Confocal micro-Raman spectroscopy: A tool for the allocation of organic and inorganic components in calcified biominerals

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Mineralized biological composites have attracted increasing interest because of their outstanding mechanical properties that are well adapted to their function. Microcharacterization of these materials allows a better understanding of the structure-properties relation ship of these materials. Since the mineralized exoskeleton (cuticle) of crustaceans is composed of various types of calcified biominerals e.g. calcium phosphate, calcite, and amorphous calcium carbonate (ACC) embedded in a chitin matrix this material is an excellent model for a biocomposite. High resolution scanning electron microscopy has been extensively used to investigate the morphology of the crustacean cuticle. However, to allocate of the spatial resolution of the various organic and inorganic components an additional chemical characterization is required. Scanning confocal µ-Raman spectroscopy (SCµ-RS) provides complementary important information about the spatial distribution of both the orgnaic and the inorganic components because the Raman spectrum can be unequivocally attributed to a certain compound even when its concentration is very low. Additionally, SCµ-RS enables one to allocate the different calciumcarbonate polymorphs.

For these studies the cuticle of the terrestrial isopod *Porcellio scaber* is be used as a model system. The spatial distribution of minerals, elements, and organic compounds was studied on the sub-micrometer scale using cross sections of the cuticle. Calcite and amorphous calcium carbonate (ACC) were found to be the main biominerals within the cuticle. For the first time, it was shown that the minerals are arranged in distinct layers. Calcite is restricted to the outer area of the cuticle, whereas ACC is localized in the middle having only little overlap with the calcite layer. The proximal region of the cuticle mainly consists of chitin. Since the cuticle is subjected to periodic molting it is periodically decalcified and shed. A new larger cuticle, synthesized before shedding, is mineralized after every molt. These processes cause spatial and temporal variations of the mineral distribution. The change in mineral during the molting cycle was monitored with respect to the organic matrix. It was shown that the protective outer calcite layer is shed away during each molt, while ACC is recycled to quickly re-establish the protective calcite layer in the new cuticle. Thus, ACC is used as a transient reservoir for calcium and carbonate ions.

Demonstrating equilibrium Fe-isotope fractionation in Fe-Cl solutions

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A major problem when conducting isotopic fractionation experiments in aqueous solutions is determining if isotopic equilibrium has been reached. We have successfully demonstrated the attainment of equilibrium isotopic fractionation in a two-phase aqueous/ether system containing a mixture of Fe-Cl complexes, using the Fe three-isotope equilibrium method of Shahar *et al*. (2006; Matsuhisha *et al*., 1978). The immiscible liquid/spiked reversal procedure should also be applicable to other aqueous systems. These reversal experiments are part of our continuing integrated theoretical and experimental studies of the effects of changing bond environments on Fe fractionation. We use aqueous Fe-Cl complexes as a example of possible Fe-ligands because of the structural similarity of chloride to other potential iron-ligands, such as sulfides (e.g., pyrite, FeS_2) and small organic ligands (e.g., rubredoxin, FeS_4R_4 similar in structure to tetrahedral $FeCl_4^-$), and the tractability of chloride in aqueous experiments.

Our experiments consist of a series of low pH solutions of ferric chloride, with total chlorinity varying from 0.5 to 5.0 M, to which an equal amount of immiscible diethyl ether has been added. As the aqueous-ether mixture equilbrates, $FeCl_4^-$, the only Fe-Cl complex soluble in the ether, moves from the aqueous into the ether phase. We use measurements of $\delta^{56}Fe(aq) - \delta^{56}Fe(ether)$, in conjunction with a speciation model, to determine the relative fractionations among the complexes. At $[Cl^-]$=1M the aq-ether fractionation is ~0.8‰.

To establish the attainment of equilibrium between the ether and aqueous phases, we paired an aq/ether mixture of unspiked $^{56}Fe/^{54}Fe$ with an equivalent mixture prepared with ^{54}Fe spike. Once both mixtures had equilibrated, half the ether from the spiked experiment was added to the ether of the normal Fe experiment, so that spiked $FeCl_4^-$ from the ether phase would have to pass into the aqueous phase of the unspiked experiment to reach equilibrium. We removed small aliquots from both the ether and aqueous phases after 20, 30, and 40 minutes, without disburbing the equilbrium of the mixture, and measured the aqueous-ether fractionation.

At 20 minutes, the ether and aqueous solutions were ~80% equilibrated, and they were totally equilibrated by 30 minutes. Equilibrium was demonstrated by the reversal in two ways: 1) both the aqueous and ether phases arrived at the same mass fractionation line; and 2) the final aqueous-ether fractionation of the reversal experiment was ~0.8‰, in agreement with the unspiked forward experiments.

References

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