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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Gallium interaction with wet synthetic analogues to bone mineral model for understanding Ga role in bone metabolism.	**Experiment number**: SC-1355
Beamline:	**Date of experiment**: from: 04 feb 2004 to: 07 feb 2004	**Date of report**:
Shifts:	**Local contact(s)**: Dr. Bohic Sylvain	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

D. Eichert* (ID 21, ESRF, Grenoble)

Report:

Introduction:

The surface properties of apatites play a crucial role in their biological behavior, however the alterations of the surface structure in aqueous media have not been extensively studied. The study of poorly crystalline apatites (PCA) analogue to bone mineral has revealed the existence of a hydrated layer probably located on the surface of the nanocrystals, associated with very specific spectroscopic characteristics observed by FTIR and MAS-NMR [1]. This hydrated unstable layer is especially developped at early stages of apatite formation and decreases with ageing due to the development of more stable apatitic domains. This evolution seems however to be limited and never reach an end: the surface layer seems always present even in mature apatites. The ions of the hydrated layer can be easily replaced by other ions from the solution [2,3] and several ion exchange reactions have been described. The object of the present experiment was to analyze the alterations of the surface layer by ionic substitution with gallium, to determine its introduction palthway into the apatitic phase and the interation between PCA and gallium. Indeed, gallium has been used in medical practice for many years: gallium nitrate is biologically active in blocking bone resorption, then diminishing bone pains and pathological fractures [4], gallium has also an anticancer activity, but its mechanism of absorption in bone is unknown.

Samples analyzed:

Firstable, some gallium standards were prepared and analyzed: different concentrations of $Ga(NO_3)_3$, (used in the exchange reactions) embedded in a cellulose binder (1, 5, 10 mg, completed at 100 mg with cellulose); some ICP standard gallium solutions (1 to 100 ppm), put in capillaries, but also metallic gallium and Ga_2O_3 (EXAFS standards). So that we could already determine the detection limit, the sensitivity to the Ga ion environment, and check the position of the Ga K-edge (10.367 keV).

Poorly crystalline apatites have been precipitated by double decomposition between a calcium and a phosphate solution or between a calcium and a phosphate-carbonate solution in order to obtain carbonated PCA. Several types of synthetic bone mineral analogues, at different maturation stages (13 maturation times for each sort of PCA, from 0 to 6 months) were obtained and analyzed in a dry state after freeze-drying, which is the common preparation and preservation method of biological mineralized samples. All samples were checked by x-ray diffraction and FTIR spectroscopy and furnished the expected patterns.

All the samples were then submitted to an ionic exchange experiment with gallium. These exchanges are fast (completed within 5 minutes), and generally reversible. The reversibility of the reaction was also performed

in order to assess if the gallium was re-exchangeable and in which proportion. Therefore, all the samples of different maturation time were submitted to a first ion exchange with gallium and a second exchange (reversibility) with calcium.
Samples were analyzed by chemical analysis, FTIR spectroscopy and x-ray diffraction to have the most precise characterization possible and to correlate these results with those obtained in the framework of SC-1355. For the fluorescence and EXAFS experiments, pellets of a precise sample quantity (100 mg) with an homogeneous thickness were submitted to the beam, so that the results of the experiment could be as quantitative as possible.

Results:

Firstable, the standards of different gallium concentrations allow us to check the linearity of the fluorescence detector and to construct a calibration curve plotting the intensity of the fluorescence as a fonction of the concentration; the quantity of gallium exchanged with calcium was then possible to determine quantitatively and to compare with the results of the chemical analysis.
For each sample, multiple points were recorded in order to improve the statistic of the measurements (around 80 points for the fluorescence), and at least 3 scans were recorded for the EXAFS experiments.
We noticed that more than 90% of the gallium content was exchanged with the calcium, and this whatever the stage of maturation. The proportion of sites occupied by gallium is decreasing exponentially with the maturation time as it was expected. The same tendency occurs whether the apatite is carbonated or not, proving that there is no interaction and no competition between the gallium and carbonate ions into the structure, which was suspected.
After the reaction of reversibility, more that 80% of the gallium is released in solution. This proportion seems independent of the maturation state of the sample: the residue of gallium is irremediably fixed into the apatitic structure and occupies around 10% of the cationic sites. This already means that gallium is stable and is incorporated into the apatitic structure. These facts were confirmed by the EXAFS experiments. We indeed see a modification of the short range order around the gallium atoms depending if PCA is carbonated or not and we also see some differences following the concentration of gallium in our samples. We clearly see the effect of the ionic exchange and reversibility on interactomic distances. The structure disorder should be linked with the proportion of gallium in our structure. EXAFS fits with the FEFF software are on the way and should give more details about the precise structure, i.e. the interatomic distances and the coordination numbers. Comparison with x-ray diffraction patterns, where we already see a increase of the structure disorder between samples exchanged or not with gallium, will be interesting as well. No other method but EXAFS was able to give reliable information of the atomic environment of that ion. EXAFS of the Ga K-edge on these samples to obtain information on the gallium environment seems particularly attractive as we can access the local environment of this peculiar ion and see if it induces some modifications of the structure of our PCA. Comparison with EXAFS performed at the calcium K-edge is important for complementarity and the experiment is already scheduled on ID26.

The work performed at ID22 opens new possibilities to follow the modifications of the mineral crystals involved in biological calcification and to follow the exchange reactions with the surrounding environment (blood, physiologic liquids) which occurs continously in bone (ex: homeostasia). The possibility to evaluate the concentration at one particular point of the sample in microfocus configuration is also very interesting as it allows us determining if there are some particular places in bone where the ionic exchanges are favorised (like mineralization front or osteons environments) and where the ion could be stored. Here, the implications in anti-cancer drug development are very important as experiments can show exactly what the consequences of the treatment are and at what level they have an efficiency on bone concern.

Reference:

[1]: D. Eichert, H. Sfihi, C. Combes, C. Rey, Key engineering Materials, 254-256 (2004) 927-930.
[2]: S. Cazalbou, C. Combes, D. Eichert, C. Rey, Journal of Materials Chemistry 14 (2004) 2148-2153.
[3]: C. Rey, A. Hina, A. Tofighi, M.J. Glimcher, Cells and Materials 5 (1995) 345-356.
[4]: P. Collery, B. Keppler, C. Madoulet, B. Desoize. Crit. Rev. Oncology/Hematology 42 (2002) 283-296.