

THESIS

TOWARDS UNDERSTANDING THE ATOMISTIC DISORDER OF SYNTHETIC BONE

MINERAL

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

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Spring 2018

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ABSTRACT

TOWARDS UNDERSTANDING THE ATOMISTIC DISORDER OF SYNTHETIC BONE MINERAL

Biomaterials are an interesting class of materials due to their complex structures and superior properties as compared to similar materials produced under laboratory settings. These complex structures often demonstrate a high level of control from the nano- to macroscopic scale. As a result, it is very difficult to create mimetic materials with hierarchical structures under laboratory conditions.

Bone mineral, nominally calcium hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, shows a distinct, well known hierarchical structure from the individual nanoparticles of hydroxyapatite in the collagen matrix to the macroscopic bone. However, the atomistic structure of the apatite is not as well understood. This is due to the high level of chemical substitution and atomistic disorder. One of the most common chemical substitutions in bone mineral is the replacement of the tetrahedral phosphate ion with a planar carbonate ion. While several studies have attempted to understand this chemical substitution, there is not a consensus on the orientation of the carbonate ion in the phosphate site. Using X-ray or neutron diffraction as a structural determination tool is very useful for highly crystalline materials. However, the usefulness of these diffraction techniques decreases with increased disorder due to broadening of reflections which can obscure structural information. Instead, a total scattering technique, such as pair distribution function analysis, can be used to obtain

an understanding of the local coordination environment. This, in conjunction with calculations of energy, can be used to identify the most likely substitution orientation. Using this method of structural determination, it is possible to conclude that the lowest energy substitution is the substitution of the planar ion into the mirror plane of the tetrahedral phosphate.

Many biominerals formed in aqueous media, such as those found in bone, are synthesized via metastable or amorphous precursors. Crystallization pathways can be dependent on the species initially present in solution and other chemical factors such as pH. Bone mineral is of importance because of the medical implications in connection with various bone tissue diseases. Understanding the pathway through which biomimetic bone mineral is formed may inform targets for bone disease or improve processing for synthetic grafting materials. Here, the crystallization of biomimetic bone mineral is monitored via *ex situ* X-ray diffraction to determine the precursor phases. Samples prepared with and without exogenous carbonate are studied to determine possible factors which influence the rate of crystallization. Carbonate is chosen because of the known substitution for phosphate in bone mineral. This synthesis pathway from low pH to high pH shows that brushite, a hydrated calcium phosphate phase, is initially formed prior to precipitation of the desired apatite phase. However, the apatite phase appears more slowly in the carbonated samples. Analysis of the phosphate concentration via an ammonium molybdate assay shows that the non-carbonated synthesis has a steady decline of the phosphate throughout the reaction while the carbonated synthesis shows an induction period during which the phosphate concentration remains steady before having a sharp decrease.

ACKNOWLEDGEMENTS

My thanks go to everyone who has encouraged me on this journey. It has been an adventure with many highs and lows and so many people have been there to pick me up and help me keep moving towards my goal. I'd like to specifically thank some of those people.

First, I'd like to thank Jamie who has been the most supportive and understanding advisor. He really encouraged me when things got tough and helped me pull through to the other side. Additionally, I'd like to thank the rest of the Neilson group who offered words of support and encouragement along the way. You're doing a great job.

Secondly, I'd like to thank my family, especially my mom. You've all reminded me of who I am and how capable I am when I needed it. And Mike, we may now be tied, but the game isn't over yet.

Lastly, I'd like to thank my friends who have been there for me, especially Cheryle, T, Brass, and Irish. Each of you has provided me with endless laughter and had my back when I needed you. I love you all.

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1. Introduction

1.1. Biomaterials

Biomaterials are inorganic materials produced by biological organisms. These range from single-celled organisms that produce exoskeletons to complex organisms, such as humans, to form their bones. The most common materials utilized by nature are calcium carbonate, calcium phosphate, and silica. Biomaterials are used for functions from exoskeletons to reproduction throughout nature.¹

Biomaterials are an interesting class of materials that have superior properties, such as increased strength, over their synthetic counterparts. Many of these improved properties are due to hierarchical structures which are difficult to reproduce under laboratory settings. However, the biomaterialization process is still not well understood, especially in creating the multi-dimensional control seen in these materials which produce the hierarchical structures.¹ While some synthesis routes have been developed to control growth and self-assembly, they often lack the desired level of reproducibility.² In fact, in order to achieve the level of reproducibility desired requires complete control of many experimental variables in the crystallization process.³ An example of this can be seen in the work done by Noorduyn et al.² where different pH conditions were employed to reliably obtain specific structure types which can be manipulated to achieve a hierarchical structure.

1.2. Bone Grafting and Structure Overview

Bone grafting and fixation is a nearly \$2.5 billion industry with over two million procedures being completed annually, nearly 500,000 of which are purely grafting.⁴ The combining effects of over 18 million treated fractures in 2010⁵ and 54 million people currently affected by osteoporosis⁶ in the United States have increased the efforts to develop bone grafting materials that are readily available and easily integrate into the human body. Three main attributes are taken into consideration when determining the efficacy of grafts: osteoconduction (scaffold), osteoinduction (cell recruiting), and osteogenesis (bone production).^{7,8} While autografts (from self) and allografts (from donor) are generally favored, they are difficult to work with due to limited availability and strict time windows for implantation. Additionally, concern for possible disease transfer or immune response due to donor tissue makes allograft materials non-ideal.⁷ Synthetic approaches to bone grafts through ceramics, bioglasses, and cements have clear advantages over their natural graft counterparts such as a nearly unlimited shelf life and controllable physical properties. However, many of these materials are difficult to synthesize or process, have very low resorption into the body, and are almost never osteogenic or osteoinductive on their own, thus requiring a composite material for biological use.^{7,8}

Natural bone is constantly being resorbed and deposited by the body such that a new mineral skeleton is created approximately every 7 years.⁹ One possible reason for the lack of resorption of synthetic grafts could be due to structural differences between biological bone mineral and the synthetic replacements. However, any structural differences would be difficult to identify due to the disordered nature of both synthetic and natural bone.

Biological bone has the additional complication of containing protein which interferes with most types of analysis. Various methods of deproteinization of bone have been developed, but the possible structural effect of these deproteinization methods are rarely studied.

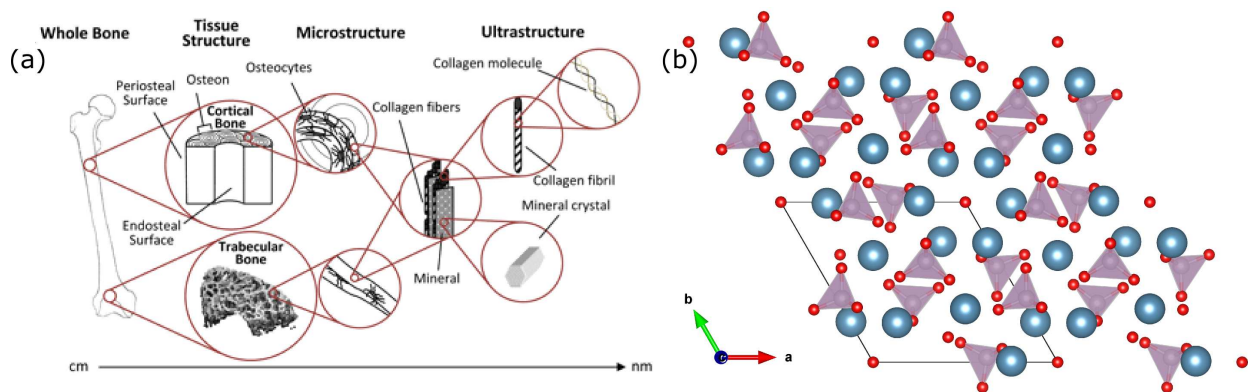


Figure 1.1: (a) The hierarchical structure of bone from collagen molecules to total bone structure. Highly organized and controlled structure of the collagen fibrils leads to strong bones. Figure reproduced from Boskey and Coleman¹⁰ with permission. (b) The ideal crystal structure for stoichiometric calcium hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. Blue atoms are calcium, red are oxygen, and purple are phosphorous. The purple polygons represent the phosphate tetrahedra.

Bone is a composite material with a highly ordered hierarchical structure (Figure 1.1a); however, the mineral portion remains disordered and poorly understood at the atomic and molecular scale. The disordered mineral phase is identified as calcium hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, Figure 1.1b], but broad reflections in diffraction experiments make structural elucidation difficult. It is also known that the apatite found in bones is substituted and can contain other metals (Mg^{2+} , Na^+ , K^+) as well as other polyatomic anions, such as carbonate, CO_3^{2-} .^{1,11} In order to maintain charge neutrality within the mineral, these substitutions may be compounded (e.g., Na^+ and CO_3^{2-}) or accompanied by a lattice vacancy. As a result, bone mineral is generally referred to as a carbonated apatite with the formula $(\text{Ca}, \text{Sr}, \text{Mg}, \text{Na}, \text{H}_2\text{O}, [])_{10}(\text{PO}_4, \text{HPO}_4, \text{CO}_3, \text{P}_2\text{O}_7)_6(\text{OH}, \text{F}, \text{Cl}, \text{H}_2\text{O}, \text{O}, [])_2$ where [] indicates a site vacancy.¹ In addition to the various chemical species differences found

in bone mineral, biological apatites vary in crystallinity and composition based upon age of the mineral and/or the organism as well as biological function (i.e., bone, dentin, enamel, Figure 1.2). However, the factors which may control them are unknown.^{9,12} The most probable sites of various substitutions are difficult to discern and are generally determined by computation,^{13–16} not experimentation. Much research has been conducted in order to identify and quantify the substitution and vacancy locations in the lattice.^{13–29} Two of the most studied vacancies and substitutions are that of the hydroxide ion and the carbonate ion.

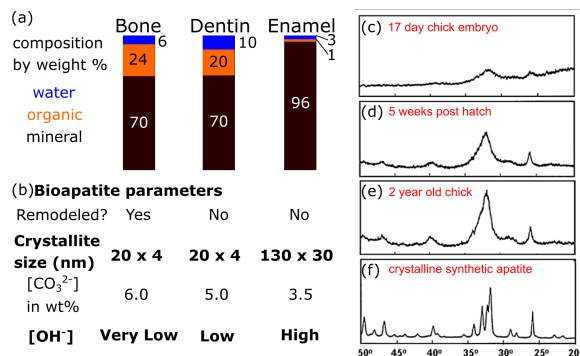


Figure 1.2: (a) and (b) show how various biological tissues have different compositions based on their functions. These different compositions effect the size and remodeling ability of the mineral portion of the tissue.⁹ (c-e) show increasing crystallinity of apatite mineral in chicken bones as the chicken ages. (f) shows a crystalline, synthetic apatite for comparison.¹⁷

1.3. Common Structural Determination Methods

Carbonate substitutions have been thought to occur in two ways, resulting in type-A and type-B substitutions. In type-A, the carbonate has replaced the hydroxide ion and is associated with high temperature synthesis. Type-B substitution is associated with precipitation of the product resulting in the substitution of phosphate ions and is considered to be more likely in biological systems. Previous work^{13–15,18,19,22,24,25,29} has been done in

an attempt to describe the carbonate location and orientation within the apatite lattice. While there is some debate remaining about the exact orientation of the carbonate ion in apatitic structures, most agree that the orientation of B-type substitution is related to the mirror symmetry of the phosphate. This has led to the suggestion that the most likely location of the carbonate ion is replacing the phosphate ion with the carbonate residing on the mirror plane of the phosphate tetrahedron^{22,24} or on the symmetry related faces of tetrahedron^{25,29} in the apatite lattice (Figure 1.3). Interestingly, the work done by both Leventouri *et al.*²⁴ and Wilson *et al.*²⁹ both relied on powder neutron diffraction data to determine the structure and location of the carbonate ion substitution. Using very similar arguments, each group came to different conclusions regarding the orientation of the carbonate ion on the phosphate site in the crystal structure. However, it is also important to note that each group was evaluating synthetic samples which were prepared under different conditions and with different starting materials.

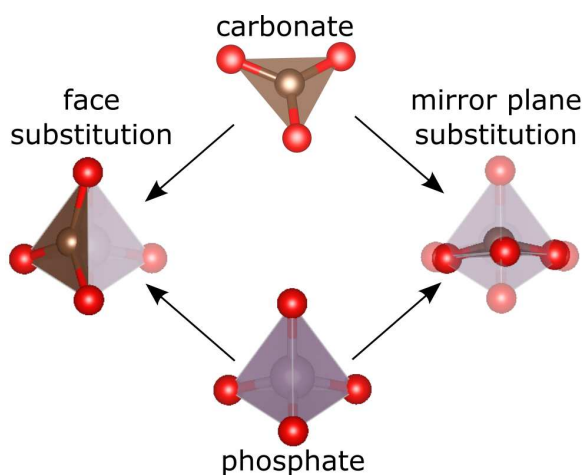


Figure 1.3: The respective structures of carbonate and phosphate ions are shown. Also shown are the superimpositions for face substitution (left) and mirror plane substitution (right).

Diffraction techniques are excellent structural determination tools for highly ordered materials, but becomes less informative with finite size (nanoparticles) and disorder in the

material.³⁰ The most basic assumption for diffraction is periodicity of the sample which produces Bragg reflections based on Bragg's law (Equation 1.1):

$$n\lambda = 2d \sin \theta. \quad (1.1)$$

Because the technique depends on constructive interference of X-rays caused by atoms in a periodic structure, disruption of this interference caused by disorder can lead to broadening of the observed reflections or a reduction in the intensity of the reflection (Figure 1.4).

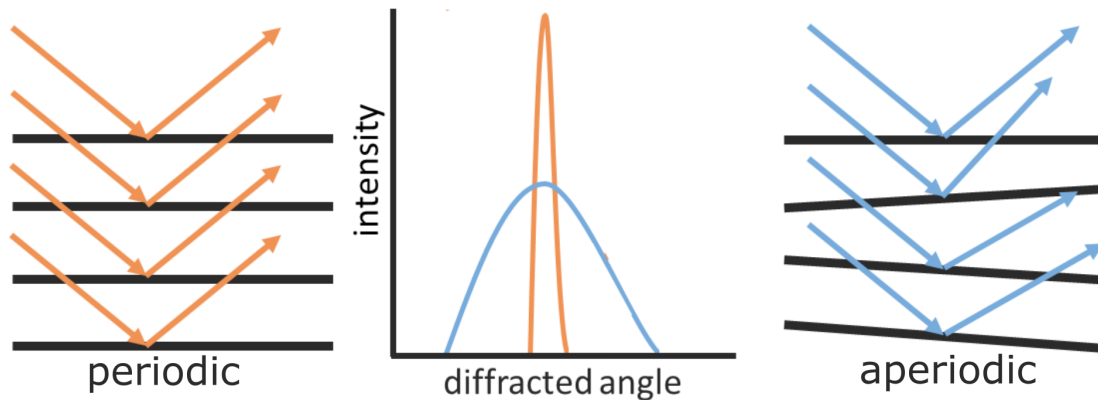


Figure 1.4: When X-rays interact with a periodic structure (orange), the diffraction pattern shows an intense, narrow peak. If the structure is aperiodic (blue), the diffraction pattern will show a weak, broad peak.

For example, the diffraction pattern of an amorphous solid would simply be a series of overlapping broad, uninformative reflections. Similarly, nanoparticles can have a similar broadening effect due to the finite size and thus finite layers to create the necessary interference. In this case, the amount of broadening can be related to the size of the particle through the Scherrer equation. However, when both disorder and finite size effects are present, it becomes nontrivial to determine the amount of broadening due to only one of those possibilities. As seen in Figure 1.2c-e, the reflections of bone mineral are very broad and do not allow for facile delineation of the reflections and provides extremely limited

structural information as compared to the crystalline synthetic apatite. Using neutrons instead of X-rays can be advantageous because of an increased sensitivity to light atoms, but the diffraction is still bound by Bragg's law. It would be unlikely that neutron powder diffraction would truly be sensitive to the orientation of carbonate ions, especially when carbonate incorporation is at less than 10 wt.% in the sample. In the specific case of biological bone, not only does one have to consider finite size and disorder of the mineral phase, but also the disorder which arises from the protein structure.

1.4. Pair Distribution Function Analysis

The pair distribution function (PDF), $G(r)$, yields atomistic details of the structure of a nanoparticle when Bragg's law can no longer provide useful information due to paracrystallinity or general disorder of a material. Understanding the local structure of a non-periodic material, like that of a liquid or glass, can still inform the observed physical properties associated with the material. Where many X-ray diffraction (XRD) studies only consider structural information as provided by Bragg's law, PDF analysis allows for the analysis of diffuse scattering. This experiment is referred to as total scattering because diffraction and diffuse scattering from the sample is collected and analyzed.³¹ The form of the PDF in reciprocal space is called the total scattering function, $S(Q)$. This total scattering function is the normalized scattering intensity which takes the atom scattering amplitudes into consideration and is derived from the experimental scattered intensity. The total scattering

function can be transformed to $G(r)$ by using a Fourier transform (Equation 1.2),

$$G(r) = \left(\frac{2}{\pi}\right) \int_{Q_{min}}^{Q_{max}} Q[S(Q) - 1] \sin(Qr) dQ. \quad (1.2)$$

The PDF can be obtained by using a synchrotron X-ray source by transformation of the collected diffraction data, allowing the PDF to give real space information from the reciprocal space data. Functionally, $G(r)$ shows a histogram of atom–atom distances in the structure (Figure 1.5). The PDF does not give the identity of the atom–atom pairs, but can be easily modeled computationally to determine the structure.

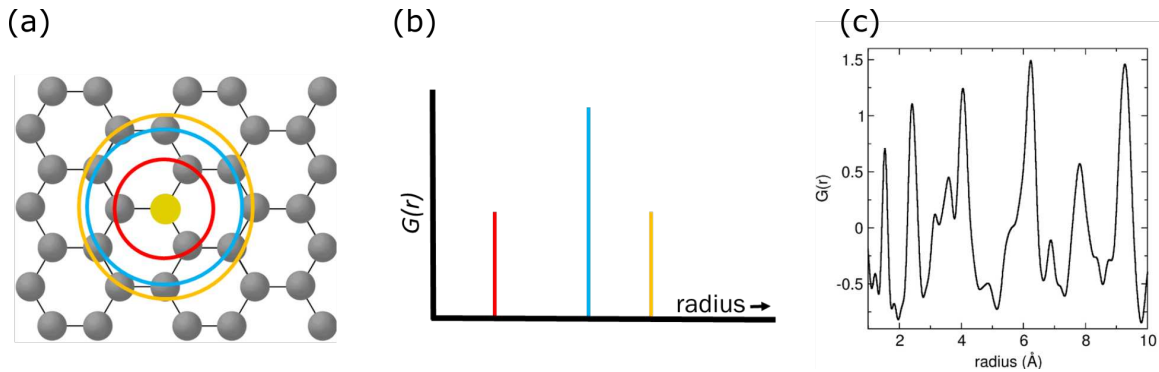


Figure 1.5: Considering the 2D example in (a), from the center yellow atom, the nearest neighbor atoms are shown in the red ring. The second and third nearest neighbor atoms are shown with the blue and orange rings, respectively. The nearest neighbors are shown in histogram form in (b). However, since atoms have some thermal motion, the resulting peaks have some broadness as seen in the example PDF in (c).

For this work, the PDF analysis will provide information about the local structure of the synthesized materials. This will help provide a foundation for understanding how different experimental conditions change the structure at smaller distances than is possible to identify by Bragg reflections. Through comparison of PDF patterns, the similarities and differences between experimental conditions can be more easily identified. Once the differences have been found, structural models which are consistent with proposed sub-

stitutions can be created. These models can then be used to calculate PDF patterns to determine which structural hypotheses are consistent with the experimental data.

1.5. Reverse Monte Carlo Modeling

In disordered materials, PDF analysis can yield a model of the local structure of materials, but does not give information about how local order modulates throughout a crystallite. A three dimensional model which is consistent with experimental data can be constructed using Reverse Monte Carlo (RMC) modeling. RMC modeling is similar to Monte Carlo modeling (simulated annealing) in that randomized atom movements are used to create a model which fits the data. However, the probability that movements in Monte Carlo simulations are accepted or rejected is based upon a Boltzmann distribution of energies,³² while the probability of acceptance in an RMC simulation is driven by how well the model fits the data (Equation 1.3):

$$P = \exp\left(\frac{-(\chi_n^2 - \chi_o^2)}{\sigma^2}\right). \quad (1.3)$$

When χ_n^2 is smaller than χ_o^2 , meaning that the new structure has better fit to the data, that atom movement is always accepted. When $\chi_n^2 > \chi_o^2$, there is a non-zero probability that the movement will be accepted which is given by Equation 1.3. This nonzero probability allows for the resulting structure to be independent of the initial configuration for the simulation. Multiple data sets can be fit simultaneously, as long as the data can be calculated, to develop the most accurate model. When this is done, the χ^2 for each data set are summed and can be weighted using different σ -values.

For this work, simultaneous fitting of real space information (PDF) and reciprocal space data ($S(Q)$) will be completed. Modeling through RMC uses a supercell containing thousands of atoms which makes fitting the $S(Q)$ data possible by convolving the size of the supercell with the experimental data. While this makes RMC a useful tool in developing a model which is consistent with the data, it is important to note that the structure determined by RMC simulations is not unique and may be unphysical.³² Because this method does not produce a unique model, meaning different simulations do not result in identical structures, many configurations which are structurally consistent with the data may be sampled. This may lead to identifying structural motifs which may inform properties of the material. However, not all the configurations will be consistent with the physical world due to no energy constraints being used thus allowing unphysical atom positions in the simulation as long as these positions fit the experimental data.

2. Paracrystalline Disorder from Phosphate Ion Orientation and Substitution in Synthetic Bone Mineral*

2.1. Introduction

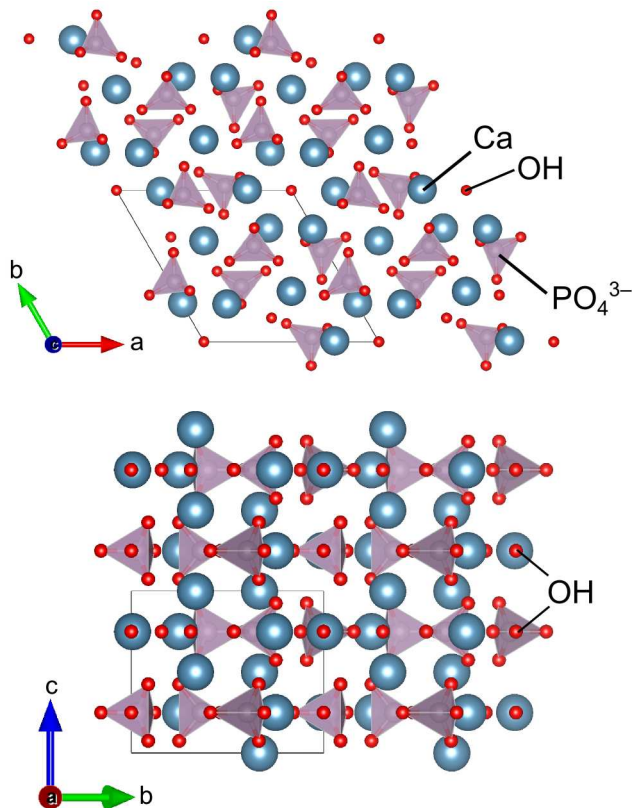


Figure 2.1: Ideal crystallographic structure for the mineral, calcium hydroxyapatite. Calcium atoms are blue, oxygen are red, and phosphorous are purple.

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Bone is a composite material with a highly-ordered hierarchical structure from cm to nm length scales; however, the mineral component remains poorly understood due to the crystallographic disorder and small crystallite sizes.^{1,33} Calcium hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, Figure 2.1], as found in bone mineral, is a disordered crystal; it is paracrystalline. X-ray diffraction experiments from these minerals reveal broad Bragg reflections, which are suggestive of short crystalline coherence lengths.³⁴ It is also known that the apatite found in bones is substituted and can contain other metal ions (e.g., Mg^{2+} , Na^+ , K^+) as well as other polyatomic anions, such as carbonate, CO_3^{2-} .^{1,11} In order to maintain neutrality within the mineral, aliovalent substitutions may be compounded (e.g., Na^+ and CO_3^{2-}) or accompanied by lattice vacancies. As a result, bone mineral is more generally referred to as a “carbonated apatite” with a general formula of $(\text{Ca}, \text{Sr}, \text{Mg}, \text{Na}, \text{H}_2\text{O}, [\])_{10}(\text{PO}_4, \text{HPO}_4, \text{CO}_3, \text{P}_2\text{O}_7)_6(\text{OH}, \text{F}, \text{Cl}, \text{H}_2\text{O}, \text{O}, [\])_2$, where $[\]$ indicates a site vacancy.¹ Biological apatites vary in crystallinity and composition based upon: age of the mineral and/or the organism, biological localization (i.e., bone, dentin, enamel), and chemical species available during synthesis. Understanding the chemical nature of these effects and the factors which may control them remains an active area of research.^{9,12} In the case of CaCO_3 crystallization, the inclusion of exogenous constituents, such as amino acids³⁵ or micelles,³⁶ need not disrupt single crystal formation. However, it appears that chemical substitution or inclusion disrupts the crystalline lattice of biological apatites.

Carbonate substitutions occur in two ways, denoted as type-A and type-B substitutions (Figure 2.2).^{18,19} Type-A substitution is defined by when the carbonate replaces the hydroxide ion found in the ideal hydroxyapatite structure and is associated with ceramic hydroxyapatite prepared at high temperatures (Figure 2.2a). Type-B substitution is de-

scribed by substitution of phosphate ions and is typically associated with specimens produced from precipitation (Figure 2.2b). Because biological apatites are formed through precipitation, type-B substitution is most likely in bone mineral.^{18,37} Previous studies using X-ray diffraction,^{18,19,25} neutron diffraction,^{22,24,29} and theory-based simulation^{13–15} have described various carbonate locations and orientations within the apatite lattice. While the exact orientation of the carbonate ion in apatitic structures is debated, there have been suggestions that the carbonate substitutes on the mirror plane of the phosphate tetrahedron^{22,24} or on the symmetry-related, triangular faces of the tetrahedron^{25,29} (Figure 2.2c). However, Leventouri *et al.*²⁴ and Wilson *et al.*²⁹ arrived at different conclusions about the orientation of the carbonate ion through Rietveld analysis of powder neutron diffraction data. The contradiction between these reports strongly suggests that crystallographic diffraction methods are insensitive to the nature of carbonate substitution due to the crystallographic disorder.

Here, we study various forms of synthetic apatite using synchrotron X-ray total scattering and pair distribution function (PDF) analysis. Samples were prepared using an established “biomimetic” method,^{11,38} which allows for variation of the hydrolysis rate and carbonate concentration to separately influence the level of crystalline disorder and level of carbonation in the lattice. Synchrotron X-ray total scattering data with PDF analysis reveals that the paracrystalline disorder in bone mineral can be attributed to variations in the phosphate group orientation. Advanced simulations, constrained by both the experimental total scattering data and density functional theory (DFT), provide insight into possible substitution mechanisms of the carbonate ion and the subsequent propagation of this disorder throughout the bulk mineral. The structural effect of the substitutional disorder

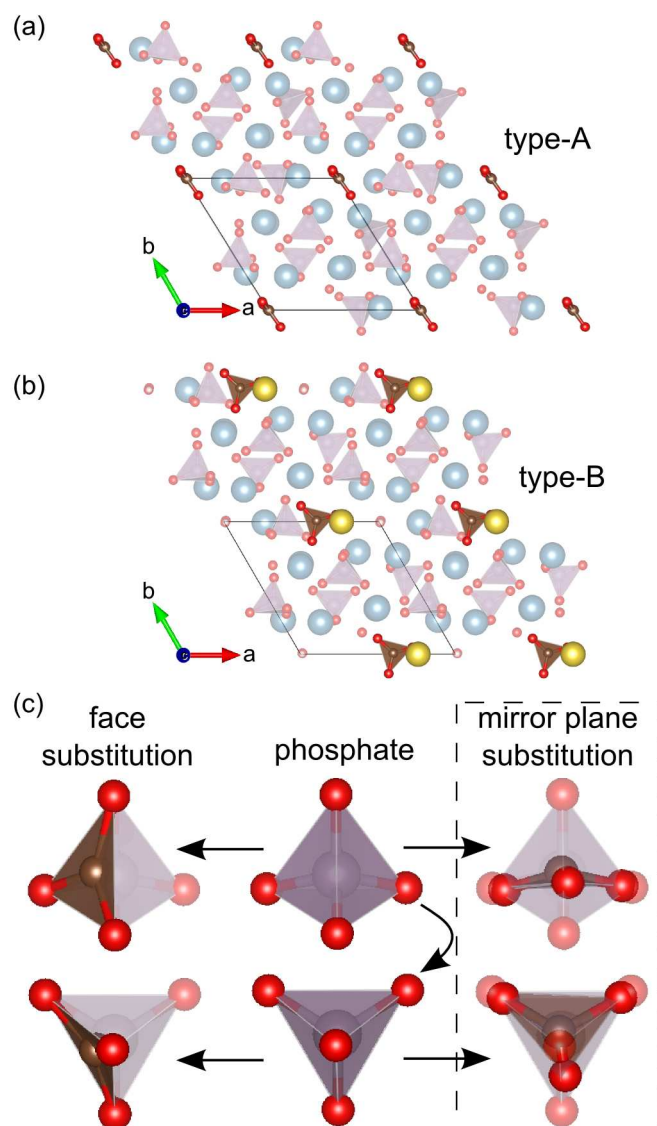


Figure 2.2: (a) Idealized type-A substitution: Calculated crystal structure for carbonate (brown atom) substitution in the calcium channel, as based on DFT calculations.¹³ (b) Idealized type-B substitution: DFT calculated crystal structure for a carbonate substitution in a phosphate site. In this case, the charge difference between carbonate and phosphate was accounted for by replacing a calcium ion with a sodium ion (yellow atom).¹⁴ (c) Cartoons of the two proposed carbonate orientations are shown superimposed on the phosphate tetrahedron, as compatible with type-B substitution. Carbonate substitution into the face of the tetrahedron is on the left and substitution into the mirror plane is shown on the right.

der of these synthetic apatites has the potential to influence both bone grafting purposes as well as biological mineralization processes.

2.2. Experimental

Calcium chloride dihydrate (Fisher Scientific), ammonium phosphate monobasic (JT Baker), sodium carbonate anhydrous (Macron Chemicals), glacial acetic acid (EMD Chemicals), and ammonium hydroxide (Fisher Scientific, 29.32% NH_3 assay) were used as received. All reagents were ACS certified and of 99% or greater purity, except where noted. All reagent water used in these experiments were purified using a Milli-Q purification system to a resistivity greater than $16 \text{ M}\Omega\cdot\text{cm}$.

Synthetic apatite was prepared through a biomimetic pathway as previously described by Nassif, *et al.*¹¹ In this procedure, an acidic solution (pH 2-3) of Ca^{2+} and PO_4^{3-} in a molar ratio of approximately 1.67 Ca/P in acetic acid was placed in a small dish (15×60 mm) in a chamber (approximately $3.5 \times 5.5 \times 2$ in). An additional dish containing NH_4OH was added to the chamber before sealing the chamber for 72 hours. For comparison, experiments were also conducted using CO_3^{2-} , as provided by Na_2CO_3 , while maintaining a Ca/(P+C) ratio of 1.67. The effective hydrolysis rate was controlled by changing the concentration of NH_3 in the chamber.³⁹ The product was collected by centrifugation and washed with water then ethanol before drying in air.

Compositional analyses of the samples were carried out using energy dispersive X-ray spectroscopy (EDS) inside a scanning electron microscope (Hitachi TM-3000 SEM with Bruker EDS). EDS was completed using a 15 kV accelerating voltage with a magnification of $80\times$ or $100\times$ and a working distance between 9.0 mm and 10.0 mm. Three control samples with known and varied molar ratios of Ca:P:Cl (A: 1.3:1.0:2.5, B: 5.72:3.4:1.0, C: 1.7:1.0:0) were made using $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NH}_4\text{H}_2\text{PO}_4$, KCl (99.6%), and CaCO_3 (Alfa

Aesar, 99%). Reagent chemicals were massed (total mass 0.2 g) and mixed in an agate before being pressed into a pellet. Each control pellet was measured in three locations to determine the uncertainty of the EDS measurement while sample pellets were only measured in one spot. Combustion analysis for carbon, hydrogen, and nitrogen was carried out using a Flash 2000 CHNS Analyzer coupled with MAS 200R autosampler.

X-ray scattering data were collected on the 11-ID-B beam-line at the Advanced Photon Source, Argonne National Laboratory, using 58 keV radiation ($\lambda = 0.2114 \text{ \AA}$). Two data sets were collected using a 2048×2048 pixel PerkinElmer 2D plate detector, one with low Q -resolution and large Q -range (i.e., total scattering) and one with limited Q -range but higher Q -resolution (i.e., diffraction). The large Q -range data set was used for PDF analysis and had a sample-to-detector distance of 17 cm using 1.3 second exposures with 480 summed exposures. The higher resolution data was used for Rietveld analysis of diffraction data with a sample-to-detector distance of 90 cm using 0.1 second exposures with 60 summed exposures ($Q_{min} = 1 \text{ \AA}^{-1}$, $Q_{max} = 9 \text{ \AA}^{-1}$). Integration of the 2D images were carried out using FIT2D.⁴⁰ The experimental pair distribution functions were extracted using PDFGETX3⁴¹ with $Q_{min} = 0.5 \text{ \AA}^{-1}$ and $Q_{max} = 30 \text{ \AA}^{-1}$. For extraction purposes, the composition of the samples were assumed to be $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ and $\text{Ca}_{10}(\text{PO}_4)_4(\text{CO}_3)_2(\text{OH})_4$ for the non-carbonated and carbonated samples, respectively. Fits and data analysis of the XRD and PDF data were completed using EXPGUI/GSAS^{42,43} and PDFGUI,⁴⁴ respectively. Instrumental resolution for PXRD refinement was convoluted with the model via prior refinement against CeO_2 powder (NIST standard 674b). For PDF analysis, the instrumental dampening was determined by prior refinement of Ni using the same instrumental conditions.

Atomistic simulations of the total scattering and PDF data to determine the disorder of the material were carried out using the Reverse Monte Carlo (RMC) algorithm of RMCProfile (version 6.5.2).⁴⁵ In order to approximate isotropic periodic boundary conditions, the hexagonal unit cell was converted to a P1 orthorhombic cell (84 atoms). Simulations were completed using a supercell containing either 3,360 or 8,820 atoms.

Density functional theory (DFT) calculations for structural relaxation of stoichiometrically carbonated apatite with the formula $\text{Ca}_{10}(\text{PO}_4)_4(\text{CO}_3)_2(\text{OH})_4$ (space group: *P*1) were carried out using the *Vienna Ab Initio Simulation Package* (VASP).^{46–49} The generalized gradient approximation was used to account for the exchange-correlation energy functional (PBE).⁵⁰ A $4 \times 4 \times 6$ *k*-point mesh was used for all DFT calculations after checking for convergence with respect to the *k*-point mesh. Relaxations were performed using a 520 eV cutoff for the plane-wave basis set and had a 0.01 eV Å^{−1} convergence criterion for the forces on all atoms in the calculation. Each force relaxation step required at least four electronic self-consistency steps.

2.3. Results and Discussion

2.3.1 Elemental composition

Compositional analysis of the apatite samples reveals the majority components of phosphorous, calcium, carbon, hydrogen, and trace elements present during synthesis (Figure 2.3). The SEM-EDS analysis for Ca and P content shows a subtle trend with the preparation conditions that is more significant than the compositional variability of known standards. After an initial increase of the Ca/P ratio, the non-carbonated samples show a

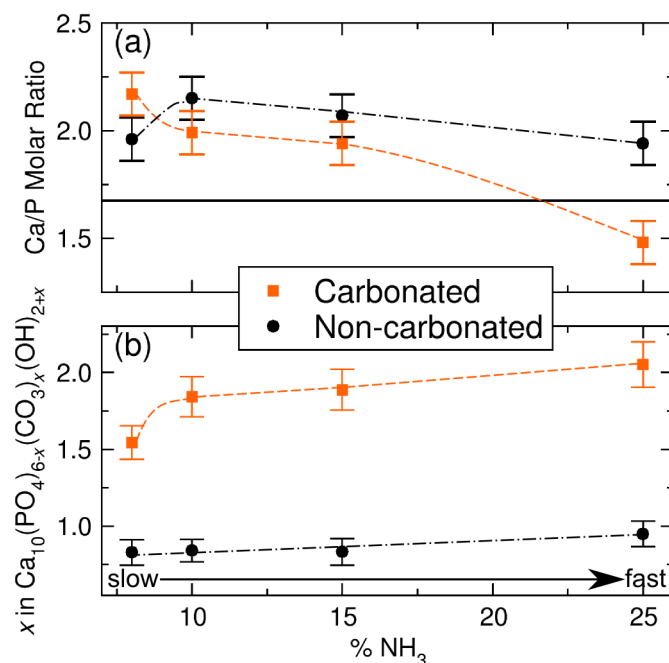


Figure 2.3: (a) Average values for Ca/P molar ratios as determined by EDS. The uncertainty of each measurement was 5% as determined by the relative standard deviation of known control samples. The solid black line shows the ideal hydroxyapatite value of the Ca/P ratio of 1.67. (b) Carbon content was determined by combustion analysis and converted into molar equivalents of carbonate assuming total calcium occupancy according to the formula $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{OH})_{2+x}$. Dashed lines in (a) and (b) guide the eye.

decreasing Ca/P ratio with increased $[\text{NH}_3]$ (Figure 2.3). Intentionally-carbonated samples also show a decreasing Ca/P ratio with increased $[\text{NH}_3]$ used during precipitation, and to a greater extent than the non-carbonated samples. Most samples are found to have a Ca/P ratio that is greater than the ideal hydroxyapatite ratio of 1.67, which is consistent with phosphate deficiency. This is consistent with the incorporation of carbonate ions into the structure at the expense of phosphate. Trace chlorine is present in all samples (<0.75 wt.%), and its presence is likely due to incidental inclusion from the reactants (HCl or CaCl_2). Although alkali metals can be easily incorporated into the apatite lattice, no Na^+ was detected by EDS in the intentionally-carbonated samples.

Combustion analysis data for H, C, and N content shows an average of approximately 1.3 wt.% carbon in the non-carbonated samples and 2.5 wt.% in the carbonated sam-

ples. These values were converted into molar equivalents of CO_3^{2-} per formula unit of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, assuming complete calcium occupancy, resulting in $\text{Ca}_{10}(\text{PO}_4)_{6-x}(\text{CO}_3)_x(\text{OH})_{2+x}$. The presence of carbon in the non-carbonated samples could be attributed to residual dissolved carbonate in the water while sitting in a carboy after purification. Alternatively, during harvest and washing of the product, air/ CO_2 exchange may have resulted in the addition of CO_3^{2-} to the product as carbonate is notoriously difficult to remove in many aqueously precipitated clays and minerals.⁵¹ The amount of carbon present in the apatite appears to increase for all samples as the $[\text{NH}_3]$ increases in the reaction chamber. Carbonate or bicarbonate are the most likely carbon-containing ions to be present in these materials.

2.3.2 Synchrotron Powder X-ray diffraction

Synchrotron powder X-ray diffraction of the apatite samples (Figure 2.4a) are consistent with those reported in the literature.^{11,34} Both the carbonated and non-carbonated samples are consistent with the hydroxyapatite crystal structure⁵² and there are no major differences observable due to the presence of exogenously added CO_3^{2-} . The broad, overlapping reflections in the XRD data suggest the presence of extensive disorder and short crystalline coherence lengths. However, the relatively narrow full-width at half maximum (FWHM) of the (002) reflection ($Q = 1.8 \text{ \AA}^{-1}$) indicates anisotropy in the nature of this disorder, such as by anisotropic crystallite sizes or strain. Due to the highly overlapping Bragg reflections, it is not possible to uniquely assign a width to each (hkl) reflection. Therefore, the anisotropy of the XRD data was modeled using a convolution of a pseudo-Voigt function and asymmetry to accommodate axial divergence of the beam^{53,54} while also including a

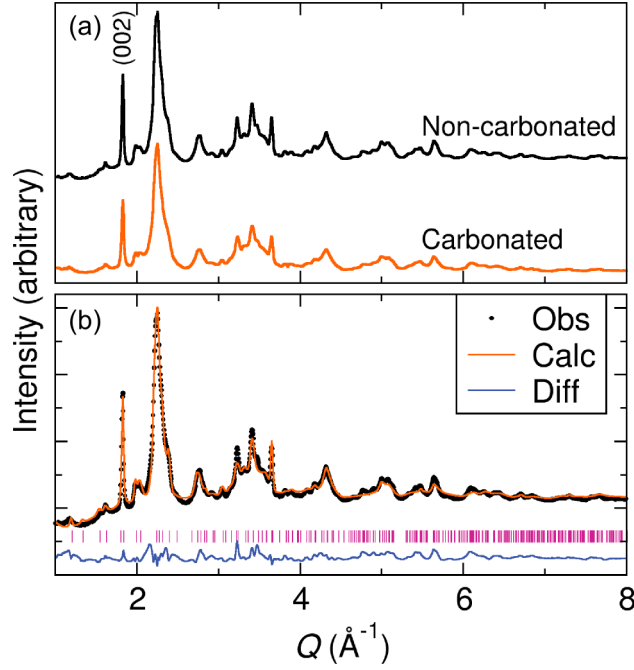


Figure 2.4: (a) Characteristic synchrotron X-ray diffraction patterns of apatite samples prepared under identical conditions, except for the absence and presence of exogenously added CO_3^{2-} . These patterns are consistent with hydroxyapatite and do not contain major differences between samples. (b) An exemplary Rietveld refinement of the synchrotron XRD data for the non-carbonated sample prepared with 25% NH_3 ; the use of anisotropic strain broadening and large thermal displacement parameters in the refinement indicates the presence of extensive crystalline disorder and only a moderate quality of fit.

description of microstrain described in a semi-empirical form.⁵⁵ This microstrain description is constrained by the symmetry of the unit cell (space group: $P63/m$). When the individual peak widths are analyzed using the method of Williamson and Hall,⁵⁶ it is apparent that the $(h00)$ reflections are significantly broadened relative to the $(00l)$ reflections (Figure A.2). However, our analysis of the overlapping peaks cannot differentiate between peak broadening due to size or strain of the particles due to the method by which the individual FWHM of each peak were extracted. The high intensity of the (002) reflection could suggest a preferred orientation of the mineral as previously suggested;¹¹ however, the analysis presented here does not require the use of a preferred orientation correction. Previous studies have shown that the apatite shows a preferred growth direction along the

[001] crystal direction, forming platelet-shaped crystallites (large in hexagonal ab plane, but narrow along the c -axis); the platelets then preferentially orient and stack along the c -axis, as aligned by hydration.^{11,57,58} The preferential ordering/disordering along specific crystallographic directions, rather than experimental artifact of particle orientation is more consistent with the results presented here.

Rietveld refinements (Figure 2.4b) of the diffraction data reveal large atomic displacement parameters (ADPs, Table 2.1) for many atom positions. ADPs typically provide a measure of thermal displacement of the atom from the average position. The large ADPs determined from Rietveld analysis here are unphysically large and likely masquerade for extensive paracrystalline disorder. The phosphorous-bound oxygen atoms were significantly large, which suggests extensive disorder is generated from the phosphate ion orientation. These anomalously large ADP values could, in part, be due to CO_3^{2-} inclusion on the phosphate ion lattice site. The smaller ADP value for the phosphorous atoms is consistent with this inference. Generally, this analysis is consistent with previous studies,^{11,34} but does not provide additional insight into the nature of the paracrystallinity.

LeBail refinements of the diffraction data from each sample reveal that the lattice parameter, a , increases as a function of increasing hydrolysis rate, as controlled by the NH_3 concentration used for preparation of the material (NH_3 concentration $\propto$ rate, Figure 2.5a). The smaller a -axis parameter for the carbonated samples is consistent with previously reported trends in precipitated apatites¹⁸ and may be indicative of a slower hydrolysis rate. This slower hydrolysis rate is to be expected as carbonate buffers the pH change. The overall change in the a lattice parameter from fastest to slowest hydrolysis rates in this work is $\Delta a \sim 0.05 \text{ \AA}$ for non-carbonated samples and only $\Delta a \sim 0.01 \text{ \AA}$ for

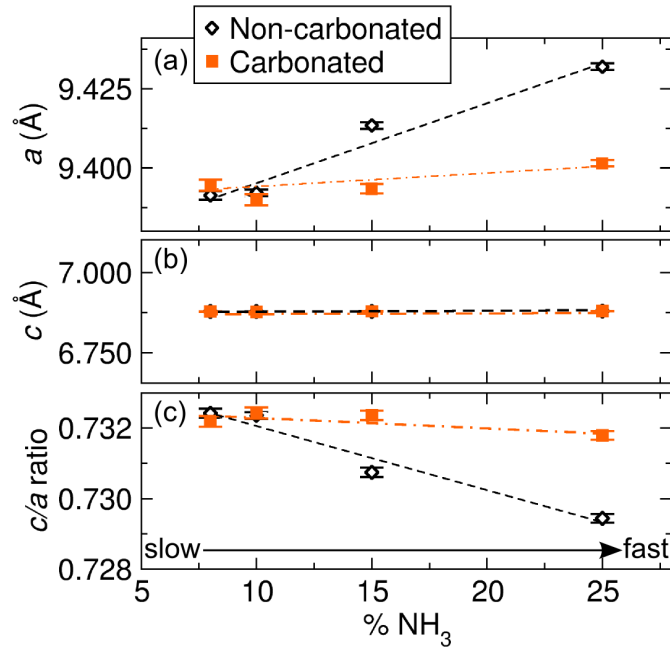


Figure 2.5: (a) a and (b) c unit cell parameters, as determined by LeBail analysis of the XRD data, show an increase in the a -axis for increasing ammonia concentrations while the c -axis remains constant. (c) The c/a ratio shows that the change in the a -axis is independent of instrumental changes during measurement. Error bars are the statistical uncertainty based from the quality of fit in the LeBail analysis. Dashed lines are a guide to the eye.

carbonated samples; this strongly suggests that carbonate incorporation is somehow involved with the lattice parameter change. More interestingly, the difference in the a lattice parameter between non-carbonated and carbonated samples with the fastest hydrolysis rate is about $\Delta a \sim 0.04$ Å. This difference decreases until the a lattice parameter becomes the same within the statistical uncertainty of the refinement at 10% NH₃. The steeper slope of the increasing lattice parameters of the non-carbonated samples as compared to the carbonated samples indicates that the hydrolysis rate has a greater effect on the crystal structure of the non-carbonated samples.

While it is not clear exactly what is causing the increase in a with respect to hydrolysis rate, one possible explanation is that the increased hydrolysis rate results in a lower density structure. However, the more significant effect of hydrolysis rate on lattice parameters seen

in the non-carbonated samples may be an indication that orientational disorder of the phosphate and/or carbonate ions causes a deformation of the lattice. The particle sizes determined using the Scherrer equation for the (002) reflection span values from 215 Å to 257 Å which are in general agreement with previous work.^{9,17,28,57} The non-carbonated samples have larger particles that generally decrease with decreasing concentration of NH₃ used; however, carbonated samples, which have smaller particle sizes, display an increase in sizes with decreasing NH₃ concentration used with the 10% NH₃ reactions resulting in the same sized particles.

Because solution-based syntheses of a material may form metastable precursors during synthesis,^{59–61} the rate at which the pH of the reaction mixture increases during hydrolysis can have a strong influence on the structure of the material being prepared.^{39,62} Both phosphate and carbonate are polyprotic, and therefore they have buffering capabilities that can influence the hydrolysis and condensation reactions in the process of precipitation. As the local atmosphere in the chamber becomes saturated with NH₃, which begins to dissolve into the reaction mixture to raise the pH, reaction solutions with both phosphate and carbonate have a greater buffering capacity over the non-carbonated counterparts. The increased buffering capacity can slow the effective rate of hydrolysis and precipitation by requiring more ammonia to be dissolved in the reaction solution before reaching the pH where precipitation of apatite begins. The more gradual pH change caused by lower concentrations of NH₃ may then result in a smaller lattice parameter as the final product is reached for a particular pH-range.⁵⁹

2.3.3 Pair Distribution Function Analysis

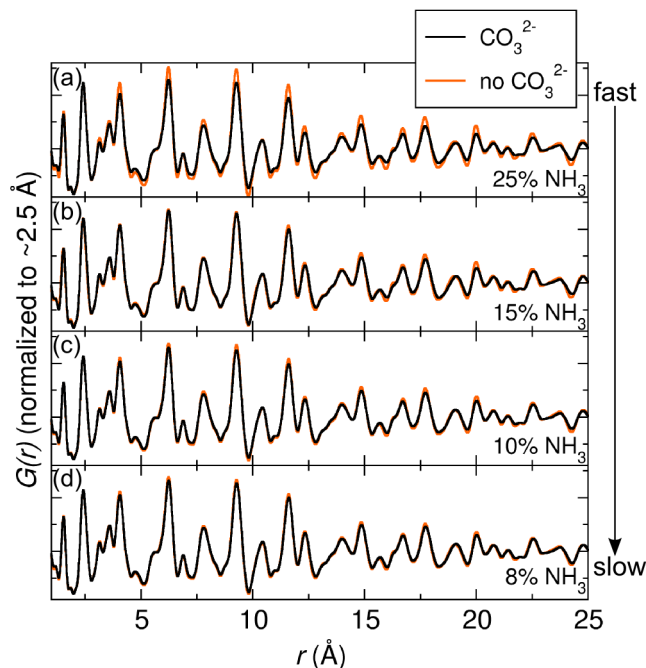


Figure 2.6: (a)-(d) Experimental PDFs for non-carbonated (orange lines) and carbonated (black lines) samples showing that the local coordination environment is maintained even when carbonate is present in the structure. Experimental PDFs have been normalized so that the assumed Ca-O peak at $r = 2.5 \text{ \AA}$ have the same intensity. The pair correlations extend to higher values of r in the non-carbonated samples; this effect is more pronounced for samples prepared with higher NH_3 concentrations.

PDF analysis reveals a local coordination environment of the synthetic apatites that closely resembles the average crystal structure of hydroxyapatite to $r \sim 12 \text{ \AA}$. Visual inspection of the experimental PDFs (Figure 2.6) shows subtle differences between carbonated and non-carbonated samples. This suggests that there is a systematic variability of the paracrystallinity in the samples prepared under faster hydrolysis rates. The increased intensity of pair correlations in $G(r)$ of the non-carbonated samples, especially for $r > 12 \text{ \AA}$, suggests that these samples have less paracrystalline disorder than the carbonated samples. However, the difference between carbonated and non-carbonated samples becomes negligible for specimens prepared at a low NH_3 concentration. To ensure that the small

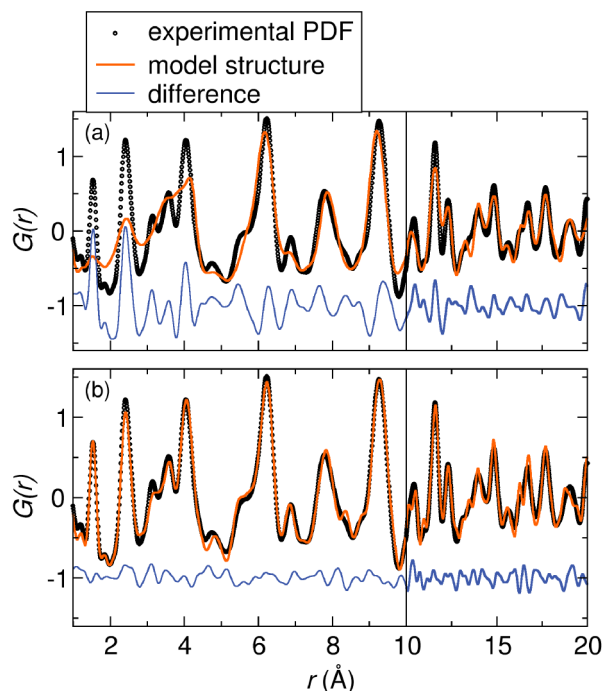


Figure 2.7: Modeling the experimental PDF ($0.99 \text{ \AA} > r > 10.0 \text{ \AA}$) of the non-carbonated sample prepared with the fastest hydrolysis rate, 25% NH_3 . (a) Use of the idealized crystal structure determined from the XRD Rietveld refinement shows that the model determined by XRD poorly describes the local coordination of the material, though the fit improves at higher values of r . (b) Least-squares refinement of the crystallographic structure of apatite against the PDF provides an improved representation of the local bonding configuration.

Table 2.1: Refined isotropic ADP values for XRD and PDF refinements using the stoichiometric calcium hydroxyapatite crystal structure modeled to the non-carbonated sample prepared with the highest concentration of NH_3 , 25%. Also shown are the ADPs for the PDF refinement using an explicitly-carbonated apatite unit cell used as the model for the carbonated sample prepared with 25% NH_3 .

Atom	XRD ^a	PDF ^{a,c}	Explicit Carbonate ^{b,c}
O1	0.043(6)	0.010(4)	0.0035
O2	0.036(6)	0.010(4)	0.0035
O3	0.094(5)	0.010(4)	0.0035
P	0.034(2)	0.006(4)	0.0035
Ca1	0.023(1)	0.008(3)	0.0035
Ca2	0.0091(6)	0.007(3)	0.0035
O(H)	0.020(9)	0.01(1)	0.02
C			0.02
O(C)			0.02

^aSpace group: $P63/m$. Atom positions for the XRD and PDF models can be found in Tables A.4 and A.7, respectively, of Appendix A.

^bSpace group: $P1$. ADP values were not refined but were set to a value; hydrogen atoms were assigned an ADP of $0.0025 \text{ \AA}^2$. Atom positions can be found in Table A.9 of Appendix A.

^cDuring refinement of the PDF models, all phosphorous-bound oxygens were assigned the same ADP parameter.

differences between PDFs from different samples are not artifacts introduced by the finite integration used for the PDF analysis, the integration parameter, Q_{max} , was varied to ensure that the features of interest did not shift position with Q_{max} .^{63,64}

The decreased pairwise correlations seen at $r \sim 12$ Å of the carbonated sample from the non-carbonated sample appears to result from paracrystallinity, as described by the atomic ADPs. When using the structure determined by Rietveld refinement of the XRD data (Figure 2.7a) the experimental PDF is poorly described below $r \sim 6.0$ Å, but fits reasonably well for $r > 10$ Å. Instead, the structural model was refined against the experimental PDF (Figure 2.7b) for $r < 10$ Å. The residual curve after subtraction of the crystalline model reveals only termination ripples from Fourier transformation of the data; therefore, we assume any amorphous material present is below the limit of detection of 2.5 mol%.^{65,66} Large ADP values are revealed when modeling the experimental PDF for $r < 10$ Å (Table 2.1). As with the parameters determined by the XRD data, the oxygen atoms result with large ADP values. In the case of the phosphorous-bound oxygen atoms, the large ADP values masquerade for significant chemical disorder rather than thermal motion. It is likely that these results arise from carbonate incorporation into the crystal structure for both carbonated and non-carbonated samples. As a result, the ADP values revealed by both XRD and PDF refinements do not provide the sensitivity required to determine the orientation of the carbonate ion within the lattice by themselves. This is probably why powder diffraction experiments led to different conclusions about the locations and orientations of carbonate ions in the lattice.^{24,29}

The differences in the experimental PDFs between carbonated and non-carbonated samples do not directly reveal how carbonate inclusion distorts the lattice; however, com-

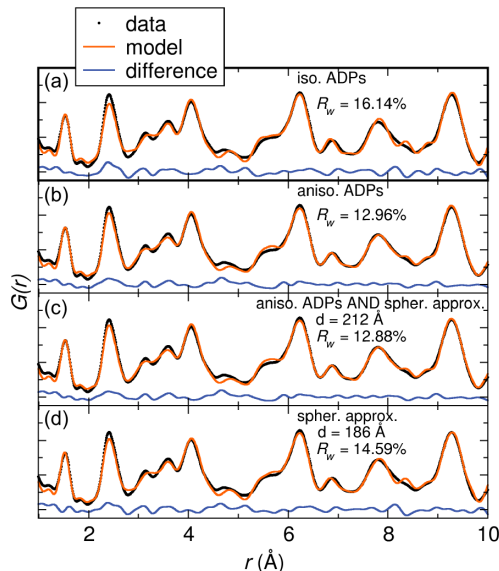


Figure 2.8: Modeling the experimental PDF of the carbonated apatite sample prepared with 25% NH_3 . (a) Using the final parameters determined from the non-carbonated sample prepared under identical conditions, some portions of the experimental PDF, especially the second nearest-neighbor peak ($2.5 \text{ \AA}$), are poorly described. This model only uses isotropic ADPs and is the initial point for the fits of alternative models in (b-d). (b) A significant decrease in the R_w value as compared to the starting model is seen when anisotropic ADP values are used for phosphorous-bound oxygen atoms and non-channel forming calcium atoms. (c) Use of the spherical particle approximation in addition to the anisotropic ADP values used in the previous model. Here, the negligible change in the R_w values suggests that approximation of the crystalline coherence length is not integral to understanding the structural differences caused by adding carbonate to the apatite material. (d) Use of the spherical particle approximation shows that carbonate does cause the crystalline coherence length to change upon inclusion into the material.

parison of multiple models fit to the experimental PDFs provides insight into these structural changes (Figure 2.8). The comparison models discussed herein were all initialized from the same set of parameters determined by the least squares refinement of the non-carbonated sample prepared using 25% NH_3 . One hypothesis for the structural differences seen in carbonated samples is significant paracrystallinity due to an anisotropic distribution of atom positions. This can be caused by the inclusion of a planar ion (CO_3^{2-}) on the site of a tetrahedral ion (PO_4^{3-}) which, when superimposed, would cause an anisotropic distribution of atomic coordinates. For this reason, the atom positions can be modeled using large, anisotropic ADPs (Figure 2.8b). The resulting structural model improves the PDF refinement by providing a better fit to the second nearest-neighbor peak ($r = 2.5 \text{ \AA}$)

and each of the three peaks in over $3 \text{ \AA} < r < 4.5 \text{ \AA}$. The anisotropic ADPs determined by this model appear to be most consistent with carbonate substitution into the mirror plane, but they are also consistent with the substitution into a face of the tetrahedron or orientational disorder. By using the anisotropic ADPs, the model is more representative by judging from the improved goodness-of-fit parameter: $R_w = 16.1\%$ to $R_w = 13.0\%$. An alternative hypothesis is that a nearly perfect crystalline order with well-defined atom positions (e.g., small U_{iso} 's), but with a short crystalline coherence length. This can be modeled using a spherical particle approximation (Figure 2.8d). The resulting structural model marginally improves the fit of the second nearest-neighbor peak ($r = 2.5 \text{ \AA}$) and somewhat corrects the fitting of the peak at $r = 7.8 \text{ \AA}$ but is otherwise nearly identical to the fit of the initial parameters to the carbonated experimental PDF. There is a moderate improvement of the R_w value from 16.1% to 14.6% with a spherical particle size of $186 \text{ \AA}$. The final hypothesis is the inclusion of both paracrystallinity and short crystalline coherence lengths which are modeled using both anisotropic ADPs and spherical particle approximation, respectively (Figure 2.8c). The resulting PDF fit describes the second nearest-neighbor peak only slightly better than when using anisotropic ADPs alone. However, comparison of the R_w values show that both models are essentially identical at $\sim 12.9\%$. In this model, the spherical particle size was determined to be $212 \text{ \AA}$. Using the Debye-Scherrer equation for particle size broadening in XRD, the particles from the carbonated sample prepared with $25\% \text{ NH}_3$ were determined to be $215 \text{ \AA}$ which is most consistent with the model using both anisotropic ADPs and the spherical particle approximations. With these control refinements resulting in imperfect models of the structure, it is clear that the orientational

disorder and anisotropic substitution of the phosphate ions results in a much better description of the observed PDF than a changing particle size.

2.3.4 Explicit Carbonate Modeling

In order to determine the locations and orientations of the carbonate ion in the apatite crystal structure which would be consistent with the experimental PDFs, several unit cells, each with a stoichiometric substitution of carbonate ions [$\text{Ca}_{10}(\text{PO}_4)_4(\text{CO}_3)_2(\text{OH})_4$, or 2.5 wt.% C], were constructed and tested for their theoretical chemical rationality with DFT, as well as their ability to describe the data. The forces on the atoms in each unit cell model were relaxed, with the total energy calculated from DFT, before the structures were used to fit the experimental PDF using a least squares refinement with ADP values fixed to approximate the certainty with which the atoms are placed (i.e., atoms most similar to ideal hydroxyapatite had small ADPs while those which were substituted were assigned larger values).⁶⁷ ADP values were set to either $0.0035 \text{ \AA}^2$ (Ca, P, O(P)), $0.0025 \text{ \AA}^2$ (H), or $0.02 \text{ \AA}^2$ (C, O(H), O(C)). Four initial structures were rationally constructed with type-B carbonate substitution in the place of a phosphate ion to test the hypothesized substitution on either (a) the mirror plane of the phosphate or (b) triangular faces of the phosphate tetrahedron. Additionally, the substitution was defined as occurring on adjacent (P-P distance = $4.1 \text{ \AA}$) or nonadjacent (P-P distance = $8.7 \text{ \AA}$) phosphates in crystallographic unit cell. The additional hydroxide ions necessary for the substitution stoichiometry were added in voids left after replacing the phosphate ion, allowing the orientation to be calculated in the DFT relaxation. After minimization of the forces on all atoms to below $0.01 \text{ eV \AA}^{-1}$, structures

were manually inspected to ensure that phosphate and carbonate bond lengths and angles were consistent with ideal structures of these ions.

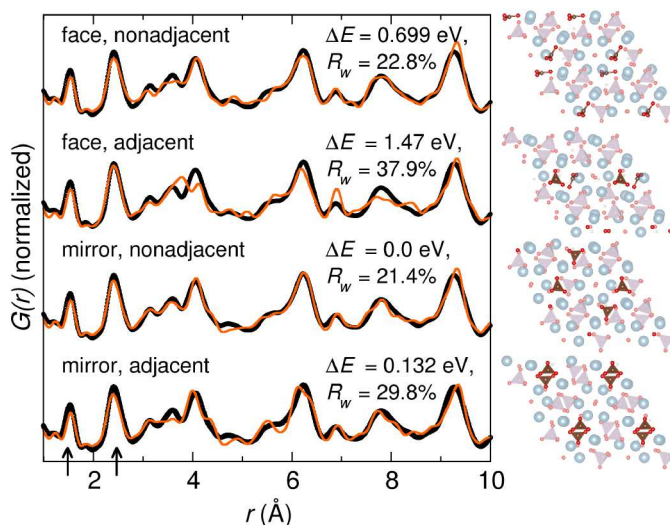


Figure 2.9: Calculated PDFs for each stoichiometrically carbonated apatite structure after relaxation of the DFT-calculated forces shows that each resulting structure is distinct from each other. This differentiation is especially obvious in the region between $r = 3.0 \text{ \AA}$ – $4.5 \text{ \AA}$. Changes in the height ratio of the first and second nearest neighbors, denoted with arrows, are also seen when comparing between substitution of adjacent or nonadjacent phosphate tetrahedra. These calculations show that carbonate substitution in the mirror plane of nonadjacent phosphate tetrahedrons is most consistent with the collected data. The total energy difference (ΔE) is relative to the most stable configuration, substitution in the mirror plane of non-adjacent phosphate sites. The thick black lines show data from the carbonated sample prepared using 25% NH_3 . ADP values were set to either $0.0035 \text{ \AA}^2$ (Ca, P, O(P)), $0.0025 \text{ \AA}^2$ (H), or $0.02 \text{ \AA}^2$ (C, O(H), O(C)).

The first DFT-relaxed structure tested against the experimental PDF of the carbonated sample prepared with 25% NH_3 (Figure 2.9) contained carbonate ion substitution onto the triangular faces of nonadjacent phosphate ions. This structure had a resulting energy of $\Delta E = 0.669 \text{ eV}$ (relative to the lowest energy substituted structure, non-adjacent substitution of mirror planes) and does a reasonable job of describing the first two nearest-neighbor peaks. While the calculated PDF follows the general features of the PDF at $r > 5 \text{ \AA}$ resulting in an $R_w = 22.8\%$, only the third of the three peaks between $r = 3.0 \text{ \AA}$ and $4.5 \text{ \AA}$ appears in the model calculation. The second structure with carbonate substitution on the face of the tetrahedron of adjacent phosphate sites resulted in the highest energy of the

tested structures at $\Delta E = 1.47$ eV. The calculated PDF for this structure captured the first two nearest-neighbors most accurately of each of the four structures tested. However, the fit above $r \sim 3$ Å poorly describes the data resulting in an $R_w = 37.9\%$. Most notably, the while three peaks can be seen in the calculated PDF between $r = 3.0$ Å and $r = 4.5$ Å, only the first peak is centered approximately while the rest are broad and poorly defined. The second of the substitution orientations, substitution into the mirror plane of the phosphate tetrahedron, resulted in the two structures with the lowest energy with $\Delta E = 0.0$ and 0.132 eV for nonadjacent and adjacent substitution methods, respectively. The most stable configuration, nonadjacent substitution into the mirror plane, also has the best fit to the experimental PDF with $R_w = 21.4\%$. While the first two nearest-neighbor peaks lack some of the intensity and shape of the experimental PDF, the following three peaks are well centered and defined in the calculated PDF. The final structure, adjacent carbonate substitution into the mirror plane, underestimates the intensity of the first two nearest-neighbor peaks and both peaks in the calculated PDF are not centered correctly ($R_w = 29.8\%$). Additionally, the following three peaks are distinguishable in the calculated PDF, but do not align with the experimental PDF.

Because a model with heterogeneous disorder, rather than small domains of a well-ordered structure, are most consistent with the analysis presented in Figure 2.8, a linear combination of these DFT-relaxed structures was used to describe the experimental PDF of the material. The best fit to the data was provided with a linear combination of the non-adjacent mirror and non-adjacent face substitution models (Figure 2.10), in a phase fraction of $57(9)$ mol% to $43(9)$ mol% ($R_w = 19.3\%$, as opposed to $R_w = 21.4\%$ for the mirror substitution alone). The large uncertainty in the mol% is indicative of the over-

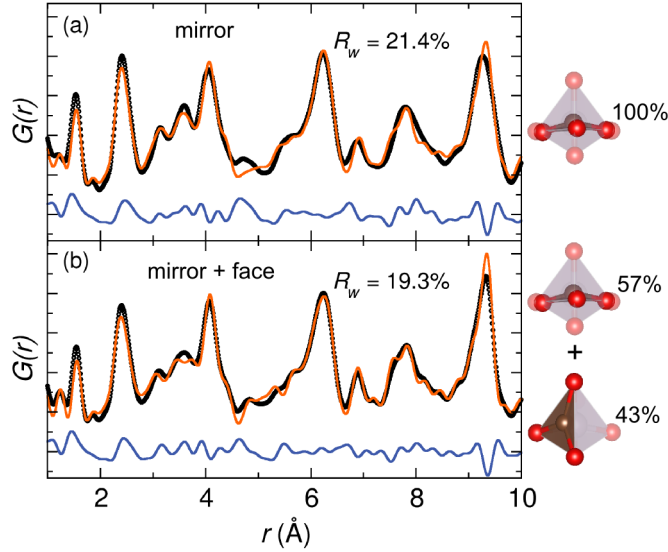


Figure 2.10: (a) Experimental PDF of the carbonated sample prepared with 25% NH_3 and structural model using only a mirror-plane substitution of CO_3^{2-} in non-adjacent phosphate tetrahedra. (b) Experimental PDF and structural model using the combined mirror plane and face substitution of non-adjacent phosphate tetrahedra. This linear combination model provides the best description of the data and suggests about 87 mol% of mirror and 13 mol% of face substitution of the phosphate sites.

simplification provided by this model. Regardless, the linear combination model improves the quality of fit, especially near the two nearest-neighbors, as well as those at $r = 4.0 \text{ \AA}$ and $r = 9.2 \text{ \AA}$. The type of substitutional disorder is consistent with the large, anisotropic atomistic displacement parameters extracted from Rietveld analysis. However, since this substitution does not carry any long range order, powder diffraction analysis (excluding PDF analysis) is insensitive to the nature of this disorder.

2.4. Conclusion

While traditional crystallographic methods have been insensitive to the type of disorder which is prevalent in biological apatite materials, the use of PDF analysis provides insight into the paracrystalline nature of the disorder. Comparison of the experimental PDFs for carbonated and non-carbonated samples prepared under identical conditions indicate that

the carbonate inclusion perturbs the lattice by incorporating into phosphate lattice sites. Calculation of the PDFs for rationally-constructed, DFT-relaxed carbonated apatite structures show that the data is best represented by a linear combination of carbonate ion substitution into the mirror plane and/or face of non-adjacent phosphate sites in the lattice. Although it is not possible to determine an exact structure for these materials due to extensive disorder, the experimental PDFs and DFT-relaxed carbonated structures have shown that CO_3^{2-} incorporation in these materials supports a B-type substitution with the planar ion in the mirror plane of the phosphate tetrahedron. The structural changes caused by the incorporation of CO_3^{2-} in the PO_4^{3-} site may indicate that species present during the preparation of the apatite mineral may have an impact on the crystallographic structure of the collected material. The work presented here provides a framework for further analysis of analogous bone mineral materials.

3. Understanding the Effect of Carbonate on the Crystallization of Synthetic Bone Mineral

3.1. Introduction

It is generally accepted that many biominerals are formed via metastable precursor phases.^{3,59,60,68-70} This is thought to occur due to the lower energy barrier to formation of the metastable phase as compared to the bulk stable phase. However, the type of crystallization process - classical or non-classical - is an area of debate.

Classical nucleation processes from a supersaturated solution utilize monomer-by-monomer attachment.^{69,71} This attachment proceeds in order to reduce the Gibbs free energy of the system with the earliest clusters considered to be of a critical size balancing the bulk and surface free energy terms.^{69,72} Experimental and theoretical deviations from classical nucleation theory have led to the development of non-classical nucleation theory. Non-classical nucleation theory includes the existence of pre-nucleation clusters, dense liquid phases, and solid amorphous precursors.^{69,71,72} However, because of Ostwald's Rule of Stages,⁷³ it has been argued that there is no need to turn to non-classical nucleation to explain the nucleation pathways of biominerals and biomimetic systems.⁷⁰

Ostwald's Rule of Stages states that the next most stable phase will form in a consecutive manner until the thermodynamic phase has been reached.^{69,70,73} Particle growth of the current phase takes place at the expense of smaller particles or the previous phase as dissolution and re-precipitation takes place. Sun and Ceder⁷⁰ argue that, instead of requiring

non-classical nucleation theory to describe biomineralization events, the pre-nucleation clusters are simply an early precursor phase that has formed.

For bone mineral, the formation pathway is not well defined. Understanding this pathway may help illuminate possible targets for diseases such as osteoporosis. Although many studies have been completed, they often focus on basic systems where only apatite formation is favorable. However, bone mineral is produced by beginning in an acidic environment and slowly changing to a basic environment. By using a biomimetic synthesis, the possible pathway to bone formation may be elucidated more clearly. Here, biomimetic apatite is synthesized utilizing a low to high pH pathway. The reaction is stopped at various time points to observe the solid phase(s) present. This reveals initial brushite formation prior to subsequent formation of apatite.

3.2. Experimental

Calcium chloride dihydrate (Fisher Scientific), ammonium phosphate monobasic (JT Baker), sodium carbonate anhydrous (Macron Chemicals), glacial acetic acid (EMD Chemicals), ammonium hydroxide (Fisher Scientific, 29.32% NH_3 assay), ammonium molybdate tetrahydrate (Strem Chemicals), and L-Ascorbic acid (Fisher Chemical) were used as received. All reagents were ACS certified and of 99% or greater purity, except where noted. All reagent water used in these experiments were purified using a Milli-Q purification system to a resistivity greater than 16 $\text{M}\Omega\cdot\text{cm}$.

Biomimetic hydroxyapatite was synthesized using a biomimetic method adapted from previous work.^{11,74} Briefly, in a small container, a dish containing a $\text{Ca}/(\text{PO}_4 + \text{CO}_3)$ so-

lution was stirred. A second dish containing 8 mL 10% NH_4OH was also placed in the container which was then sealed for an amount of time between 2 min and 45 min. The pH of the reaction solution was measured using litmus paper immediately after opening the container. The contents of the Ca/(P+C) dish was transferred to a centrifuge tube and centrifuged for about 10 minutes. Solid precipitates were washed with water before drying in air prior to characterization by X-ray diffraction.

X-ray diffraction was carried out using a PANalytical Empyrean instrument. Data was collected from 20 to 60 degrees 2θ using $\text{Cu K}\alpha$ radiation using the position-sensitive GaliPIX^{3D} detector measured for 3 seconds per step. Fits and data analysis of the XRD data were completed using EXPGUI/GSAS.^{42,43}

The amount of phosphate in solution was determined by ammonium molybdate assay.⁷⁵ At each time point, 10 μL of the supernatant was diluted to 10 mL. Using 5 mL of this solution, 10 mL water was added with approximately 0.1 g ascorbic acid and 1 mL of a $(\text{NH}_4)_2\text{MoO}_4$ solution. This mixture was heated for about five minutes (to almost boiling) to develop the blue color. The assay was carried out using a Cary 500 Scan UV-Vis-NIR spectrometer and absorbance was measured at 811 nm.

3.3. Results and Discussion

This synthesis pathway from low pH to high pH shows that brushite, a hydrated calcium phosphate phase, is initially formed prior to formation of the desired apatite phase (Figure 3.1). However, the apatite phase appears more slowly in the carbonated samples as determined by the amount of the apatite phase after 25 minutes. Initial formation of

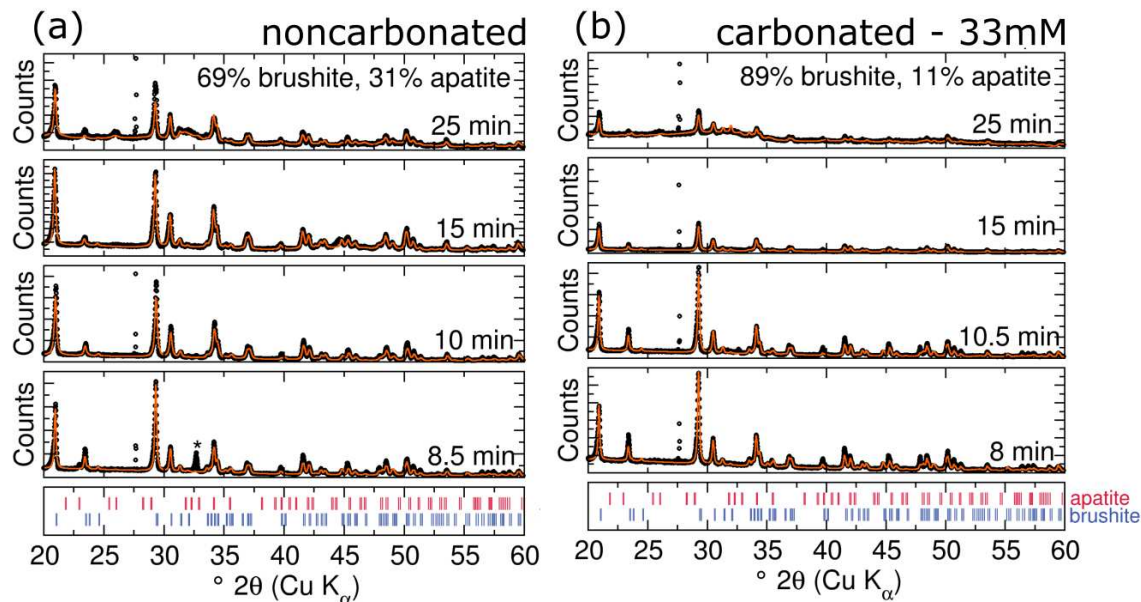


Figure 3.1: XRD Rietveld refinements of the stirred syntheses of apatite for noncarbonated (a) and carbonated - 33 mM (b). The desired apatite phase does not appear until 25 minutes have elapsed, but smaller amounts of apatite appear in samples with more exogenous carbonate present. The unindexed reflection at $27.5^\circ 2\theta$ is an artifact from the sample holder.

brushite is to be expected because it is the thermodynamically stable phase at low pH.⁵⁹ The slower development of the apatite phase in the carbonated samples may be an indication that the carbonate acts to slow the conversion of brushite to apatite. While this could be an indication of the buffering effect of the carbonate, it can be seen in the pH curve (Figure 3.2) that the carbonated samples actually reach a more basic pH faster than the non-carbonated samples.

While similar molar values of brushite are seen for both carbonated and non-carbonated samples, less of the carbonated apatite is formed than of the non-carbonated (Figure 3.2). This is likely simply due to the smaller molar mass of the carbonated apatite as compared to the non-carbonated apatite. However, it is possible that this discrepancy is due to the loss of carbonate during the acidification of the $\text{Ca}/(\text{PO}_4 + \text{CO}_3)$ solution. Attempts to quantify the amount of carbonate lost during acidification have not yet been successful.

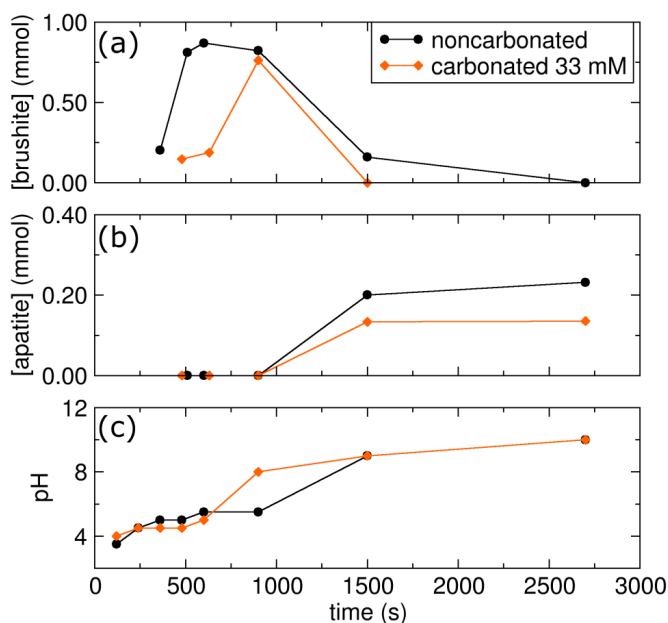


Figure 3.2: The molar amounts of brushite (a) and apatite (b) yielded from a timed reaction of the non-carbonated (black) and carbonated (orange) syntheses. As the amount of brushite decreases, the amount of apatite increases. The change in pH (c) as a function of reaction time. pH was measured *ex situ* with litmus paper.

Analysis of the phosphate concentration via an ammonium molybdate assay shows that the non-carbonated synthesis has a steady decline of the phosphate throughout the reaction. However, the carbonated synthesis shows an induction period during which the phosphate concentration remains steady before having a sharp decrease (Figure 3.3). This sharp decrease in phosphate concentration is accompanied by an increase in the amount of brushite that is recovered from the reaction. The induction period may be an indication that the carbonate species is being used first before the use of phosphate in the formation of brushite. Additionally, the continuous decrease of the phosphate concentration in the non-carbonated samples may be an indicator that phosphate clusters are forming. It can be shown that when the phosphate assay is conducted in the presence of pyrophosphate, P_2O_7 , almost no absorption is recorded (Figure 3.4). The absorption that is registered

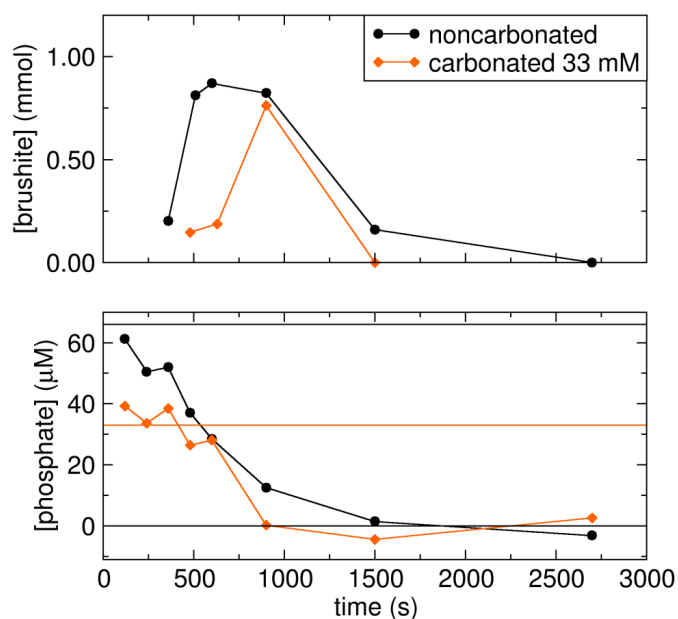


Figure 3.3: The molar amounts of brushite (top) compared to the evolution of the phosphate concentration (bottom) for carbonated and non-carbonated samples. The non-carbonated sample shows a steady decrease in the phosphate concentration while the carbonated samples show an induction period prior to a sudden drop in concentration.

is likely the result of some spontaneous hydrolysis of the pyrophosphate due to acidic environment of the assay.

From this phosphate concentration data, it can be seen that the initiation of the apatite phase does not occur until the phosphate concentration has nearly been depleted. This suggests that there is some type of conversion from brushite to apatite occurring, but does not give a hint as to if this is a solid-to-solid or solid-to-aqueous-to-solid conversion. However, it is also possible that apatite is nucleating without going through the brushite phase.⁷⁰

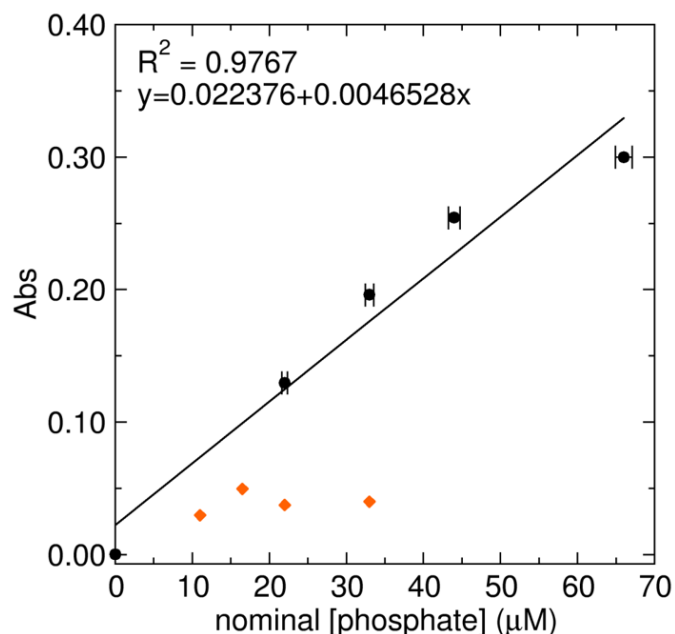


Figure 3.4: A set of phosphate standards (black data, line shows fit) compared to pyrophosphate (orange data). This shows that the molybdate assay is selective for free phosphate ions in solution because the pyrophosphate does not show any trend similar to that of the phosphate standard.

3.4. Conclusion and Future Directions

Biomimetic apatite samples were successfully prepared showing the original phase formed to be brushite. This brushite phase then converts to apatite at a rate slower than the initial formation of brushite. However, this does not preclude the nucleation event of apatite even though the energy barrier to doing so would initially be higher than to nucleate brushite. Additionally, following the concentration of phosphate through the reaction suggests the formation of $(\text{PO}_4)_x$ prenucleation clusters in non-carbonated samples. This indicates that Ostwald's Rule of Stages is being followed in the formation of samples. Because carbonate prenucleation clusters can also be formed,⁷² it is possible that these clusters are also forming prior to nucleation in the carbonated samples.

Going forward, it will be necessary to quantify the amount of carbonate in the reaction solution after acidification as well as the amount of carbonate in the initial solids formed during the reaction. One possible way to do this would be to use the Conway diffusion method^{29,76}. This method utilizes the fact that carbonate reacts with acid to form CO₂ gas. Once in the gas phase, the CO₂ can then diffuse and mix with a barium hydroxide solution causing precipitation of BaCO₃. The concentration of carbonate can then be back calculated by titration of the Ba(OH)₂ solution. This will also be helpful to quantify the amount of carbonate in the precipitated solids which may give some indication of how the carbonate is used in the synthesis process.

In situ studies would be very valuable in this system. Probably the easiest would be to do *in situ* pH monitoring. Because changes to the reaction setup can influence how the reaction proceeds,,⁷⁷ this would require a small pH sensing device such as indium-doped tin oxide (ITO)⁷⁸ to avoid making changes to the setup. Another study that would be beneficial would be *in situ* studies of the solid phase. However, this is non-trivial because of the solution phase and the necessary requirements to slowly raise the pH.

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A. Appendix A

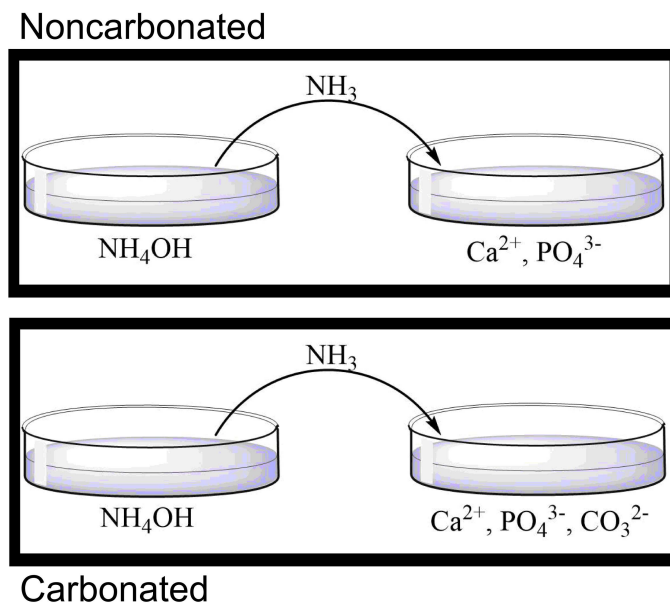


Figure A.1: Experimental setup for the preparation of biomimetic apatites.¹¹ The black box represents the reaction chamber in which synthesis occurred. The ratio of Ca/P or Ca/P+C was set to the stoichiometric ratio of 1.67. Precipitation of the desired phase occurs as NH_3 fills the local atmosphere, dissolves into the Ca/P solution, and increases the pH. The change in pH induces the precipitation of the apatite phase near $\text{pH}=4$.

Table A.1: Amounts and sources of Ca, P, and Cl for the standardized pellets used to determine the uncertainty of EDS measurements.

Standard	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (g)	$\text{NH}_4\text{H}_2\text{PO}_4$ (g)	CaCO_3 (g)	KCl (g)
A	0.1231	0.0768		
B		0.0758	0.1098	0.0143
C		0.0810	0.1189	
	Ca (mmol)	P (mmol)	Cl (mmol)	
A	0.837	0.668	1.675	
B	1.097	0.659	0.192	
C	1.188	0.704		

Table A.2: Weight percents of carbon, hydrogen, and nitrogen determined from HCNS combustion analysis. Sulfur was not detected in any sample. Values presented are the average measurement from two samples.

	wt.% C	wt.% H	wt.% N
Noncarbonated			
25% NH ₃	1.46(6)	0.99(5)	0.00
15% NH ₃	1.3(4)	0.99(1)	0.00
10% NH ₃	1.3(3)	1.014(1)	0.3(3)
8% NH ₃	1.08(7)	1.03(5)	0.00
Carbonated			
25% NH ₃	2.8(4)	1.26(8)	0.5(5)
15% NH ₃	2.57(98)	1.09(6)	0.3(3)
10% NH ₃	2.4(4)	1.03(5)	0.00
8% NH ₃	2.1(1)	1.066(6)	0.00

Table A.3: Molar values of calcium, phosphorous, and chlorine determined via energy dispersive spectroscopy (EDS). No other elements were detected in these samples. Values are the average measurement from two samples, each with a 5% error.

	mol Ca	mol P	mol Cl
Noncarbonated			
25% NH ₃	1.18(6)	0.61(3)	0.015(1)
15% NH ₃	1.16(6)	0.56(3)	0.013(1)
10% NH ₃	1.20(6)	0.56(3)	0.006(1)
8% NH ₃	1.10(6)	0.57(3)	0.008(1)
Carbonated			
25% NH ₃	1.15(6)	0.53(3)	0.006(1)
15% NH ₃	1.13(6)	0.59(3)	0.006(1)
10% NH ₃	1.09(6)	0.56(3)	0.012(1)
8% NH ₃	1.13(6)	0.52(3)	0.005(1)

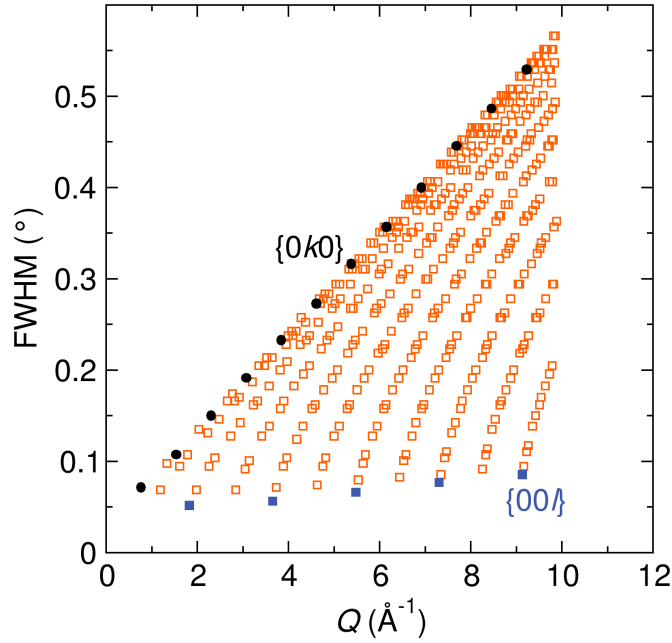


Figure A.2: Williamson-Hall analysis⁵⁶ of the Bragg reflection peak widths illustrates that the broader and narrowest reflections in the apatite material are the $(0k0)$ and $(00l)$ families of planes, respectively. While this analysis suggests that these reflections correspond to the same sizes, our analysis cannot differentiate between size and strain due to the method used for peak deconvolution (i.e., microstrain broadening).

Table A.4: Refined atomic positions, ADPs, and profile terms determined from Rietveld refinement of the XRD data for the non-carbonated sample prepared with 25% NH_3 . Lattice parameters: $a = b = 9.437(2)$ Å, $c = 6.8797(6)$ Å. Space group: $P63/m$

Atom	x	y	z	U_{iso}
O1	0.343(2)	0.514(1)	1/4	0.043(6)
O2	0.542(1)	0.435(2)	1/4	0.036(6)
O3	0.339(2)	0.261(1)	0.063(1)	0.094(5)
P	0.3664(8)	0.4013(9)	1/4	0.034(2)
Ca1	1/3	2/3	0.998(1)	0.023(1)
Ca2	0.2536(5)	0.9903(8)	1/4	0.0091(6)
O(H)	0.0	0.0	1/4	0.020(9)
scale	0.1510(6)			
size	234 Å			
LX	2.74(7)			
η	0.47(2)			
S_{400}	10.1(3)			
S_{004}	0.24(6)			
S_{202}	3.5(3)			

Table A.5: Refined values from LeBail refinement of the non-carbonated samples. Particle size was calculated using the Scherrer equation based on the FWHM of the (002) reflection.

% NH ₃	$a = b$ (Å)	c (Å)	LX	η	S ₄₀₀	S ₀₀₄	S ₂₀₂	scale	size (Å)
25	9.432(1)	6.8800(4)	2.31(5)	0.64(1)	16.9(3)	0.16(4)	5.1(2)	0.162210	257
15	9.413(1)	6.8788(4)	1.41(5)	0.351(9)	14.5(2)	0.71(5)	2.3(2)	0.121960	257
10	9.392(1)	6.8784(5)	5.15(5)	0.286(9)	14.5(2)	0.56(5)	2.0(2)	0.162130	245
8	9.391(1)	6.8784(6)	2.52(7)	0.35(1)	19.3(4)	0.40(6)	2.2(3)	0.270230	234

Table A.6: Refined values from LeBail refinement of the carbonated samples. Particle size was calculated using the Scherrer equation based on the FWHM of the (002) reflection.

% NH ₃	$a = b$ (Å)	c (Å)	LX	η	S ₄₀₀	S ₀₀₄	S ₂₀₂	scale	size (Å)
25	9.402(1)	6.8780(5)	2.39(5)	0.141(8)	18.7(2)	0.87(5)	0.3(1)	0.0604570	215
15	9.394(2)	6.8794(6)	1.9(2)	0.37(2)	28(1)	1.0(2)	1.2(4)	0.0158070	234
10	9.390(2)	6.8775(7)	1.9(2)	0.41(2)	26(1)	0.9(2)	2.3(5)	0.0212560	245
8	9.395(2)	6.8786(7)	2.06(8)	0.42(1)	25.2(5)	0.59(8)	3.6(4)	0.0177840	245

Table A.7: Refined values for applying the XRD model to the experimental PDF (Figure 7a) and isotropic ADP values and atom positions for the model based on refinement of the experimental PDF (Figure 7b).

	XRD Model	Refined PDF Model			
scale factor	0.47(3)	0.42(3)			
$a = b$ (Å)	9.437	9.41(4)			
c (Å)	6.8797	6.88(4)			
delta2	2(1)	1.8(3)			
R_w	47.6%	11.9%			
		x	y	z	U_{iso}
O1		0.32(1)	0.48(2)	1/4	0.010(4)
O2		0.59(2)	0.47(2)	1/4	0.010(4)
O3		0.345(8)	0.256(8)	0.074(9)	0.010(4)
P		0.398(6)	0.367(7)	1/4	0.006(4)
Ca1		1/3	2/3	0.001(01)	0.008(3)
Ca2		0.249(4)	0.994(6)	1/4	0.007(3)
O(H)		0.0	0.0	1/4	0.01(1)

Table A.8: Refined parameters for modeling the changes between non-carbonated and carbonated experimental PDF (Figure 8). No atom positions were refined.

	Non-carbonated Model	Anisotropic	Anisotropic + Spherical	Spherical
scale factor	0.36(3)	0.37(4)	0.39(7)	0.39(7)
$a = b$ (Å)	9.42(4)	9.43(6)	9.42(6)	9.41(4)
c (Å)	6.88(4)	6.87(6)	6.88(7)	6.55(5)
delta2	1.8(4)	2(1)	2(1)	1.7(5)
sp diameter			212 (900)	186(580)
R_w	16.14%	12.96%	12.88%	14.59%
O1 (U_{11})	0.010	0.01(2)	0.01(2)	0.009(4)
O1 (U_{22})	0.010	0.01(2)	0.01(2)	0.009(4)
O1 (U_{33})	0.010	0.01(3)	0.01(3)	0.009(4)
O1 (U_{12})		0.003(19)	0.005(22)	
O2 (U_{11})	0.010	0.01(2)	0.01(2)	0.009(4)
O2 (U_{22})	0.010	0.01(3)	0.02(3)	0.009(4)
O2 (U_{33})	0.010	0.01(4)	0.01(5)	0.009(4)
O2 (U_{12})		0.01(2)	0.01(2)	
O3 (U_{11})	0.010	0.03(3)	0.03(3)	0.009(4)
O3 (U_{22})	0.010	0.01(2)	0.01(3)	0.009(4)
O3 (U_{33})	0.010	0.01(2)	0.01(2)	0.009(4)
O3 (U_{12})		0.01(3)	0.01(3)	
O3 (U_{13})		-0.01(3)	-0.01(3)	
O3 (U_{23})		-0.01(2)	-0.005(20)	
P	0.006(4)	0.007(6)	0.007(6)	0.007(5)
Ca1 (U_{11})	0.008(3)	0.015(7)	0.015(7)	0.011(5)
Ca1 (U_{22})	0.008(3)	0.015(7)	0.015(7)	0.011(5)
Ca1 (U_{33})	0.008(3)	0.01(2)	0.01(2)	0.011(5)
Ca1 (U_{12})		0.008(7)	0.008(7)	
Ca2	0.007(3)	0.008(4)	0.008(4)	0.0058(3)
O(H)	0.01(1)	0.01(2)	0.01(2)	0.01(1)

Table A.9: Calculated atom positions and selected ADP values for the explicitly carbonated model using non-adjacent substitution into the mirror plane. Atom positions were not refined and remain as calculated following the DFT structure calculation.

Atom	x	y	z	U_{iso}
O(P)	0.328740	0.483801	0.256939	0.0035
O(P)	0.616631	0.470752	0.738433	0.0035
O(P)	0.507038	0.838650	0.241234	0.0035
O(P)	0.839847	0.319594	0.753468	0.0035
O(P)	0.589040	0.459704	0.261902	0.0035
O(P)	0.405829	0.553712	0.710724	0.0035
O(P)	0.540480	0.124521	0.232382	0.0035
O(P)	0.118100	0.585118	0.739512	0.0035
O(P)	0.347619	0.264374	0.063152	0.0035
O(P)	0.639439	0.721148	0.929195	0.0035
O(P)	0.751157	0.075667	0.067971	0.0035
O(P)	0.094008	0.348940	0.929193	0.0035
O(P)	0.692798	0.743780	0.571382	0.0035
O(P)	0.335669	0.243826	0.425663	0.0035
O(P)	0.736206	0.077230	0.431278	0.0035
O(P)	0.077478	0.331000	0.565908	0.0035
P	0.39909	0.36740	0.25288	0.0035
P	0.59389	0.62250	0.73647	0.0035
P	0.63176	0.02469	0.24239	0.0035
P	0.02834	0.39470	0.74589	0.0035
Ca1	0.328540	0.652338	0.994674	0.0035
Ca1	0.682449	0.355334	0.999632	0.0035
Ca1	0.657502	0.311931	0.502739	0.0035
Ca1	0.325005	0.688729	0.491657	0.0035
Ca2	0.248940	0.002405	0.217099	0.0035
Ca2	0.760726	0.000124	0.747969	0.0035
Ca2	0.007936	0.246535	0.245292	0.0035
Ca2	0.015311	0.765643	0.709579	0.0035
Ca2	0.760589	0.736977	0.231694	0.0035
Ca2	0.270085	0.266017	0.746765	0.0035
O(H)	0.999920	0.993341	0.200563	0.02
O(H)	0.020662	0.014822	0.722612	0.02
O(H)	0.889033	0.649476	0.998618	0.02
O(H)	0.240986	0.890061	0.503515	0.02
C	0.409430	0.973536	0.821182	0.02
C	0.970846	0.551726	0.311880	0.02
O(C)	0.472920	0.125238	0.767886	0.02
O(C)	0.120085	0.627100	0.247816	0.02
O(C)	0.474406	0.886180	0.769116	0.02
O(C)	0.873403	0.399411	0.262496	0.02
O(C)	0.276980	0.901999	0.926176	0.02
O(C)	0.911931	0.619851	0.423519	0.02

^aSpace group: *P1*. Hydrogen atom positions were not refined. ADP values were not refined but were set to a value ($H = 0.0025$).

Table A.10: Refined values for DFT-relaxed structures used to model the experimental PDF of the carbonated sample prepared using 25% NH_3 . ADP values were not refined and remain constant for all DFT structures. Ca1, Ca2, P, O(P) = 0.0035; C, O(H), O(C) = 0.02.

	scale factor	$a = b$ (Å)	c (Å)	delta2	R_w
Face, nonadjacent	0.85(2)	9.36(1)	6.90(2)	1.4(3)	22.8%
Face, adjacent	1.02(3)	9.349(1)	6.89(1)	1.0(4)	37.9%
Mirror, nonadjacent	0.87(2)	9.36(1)	6.90(2)	1.4(3)	21.4%
Mirror, adjacent	0.85(2)	9.39(1)	6.89(1)	1.3(4)	29.8%

Table A.11: Refined values for the best linear combination of DFT-relaxed structures to describe the experimental PDF of the carbonated sample prepared using 35% NH_3 . The linear combination combines the nonadjacent carbonate substitutions into both the mirror plane and face of the phosphate ion. The atom positions and ADPs were not refined.

Linear Combination	
scale factor (mirror)	0.5(1)
scale factor (face)	0.4(1)
$a = b$ (Å)	9.37(2)
c (Å)	6.89(2)
delta2	1.4(3)
mol% mirror	57(9)
mol% face	43(9)

B. Appendix B

B.I. Reverse Monte Carlo Simulations

Because PDF is an ensemble average of the local structure and XRD is an average of long-range crystallographic order, Reverse Monte Carlo (RMC) was employed to model both long-range ordered components of the structure (as informed by the total scattering), as well as components with short-range order (as informed by experimental PDF) that do not possess crystallographic order. These simulations were consistent with both the total scattering and PDF data and show reasonable fits (Figure B.1). The RMC simulation shows displacements consistent with thermal disorder is apparent in all atom types and sites, the Ca and P atoms seem more precisely grouped as compared to the phosphate-linked oxygen atoms. The locations of the phosphate-linked oxygens and Ca1, non-channel-forming atom sites, also seem to be more anisotropic than the other atoms in the simulated cell which gives some justification to allowing those sites to refine anisotropically in the PDF refinements of carbonated samples. Interestingly, there is only a very small amount of displacement of the hydroxide oxygen site through this simulation such that atoms were placed on the expected site or within an area of the radius of an oxygen atom. Although this was not seen in refinements of the individual PDF and XRD data, it is considered a robust structural motif of the sample because a large uncertainty in the position should create a lot of variation in the location of this atom, not the small amount seen here. However, the observation of an atom consistently in this location does not conclusively show the presence of the hydroxide ion in the lattice. In the apatite family, this lattice site is

often occupied by monatomic ions such as Cl^- , which is also present in the reaction mixture due to acidification with HCl and the Ca^{2+} source and could be easily incorporated into the lattice. However, at less than 1% detection by SEM-EDS, it is unlikely that PDF would be sensitive to this level of substitution. Instead, it is possible that the large ADP values refined from PDF and XRD data may instead be the result of an inconsistent hydrogen location. Because X-rays are insensitive to hydrogen atoms, simulations, refinements, and fits were completed using structures that did not include hydrogen atoms. Thus, it is possible that small pockets of electron density caused by hydrogen may be falsely inflating the ADP value of the hydroxide oxygen.

Per visual inspection of the simulated supercell structure, some of the phosphate sites looked to have only three neighboring oxygens within the appropriate range to bond. Statistical analysis of the bond lengths and bond angles of phosphorous-oxygen nearest neighbors revealed the possibility of carbonate-type geometry in about 10% to 20% of phosphate sites (Figure B.2). In the medium-sized box (8,820 atoms, 1,260 phosphorous atoms) the average bond angle for the phosphate-type coordination was 108° with an average bond length of 1.497 Å while carbonate-type coordinations had an average bond angle of 116° and bond length of 1.403 Å. The phosphate-type bond angle is very similar to the expected tetrahedral bond angle of 109.5° and the carbonate-type angle is fairly close to the expected bond angle of 120° showing that this analysis does suggest that there is carbonate substitution in the phosphate location that would be consistent with the experimental scattering data. While the bond lengths are not the ideal 1.6 Å or 1.5 Å expected for phosphate or carbonate bonds, respectively, the small difference in the calculated bond lengths does further the argument of distinctly different bonding environments. Further-

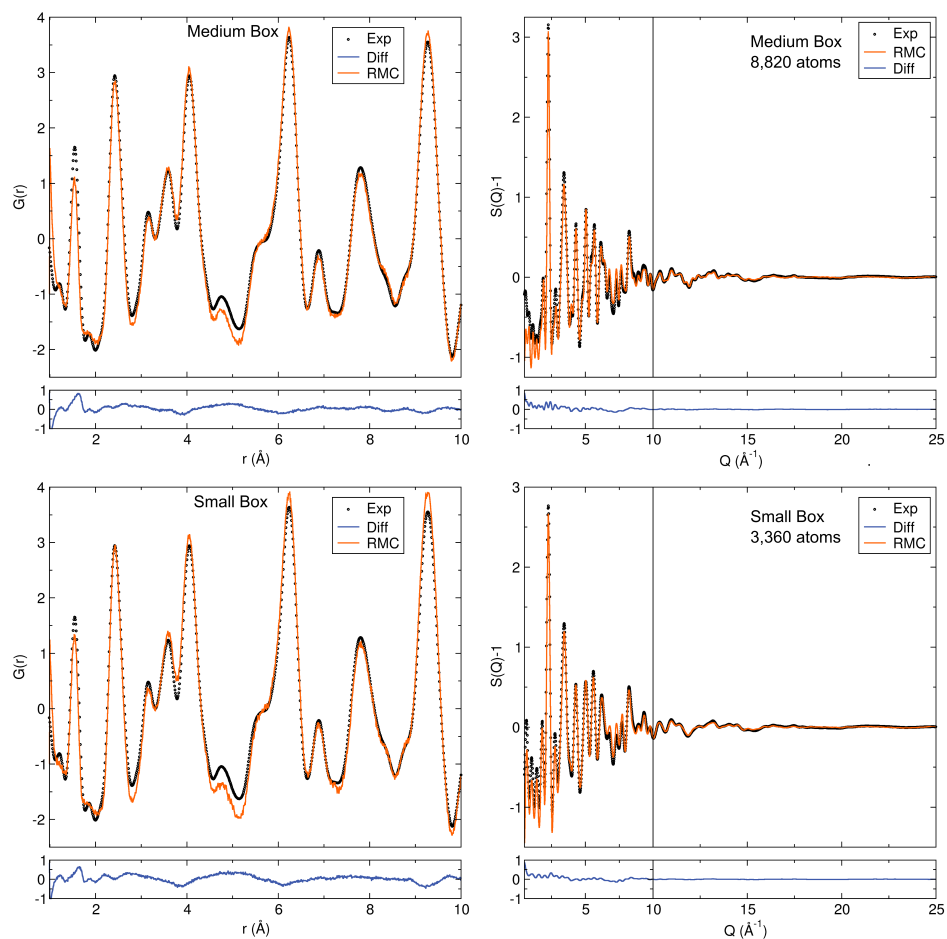


Figure B.1: Calculated RMC fits for the "medium" (top) and "small" (bottom) box simulations. Total scattering, $S(Q)$ fits are on right and $G(r)$, PDF, fits are on the left. These fits show that the simulation is creating a structure which is fitting the data appropriately.

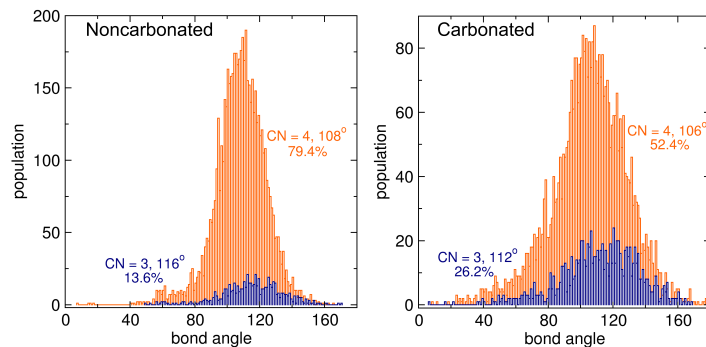


Figure B.2: Shows the histograms for noncarbonated (left) and carbonated (right) medium box simulations to determine if the simulation creates more carbonate-type environments when the data is from a carbonated sample.

more, the non-ideal bond lengths may simply be a result of the nonideal geometries that have been induced by the RMC simulation via RMCProfile. This procedure was repeated with a simulation completed using data from a carbonated sample. It was determined that when the data was from a carbonated sample, the same variation of coordination numbers for phosphorous atoms was found, but the number of atoms with CN=4 decreased and CN=3 increased. This shows that the simulations are consistent with the possibility of carbonate incorporation into the material and suggests that the carbonate is incorporating into the phosphate location.