

## Synthesis and Characterization of Type AB Carbonated Hydroxyapatite

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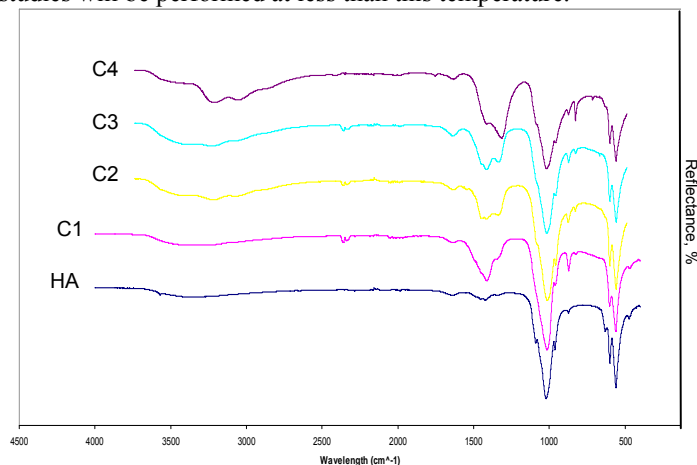
**Statement of Purpose:** Carbonated hydroxyapatite is the mineral phase present in bone, dentin and dental enamel. Carbonate ions can occupy two different sites in hydroxyapatite (HA): site A, where they substitute for OH<sup>-</sup> ions and site B where they replace PO<sub>4</sub><sup>3-</sup>. These two types of substitution can occur separately as type A or B carbonated HA or simultaneously resulting in a mixed AB-type carbonated HA similar to that of biological bone [1]. The presence of type B substituted carbonate in the apatite lattice results in an increase in solubility compared to pure HA both *in vitro* and *in vivo* [2], which enables the material to be better integrated into the natural bone. However, type-A carbonated HA results in lower cell attachment and collagen production compared to pure HA [3]. The cell adhesion characteristics of type AB carbonated HA have not yet been investigated, but are expected to be superior to pure HA. Thus, the aim of this study was to prepare and characterize a range of type AB carbonate-substituted HA (ranging from 4-6 wt%) via FT-IR and XRD. Later *in vitro* experiments will investigate the biological responses of osteoblasts to these materials.

**Methods:** Stoichiometric HA and carbonated HA were prepared by an aqueous precipitation reaction between a calcium nitrate solution, diammonium hydrogen phosphate (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> solutions, and four different percentages of ammonium carbonate (3.8, 4.8, 5.5 and 5.8 wt%), designated as C1, C2, C3 and C4, respectively. The reaction temperature was maintained at 60°C, and the pH was maintained at 11. The settled precipitate was aged for 1 hour. After aging, the obtained white precipitate was left overnight and then decanted, centrifuged, and washed with NH<sub>4</sub>NO<sub>3</sub> and distilled water until the ammonia and all residual free calcium salts were removed. Each of the above prepared precipitates were dried at 80°C and then milled to a particle size between 1 and 8 μm. The prepared powders were characterized by FT-IR and XRD to identify the location of the substituted carbonate groups. Since heating can decrease the carbonate content, XRD was also performed on the carbonated HA after heat treatment to ensure that the amount of carbonate remained similar to that of biological HA (4-8 wt%) [4].

**Results:** The FT-IR results are shown in Figure 1. The spectra for all of the samples show the bands typical of calcium phosphates. The stretching mode of the OH<sup>-</sup> vibration band indicates significant differences between the prepared samples. In the pure HA sample, this band is observed at 3571 cm<sup>-1</sup>, whereas in the carbonate substituted samples, this band is partially obscured by a broad band, which is shifted and split by increasing the carbonate content. The characteristic phosphate peaks appear as three peaks at 1094, 1021 and 960 cm<sup>-1</sup> and two

peaks at 600 and 559 cm<sup>-1</sup>. The phosphate band located at 960 cm<sup>-1</sup> appeared in all the spectra with a minor shift caused by the substitution of carbonate. The phosphate band located at 1090 cm<sup>-1</sup> for pure HA appears as a shoulder in the C1 sample and is absent for the C2, C3 and C4 samples. The characteristic carbonate bands were present between 1650 - 1300 cm<sup>-1</sup> and at 875 cm<sup>-1</sup> showing type B substitutions. There was a noticeable increase in the peak intensity of these bands as carbonate content was increased. Type A substitutions are shown by weak hydroxyl bands at 3570 cm<sup>-1</sup>. Thus, these are type AB carbonated HA compounds.

XRD analysis of the samples indicated only the presence of HA-based compounds showing no presence of CaCO<sub>3</sub> as aragonite or calcite forms. XRD analysis performed after heat treatment showed that the carbonate began to dissociate from the HA at 750 °C forming CaO (data not shown). Thus, heat treatment of tablets for cell culture studies will be performed at less than this temperature.



**Figure 1.** FT-IR spectra for pure HA and carbonated HA.

**Discussion:** This work demonstrates the characterization of a physiological formulation of type AB carbonated HA via the partial replacement of OH<sup>-</sup> ions and PO<sub>4</sub> with carbonate ions. Future work includes growing osteoblasts on pressed and sintered tablets produced from this powder and characterizing their biological responses.

### References:

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