ESRF	**Experiment title: Study of poorly crystalline apatites analogous to bone mineral crystals**	**Experiment number**: CH1266
Beamline: ID21	**Date of experiment**: from: 08-nov-02 7:00 to: 12-nov-02 7:00	**Date of report**: 25-aug-03
Shifts:	**Local contact(s)**: M. Salome and D. Eichert	*Received at ESRF:*

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Report:

Introduction: Recent progresses in the investigation of the short range ionic organization of poorly crystalline apatitic calcium phosphates (PCA) involved in biomaterials and biological mineralizations have revealed the existence of non-apatitic environments of the mineral ions especially phosphate and carbonate. The non-apatitic ionic environments are believed to correspond to ions in a hydrated layer on the surface of the mineral crystals and they are modified by drying (Eichert, 2001). However, unlike for phosphate and carbonate ions it did not exist any direct spectroscopic evidence for a discrimination of calcium environment in apatitic compounds. Recently however a study of bone crystals by X-ray emission spectroscopy suggested the existence additional coordination types of P and Ca polyedra in PCA structure (Shpak 1998). The aim of this study was to determine accurately the spectrocopic characteristics of wet and dry calcium and phosphate ions in several apatitic and non-apatitic compounds of biological interest and to connect these observation the data already obtained on the non-apatitic environements of phosphate ions in poorly crystalline apatites analogous to bone mineral.

Samples analyzed: Several Ca-P standard samples of biological interest have been prepared (Dicalcium phosphate dihydrate (DCPD), Anhydrous dicalcium phosphate (DCPA), octacalcium phosphate (OCP), amorphous calcium phosphate, alpha and beta tricalcium phosphate (α- or β-TCP), several stoichiometric apatite (Hydroxyapatite, Chlorapatite, Fluorapatite), several non-stoichiometric well crystallized apatites containing different types of ionic vacancies such as type A and B carbonate apatites, and HPO_4^{2-}-containing apatites. Several types of synthetic bone mineral analogues at different maturation stages were obtained and analyzed either as wet precipitates of in a dry state after freeze-drying, which is the common preparation and preservation method of biological mineralized samples.

Bone samples from chicken at different ages were also analyzed and compared to synthetic standards. A mapping was accomplished on several bone slices by XANES to analyze the variations of the environment of Ca and P atoms during bone formation, ageing and remodelling.

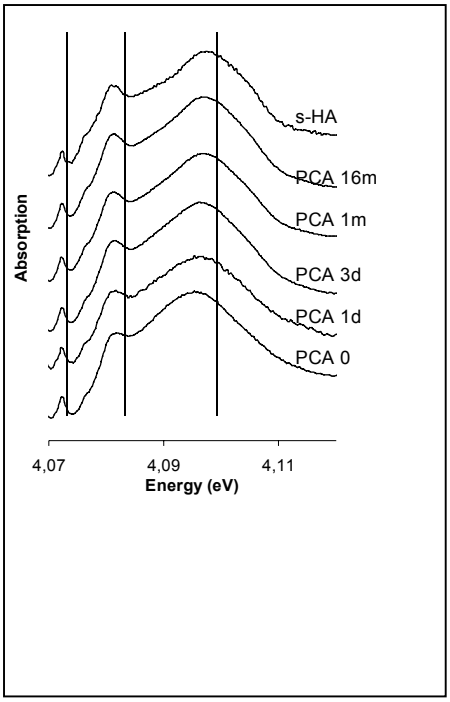
Results: The spectra obtained on well crystallized synthetic samples showed faint but distinct differences at Ca K-edge indicating that XANES allows the identification of well crystallized calcium phosphates. At P K-edge, however, the differences appeared very weak and are probably not usable for identification of Ca-P salts.

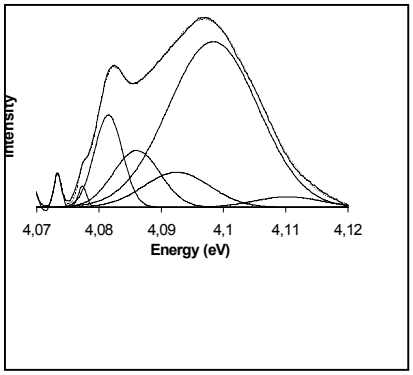
In addition faint variations were observed at Ca K-edge related to the maturation of PCA (Figure 1). A decomposition of the spectral data allowed the identification of a very specific peak directly related to the maturation of Ca salts (Figure 2). The variations of the specific peak intensity corresponded to the evolution of non-apatitic environments detected by other techniques and can be considered as the first direct evidence for the existence of non-apatitic environments of cations in PCA. The relative quantitative amount of non-apatitic environments of calcium could be determined (Figure 3) and followed that obtained by other techniques (Figure 4).

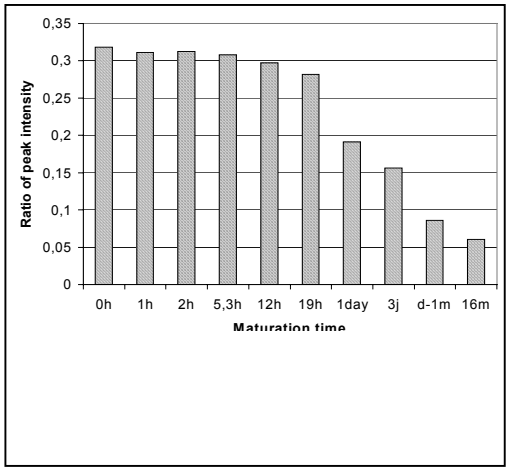
The detailed analysis of the spectra suggest that the non-apatitic environment of Ca ions is related to that observed in hydrated Ca salts such as DCPD and OCP. However we did not detect any difference between wet and dry PCA samples. It may be suggested that the hydrated environment of Ca ions is maintained on drying which is not the case for phosphate ions. XANES data suggest thus that the hydration sphere around Ca ions is not modified by freeze-drying This work thus confirms the existence of a hydrated layer probably located at the surface of PCA nanocrystals which could probably reduce their surface energy and allow their existence in biological media.

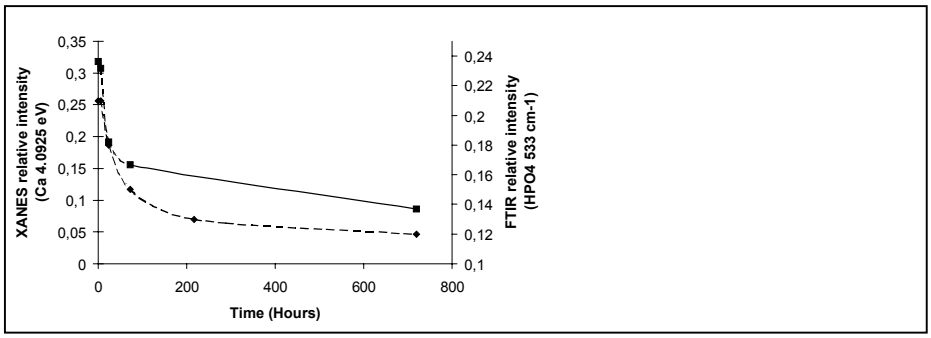
The analysis of bone samples has been accomplished recently at ESRF (May 2003) and confirms the possibility to follow the maturity of bone by using its Ca K-edge XANES spectrum. Mapping of bone at different maturation stages corresponding to aging and remodelling was attempted.

The work performed at ESRF opens new possibilities to follow the modifications of the mineral crystals involved in hard tissues or in ectopic calcifications on aging or in several disease states. As only very scarce data are collected on mineral crystals of biological origin this opportunity to obtain information on the Ca environment seems particularly attractive.









References:

D. Eichert, Thesis, INPT, 2001

A.P. Shpak, V.L. Karbovskii, V.V. Trachevskii. J. Elect. Spect. Rel. Phen. 88-91, 973-976, 1998