

Introducing a New Method for *In Situ* X-ray Scattering to Quantify Structural and Chemical Controls on Amorphous Calcium Phosphate

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Understanding the fundamental processes behind crystal nucleation and growth is essential for understanding the formation of natural materials and developing synthetic analogues. For example, calcium phosphate minerals are the main constituents of bones and teeth in humans and there is extensive research to create biocompatible materials for medicinal advances. Over the last decade, advancements in crystal growth research have led to the realization that there are multiple pathways to crystal growth for a single mineral. These pathways are not fully understood and are highly sensitive to initial system composition. Further, many pathways include the formation of metastable, short-lived intermediates, such as amorphous calcium phosphate (ACP). Previous methods have not provided adequate controls to examine the structure and evolution of the intermediates over a dynamic range of solution conditions and time frames.

Our study uses a novel mixed-flow reactor (MFR) synthesis method to examine the precursor phases *in situ*. The precipitate flows through the x-ray beam while still in suspension and high intensity synchrotron x-ray total scattering is used to collect structural information for pair distribution function analysis. The MFR set-up enables precise control of system chemistry and sample age at the time of analysis without concern of sample evolution during data collection. Data are collected at a set point after initial mixing, facilitating measurements of the structure and evolution of phases that occur early in the crystallization process. Our investigation focuses on how the initial ratio of calcium to phosphate and pH during mixing, can affect the structure and evolution of the precursor phases.

Results show that the short-range structure of ACP varies due to solution chemistry. With a high Ca/P ratio of 5.0, the bonding geometry between calcium and phosphate is predominantly bidentate. At intermediate and low ratios of 1.0 and 0.2 there is a mix of bidentate and monodentate geometries. These findings were consistent for pH's between 6 and 10. While the literature has hypothesized the existence of multiple types of ACP, this is the first structural evidence to be presented. The method presented here can easily be adapted for other mineral chemistries to examine controls on the evolution and transformation of metastable phases during crystal growth.