{\text{Tr}/\text{Ca}} \) may vary either as a function of the solution composition or that some compositions of the aqueous – solid solutions may be metastable.

The chemical thermodynamic system was set up for Ba, C, Ca, H, O, Sr, and charge. Overall charge neutrality was assumed. The thermodynamic parameters of aqueous species, gasses and minerals come from the GEM-Selektor variant of the Nagra-PSI data base [14], except those for rhombohedral (RH) SrCO\(_3\) and BaCO\(_3\) which come from [15]. They are shown in Table 1 of the Sup Info file. Aqueous activity coefficients were calculated using the extended Debbye–Hückel equation (common ion size parameter of 0.372 nm and the third parameter \( b_g \) of 0.064 as for NaCl electrolyte were used). All the calculations have been performed at 25°C because most of the data are available at this temperature.

The interaction parameters, \( W_g \), were taken from the literature if available. When only an experimental value of \( \Delta_{\text{Tr}/\text{Ca}} \) supposed to be at equilibrium is known, the corresponding \( W_g \) can be recalculated by using GEM-Selektor. The interaction parameters of the different aqueous – solid solutions used in this study are summarized in Table 1 of the Sup Info file. The Ca–Sr carbonate system has been investigated in detail by Kulik and co-workers [13]. They derived \( W_g \) values from theoretical considerations. For the calcite – SrCO\(_3\) (RH) solid solution, we recalculated \( \Delta_{\text{Sr}/\text{Ca}} \) and it exactly coincides with the value by Tesoriero and Pankow [16]. For the aragonite – SrCO\(_3\) (OR) (strontianite) solid solution, no relevant experimental data were available for comparison. There is scant data for the Ca–Ba carbonate system. For calcite – BaCO\(_3\) (RH), \( \Delta_{\text{Ba}/\text{Ca}} \) has been measured: 0.012 [16] and 0.016 [17]. We therefore considered an average value of 0.014 and derived the value of the interaction parameter. No such studies exist for the aragonite – BaCO\(_3\) (OR) (witherite) solid solution. \( \Delta_{\text{Ba}/\text{Ca}} \) have been measured for sea-carbonates [18] but the high values of \( \Delta_{\text{Sr}/\text{Ca}} \) obtained in the study suggest a high uptake kinetic effect. Therefore those values are not at equilibrium and cannot be considered to establish \( W_g \). Wang and Xu [5] performed theoretical calculations in order to establish equilibrium \( \Delta_{\text{Tr}/\text{Ca}} \) in carbonates. Although their results were not consistent with experimental observations for Sr in calcite, they were for Ba in calcite and Sr in aragonite. Since no better values are available, we considered this study for the value of \( \Delta_{\text{Ba}/\text{Ca}} \) at equilibrium in the aragonite – witherite solid solution.

It should be mentioned that the carbonates in the phytoplankton are amorphous [1] whereas the studies cited concern well-crystallized carbonates. Values of distribution coefficients in amorphous carbonates (as is the case for thermodynamic data) are quasi non-existent in the literature but it has been shown that amorphous calcium carbonate is a precursor of crystalline carbonate and that a continuum exists between these two stages [19]. In addition, Politi et al. [20] showed that the amorphous precursor phase of calcite in sea urchin embryos exhibit a short-range order (i.e. a non-random distribution) similar to calcite. This is why this study considers the values for crystallized carbonates, on the assumption that they are not much different from amorphous ones [21].

**Results and discussion**

**Composition of the carbonates**

In Lake Geneva waters, Sr and Ba concentrations are much lower than Ca ones and well below the saturation values of their carbonates (Fig. 2 in Sup Info file). In comparison, the carbonate micropearls found in the phytoplankton contain significant amounts of both elements. Calculation of \( \Delta_{\text{Sr}/\text{Ca}} \) considering the micropearls and lake water concentrations gives values ranging from 10.5 to 27863 (Table 1). Published \( \Delta_{\text{Sr}/\text{Ca}} \) and \( \Delta_{\text{Ba}/\text{Ca}} \) values for Ca carbonates range from 0.02 to 1.25 and 0.003 to 3 [4, 18], respectively. Observed variations are function of temperature, pH and mineral growth rate. Thus the carbonates in the phytoplankton contain higher concentrations of these trace elements than it is possible to incorporate directly from lake water.
Enrichment in trace elements inside the microorganisms

For micropearls to form, phytoplankters must concentrate Ba, Sr and Ca inside the cell. Although the actual mechanism is not known, it is a plausible hypothesis that these hard-acid type elements are concentrated as they would be if complexed by carboxylate groups, which are the most common complexing groups in carbohydrates. By considering the equilibrium constant of acetate with Ca, Ba and Sr (values in Table 3 of Sup Info file), we calculated a possible composition of the aqueous solution inside the microorganisms (Table 1). It is important to note that such calculations do not provide absolute concentrations of the elements, but only concentration ratios. By considering that the carbonates grow from this solution, the corresponding $\Delta_{\text{Tr}/\text{Ca}}$ can be recalculated. The values obtained are given in Table 1. They fall exactly in the range given in the literature. Although it is worth pointing out that it is not yet known whether the micropearls are contained in the cell cytoplasm or in specific compartments (i.e. vacuoles), this has no impact on what follows.

Aqueous – solid solution and uptake kinetics

The next step is to investigate whether the measured composition of the micropearls and their composition calculated from the estimated composition of the internal cell solution are in accordance with the theory of aqueous solid solutions.

An important point, usually neglected in the calculation of fractionation coefficients at equilibrium when applying solid solution theory, is that they can vary as a function of the composition of the solution and/or the solid [22]. All literature $\Delta$ values discussed above correspond to a situation where $[\text{Tr}] << [\text{Ca}]$. In our case, this condition does not apply inside the cells and, thus, we took it into account when calculating the theoretical values of $\Delta_{\text{Tr}/\text{Ca}}$ related to the micropearls (i.e. by considering the possible composition of the aqueous solution inside the microorganisms). The results (Table 2) indicate that the variation of $\Delta_{\text{Tr}/\text{Ca}}$ as a function of the solution composition is negligible for calcite-like structure aqueous – solid solutions, but becomes significant in aragonite-like structure aqueous – solid solutions. More details about the calculations

Table 1: Molar ratios and fractionation coefficients $\Delta$.

<table>
<thead>
<tr>
<th>Molar ratios</th>
<th>$\Delta_{\text{Sr}/\text{Ca}}$</th>
<th>$\Delta_{\text{Ba}/\text{Ca}}$</th>
<th>$\Delta_{\text{Sr}/\text{Ca}}$</th>
<th>$\Delta_{\text{Ba}/\text{Ca}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake water*</td>
<td>$4.65 \times 10^{-3}$</td>
<td>$1.17 \times 10^{-4}$</td>
<td>1.23</td>
<td>1.36</td>
</tr>
<tr>
<td>Solution inside MO</td>
<td>0.075</td>
<td>–</td>
<td>16.1</td>
<td>–</td>
</tr>
<tr>
<td>Ca–Sr micropearls</td>
<td>0.087</td>
<td>3.26</td>
<td>18.7</td>
<td>27,863</td>
</tr>
<tr>
<td>Ca–Sr–Ba micropearls (smaller diameter)</td>
<td>0.049</td>
<td>0.59</td>
<td>10.5</td>
<td>5043</td>
</tr>
<tr>
<td>Ca–Sr–Ba micropearls (larger diameter)</td>
<td>0.049</td>
<td>0.59</td>
<td>10.5</td>
<td>5043</td>
</tr>
</tbody>
</table>

*aAverage Lake Geneva waters (2012–2014). Values at the GE3 station. See Fig. 2 in Sup Info file. Molar ratios were measured, except those for the solution inside the microorganisms (MO) that were calculated according to the hypothesis explained in the text. $\Delta$ were recalculated, for both lake water and solution inside the MO.

Table 2: Equilibrium fractionation coefficients of the different aqueous – solid solutions considered in this study.

<table>
<thead>
<tr>
<th>Systems</th>
<th>$\Delta_{\text{Tr}/\text{Ca}}$, eq. lake water</th>
<th>$\Delta_{\text{Tr}/\text{Ca}}$, eq. MO water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcite – SrCO$_3$(RH)</td>
<td>0.021</td>
<td>0.025</td>
</tr>
<tr>
<td>Aragonite – SrCO$_3$(OR) (strontianite)</td>
<td>0.50</td>
<td>129</td>
</tr>
<tr>
<td>Calcite – BaCO$_3$(RH)</td>
<td>0.014</td>
<td>0.015</td>
</tr>
<tr>
<td>Aragonite – BaCO$_3$(OR) (witherite)</td>
<td>0.14</td>
<td>14</td>
</tr>
</tbody>
</table>

(RH) means rhombohedral structure (i.e. calcite-like structure), and OR means orthorhombic structure (i.e. aragonite-like structure).
along with their visualizations are given in Fig. 3 of Sup Info file. Although the calculated \( \Delta_{\text{Tr/Ca}} \) values (Table 1) in the micropearls differ from the equilibrium values (Table 2), in calcite-like structures they remain within the same order of magnitude, while in aragonite-like structures the differences are very large. Therefore, these results clearly point to micropearls having calcite-like structures only.

The difference between \( \Delta_{\text{Sr/Ca}} \) at equilibrium and \( \Delta_{\text{Sr/Ca}} \) calculated in the micropearls can be explained by the uptake kinetic effect mentioned previously. When such a difference exists, an enrichment factor (i.e. the ratio between the measured value and the equilibrium value) can be calculated. It has been experimentally observed that \( \Delta_{\text{Sr/Ca}} \) increases with the growth rate in the calcite – \( \text{SrCO}_3 \) (RH) solid solution with a maximum enrichment factor value around 6 [6, 16]. A similar effect has been observed for calcite – \( \text{BaCO}_3 \) (RH) solid solution with a maximum enrichment factor of 10 [16]. No such studies exist for the aragonite systems but enrichment factors of 2.3 for aragonite – strontianite and 15.5 for aragonite – witherite solid solutions in seawater can be estimated from measured \( \Delta_{\text{Sr/Ca}} = 1.17 \) and \( \Delta_{\text{Ba/Ca}} = 2.17 \) values for aragonite in seawater [18] and the assumption that Sr/Ca ratio in seawater is similar to that of Lake Geneva (i.e. equilibrium values of \( \Delta_{\text{Sr/Ca}} \) for diluted systems given on Table 2 can be used).

Based on equilibrium \( \Delta_{\text{Sr/Ca}} \) (Table 2) and estimated \( \Delta_{\text{Sr/Ca}} \) (Table 1), the enrichment in the micropearls can be calculated. Let us first consider the calcite-like structures. The enrichment ranges between 1.6 and 2.8 for Sr; and between 29 and 160 for Ba. The values of 29 and 160 look too high when compared with the enrichment factor of 10 for Ba in calcite mentioned above, but it has been shown that, at very high growth rates, the enrichment can be higher than measured values of the enrichment factor based on sorption [23]. When considering an aragonite-like structure, the enrichment is always lower than one (i.e. depletion instead of enrichment) whereas the data of Gaetani and Cohen [18] might suggest that Sr is enriched rather than depleted in aragonite. Thus, we can again conclude that the micropearls consist of a calcite-like structure. Proper alignment of the Ca–Ba–Sr micropearls with equilibrium calcite in Fig. 2 reinforces the hypothesis of a calcite-like structure of the micropearls. Moreover, the correlation between the enrichment in Sr and in Ba suggests that the same enrichment mechanism occurs. These conclusions are fully in line with the experimental observations [1].

### Are results valid at temperatures other than 25 °C?

The microorganisms form in the photic zone of the lake, where the temperature reaches 25 °C in summer, but can drop to as low as 4 °C in winter (Fig. 4 in Sup. Info). However, as mentioned above, we considered a temperature of 25 °C for this study and, thus, the results obtained are only valid for the carbonates formed at 25 °C unless the potential impact of lower temperatures is clarified. In fact, \( \Delta_{\text{Sr/Ca}} \) decreases with an increase of temperature for Sr and Ba in aragonite [18, 24] and for Sr in calcite [25]. There is no data available for Ba in calcite but there is no reason why it should not follow the same behavior as Sr. The data reported in these
studies point to a maximum increase of $\Delta_{\text{Tr/Ca}}$ from 25 °C to 5 °C of 1.8 (at a similar growth rate). So, even if some of the microorganisms form at temperatures below 25 °C, this has no impact on the conclusions drawn in this study.

**Effect of organism size on micropearl composition**

The Ba-micropearls observed in the smaller organisms are more enriched in Sr and Ba than those formed in the larger ones. The reasons behind the existence of two different-sized organisms (i.e. different stages of their growing cycle versus different taxa) are outside the scope of this study. It can be mentioned however that the hypothesis of different growing stages is unlikely because, in this case, more than two sizes would be observed. On the other hand, and irrespective of the reasons behind the existence of organisms of two sizes, the effect of organism size on the micropearl composition is of interest.

This could be nicely explained by the interlinkage between precipitation rates and trace element solution concentrations in the frame of the solid solution approach: when considering a closed medium, the internal solution in the smaller microorganisms should get depleted in Ca sooner than in the bigger ones, and this would trigger a decrease in the micropearl precipitation rate (i.e. a decrease of the saturation index of the carbonates). Thus, the micropearls would automatically be less concentrated in Sr and Ba. Interestingly, even if the Ca depletion was not sufficient to trigger a significant decrease in the precipitation rate, the depletion of the solution in Sr and Ba would in any case lead to less enriched micropearls. And yet we observe that the micropearls in the small microorganisms are nonetheless enriched in Sr and Ba! In fact, the space inside the microorganisms is probably not a closed medium. If the microorganisms concentrate the elements from the lake water, it is reasonable to assume a capacity to reload the depleted solution. It has been observed [1] that the internal structure of the micropearls is not uniform but is constituted of several layers, suggesting the existence of depletion/reloading cycles (Fig. 1c). Such a mechanism is certainly not instantaneous but rather subject to a certain kinetic rate. In such a case, the capacity of the microorganism to bring the elements inside depends on the surface area of the cell membrane. The higher the surface/volume ratio is, the faster the replenishment process. By assuming a spherical shape of the space inside the microorganisms, the surface/volume ratio is equal to $3/r$ where $r$ is the radius. Therefore, the smaller the microorganism (or the vacuole), the lower the depletion of the solution inside and the more enriched the micropearls will be.

**The time scale of formation of micropearls**

The time necessary to precipitate the carbonate micropearls can be estimated by using a kinetic equation (e.g. [26]) but it requires the saturation index of the mineral to be calculated. This is not possible as we cannot measure the composition of the solution inside the cells. Therefore, other methods need to be used to estimate the precipitation rate. For instance, $\Delta_{\text{Sr/Ca}}$ can be used because $\Delta_{\text{Sr/Ca}}$ in calcite increases as a function of the growth rate and, thus, a given value of $\Delta_{\text{Sr/Ca}}$ should correspond to a certain value of the growth rate. However, different studies report significant variations of $\Delta_{\text{Sr/Ca}}$ for a similar growth rate [9]. We use here the experimental data of Tang and co-workers [25] to estimate micropearl growth rates because their average pH values are in line with those of the lake (ca. 8). The recalculated growth rates corresponding to the $\Delta_{\text{Sr/Ca}}$ values under consideration are given in Table 3. If the size of the micropearls ranges between 0.3 and 2.5 μm, their potential growth time would then be between 0.6 and 72 days.

Since $\Delta_{\text{Ba/Ca}}$ decreases with the temperature and larger microorganisms form micropearls which are less concentrated in Ba, it would be tempting to formulate the hypothesis that micropearls in the larger microorganisms form at higher temperatures than in the smaller ones. Such an hypothesis would imply a growth time of micropearls of 1.6–13 days at 5 °C and of 9–72 days at 25 °C. This hypothesis is supported by the experimental observation that higher numbers of smaller microorganisms have been observed in winter than in summer [1]. However, processes in nature are probably more complex than this hypothesis suggests because bigger
and smaller organisms have also been observed simultaneously in summer. Thus, other physico-chemical temperature-dependent factors such as higher content of dissolved CO\textsubscript{2} in cold water (which favors the precipitation of carbonates) or the Arrhenius effect (the higher the temperature, the faster the carbonate precipitation rate) very probably play a variable and simultaneous role. Finally, it should also not be forgotten that temperature has a direct effect on the presence and life cycle of the microorganisms, irrespective of the formation of micropearls.

**Conclusions**

Amorphous calcium carbonate micropearls growing inside phytoplanktonic microorganisms in Lake Geneva exhibit very high concentrations of Sr and Ba compared to the lake water. Such extreme enrichment can be explained by the probable pre-accumulation of those elements in the internal solution inside the microorganisms combined with a growth entrapment effect. On the basis of our solid solution thermodynamic modeling, we can conclude that the micropearls have a calcite-like structure and estimate a formation time of the order of a few days. Our results also provide an insight into the reasons why the micropearls in the smaller microorganisms are more enriched in Sr and Ba than those in the larger ones. This study shows that physico-chemical modeling provides a useful explanatory tool for understanding observed complex natural phenomena.

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**References**


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