# Characterization of Synthetic and Biogenic Hydroxyapatite Using Modern Synchrotron Pair Distribution Function Techniques

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The composition of diseased bone (such as osteoporosis) is the subject of much debate. Recent work demonstrates that the amount of carbonate substitution within the apatite lattice of osteoporotic mineral is significantly modified compared to that of non-diseased tissue [1]. In general, increasing amounts of carbonate substitution increases dissolution rates, decreases average crystallite size and moderates crystal morphology, which could contribute to the greater rates of bone loss observed with osteoporosis. Carbonate ($CO_3^{2-}$) occupies two non-equivalent sites within biological hydroxyapatite (HA) through substitution of phosphate ions (B-type) and hydroxyl ions (A-type). Previous work to quantify the site distribution is heavily reliant on Fourier transform infrared spectroscopy (FTIR) studies which exploit lattice site degeneracy of the $\nu_2CO_3^{2-}$ absorption band. However, the significant overlapping of bands at this wavenumber produces equivocal quantification of carbonate that is not mitigated by peak fitting algorithms. Thus we have been exploring alternative techniques for determining the carbonate distribution.

Initially, we have applied pair distribution function (PDF) studies measured with a relatively high $Q$ range ($Q_{max}$ = 30 Å$^{-1}$) to investigate relationships between crystallite size and carbonate substitutions. We have compared data from conventional line profile analysis with that determinized from the mineralization ratio (MR, [2]):

$$MR = \frac{\sqrt{\sum_{r=15}^{25} G(r_m)^2/m}}{\sqrt{\sum_{r=1}^{7} G(r_n)^2/n}}$$

where $G(r_n)$ is the amplitude of the PDF at point $n$ for the set of values $\{r_1 = 1, r_2 = 1 + \delta, \ldots, r_n = 7\}$ (where $\delta$ is the PDF spacing), $n$ is the total number of values summed, $G(r_m)$ is the amplitude of the PDF at point $m$ for the set of values $\{r_1 = 15, r_2 = 15 + \delta, \ldots, r_n = 25\}$, and $m$ is the total number of values summed. Additionally small box modelling using PDFGui [3] and DiffPy-CMI [4] was employed.

Samples including standard reference materials, synthetic HA with a range of $CO_3^{2-}$ substitution levels, and biological HA taken from eight species with varying known physicochemical properties (such as crystallite size and $CO_3^{2-}$ substitution) were measured. PDFs were produced at Diamond Light Source on Beamline I15-1 and processed using GudrunX [5], then compared with those in literature (previously collected to lower $Q$ ranges between 8 Å$^{-1}$ and 16 Å$^{-1}$ [2,6]).

Size was determined by Williamson-Hall analysis and compared to MR, particle diameter determined by PDFGui when a spherical envelope is used, and crystallite size determined by DiffPy-CMI when a rod-shape envelope was applied. Additionally, carbonate wt% was determined by fitting distinct A-type and B-type structures to PDFs and compared to FTIR analysis. PCA was also performed on biological and synthetic samples to identify systematic differences. PCA was applied to both PDFs and Bragg data (measured simultaneously) as well as individually to determine if any differences seen can be detected in both data sets.

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