The incorporation of small cations in hydroxyapatite : synthesis, crystal chemistry and functionality

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Abstract

Hydroxyapatite (HAp) is an important oxide in biotechnology, and also holds promise as a heterogeneous catalyst. Its ideal composition is $[(\text{Ca}^{\text{F}}_4)(\text{Ca}^{\text{T}}_6)(\text{PO}_4)_6(\text{OH})_2]$ where an adaptable framework (F) structure creates one-dimensional tunnels (T) that provide multiple ionic acceptor sites ($\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}/\text{P}/\text{OH}$) which can be adjusted to deliver specific functionality. Modification of cation and anion type and proportions, together with selection of appropriate syntheses, can be used to regulate structural distortions and phase transformations. However, first-principles crystallographic adaptation to optimize performance is less explored, especially for tailoring excipient responses and catalytic activity, which are the primary concern of this study.

Bioactive and catalytic HAp is nanocrystalline and usually nonstoichiometric, especially with respect to cation vacancies, carbonate and water incorporation, and oxygen displacement of hydroxyl ions. The hypothesis explored in this thesis is that controlled doping of HAp by small cations (Fe^{3+} , Zn^{2+} , Mg^{2+}) will alter the crystal chemistry in a manner that enhances cell proliferation (biological application) and product yield (catalytic application). The selection of small cations for investigation was deduced from their abundance and essential role in bone metabolism and the potential of these metals for enhancing chemical conversion of alcohol while fixed in an environmentally benign HAp matrix. To this end, methods to synthesize homogeneous ceramic powders of single phase Fe-, Zn-, and Mg-HAps to the solid solubility limits of these metals were developed from which structure-property correlations were established. All three series were characterized with X-ray and neutron diffraction, electron microscopy, and several spectroscopies, including FTIR and NMR techniques, to corroborate crystallographic incorporation and concomitant symmetry transformations, determine phase proportions (crystalline and amorphous), and metal partitioning preferences across the $\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}$ sites.

Crystal structure refinement was challenging due to the fine-grain size, although in this physical form, large reactive surfaces are produced that are preferable for the targeted applications. It was found that the solid solution limit of Fe-HAps {nominally $[(\text{Ca}_{10-x}\text{Fe}_x)(\text{PO}_4)_6(\text{OH}_{2-x}\text{O}_x)]$ } was $x = 0.5$, with Fe^{3+} entering the Ca^{F} site at low loadings ($x = 0.1$) and subsequently residing in both the Ca^{F} and Ca^{T} at $x = 0.5$.

As iron content increased, a pseudomorphic phase transformation from hexagonal $P6_3/m$ (apatite- $2H$) to monoclinic $P2_1/m$ (apatite- $2M$) resulted from framework distortion driven by tunnel-site hydroxyl nonstoichiometry. In Zn-HAps {nominally $[(Ca_{10-x}Zn_x)(PO_4)_6(OH)_2]$ }, the solid solubility was $x = 0.5$ with Zn^{2+} located in the Ca^T site and the possible existence of $2H$ and $2M$ pseudomorphs. Simultaneous incorporation of carbonate ($x = 0.1$) and water ($0 < x < 0.5$) was significant and contributed to the stabilization of an amorphous phase. The solid solubility of Mg-HAps {nominally $[(Ca_{10-x}Mg_x)(PO_4)_6(OH)_2]$ } was not breached in the present experiments, with Mg^{2+} exclusively entering the Ca^F site (up to $x = 0.4$). The gradual transformation from hydroxy- into oxy-apatite with increasing Mg loading was observed, while retaining hexagonal $2H$ symmetry. In addition, a novel method was employed to fabricate a porous Mg-HAp ceramic foam, as a scaffold for bone regeneration.

Both Fe-Ap and Mg-HAp show superior bioactivity with respect to pure HAps, even without the participation of carbonate which is generally regarded as an essential component of bone. The Mg-HAp foams were found suitable for enhancing cell proliferation. Alcohol decomposition was selected as a model reaction to probe catalytic activity. Fe-Aps ($x = 0.1$ and 0.5) were effective novel catalysts for ethanol decomposition, while Zn-HAps ($x = 0.1$ and 0.5) and Mg-HAps ($x = 0.2$ and 0.4) were screened in 2-propanol decomposition. These reconnaissance studies confirmed the potential of these materials as heterogeneous catalysts, with further optimization required to fully describe the reaction mechanisms.

These studies have lead to a systemization of hydroxyapatite crystal chemistry for the insertion of small cations. The metaprism twist angle (φ) was employed throughout this study to examine the crystal chemistry, and authenticate the crystallographic data of these apatite series extracted by diffraction, microscopy and spectroscopy. A critical review of published data, in combination with the experiments describe here, rationalized the behavior of small cations HAp, allowing the recognition of deviations in space group and composition.

The central hypothesis of this thesis, that the incorporation of small metals can be tailored to crystallographically modify HAps as prospective biomaterials and catalysts, is found to be feasible.

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Figure 4-12. Desorption profiles of (a) as synthesized pure HAp, (b) Zn-HAp ($x = 0.1$) and (c) Zn-HAp ($x = 0.5$). The presence of zinc did not introduce new surface adsorption sites and also no significant changes to the desorption temperature.

Figure 4-13. Catalytic decomposition of IPA with Zn-HAp show advantageous in the selectivity (%) towards propene (C_3H_6) but comparable yield (%) at various reaction temperature. The abrupt increase of yield for Zn-HAp ($x = 0.1$) beyond 250 °C attributed to leaching of Zn^{2+} forming of zinc oxide (ZnO) that increased the surface active sites.

Figure 5-1. Compilation of published XRD data on Mg-HAp series compared against this work (◆: 700 °C and ▲: 600 °C) [53, 114-117, 158]. The variations in (a) a -edge and (b) unit cell volume are in accord with the general systematics reported although the solid solubility limit was variable. A decrease in lattice constant and volume is indicative of preferential framework Ca^{F} partitioning of Mg^{2+} , in contrast to Bigi *et al.*

Figure 5-2. FTIR spectra of Mg-HAp calcined at (a) 600 °C, (b) 700 °C showing signature hydroxyl and mixed A-B type carbonate bands. (c) The hydroxyl feature progressively diminishes with magnesium loading, while only a minute portion of carbonate is crystallographically bound at the onset of magnesium incorporation (dotted lines refer to the 700 °C series and solid lines are 600 °C). Similar trends of hydroxyl band found in (d) macroporous Mg-HAp with increasing magnesium, but carbonate is below detection limit. Fabrication of the foam materials introduced an extra feature in the vicinity of the original hydroxyl band ($\sim 3570 \text{ cm}^{-1}$).

Figure 5-3. Unit cell trends in Mg-HAp refined from XRD data. (a) The incorporation of Mg^{2+} leads to a decrease in a -edge and volume contraction (inset), in accord with a framework partitioning. For a given composition, higher calcination temperatures confer to a longer a -axis and lattice expansion. (b) The c -axis converges towards ~ 6.88 with increasing magnesium loading, reflecting an insignificant modification along the tunnel. Amorphous content arising from microabsorption are in the range of 30 and 40 wt% in all samples (inset). (d) crystal size were marginally larger at higher calcinations temperature, although increasing magnesium inhibited crystal growth ($x = 0.4$).

Figure 5-4. Partial XRD pattern ($20\text{--}45^\circ 2\theta$) of (a) Mg-HAp ($x = 0.1$ and 0.4) calcined at 700 °C. Higher magnesium content lead to poor crystallinity as reflected in loss of diffracted intensity especially. The sharp peak at $\sim 28^\circ$ originated from fluorite. (b) Fabrication of Mg-HAp ($x = 0.4$, 600 °C) foam require further heat treatment that produce marginally larger crystal size, improving total counts of the diffracted peaks. Note the separation at $\sim 32^\circ$ and 38° (marked with *).

Figure 5-5. Rietveld plot of the neutron diffraction data for Mg-HAp ($x = 0.1$) prepared via sintering under N_2 (700 °C/ 2h) and spiked with CaF_2 (10 wt %). The upper trace is an overlay of the experimental pattern (circles) and calculated intensity (continuous line) while the lower trace shows the

intensity differences between the experimental and calculated patterns. Refinement is carried out in the 10-150° region and bottom-most are the Bragg markers for CaF₂ and Mg-HAp, respectively.

Figure 5-6. Crystallographic information of Mg-HAp series refined from PND data.. The incorporation of Mg²⁺ in place of larger Ca²⁺ at the framework site leads to (a) *a*-edge and (b) lattice volume contraction consistent with XRD analysis. For any given composition, the insertion of Mg²⁺ is stabilized at higher calcinations temperature with B_{iso}-Ca^F smaller, when the crystal size is marginally larger (inset). (c) The *c*-axis dilates gradually with increasing magnesium loading, while the tunnel is more stabilized as depicted by a decreasing thermal parameters [B_{iso}-O(4)](inset). Pure HAp containing both A and B type carbonate significantly decreases the lattice parameters. (c) The unit cell contracts in accord with the reduction in the volume of apatite framework. (d) The simultaneous accommodation of smaller Mg²⁺ and tunnel expansion leading to more acute metaprisism twist angle (ϕ).

Figure 5-7. Polyhedral volume (Å³) adjusts accordingly with increasing Mg²⁺ loading *x* (at %) (inset). (a) The channel Ca^TO₆X polyhedra size increases as the unit cell expands. This tunnel expansion induced framework adjustment to a more acute ϕ . The framework (b) Ca^FO₆ is invariant and (c) PO₄ tetrahedra are refined as semi-rigid bodies, both indiscreet to unit cell volume changes.

Figure 5-8. Relationship between metaprismatic bond lengths [Ca^F-O(1) and Ca^F-O(2)] (Å) and twist angle ϕ (°). The insertion of Mg²⁺ induce a preferential displacement of the cation position that lead to a shorter Ca^F-O(1) and Ca^F-O(2) elongation with increasing Mg²⁺ loading, in which ϕ varies accordingly.

Figure 5-9. Microstructure of the porous Mg-HAp (*x* = 0.4). The architecture was developed from an annealing process that promoted crystal growth “bridging” the nano-crystals.

Figure 5-10. Microanalysis of Mg-HAp showing (a) Magnesium (Mg) and calcium (Ca) content (wt %) (inset) normalized by weight percent oxide plotted against nominal Mg content. (b) The amount is somewhat lower than nominal content when normalized to 6 apfu of phosphorous, but the trend is in accord with Mg incorporation leading to a shorter lattice edge. Pure HAp would contain Ca 39.9, P 18.5, O 41.4, H 0.2 wt%, respectively.

Figure 5-11. Selectivity (%) and yield (%) of hydrogen (H₂) and propene (C₃H₆) at various reaction temperature, utilizing Mg-HAp as catalysts. The result of pure HAp is included for comparison. Mg-HAps are marginally superior to pure HAp in the production of propene beyond 150 °C.

Figure 5-12. MG-63 Osteosarcoma cell viability of nano-crystalline powder and macroporous foam of Mg-HAp (*x* = 0, 0.2, 0.4) as a function of incubation period. The porous structure show enhancing effect in cell proliferation that is further improved by the incorporation of magnesium.

Figure 5-13. Light microscopic observations of osteosarcoma cells in PBS incubated with (a), (b) control, (c), (d) powder pure HAp, (e), (f) foam pure HAp, and (g), (h) powder Mg-HAp (*x* = 0.2) for 1 and 7 days. The cell proliferation was active after 7 days with respect to control sample. No adverse effect was observed in the foam materials, and the subtle change in cell morphologies is attributed to Mg²⁺ introduction that altered the biological response of these cells. Scale bar is 50 μm.

Figure 5-14. Confocal laser micrographs of the (a), (b) control, (c), (d) powder pure HAp, (e), (f) powder Mg-HAp (*x* = 0.2), and (g), (h) foam Mg-HAp (*x* = 0.4) samples after 7 days incubation with osteosarcoma cells that were dyed with fluorescence red. Merged image of the same focal height (*z*) is included to locate the powder and foam materials. The findings were consistent with light microscopy where cells morphology is more isotropic in Mg-HAps while the cytoskeleton fibres are more defined with Mg²⁺ incorporation. Scale bar is 75 μm.

Figure 6-1. Mechanism of systematic incorporation of carbonate in the synthesis protocol.

Figure 6-2. Various crystallographic orientation of A and B-type carbonate reported. The orientation of A-type carbonate is similar as reported by Fleet *et al* [123] and Suetsugu *et al* [179], note that the A2-type which is a high pressure feature. B-type carbonate generally occupies two of the random faces of the phosphate tetrahedra. The different models proposed by Fleet *et al* [123], Ivanova *et al* [122] and Wilson *et al* [184] is indicative of the difficulty in completely understanding the condition in which these model prevails.

Figure 6-3. Band area of water vibration ($\sim 2500\text{--}4000\text{ cm}^{-1}$) and carbonate vibration ($\sim 1350\text{--}1550\text{ cm}^{-1}$) from FTIR spectra decrease with increasing Fe, Zn and Mg loading. The absolute amount could not be quantified from the figures but estimation of relative proportions provided insights to the extent of extra stoichiometric modification.

Figure 6-4. Largest metaprism twist angle ($\phi_{\max}$) versus framework to tunnel ionic radius ratio (F:T) in this work and various apatite collated for comparison. (a) In general a larger F:T would increase $\phi_{\max}$ and transformation to lower symmetry pseudomorph is expected when $\phi_{\max} > 27^\circ$. (b) The incorporation of carbonate does not alter ϕ significantly, but (c) show sensitivity to tunnel contents.

Figure 6-5. Structure drawings of $P6_3/m [Ca^F_4Ca^T_6](PO_4)_6(OH)_2$ hydroxyapatite. (a) An [001] projection of the zeolitic $[Ca^F_4(PO_4)_6]^{10-}$ framework that is constructed from Ca^FO_6 metaprisms (grey polyhedra) and PO_4 tetrahedra (green polyhedra). The metaprism is a solid figure intermediate between an octahedron and trigonal prism. The one-dimensional channels are occupied by Ca^T (green cation) and OH (yellow oxy-anion) that combine as $[Ca^T_6(OH)_2]^{10+}$ counter ions. The framework is flexible and during substitution of Ca, P or OH by other species, the metaprisms twist co-operatively to dilate or constrict the channel according to the size and occupation factors of the substituents. (b) The [110] projection shows the alternation of twist along [001] between successive metaprisms and emphasizes the isolation of the PO_4 tetrahedra. (c) A clinographic projection of apatite illustrating the spatial relationship between the framework polyhedra and channel ions.

Figure 6-6. Bond length changes with degree of crystallographic modifications. (a) The average framework Ca-O bond length $[(Ca-O)^F_{ave}]$ is shorter when a relatively larger cation ($Fe^{3+} < Mg^{2+} < Zn^{2+}$) is incorporated. The average P-O bond length $[(P-O)^{ave}]$ behaves in the opposite way where $(P-O)^{ave}$ of Fe-Aps is shorter compared. Note the three distinct PO_4 polyhedra in monoclinic $P2_1/m$ Fe-Ap ($x = 0.2$ and 0.5).

Figure 6-7. Polyhedral volume ($\text{\AA}^3$) changes against Fe, Zn and Mg loading (at%). Incorporation of Fe and Zn modifies the Ca^FO_6 and Ca^TO_6X polyhedra considerably, but invariant with Mg loading. Distinctive regions with increasing lattice parameter a is correlated with increasing ionic radius of the substituted cations, with an anomaly in Zn-HAp ($x = 0.1$) that is ascribed to crystallographically bound carbonate and water.

Figure 6-8. PO_4 , Ca^FO_6 and Ca^TO_6X polyhedral volume ($\text{\AA}^3$) versus unit cell volume ($\text{\AA}^3$) of all three apatite series. The trend is consistent with increasing ionic radius of substituted cations dilating the unit cell volume, with PO_4 being relatively rigid. The elongation in c -axis is associated with tunnel Ca^TO_6X .

Chapter 1

Introduction

Cation-doped hydroxyapatites are widely used as prosthetic materials and are under development as heterogeneous catalysts, but the role of crystallochemical modifications in regulating excipient and catalytic functionalities are poorly understood. This thesis describes the synthesis of apatites of prescribed chemistries and architectures for deployment as biomaterials and in chemical synthesis. The properties of these fine-grained apatites were investigated through comprehensive crystallographic, microscopic and spectroscopic characterization, that was systematically correlated with functionality.

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1.1 Problem Statement

A substantial literature exists concerning the fabrication of nano-scale hydroxyapatite (HAp) for prosthetic [1] and catalytic applications [2]. The precise nature of these materials is not readily ascertained, and optimization of performance employs a range of strategies including crystallochemical modification through the incorporation of cations and/or anion radicals [3,4]; introduction of bio-molecules [5], polymer coatings [6] and organic matrices [7]; and customization of shape as porous monoliths [8] or thin films [9]. These approaches, when deployed in combination, make HAp product validation procedurally demanding.

The apatite family of materials, particularly hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$, has been synthesized by many methods to suit the end application, with numerous permutations of wet chemical [10], solid state [11], sol-gel [12], and hydrothermal reactions [13] developed. The performance and properties of apatites can be regulated by inserting smaller cations in place of Ca^{2+} (ionic radius = $1.00\text{\AA}$), particularly first row transition metals, whose radii are 35-45% smaller [14]. Accommodating this size difference while simultaneously satisfying charge balance, leads to changes in structure and symmetry, often accompanied by non-stoichiometry [15]. In practice, controlling the overall composition precisely can be challenging, with atmospheric moisture and carbon dioxide readily taken up [16, 17]. Although the solid solubility of small cations in HAp is limited, their presence invariably inhibits crystal growth, and often favors the formation of transition metal rich compounds, especially tri-calcium phosphate (β -TCP) [18], that coexists with amorphous phases. Controlling synthesis parameters to obtain single-phase, polycrystalline apatites of complex chemistry remains challenging.

This research is concerned with processes to synthesize powders and macroporous foams of HAp lightly doped with iron, zinc and magnesium to enhance their biological response and catalytic activity.

The general formula of hexagonal $P6_3/m$ apatite is $[A_4^F][A_6^T][(BO_4)_6]X_2$ where A^F , A^T and B are discrete cation sites and X is an anion or oxyanion. The structure is ‘adaptive’, in the sense that one-dimensional channels are constructed from columns of framework (F) metaprisms ($A^F O_6$) that are corner-connected to BO_4 tetrahedra. The tunnels (T) are occupied by larger cations (A^T) and anions (X) of ideal stoichiometry $A_6^T X_2$ [19]. Framework flexibility is achieved by adjustment of the metaprism twist angle (ϕ), with more complex apatite varieties derived by lowering the symmetry from hexagonal to monoclinic or triclinic that creates polymorphs and pseudomorphs [20]. For example, HAp is trimorphic ($P6_3/m$, $P2_1/b$ and PI) as a result of small compositional deviations such as the incorporation of carbonate (CO_3^{2-}) and/or dehydroxylation ($2OH^- \rightarrow O^{2-}$) [21], or inter-tunnel order correlations of the X-site anions [22]. Moreover, polysomatic apatite structures can be described as c -stacking rotation twins of fundamental (001) modules ($A_2^F A_3^T B_3 O_{18} X_6$) [23]; to be completely general, the hexagonal, monoclinic and triclinic trimorphs of HAp are all bimodular and designated *apatite-2H*, *apatite-2M* and *apatite-2A*, respectively. HAp functionality can be modified by controlling pseudomorphic and polymorphic transformations through compositional adjustments.

This thesis examines the hypothesis that small-cation doping (Fe^{3+} , Zn^{2+} , Mg^{2+}) systematically alters HAp symmetry by topological framework distortion, cation ordering, and non-stoichiometry, and that such changes can be tailored to improve the bioactivity and catalytic performance of these materials.

1.2 Scope and Objectives

The premise of these investigations is that low concentrations of small metal ions alter the physical form and functionality of HAp to promote cell proliferation and catalytic reactions. The objectives of this research are to:

- (i) *Recognize and compile generic relationships* between apatite crystal chemistry, morphology and functionality; and
- (ii) *Demonstrate the bioactivity and catalytic performance* of ferric-, zinc- and magnesium-HAp systems.

More generally, these applications in biomedicine and green chemistry illustrate the diverse functionality of apatite chemical analogues.

The scope of this work includes:

- (i) Establishing maps of topological distortions and structural modification as a function of composition.
- (ii) Fabricating apatites through a variety of routes, the principle being:
 - a. Co-precipitation of stoichiometric proportions of reagents [including calcium hydroxide ($\text{Ca}(\text{OH})_2$), chlorides (MX_2 , M:Fe, Zn, Mg) and phosphoric acid ($\text{H}_3(\text{PO})_4$)] and calcination in flowing nitrogen at various temperatures to obtain nano-crystalline powders;
 - b. A 2-step synthesis of a macroporous ceramic by foaming methyl cellulose in water and adding apatite powders with subsequent high temperature treatment to remove precursors/residues; and
 - c. Solid state reaction by sintering stoichiometric precursors (chlorides and tri-calcium phosphate) at high temperatures for extended periods to enhance yield.
- (iii) Comprehensive characterization by:
 - a. Powder diffraction using both fixed wavelength neutron (PND) and laboratory X-ray (XRD) sources for quantitative phase analysis and the extraction of lattice parameters, symmetry, and site occupancy;
 - b. Scanning and transmission electron microscopy to study nano-structures, the effect of electron beam damage, and conduct quantitative microanalysis by energy dispersive X-ray spectroscopy (EDS), high resolution transmission electron microscopy (HRTEM), and selected area electron diffraction (SAED); and

- c. Spectroscopic methods including Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), and Mössbauer spectroscopy (MS) to probe atomic coordination and bonding, chemical state and magnetism.
- (iv) Catalytic activity
 - a. In-situ diffuse reflectance infra-red Fourier transform spectroscopy (DRIFTS) monitored high temperature carbon monoxide adsorption, and temperature programmed desorption (TPD) of carbon dioxide was used to determine active base site abundance and desorption kinetics.
 - b. Dehydration and dehydrogenation of alcohols (ethanol and 2-propanol) was chosen to evaluate the catalytic activity (conversion and selectivity). The reaction was carried out in a fixed bed reactor and mass spectrometry measured the production of acetaldehyde, acetone, propylene, water and hydrogen as a function of temperature.
- (v) Biocompatibility

Mouse embryonic fibroblasts cells and osteosarcoma cells were seeded into media containing apatite and incubated to assess cell proliferation and viability as a function of dosage and incubation time. Cell proliferation was evaluated using confocal microscopy where cells were stained fluorescent-red with F-actin phalloidin. Cell viability tests were performed in triplicate and analyzed using MTS assay [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] that determines the number of metabolically active cells by monitoring the absorbance of formazan, an enzymatic reaction product.

1.3 Dissertation Overview

This dissertation describes fundamental studies that establish HAp phase boundaries and a compositional-structural hierarchy, followed by the

correlation of apatite crystal chemistry with prosthetic and catalytic performance. *Chapter 1* outlines the research justification, objective and scope directed towards tailoring apatites of optimal functionality.

Chapter 2 provides a state-of-the-art review of the crystallochemical adaptability of hydroxyapatite, especially the incorporation of small cations and the role of non-stoichiometry and phase transformations, together with comparative excipient and catalytic performance with respect to composition. The space group hierarchy of apatites, from hexagonal to triclinic lattice symmetries, and the relationship to the metaprism twist angle and stoichiometry are briefly described. Also included are the strengths and weaknesses of the characterization techniques used to study the crystallographic, mechanistic and functional aspects of HAp.

Chapter 3 examines iron (Fe^{3+}) and carbonate (CO_3^{2-}) incorporation in hydroxyapatite using X-ray and neutron diffraction, and electron microscopy. The partitioning between Ca^{F} and Ca^{T} sites, substitution mechanisms, and a phase transformation from hexagonal to monoclinic pseudomorphs are established. In addition, the bioactivity of this material is assessed and the chemical state of iron verified using a combination of spectroscopic techniques including FTIR, EPR, NMR and Mössbauer.

Chapter 4 considers a possible phase transformation in zinc (Zn^{2+}) substituted hydroxyapatite and the topological distortions related to its incorporation into Ca^{T} and attendant nonstoichiometry. The bonding environment and surface chemistry was probed by FTIR, DRIFTS and temperature programmed desorption (TPD). The catalytic dehydrogenation of 2-propanol favored the production of propene.

Chapter 5 describes the crystal chemistry of magnesium (Mg^{2+}) substituted hydroxyapatite. The site preference for Mg was completely towards the Ca^{F} site, with the average composition determined by EDS. A macroporous architecture was synthesized from nano-crystalline powder and studied *in vivo*

to determine cell viability, with confocal laser microscopy used to directly observe cell proliferation.

Chapter 6 provides a critical review of the synthesis, characterization and crystal chemical systematics of small cations (iron, zinc and magnesium) in hydroxyapatite. Deficiencies in previous descriptions are identified and reformulations devised to better describe the materials. The bond lengths, polyhedral volumes and metaprisim twist angles were employed to monitor excursions in compositional and symmetry.

Chapter 7 proposes future designs and tailoring of hydroxyapatite based upon the understanding of these materials class developed in this thesis. Suggestions are made to exploit and expand these new ideas through association of crystal chemistry, architecture fabrication and the targeted functionality.

The objectives of this research have been achieved by:

- (i) *Establishing a comprehensive understanding of the crystallochemical features of HAp* that permit accommodation of small cations;
- (ii) *Correlating bioactivity and catalytic functionality* of HAp with composition and physical form.

Novelty arises from:

- (i) Designing and demonstrating a co-precipitation method to synthesize nano-sized and single phase iron, zinc and magnesium incorporated HAp powders;
- (ii) Fabricating macroporous foams from nano-crystalline powder apatites to improve reactivity and mass transfer;
- (iii) Characterizing the iron-, zinc- and magnesium-HAp solid solution series by neutron diffraction, complemented by electron microscopy and spectroscopy;

- (iv) Showing that the bioactivity of HAp, in both nano-powders and macroporous form, are enhanced with the incorporation of iron and magnesium; and
- (v) Demonstrating the catalytic performance of zinc and magnesium bearing HAp for dehydration and dehydrogenation of isopropanol.

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Chapter 2

Apatites as

Biomaterials and Catalysts

Chemically and morphologically tailored hydroxyapatites are an important class of biomaterials that also show promise as heterogeneous catalysts. The properties of these nano-sized powders can be optimized by adjusting crystal size and composition, with small cation incorporation, especially the first row transition metals, providing a means to inhibit crystal growth and promote pseudomorphism. This state-of-the-art review shows that assessing the functionalization of these materials is challenging and a suite of complementary characterization methodologies is essential to unambiguously correlate structure with performance.

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The apatite family of materials can be adapted for deployment in a wide range of applications including biomaterials, heterogeneous catalysis, phosphors, toxic metal stabilization, and solid oxide electrolytes [24-28]. This diversity of functionality arises because multiple ionic acceptor sites allow a great variety of partial or complete substitutions through isovalent or altermant mechanisms. However, this accommodation of compositional variability has led to confusing and/or conflicting reports concerning absolute apatite chemistries, especially in technological materials, where crystallochemical characterization can be less complete due to small grain size, coexisting apatite phases, unexpected departures in stoichiometry, or polymorphic and polysomatic modifications [20-23]. Many questions remain, and in this chapter, the scientific basis for developing cation-doped hydroxyapatite (HAp) as a biomaterial and heterogeneous catalyst is reviewed.

2.1 Crystal Chemistry and Nomenclature

As noted earlier, the $P6_3/m$ apatite aristotype conforms to the general formula $[A_4^F][A_6^T][(BO_4)_6]X_2$. Hydroxyapatite (HAp), where $A = Ca$, $B = P$ and $X = OH$, can be described as a $[Ca_4^F(PO_4)_6]^{10-}$ framework surrounding one-dimensional channels with $[Ca_6^T(OH)_2]^{10+}$ as counter ions. The flexible framework twists to dilate or constrict the channel to match the size and fractional occupancy of the tunnel substituents. Apatites can crystallize in maximal non-isomorphic subgroups of $P6_3/m$ including $P6_3$, $P3$, $P6$, $P2_1/m$, $P2_1$ and $P1$ [29] (Figure 2-1). These symmetries can accommodate *framework distortion* [21] where tunnel sub-stoichiometry leads to framework contraction; *cation ordering* [30] in complex substitutions; and *oxygen nonstoichiometry* [11] that leads to partial or complete replacement of BO_4 tetrahedra by BO_5 and BO_3 polyhedra (Figure 2-2). The well known representation of HAp in $P2_1/b$ can be treated as distinct, since this modification does not directly involve framework adaptation, but rather the hydroxyl groups are slightly displaced from the $Ca^T-Ca^T-Ca^T$ triangular planes at $z = 0, \frac{1}{2}$ to $z \sim \pm(0.02, 0.52)$ with perfect inter-tunnel correlation and statistical occupancy [22]. This

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three-dimensional space group representation provides a reasonable approximation of incommensuration, that offers a more complete analysis of hydroxyl group displacement [31].

While the taxonomic relationships between apatites are clear, arriving at unambiguous crystal chemical descriptions from powder diffraction is challenging, especially for bio- and lithogenic species that are chemically intricate [32, 33] (see for example, section 6.3 for CO_3^{2-} substitution), or for sub-stoichiometric and disordered materials [34], as these modifications can prove difficult to deconvolute from the characteristics of lower symmetries ($P6_3$, $P3$, $P6$, $P2_1/m$). Some of the more common chemical replacements in natural HAp include:

It should be noted that these equations do not differentiate the A^{F} and A^{T} sites, although these show specific predilections for substituent types. For example, Na^+ will invariably displace Ca^{F} [35], while Sr^{2+} is tenanted in the Ca^{T} position [36].

Figure 2-1. Space group hierarchy for apatites and its relationship to crystallochemical adaptations. Note that $P2_1/b$ is not a maximal isomorphic subgroup of $P6_3/m$, although the former is used to described well-ordered HAp and chlorapatite (ClAp).

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To concisely capture the primary features of chemically modified varieties the nomenclature *apatite*-(*ABX*)-*NS* was devised, where *ABX* are the most abundant atoms on these sites, *N* is the number of layers in the (001) crystallographic repeat ($A_2^F A_3^T B_3 O_{18} X$), and *S* is the lattice symmetry [37]. For example, hexagonal $P6_3/m$ HAp is *hydroxyapatite*-(*CaPOH*)-*2H* while monoclinic mimetite [$Pb_{10}(AsO_4)_6 Cl_2$] would be *mimetite*-(*PbAsCl*)-*2M*. In addition to framework adaptation, the apatite family also extends to polymorphism, pseudomorphism and polysomatism. For the materials examined in this thesis pseudomorphic modifications prove to be the most important.

2.1.1 Framework Adaptation and Twist Angle Systematics

The metaprism twist angle (φ) in $P6_3/m$ HAp is defined as the angle between $O(1)-A^F-O(2)$ of the $A^F O_6$ metaprism when projected along [001] as calculated from the absolute fractional coordinates [19] (Figure 2-3). In $P6_3/m$ every $A^F O_6$ metaprism contains one φ , but in monoclinic $P2_1/m$ there are three discrete twist angles ($\varphi_1, \varphi_2, \varphi_3$) for each metaprism. Further transformation to triclinic ($P\bar{1}$) creates two metaprism types, each with three unique twist angles. It is observed that φ adjusts systematically as a function of composition, increasing to constrict the tunnel when the framework ions (A^F, B, O) are large relative to those in the tunnel (A^T, X). Conversely, a smaller φ opens the tunnel when it is occupied by larger species. The PO_4 tetrahedra remain essentially invariant, but rotate about $\langle 100 \rangle$ in $P2_1/m$ and $P\bar{1}$ [38].

This analysis of φ in apatites is analogous to the widely studied ABO_3 perovskites where BO_6 octahedral tilting is systematically modified according to symmetry and composition (i.e. larger BO_6 tilting favors lower symmetry). Metaprism twisting is a reliable approach for assessing the quality of apatite structure refinements and recognizing excursions in symmetry or composition.

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Figure 2-2. The apatite crystallographic family for bimodular polysomes. Examples of the genera are labelled with #.

Figure 2-3. Polyhedral drawing of $P6_3/m$ HAp illustrating the framework $\text{Ca}^{\text{F}}\text{O}_6$ metaprism (red) and PO_4 tetrahedron (blue), surrounding one-dimensional channels that contain $[\text{Ca}^{\text{T}}_6(\text{OH})_2]^{10+}$. The unit cell and metaprism twist angle (φ) are also indicated.

2.1.2 Polymorphism

Polymorphic transformations arise from changes in symmetry without adjustment of composition: both temperature (high temperature promotes high symmetry) and pressure (high pressure promotes lower symmetry) will alter φ due to changes in relative ionic size that promote phase changes through flexing of framework modules. For example, $[\text{La}_9\text{Sr}_1][(\text{GeO}_4)_6]\text{O}_2$ transforms from $2A$ (623-853 K) $\rightarrow$ $2M$ (853-933 K) $\rightarrow$ $2H$ (933-1023 K) polymorphs as a function of temperature, in a process of tunnel expansion with φ becoming more acute [39].

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2.1.3 Pseudomorphism

Pseudomorphic phase transitions are driven by compositional adjustments that lead to structural relaxations to satisfy bond valence criterion. As for polymorphs, all pseudomorphs contain two fundamental modules ($A_2^F A_3^T B_3 O_{18} X$) in their [001] crystallographic repeat. Typically the transformation sequence $2H \rightarrow 2M \rightarrow 2A$ arises from framework B -site substitutions or tunnel X -site sub-stoichiometry. For example, powder X-ray diffraction of the series $Ca_{10}(P_{1-x}V_xO_4)_6F_2$ confirm a departure from the hexagonal symmetry with increasing x [40]. In oxy-apatite, $[Ca_{10}(PO_4)_6(OH)_{2-x}O_{x/2}]$ ($0.78 < x < 1.56$), $P1$] dehydroxylation stabilizes the $2A$ pseudomorph and leads to obtuse twist angles ($\varphi_{\max} \sim 28^\circ$) [21].

2.1.4 Polysomatism

Polysomatic apatites are generated through [001] rotations (0° or 60°) of the stacking modules ($A_2^F A_3^T B_3 O_{18} X$) in different sequences, to create a variety of long period polysomes of general formula $A_{5N}B_{3N}O_{9N+6}X_{N\delta}$ ($2 \leq N \leq \infty$). When the twin rotation angle is 0° , φ is quite acute (the metaprisms are almost ideal trigonal prisms). For example, the nasonite polysome $[(Ca_8)(Pb_{12})(Si_2O_7)_6Cl_4]$ is hexagonal with four layers in its polysomatic repetition and represented as *nasonite-(PbSiCl)-4H* [23]. In the present studies, polysomatism is not significant as lead (Pb) or bismuth (Bi) are essential components of the polysomes documented to date.

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2.2 Harnessing Crystallochemical Adaption to Enhance Functionality in HAp

2.2.1 HAp as a Biomaterial

Principles

Biological HAp is the major inorganic constituent of bone and enamel, typified by poor crystallinity and a wide compositional range, especially with respect to carbonates, water, F^- , Na^+ , Mg^{2+} , Zn^{2+} , Sr^{2+} and Si^{4+} [4]; gross non-stoichiometry (cation vacancies) is also present [41]. Synthetic analogues of HAp for biomedical applications have been extensively studied, and the fluorination of water to reduce dental caries is the best known example of *in situ* modification of apatite chemistry [42]. It has been reported that the tailored incorporation of small cations not only stimulates cellular response at the molecular level, but also improves mechanical properties [43]. In addition, HAp has been coated on titanium alloys and stainless steels to create objects with mechanical strength encased in a bioactive coating [44]. Elsewhere, magnetite-HAp core-shell architectures have been fabricated as drug delivery systems with magnetic nanoparticles as carriers [45], and macroporous textures allow the incorporation of biomolecules that significantly accelerate cell proliferation [46] or adapted for controlled drug release [47]. Chemically modified HAp enhance biomineralization of hard tissue and can be osteoconductive (promote bone growth), osteoinductive (stimulate cell proliferation on new bone materials) and osseointegrative (bonding with existing bone structure) [48]. There have been many attempts to mimic biogenic apatites, however, much remains to be documented, especially the role of coexisting amorphous calcium phosphate (ACP) phases, that are difficult to analyze and quantify [49].

Technology

Calcium phosphate ceramics are exploited for medical applications requiring bioactive (HAp) and bioresorbable (β -TCP) materials (Figure 2-4). Bioglasses enhance the precipitation of HAp in physiological environments to enable strong adherence to mechanical supports (e.g titanium prosthesis) [50].

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Bioinert materials such as alumina and zirconia continue to be used in hip joint prostheses because of their rigidity and toughness [50], while bioactive HAp remains the preferred material for osteopathic repair and replacement due to its “mineralogical” similarity to bone [51].

The influence of chemical modification on the excipient properties of HAp are understood in a general sense. *Carbonate* (CO_3^{2-}) is the most abundant extrastochiometric species found in biological HAp (~8 wt%) where it enhances dissolution rates, without significantly altering crystallinity, so that osteoclasts resorb more efficiently on surfaces of carbonate-apatites (CAps) to enhance bone growth [52]. *Secondary phosphates* (HPO_4^{2-}) are usually present in enamel and non-stoichiometric-HAps, altering dissolution rates and playing an important role in the formation of hard tissue [4]. *Fluorine* (F) is another common substituent found in bone and enamel that prevents dental cavities and tooth decay [42]. *Magnesium* (Mg^{2+}) is implicated in the calcification process, presumably by stimulating osteoblast activity, and accelerating bone remodeling processes. However, the incorporation of Mg is intriguing, as higher concentrations affect the extent of dissolution and the rate of mineral metabolism. Ultimately, Mg will destabilize HAp in favor of β -TCP [53]. *Zinc* (Zn^{2+}) substitution in HAp increases osteoblastic adhesion at low concentration (< 1.2 wt%) and inhibits osteoclastic resorption *in vitro* [54]. The presence of zinc in all biological tissues (including teeth) is indicative of its diverse roles in enzyme activity, metabolism and protein adsorption [5]. Despite the importance of zinc in bio-minerals, the local structure and site preference in HAp remains speculative [55]. *Iron* ($\text{Fe}^{2+/3+}$) is also found (< 1 wt%) in hard tissues, and iron-bearing HAp shows therapeutic functions as a component of bioglasses and biocements that accelerate bone regeneration [56]. Controlling the chemical state ($\text{Fe}^{2+/3+}$) will alter the excipient properties. Of course, not all substituents are beneficial, at least beyond certain concentrations. For example, humans metabolise *strontium* due to its crystallochemical similarity with calcium, and trace quantities in natural isotopic abundance reduces caries [57], but accumulation of the radioactive fission product ^{90}Sr initiates metastasis [58].

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Figure 2-4. Classification of biomaterials.

In summary, low concentrations of crystallochemically incorporated cation and anion species in HAp may enhance its preventative and therapeutic properties by stimulating cell response. However, the approach to date has been empirical and little attention has been paid to correlating biological responses to the crystal chemistry of first row transition metal cation-substituted HAp.

A goal of these studies is to systematically associate the excipient properties of (especially bioactivity) small cation doped HAp, specifically Mg^{2+} and Fe^{3+} , with crystal chemical modifications.

2.2.2 HAp as a Heterogeneous Catalyst

Principles

Catalytic reactions are essential in petrochemistry, effluent remediation and fuel cell technologies that reduce waste, minimize energy consumption and contain costs [59, 60]. Homogeneous catalysts promote reactions that take place in a single phase, usually a miscible liquid. In contrast, heterogeneous catalysts are solids that allow specific molecules to temporarily adsorb, react and create weakly bonded products that are readily released. Most solid phase heterogeneous catalysts, including metal phosphates, alumina, zeolites, and montmorillonite clay [60] are well developed, highly effective, and easily separated from the reaction products. HAp is attracting increasing interest as a catalyst (Figure 2-5) due to its acid-base (“amphoteric”) properties and well-

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defined active sites [61]. Moreover, its zeolitic-like framework can be modified with counter ions to produce novel catalysts [62], or used without chemical tailoring as a support for gold, titania and other co-catalysts [63-65]. For the latter, catalytic activity is controlled by modifying the chemical speciation of the decorating material, while in the former, crystallochemical modification of apatite can enhance the rate or selectivity of chemical reactions. This thesis is solely concerned with the direct modification of HAp chemistry.

“Tailored HAp” possess vastly different catalytic properties. To optimize these materials a breadth of disciplines are involved (materials science, crystallography, catalysis) and the terminologies in common use are often conflicting. For example, metal “doping” in catalytic literature usually implies a mixture of co-catalysts (e.g. ruthenium and apatite), but in solid state chemistry the assumption is crystallographic incorporation. Similarly, there should be clear differentiation of surface modification from crystallochemical adjustment. Here, the principle means of modifying HAp have been categorized as:

- (a) “Cation exchange” and “ligand exchange” (e.g. ruthenium, palladium, nickel) [66-68] where monomeric metal-phosphate complexes are deposited at the HAp surface (but not in the bulk) via chemical treatment, and these highly stable species provide accessible active sites for catalytic reaction;
- (b) “Surface adsorption” in which catalytically active nanoparticles (e.g. gold, silver, titania) [63-65] are decorated on HAp that serves as a stable support and on occasion, as a co-catalyst; and
- (c) “Crystallochemical incorporation” where there is systematic replacement of cation/anion groups in bulk HAp, as for example, strontium and barium substituting for calcium [69], or vanadate replacing phosphate [70], and fluorine displacing hydroxyl [71].

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Figure 2-5. Growth in publications over the past 20 years, that describe HAp or modified HAp as catalysts. (Retrieved from ISI Web of Science, search terms “hydroxyapatite” + “catalysts”)

Generally, little attention has been paid to differentiate cation-“bound”-HAp [i.e. type (a) and (b)] from cation-“substituted”-HAp [type (c)] catalysts, and these common descriptors are often used interchangeably leading to some confusion. Naturally, surface properties are of great significance and require particular scrutiny through experimentation and computation (Figure 2-6).

Technology

HAp was first employed as a catalyst for the dehydration and dehydrogenation of alcohol more than 40 years ago, with the general conclusion that incorporation of larger alkaline earths (Sr^{2+} , Ba^{2+}) supplies basic sites associated with dehydrogenation, while dehydration takes place at the acidic phosphate sites [69]. This concept was exploited for the conversion of alcohol to carbonyl compounds by aerobic oxidation for the synthesis of fine chemicals, through the development of modified HAp [types (a) and (b)] and maghemite-HAp core-shell architectures as supports for active nanocrystalline metals such as ruthenium, palladium, chromium and nickel [66-68]. Titania, silver and gold nanoparticles have been decorated on HAp for photocatalytic degradation and carbon monoxide oxidation [63-65].

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Separately, the Sugiyama group pioneered the investigation of cation substituted HAp [type (c)] by introducing strontium, barium and lead for the remediation of chlorinated hydrocarbons (e.g. trichloroethylene, tetrachloromethane) [71, 72] and oxidative dehydrogenation (ODH) of methane to carbon oxides (CO or CO₂) and hydrogen [73]. Subsequently, vanadate substituted HAp (VHAp) was developed for ODH of propane to propylene - an important raw material for petrochemical synthesis [70]. To date, VHAp has also been exploited in the Michael reaction, aldol condensation, and epoxidation of carbonyl compounds [74-76]. An attempt to replace calcium with cobalt in HAp for ODH of propane found the extent of the substitution to be very limited, and speculative, from a crystallochemical perspective [77]. In addition, ferric species introduced via “cation exchange” show promising catalytic performances in propane ODH and butan-2-ol conversion [78]. Most recently, attention has been paid to biogasoline production and carbon dioxide (CO₂) fixation to reduce greenhouse gas emissions [59, 79]. In this context, “surface adsorbed” zinc-HAp was discovered to be highly efficient for cyclo-addition of CO₂ to styrene oxide [80], that traditionally used magnesium oxide based catalysts [81].

In summary, it is clear that transition metals whether physically or chemically adsorbed, or crystallochemically incorporated in HAp, are potentially promising catalysts. However, there has been limited investigation of fabricating first row transition metal cation-substituted HAp as a green catalyst for alcohol (ethanol and 2-propanol) oxidative dehydration and dehydrogenation.

The aim of this research is to correlate catalytic activity with small cation incorporation in HAp [type (c)], specifically for Mg²⁺, Fe³⁺ and Zn²⁺, and systemize the crystallographic modifications that accompany chemical tailoring.

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H																	He	
Li	Be	A ^F /A ^T				B		X		B				C	N	O	F	Ne
Surface Adsorption										Al				Si	P	S	Cl	Ar
Na	Mg																	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr	
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe	
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn	
Fr	Ra	Ac																
lanthanides			Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu		
actinides			Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw		

Alkane oxidative dehydrogenation

CO₂ fixation

Michael reaction

Alcohol oxidation/decomposition

CO oxidation

Photocatalysts

Figure 2-6. Major substitutions and surface adsorbed species in HAp for several common catalytic reactions.

2.3 Crystal Chemistry of Small Cations in Hydroxyapatite

The crystal chemistry of small cations in HAp is inherently complex due to the multiple acceptor sites that readily incorporate a wide range of ionic sizes, however an empirical method for evaluating the validity of crystal structures and the likelihood of substitution is bond valence summing (BVS). The total valence strength of the bonds which reach a central ion (usually a cation) from all neighboring ions (usually anions) equals the charge of the cation [82]. The BVS (the sum of partial bond valences) give an indication of the oxidation state, and where the departure from formal valence is large [> 0.1 valence units (vu)] the structural model is possibly incorrect.

In pure HAp, the BVS of Ca^{F} is 1.97 and 2.17 for Ca^{T} . If, for the moment, structural relaxation is ignored, smaller divalent species are more likely to occupy the less voluminous framework site ($A^{\text{F}} \sim 15.6 \text{ \AA}^3$) which is marginally underbonded, while the tunnel site ($A^{\text{T}} \sim 19.8 \text{ \AA}^3$) will usually host cations of higher formal valence to distribute the charge over the near neighbor anions [19]. As discussed, first row transition metals in association with HAp are attractive for a wide range of applications, but the exploration of these crystal chemistries is limited. Owing to the vast spread in ionic radii across the row (Figure 2-7), the most ready substitutions in the A^{F} (e.g. Fe, Co), B (e.g. V, Cr),

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and X-sites (e.g. Ni, Cu) can be predicted, and although largely confirmed experimentally [14, 83-86], the products are often polyphase assemblages.

The present investigations are concerned with synthesizing single phase, transition metal substituted HAp, determining solid solubility limits, and establishing the substitution mechanisms and partitioning preferences. However, in open reaction systems as used here, atmospheric carbonate (CO_3^{2-}) is sometimes incorporated to produce carbonate-bearing HAp (CHAp). It is well appreciated that CO_3^{2-} can replace both tunnel X anions (type A) and framework B cations (type B) as [87]

In A-type CHAps the C-O triangular plane is approximately parallel to the tunnel axis while B-type CO_3^{2-} ions randomly occupy either of the two opposing faces of the “phosphate” tetrahedron (see section 6.2.2). For biogenic apatites that contain trace Na^+ a common replacement is [88]:

The variable oxidation state of the transition metals is an added complication. For example, divalent manganese (Mn^{2+} , IR = 0.83 Å) can substitute into the A-site [e.g. $\text{Mn}_{10}^{2+}(\text{PO}_4)_6(\text{OH})_2$] [89], or in the pentavalent form (Mn^{5+} , IR = 0.33 Å) can enter the B-site [e.g. $\text{Ba}_{10}(\text{Mn}^{5+}\text{O}_4)_6(\text{OH})_2$] [90]. Iron is not an obvious substituent in HAp, and the ferrous (Fe^{2+} , IR = 0.78 Å) and ferric (Fe^{3+} , IR = 0.65 Å) ions evidently do not meet the usual bond valence criteria for insertion. The solid solution limits and substitution mechanism of Fe^{2+} in fluorapatite (FAp) was examined by Khudolozhkin *et al* using powder XRD [91]. A unit cell contraction with increasing iron content, was consistent with Fe^{2+} preferentially displacing Ca^{F} to a solid solution limit of $x = 1.5$ via a simple isovalent substitution in $[\text{Ca}_{4-x}^{\text{F}}\text{Fe}_x][\text{Ca}_6^{\text{T}}][(\text{PO}_4)_6]\text{F}_2$. On the other hand, the incorporation of ferric iron in HAp is restricted because introducing

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Fe^{3+} leads to unrealistic BVSs of 1.1 and 1.2 on the Ca^{F} and Ca^{T} sites, respectively. Many synthesis routes have been developed to incorporate Fe^{3+} in HAp that have met with varying degrees of success in obtaining a single phase product [92-97]. Generally, the crystal chemistry was not considered in detail [98-101]. Consequently, the solubility limit and local environment of Fe^{3+} in HAp remains poorly defined. Jiang *et al* [15] reported the chemical state and conducted phase analysis to differentiate surface adsorption from crystallographic incorporation, and found that Fe^{3+} preferentially occupies A^{F} sites at low concentrations (< 5 wt%) via

together with possible A and B type carbonate substitutions.

Studies to date [54, 55] suggest zinc readily modifies HAp function, but even at low concentrations Zn^{2+} inhibits crystal growth and disrupts crystallization, so substitution is minimal. The local structure of zinc in biological apatite was examined using extended X-ray fine structure spectroscopy (EXAFS). This study found that Zn^{2+} is more likely to be surface adsorbed on HAp or accumulate in amorphous phases [102]. Where substitution does occur, lattice contraction is anticipated as Zn^{2+} ($\text{IR} = 0.74\text{\AA}$) will replace Ca^{2+} ($\text{IR} = 1.00\text{\AA}$) in an isovalent substitution

Simulation and theoretical studies by Terra *et al* [103] and Matsunaga *et al* [104] showed the preference of Zn^{2+} for six-fold coordination to oxygen, making it energetically favorable to occupy the Ca^{T} site. Silva *et al* [105] synthesized single-phase FAp with zinc-substitution up to ~ 6 at% with decreasing lattice parameters, while Ren *et al* [106] precipitated single phase Zn-HAp incorporating up to 3 at% Zn^{2+} , where conflicting unit cell trends suggest the substitution is not direct. Recently, Tang *et al* [107] combined X-ray absorption near edge structure (XANES) spectroscopy and modeling to

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demonstrate that Zn^{2+} prefers the Ca^{F} over Ca^{T} site in a heavily distorted tetrahedral coordination. This leads to an extremely low BVS of 1.77 on Ca^{T} site, which is probably feasible only at quite low concentrations (<1000ppm). It is evident that crystallochemical reports of Zn-HAp are contradictory, possibly because of materials differences [108-110], and the systematics of Zn^{2+} incorporation remain to be resolved.

Figure 2-7. Ionic radii of first row transition metal cations (6-coordination to oxygen) in common oxidation states. Values extracted from Shannon and Prewitt [111].

As magnesium is one of the most important and widely studied divalent cations associated with biological apatites, the replacement of Ca^{2+} with Mg^{2+} in HAp is conclusive [112]. The substitution mechanism is straightforward as Mg^{2+} is expected to occupy the framework site by replacing Ca^{2+} , but increasing amounts of Mg^{2+} enhance dissolution, and at high levels (>10 at%) eventually stabilizes β -TCP.

The dual phase nature of many Mg-HAp synthetics complicates analysis [113]. Early work describing cell constant trends with magnesium doping were inconsistent, as possible carbonate incorporation and co-existence of an amorphous phase were not considered. Various synthesis methods reportedly lead to different substitution limits of Mg^{2+} for Ca^{2+} , with a reduction in unit

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cell volume [114-116]. Bigi *et al* [117] extended the incorporation to ~10 at% but Rietveld refinement from XRD suggests preference over the tunnel Ca^{T} site, which is at odds with the crystallographic precept that smaller divalent cations will prefer the less voluminous Ca^{F} site. These inconsistencies are comprehensively resolved by the present studies (Chapter 5).

2.4 Characterization Methodology

The characterization of small-cation doped-HAp is not straightforward as a result of pseudomorphism, non-stoichiometry, and co-existing amorphous forms. A combination of microscopic, diffraction and spectroscopic techniques are required to fully describe the features of these nanomaterials and validate performance (Figure 2-8). The rationalization of information collected from analytical methods based on physically discrete phenomena, provide the greatest assurance of holistic and consistent descriptions. A multi-technique approach to examining these nanomaterials is essential. The short descriptions that follow do not detail the principles of the analytical tools, but rather, highlight the strengths and weaknesses of these methods when applied to HAp investigation. Specific characterization and operating methods employed for each of the apatite series examined are given in subsequent chapters.

2.4.1 Electron Microscopy

Scanning Electron Microscopy (SEM)

SEM provides a range of microstructural information which includes phase assemblage, particle size, pore size, and particle morphology, that compliments micron-scale information derived from Rietveld analysis. In this study, powders of small cation doped-HAp were mounted on carbon tape and carbon coated before observation to minimize charging that degrades image quality.

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Figure 2-8. A combination of diffraction, microscopic and spectroscopic techniques is required to develop comprehensive crystallographic models of fine-grained bioactive and catalytic apatite powders.

Analytical Scanning Electron Microscopy (ASEM)

Energy dispersive X-ray spectroscopy (EDS) when used in conjunction with SEM can perform microanalysis to determine the abundance and relative proportions of elements present. EDS suffers from two primary limitations: (1) only heavier elements ($> \text{Na}$) can be reliably detected and quantified, and (2) the interaction volume is usually significantly larger than the individual apatite crystals. Nonetheless, this method can assess if the dopant series (Fe, Zn, Mg) are retained during synthesis. The powders were embedded in resin and polished to a mirror finish prior to the analysis.

Transmission Electron Microscopy (TEM)

TEM allows direct observation of nano-scale details and crystal texture via electron diffraction. Atomic scale resolution can be achieved by operating at high voltages (125 or 200kV in these studies). The materials were ultrasonically dispersed in ethanol for 5 min with a drop of suspension deposited onto holey carbon-coated grids. However, collecting images and diffraction patterns of small cation doped-HAp nanocrystals is not straightforward as electron beam damage rapidly accumulates leading to structural disintegration. Although dosage can be minimized by focusing off-specimen then rapidly translating to the target area, this approach is only

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partially effective. Thus, the powders were embedded in epoxy resin and sectioned using microtome to preserve the structure. Images were collected digitally with a double tilt holder employed to orient the crystals.

2.4.2 Diffraction

Powder X-ray Diffraction (XRD)

XRD provides a means of quantitative phase analysis (QPA) where phase compositions, unit cell parameters and crystallite size can be determined. An internal standard (fluorite) is introduced to extract absolute lattice constants, amorphous contents and other crystallochemical information of small cation doped-HAp by Rietveld analysis. The amorphous phase is a non-diffracting component of the bulk crystalline sample. However, X-rays are often attenuated by microabsorption, the relative impact being determined by the X-ray mass absorption coefficient, and this can cause very large errors during QPA. This artifact can be avoided by neutron diffraction where the scattered intensities are not dependent on mass absorption of the nanomaterials. Prior to XRD the materials were manually ground to achieve homogeneous size distribution and firmly packed into a holder.

Neutron Diffraction

Fixed wavelength powder neutron diffraction (PND) is a powerful tool in which neutrons are elastically scattered after interaction with the nucleus of an atom. In addition to crystallographic data that can be obtained with Rietveld refinements, this highly penetrating method is unaffected by microabsorption because neutrons interact weakly with the condensed matters, permitting accurate determination of oxygen positions, amorphicity and the site preference of small cations in HAp. However, accessibility to neutron sources is limited, with a large amount of sample required to improve diffracted intensity, and activation after interaction with neutrons can delay later examination by other techniques.

2.4.3 Spectroscopy

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Infra-red spectroscopy

Fourier transform infrared (FTIR) spectroscopy was used to identify and quantify the abundance of anion groups. FTIR proved more sensitive to carbonate and hydroxyl than diffraction, partly as the infrared signal is collected from shallower depths and therefore detects absorbed species, and also because the lower detection limit for carbon and oxygen by PND is ~1 at%, as shown later (Chapter 3). The HAp powders were mixed with potassium bromide and pressed into pellets. Atmospheric carbonate and moisture can be incorporated during the synthesis. To distinguish sorbed carbonate and hydroxyls from structurally bound entities, the ceramic powders were stirred with dilute hydrochloric acid, washed, centrifuged, dried and a second spectrum was acquired. Surface species and co-existing CaCO_3 were readily removed, but lattice species persists [8].

In situ diffuse reflectance infrared transmission spectroscopy (DRIFTS) was used to study adsorption properties by flowing carbon monoxide through a catalyst bed, and the spectra acquired at room temperature.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy probes changes in the local structure of selected species when the neighboring atoms are chemically discrete or topologically modified. The changes in environment when a small cation is introduced, causes modification to the nuclear magnetic moment that are readily detected. This provides insights to geometric distortions and coordination, and allows direct monitoring of structural adjustments during metal insertion. For example, phosphorous magic angle spinning NMR spectra acquired using primary and secondary standards [69] permit investigation of changes in phosphorous coordination to oxygen.

Magnetic spectroscopy

Several spectroscopic methods can provide information on the chemical state and magnetic properties of nanomaterials. In these investigations, Mössbauer spectroscopy, electron paramagnetic resonance (EPR) spectroscopy and a

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superconducting quantum interference device (SQUID magnetometer) were used to determine the chemical state, local environment and spin state of magnetic species, and magnetic susceptibility. This was only applicable to Fe-HAp because only Fe^{3+} possesses magnetism detectable by these techniques.

2.5 Bioactivity and Catalytic Performance

2.5.1 Protocols for Testing Cell Proliferation and Viability

Cells (mouse embryonic fibroblast L929 and osteosarcoma MG63) were cultivated in a medium containing serum and antibiotics before transferring to a culture plate for seeding and attachment. All HAp samples were sterilized under ultraviolet light prior to experiments.

The cytotoxicity test was carried out by seeding a predetermined number of cells in a well plate and using a cell proliferation assay that determines the number of metabolically active cells by measuring the absorbance directly from the plate at different time point (from 24 h to 168 h). Each experiment was performed in triplicate and a single factor analysis of variance was performed to test for statistical significance.

For SEM and confocal microscope observation, cells were seeded on a thin cover slip (2.54 cm^2) in well plates. SEM samples were prepared by washing the cover slips with phosphate buffer solution (PBS), chemically affixing the cells with glutaraldehyde, and then sterilized by flushing with ethanol of various concentrations (50, 70, 95 and 100%) before finally air dried and carbon coated. For confocal laser microscopy, samples were washed with PBS, with the addition of paraformaldehyde and Triton X-100 before fluorescently stained red and finally removed from the well plate and mounted onto glass slides for observation.

2.5.2 Protocols for Catalytic Testing

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Temperature programmed desorption (TPD) of carbon dioxide (CO₂) was performed in a micro-fixed-bed reactor connected to a gas chromatograph with multiple ion detector. Powder samples were stuffed in between quartz wools in a glass tube lying vertically. All samples were pre-treated under helium (He) at a flow rate of 20 mL/min, heated to 550 °C (10 °C/min) for 2 h, and air-cooled to ambient before purging with He+CO₂ at a flow rate of 25+5 mL/min for 1 h. Desorption was preceded by heating to 550 °C (10 °C/min) for 1 h and TPD quantitative analyses were calibrated against 250 mg of lime (CaO, Merck) under the same experimental condition.

Ethanol (EtOH) decomposition was carried out in a micro-fixed-bed reactor connected to a gas chromatograph with EtOH bubbled through nitrogen at 50 mL/min. Iso-propanol (IPA) decomposition took place in a different micro-fixed-bed reactor with IPA vapor flowing at 0.1 mL/min, and heating at various temperatures (from 100 to 350 °C) for 1 h to observe conversion rate and selectivity.

2.6 Outstanding Questions

This literature review shows that apatites generally, and hydroxyapatite in particular, have been widely studied for technological applications. The crystal structure is complex, with several atom acceptor sites and pseudomorphic modification is common. This adaptability allows apatites of wide functionality to be synthesized, but at the same time, makes tailoring of composition and functionality more demanding. This thesis seeks to address these outstanding questions for iron, magnesium and zinc HAp through developing methods to prepare single phase products, characterized using multiple techniques, and correlating bioactivity and catalytic performance with crystal chemical modification.

Chapter 3

Ferric Hydroxyapatite

The incorporation of ferric iron into $P6_3/m$ hexagonal hydroxyapatite is coincident with, and perhaps reliant upon, carbonate displacement of hydroxyls (OH^-) and phosphate $[(PO_4)^{3-}]$ especially at lower Fe^{3+} concentrations. Higher iron loadings (0.5 at %) promote the crystallization of lower symmetry ($P2_1/m$) pseudomorphs. Spectroscopic methods confirmed the trivalent chemical state of iron and identified a paramagnetic phase transition. Powder X-ray and neutron diffraction show systematic crystallographic modifications with composition. The materials are biocompatible, and while an amorphous component is evident, iron is principally found in apatite.

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Iron-bearing apatites (Fe-Ap) show potential as medical and catalytic materials. Introducing ferric ions into HAp-based bioglasses and biocements accelerates bone regeneration by enhancing osteoblast proliferation [118], and these nano-crystalline powders are under development for drug delivery [119]. Iron-phosphate-based materials show high catalytic activity towards some important petrochemical reactions, including alkane oxidation and oxidative dehydrogenation of organic acid [78, 120]. Here, a reliable method for synthesizing Fe-HAp is described and the crystal chemistry of the products evaluated. The excipient property of the apatite powders with respect to fibroblast cell viability is explored.

3.1 Iron Incorporation in Apatite

In Chapter 2, it was noted that the solid solubility of iron in HAp is restricted as the ionic radii of $\text{Fe}^{2+/3+}$ (0.78/0.65 Å) are small relative to Ca^{2+} (1.00 Å). In addition, Fe destabilizes apatite in favor of tricalcium phosphate (β -TCP) at higher loadings and calcination temperatures [29]. Powder X-ray diffraction (XRD) of ferrous-HAp found that Fe^{2+} preferentially replaces Ca^{2+} in the Ca^{F} metaprism in $[\text{Ca}_{10-x}\text{Fe}_x^{2+}][(\text{PO}_4)_6][\text{F}_2]$ ($0 \leq x \leq 1.5$) with the unit cell volume contracting with increasing Fe^{2+} content. The isotropic contraction of the cell edges is in accord with the isovalent substitution [88]

On the other hand, the incorporation of ferric (Fe^{3+}) iron into HAp requires structural relaxation and insertion of counter ions. An earlier powder XRD study suggested that Fe^{3+} prefers the framework Ca^{F} sites [15]. While it is generally assumed that Fe^{3+} will replace Ca^{2+} the discrepancy in size is large, and mechanisms for altermvalent charge balance have not to date been widely examined. Depending on the synthesis method, it is postulated that oxygen nonstoichiometry (e.g. atmospheric carbonate, ‘interstitial’ oxygen) can play a role [121]:

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However, these mechanisms cannot be differentiated by X-ray diffraction due to the poor crystallinity (<100 nm) and negligible diffraction sensitivity. The analysis will be further complicated if pseudomorphism and polysomatism are present. The chemical states of iron in HAp prepared using co-precipitation, solid state reaction and microwave irradiation are reported [91-93], but less emphasis has been given to defining the crystallographic location and coordination of the metal.

In this chapter, X-ray and neutron diffraction were used in combination with spectroscopic methods to examine the partitioning of ferric ions between the framework and tunnel A cation sites, confirm an hexagonal to monoclinic phase transformation, determine the extent of amorphicity and the distribution of carbonate. Analysis of the metaprisim twist angles was also employed to monitor the relative sizes of the framework and tunnel, which provides direct information on the incorporation mechanisms. Finally, a reconnaissance study of Fe-HAp bioactivity was undertaken.

3.2 Experimental Methods

3.2.1 Powder Preparation

Fe-Ap of nominal composition $[\text{Ca}_{10-x}\text{Fe}_x^{3+}][(\text{PO}_4)_6][(\text{OH})_{2-x}\text{O}_x]$ ($0 \leq x \leq 0.5$) was synthesized from stoichiometric proportions of the reagents; the compositional limit was set from preliminary experiments that showed higher iron contents lead to β -TCP as the dominant phase. A 0.1M Fe^{3+} solution was prepared by dissolving 2.5 mmol iron chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) in 25 mL of de-ionized water. This solution was stirred for 10 mins at room temperature with an aqueous suspension of calcium hydroxide $[\text{Ca}(\text{OH})_2]$ (0.064 moles/128 mL). Phosphate crystallization was initiated by adding 0.2 M orthophosphoric acid (H_3PO_4) dropwise to the suspension (18 mL/min) with continuous mixing. The slurry was stirred at 65°C for 90 min and

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maintained at 100°C for a further 2 hr before settling the precipitate overnight at ambient. This product was separated using vacuum filtration and washed several times to remove excess chloride prior to drying overnight at 100°C. These precursors were calcined (600°C-1000°C) under nitrogen using a heating rate of 1°C/min, dwell time of 2 hr, and finally cooling at a rate of 5°C/min. Sintering in air proved inappropriate as this yielded a mixture of apatite and hematite (Fe_2O_3).

Solid state reaction by stoichiometric precursors (calcium chloride, ferric chloride and tri-calcium phosphate) was unsuccessful as this also yielded a mixture of apatite and hematite.

3.2.2 Powder X-ray and Neutron Diffraction

The Fe-HAp products were manually ground in an agate mortar and pestle prior to XRD with the pattern recorded using Ni-filtered $\text{Cu K}\alpha$ radiation generated by a Shimadzu X-ray Diffractometer-6000 generator operating at 40 kV and 30 mA. Data were accumulated with a step scan increment of 0.02° 2θ and dwell time of 2 s over the angular range 10 to 130° . All specimens were spiked with a silicon standard (10 wt%, NIST660a, $a = 5.40825\text{\AA}$) to determine the unit cell constants absolutely, while amorphous content was scaled against a standard addition of fluorite (CaF_2 , 20 wt%), chosen to have an X-ray mass adsorption coefficient similar to HAp. Rietveld refinement was performed using the fundamental parameters approach with TOPAS V3 (Bruker-AXS) and $\text{P6}_3/m$ hydroxyapatite as the starting model. Crystallographic parameters were ultimately refined simultaneously, but initially, these variables were released in the sequence: scale factor, zero shift, Chebyshev background polynomial (4th order), crystallite size (i.e. coherently scattering domain size), and lattice parameters.

PND patterns were collected on the high resolution two-axis diffractometer (D1A) at the Institut Laue-Langevin (ILL) Grenoble. Diffraction data were accumulated at room temperature from 0° to 157.9° 2θ in 0.1° steps using a

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neutron wavelength of 1.38 Å, where 2 g of sample was loaded in a 9 mm diameter vanadium can. The diffractometer was equipped with a focusing Ge monochromator at a take-off angle of 123° and 25 standard ^3He counter detectors. The absolute phase proportions and cell metrics were calibrated against 20 wt% fluorite as an internal standard. Rietveld analysis was performed using TOPAS V3.0 from 10° to 150° 2 θ with a pseudo-Voigt peak shape profile function where the parameters U, V, W (0.12, -0.21, 0.17) were fixed to values supplied by ILL. For each data set, a five coefficient Chebychev polynomial and 1/x background, zero error, unit cell parameters, scale factors, and crystal size were refined successively. The cation positions were established first, followed by oxygen, in order to achieve a smooth convergence. Site occupancies of all crystallographic positions were determined with constraints in accord with the substitution mechanism and stoichiometric crystallochemical formula (e.g. framework PO_4 and type B carbonate were totaled to 6 per formula unit). The PO_4 tetrahedra were refined as semi-rigid bodies. Type A (tunnel) and type B (framework) carbonate were introduced according to the models of Ivanova *et al* [122] and Fleet *et al* [123] where the atomic positions of carbon were fixed while oxygen were refined.

3.2.3 Transmission Electron Microscopy

High resolution transmission electron microscope (HRTEM) images and energy dispersive X-ray spectroscopy (EDS) were acquired from powders ultrasonically dispersed in ethanol for 5 mins with a drop of suspension deposited on holey carbon-coated copper grids. Data were collected digitally using a JEOL-2100F field emission transmission electron microscope ($C_s = 0.5$ mm and $C_c = 1.1$ mm) operating at 200 kV and fitted with a low-background Gatan double tilt holder. As Fe-HAp and Fe-OAp can amorphize after 1-2 min electron irradiation, the dose was minimized by focusing off-specimen then rapidly translating to the target area. In addition, EDS signals were accumulated over 100 s using the same microscope fitted with a Si(Li) X-ray detector.

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3.2.4 Fourier Transform Infrared Spectroscopy

Powders were mixed with potassium bromide (KBr) and pressed into pellets prior to Fourier transform infra red (FTIR) absorption measurements performed over 16 scans in the wavenumber range of 400 and 4000 cm^{-1} with a Perkin Elmer (Spectrum 2000) spectrophotometer. To differentiate crystallographic hydroxyl and carbonate from surface sorbed species, 30mg Fe-HAp was stirred with 30mL of 0.05M hydrochloric acid for 5 mins, washed and centrifuged with de-ionized water, and oven dried at 100°C before acquiring FTIR spectrum. Spectral manipulation included baseline adjustment, smoothing and normalization followed by band fitting using 4 pure Gaussian functions of fixed width over the carbonate ν_3 asymmetric stretch (1400-1600 cm^{-1}) and ν_2 out-of-plane bend (873-880 cm^{-1}) regions, with a least-squares convergence of $r^2 > 0.995$.

3.2.5 NMR Spectroscopy

Phosphorous magic angle spinning nuclear magnetic resonance (^{31}P MAS NMR) spectra were acquired at 10 kHz with a Bruker Avance 400 NMR spectrometer using 85% H_3PO_4 as a primary standard at 0 ppm and ammonium dihydrogen phosphate (ADP) as a secondary standard at +0.9 ppm. Experimental resonances were fitted with Gaussian and/or Lorentzian lines using DMfit [124].

3.2.6 Mössbauer and Electron Paramagnetic Resonance Spectroscopies

^{57}Fe -Mössbauer spectra were recorded at 300 K using a commercial drive unit and transducer (Wissel) operating in sinusoidal oscillation mode with a 25 mCi source of $^{57}\text{Co}:\text{Rh}$, and the 14.4 keV γ -rays were detected with a Kr proportional counter. The drive velocity was calibrated against the 300 K spectrum of α -Fe. EPR spectra were recorded at 300 K with an ESPRIT-425 vol604 spectrometer equipped with X band (9GHz microwave frequency).

3.2.7 Magnetic Susceptibility

Magnetic susceptibility measurements were conducted in a quantum design superconducting quantum interference device (SQUID magnetometer) over the temperature range 4-300 K with a magnetic field of 3 kOe.

3.2.8 Catalytic Performance

Fe-Ap samples (50 mg each) were loaded in a micro-fixed-bed reactor connected to a gas phase chromatograph equipped with a flame-ionizer-detector (GC-FID) utilizing hydrogen (H₂) as fuel gas. Powders were pre-treated with 50 mL/min flowing nitrogen (N₂) at 250 °C to remove moisture and surface contamination. Ethanol was bubbled through nitrogen (N₂) at 50 mL/min while the reactor was heated to various temperatures (250, 300, 350, 400, 450 and 500 °C) at a rate of 20°C/min to detect residue reactants and products. Conversion is calculated based on the percentage changes in the peak area of ethanol, while selectivity is determined from the peak area ratio of the product and the reactant.

3.2.9 Biocompatibility

Mouse embryonic fibroblast cells (L929, ATCC) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and incubated overnight at 37°C in a humidified (5% CO₂/ 95% air) atmosphere in a T-75 flask. The medium was subsequently aspirated from the flask and washed with a phosphate buffer saline (PBS) before adding trypsin-EDTA to allow cell detachment. The cells were suspended in fresh medium and centrifuged to create pellets. The supernatant was discarded and the cell count performed with 20µL of the cell suspension using a hemacytometer. A 24-well culture plate was used for cell seeding at a density of 3×10^4 cells per well and cultivated in 500 µL of DMEM. The well plate was incubated as before to allow cell attachment, with the medium containing Fe-Ap (0 to 4 mg/mL), that was sterilized under

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ultraviolet light for 2 h, and replaced every two days with total incubation times ranging from 24 h to 168 h.

Cell viability as a function of Fe-OAp was analyzed using Owen's reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium] as the inner salt (MTS assay, CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega). This colorimetric method determines the number of metabolically active cells by monitoring the reduction of the tetrazolium salt by dehydrogenase enzymes to a soluble formazan, in the presence of an electron-coupling agent, and measuring the absorbance of formazan (490 nm) directly from the plate. After each incubation interval (24, 72, 120, 168 h), 100 μ L of Owen's reagent was added to the wells and incubated under the same condition for 4 hr prior to transferring the supernatants to a 96-well plate for absorbance readings in a MicroKinetics plate reader (TECAN). The experiment was performed in triplicate for each Fe-HAp concentration. Single factor ANalysis Of VAriances (ANOVA) was performed to test for statistical significance.

3.3 Crystal Chemistry of the Ferric-Apatite Series

3.3.1 Unit Cell Systematics

FTIR

FTIR spectroscopy of the Fe-Ap series reveal a systematic diminution in the characteristic hydroxyl (OH^- , $\sim 3500 \text{ cm}^{-1}$) and carbonate (CO_3^{2-} , ~ 870 and 1400 cm^{-1}) vibrations with increasing Fe content [Figure 3-1(a)] accompanying a phase transformation from Fe-HAp to Fe-OAp, and for $x = 0.5$ these species are at, or below, the detection limit. At a given iron loading ($x = 0.1$ and 0.2), the spectra were invariant with calcination temperature [Figure 3-1(b)]. For $x = 0$ the fingerprint bands of OH^- and CO_3^{2-} peaks remain with diminished intensity after acid dissolution demonstrating that a portion of these species are bound crystallographically. The planar carbonate ion vibrates in two modes in the infrared region: non-degenerative ν_2 at 879

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cm^{-1} and doubly degenerate ν_3 at 1415 cm^{-1} . The relative proportions of type A and type B CO_3^{2-} are collated (Table 3-1, Figure 3-2). However, the diminishing of characteristic vibrations due to reduction in crystallinity should be considered, although this could not be established unequivocally.

Figure 3-1. FTIR spectra of Fe-HAP calcined at (a) 600°C and (b) 700°C show the signature hydroxyl and carbonate bands of a mixed A-B type apatite at low iron contents. These features progressively diminish with iron loading because the inclusion of OH^- and CO_3^{2-} operate against the inclusion of the higher valence Fe^{3+} . The broad band at $\sim 3450 \text{ cm}^{-1}$ is from atmospheric water adsorbed to the KBr specimen pellet.

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Table 3-1. FTIR ν_3 and ν_2 wavenumbers (cm^{-1}), area proportion (%) and area ratio of type B and type A carbonate species in Fe-Aps.

type	Temperature ($^{\circ}\text{C}$)	600			700		
	iron loading (x at%)	0	0.1	0.2	0	0.1	0.2
A	ν_3 bands (cm^{-1})	1463	1460	1464	1465	1457	1461
	area proportions (%)	56	38	63	56.5	37	66
B	ν_3 bands (cm^{-1})	1410	1417	1416	1412	1416	1414
	area proportions (%)	44	62	37	43.5	63	34
$\frac{B}{A}$	area ratio	0.8	1.6	0.6	0.8	1.7	0.5
A	ν_2 band (cm^{-1})	876	877	876	875	876	875
	area proportions (%)	58	36	72	58	36	75
B	ν_2 band (cm^{-1})	868	869	869	868	869	867
	area proportions (%)	42	64	28	42	64	25
$\frac{B}{A}$	area ratio	0.7	1.8	0.4	0.7	1.8	0.3

Figure 3-2. Spectral deconvolutions of Fe-Aps for $x = 0.1$ [(a) and (b)], and $x = 0.2$ [(c) and (d)] calcined at 700°C in the regions $1400-1600 \text{ cm}^{-1}$ (ν_3 ; asymmetric stretch vibration) and $873-880 \text{ cm}^{-1}$ (ν_2 ; out-of-plane bend vibration).

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XRD

For all Fe-Ap compositions ($0 < x \leq 0.5$) crystallinity was enhanced at elevated sintering temperatures (Figure 3-3). Higher iron content inhibited crystal growth at 600-700°C, but this effect was less significant from 800-1000°C, with iron-rich crystals marginally larger. The HAp-OAp-(β -TCP) phase boundaries were dependent on iron loading and heat treatment. The solid solution limit decreased from $x = 0.5$ after firing at 600°C to $x = 0.2$ at 1000°C. For a given composition, the appearance of more crystalline Fe-OAp was accompanied by an increase in unit cell volume, especially at lower iron loadings [Figure 3-4(c)]. The displacement of hydroxyl in the course of the transformation to Fe-OAp was in accord with FTIR.

For powders calcined at 600 °C, even though smaller Fe^{3+} replaces Ca^{2+} , the unit cell dilates anisotropically along the a -edge with increasing iron loading [Figure 3-4(a)]. A discontinuity in volume expansion as a function of composition, especially at $x \sim 0.2$, with a coincident reduction in the c -axis, is postulated to arise by transformation from the hexagonal to a monoclinic pseudomorph [Table 3-2, Figure 3-4(b)].

Rietveld analysis of the X-ray data could not refine the occupation of Fe^{3+} over the calcium sites, although least squares fitting invariably yielded a scattering power >18 electrons (i.e. Ca^{2+}) on both Ca^{F} and Ca^{T} positions. However, Mössbauer and EPR spectroscopies contained multiple components suggestive of distributed iron content (see section 3.3.3). Quantitative XRD yielded a constant amorphous content in all the powders independent of iron loading, however the value obtained (~ 30 wt%) is significantly overestimated due to microabsorption [125]. Nonetheless, a non X-ray diffracting component is present and was directly identified by electron microscopy (see Section 3.3.4).

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Temp (°C)	Nominal Fe loading (at%)					
	0	0.1	0.2	0.3	0.4	0.5
600	29 nm	21 nm	22 nm	17 nm	13 nm	13 nm
700	35 nm	28 nm	33 nm	21 nm		
800	44 nm	48 nm	53 nm			
1000	114 nm	101 nm	116 nm			

Fe-HAp
 Fe-OAp
 TCP + Fe-OAp

Figure 3-3. Phase boundary of carbonate iron hydroxyapatite (Fe-HAp), iron oxyapatite (Fe-OAp) and tricalcium phosphate (β -TCP) phase regions as a function of iron loading and sintering temperature. The crystal domain size as determined by powder X-ray diffraction is given for the single phase apatite zones.

Figure 3-4. Unit cell trends in Fe-HAp. (a) The incorporation of Fe^{3+} leads to a dilation of the a -axis indicative of an increase in the volume of the apatite framework. The c -axis shows a discontinuity at $x \sim 0.2$ reflecting a change in the mechanism of iron incorporation. In contrast, substitution of Fe^{2+} is accompanied by a linear decrease of lattice constants. (b) For the materials sintered at 600°C , the unit cell volume dilates with Fe^{3+} substitution to a solid solution limit of $x \sim 0.5$, while Fe^{2+} can be accommodated to $x = 1.5$ with a decrease in cell volume²³. (c) For a given composition of Fe^{3+} -HAp higher temperature sintering leads to a slight increase in unit cell volume, especially at low Fe^{3+} loadings.

Table 3-2. Lattice parameters of Fe-Aps calcined at 600°C .

nominal Fe (x)	a ($\text{\AA}$)	c ($\text{\AA}$)	volume ($\text{\AA}^3$)
0	9.3948(4)	6.8906(4)	526.8
0.1	9.4177(5)	6.8866(5)	529.0
0.2	9.4138(5)	6.8896(5)	528.8
0.3	9.4329(7)	6.8867(7)	530.7
0.4	9.456(1)	6.885(1)	533.2
0.5	9.464(1)	6.879(1)	533.6

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PND

Rietveld refinements of the PND data show that at very low Fe^{3+} levels ($x = 0.1$), carbonate is mixed A-type [mechanism (3-2)] and B-type [mechanism (2-8)] to yield the formula $[(\text{Ca}_{3.9}\text{Fe}_{0.1})^{\text{F}}(\text{Ca}_{5.87}^{\text{T}}\square_{0.13})^{\text{T}}][(\text{PO}_4)_{5.80}(\text{CO}_3)_{0.20}][(\text{CO}_3)_{0.08}(\text{OH})_{1.92}]$ (Table 3-4). For this material, the relatively large proportion of B-type carbonate masked the small A-type contribution in FTIR spectra (Figure 3-2). For $x = 0.2$, B-type carbonate is eliminated and Fe^{3+} is predominantly incorporated by mechanism (3-2) as $[(\text{Ca}_{3.80}\text{Fe}_{0.20})^{\text{F}}(\text{Ca}_6)^{\text{T}}][(\text{PO}_4)_6][(\text{CO}_3)_{0.24}(\text{OH})_{1.76}]$ (Table 3-5). The proportions of B-type and A-type carbonate are consistent with the semi-quantitative analysis of FTIR spectra and the substitution mechanisms. Beyond $x \sim 0.2$, HAp transforms to OAp as

by partially oxidizing the tunnel hydroxyl group that gives rise to $[(\text{Ca}_{3.77}\text{Fe}_{0.23})^{\text{F}}(\text{Ca}_{5.72}\text{Fe}_{0.28})^{\text{T}}][(\text{PO}_4)_6][\text{O}_{0.52}\text{OH}_{1.48}]$ and a solid solution limit of $x \sim 0.5$ (Table 3-6). The presumption of oxygen nonstoichiometry [mechanism (3-3)] promoting phase transformation to a triclinic 2A pseudomorph was not supported.

For $x = 0.1$ and 0.2 Fe^{3+} partitions exclusively to the framework, within the sensitivity of PND, but for $x = 0.5$ it enters both the Ca^{F} and Ca^{T} positions in the ratio of 2:3 (Table 3-3). β -TCP appears as a minor phase up to $x = 0.2$ at%. In the 700°C series, the amorphous component, if present, is below the detection limit of neutron diffraction (Table 3-3).

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Figure 3-5. Rietveld plot of the neutron diffraction data for Fe-CHAp ($x = 0.1$) prepared via sintering under N_2 (600 °C/ 2h) and spiked with CaF_2 (20 wt %). The upper trace is an overlay of the experimental pattern (circles) and calculated intensity (continuous line) while the lower trace shows the intensity differences between the experimental and calculated patterns. An excluded region is shaded and bottom-most are the Bragg markers for CaF_2 , Fe-CHAp and β -TCP, respectively.

Table 3-3. Crystallographic information of the refined patterns for Fe-Aps.

	$x = 0.1$	$x = 0.2$	$x = 0.5$
Phase	CHAp		OAp
Space group	$P6_3/m$	$P2_1/m$	$P2_1/m$
Pseudomorph	$2H$	$2M$	$2M$
R_b	1.47	12.18	1.13
S_{wp}	0.24	0.30	0.31
R_{wp}	2.72	3.27	2.79
Crystal size (nm)	32.0(10)	17.4(7)	15.4(6)
Density (g/cm ³)	3.182(8)	3.202(4)	3.207(3)
a (Å)	9.371(1)	9.432(6)	9.422(5)
b (Å)	9.371(1)	9.368(7)	9.362(5)
c (Å)	6.837(1)	6.827(3)	6.821(2)
γ (°)	120	120.30(6)	120.21(6)
volume (Å ³)	519.9(1)	520.8(6)	520.0(5)
wt % Fe-Ap	67	65	79
wt % β -TCP	15	16	0
metamorphism twist angle (°)			
φ_1	25.6	24.4	24.6
φ_2		14.3	14.3
φ_3		30.6	29.4
φ_{ave}	25.6	23.1	22.8
carbonate ratio (B/A) determined via PND	2.4	0.3	--
%Fe on A ^F	100	100	40
%Fe on A ^T	0	0	60

† Agreement measures are $R_{wp} = \sqrt{\frac{\sum_i w_i (y_i(obs) - y_i(calc))^2}{\sum_i w_i (y_i(obs))^2}}$ and $R_B = \frac{\sum_k I_k(obs) - I_k(calc)}{\sum_k I_k(obs)}$.

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Table 3-4. Atomic coordinates and isotropic displacement parameters ($\text{\AA}^2$) of Fe-CHAp ($x = 0.1$).

	Wyckoff symbol	occupancy	x	y	z	B_{eq}
Ca1/Fe1	4f	0.975/0.025 *	2/3	1/3	-0.004(2)	0.9(3)
Ca2	6h	0.968(17)	0.992(2)	0.239(2)	0.25	1.6(2)
P	6h	0.967(1)	0.370(1)	0.404(1)	0.25	1.4(8)
O1	6h	0.967(1)	0.471(1)	0.315(1)	0.25	1.4(8)
O2	6h	0.967(1)	0.470(1)	0.590(1)	0.25	1.4(8)
O3	12i	0.967(1)	0.254(1)	0.3414(8)	0.0740(9)	1.4(8)
O4	4e	0.48(1)	0	0	0.190(3)	2.1(4)
H	4e	0.48(1)	0	0	0.084(6)	2.1(4)
C1 (type B)	6h	0.017(5)	0.3618*	0.3522*	0.25	0.05 *
C2 (type B)	6h	0.017(5)	0.3550*	0.4380*	0.25	0.05 *
O5 (type B)	12i	0.05(1)	0.306(8)	0.364(8)	0.090(1)	0.05 *
C3 (type A)	2e	0.04(2)	0	0	0	0.05 *
O6 (type A)	12i	0.01(5)	0.03(6)	1.01(9)	0.68(4)	0.05 *
O7 (type A)	12i	0.01(5)	0.97(4)	0.12(4)	0.52(8)	0.05 *

* Not refined.

Table 3-5. Atomic coordinates and isotropic displacement parameters ($\text{\AA}^2$) of Fe-CHAp ($x = 0.2$).

	Wyckoff symbol	occupancy	x	y	z	B_{eq}
Ca1/Fe1	4f	0.948(48)/0.052(48)	0.661(6)	0.331(6)	0.002(3)	0.4(3)
Ca2	2e	1 *	0.259(6)	0.035(8)	0.25	0.9(8)
Ca3	2e	1 *	0.773(7)	0.788(7)	0.25	1.0(7)
Ca4	2e	1 *	0.979(8)	0.236(8)	0.25	1.0(8)
P1	2e	0.991(22)	0.030(4)	-0.360(4)	0.25	0.8(1)
P2	2e	0.991(22)	-0.425(4)	0.965(5)	0.25	0.8(1)
P3	2e	0.991(22)	0.368(5)	0.387(5)	0.25	0.8(1)
O1	2e	0.991(22)	0.494(5)	0.331(3)	0.25	0.8(1)
O2	2e	0.991(22)	0.842(4)	-0.444(5)	0.25	0.8(1)
O3	2e	0.991(22)	-0.333(5)	0.153(4)	0.25	0.8(1)
O4	2e	0.991(22)	0.466(4)	0.578(4)	0.25	0.8(1)
O5	2e	0.991(22)	0.132(4)	0.554(4)	0.25	0.8(1)
O6	2e	0.991(22)	0.387(4)	0.880(4)	0.25	0.8(1)
O7	4f	0.991(22)	0.255(3)	0.355(3)	0.074(3)	0.8(1)
O8	4f	0.991(22)	0.076(3)	0.755(3)	0.076(5)	0.8(1)
O9	4f	0.991(22)	0.635(4)	-0.085(3)	0.071(3)	0.8(1)
O10	4f	0.44(2)	0.01(2)	-0.01(2)	0.173(8)	0.6(5)
H	4f	0.44(2)	-0.00(2)	-0.00(2)	0.08(1)	0.6(5)
C (type A)	2e	0.12(3)	0	0	0	0.05 *
O11 (type A)	4f	0.03(1)	0.97(2)	0.11(2)	0.55(3)	0.05 *
O12 (type A)	4f	0.03(1)	-0.13(3)	0.86(2)	0.52(2)	0.05 *
O13 (type A)	4f	0.03(1)	0.14(2)	-0.97(2)	0.55(4)	0.05 *
O14 (type A)	4f	0.03(1)	0.00(3)	0.05(2)	0.67(2)	0.05 *
O15 (type A)	4f	0.03(1)	-0.01(4)	-0.98(3)	0.68(2)	0.05 *
O16 (type A)	4f	0.03(1)	0.06(2)	-0.05(2)	0.62(2)	0.05 *

* Not refined.

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Table 3-6. Atomic coordinates and isotropic displacement parameters ($\text{\AA}^2$) of Fe-OAp ($x = 0.5$).

	Wyckoff symbol	occupancy	x	y	z	B_{eq}
Ca1/Fe1	4f	0.942(40)/0.058(40)	0.662(4)	0.332(5)	-0.002(2)	1.0(2)
Ca2	2e	0.993(70)	0.256(6)	0.029(6)	0.25	0.9(6)
Ca3	2e	1.000(46)	0.739(5)	0.746(6)	0.25	1.1(5)
Ca4/Fe2	2e	0.860(54)/0.140(54)	0.980(4)	0.216(4)	0.25	0.24(5)
P1	2e	1.000(20)	0.030(3)	-0.360(4)	0.25	0.92(9)
P2	2e	1.000(20)	-0.432(4)	0.965(4)	0.25	0.92(9)
P3	2e	1.000(20)	0.361(3)	0.385(3)	0.25	0.92(9)
O1	2e	1.000(20)	0.479(3)	0.316(3)	0.25	0.92(9)
O2	2e	1.000(20)	0.850(4)	-0.493(3)	0.25	0.92(9)
O3	2e	1.000(20)	-0.329(3)	0.153(3)	0.25	0.92(9)
O4	2e	1.000(20)	0.462(3)	0.576(3)	0.25	0.92(9)
O5	2e	1.000(20)	0.135(3)	0.561(3)	0.25	0.92(9)
O6	2e	1.000(20)	0.382(3)	0.874(3)	0.25	0.92(9)
O7	4f	1.000(20)	0.243(3)	0.340(3)	0.076(3)	0.92(9)
O8	4f	1.000(20)	0.053(3)	0.752(2)	0.075(3)	0.92(9)
O9	4f	1.000(20)	0.629(2)	-0.086(2)	0.070(2)	0.92(9)
O10	4f	0.370(79)	0.002(5)	-0.005(6)	0.160(5)	0.05 *
H	4f	0.370(79)	-0.008(7)	-0.047(6)	0.115(8)	0.05 *
O11	4f	0.130(79)	-0.000(2)	-0.01(2)	0.32(2)	0.05 *

* Not refined.

3.3.2 Crystal Chemistry

Iron incorporation

At its putative limit of $x = 0.5$, Fe^{3+} is nearly evenly distributed over the Ca^{F} and Ca^{T} site after transformation from $P6_3/m$ Fe-CHAp to $P2_1/m$ Fe-OAp. This is consistent with the shorter average $\text{Ca}^{\text{F}}\text{-O}$ bond lengths of the metaprisms, while the P-O bonds are relatively fixed (Table 3-7). Coherent crystal size peak broadening of the neutron data means the triclinic metric (PI) cannot be completely excluded for $x = 0.2$ and 0.5 , but these materials are certainly not hexagonal. Moreover, as oxygen nonstoichiometry [mechanism (3-3)] is not required to charge balance Fe^{3+} , the 2A pseudomorph is unlikely to exist under the present synthesis conditions.

Up to $x \sim 0.2$, iron is introduced into HAp in tandem with A-type tunnel carbonate [mechanism (3-2)] that reduces channel anion content, decreases the twist angle and lowers the symmetry to $P2_1/m$ [Figure 3-6(a), (b)]. When iron

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is less abundant ($x = 0.1$) $P6_3/m$ is preserved because the carbonate is principally B-type [mechanism (3-4)] and the tunnel is nearly completely filled. In Fe-CHAp with $x \leq 0.2$, the ferric ion is incorporated in the framework and distorts the $\text{Ca}^{\text{F}}\text{O}_6$ metaprisms, such that the average $\text{Ca}^{\text{F}}\text{-O}$ bond length shortens [Table 3-7, Figure 3-7(a)], and for $x = 0.2$ the twist angles are comparable to the HAp-2M pseudomorph while the PO_4 tetrahedra is essentially a rigid-body and resists distortion [Figure 3-7(b)]. At the solubility limit ($x = 0.5$), iron partitions to both the Ca^{F} and Ca^{T} position, but the smaller average crystal radii causes framework contraction in order to satisfy the shorter bond-length requirements, while tunnel nonstoichiometry led to a partially occupied mixed anion tunnel containing disorder (Table 3-8). Chemical substitutions in the tunnel [mechanism (2-2)] and framework (Ca^{F} and Fe^{3+}) promote structural relaxation of Ca^{T} into three distinct crystallographic sites, with a change in the multiplicity of the framework $6h$ and $12i$ sites (in 2H) to $3 \times 2e$ and $3 \times 4f$ (in 2M).

Twist angle systematics

For Fe-HAp φ_{max} in the 2M pseudomorph becomes more evidentially obtuse with increasing iron and/or carbonate content as the average crystal radii decreases in accord with apatite systematics. The 2M pseudomorphs ($x = 0.2$ and 0.5) have φ_{max} of 30.6° and 29.4° , while apatite-2H ($x = 0.1$) possesses a moderately acute $\varphi \sim 25.6^\circ$ (Table 3-3). In this study, B-type carbonate incorporation and tunnel sub-stoichiometry partially empties the tunnel, and the channel collapses by framework twisting to give an obtuse twist angle. Consequently, Fe-CHAp ($x = 0.2$) that crystallizes as monoclinic $P2_1/m$ contains a single large twist angle ($\varphi_3 = 30.6^\circ$), with the remaining angles more acute (14.3 and 24.4°) to compensate [Figure 3-6 (a)-(c)]. Large twist angles ($\varphi > 27^\circ$) are not unusual, for example an electron diffraction study of a triclinic ($P\bar{1}$) oxy-apatite gave $\varphi_{\text{max}} \sim 28^\circ$ attributed to tunnel sub-stoichiometry [21].

Carbonate

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The Rietveld refinements of framework and tunnel carbonate were to some extent compromised by peak broadening, leading to poorer accuracy in O-O bond lengths and O-C-O bond angles as compared to single crystal studies [123] [Table 3-7, Figure 3-7 (d)-(f)]. X-ray absorption spectroscopy (XAS) should be utilized to correlate crystal chemical composition to the local environment of Fe^{3+} and CO_3^{2-} , but at present the matter is unresolved. In addition, there are insufficient data sets from apatites of unique composition to clearly correlate the relationship between carbonate content and unit cell metrics; this could be addressed by growing single crystals Fe-CHAp, however, preliminary attempts to prepare these materials were unsuccessful.

Table 3-7. Selected bond lengths (Å) of Fe-Aps.

$P6_3/m$		$x = 0.1$	$P2_1/m$		$x = 0.2$	$x = 0.5$
Framework						
Ca1-O1	x 3	2.465(3)	Ca1-O1		2.315(5)	2.376(3)
Ca1-O2	x 3	2.433(7)	Ca1-O2		2.568(3)	2.399(2)
Mean		2.449	Ca1-O3		2.390(5)	2.411(4)
			Ca1-O4		2.488(5)	2.464(4)
			Ca1-O5		2.413(4)	2.379(3)
			Ca1-O6		2.479(4)	2.440(4)
			Mean		2.442	2.412
P-O1		1.542(7)	P1-O2		1.538(5)	1.544(2)
P-O2		1.513(8)	P1-O5		1.533(3)	1.511(3)
P-O3	x 2	1.523(5)	P1-O8	x 2	1.513(3)	1.529(4)
Mean		1.525	Mean		1.524	1.528
C1-O1	x 2	1.238(6)	P2-O3		1.527(4)	1.520(2)
C1-O5		1.269(3)	P2-O6		1.536(4)	1.519(1)
Mean		1.248	P2-O9	x 2	1.513(2)	1.528(8)
			Mean		1.522	1.524
C2-O2	x 2	1.287(3)	P3-O1		1.524(2)	1.537(4)
C2-O5		1.279(4)	P3-O4		1.545(2)	1.547(2)
Mean		1.284	P3-O7	x 2	1.534(5)	1.519(1)
			Mean		1.534	1.531
tunnel						
C3-O6	x 2	1.230(1)	C3-O11		1.247(2)	
C3-O7		1.261(7)	C3-O12		1.294(4)	
Mean		1.240	C3-O13		1.248(1)	
			C3-O14		1.229(5)	
			C3-O15		1.274(7)	
			C3-O16		1.256(5)	
			Mean		1.258	

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Table 3-8. Bond valence sum (BVS) of Ca^{2+} and $\text{Fe}^{2+}/\text{Fe}^{3+}$ in Fe-Aps without considering structural relaxation and oxygen nonstoichiometry.^a

BVS	iron loading (x)		
	0.1	0.2	0.5
framework			
Ca^{2+}	1.95	2.09	2.17
Fe^{2+}	1.05	1.12	1.16
Fe^{3+}	1.11	1.19	1.24
tunnel			
$\text{Ca}^{\text{T}}(1)$			
Ca^{2+}	2.54	2.53	2.41
Fe^{2+}	1.35	1.35	1.29
Fe^{3+}	1.45	1.44	1.38
$\text{Ca}^{\text{T}}(2)$			
Ca^{2+}		2.63	2.08
Fe^{2+}	N.A.	1.40	1.11
Fe^{3+}		1.50	1.19
$\text{Ca}^{\text{T}}(3)$			
Ca^{2+}		2.12	2.57
Fe^{2+}	N.A.	1.13	1.37
Fe^{3+}		1.21	1.46

^aThe bond lengths were calculated from the refined PND data and the bond valence parameters for the calculation were taken from Brown and Altermatt [82].

Figure 3-6. Polyhedral drawings of the $\text{Ca}^{\text{F}}\text{O}_6$ metaprisms in the $2H$ and $2M$ pseudomorphs showing twist angle of Fe-Aps (a) $x = 0.1$ ($P6_3/m$), (b) $x = 0.2$ ($P2_1/m$) and (c) $x = 0.5$ ($P2_1/m$), respectively. (d) Location of type A and type B carbonate in $[001]$ projection showing the crystallochemical adaptation during the substitution of PO_4^{3-} by CO_3^{2-} , while tunnel CO_3^{2-} insertion consists of six different crystallographic sites randomly occupied. (e) The $[110]$ projection of the two types of CO_3 and (f) one of the tunnel CO_3 with bond lengths shown.

Figure 3-7. Polyhedral volume (Å³) changes with increasing iron loading (at %). Framework PO₄ tetrahedra are relatively more rigid than the Ca^FO₆ metaprism. The Ca^FO₆ metaprisms contract in line with framework iron substitution while the tunnel polyhedral (Ca^TO₆X) expand due to partial oxidation of tunnel hydroxyl.

3.3.3 Nanostructure

Although EDS confirms iron incorporation (Figure 3-8), the nanostructure of Fe-Aps was invariably complex, even for those samples that were nominally single phase according to XRD. Lattice imaging reveals a combination of crystalline and amorphous regions, where cryptocrystalline agglomerates gradually order as larger acicular HAp and OAp crystals with extension along *c* [Figure 3-9(a)]. Apatites treated at lower temperatures, and therefore containing more hydroxyl groups, were highly susceptible to damage and rapidly developed etch pits and cavities prior to amorphization [Figure 3-9(b), (c)]. Large acicular crystals of Fe-OAp proved to be less robust under electron irradiation than equivalent pure HAp. Iron-rich precipitates such as Fe₂O₃ or FeOOH were not detected.

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Figure 3-8. Energy dispersive X-ray spectra of Fe-Aps calcined at 600 °C showing an increase in iron content with doping and decrease in calcium signal consistent with the replacement by iron.

Figure 3-9. (a) Lattice image of Fe-HAp ($x = 0.5$ sintered at 600°C) showing distinctive amorphous (region A) and crystalline (region B) areas collected using the low electron dose technique. The Fast Fourier transform (FFT) of the whole image is shown top left, while local order within the outlined regions is illustrated bottom right. The nanocrystal (B) is in [100] orientation. The developing acicular morphology with extension along $\sim c$ is evident, and as annealing continues, the small domains become increasingly coherent. In general, electron beam damage was rapid as shown by the bright field images for a collection of Fe-HAp crystals collected after (b) 1 min and (c) 3 min irradiation.

3.3.4 Chemical State and Magnetism

NMR

The ^{31}P NMR resonance broadened with increasing iron loading in the Fe-HAp region [Figure 3-10(a)] primarily due to paramagnetic relaxation, suggesting Fe^{3+} is in close proximity to the phosphate groups. In the Fe-OAp/ β -TCP mixed phase region [Figure 3-10(b)], the 800°C peak is asymmetric on the low frequency side and shifted by about 0.8 ppm, relative to those at 600°C and 700°C, probably as a result of overlapping close-spaced resonances arising from Fe-OAp and β -TCP that cannot be resolved due to paramagnetic broadening [126] (Table 3-9). In addition, a broad background appears at 800°C which reflects the reconstructive phase transformation of Fe-OAp to β -TCP and the probable appearance of a transient intermediate amorphous phase, since the conversion of apatite to β -TCP is reconstructive.

Mössbauer spectroscopy

The room temperature ^{57}Fe -Mössbauer spectrum recorded for Fe-HAp ($x = 0.5$) prepared at 600 °C could be fitted with 4 quadrupole-split doublets associated with Fe in different sites and environments (Figure 3-11). The isomer shifts (IS, relative to α -Fe) and quadrupole splittings (QS) are presented in Table 3-9. For all four doublets, the IS value is in the range of 0.27 – 0.33 mm/s which is indicative of Fe^{3+} (by contrast, the IS for Fe^{2+} is typically in excess of 1 mm/s). The doublets labelled AA*, BB* and CC* correspond to D3 (six-fold coordination), D4 (four-fold coordination) and D5 (surface iron) of reference values [15] (Table 3-10). The fourth doublet, DD* provides an approximate description of a weak, broad feature in the shoulders of the spectrum and is attributed to an amorphous phosphate phase.

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Figure 3-10. ^{31}P MAS NMR spectra of (a) Fe-HAp sintered at 800°C as a function of iron loading showing the progressive broadening of the phosphorous resonance due to a combination of paramagnetic effects and the partial conversion of PO_4 tetrahedra to PO_5 trigonal bipyramids that accompanies replacement of Ca^{2+} by Fe^{3+} , and (b) the asymmetric broadening arising from overlapping signals from Fe-OAp ($x = 0.5$) mixed with β -TCP that becomes more evident at higher temperatures as the phase transformation proceeds.

Table 3-9. Fitted parameters of experimental ^{31}P NMR peaks recorded at 10 kHz for pure HAp and Fe-HAp ($x = 0.4$ and 0.5) sintered at 800°C .

X	Shift (Hz)	Width (Hz)	$xG/(1-x)L$	Integrated (%)	Assignment
0	438	95	0	100	Fe-HAp
0.4	425	430	0.4	37.0	Fe-HAp
	520	3000	0.0	63.0	β -TCP
0.5	324	330	0.4	31.5	Fe-HAp
	520	37	0.9	10.5	β -TCP
	560	2800	0.0	58.0	

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Figure 3-11. Room temperature ^{57}Fe -Mössbauer spectrum for Fe-HAp ($x = 0.5$ / 600°C) fitted with 4 quadrupole-split doublets attributed to Fe^{3+} in six-fold co-ordination (AA*), four-fold co-ordination (BB*), surface iron (CC*) and an amorphous phase (DD*).

Table 3-10. Fitted parameters of a 4-doublet analysis low speed ^{57}Fe -Mössbauer spectrum for Fe-HAp ($x = 0.5$) sintered at 600°C .

Sub spectra	IS (mm/s)	QS (mm/s)	Line width (mm/s)	Area (%)	Possible Assignment
AA*	0.33(6)	1.65(23)	0.60(20)	37.7	Fe^{3+} in A ^I
BB*	0.27(4)	1.02(16)	0.45(7)	33.8	Fe^{3+} in A ^{II}
CC*	0.28(7)	0.57(10)	0.4	24.7	Surface Fe^{3+}
DD*	0.30(7)	2.7(1.3)	0.9(4)	3.9	Amorphous Fe^{3+}

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EPR

EPR spectra collected from the $x = 0.2$ and $x = 0.5$ materials (also sintered at 600°C) contain parallel ($g_{\parallel}$) and perpendicular ($g_{\perp}$) components at 8.89 mT and 4.31 mT which according to Sutter *et al.* [127] indicates high spin Fe^{3+} in distorted octahedral or tetrahedral coordination respectively, although the latter assignment appears crystallochemically unlikely. A broad signal at $g = 2.05$ increases with iron loading and may be due to an amorphous phosphate phase or surface superparamagnetic Fe^{3+} [128] (Figure 3-12). The proximity of Fe^{3+} to the charge balancing groups is unknown, and clustering of these species should be examined by temperature dependant Mössbauer, NMR and EPR spectroscopy.

Figure 3-12. EPR spectra of Fe-HAp ($x = 0.2$ and 0.5) are consistent with Fe^{3+} with the broad feature possibly associated with amorphous material that may be more abundant near reconstructive HAp to β -TCP phase boundary.

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Magnetism

All the materials were paramagnetic above 100 K with the degree of magnetization greatest at low iron loadings. In addition, for $x = 0.2$ a transition with onset at 90 K appears that may be due to spin coupling combined with crystallochemical modification (Figure 3-13). Clarification of the origin of this behavior would require additional experiments at different applied magnetic fields, but it is suggested that higher iron levels ($x = 0.3$ onwards) lead to clustering of Fe^{3+} that decreases susceptibility, whereas for $x = 0.2$ long range magnetic ordering is possible. The magnetic phase transition observed at low iron content ($x = 0.2$) has not been observed previously in apatite, and not only reflects modification of spin order, but also the crystallochemical conversion from a type A-B apatite to an oxidized structure with framework oxygen.

Nonetheless, it is clear that neither Fe-HAp nor Fe-OAp is strongly magnetic and will not intrinsically aid the functionality of biomagnetic nanoparticles (BNP), where superparamagnetism is essential to control placement by an external field. The contribution from amorphous phosphates to the overall magnetism could not be assessed quantitatively.

Figure 3-13. The (a) magnetic susceptibility (χ) and (b) inverse magnetic susceptibility (χ^{-1}) versus temperature in a magnetic field of 3 kOe are typical of paramagnetism above 100K. A transition with an onset at 90 K reflects a change in spin order associated with a crystallographic phase transition (see Figure 3).

3.4 Functionalities

3.4.1 Catalytic Activity

The catalytic activities of Fe-Aps towards the decomposition of ethanol were monitored by evaluating the conversion of reactant and selectivity of major products, including acetaldehyde ($\text{CH}_3\text{CH}=\text{O}$) and 1-butanol [$\text{CH}_3(\text{CH}_2)_3(\text{OH})$]. For Fe-HAp ($x = 0.1$), the conversion was not improved with increasing reaction temperature, with the best performance achieved at 500 °C (~55%) [Figure 3-14 (a)]. The concomitant incorporation of carbonates is speculated to contribute to the reduction by decreasing the number of active sites. However, the results are promising for Fe-OAp ($x = 0.5$) as the conversion is superior compared to pure HAp beyond 250 °C, with a maximum of ~75% achieved at 500 °C. The selectivity towards 1-butanol was not improved at higher reaction temperature, but instead, show a sharp reversal in 1-butanol production when heated beyond 250 °C [Figure 3-14 (b)].

Catalytic dehydration or dehydrogenation of ethanol is inherently intricate due to the multi reaction pathway allowing reactants to decompose into various products. Formation of 1-butanol is possible either via direct condensation or a Guebert reaction that consumes acetaldehyde in ethanol dehydrogenation [129, 130] (Figure 3-15). The selectivity towards acetaldehyde and 1-butanol varied similarly with increasing ferric concentration. However, for pure HAp and Fe-Aps, the former route is not substantiated as the favored path because the selectivity towards acetaldehyde decreases with reaction temperature, suggesting participation of acetaldehyde in the subsequent reactions to give 1-butanol (Figure 3-16). Thus, a careful quantification of the byproducts (e.g. aldol intermediates and unsaturated aldehydes) and surface reactivity testing (e.g. TPD) should be carried out to completely ascertain the reaction chemistry.

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Figure 3-14. (a) Conversion (%) and (b) selectivity towards butanol (%) for dehydration of ethanol for pure HAp and Fe-Aps ($x = 0.1$ and 0.5). Increasing reaction temperature improved ethanol conversion but selectivity towards butanol is compromised. Maximum conversion (~75%) is achieved at 500 °C when iron loading reaches its putative limit.

Figure 3-15. Schematic of a Guebert reaction to synthesize 1-butanol.

Figure 3-16. (a) Selectivity towards acetaldehyde ($\text{CH}_3\text{CH}=\text{O}$) at various reaction temperature. A disfavor in the production of acetaldehyde is analogous to 1-butanol, indicating a preference for ethanol dehydration with Fe-Ap as catalysts. (b) Modified Arrhenius plot of ethanol dehydration utilizing Fe-OAp ($x = 0.5$) as catalysts. The linear best fit curve ($R^2 = 0.98$) is indicative of a single step rate limiting reaction.

3.4.2 Biological Response

The biocompatibility of ferric Fe-OAp was superior to that in pure HAp up to a concentration of 4 mg/mL with significant cell proliferation after 7 days incubation (Figure 3-17). The enhanced viability of fibroblast cells compared to pure HAp shows potential for exploitation in bone regeneration where scaffolds and fillers could be fabricated from Fe-OAp and incorporated in calcium phosphate glass or cements [131]. It is clear that the surface chemistry of Fe-OAp is different and the smaller crystallite size may be advantageous for excipient applications that require high surface area.

Biological apatites in bone and enamel predominantly contain trace elements (e.g. Mg^{2+} , Na^+) and are believed to be substantially type-B CHAp, from single crystal X-ray diffraction and infrared studies [132]. From this, it is inferred that tunnel substitutions are likely to be most reactive *in vivo*. On this basis, Fe-CHAp ($x = 0.2$) which contains the highest type-A carbonate may be optimal for enhancing the excipient properties of apatites and at lower Fe

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content ($x = 0.1$) the apatite- $2H$ tunnel is less dilated which may reduce reactivity. The biological response of oxy-apatite $2M$ ($x = 0.5$) shows improvement, even when carbonate is excluded, demonstrating the importance of Fe^{3+} to bioactivity.

By focusing on crystal chemistry, this investigation complements earlier studies that examined surface adsorption of iron oxides (e.g. Fe_3O_4) or oxy-hydroxides (e.g. FeOOH), and will underpin the design of hybrid architectures, such as core-shell Fe_3O_4 -(Fe-HAp) nanopebbles for drug delivery or contrast agents in magnetic resonance imaging and biosensors [133, 134].

Figure 3-17. L929 fibroblast cell viability as a function of (a) concentration of pure HAp and Fe-OAp ($x = 0.5$) sintered at 600°C and (b) incubation period.

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3.5 Conclusions

Crystal chemistry

The crystal structures of Fe-CHAp and Fe-OAp pseudomorphs have been studied by combination of diffraction, electron microscopy and spectroscopy. The space group for Fe-CHAp-2H is $P6_3/m$ and $P2_1/m$ for Fe-OAp-2M. From PND refinements and FTIR, charge balance is maintained through displacing phosphate and hydroxyl groups by atmospheric carbonate across both framework and tunnel [mechanisms (3-2) and (3-4)] at low Fe^{3+} loadings ($x = 0.1$ and 0.2), to yield the ideal crystal chemical formula

while at the same time transforming apatite-2H to 2M at $x \sim 0.2$. At the solid solubility limit of Fe^{3+} ($x = 0.5$), the 2M pseudomorph is stabilized as an oxyhydroxyapatite via tunnel non-stoichiometry [mechanism (3-5)]:

The framework site (Ca^{F}) is the preferred location for Fe^{3+} at low loadings (up to $x = 0.2$), beyond which, there is partial substitution into the tunnel (Ca^{T}). Spectroscopies (NMR, EPR, Mössbauer) and magnetic measurements were consistent with the PND refinements in addition to validation of chemical state and observation of paramagnetism. The amorphous content is below detection, in contrast to ~ 20 wt% non-diffracting content quantified from PXRD, with the latter anomalous result arising from microabsorption.

Functionality

The catalytic performance of Fe-Aps shows promise by favoring an ethanol dehydrogenation reaction producing acetaldehyde and 1-butanol. Optimizing the reaction parameters to improve selectivity and control would be required to properly evaluate its potential.

The bioactivity of Fe-HAp is superior to HAp because of the concomitant incorporation of Fe^{3+} and CO_3^{2-} that stimulates cell proliferation. However, these materials are weakly paramagnetic and not intrinsically suitable for localized hyperthermic therapies [135].

Chapter 4

Zinc Hydroxyapatite

The crystallographic modification of HAp through incorporation of zinc (Zn^{2+}) was studied using diffraction and spectroscopic techniques. The preferential displacement of tunnel calcium (Ca^{2+}) by Zn^{2+} inhibits crystal growth significantly and promotes the retention of 20 wt% amorphous component. Carbonate is incorporated (~ 0.1 at%) at lower Zn^{2+} content ($x = 0.1$), but is absent at higher zinc levels. These Zn-HAp materials effectively absorb carbon monoxide (CO) and catalyze the decomposition of 2-propanol.

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Zinc (Zn) is an essential element for human metabolism that promotes bone formation via osteogenesis [136]. In addition, zinc-doped hydroxyapatite and tricalcium phosphate composites (Zn-HAp/ β -TCP) demonstrate superior bioactivity for protein adsorption and can serve as an antimicrobial agent [5, 137]. Zn-containing catalysts are also widely studied for methanol synthesis, Friedel-Crafts acylation and carbon monoxide oxidation [138-140]. In the following, Zn-HAp is synthesized in an analogous fashion to Fe-HAp (Chapter 3) with the crystal chemistry examined by diffraction and spectroscopic methods. In addition, the catalytic performance of these materials towards dehydrogenation of 2-propanol (IPA) is examined.

4.1 Zinc in Hydroxyapatite

In common with other small cations, the incorporation of zinc into HAp inhibits crystal growth, and promotes β -TCP formation at higher calcination temperatures [18]. Functionality can be manipulated through an amorphous calcium phosphate (ACP) phase formed during the incorporation of Zn^{2+} , that leads to enhanced surface adsorption and greater reactivity. The solid solubility limit of Zn^{2+} , which will displace Ca^{2+} and can potentially partition to either the framework or tunnel, is expected to be low to satisfy the bond valence criteria. It is not known if the symmetry of Zn-HAp departs from hexagonal to monoclinic or triclinic metrics through distortion to better accommodate the $\text{Ca}^{2+} \rightarrow \text{Zn}^{2+}$ isovalent substitution (mechanism 2-11). Because Zn^{2+} (ionic radius = 0.74Å) is smaller than Ca^{2+} (ionic radius = 1.00Å), unit cell contraction is expected with the reduction in a greater than c to best alleviate stress arising from the shorter Zn-O bond length. To date, the incorporation mechanism and true symmetry of Zn-HAp remain speculative due to poor crystallinity and limited Zn solubility [106, 141, 142]. The analysis is further complicated should atmospheric carbonate and/or water enter the system during synthesis:

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A range of scenarios have been suggested for Zn incorporation based on both computational and experimental studies (Figure 4-1). Earlier atomic simulations suggest that six-fold coordination is energetically favorable for Zn^{2+} in HAp, and the Ca^{T} site is preferred over the Ca^{F} site [103, 104]. Recent studies combining theoretical modeling and X-ray absorption near edge structure (XANES) spectroscopy also support preferential entry to the Ca^{T} site [107], and at low concentrations ($x \leq 0.1$), local defects and counter ions cause the framework to expand relative to the tunnel contents:

In these models, the underbonded Zn^{2+} (BVS = 1.77) positioned on the Ca^{T} site anticipates limited substitution, and possible pseudomorphism.

Zn-HAp can be prepared by co-precipitation routes, but depending on the precursors used, product purity can vary [106, 143]. In addition, the possible coexistence of cation vacancies, extrastochiometric groups (HPO_4^{2-} , CO_3^{2-}) [108], and the less considered ACP, should not be disregarded [144]. Thus, it is a challenging, but important task to synthesize and characterize single phase products, with an emphasis on differentiating “crystallochemical incorporation” from “surface adsorption” [145] to systematically analyze the crystal structure and tailor the functionalities of Zn-HAp.

In this chapter, X-ray and neutron diffraction combined with FTIR spectroscopy are used to examine the partitioning of zinc between the Ca^{F} and Ca^{T} sites, confirm the transformation to a lower symmetry pseudomorph, examine the distribution of extrastochiometric groups (HPO_4^{2-} , CO_3^{2-}), and verify the presence of an amorphous phase. Preliminary testing of the catalytic decomposition of 2-propanol (IPA) is also reported.

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Figure 4-1. Compilation of published XRD data for Zn-HAp showing variations in (a) lattice parameters a , c (inset) and (b) unit cell volume [18, 106, 108, 143]. The reported lattice systematics and solid solubility limit show degree of unpredictability and variation, demonstrating the difficulty of analyzing the crystal structure when zinc is incorporated.

4.2 Experimental Methods

4.2.1 Powder Preparation

Zn-HAp of nominal composition $[\text{Ca}_{10-x}\text{Zn}_x][(\text{PO}_4)_6][(\text{OH})_2]$ ($0 \leq x \leq 0.5$) was synthesized from stoichiometric proportions of zinc chloride hexahydrate ($\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$), calcium hydroxide $[\text{Ca}(\text{OH})_2]$ and orthophosphoric acid (H_3PO_4). A 0.1M Zn^{2+} solution was stirred for 10 mins at room temperature with an aqueous suspension of $\text{Ca}(\text{OH})_2$ (0.064 moles/128 mL) before dropwise addition of 0.2 M H_3PO_4 (18 mL/min) with continuous mixing to initiate phosphate crystallization. The slurry was stirred at 65°C for 90 min and maintained at 100°C for a further 2 hr before settling the precipitate overnight at ambient. The product was separated using vacuum filtration and washed several times to remove excess chloride prior to drying overnight at 100°C. Finally, these precursors were calcined (600°C-700°C) under nitrogen using a heating rate of 1°C/min, dwell time of 2 hr, and finally cooling at 5°C/min. Reconnaissance syntheses found that sintering at higher temperature favored the formation of β -TCP.

4.2.2 Diffraction

Laboratory X-ray diffraction (XRD)

Zn-HAp products were manually ground with an agate mortar and pestle prior to XRD with the pattern recorded using Cu K α radiation generated by a Bruker D8-ADVANCE X-ray diffractometer operating at 40 kV and 40 mA. Data were accumulated with a step scan increment of 0.02° 2 θ and dwell time of 2 s over the angular range 10 to 130°. The samples were spiked with a standard addition of fluorite (CaF₂, 20 wt%) to determine the unit cell constants and amorphous content absolutely. Rietveld refinement was performed using the fundamental parameters approach with TOPAS V3.0 (Bruker-AXS) and P6₃/m hydroxyapatite as the starting model. Crystallographic parameters were ultimately refined simultaneously, but initially, these variables were released in the sequence: scale factor, zero shift, six coefficient Chebyshev background polynomial, coherent scattering domain size (i.e. Lorentzian and Gaussian crystallite size), and lattice parameters.

Powder Neutron Diffraction (PND)

PND patterns were collected on the high resolution two-axis diffractometer (Echidna) at the Australian Nuclear Science and Technology Organization (ANSTO) Lucas Heights. Approximately 1 g of sample (including 10 wt% fluorite as internal standard) was loaded in 5 mm diameter vanadium cans, and diffraction data accumulated at room temperature from 5° to 159° 2 θ in 0.05° steps using a neutron wavelength of 1.622 Å. The diffractometer was equipped with a focusing Ge-(335) monochromator and 128 standard ³He counter detectors. Rietveld analysis was performed using TOPAS V3.0 from 10° to 150° 2 θ with a pseudo-Voigt peak shape profile function where the parameters U, V, W were constrained to be the same in both fluorite and Zn-HAp phases. For each data set, a six coefficient Chebychev polynomial and 1/x background, zero error, unit cell parameters, scale factors, and crystallite size were refined successively. All crystallographic positions and site occupancies converged smoothly with constraints (in accord with stoichiometric formulae) by applying a similar strategy as in the Fe-HAp

series. Type A (tunnel) and type B (framework) carbonate were introduced according to the models of Ivanova *et al* [122] and Fleet *et al* [123]. Joint XRD and PND refinements were unsuccessful due to overwhelming diffracted intensity from X-rays dominating the analysis.

4.2.3 Electron Microscopy and Microanalysis

TEM

Transmission electron micrographs were acquired from powders embedded in epoxy resin and sectioned to 5 μm using a Leica microtome. Images were digitally recorded using a Hitachi H7100FA LaB₆ transmission electron microscope (TEM) operating at 125 kV. Energy dispersive X-ray spectra (EDS) were accumulated for 60 s, and averaged over 12 area scans.

Analytical Scanning Electron Microscopy (ASEM)

Chemical analyses of Zn-HAps ($x = 0.4$ and 0.5) were determined by energy dispersive X-ray spectroscopy (EDS) at 15 kV and 1 nA using a SEM (Hitachi 4300) equipped with an Oxford Instruments light element EDS detector and INCA software. Data was calibrated against ASTM Mineral apatite and diopside, and averaged over 12 area scans. All powder samples were embedded in resin, polished to a near mirror finish, and carbon coated prior to analysis.

4.2.4 Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR)

Powders were mixed with potassium bromide (KBr) and pressed into pellets prior to FTIR absorption measurements performed over 16 scans in the wavenumber range 400 to 4000 cm^{-1} with a Perkin Elmer (Spectrum 2000) spectrophotometer.

In situ Diffuse Reflectance Infrared Transmission Spectroscopy (DRIFTS)

DRIFTS for the CO adsorption on Zn-HAps (in helium at 400 °C at a flow rate of 20 mL/min) were conducted in a reaction cell (modified Harricks model HV-DR2) in order to allow gas to flow continuously through the catalyst bed (ca. 0.1 g) during spectral acquisition. The spectra were recorded with a Bio-Rad FT-IR 3000 MX spectrometer at a resolution of 4 cm⁻¹ at room temperature.

4.2.5 Temperature Programmed Desorption (TPD)

TPD of carbon dioxide (CO₂) was performed in a micro-fixed-bed reactor connected to a gas chromatograph (QMS Hiden Analytical HPR-20) with multiple ion detector (mass to charge ratios of 2, 16, 17, 18, 28 and 44). Pre-treatment of Zn-HAp (150 mg) powders was carried out under helium (He) at a flow rate of 20 mL/min, and heated to 550 °C (10 °C/min) for 2 h before air-cooling to room temperature. Samples were then purged with He+CO₂ at a flow rate of 25+5 mL/min for 1 h, and the desorption preceded by heating to 550 °C (10 °C/min) for 1 h. The TPD quantitative analysis was calibrated against 250 mg of lime (CaO, Merck) under the same experimental conditions.

4.2.6 Catalytic decomposition of iso-propanol (IPA)

Zn-HAp samples were loaded in a micro-fixed-bed reactor, pre-treated at 300 °C in He at a flow rate of 20 mL/min for 2 h and air-cooled to ambient prior to catalytic testing. IPA was fed at a rate of 0.2 mL/min and the reactor heated to various fixed temperatures (100, 150, 200, 250, 300 and 350 °C) for 1 h to equilibrate. Conversion rate and selectivity were calculated based on the hydrogen and propene detected.

4.3 Crystal Structures of Zinc-apatites

4.3.1 Lattice Trends and Systematics

FTIR

FTIR spectroscopy of the Zn-HAp series reveals a significant diminution in the characteristic carbonate vibrations ($\nu_2 \sim 870$ and $\nu_3 \sim 1400$ cm^{-1}) at higher zinc content, with a minor contribution attributed to surface adsorption of atmospheric CO_2 . The relative strength of the type A and type B CO_3^{2-} stretching bands are fixed and independent of zinc loading, and below the detection limit when $x > 0.1$ Zn^{2+} is incorporated. The hydroxyl feature (OH^- , $\nu_s \sim 3570$ cm^{-1}) is slightly diminished as zinc loading increases (Figure 4-2), that can also be ascribed to a reduction in crystallinity. The broad bands at ~ 3570 cm^{-1} and ~ 1640 cm^{-1} are attributed to surface adsorbed and/or crystallographically incorporated water molecules [4]. This hygroscopic feature is also associated with an amorphous phase quantified during diffraction (see below).

Figure 4-2. FTIR spectra of Zn-HAp series calcined at 600°C demonstrating the signature hydroxyl and carbonate bands of a mixed A-B type apatite at low zinc contents ($x = 0.1$). These features weaken with zinc loading at the onset of $x = 0.1$. The broad band at ~ 3450 cm^{-1} and ~ 1640 cm^{-1} are attributed to surface-adsorbed water molecules.

XRD

For all Zn-HAp compositions, higher zinc loadings progressively destabilize apatite and inhibit crystal growth. The phase boundary with β -TCP was dependent on calcination temperature and zinc content. At $x = 0.1$, zinc is partially eliminated from HAp as zinc oxide (ZnO) when treated at $>700^\circ\text{C}$; this process promotes apatite crystal growth. β -TCP is stabilized at higher zinc loadings ($0.2 \leq x \leq 0.5$) (Table 4-1).

Using hexagonal $P6_3/m$ as a starting model, the a_{hex} -edges increase with zinc loading up to $x = 0.3$, but contracts slightly for $x > 0.3$ up to the solid solubility limit. The unit cell volume contracts and also shows a clear discontinuity at $x = 0.3$, although smaller Zn^{2+} is incorporated in place of Ca^{2+} , suggesting a complex substitution mechanism. Refinements using monoclinic $P2_1/m$ show that a_{mono} increases almost linearly with composition while b is invariant at $\sim 9.38 \text{ \AA}$ (except for $x = 0.1$ which contain A-B type carbonate). The unit cell volume shows inflection at $x = 0.3$, but in the opposite sense to $P6_3/m$ (Figure 4-4). These cell metric behaviors suggest the large amount of crystallographic CO_3^{2-} and H_2O detected in FTIR overwhelm the contrastive lattice metric effect of Zn^{2+} substitution.

Rietveld analysis of the X-ray data indicates preferential entry of Zn^{2+} into the tunnel Ca^{T} site, but this could not be examined quantitatively due to particle size broadening of the diffracted peaks. Quantitative XRD yielded a significant proportion of amorphous content ($\sim 20\text{-}40 \text{ wt\%}$) in all the powders independent of zinc loading, and a non-diffracting component was also observed in polyphase materials (Zn-HAp and β -TCP) at higher calcination temperature (Table 4-1). These findings are in close agreement with PND quantitative phase analysis, although the latter are more reliable (see next section) [125].

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Table 4-1. The approximate phase boundary of Zn-HAp (Ap) and tricalcium phosphate (β -TCP) phase regions as a function of zinc loading and calcination temperature. The amorphous content (wt%) is estimated by spiking the powders with 10 wt% fluorite. Zinc oxide (ZnO) crystallizes exclusively when sintering Zn-HAp ($x = 0.1$) above 600 °C.

Temp (°C)	Nominal x (at%)				
	0.1	0.2	0.3	0.4	0.5
600	18 Ap	27 Ap	28 Ap	25 Ap	41 Ap
700	17 Ap + ZnO	18 TCP + Ap	18 TCP + Ap	22 TCP	30 TCP
800	25 Ap + ZnO	20 TCP + Ap

Figure 4-3. Rietveld plot of the (a) X-ray and (b) neutron diffraction data for Zn-HAp ($x = 0.1$) prepared via sintering under N_2 (600 °C/ 2h) and spiked with CaF_2 (10 wt %). The upper trace is an overlay of the experimental pattern (circles) and calculated intensity (continuous line) while the lower trace shows the intensity differences between the experimental and calculated patterns. Refinement is carried out in the 10-130° region and bottom-most are the Bragg markers for CaF_2 and Zn-HAp, respectively.

Figure 4-4. Unit cell trends in Zn-HAp from XRD data. Refinements were carried out by employing both hexagonal and monoclinic HAp models separately. The incorporation of Zn^{2+} led to an expansion in (a) a -edge and (b) unit cell dilation. The discontinuity in the unit cell trends observed in the hexagonal refinement model inferred that the crystal structure is inherently complex. However, increasing zinc loading did not modify b -axis at $x > 0.2$ when refined employing the monoclinic system, suggesting a preferential site displacement, where crystal growth is greatly inhibited.

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PND

Rietveld refinements of PND data for $x = 0.1$ are consistent with carbonate incorporation as a mixed A-B-type [mechanism (4-3)], and water (H_2O) is simultaneously inserted to yield the composition $[\text{Ca}_4]^{\text{F}}[\text{Ca}_{5.9}\text{Zn}_{0.1}]^{\text{T}}[(\text{PO}_4)_{5.916}(\text{CO}_3)_{0.084}][(\text{OH})_{1.8-y}(\text{CO}_3)_{0.099}(\text{H}_2\text{O})_y]$ (Table 4-2). The proportions of A and B-type carbonate are consistent with FTIR spectroscopy.

The a -edge increases initially ($0 < x < 0.3$) due to the cooperative removal of CO_3^{2-} and H_2O masking the effect of Zn^{2+} substitution. From $x = 0.3$ to the solid solubility limit ($x \sim 0.5$), participation of carbonate is generally restricted and zinc (Zn^{2+}) is incorporated into the tunnel Ca^{T} site by a simple isovalent substitution ($\text{Ca}^{2+} \rightarrow \text{Zn}^{2+}$) to form $[\text{Ca}_4]^{\text{F}}[\text{Ca}_{10-x}\text{Zn}_x]^{\text{T}}[(\text{PO}_4)_6][(\text{OH})_{2-y}(\text{H}_2\text{O})_y]$ ($0.2 < x \leq 0.5$) (Table 4-3), leading to a decrease in the lattice parameter a and unit cell volume as smaller Zn^{2+} replaces Ca^{2+} [Figure 4-5 (a)]. Transformation to an oxyhydroxyapatite (OAp) as observed by FTIR was not confirmed and possible phase transformation from $2H$ to $2M$ or $2A$ pseudomorphs could not be validated by PND.

An amorphous component was detected in all low-carbonate-content-Zn-HAps ($0.2 \leq x \leq 0.5$) (10 to 20 wt%) [Figure 4-5 (c) inset]. The c -axis contracts and the crystal size decreases [Figure 4-5 (b) and (c)] with increasing zinc loading. The fractional coordinates and bond lengths are within anticipated ranges [Tables 4-3 and 4-4]. PND refinements show strong preference for Zn^{2+} to enter the tunnel Ca^{T} position, with particle size broadening of reflections peaks, and the amorphous phase limiting accuracy. Although FTIR revealed trace carbonate at higher zinc loading, the content falls below the detection limit of neutron diffraction.

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Figure 4-5. Crystallographic information of Zn-HAp series refined from PND data. The replacement of smaller Zn^{2+} in place of Ca^{2+} is inevitably intricate. The (a) a -edge and (b) unit cell volume show clear discontinuity, increasing up to $x = 0.3$ and contracts with further zinc incorporation. The reduction in crystal size is consistent with zinc substitution and X-ray investigations (inset). (c) The c -edge contract systematically with increasing zinc, while a non-diffracting amorphous phase is present at $x > 0.1$ ranging from 10 to 20 wt% (inset). (d) Presence of Zn^{2+} lead to a more acute metaprisim twist angle ($\phi \approx 23^\circ$), and additional crystallographic H_2O is associated with a smaller ϕ (inset) that is evidenced in Zn-CHAp ($x = 0.1$).

Table 4-2. Atomic coordinates, site occupancy and isotropic displacement parameters (Å²) of Zn-HAp ($x = 0.1$).

	Wyckoff symbol	site occupancy	x	y	z	B _{iso}
Ca ^F	4f	1.0 *	2/3	1/3	0.002(2)	1.7(3)
Ca ^T	6h	0.95(6)	0.991(1)	0.2429(9)	0.25	0.7(1)
P	6h	0.986(11)	0.3682(8)	0.3974(8)	0.25	1.31(6)
O1	6h	0.986(11)	0.4877(8)	0.3296(6)	0.25	1.31(6)
O2	6h	0.986(11)	0.4660(8)	0.5873(5)	0.25	1.31(6)
O3	12i	0.986(11)	0.2574(6)	0.3425(6)	0.0689(6)	1.31(6)
O4	4e	0.451(5)	0	0	0.189(3)	1.9(3)
H	4e	0.451(5)	0	0	0.056(4)	1.9(3)
C1 (type B)	6h	0.007(1)	0.352*	0.362*	0.25	0.05 *
C2 (type B)	6h	0.007(1)	0.438*	0.355*	0.25	0.05 *
O(B)	12i	0.021(1)	0.36*	0.28*	0.09*	0.05 *
C3 (type A)	2e	0.049(5)	0	0	0	0.05 *
O (A1)	12i	0.025(5)	0.99(3)	1.00(5)	0.66(2)	0.05 *
O (A2)	12i	0.025(5)	0.93(2)	0.13(2)	0.55(3)	0.05 *

*Not refined

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Table 4-3. Lattice parameters and crystallographic data for $[\text{Ca}_{10-x}\text{Zn}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (600 °C).

Composition x	0.1	0.2	0.3	0.4	0.5
Space Group	$P6_3/m$	$P6_3/m$	$P6_3/m$	$P6_3/m$	$P6_3/m$
a (Å)	9.398(1)	9.427(1)	9.442(2)	9.441(2)	9.427(2)
c (Å)	6.877(1)	6.876(1)	6.873(2)	6.870(2)	6.864(2)
V (Å ³)	526.0(2)	529.2(2)	530.7(3)	530.3(3)	528.3(3)
R_p	2.37	2.41	2.71	2.73	2.42
R_{wp}	3.12	3.10	3.48	3.47	3.16
GOF	1.37	1.29	1.45	1.38	1.39
R_B	0.92	1.00	1.29	1.19	0.98
Crystal Size (nm)	19.1(5)	20.3(5)	13.7(3)	14.2(4)	13.1(4)
Ca^F , $4f$ (1/3, 2/3, z)					
z		-0.003(2)	-0.003(2)	-0.003(2)	0.001(2)
B iso		1.70(28)	1.78(34)	1.37(32)	2.05(39)
Ca^T , $6h$ (x, y, 1/4)					
x		0.243(1)	0.243(1)	0.239(1)	0.244(1)
y		0.993(1)	0.989(1)	0.991(1)	0.988(1)
Occ		0.906(64)	0.859(74)	0.806(60)	0.758(60)
Occ (Zn)		0.094	0.141	0.194	0.242
B iso		0.80(13)	0.95(16)	1.11(16)	0.97(17)
P, $6h$ (x, y, 1/4)					
x		0.3968(9)	0.393(1)	0.394(1)	0.397(1)
y		0.3682(8)	0.366(1)	0.367(1)	0.367(1)
Occ		1.00(1)	1.00(1)	1.00(1)	1.00(1)
B iso		1.14(6)	1.34(7)	1.35(7)	1.36(8)
O(1), $6h$ (x, y, 1/4)					
x		0.3297(6)	0.3342(8)	0.3320(8)	0.3330(9)
y		0.4859(8)	0.4893(9)	0.4885(9)	0.4884(11)
B iso		1.14(6)	1.34(7)	1.35(7)	1.36(8)
O(2), $6h$ (x, y, 1/4)					
x		0.5863(7)	0.5823(8)	0.5830(8)	0.5856(9)
y		0.4598(9)	0.4585(10)	0.457(1)	0.460(1)
B iso		1.14(6)	1.34(7)	1.35(7)	1.36(8)
O(3), $12i$ (x, y, z)					
x		0.3433(6)	0.3405(7)	0.3445(7)	0.3446(7)
y		0.2538(6)	0.2486(7)	0.2541(7)	0.2542(8)
z		0.0717(6)	0.0750(7)	0.0715(7)	0.0696(8)
B iso		1.14(6)	1.34(7)	1.35(7)	1.36(8)
O(4), $4e$ (0, 0, z)					
z		0.187(3)	0.170(3)	0.170(3)	0.172(4)
Occ		0.49(2)	0.50(2)	0.50(2)	0.50(2)
B iso		1.38(26)	2.96(45)	2.09(35)	2.39(43)
H, $4e$ (0, 0, z)					
z		0.062(4)	0.072(5)	0.075(5)	0.074(5)
B iso		1.38(26)	2.96(45)	2.09(35)	2.39(43)

† Agreement measures are $R_{wp} = \sqrt{\frac{\sum_i w_i(y_i(obs) - y_i(calc))^2}{\sum_i w_i(y_i(obs))^2}}$ and $R_B = \frac{\sum_k I_k(obs) - I_k(calc)}{\sum_k I_k(obs)}$.

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Table 4-4. Metaprism twist angle (φ), bond length and bond angle for $[\text{Ca}_{10-x}\text{Zn}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (600 °C).

Composition x	0.1	0.2	0.3	0.4	0.5
φ	23.0	23.7	23.3	23.1	22.9
$\text{Ca}^{\text{F}}\text{-O}(1)$	2.383(2)	2.422(5)	2.415(6)	2.412(3)	2.397(3)
$\text{Ca}^{\text{F}}\text{-O}(2)$	2.454(5)	2.407(5)	2.420(6)	2.408(4)	2.427(7)
$\text{Ca}^{\text{F}}\text{-O}(3)$	2.802(8)	2.792(8)	2.811(7)	2.785(1)	2.783(7)
$\text{Ca}^{\text{T}}\text{-O}(4)$	2.348(3)	2.362(3)	2.415(5)	2.360(5)	2.418(5)
P-O(1)	1.535(5)	1.527(7)	1.522(9)	1.526(6)	1.533(6)
P-O(2)	1.546(3)	1.547(1)	1.545(2)	1.548(7)	1.541(2)
P-O(3) x 2	1.545(3)	1.542(3)	1.540(4)	1.536(4)	1.545(4)
mean P-O	1.543	1.540	1.537	1.537	1.541
O(1)-P-O(3)	112.06(7)	113.54(5)	112.6(4)	115.15(9)	113.9(1)
O(1)-P-O(2)	110.8(2)	112.13(9)	109.2(1)	111.26(1)	110.5(2)
O(3)-P-O(2)	106.98(4)	105.83(4)	105.86(4)	104.30(7)	105.5(2)
O(3)-P-O(3)	107.8(2)	105.3(2)	102.7(4)	106.0(2)	106.52(4)

4.3.2 Crystal Chemistry

Zinc in Hydroxyapatite

Crystal chemical percepts suggest that Zn^{2+} will preferentially occupy the framework Ca^{F} site. As examined in Chapter 2, the relatively low bond valence sum (BVS) of Zn^{2+} at both framework and tunnel sites is indicative of an underbonded environment. Thus, a degree of structural relaxation will be required, even though Zn^{2+} is incorporated via a simple isovalent substitution, without the need of charge coupled replacements, vacancies or interstitial species.

In this study, Zn^{2+} was introduced into apatite up to $x = 0.5$, with preferential partitioning to the tunnel Ca^{T} site. With the exception of $x = 0.1$ where A-B-type carbonate masked the average coordination of framework oxygens, the average $\text{Ca}^{\text{F}}\text{-O}(1)$ bond lengths decreases, although the $\text{Ca}^{\text{F}}\text{O}_6$ polyhedral volume remains invariant with zinc loading. On the other hand, an expansion in $\text{Ca}^{\text{T}}\text{O}_6\text{X}$ polyhedral volume is indicative of a Ca^{T} site distortion. The PO_4 tetrahedra resist distortion due to relatively rigid P-O bonds (Figure 4-6, Table 4-4). These bond length analyses were reliable with a precision of $\pm 0.001 \text{ \AA}$ (excluding $x = 0.1$), that possibly overestimates the absolute accuracy.

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The incorporation of zinc synergistically excludes both A and B-type carbonates which preserves overall framework and tunnel stoichiometry. These crystallographic modifications modify bond lengths, and a transformation to lower symmetry ($2M$ or $2A$) pseudomorphs cannot be excluded. Although the diffraction patterns showed neither splitting of the Bragg reflections, or anisotropic peak broadening, indicative of a reduction in symmetry, the broad peaks would mask phase transformations (Figure 4-7). Refinements using lower symmetry space group models improve fitting quality slightly (reflected in agreement factors such as R_{bragg}) due to the greater number of refineable parameters. Although $2M$ or $2A$ pseudomorphs cannot be observed unambiguously, the anomalies in unit cell parameters require further investigations (e.g. single crystal growth) to completely assess the crystal structure.

Twist Angle Systematics

Should Zn^{2+} (ionic radius $\text{Zn}^{2+} = 0.74\text{\AA}$) partition to the tunnel Ca^{T} site without the participation of extra-stoichiometric species (e.g. A and B-type carbonate) φ is expected to become obtuse as observed for $0 < x < 0.2$. However, for $x > 0.2$ the incorporation of water within the tunnel proved dominant and lead to tunnel dilation ($\varphi \sim 23^\circ$) [Figure 4-5 (d)]. In the $2H$ pseudomorphs where smaller cations are replacing the tunnel Ca^{2+} , it would be expected that φ values will be less than pure HAp $[\text{Ca}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$ ($\varphi = 24.8^\circ$) [146]. For example in lithogenic ferrous-HAp (ionic radius $\text{Fe}^{2+} = 0.78\text{\AA}$) $[\text{Ca}_{9.8}\text{Fe}^{2+}_{0.2}][(\text{PO}_4)_6][(\text{OH})_2]$ it is found that $\varphi = 22.9^\circ$ [147]. The inclusion of tunnel water that expands the channel is also associated with more acute φ ; for example, in $[\text{Ca}_{10-x}][(\text{PO}_4)_6][(\text{H}_2\text{O})_y(\text{OH})_{2-y}]$ ($1.8 < y < 2.2$), φ decreases from 24.4° to 23.0° with more water molecules per formula unit (pfu) [16] [Figure 4-5 (d) inset].

Carbonate

The study of carbonate apatites (CAp) is limited due to the inherently small crystallite size, and complicated by crystallographically incorporated and

surface adsorbed species [4]. Carbonate ordering can promote the transformation to lower symmetry [148] but in general is not observed. Carbonate (both A and B-type) was introduced only in the PND Rietveld refinement of Zn-HAp ($x = 0.1$) where a significant vibration band was detected in the infra-red spectroscopy. The atomic coordinates of carbon were fixed while the occupancy was constrained to stoichiometric proportions in the FTIR analysis. A correlation between Zn^{2+} and CO_3^{2-} content could not be established, and a combination of diffraction and X-ray spectroscopies (e.g. XANES) would be required to extract such information.

4.3.3 Nanostructures and Chemical Analysis

Both electron imaging and X-ray microanalysis of Zn-HAp by electron microscopy are challenging. Lattice imaging of the Zn-HAp series, similar to that employed for Fe-HAp could have confirmed crystal size, but vulnerability to electron beam damage, usually accompanied by amorphization, prevented such observations. For $x = 0.4$, embedding in epoxy resins prior to TEM preserved the structure for an extended period, although image quality was slightly compromised, as the samples were thicker than usual, and consequently, crystalline and amorphous regions could not be differentiated. However, TEM-EDS confirmed the absence of Zn-rich phases (e.g. ZnO), and the semi-quantitative data averaged over 10 area scans are indicative of zinc incorporation into apatite (Figure 4-8).

Microanalysis of Zn-HAp ($x = 0.4$ and 0.5) by SEM combined with EDS confirms zinc incorporation, but quantification was not possible. A systematic overestimation of zinc content (at%) is revealed in data normalized to stoichiometric proportions (6 apfu of phosphorous), in addition to magnesium contamination (~ 0.03 at%) (Table 4-5). The powders were pelletized prior to embedding into epoxy resins to maximize the characteristic X-ray signal that is observed in the chemical analyses of Mg-HAps (see section 5.3.4). However, these porous materials are susceptible to phase transformation under

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electron irradiation, which may contribute to the variations in the analysis (e.g. large standard deviations) and underestimation of the metal content.

Figure 4-6. Polyhedral volume (Å³) reflects changes in Zn²⁺ loading x (at %) systematically. (a) The channel Ca^TO₆X polyhedra dilates with increasing zinc incorporation, while (b) the Ca^FO₆ metaprisms is invariant, suggesting no major framework modification is taking place. The PO₄ tetrahedra are generally indiscreet to unit cell volume changes as it is refined as semi-rigid bodies (inset). (c) The trends are consistent with unit cell volume adjustments.

Figure 4-7. Partial PND pattern (25-29 and 39-45° 2 θ) of Zn-HAp ($x = 0, 0.1$ and 0.5) calcined at 600 °C showing the (a) (0 0 2) and (b) (1 3 0) peaks. Diminishing diffracted intensity and peak broadening are attributed to zinc incorporation which inhibited crystal growth. No peak separation was observed at the strongest (1 3 0) diffraction.

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Figure 4-8. An electron transparent section of Zn-HAp ($x = 0.4$) prepared from powder dispersed in epoxy resin. The acicular morphology is evidential of an apatite phase, and the nanocrystals are porous although electron beam damage is contributing to the effect. The semi-quantitative EDS analysis (inset, normalized to wt% oxides) is indicative of zinc incorporation.

Table 4-5. Normalized microanalysis data of Zn-HAp ($x = 0.4$ and 0.5) obtained from EDS. The mass percentage is normalized to stoichiometric phosphorous proportions (i.e. 6 atoms). Large variations in the standard deviations are indicative of the shortcoming in this technique, possessing difficulties for analysis nanocrystalline materials.

element	$x = 0.4$	$x = 0.5$
	atoms per formula unit (apfu)	
Ca	8.0 ± 0.2	8.2 ± 0.7
Zn	0.70 ± 0.03	0.77 ± 0.08
O	23.7 ± 0.2	24.0 ± 0.8
P	6	6
Mg	0.04 ± 0.09	0.04 ± 0.06

4.4 Functionality: Catalytic Properties

DRIFTS

The *in situ* DRIFTS spectra of Zn-HAp ($x = 0.1$) recorded during heat treatment are analogous to conventional FTIR scans. As no significant diminution of carbonate (CO_3^{2-} , $\nu_2 \sim 870$ and $\nu_3 \sim 1400 \text{ cm}^{-1}$) accompanies heating at 400°C , it is presumed these species are crystallographically incorporated. In contrast, moisture removal and dehydroxylation are consistent with diminished bands at $\sim 1640 \text{ cm}^{-1}$ (H-O-H bending vibration of H_2O) and $\sim 3570 \text{ cm}^{-1}$. An additional peak, that appeared at $\sim 1710 \text{ cm}^{-1}$ after heat treatment (* in Figure 4-9), is attributable to surface adsorption of helium

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(He) on Zn-HAp ($x = 0.1$), that is later desorbed at $> 300\text{ }^{\circ}\text{C}$ (Figure 4-9). The active site for helium attachment is regenerated from the removal of surface-adsorbed water molecules.

Using the same sample, *in situ* DRIFTS measurement of carbon monoxide (CO) adsorption was examined (Figure 4-10). In the spectrum obtained via KM-corrected background removal, a peak appearing at $\sim 1711\text{ cm}^{-1}$ is attributed to surface adsorption of CO analogous to the Zn-HAp–He interaction [149], suggesting a similar bonding environment between Zn-HAp and CO. When the same spectrum was corrected using the heat-treated-sample (‘after $400\text{ }^{\circ}\text{C} + 2\text{h}$ ’) to remove background (Figure 4-11) peaks at $\sim 2174\text{ cm}^{-1}$ and $\sim 2183\text{ cm}^{-1}$ could be assigned to Ca^{2+} – CO carbonyls, with splitting arising from the electrostatic interaction of surface and bulk species [150]. The peaks at $\sim 1711\text{ cm}^{-1}$ and $\sim 1720\text{ cm}^{-1}$ are assigned to a Zn^{2+} –CO bridged carbonyl vibration [145]. Features at $\sim 2108\text{ cm}^{-1}$ and $\sim 2118\text{ cm}^{-1}$ are interpreted as adsorption of CO on surface active Lewis acid sites (P – OH) by carbon [150]. Surface adsorbed water appears at $\sim 1650\text{ cm}^{-1}$, while bands at $\sim 1692\text{ cm}^{-1}$, $\sim 2336\text{ cm}^{-1}$ and $\sim 2360\text{ cm}^{-1}$ are assigned to carbon dioxide (CO_2) adsorbed on cationic species ($\text{Ca}^{2+}/\text{Zn}^{2+}$) by dipole interactions [151].

The restoration of the hydroxyl band at $\sim 3570\text{ cm}^{-1}$ suggests the presence of surface protonic acidity creating additional active OH sites [150]. The formation of CO_2 , which suggests oxidation of adsorbed species is an indication of CO adsorption over the transition metal modified surface. From this, it is inferred that a proportional amount of hydroxyl group is accessible by CO for adsorption and/or available in the form of surface acidic P – OH sites, promoting the formation of H_2O and CO_2 .

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Figure 4-9. Sequence of in situ DRIFTS spectra for Zn-HAp ($x = 0.1$) recorded at various temperature. Heat treatment was instigated from 25 °C (RT) to 120 °C and dwell for 2 h (denoted as '120 °C + 2h') to remove surface adsorbed species. After cooling to RT, the sample was heated to 100, 200, 300, and 400 °C, respectively, at a rate of 5 °C/min. * is attributed to surface adsorbed helium (He) on Zn-HAp ($x = 0.1$).

Figure 4-10. In situ DRIFTS spectrum of room temperature carbon monoxide (CO) adsorption on Zn-HAp ($x = 0.1$) recorded after 5 min (denoted as 'after 5 min CO'). The peak at 1711 cm^{-1} is attributed to a Zn^{2+} -CO bonding environment. Background corrected spectra of the as-synthesized sample before ('no treatment') and after heat treatment ('after 400 °C + 2h') in Helium are inserted for comparison. The removal of water is depicted in the diminishing band at $\sim 1640 \text{ cm}^{-1}$.

Figure 4-11. In situ DRIFTS spectrum of room temperature carbon monoxide (CO) adsorption on Zn-HAp ($x = 0.1$), background corrected by spectrum of the heat treated sample ('after 400 °C + 2h'). All peaks present thereafter are characteristics of CO adsorption on different active sites of Zn-HAp ($x = 0.1$).

TPD

Temperature programmed desorption (TPD) of carbon dioxide (CO_2) was carried out to examine the surface activity of Zn-HAp, particularly with respect to the proportion of basic sites. Water ($m/e = 18$) is always released simultaneously with the desorption of CO_2 ($m/e = 44$) at ~ 72 °C, suggesting moisture is adsorbed onto a substantial proportion of surface active sites, that do not favor the attachment of CO_2 (Figure 4-12). As-synthesized pure HAp and Zn-HAp exhibit higher surface activity due to the porous nature of the materials compared to commercially available pure Hap (Merck), and can capture more CO_2 (Table 4-6). However, increasing zinc loading shows no improvement in CO_2 uptake, implying the local environment of Zn^{2+} is not basic. For comparison lime (CaO , Merck) which is a typical basic catalyst effectively takes up CO_2 (0.167 mmol/g). No additional products were detected in the desorption profile beyond 200 °C, that would be indicative of a specific active site available for adsorption/desorption of CO_2 . However, XRD analysis of Zn-HAp samples after one TPD cycle found ZnO ($x = 0.1$) and β -TCP ($x = 0.5$) (Table 4-7).

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In the second desorption cycle, the poorer adsorption characteristics for pure HAp ($x = 0$) would be consistent with saturation of the surface active site due to accumulation of CO_2 from the previous cycle. But for $x = 0.1$, the increase in CO_2 uptake in the second cycle is attributed to the coexisting nanocrystalline ZnO, and the relatively few basic sites of Zn- β -TCP ($x = 0.5$) [152].

It is inferred from the DRIFTS and TPD experiments that the incorporation of Zn^{2+} into HAp generates surface acidic sites, in addition to the existing Ca^{2+} basic site and the P – OH acidic site for catalytic reaction. Although the two crystallographic Ca^{2+} sites could not be differentiated, the results demonstrate modification of the amphoteric properties of HAp in catalytic reactions.

Figure 4-12. Desorption profiles of (a) as synthesized pure HAp, (b) Zn-HAp ($x = 0.1$) and (c) Zn-HAp ($x = 0.5$). The presence of zinc did not introduce new surface adsorption sites and also no significant changes to the desorption temperature.

Table 4-6. Total CO_2 uptake of various catalysts calculated from TPD experiments. Commercial HAp contains relatively less number of surface basicity sites than synthesized powders. Introduction of Zn^{2+} ($x = 0.1$ and 0.5) does not significantly improve the amount of active basic sites.

Nominal x (at%)	CO_2 concentration (mmol/g)	
	cycle 1	cycle 2
0 (commercial)	2.09×10^{-5}	N.A.
0 (synthesized)	4.92×10^{-3}	3.33×10^{-3}
0.1	1.16×10^{-4}	6.13×10^{-3}
0.5	1.48×10^{-4}	4.06×10^{-5}

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Table 4-7. X-ray analysis of the catalysts before and after TPD experiments. Quantitative phase analysis (QPA) for samples obtained after TPD was not spiked with fluorite. For $x = 0.1$, the crystallization of ZnO and crystal growth of Zn-free-HAp are similarly observed when the powders underwent heat treatment (>600 °C, see section 4.3.1).

Nominal x (at%)		CO ₂ TPD (550 °C)	
		before	after
0.1	Crystal size (nm)	20	34
	QPA (wt%)	100Ap	98.5Ap + 1.5ZnO
0.5	Crystal size (nm)	13	89
	QPA (wt%)	100Ap	100TCP

IPA decomposition

Catalytic dehydration or dehydrogenation of 2-propanol (IPA) is controlled by the surface reactivity of the catalyst, and both first-order reactions proceed in parallel. Dehydration that produces propene ($\text{CH}_3\text{CH}=\text{CH}_2$) and water, occurs on the acidic sites, while dehydrogenation is favored on basic sites generating acetone [$(\text{CH}_3)_2\text{CH}=\text{O}$] and hydrogen (H_2) [153, 154].

The catalytic activities of pure HAp and Zn-HAp were studied by detecting the gaseous products in the reaction, i.e. hydrogen (H_2) in dehydrogenation and propene ($\text{CH}_3\text{CH}=\text{CH}_2$) in dehydration. Increasing zinc loading ($x = 0.5$) enhances the dehydration reaction, that is reflected in the selectivity towards propene [Zn-HAp ($x = 0.5$) > pure HAp] [Figure 4-13 (a), (b)]. The overall activity (selectivity and yield) of Zn-HAp ($x = 0.1$) closely resemble pure HAp, probably due to the presence of carbonate that inhibited the regeneration of surface active sites. Higher reaction temperatures moderately improve yield and selectivity towards H_2 , but for Zn-HAp ($x = 0.1$), the superior yield beyond 250 °C is attributed to the precipitation of zinc oxide (ZnO) that could increase the specific surface area and generate additional active sites for the reaction [Figure 4-13 (c), (d)] [155].

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From this, it is deduced that the introduction of zinc into HAp proved beneficial in catalyzing the dehydration of IPA, but the contribution might be over estimated because crystal growth is inhibited in Zn-HAp that increased the specific surface area (SSA) of these nanocrystalline materials and improved performance [155]. The role of ZnO as co-catalysts requires further clarification and investigation (e.g. SSA measurement, IPA-TPD) to better correlate the effect of Zn^{2+} in the reaction.

Figure 4-13. Catalytic decomposition of IPA with Zn-HAp show advantageous in the selectivity (%) towards propene (C_3H_6) but comparable yield (%) at various reaction temperature. The abrupt increase of yield for Zn-HAp ($x = 0.1$) beyond 250 °C attributed to leaching of Zn^{2+} forming of zinc oxide (ZnO) that increased the surface active sites.

4.5 Conclusions

Crystal chemistry

The crystal structures of Zn-HAps have been studied by diffraction, electron microscopy and spectroscopy. The Zn-HAp phase boundary is dependent on zinc loading and calcination temperature. Crystal growth is greatly inhibited when Zn^{2+} preferentially enters the tunnel Ca^{T} site. At low zinc content, atmospheric carbonate is incorporated concomitantly displacing both the phosphate and hydroxyl groups to yield

Carbonate is eliminated with increasing zinc loading stabilizing a *2H* pseudomorph of an ideal crystal chemical formula

Stabilization of a *2M* pseudomorph is possible, but could not be substantiated presently, and the space group of Zn-HAp-*2H* is $P6_3/m$. The presence of amorphous content (~10-20 wt%) is detected consistently in quantitative PND.

Catalytic Functionality

The surface chemistry of Zn-HAps was studied using DRIFTS and TPD. The catalytic performance of Zn-HAp in decomposition of IPA is distinguishable from pure HAp because of the introduction of new active site for surface adsorption. However, these materials possess low thermal stability that should be overcome in order to fully explore its potential as a novel base catalyst.

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Magnesium Hydroxyapatite

Magnesium (Mg^{2+}) enters hydroxyapatite by a direct isovalent displacement of framework calcium (Ca^{2+}). Infra-red spectroscopy shows a gradual transformation into oxy-apatite with increasing Mg^{2+} loading, while powder neutron diffraction confirmed retention of hexagonal $P6_3/m$ symmetry. Both the nano-crystalline powder, and a macroporous foam scaffold for bone regeneration, promote osteoblast proliferation more effectively than HAp.

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Magnesium hydroxyapatite (Mg-HAp) has been extensively studied due to its essential role in bone health and metabolism [112]. Found in enamel, dentin and skeletal tissue, magnesium (Mg^{2+}) inhibits dental caries and promotes biomineralization [156]. Mg^{2+} -based catalysts are also effective in oxidative coupling of methane and decomposition of volatile organic compounds (VOCs) [157, 158]. Here, a method for fabricating nano-crystalline and macroporous Mg-HAp is described and the crystal chemistry investigated. The bioactivity and catalytic efficacy of these materials are also explored.

5.1 Magnesium in Hydroxyapatites

In *Chapter 2* it was pointed out that, in common with first row transition metals, Mg^{2+} inhibits HAp crystal growth, decreases thermal stability, and favors the formation β -TCP at higher concentrations. The excipient properties of HAp can be manipulated through the systematic incorporation of magnesium via a simple isovalent substitution $\text{Ca}^{2+} \rightarrow \text{Mg}^{2+}$ (mechanism 2-12).

Unlike Fe^{3+} (*Chapter 3*) and Zn^{2+} (*Chapter 4*) pseudomorphism is not anticipated. Crystal chemistry suggests that the solid solubility limit will be low to satisfy bond valence criteria, with Mg^{2+} (0.72 Å) showing typical alkali earth characteristics. The site preference (Ca^{F} versus Ca^{T}) of Mg^{2+} remains arguable due to poor crystallinity and coexistence with amorphous phases [159, 160]. Prior studies yield a range of Mg solid solution limits [115, 116], and few investigations have examined site preference or lattice symmetry. Specifically, the incorporation of carbonate and water requires clarification [114], since the former is known to offset the destabilizing effects trace metals in biological HAp [111]. Bigi *et al* [117] using XRD Rietveld refinement found tunnel Ca^{T} site preference of Mg^{2+} in HAp for up to 10 at%, but this result contradicts all other crystallographic observations. A recent study combining *ab initio* simulation and X-ray techniques found the Ca^{F} site is preferred over the tunnel Ca^{T} site, with the expected contraction of unit cell

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parameters [53] (Figure 5-1). Mg-HAp has been prepared by precipitation from a variety of precursors in accord with the reaction,

although $x \leq 1$ is probably the limit of Mg uptake. In some studies, the solid solubility may have been overestimated because unrecognized β -TCP formation becomes prevalent with increasing magnesium content and higher temperature heat treatment [113]. Mg-HAp is challenging to synthesize single phase for baseline studies.

In this chapter, X-ray and neutron diffraction are combined with analytical scanning electron microscopy (ASEM) and FTIR spectroscopy to confirm the incorporation of magnesium in HAp, examine the distribution of Mg^{2+} between the Ca^{F} and Ca^{T} sites, and recognize tunnel non-stoichiometry. In addition, a novel macroporous ceramic foam was synthesized as a biomaterials, with cell growth monitored using confocal laser microscopy.

Figure 5-1. Compilation of published XRD data on Mg-HAp series compared against this work (♦: 700 °C and ▲: 600 °C) [53, 114-117, 158]. The variations in (a) a -edge and (b) unit cell volume are in accord with the general systematics reported although the solid solubility limit was variable. A decrease in lattice constant and volume is indicative of preferential framework Ca^{F} partitioning of Mg^{2+} , in contrast to Bigi *et al.*

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5.2 Experimental Methods

5.2.1 Synthesis

Nanocrystalline Powder

Mg-HAp of nominal composition $[\text{Ca}_{10-x}\text{Mg}_x^{2+}][(\text{PO}_4)_6][(\text{OH})_2]$ ($0 \leq x \leq 0.5$) was prepared from stoichiometric proportions of magnesium chloride tetrahydrate ($\text{MgCl}_2 \cdot 4\text{H}_2\text{O}$), calcium hydroxide $[\text{Ca}(\text{OH})_2]$ and orthophosphoric acid (H_3PO_4). The precursor was synthesized using the same approach as for Zn-HAp (Chapter 4) before calcination (600°C - 700°C) under flowing nitrogen using a heating rate of $1^\circ\text{C}/\text{min}$, dwell time of 2 hr, and finally cooling at $5^\circ\text{C}/\text{min}$.

Solid state reaction by stoichiometric precursors (calcium chloride, magnesium chloride and tri-calcium phosphate) was unsuccessful as this yielded a mixture of apatite and magnesium oxide (MgO).

Macroporous Foam

The porous Mg-HAp was fabricated in two-steps [65]: 1g of methyl cellulose was dissolved in 45 mL of deionized water by stirring vigorously overnight before mixing with an aqueous suspension of Mg-HAp (0.5 g/5 mL H_2O) using a stainless steel blade agitator for 15 mins at 2000 rpm. The foam was calcined in air (250°C , $0.7^\circ\text{C}/\text{min}$) for 0.5 h, then treated at 600°C ($3.33^\circ\text{C}/\text{min}$) for 5 h before cooling to ambient.

5.2.2 X-ray and Neutron Diffraction

Powder X-ray (XRD) and neutron diffraction (PND)

Mg-HAp products were manually ground with an agate mortar and pestle prior to XRD with the pattern recorded using Cu $K\alpha$ radiation generated by a Bruker D8-ADVANCE X-ray Diffractometer operating at 40 kV and 40 mA. Data were accumulated with a step scan increment of $0.02^\circ 2\theta$ and dwell time of 2 s over the angular range 10 to 130° . PND patterns were collected on the

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high resolution two-axis diffractometer (Echidna) at the Australian Nuclear Science and Technology Organization (ANSTO) Lucas Heights. Approximately 2 g of sample was loaded in 9 mm diameter vanadium cans, and diffraction data accumulated at room temperature from 5° to $159^\circ 2\theta$ in 0.05° steps using a neutron wavelength of 1.622 Å. The diffractometer was equipped with a focusing Ge-(335) monochromator and 128 standard ^3He counter detectors. The absolute phase proportions and cell metrics were calibrated against 10 wt% fluorite (CaF_2) as an internal standard in both XRD and PND experiments.

Rietveld Refinements

Rietveld analysis of the XRD data was performed with TOPAS V3.0 (Bruker-AXS) using the fundamental parameters approach, and $\text{P6}_3/m$ hydroxyapatite as the starting model. PND data were refined with a pseudo-Voigt peak shape profile from 10° to $150^\circ 2\theta$, and the parameters U, V, W constrained to be the same in both fluorite and Mg-HAp phases. For each data set, the refineable parameters were released in sequence: scale factor, zero shift, a six coefficient Chebychev polynomial, $1/x$ background, coherent scattering domain size (i.e. Lorentzian and Gaussian crystallite size), and unit cell parameters. The cation positions were established first, followed by oxygen, in order to achieve a smooth convergence. Site occupancies of all crystallographic positions were determined with constraints in accord with the substitution mechanism and stoichiometric crystallochemical formula (e.g. framework PO_4 and type B carbonate were totaled to 6 apfu). The PO_4 tetrahedra were refined as semi-rigid bodies. Tunnel A-type carbonate was introduced in the refinement of pure HAp ($x = 0$) according to the model of Fleet *et al* [123].

5.2.3 Fourier Transform Infrared Spectroscopy (FTIR)

Powders were mixed with oven dried potassium bromide (KBr) and pressed into pellets prior to FTIR absorption measurements performed over 16 scans across the wavenumber range of 400 and 4000 cm^{-1} with a Perkin Elmer (Spectrum 2000) spectrophotometer.

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5.2.4 Electron Microscopy and Microanalysis

The morphology of the macroporous Mg-HAp was examined by field emission scanning electron microscopy (JEOL 7600F) in secondary electron imaging mode at 15 kV. Compositions of the Mg-HAp series were determined by energy dispersive X-ray spectroscopy (EDS) at 15 kV and 1 nA using a SEM (Hitachi 4300) equipped with an Oxford Instruments light element EDS detector and INCA software. ASTM Mineral apatite and diopside were used as calibrants. All samples were embedded in epoxy resins, polished to near mirror finish and carbon coated prior to data accumulation that was averaged over 12 area scans.

5.2.5 Catalytic Activity

Mg-HAp samples were loaded in a micro-fixed-bed reactor configured similarly for catalytic testing of Zn-HAp. Samples were pre-treated at 300 °C in He at a flow rate of 20 mL/min for 2 h before air-cooling to ambient. IPA was fed at a rate of 0.2 mL/min and the reactor heated to various fixed temperatures (100, 150, 200, 250, 300 and 350 °C) for 1 h to equilibrate. Conversion rate and selectivity were calculated based on hydrogen and propene detected.

5.2.6 Bioactivity

Cell culture

MG-63 Osteosarcoma cells were cultured in Dubelcco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% streptomycin and incubated overnight at 37 °C in a humidified (5% CO₂/ 95% air) atmosphere in a T-75 flask. The medium was subsequently aspirated from the flask and washed with a phosphate buffer saline (PBS) before adding trypsin-EDTA to allow cell detachment. The cells were suspended in fresh

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medium and centrifuged to create pellets. The supernatant was discarded and the number of cells were counted using a hemacytometer.

Biocompatibility Testing

A 96-well culture plate was used for cell seeding at a density of 2×10^3 cells per well and cultivated in 100 μ L of DMEM. The cells were incubated with the medium containing Mg-HAp ($x = 0$ and 0.4, both nano-crystalline and macroporous architecture), that was sterilized under ultraviolet light overnight prior to cell seeding, and the medium replaced every two days with total incubation times ranging from 24 h to 168 h. Cell viability of Mg-HAp was analyzed using Owen's reagent [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] as the inner salt (MTS assay, CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega). This colorimetric method determines the number of metabolically active cells by monitoring the reduction of the tetrazolium salt by dehydrogenase enzymes to a soluble formazan, in the presence of an electron-coupling agent, and measuring the absorbance of formazan (490 nm) directly from the plate. After each incubation interval (24, 72, 120, 168 h), 100 μ L of Owen's reagent was added to the wells and incubated under the same condition for 4 hr prior to transferring the supernatants to a 96-well plate for absorbance readings in a MicroKinetics plate reader (TECAN). The experiments were performed in triplicate.

Cell Proliferation Studies and Confocal Microscopy

For SEM and confocal laser microscope observation, a 6-well culture plate was used for cell seeding at a density of 1×10^4 cells per well on a thin cover slip (1 in²) and cultivated in 400 μ L of DMEM. Powder Mg-HAp ($x = 0$, 0.2 and 0.4, both nano-crystalline and macroporous architecture) was sterilized under ultraviolet light overnight prior to cell seeding. The well plate was incubated in CO₂, and an additional 600 μ L of medium was added to fully immerse the glass slides after 2 h, subsequently replaced every two days with total incubation times up to 168 h.

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SEM samples were prepared by first aspirating the medium and washing with 1000 μL of PBS three times before adding 300 μL of glutaraldehyde (2.5%) to allow chemical fixation for 1h. This was followed by thrice flushing with 1000 μL of PBS, immersing in 1 mL of ethanol (50%) for 15 mins, 1 mL of ethanol (70%) overnight, 1 mL of ethanol (95%) twice for 15 mins, and finally 1 mL of ethanol (100%) twice for 30 mins, before air drying after aspirating. The cover slips were removed from the well plate and carbon-coated prior to observation using a field emission SEM (7600F, JEOL) operating at 5 kV.

For confocal laser microscopy the samples were prepared by aspirating the medium and washed with 1000 μL of PBS twice before adding 500 μL of paraformaldehyde (4%) to allow chemical fixation for 15 mins. Next, paraformaldehyde was aspirated and rinsed with 500 μL and 1000 μL of PBS for 5 mins before adding 500 μL Triton X-100 (1%) for 10 mins. The F-actin of cultured cells on cover slips were fluorescence red stained with Alexa Fluor-633 phalloidin [Molecular Probes (Invitrogen)] diluted 1:100 in PBS prior to removal from the well plate, and mounted onto glass slides with antifade medium for observation using a confocal laser microscope (Leica TCS-SP). The cells were observed under a 63X objective oil immersion lens, beam splitter TT 488/568/630 with excitation and emission wavelength at 568 and 610 nm, respectively. The F-actin fibers were assessed by red fluorescence mode and the powder and foam images were captured in bright field (BF) mode using Leica software.

5.3 Crystal Structures of Magnesium-HAp Series

5.3.1 Chemical and Crystal Lattice Systematics

In the Mg-Ap series, the characteristic carbonate (CO_3^{2-} , $\nu_2 \sim 870$ and $\nu_3 \sim 1400$ cm^{-1}) and hydroxyl (OH^- , $\nu_s \sim 3500$ cm^{-1}) bands, for a given magnesium loading, were invariant in wavenumber with calcination temperature (600 and 700 $^\circ\text{C}$) [Figure 5-2 (a) and (b)]. However, the carbonate vibrations significantly attenuate at the onset of Mg^{2+} incorporation, and these lower intensity bands

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are consistent with A and B type carbonate. The hydroxyl feature progressively diminishes with increasing magnesium [Figure 5-2 (c)], due to tunnel sub-stoichiometry and a phase transformation from Mg-HAp to an oxyapatite Mg-OAp. Although decreasing crystal size may lead to diminution of characteristic vibration, the former is established by PND (see section 5.3.2).

FTIR spectroscopy of the macroporous Mg-HAp series show weaker CO_3^{2-} and OH^- fingerprint bands as compared to the powders [Figure 5-2 (d)]. This suggests the additional calcination process to oxidize the polymer foaming agent also removed carbonate, and modified the crystallographic orientation of the hydroxyl group, with attribution to an additional feature in the vicinity of the original hydroxyl vibration band ($\sim 3570 \text{ cm}^{-1}$) [4]. With increasing magnesium, diminution of these species fell to the detection limit of FTIR.

Figure 5-2. FTIR spectra of Mg-HAp calcined at (a) 600 °C, (b) 700 °C showing signature hydroxyl and mixed A-B type carbonate bands. (c) The hydroxyl feature diminishes with magnesium loading, while only a minute portion of carbonate is crystallographically bound at the onset of magnesium incorporation (dotted lines: 700 °C series; solid lines: 600 °C). Similar trends of hydroxyl band found in (d) macroporous Mg-HAp with increasing magnesium, but carbonate is below detection limit. Fabrication of the foam materials introduced an extra feature in the vicinity of the original hydroxyl band ($\sim 3570 \text{ cm}^{-1}$).

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The crystallinity of Mg-Ap for compositions $x = 0.1$ to 0.3 was enhanced at elevated calcination temperature, although higher magnesium content inhibited crystal growth ($x = 0.4$) [Figure 5-3 (c)]. For a given composition, greater crystallinity is accompanied by an increase in unit cell volume [Figure 5-3 (a)]. The apatite- β -TCP phase boundary was dependent on magnesium loading and heat treatment.

For all calcination temperatures, the lattice contracts isotropically along the a -edge with increasing magnesium loading as Mg^{2+} is smaller than Ca^{2+} . The c -edge is invariant, and overall the unit cell volume is larger at higher temperature [Figure 5-3 (a) and (b)]. The removal of carbonate in FTIR correlates with an inflection in lattice parameters upon introducing magnesium ($x > 0$). Rietveld analysis of the X-ray data indicated framework Ca^{F} site preference of Mg^{2+} , although the extent of substitution could not be reliably determined. Quantitative XRD revealed a constant amorphous content in all the powders, although X-ray microabsorption leads to an overestimation (< 35 wt%) of content.

All Mg-Ap foams were single-phase apatite after calcination, but with larger crystal size as confirmed by narrower diffraction peaks [Figure 5-4 (b)]. The lattice parameters (a and c) of the Mg-Ap foams are in accord with the systematics of the powder Mg-HAp samples (Table 5-1).

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Figure 5-3. Unit cell trends in Mg-HAp refined from XRD data. (a) The incorporation of Mg^{2+} leads to a decrease in a -edge and volume contraction (inset), in accord with a framework partitioning. For a given composition, higher calcination temperatures confer to a longer a -axis and lattice expansion. (b) The c -axis converges towards ~ 6.88 with increasing magnesium loading, reflecting an insignificant modification along the tunnel. Amorphous content arising from microabsorption are in the range of 30 and 40 wt% in all samples (inset). (d) crystal size were marginally larger at higher calcinations temperature, although increasing magnesium inhibited crystal growth ($x = 0.4$).

Figure 5-4. Partial XRD pattern ($20\text{--}45^\circ 2\theta$) of (a) Mg-HAp ($x = 0.1$ and 0.4) calcined at 700°C . Higher magnesium content lead to poor crystallinity as reflected in loss of diffracted intensity especially. The sharp peak at $\sim 28^\circ$ originated from fluorite. (b) Fabrication of Mg-HAp ($x = 0.4$, 600°C) foam require further heat treatment that produce marginally larger crystal size, improving total counts of the diffracted peaks. Note the separation at $\sim 32^\circ$ and 38° (marked with *).

Table 5-1. Refined lattice parameters from XRD data of Mg-HAp foam at different magnesium loading (x).

x	0	0.2	0.4
a (Å)	9.429(1)	9.4218(4)	9.4213(2)
c (Å)	6.8821(9)	6.8800(4)	6.8795(5)
V (Å ³)	529.9(1)	528.91(6)	528.82(7)
Crystal size (nm)	36(2)	51.2(8)	48(2)
R_{bragg}	2.2	4.1	3.8

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5.3.2 Structure Refinement by PND

At $x = 0$ (i.e. pure HAp), Rietveld refinements of PND data for powders calcined at 600 °C show that carbonate is incorporated as A-type [mechanism (2-7)] to yield the formula $[\text{Ca}_4^{\text{F}}\text{Ca}_6^{\text{T}}][(\text{PO}_4)_6][(\text{OH})_{1.48}(\text{CO}_3)_{0.26}]$. However, for powders calcined at 700 °C, a proportion of A-type carbonate is removed to yield the formula $[\text{Ca}_4^{\text{F}}\text{Ca}_6^{\text{T}}][(\text{PO}_4)_6][(\text{OH})_{1.74}(\text{CO}_3)_{0.13}]$ (Tables 5-2 and 5-3).

Up to $x = 0.3$, magnesium (Mg^{2+}) is incorporated into the lattice by an isovalent substitution [mechanism (5-1)] that gives rise to $[\text{Ca}_{4-x}\text{Mg}_x]^{\text{F}}[\text{Ca}_6]^{\text{T}}[(\text{PO}_4)_6][(\text{OH})_2]$ ($0 \leq x \leq 0.3$). However, beyond $x = 0.3$, the tunnel hydroxyl group is partially displaced by oxygen to yield an oxyhydroxyapatite (OAp): $[\text{Ca}_{3.6}\text{Mg}_{0.4}]^{\text{F}}[\text{Ca}_6]^{\text{T}}[(\text{PO}_4)_6][(\text{OH})_{2-y}\text{O}_y]$ ($0.08 \leq y \leq 0.12$) [Table 5-4 and 5-5]. Higher calcination temperatures enhance the crystallinity of these materials and lead to unit cell dilation, that improved diffraction pattern quality. Overall the unit cell contracts when smaller Mg^{2+} is incorporated, lead by a decrease in the a -edge, and with a lesser expansion of the c -axis. Isotropic displacement parameters (B_{iso}) for the framework Ca^{F} and tunnel O(4) sites also decrease with increasing magnesium loading [Figure 5-5 (a), (b) and (c)]. For all data sets, the fractional coordinates and bond lengths are within expected ranges [Table 5-6 and 5-7]. A phase transformation from $2H$ to $2M$ or $2A$ pseudomorphs was not indicated.

Both the lattice parameter trend and occupancy refinement suggest Mg^{2+} enters the Ca^{F} position, although the overall refined Mg content is possibly an overestimation, due to the comparable coherent neutron scattering lengths of $b_c(^{20}\text{Ca}) = 4.70$ and $b_c(^{12}\text{Mg}) = 5.38$. Both A and B-type carbonates are below the PND detection limit, although FTIR spectra show a minor contribution that might be attributed to surface adsorbed species. β -TCP and an amorphous component, if present, are below the sensitivity of PND.

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Figure 5-5. Crystallographic information of Mg-HAp series refined from PND data.. The incorporation of Mg^{2+} in place of larger Ca^{2+} at the framework site leads to (a) a -edge and (b) lattice volume contraction consistent with XRD analysis. For any given composition, the insertion of Mg^{2+} is stabilized at higher calcinations temperature with $B_{iso}-Ca^F$ smaller, when the crystal size is marginally larger (inset). (c) The c -axis dilates gradually with increasing magnesium loading, while the tunnel is more stabilized as depicted by a decreasing thermal parameters [$B_{iso}-O(4)$](inset). Pure HAp containing both A and B type carbonate significantly decreases the lattice parameters. (c) The unit cell contracts in accord with the reduction in the volume of apatite framework. (d) The simultaneous accommodation of smaller Mg^{2+} and tunnel expansion leading to more acute metaprisim twist angle (ϕ).

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Figure 5-6. Rietveld plot of the neutron diffraction data for Mg-HAp ($x = 0.1$) prepared via sintering under N_2 (700 °C/ 2h) and spiked with CaF_2 (10 wt %). The upper trace is an overlay of the experimental pattern (circles) and calculated intensity (continuous line) while the lower trace shows the intensity differences between the experimental and calculated patterns. Refinement is carried out in the 10-150° region and bottom-most are the Bragg markers for CaF_2 and Mg-HAp, respectively.

Table 5-2. Crystallographic data and selected carbonate bond lengths of pure HAp.

Calcination temperature (°C)	600	700
Space Group	$P6_3/m$	$P6_3/m$
a (Å)	9.387(1)	9.4002(6)
c (Å)	6.882(2)	6.8722(7)
V (Å ³)	525.2(2)	525.90(8)
R _p	3.23	18.98
R _{wp}	5.69	24.58
GOF	2.66	2.47
R _B	0.938	1.595
Crystal Size (nm)	19.6(6)	32.1(1)
A-type carbonate bond length (Å)		
C-O(A)	1.19(11)	1.13(10)
C-O(4)	1.30(10)	1.322(4)

† Agreement measures are $R_{wp} = \sqrt{\frac{\sum_i w_i(y_i(obs) - y_i(calc))^2}{\sum_i w_i(y_i(obs))^2}}$ and $R_B = \frac{\sum_k I_k(obs) - I_k(calc)}{\sum_k I_k(obs)}$.

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Table 5-3. Atomic coordinates, site occupancy and isotropic displacement parameters ($\text{\AA}^2$) of pure HAp.

	Wykoff symbol	site occupancy	x	y	z	B _{iso}
<u>700 °C</u>						
Ca ^F	4f	1.0 *	2/3	1/3	0.008(1)	1.5(2)
Ca ^T	6h	1.0 *	0.990(1)	0.2449(9)	0.25	0.8(9)
P	6h	0.979(8)	0.3684(5)	0.4001(6)	0.25	0.8(1)
O1	6h	0.979(8)	0.4815(6)	0.3251(5)	0.25	0.64(9)
O2	6h	0.979(8)	0.4636(6)	0.5880(5)	0.25	1.0(1)
O3	12i	0.979(8)	0.2527(4)	0.3410(4)	0.0710(5)	1.46(7)
O4	4e	0.468(4)	0	0	0.190(2)	1.9(2)
H	4e	0.468(4)	0	0	0.051(3)	1.9(2)
C (type A)	2e	0.031(4)	0	0	0	0.05 *
O (A1)	12i	0.016(4)	0.99(6)	1.00(2)	0.66(2)	0.05 *
O (A2)	12i	0.016(4)	0.95(2)	0.12(2)	0.55(3)	0.05 *
<u>600 °C</u>						
Ca ^F	4f	1.0 *	2/3	1/3	0.013(2)	0.9(3)
Ca ^T	6h	1.0 *	0.995(1)	0.2505(12)	0.25	0.3(2)
P	6h	1.00(9)	0.3684(9)	0.400(1)	0.25	1.4(2)
O1	6h	1.00(9)	0.4808(9)	0.3251(8)	0.25	0.8(2)
O2	6h	1.00(9)	0.4565(10)	0.5875(9)	0.25	1.0(2)
O3	12i	1.00(9)	0.2538(7)	0.3434(6)	0.0720(7)	1.6(1)
O4	4e	0.435(6)	0	0	0.188(4)	2.2(4)
H	4e	0.435(6)	0	0	0.052(5)	2.2(4)
C (type A)	2e	0.065(6)	0	0	0	0.05 *
O (A1)	12i	0.033(6)	0.978(14)	0.991(18)	0.666(17)	0.05 *
O (A2)	12i	0.033(6)	0.940(14)	0.165(16)	0.589(21)	0.05 *

*Not refined

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Table 5-4. Lattice parameters and crystallographic data for $[\text{Ca}_{10-x}\text{Mg}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (700 °C).

Composition x	0.1	0.2	0.3	0.4
Space Group	$P6_3/m$	$P6_3/m$	$P6_3/m$	$P6_3/m$
a (Å)	9.4104(9)	9.4084(8)	9.406(1)	9.405(1)
c (Å)	6.870(1)	6.8746(9)	6.875(1)	6.877(2)
V (Å ³)	526.9(1)	527.0(1)	526.8(1)	526.8(2)
R _p	4.27	4.11	3.51	3.55
R _{wp}	6.44	6.43	5.75	6.10
GOF	2.64	2.59	2.46	2.62
R _B	2.025	1.569	1.281	1.105
Crystal Size (nm)	24.4(7)	25.8(7)	21.4(6)	19.7(7)
Ca ^F , 4f (2/3, 1/3, z)				
Z	0.0036(15)	0.009(1)	0.014(2)	0.014(2)
Occ	0.90(13)	0.87(13)	0.84(13)	0.81(13)
Occ (Mg)	0.1	0.13	0.16	0.19
B iso	1.41(23)	1.00(20)	0.75(20)	0.83(26)
Ca ^T , 6h (x, y, 1/4)				
x	0.986(1)	0.9851(9)	0.986(1)	0.987(1)
y	0.2385(9)	0.2421(8)	0.2423(9)	0.243(1)
B iso	0.41(12)	0.35(10)	0.47(11)	0.23(14)
P, 6h (x, y, 1/4)				
x	0.3682(8)	0.3689(7)	0.3691(7)	0.3688(9)
y	0.3993(8)	0.3988(7)	0.3988(8)	0.3985(10)
Occ	1.00(1)	1.00(1)	1.00(1)	1.00(2)
B iso	1.07(6)	1.05(5)	1.17(6)	1.16(7)
O(1), 6h (x, y, 1/4)				
x	0.4843(7)	0.4840(7)	0.4840(7)	0.483(1)
y	0.3278(6)	0.3274(5)	0.3268(6)	0.3262(8)
B iso	1.07(6)	1.05(5)	1.17(6)	1.16(7)
O(2), 6h (x, y, 1/4)				
x	0.4628(8)	0.4615(7)	0.4640(8)	0.465(1)
y	0.5851(7)	0.5870(6)	0.5883(7)	0.5880(8)
B iso	1.07(6)	1.05(5)	1.17(6)	1.16(7)
O(3), 12i (x, y, z)				
x	0.2543(5)	0.2550(5)	0.2541(5)	0.2531(7)
y	0.3427(5)	0.3414(5)	0.3405(5)	0.3402(7)
z	0.0706(5)	0.0703(5)	0.0700(6)	0.0715(7)
B iso	1.07(6)	1.05(5)	1.17(6)	1.16(7)
O(4), 4e (0, 0, z)				
z	0.178(3)	0.178(2)	0.180(2)	0.176(3)
Occ	0.50(2)	0.50(2)	0.50(2)	0.48(2)
B iso	1.82(25)	1.71(22)	1.49(23)	1.23(29)
H, 4e (0, 0, z)				
z	0.058(4)	0.060(3)	0.061(3)	0.066(5)
B iso	1.82(25)	1.71(22)	1.49(23)	1.23(29)

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Table 5-5. Lattice parameters and crystallographic data for $[\text{Ca}_{10-x}\text{Mg}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (600 °C).

Composition x	0.1	0.2	0.3	0.4
Space Group	$P6_3/m$	$P6_3/m$	$P6_3/m$	$P6_3/m$
a (Å)	9.408(1)	9.404(1)	9.405(1)	9.402(1)
c (Å)	6.873(2)	6.875(1)	6.874(2)	6.876(2)
V (Å ³)	526.8(2)	526.6(2)	526.5(2)	526.4(2)
R _p	4.00	3.52	3.52	3.84
R _{wp}	6.28	6.07	5.80	6.29
GOF	2.68	2.73	2.71	2.81
R _B	1.417	1.229	1.256	1.401
Crystal Size (nm)	16.9(5)	20.7(7)	18.7(6)	18.4(6)
Ca ^F , 4f (2/3, 1/3, z)				
z	0.014(2)	0.013(2)	0.015(2)	0.014(2)
Occ	0.9	0.81	0.78	0.77
Occ (Mg)	0.1	0.19	0.22	0.23
B iso	1.38(28)	1.21(27)	1.25(25)	1.61(29)
Ca ^T , 6h (x, y, 1/4)				
x	0.987(1)	0.987(1)	0.987(1)	0.986(1)
y	0.245(2)	0.244(1)	0.243(1)	0.244(1)
B iso	0.48(14)	0.44(14)	0.37(14)	0.23(14)
P, 6h (x, y, 1/4)				
x	0.3673(9)	0.3681(9)	0.3672(9)	0.3661(9)
y	0.397(1)	0.397(1)	0.396(1)	0.395(1)
Occ	1.00(2)	0.99(1)	1.00(1)	0.98(1)
B iso	1.00(7)	1.00(7)	1.00(7)	1.06(7)
O(1), 6h (x, y, 1/4)				
x	0.4834(9)	0.4819(9)	0.4834(9)	0.485(1)
y	0.3269(7)	0.3241(7)	0.3270(7)	0.3287(8)
B iso	1.00(7)	1.00(7)	1.00(7)	1.06(7)
O(2), 6h (x, y, 1/4)				
x	0.459(1)	0.461(1)	0.4612(9)	0.460(1)
y	0.5857(8)	0.5875(9)	0.5860(8)	0.5844(9)
B iso	1.00(7)	1.00(7)	1.00(7)	1.06(7)
O(3), 12i (x, y, z)				
x	0.2539(7)	0.2520(7)	0.2517(6)	0.2514(7)
y	0.3431(6)	0.3424(6)	0.3413(6)	0.3441(6)
z	0.0708(7)	0.0725(7)	0.0709(7)	0.0708(7)
B iso	1.00(7)	1.00(7)	1.00(7)	1.06(7)
O(4), 4e (0, 0, z)				
z	0.176(3)	0.179(3)	0.180(3)	0.184(4)
Occ	0.50(2)	0.50(2)	0.50(2)	0.47(2)
B iso	1.66(32)	1.60(29)	1.32(26)	1.47(30)
H, 4e (0, 0, z)				
z	0.072(5)	0.070(4)	0.072(4)	0.069(5)
B iso	1.66(32)	1.60(29)	1.32(26)	1.47(30)

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Table 5-6. Metaprism twist angle (φ), bond length and bond angle for $[\text{Ca}_{10-x}\text{Mg}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (700 °C).

Composition x	0	0.1	0.2	0.3	0.4
φ	24.1	24.1	24	23.6	23.7
$\text{Ca}^{\text{F}}\text{-O}(1)$	2.379(2)	2.428(5)	2.366(4)	2.342(5)	2.348(6)
$\text{Ca}^{\text{F}}\text{-O}(2)$	2.462(4)	2.413(5)	2.469(4)	2.502(3)	2.508(4)
$\text{Ca}^{\text{F}}\text{-O}(3)$	2.815(4)	2.7901(7)	2.8200(8)	2.8310(9)	2.833(1)
$\text{Ca}^{\text{T}}\text{-O}(4)$	2.385(2)	2.364(3)	2.402(3)	2.399(3)	2.403(4)
P-O(1)	1.542(4)	1.543(6)	1.532(6)	1.536(6)	1.531(8)
P-O(2)	1.529(2)	1.529(5)	1.534(3)	1.541(5)	1.543(2)
P-O(3) x 2	1.549(1)	1.545(3)	1.546(3)	1.552(1)	1.548(4)
mean P-O	1.542	1.541	1.540	1.545	1.543
O(1)-P-O(3)	110.9(1)	111.7(7)	111.5(6)	111.3(1)	111.6(1)
O(1)-P-O(2)	112.9(1)	111.9(2)	112.9(1)	112.4(2)	112.1(1)
O(3)-P-O(2)	108.34(5)	107.5(7)	107.3(6)	107.9(7)	108.2(1)
O(3)-P-O(3)	105.12(1)	106.4(2)	106.1(2)	105.8(2)	104.9(2)

Table 5-7. Metaprism twist angle (φ), bond length and bond angle for $[\text{Ca}_{10-x}\text{Mg}_x][(\text{PO}_4)_6][(\text{OH})_2]$ (600 °C).

Composition x	0	0.1	0.2	0.3	0.4
φ	25.1	24.6	24.8	24.2	24.3
$\text{Ca}^{\text{F}}\text{-O}(1)$	2.361(3)	2.347(3)	2.356(6)	2.343(6)	2.344(3)
$\text{Ca}^{\text{F}}\text{-O}(2)$	2.455(6)	2.487(6)	2.488(6)	2.506(4)	2.494(4)
$\text{Ca}^{\text{F}}\text{-O}(3)$	2.800(6)	2.809(1)	2.810(1)	2.823(1)	2.791(1)
$\text{Ca}^{\text{T}}\text{-O}(4)$	2.412(4)	2.424(4)	2.404(4)	2.403(4)	2.403(4)
P-O(1)	1.534(6)	1.533(8)	1.532(8)	1.526(5)	1.526(5)
P-O(2)	1.524(7)	1.536(2)	1.547(7)	1.549(6)	1.542(7)
P-O(3) x 2	1.539(4)	1.540(3)	1.545(3)	1.549(4)	1.548(3)
mean P-O	1.534	1.537	1.542	1.543	1.541
O(1)-P-O(3)	111.35(9)	112.51(6)	112.77(6)	113.1(1)	114.3(1)
O(1)-P-O(2)	115.4(2)	112.7(1)	113.4(1)	111.9(2)	110.9(2)
O(3)-P-O(2)	106.31(8)	106.2(2)	106.38(9)	106.51(8)	105.46(9)
O(3)-P-O(3)	105.47(2)	106.24(3)	104.5(2)	105.2(2)	105.5(2)

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5.3.3 Crystal Chemistry

Magnesium Incorporation

In Mg-HAp, the bond valence sum (BVS) of Ca^{2+} at Ca^{F} ranges from 2.04 to 2.09, while the BVS of Ca^{2+} at Ca^{T} lies between 2.30 to 2.45. That is, the tunnel Ca^{T} is relatively overbonded. As expected, the BVSs of Mg^{2+} at both Ca^{F} and Ca^{T} are ~ 1.00 (Table 5-8), and this significant underbonding, without consideration of local relaxation, largely explains the limited solid solubility. In addition, general crystal chemical percepts argue for less voluminous ionically bonded cations to favor the framework Ca^{F} site, although the accommodation of Mg^{2+} will distort the metaprism.

In the present experiments, the solid solubility limit was not reached, and Mg^{2+} partitions exclusively to the framework Ca^{F} site up to $x = 0.4$, leading to progressively shorter $\text{Ca}^{\text{F}}\text{-O}(1)$ bond lengths and extension of $\text{Ca}^{\text{F}}\text{-O}(2)$ (average Ca-O bond length is $2.36\text{\AA}$), consistent with preferential displacement of Mg^{2+} towards $\text{O}(1)$ in the $\text{Ca}^{\text{F}}\text{O}_6$ metaprism (Table 5-6 and 5-7). The $\text{Ca}^{\text{F}}\text{O}_6$ polyhedral volume is fixed with magnesium loading, and increase of $\text{Ca}^{\text{T}}\text{O}_6\text{X}$ volume accounts for unit cell dilation [Figure 5-7(a)]. On the other hand, the PO_4 tetrahedra also resist distortion because the P-O bonds are relatively rigid [Figure 5-7 (b) and (c)]. The trend is especially apparent when the materials were calcined at $700\text{ }^{\circ}\text{C}$ that give rise to larger, and presumably more nearly equilibrated crystallite sizes and stronger diffraction.

Although peak broadening was apparent at higher magnesium content, monoclinic ($P2_1/m$) or triclinic (PI) symmetries were not considered because the lattice metric did not deviate significantly from hexagonal values, and the agreement factor (R_{Bragg}) was not improved by using these alternate models. In addition, long range cation ordering and framework distortion were not evident even at high magnesium loadings ($x = 0.4$). Oxygen nonstoichiometry also did not participate in the substitution mechanism. Thus, $2M$ or $2A$ pseudomorphs are unlikely to exist in these materials.

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Upon introduction of magnesium, both A and B-type carbonates are excluded but the tunnel is filled and the framework intact, preserving the hexagonal ($P6_3/m$) lattice. In Mg-HAp ($x \geq 0.3$), channel X-site sub-stoichiometry promoted partial dehydroxylation to a Mg-OAp. The isotropic atomic displacement parameters (B_{iso}) decreases with increasing magnesium also suggesting ordering along the channel is stabilized through mixed tunnel content. These crystal chemical modifications (both framework distortion and tunnel nonstoichiometry) were relatively minor, precluding the need for pseudomorphic transformations.

Twist Angle Systematics

In the Mg-HAp $2H$ series ($0 \leq x \leq 0.4$), the hexagonal metric is preserved and the structure contains one unique metaprism twist angle (φ). At $x = 0$, the A-type carbonate opens the channel via a moderately acute φ . Earlier studies of single crystal CHAp found $\varphi = 22.7^\circ$ in a A-type CHAp $[Ca_{10}][(PO_4)_{6.00}][(CO_3)_{0.14}(OH)_{1.72}]$ [123] and $\varphi = 23.2^\circ$ in a B-type CHAp $[Ca_{10}][(PO_4)_{5.83}(CO_3)_{0.17}][(OH)_{2.00}]$ [123].

As the average crystal radii decreases with increasing magnesium content, φ becomes more acute [Figure 5-5 (d)]. Without the participation of extra-stoichiometric A and B-type carbonate, φ decreases almost linearly as smaller Mg^{2+} partitions to the framework Ca^F (4f) site, as also reflected in the Ca^F -O bond lengths. At the onset of magnesium substitution ($0.1 \leq x \leq 0.4$), φ decreases from 24.1° to 23.7° at $700^\circ C$ and from 24.6° to 24.3° at $600^\circ C$ [Figure 5-8]. Higher calcination temperature partially relax the framework to give an acute φ . Magnesium partitioning to Ca^F is most evident at higher temperature since solid state diffusion is enhanced and the crystals better equilibrated. Over the range of substitution, the tunnel diameter is generally invariant. With increasing Mg^{2+} tenanted in the 4f site, the average position of Ca^F is displaced along z , distorting the framework metaprism and reducing φ [Figure 5-8 (a) and (b)]. These φ values are comparable to previous observations in the hexagonal ($P6_3/m$) system, for example in pure HAp $[Ca_{10}][(PO_4)_6][(OH)_2]$ ($\varphi = 24.8^\circ$) [146] and in natural apatites that contain

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small cations $[\text{Ca}_{9.8}\text{Fe}^{2+}_{0.2}][(\text{PO}_4)_6][(\text{OH})_2]$ ($\varphi = 22.9$) [147]. Large φ ($> 25^\circ$) were not observed in this series, as anticipated in the absence of pseudomorphism.

Carbonate

Carbonate (both A and B-type) was introduced only for the PND Rietveld refinement of pure HAp. The atomic coordinates were fixed to accepted values in the analysis as the data quality was insufficient to directly extract crystallographical information (including bond length and bond angle). The removal of A-type carbonate with higher calcination temperature is a thermodynamic feature indicating the volatility of channel ions [4]. For the materials examined here carbonate is barely detectable by FTIR in the Mg-HAp, and correlations of Mg^{2+} and CO_3^{2-} may not be significant.

Figure 5-7. Polyhedral volume ($\text{\AA}^3$) adjusts accordingly with increasing Mg^{2+} loading x (at %) (inset). (a) The channel $\text{Ca}^{\text{T}}\text{O}_6\text{X}$ polyhedra size increases as the unit cell expands. This tunnel expansion induced framework adjustment to a more acute φ . The framework (b) $\text{Ca}^{\text{F}}\text{O}_6$ is invariant and (c) PO_4 tetrahedra are refined as semi-rigid bodies, both indiscreet to unit cell volume changes.

Table 5-8. Bond valence sum (BVS) of Ca^{2+} and Mg^{2+} in Mg-HAp series (700 °C and 600 °C) without considering structural relaxation and oxygen nonstoichiometry.^a

Magnesium loading (x)	framework		Tunnel	
	Ca^{2+}	Mg^{2+}	Ca^{2+}	Mg^{2+}
BVS (700 °C)				
0	2.08	-	2.36	-
0.1	2.04	0.97	2.45	1.09
0.2	2.09	1.00	2.33	1.11
0.3	2.09	0.99	2.30	1.10
0.4	2.06	0.98	2.30	1.10
BVS (600 °C)				
0	2.15	-	2.44	-
0.1	2.12	1.01	2.23	1.07
0.2	2.09	1.00	2.29	1.09
0.3	2.08	0.99	2.29	1.09
0.4	2.13	1.01	2.27	1.08

^aThe bond lengths were calculated from the refined PND data and the bond valence parameters for the calculation were taken from Brown and Altermatt [82].

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Figure 5-8. Relationship between metaprismatic bond lengths [Ca^F-O(1) and Ca^F-O(2)] (Å) and twist angle ϕ (°). The insertion of Mg²⁺ induce a preferential displacement of the cation position that lead to a shorter Ca^F-O(1) and Ca^F-O(2) elongation with increasing Mg²⁺ loading, in which ϕ varies accordingly.

5.3.4 Nanostructure

The nanostructure of Mg-HAp was intricate even though quantitative XRD and PND did not detect any Mg-rich phases [e.g. MgO, Mg₃(PO₄)₂]. Lattice imaging of apatites calcined at low temperatures (< 800 °C), was not possible as the electron beam caused rapid amorphization. Pure HAp have marginally larger crystals than Mg-HAp and are less sensitive to electron beam radiation.

The crystallinity of macroporous Mg-HAp ($x = 0.4$) improved, compared to the free powder, because additional annealing at 600 °C was required to remove the polymer precursors. The associated shrinkage due to crystal growth created interconnected “pores” that can be described as a ceramic foam. In this study, the directly observed pore size is on average ~50 nm (Figure 5-9). The surface area, or effective pore size of the macroporous nanostructure is controlled by the calcination temperature and time.

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5.3.5 Chemical Composition

Microanalysis of the Mg-HAp (700 °C) series confirms magnesium incorporation, but reliable quantification was not possible due to the small grain size. In addition, these apatites generally contain carbonate and hydroxyl groups, susceptible to thermal decomposition and/or removal under electron irradiation [161]. In both cases, overestimation of oxygen content and underestimation of hydrogen content inferred from metal content, would be anticipated and unavoidable. Moreover, the loosely packed powders when embedded in the resin creates void which reduce the X-ray signals. These experiments did reveal the unexpected presence of Mg in the $x = 0$ material (~0.43 wt%), believed to be introduced with the calcium precursors. Normalized ASEM show a systematic increase in magnesium content with the actual content always slightly higher than expected due to the magnesium contamination [Figure 5-10 (a)]. Powder XRD also confirms Mg incorporation since the a -edge contracts as calcium (Ca) content decreases, while phosphorous is fixed, the a -edge contracts [Figure 5-10 (b)]. For the nano-powders carbonate is detected by FTIR for $x = 0$, and Mg-OAp for $x = 0.5$.

Figure 5-9. Microstructure of the porous Mg-HAp ($x = 0.4$). The architecture was developed from an annealing process that promoted crystal growth “bridging” the nano-crystals.

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Figure 5-10. Microanalysis of Mg-HAp showing (a) Magnesium (Mg) and calcium (Ca) content (wt %) (inset) normalized by weight percent oxide plotted against nominal Mg content. (b) The amount is somewhat lower than nominal content when normalized to 6 apfu of phosphorous, but the trend is in accord with Mg incorporation leading to a shorter lattice edge. Pure HAp would contain Ca 39.9, P 18.5, O 41.4, H 0.2 wt%, respectively.

5.4 Functionality and Performance

5.4.1 Catalytic Activity

The catalytic activity of Mg-HAp towards the dehydration and/or dehydrogenation of IPA were studied using method established for Zn-HAp, and showed improved selectivity towards propene ($\text{CH}_3\text{CH}=\text{CH}_2$) production with increasing magnesium content, i.e. Mg-HAp ($x = 0.4$) > Mg-HAp ($x = 0.1$) > pure HAp. Hydrogen (H_2) synthesis was not enhanced, but the selectivity remained steady with temperature. Higher reaction temperatures moderately improve yield except for Mg-HAp ($x = 0.4$) at 300 °C, where a phase transformation to Mg- β -TCP may deactivate surface active sites and reduce yield. The maximum yield (>30 %) for both H_2 and $\text{CH}_3\text{CH}=\text{CH}_2$ was achieved at 250 °C (Figure 5-11).

The catalytic properties of Mg-HAp was comparable to magnesium oxide (MgO), and therefore promise as an environmentally benign material for both dehydration and/or dehydrogenation of IPA [162, 163]. The adsorption capacity of Mg-HAp could be increased with magnesium loading, however, the presence of carbonate probably inhibited the performance of Mg-HAp ($x = 0.1$) by reducing the number of surface metal-oxygen defects for adsorption.

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Nonetheless, further investigation on the surface reactivity of Mg-HAp (e.g. TPD, kinetic analysis) is required.

Figure 5-11. Selectivity (%) and yield (%) of hydrogen (H₂) and propene (C₃H₆) at various reaction temperature, utilizing Mg-HAp as catalysts. The result of pure HAp is included for comparison. Mg-HAps are marginally superior to pure HAp in the production of propene beyond 150 °C.

5.4.2 Bioactivity

Cell Viability

Neither the nano-powders nor macroporous Mg-HAp ($x = 0, 0.2$) were cytotoxic, and cell proliferation was active up to 7 days incubation. The cell viability of the control sample was marginally reduced after 5 incubation days due to rapid growth of osteosarcoma cells creating an overcrowded environment in the culture plate that impeded further proliferation. This was similarly observed in cells incubated with powder Mg-HAp ($x = 0.2$) (Figure 5-12). Magnesium (Mg²⁺) incorporation in biological or synthetic apatites is well known to enhance excipient properties compared to synthetic pure-HAp [112]. The enhanced viability in osteosarcoma cells with respect to pure HAp is consistent with the bioactive role of Mg²⁺, and show promise as components

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of bone regeneration materials utilizing Mg-HAp/ β -TCP biphasic materials to increase osteoblast activity [164, 165]. Further improvements might be achieved through the simultaneous introduction of A-type carbonate, also found in biological HAp [132]. The foam materials show no adverse response from methyl cellulose precursors, with marginally improved performance compared to the powder pure HAp (Figure 5-12). The increased effective surface area promoted cell attachment and subsequent proliferation, although A-type carbonate was removed in the synthesis of this porous architecture [Figure 5-2 (d)]. It is anticipated that fabrication of an A-type carbonate HAp foam will enhance the cell viability [24].

Cell Proliferation Studies

The cell proliferation was readily observed by conventional optical microscope. The adhered cells incubated with both nano-powders and foam show no evidence of cytotoxicity, consistent with viability studies. The cells incubated as a control and those with pure HAp (powder and foam), show isotropic growth morphologies with expanded fibrous pseudopods. The introduction of Mg^{2+} is expected to be beneficial to cell viability and the pseudopods are still visible up to 7 days incubation with powder Mg-HAp ($x = 0.2$) (Figure 5-13). Nevertheless, the cell morphologies become more isotropic (average size ~ 50 nm in width), with growth of better defined pseudopods that is ascribed to the changes in cell adhesion points and cytoskeleton growth promoted by the presence of Mg^{2+} .

Confocal laser microscopy also shows no adverse effect when the osteosarcoma cells are incubated with pure or Mg-HAp, consistent with previous findings. The stress fiber fibrous signals (red fluorescence) are emitted from the F-actin at the interface. The orientation of the control cells remain elongated with rough fibrous structure, consistent with optical microscopy observations. The cells show no reduction in proliferation rate, and remain active up to 7 days, but are less fibrous when incubated with Mg-HAp, and relatively more isotropic with respect to those with pure-HAp (Figure 5-14). At the same time, the actin filaments are more distinct and

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defined, indicating an adjustment in cytoskeleton movement that is associated with the presence of Mg^{2+} in the system, consistent with light microscopy findings. This is because Mg^{2+} contributes to the organized control of metabolism in cells [166].

No noticeable difference was found in the cells grown on Mg-HAp powder and foam. The effect of macroporous architecture could not be established although the cell proliferation was active. From this, it is inferred that the incorporation of Mg^{2+} is favorable for enhancing biological performance but the fabrication of foam HAp may only be advantageous in targeted applications that require higher surface area and defined shape, such as composite materials that combine organic materials into porous matrices [167].

Figure 5-12. MG-63 Osteosarcoma cell viability of nano-crystalline powder and macroporous foam of Mg-HAp ($x = 0, 0.2, 0.4$) as a function of incubation period. The porous structure show enhancing effect in cell proliferation that is further improved by the incorporation of magnesium.

Figure 5-13. Light microscopic observations of osteosarcoma cells in PBS incubated with (a), (b) control, (c), (d) powder pure HAp, (e), (f) foam pure HAp, and (g), (h) powder Mg-HAp ($x = 0.2$) for 1 and 7 days. The cell proliferation was active after 7 days with respect to control sample. No adverse effect was observed in the foam materials, and the subtle change in cell morphologies is attributed to Mg^{2+} introduction that altered the biological response of these cells. Scale bar is 50 μm .

Figure 5-14. Confocal laser micrographs of the (a), (b) control, (c), (d) powder pure HAp, (e), (f) powder Mg-HAp ($x = 0.2$), and (g), (h) foam Mg-HAp ($x = 0.4$) samples after 7 days incubation with osteosarcoma cells that were dyed with fluorescence red. Bright field images of the same focal height (z) are included to show Mg-HAp powder and foam. Cell morphology is more isotropic in Mg-HAps while the cytoskeleton actin fibres are more defined with Mg^{2+} incorporation, consistent with light microscopy findings. Scale bar is 75 μm .

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5.5 Conclusions

Crystal chemistry

The crystal structures of Mg-HAp 2H pseudomorphs have been studied using a combination of X-ray and neutron diffraction, electron microscopy and spectroscopy. Incorporation of Mg^{2+} is via an isovalent substitution $[\text{Ca}_{4-x}\text{Mg}_x]^\text{F}[\text{Ca}_6]^\text{T}[(\text{PO}_4)_6][(\text{OH})_2]$ ($0 \leq x \leq 0.3$), while a partial dehydroxylation transforms the materials to an oxyhydroxyapatite at higher Mg content

Microanalysis confirmed systematic incorporation of magnesium. Increasing calcination temperature promotes crystal growth with Mg^{2+} exclusively entering the framework Ca^F site. Regardless of calcination temperature, the space group for Mg-HAp-2H is $P6_3/m$ and spectroscopic studies (FTIR) showing minimal carbonate was consistent with PND refinements. Amorphous content was below the detection limit. The fabricated macroporous foam retained the apatite structure with interconnected nano-crystals creating a open morphology.

Functionality

For heterogeneous catalysis, the selectivity and yield of decomposition of IPA was distinctive with the introduction of Mg^{2+} to HAp. However, nano-powder surface area, activity and architecture require further tailoring to improve performance.

As a biomaterial, both macroporosity and the incorporation of Mg^{2+} in HAp show beneficial effects towards cell proliferation. The cell morphology was distinct in the presence of Mg^{2+} , and fabrication of a Mg-HAp based scaffold for excipient application is promising.

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The structure of calcium hydroxyapatite (HAp) is adaptable, consisting of a tunnel and framework architecture that may dilate or constrict to allow incorporation of a wide range of ions. Introducing small metals in place of calcium can initiate phase transformations, sometimes supplemented by a coexisting amorphous phase. Unlike alkalis, alkali earth and rare earths where complete displacement of calcium is possible, transition metals often show limited solubility, with extra-stoichiometric species such as carbonate and water essential components. In addition to size, the degree of covalency of the metal is also important in determining partitioning preference. A key diagnostic tool for systematic crystallographic analysis of apatites is the behavior of the metaprism twist angle that directly correlates structure with composition.

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In these studies, a novel co-precipitation method was used to introduce small cations into hydroxyapatite (HAp). However, simultaneous insertion of carbonate and water were unavoidable, especially at low loadings of Fe, Zn and Mg, and together with poor crystallinity and amorphous content, a degree of intuition was required to arrive at reasonable crystal structure solutions. Therefore, X-ray and neutron diffraction were complemented by spectroscopic investigations to more fully describe the crystal chemistries, supplemented by an analysis of metaprism twist angles (φ), bond lengths and bond valence sums to better understand the behavior of these materials. In particular, collating φ allowed correlation of the mode of modification, including changes in symmetry, with metal partitioning over framework and tunnel crystallographic sites. This comprehensive methodology can reliably scrutinize apatite crystal chemical anomalies and guide tailoring of functionalities. Here, this approach is extended to a critical review of the modifications of apatite crystal chemistry that places the current investigations within the wider context of HAp as an advanced material.

6.1 Synthesis and Characterization

6.1.1 Fabrication of Nanocrystalline Powders and Macroporous Foams

Nano-powders

The formation of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ (HAp) crystals of specified crystal size and morphology can be controlled by co-precipitation in the $\text{Ca}(\text{OH})_2 - \text{H}_3\text{PO}_4 - \text{H}_2\text{O}$ system through manipulating temperature, pH, and the Ca:P ratio [168]. Higher temperatures promote crystal growth, but may inhibit the incorporation of foreign cations and promote dehydroxylation. At $\text{pH} > 9$, with a controlled reactant addition rate under constant stirring, a yield of ~87% is achieved at production rate of ~0.83 g/min, while formation of calcium-deficient-HAp is favored at lower pH values (< 7). The balance of products is usually amorphous calcium phosphate (ACP) or calcium carbonate (CaCO_3).

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In biological HAp, deposition of an apatitic phase is often preceded by the appearance of less stable phosphate phases such as ACP [$\sim\text{Ca}_9(\text{PO}_4)_6$], octacalcium phosphate [$\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$, OCP] and brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) that can ultimately transform to apatite through several reactions [169, 170]:

Therefore, the starting point for precursor development is usually a mixture of solid ACP and small amounts of brushite nano-crystals with a high surface free energy that accelerates nucleation and growth [171]. The introduction of species that displace calcium, phosphorous or hydroxyl hinders the formation of single phase products [141]. It is believed that chemical substitutions destabilize the conventional HAp formation pathway as larger surface area increases surface free energy, and lowers the barrier for crystallization of 2M and/or 2A pseudomorphs.

Synergetic Incorporation of Carbonate

The extent of water and carbonate incorporation during synthesis of Fe-, Zn- and Mg-HAps was not readily controlled. The wet chemistry method consisted of four steps (Figure 6-1):

- (i) dropwise addition of phosphoric acid into a calcium hydroxide [$\text{Ca}(\text{OH})_2$] suspension;
- (ii) continuous stirring with addition of dissolved metal chlorides (MCl_2) (65 °C for 90 mins);
- (iii) heat treatment (100 °C / 120 mins) of the suspension prior to filtration; and
- (iv) calcination of the filtercake in flowing N_2 .

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This “open” configuration unavoidably introduced atmospheric carbonate, especially at low dopant metal concentrations, and a scenario for the mode of incorporation is hypothesized.

Crystallization of tricalcium phosphate (β -TCP) is initiated in step (i), and atmospheric carbonate is introduced into the suspension in step (ii) via carbonic acid chemical equilibrium ($\text{H}_2\text{O} + \text{CO}_2 = 2\text{H}^+ + \text{CO}_3^{2-}$). In pure HAp, this dissolved carbonate reacted with suspended $\text{Ca}(\text{OH})_2$ to form minute calcium carbonate (CaCO_3) crystals. These crystal seeds mature and stabilize in step (iii), where heating of the suspension promoted crystallization of β -TCP and conversion of $\text{Ca}(\text{OH})_2$ to CaO . However, the high decomposition temperature of CaCO_3 (Table 6-1) ensures these crystals remain stable through to step (iv). During calcination, CaCO_3 participates in the formation of HAp together with β -TCP and $\text{Ca}(\text{OH})_2$, leading ultimately to the formation of carbonated-HAp (CHAp), of the A-type.

On the other hand, in small cation substituted HAp, chlorides (MCl_2 , M: Fe, Zn, Mg) were introduced in stoichiometric proportions to displace calcium. However, these metal chlorides, unlike $\text{Ca}(\text{OH})_2$, are soluble, and may promote the rapid precipitation of MCO_3 in step (ii). By removing carbonic acid rapidly, the formation of CaCO_3 is suppressed and crystal growth of MCO_3 is dominant in step (iii). Moreover, the smaller solubility products (K_{sp}) of MCO_3 favors their precipitation over CaCO_3 (Table 6-1). Subsequently in step (iv), MCO_3 shows lower thermal stability, with respect to CaCO_3 decomposing to oxides and carbon dioxide gas released prior to the formation of HAp. For this reason, carbonate content in HAp decreases rapidly with increasing concentration of small cations, and eventually promotes the formation of an oxyhydroxyapatite (OAp). It is noteworthy that unlike Fe and Zn a diminishing carbonate concentration was not evident in the Mg-HAp series probably because of a moderately larger $K_{\text{sp}}(\text{MgCO}_3)$, and the associated transformation to Mg-OAp was not observed.

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Table 6-1. Solubility products (K_{sp}), solubility (g/ 100 g H_2O), and decomposition temperatures of metal carbonates.

	solubility product K_{sp} (25 °C)	solubility (g/100g H_2O)	decomposition temp (°C)
$CaCO_3$	3.36×10^{-9}	6.6×10^{-4}	1330
$FeCO_3$	3.13×10^{-11}	6.2×10^{-5}	N.A.
$ZnCO_3$	1.46×10^{-10}	9.1×10^{-5}	140
$MgCO_3$	6.82×10^{-6}	1.8×10^{-1}	990
HAp	1.0×10^{-36}

Figure 6-1. Postulated mechanism for the systematic incorporation of carbonate in the synthesis protocol.

Foam Architecture

Apatites are microporous materials, as are zeolites, with many structural features in common [172]. Moreover, the development of macro- and mesoporous HAp as functional materials [167] has become important to yield larger surface areas or improve mass transport, both of which render HAp more chemically reactive. Depending on pore geometry, porous networks provide additional active sites that allow specific chemical adsorption to enhance functionality; examples include improving bone adherence [173], or

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modifying selectivity in a catalytic reaction [65]. In this study, a macroporous foam material was fabricated that showed promise as a biomaterial.

6.1.2 Materials Characterization

Neutron diffraction

In all three series (Fe, Zn and Mg-Aps) a high background in the diffraction patterns arising from incoherent scattering was attributed to water/hydroxyl content, and is consistent with FTIR spectroscopy, that also showed a large water signature. This is especially apparent in the Zn-HAp series, where background accounts for ~10% of the total counts in the diffracted intensities. The amount and crystallographic location of OH⁻ could not be separated from contributions arising from water molecules (H₂O) and protons (H⁺) using PND. In other studies, deuteration was employed to exploit the substantially different coherent scattering lengths of hydrogen [$b_c(^1\text{H}) = -3.74$] and deuterium [$b_c(^2\text{D}) = 6.67$] as this allows more reliable location of hydroxyls [174].

Here, Rietveld refinement of the Fe-Aps neutron pattern effectively separated Fe from Ca due to the large difference in scattering cross sections (σ_{scatt}) (Table 6-2), allowing the determination of Fe partitioning across the Ca^F and Ca^T sites, and confirming the departure from hexagonal *2H* pseudomorph symmetry, even though the Fe solid solubility limit was low. Refinement of metal occupancies in Zn- and Mg-HAp was not as precise because the dominant isotopes of Ca, Mg and Zn show similar neutron scattering lengths (Table 6-2). The substantial amorphous phase in Zn-Ap series also detracted from the reliability of the structure refinements. In all three series, carbonate was introduced to the structure models in accord with FTIR data, but the CO₃ fractional coordinates and atomic displacement parameters (B_{iso}) were not refined because the sites were in close proximity to the more abundant framework oxygens (O1, O2 and O3). Although the light atoms (e.g. O, C)

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are readily detectable via neutron diffraction, the powder pattern is an ‘average’, and atomic positions reflect these delocalized scattering centers.

Magnesium contamination

In Mg-HAp, the observed Mg content was larger than anticipated due to contamination from the calcium precursor. In fact, for pure HAp the Mg content was found by analytical SEM to be ~0.43 wt% (Table 6-3). Trace contributions (<1 wt%) of Mg were also detected in the Fe and Zn-Ap series, confirming the introduction of impurities from reagents, most probably calcium hydroxide $[\text{Ca}(\text{OH})_2]$. It is not uncommon for slaked lime synthesized from quicklime (CaO) to contain minor Mg-based materials including periclase (MgO) and brucite $[\text{Mg}(\text{OH})_2]$ that are difficult to separate. High purity precursors would be preferred.

Table 6-2. Neutron scattering length and cross section of atoms in the Fe-, Zn, and Mg-Ap series.

Isotopes	coherent scattering length (fm) b_c	coherent cross section (barn) σ_{coh}	Incoherent cross section (barn) σ_{incoh}	total cross section (barn) σ_{scatt}
^{20}Ca	4.70(2)	2.78(2)	0.05(3)	2.83(2)
^{26}Fe	9.45(2)	11.22(5)	0.40(11)	11.6(1)
^{30}Zn	5.680(5)	4.054(7)	0.077(7)	4.13(1)
^{12}Mg	5.375(4)	3.631(5)	0.08(6)	3.71(4)
^{15}P	5.13(1)	3.31(1)	0.005(10)	3.31(2)
^{6}C	6.648(1)	5.551(2)	0.001(4)	5.551(3)

Table 6-3. Weight % impurities (Mg) in various samples, quantified by analytical scanning electron microscopy (ASEM) and X-ray fluorescence spectroscopy (XRF).

apatite	Mg (wt% oxide)	Method
pure HAp ($x = 0$)	0.43	ASEM (powder)
Fe-Ap ($x = 0.1$)	0.17	XRF
Fe-Ap ($x = 0.5$)	0.41	XRF
Zn-Ap ($x = 0.4$)	0.04	ASEM (pellet)
Zn-Ap ($x = 0.5$)	0.04	ASEM (pellet)
Mg-HAp	N.A.	

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6.2 Crystal Chemical Trends

6.2.1 Water and Hydroxyl

The crystallographic location of hydroxyl (OH^-) groups in HAp has been widely examined because human and rat cortical bone are composed of dehydroxylated (~20%) HAp, and OH^- controls solubility [175]. However, matching the excipient properties of synthetic HAp with the amount of OH^- is difficult as water (H_2O) and protons (in the form of HPO_4^{2-}) are not easily quantified [176]. A careful study of water incorporated in HAp revealed a gradual decrease in the a lattice parameter with the metaprism twist angle (φ) becoming more obtuse [16], but the correlation is not direct due to the concomitant introduction of cation vacancies (at the Ca^{F} site) to maintain charge balance [177]. Water may also contribute to the stabilization of an amorphous phase observed by diffraction (see section 4.3.1). Spectroscopic methods (IR, NMR) are better suited than diffraction and microscopy to quantify water content [178]. In particular, FTIR spectra contain an OH^- vibration at $\sim 3570\text{ cm}^{-1}$, with broad molecular water bands at $\sim 3400\text{--}3600\text{ cm}^{-1}$ and $\sim 1600\text{--}1700\text{ cm}^{-1}$ (H_2O in-plane bend) [1].

6.2.2 Carbonates

The examination of low level carbonate (CO_3^{2-}) by powder and single crystal X-ray diffraction patterns of biogenic and synthetic carbonate-bearing HAp (CHAp) proves difficult due to the small crystallite size and possible transformations to lower symmetry [179]. Furthermore, CO_3^{2-} is distributed statistically, and in the absence of long range order, not readily assessed by diffraction methods. Therefore, spectroscopic (IR, NMR) approaches to probe carbonate environments prove most informative [180]. Two principle mechanisms of CO_3^{2-} substitution (A- and B-type) are found in HAp:

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A-type CHAp

Type A ($^A\text{CO}_3^{2-}$) is dominant in biogenic or synthetic HAp and replaces tunnel X anions [mechanism (2-1)]. This form is characterized by a doublet at ~ 1545 and 1450 cm^{-1} (asymmetric stretch vibration, ν_3) and a singlet at $\sim 878\text{ cm}^{-1}$ (out-of-plane bend vibration, ν_2). An ideal A-type carbonate possesses equilateral triangular geometry, analogous to calcite, with the C-O plane approximately parallel to the tunnel and carbon located at $z \approx 0.5$, with an O-O edge of the triangles directed along the c -axis [179] (Figure 6-2). In general, progressive displacement of OH^- by $^A\text{CO}_3^{2-}$ increases a and decreases c metrics, as the tunnel dilates [4]. This adjustment of the channel wall leads to shorter $\text{Ca}^{\text{T}}\text{-O}$ bond lengths, and possibly twisting of the PO_4^{3-} group, while ordering of $^A\text{CO}_3^{2-}$ can promote lower symmetry ($P3$) structure [148]. Taken to the carbonate apatite endmember $[\text{Ca}_{10}][(\text{PO}_4)_6][\text{CO}_3]$ long range order creates a monoclinic Pb structure [181, 182].

B-type CHAp

In contrast, the B-type ($^B\text{CO}_3^{2-}$) is usually found in HAp produced at high temperature and replaces the phosphate tetrahedron [mechanism (2-2)], with characteristic vibrations at ~ 1455 , 1410 and 873 cm^{-1} . In B-type substitutions, a -edge contraction and expansion of c are observed due to less voluminous $^B\text{CO}_3^{2-}$ replacing PO_4^{3-} , and the removal of oxygen [183]. The precise location of B-type carbonate remains controversial [122, 123, 184], as $^B\text{CO}_3^{2-}$ randomly occupy either pair of the two opposing faces of the “phosphate” tetrahedron, and overlapping electron density of carbonate and phosphate oxygen perhaps make this matter impracticable to resolve. Powder XRD studies by Ivanova *et al* [122] and Wilson *et al* [184] found that carbon is located in close proximity to phosphorous position (inside the putative tetrahedron), with the carbonate oxygen occupying positions near to those of the PO_4 group and oriented almost parallel to $[001]$. However, single crystal studies by Fleet *et al* [123] resolved the average $P6_3/m$ structure with carbonate located almost at right angle to $[001]$ and external to the tetrahedron, while simultaneously displacing the phosphorous oxygen O(3) to satisfy the shorter bond length requirement (Figure 6-2). The conditions underwhich the

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models by Ivanova *et al* [122], Wilson *et al* [184] or Fleet *et al* [123] prevail requires clarification.

A2-type CHAp

Recently, a third mechanism of carbonate substitution was identified in single crystals synthesized at high pressure (> 2 GPa) [123]. In this case, a “stuffed” channel position designated the A2-type is produced with characteristic IR bands at 1563 and 1506 cm⁻¹. Charge balance is maintained as follows:

Mixed A-B type CHAp

It is possible for simultaneous A- and B-type substitution when HPO₄²⁻ and/or H₂O are present [185]:

Furthermore, in bone and minerals, Na⁺ and F⁻ may be present [186, 187]:

In mixed A-B-type CHAp and substituted CHAp (e.g. Na-CHAp) [88, 188], overlap and shift of the ν_3 bands lead to an asymmetric signature that can be deconvoluted into its symmetric components [189]. The cooperative effect of A-B substitutions on crystal structure is complicated, as reflected in lattice trends and metaprisim twist angle variations (see section 6.2.1).

Quantitative Analysis

The integrated areas of background subtracted infrared (IR) bands for A and B-type carbonate (~1350-1550 cm⁻¹) and water (~2500-4000 cm⁻¹) show quantitatively the participation of these species in Fe, Zn and Mg-Aps (Figure 6-3). In Fe and Mg-Aps, IR bands of water were consistently low, while in Zn-HAp a significant proportion of surface adsorbed and crystallographically incorporated water was detected. For Fe- and Zn-HAps, CO₃²⁻ is gradually

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eliminated with increasing substitution, as confirmed during PND refinement for Fe-HAp ($x = 0.1$) and Zn-HAp ($x = 0.1$), while in Mg-HAp the proportion of CO_3^{2-} was below the detection limit.

Figure 6-2. Various crystallographic orientation of A and B-type carbonate reported. The orientation of A-type carbonate is similar as reported by Fleet *et al* [123] and Suetsugu *et al* [179], note that the A2-type which is a high pressure feature. B-type carbonate generally occupies two of the random faces of the phosphate tetrahedra. The different models proposed by Fleet *et al* [123], Ivanova *et al* [122] and Wilson *et al* [184] is indicative of the difficulty in completely understanding the condition in which these model prevails.

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Figure 6-3. Band area of water vibration ($\sim 2500\text{--}4000\text{ cm}^{-1}$) and carbonate vibration ($\sim 1350\text{--}1550\text{ cm}^{-1}$) from FTIR spectra decrease with increasing Fe, Zn and Mg loading. The absolute amount could not be quantified from the figures but estimation of relative proportions provided insights to the extent of extra-stoichiometric modification.

6.2.3 Small cation substitutions in HAp

The distribution of transition metal cations in HAp remains debatable despite intensive studies. As discussed in Chapter 2, under specific conditions, partitioning to all the available acceptor sites ($\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}/\text{B}/\text{X}$) is possible, controlled not only by relative size and chemical state, but also chemical bonding (ionic versus covalent) and electronegativity (χ).

Fe^{3+} -Ap series

The insertion of Fe^{3+} requires charge balance through coupled substitution, and here, this was achieved through non-stoichiometry (vacancies and carbonate incorporation) and framework distortion (driven by tunnel dehydroxylation). Partitioning to the framework Ca^{F} site is preferred at lower concentrations, but with increasing loading Fe^{3+} is partially tenanted in the Ca^{T} site to allow better distribution of charge over a greater number of near neighbors. Pseudomorphism is also driven by size and charge differences. For example, a single crystal study of natural Fe^{2+} -HAp ($x \sim 0.2$) found that

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Fe^{2+} partitions exclusively to the tunnel Ca^{T} site and crystallizes in monoclinic symmetry $\text{P2}_1/\text{m}$ [143], while the contrary was found in a synthetic hexagonal $\text{P6}_3/\text{m}$ fluorapatite (FAp) $[\text{Ca}_{10-x}\text{Fe}^{2+}_x][(\text{PO}_4)_6][\text{F}_2]$ ($0 \leq x \leq 1.5$) with Fe^{2+} residing at Ca^{F} [88]. In Co-HAp $[(\text{Ca}_{3.81}\text{Co}_{0.19})^{\text{F}}(\text{Ca}_{5.46}\text{Co}_{0.54})^{\text{T}}][(\text{PO}_4)_6][\text{OH}_2]$ [190] and Mn-FAp $[(\text{Ca}_{2.88}\text{Mn}_{1.12})^{\text{F}}(\text{Ca}_{5.76}\text{Mn}_{0.24})^{\text{T}}][(\text{PO}_4)_6][\text{F}_2]$ [191] the divalent transition metals are also distributed over the Ca^{F} and Ca^{T} sites.

The present studies provide the first comprehensively substantiated ambi-site partitioning of a trivalent species (Fe^{3+}) in HAp. Chemically analogous ions are likely to show similar behavior. For example, a reconnaissance synthesis found that scandium (Sc^{3+}) mimics the crystallographic behavior of Fe^{3+} . The Sc^{3+} -HAp ($x = 0.5$) lattice volume dilated despite a smaller ionic radius (Table 6-4), indicative of a lower symmetry pseudomorph and/or the formation of an oxyhydroxyapatite (OAp) analogous to a Fe-OAp ($x = 0.5$).

Zn-Ap series

In general, the prediction of $\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}$ site partitioning assumes ionic bonding, and thus Zn^{2+} would be anticipated to enter the smaller Ca^{F} site of HAp. However, computational studies and X-ray absorption spectroscopy suggest a tunnel Ca^{T} site preference for Zn^{2+} . The current work confirms this result, although Zn incorporation is accompanied by extra-stoichiometric modification (carbonate and water). For HAp Zn^{2+} displaces Ca^{2+} , but in strontium-HAp (Sr-HAp) where the framework is expanded, entry in place of the X anion is permitted. Kazin *et al* [192] found lower amounts (0.15 mol %) of Zn^{2+} reside in the unique X-site (0, 0, 0) of Sr-HAp; Cu^{2+} and Ni^{2+} , are similar as substantiated through diffraction and various spectroscopies [193]. Moreover, the unique Cu^{2+} (electron configuration of Cu^{2+} : $1s^2 2s^2 2p^6 3s^2 3p^6 3d^7 4s^2$) may also concomitantly enter the $\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}$ sites along with Sr^{2+} [83].

Zinc and cadmium are group IIB elements and it is notable that the findings for Zn-Aps are comparable to the more readily characterized Cd-HAp series. For Cd^{2+} , substitution at the framework Ca^{F} site would be expected on the

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basis of size. However, Terra *et al* [194] using a combination of high resolution synchrotron diffraction and simulation found tenancy of Cd^{2+} on the Ca^{T} site was energetically favored, a result ascribed to the covalent nature of Cd^{2+} -O bonds [195]. As Zn^{2+} and Cd^{2+} possess almost identical electronegativity values [$\chi(\text{Zn}) = 1.65$, $\chi(\text{Cd}) = 1.69$] (Table 6-5) it is perhaps not surprising the partitioning behavior is similar. Nevertheless, solid solubility of Zn^{2+} is very limited due to significant size difference while complete substitution of Cd^{2+} for Ca^{2+} is possible, with a reported $\text{P6}_3[\text{Cd}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$ [$a = 16.1990(3) \text{ \AA}$, $c = 6.6485(1) \text{ \AA}$] [196] structure for the endmember.

Mg-HAp series

Alkali earth ions larger than Ca^{2+} (Sr^{2+} , Ba^{2+}) invariably show non-statistical distributions across the Ca^{F} and Ca^{T} sites. At lower concentration, the substituents initially occupy the Ca^{T} site until it is fully occupied before filling Ca^{F} . This has been confirmed in $[\text{Ca}_{10-x}\text{Sr}^{2+}_x][(\text{PO}_4)_6][\text{F}_2]$ [197] and $[\text{Ba}_{10-x}\text{Sr}^{2+}_x][(\text{PO}_4)_6][\text{F}_2]$ [198], where the ratio of the average ionic size show parabolic distribution across the entire solid solution. This trend is applicable to all larger isovalent cations including Pb^{2+} (ionic radius = $1.19 \text{ \AA}$) as in $[\text{Ca}_{10-x}\text{Pb}^{2+}_x][(\text{PO}_4)_6][(\text{OH})_2]$ [199]. However, because $\text{Mg}^{2+} < \text{Ca}^{2+}$ the reverse is observed in Mg-HAp, with preferential partitioning to Ca^{F} anticipated, but the solid solubility is limited (at least at standard pressures) due to the significant size difference and inhibiting effect of Mg^{2+} in HAp crystal growth.

It is generally observed that framework $\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}$ site ordering and/or composition can be modified by heat treatment, with high temperature calcination allowing equilibration of the $\text{Ca}^{\text{F}}/\text{Ca}^{\text{T}}$ partitioning. For example, the ordering of Pb^{2+} in $[\text{Ca}_5\text{Pb}^{2+}_5][(\text{VO}_4)_6][\text{F}_2]$ between the framework and tunnel required extended annealing [30, 200]. At the same time, heat treatment will evaporate tunnel anions that may result in a reduction in symmetry [21]. However, Mg-HAp could not readily equilibrate as unlike the

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other alkali earth substituents (Sr, Ba) higher temperatures and doping favor transformation to Mg- β -TCP.

B-site correlations

Transition metals may also enter the *B*-site, with pentavalent cation solid solution series including $[\text{Ca}_{10}][(\text{V}^{5+}_x\text{P}_{1-x}\text{O}_4)_6][\text{F}_2]$ [84], $[\text{Sr}_{10}][(\text{Cr}^{5+}_x\text{P}_{1-x}\text{O}_4)_6][\text{F}_2]$ [85] and $[\text{Ba}_{10}][(\text{Mn}^{5+}_x\text{P}_{1-x}\text{O}_4)_6][(\text{OH})_2]$ [90]. As V^{5+} , Cr^{5+} and Mn^{5+} are significantly larger with respect to P^{5+} , this can drive a reduction in symmetry to satisfy bond valence criteria. Nevertheless, a complete solid solution of V^{5+} in HAp is validated and it is recognized to be isostructural with common metalloid substituted apatites, such as arsenous FAp $[\text{Ca}_{10}][(\text{As}^{5+}_x\text{P}_{1-x}\text{O}_4)_6][\text{F}_2]$ [38].

Table 6-4. Lattice parameters from XRD refinements of Sc^{3+} -HAp (600 °C).

nominal Sc (x)	a (Å)	c (Å)	volume (Å ³)	crystal size (nm)
0	9.3948(4)	6.8906(4)	526.8	29
0.5	9.4202(7)	6.8789(6)	528.7	18

Table 6-5. Electronegativity values (Pauling scale) of selected atoms [201].

atom	Electronegativity (χ)
Ca	1.00
Fe	1.83
Zn	1.65
Cd	1.69
Mg	1.31

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Table 6-6. Ionic radius (Å, six-coordination with oxygen) of selected transition metal cations and the respective bond valence parameters.

species	ionic radius (Å) (6CN with O) [111]	bond valence parameters [82]
<u>Framework Ca^F/Ca^T</u>		
Ca ²⁺	1.00	1.967
Fe ²⁺	0.78	1.734
Co ²⁺	0.75	1.692
Fe ³⁺	0.65	1.759
Sc ³⁺	0.75	1.849
Zn ²⁺	0.74	1.704
Cd ²⁺	0.95	1.904
Mg ²⁺	0.72	1.693
Sr ²⁺	1.18	2.118
<u>Framework B</u>		
P ⁵⁺	0.17	1.604
V ⁵⁺	0.36	1.803
<u>Tunnel X</u>		
OH ⁻	1.40	
O ²⁻	1.37	
F ⁻	1.33	
Cl ⁻	1.81	
Br ⁻	1.96	

6.2.4 Phase Transformation Systematics

As already described (Chapter 2), $P6_3/m$ HAp consists of a $[\text{Ca}_4^{\text{F}}(\text{PO}_4)_6]^{10-}$ framework surrounding one-dimensional channels containing $[\text{Ca}_6^{\text{T}}(\text{OH})_2]^{10+}$. Changes in the relative size of the framework and tunnel constituents can be inferred from the metaprism twist angle (φ), and discontinuities in φ , imply a reduction in symmetry or compositional excursions (Figure 6-5). The metaprism twist angle φ also serves to rationalize the extended “phase space” of the apatite family, created through the application of seven adaptive mechanisms collated by Baikie *et al* [23].

- (i) inter- and intra-tunnel ordering
- (ii) framework distortion (promoted by tunnel nonstoichiometry)
- (iii) pseudomorphism
- (iv) oxygen non-stoichiometry

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- (v) polymorphism
- (vi) cation ordering
- (vii) polysomatism

Of these, the first four adaptations are significant to HAp:

(i) Inter- and Intra-tunnel Ordering

The crystallization of HAp in $P2_1/b$ (rather than $P6_3/m$) is observed in equilibrated crystals where hydroxyl groups are ordered and slightly displaced along z from the Ca^T triangular planes at $z = 0, \frac{1}{2}$. The displaced OH^- statistically occupy the $(0\ 0\ z)$ position and reflect an ‘averaged’ structure. In detail, these displaced species may be modulated, with respect to the parent, and for long range ordered samples best described as a 4D incommensurate structure [31]. The $P2_1/b$ approximation of the incommensurate structure possesses six distinct metaprisim twist angles φ that fluctuate within 1° of the average (φ_{ave}) [202]. In pure $P2_1/b$ HAp the maximum twist angle is $\varphi \sim 25^\circ$ [203] (Table 6-7), but in apatites containing mixed tunnel content ($\text{Cl}^- > \text{F}^- > \text{OH}^-$) the larger average ionic radius of X leads to a smaller $\varphi_{\text{ave}} \sim 21^\circ$ [204]. In certain varieties, such as the cadmium-halogen series, intra-tunnel anion ordering and intra-tunnel modulation is well documented in single crystals of $[\text{Cd}_{10}][(\text{PO}_4)_3][\text{Br}_2]$ and $[\text{Cd}_{10}][(\text{VO}_4)_3][\text{I}_2]$. For example, the cadmium-bromo-apatite (*CdPBr*)-2*T* is a modulated superstructure that crystallizes in $P3$ [$a = 16.932(2)\ \text{\AA}$, $c = 6.451(1)\ \text{\AA}$] [205].

In this study, inter-tunnel hydroxyl ordering was not observed because the incorporation of small cations was accompanied by tunnel nonstoichiometry (i.e. less than 2 X apfu in $A_{10}(\text{BO}_4)_6X_2$) to stabilize the structure, and the relatively low synthesis temperatures and short sintering times, did not allow complete structural equilibration.

(ii) Framework Distortion (Tunnel Nonstoichiometry)

The framework is susceptible to distortion when $A_6^T X_2$ tunnel contents are sufficiently sub-stoichiometric. A collation of twist angles in (*CaPX*)-2*H* (X : F, Cl, Br, O, OH, CO_3) hexagonal pseudomorphs found $21^\circ < \varphi < 26^\circ$ [Figure

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6-5 (c), Table 6-7]. Crystallization to lower symmetry subgroups may be stabilized with mixed tunnel site occupancies; for example, $P3$ in A-type-carbonate-HAp [148] and $P6$ in OAp have been reported [206]. In an alternate mechanism when $\varphi \geq 27^\circ$ monoclinic or triclinic $2M$ or $2A$ variants that contain 3 unique twist angles are favored, as in $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_{2-x}\text{O}_{x/2}]$ ($0.78 < x < 1.56$) which is triclinic $P1$ ($\varphi_{\max} \sim 28^\circ$) [21]. Fully vacant tunnel X sites are found in plumbous lacunar apatites [207] but this is unlikely in HAp.

(iii) Pseudomorphism

Pseudomorphic transformations ($2H \rightarrow 2M \rightarrow 2A$) are initiated by small compositional adjustments, and were frequently encountered in these studies due to the concomitant incorporation of carbonate (CO_3^{2-}), and tunnel dehydroxylation ($2\text{OH}^- \rightarrow \text{O}^{2-}$) that together increased the framework average ionic radii relative to the tunnel, promoting larger metaprism twist angles, that in turn were accommodated by a reduction in symmetry. The φ_{ave} increases as the framework distorts to allow one of the φ to become obtuse and the other two become more acute. For example, the pseudomorphic transformation: Fe-HAp- $2H \rightarrow$ Fe-Oap- $2M$ $[\text{Ca}_{9.77}\text{Fe}_{0.1}\square_{0.13}][(\text{PO}_4)_{5.80}(\text{CO}_3)_{0.20}][(\text{CO}_3)_{0.09}(\text{OH})_{1.82}] \rightarrow [\text{Ca}_{9.49}\text{Fe}_{0.51}][(\text{PO}_4)_{6.00}][\text{O}_{0.26}\text{OH}_{1.48}]$ required cooperative twisting of the framework leading to a more obtuse φ in the $2M$ pseudomorph [82]. However, the large $\varphi_{\max}$ [$\varphi_{\max} = 30.6^\circ$ in Fe-HAp ($x = 0.2$) and $\varphi_{\max} = 29.4^\circ$ in Fe-OAp ($x = 0.5$)] in Fe-Aps is indicative of a transformation beyond monoclinic $2M$ pseudomorphs, where $\varphi_{\max} = 28^\circ$ was reported previously in a triclinic $P1$ oxyapatite (OAp) [21], although this could not be established unequivocally [Figure 6-5 (d)].

In the case of Mg-HAp $[\text{Ca}_{4-x}^{\text{F}}\text{Mg}_x][\text{Ca}_6^{\text{T}}](\text{PO}_4)_6[(\text{OH})_2]$ ($0 \leq x \leq 0.4$) φ becomes progressively more acute with increasing Mg loading, consistent with the smaller Mg^{2+} replacing the framework Ca^{2+} . However, transformation to lower symmetry pseudomorphs was not observed although the material was calcined at 700°C , and is more nearly equilibrated than Fe-Ap and Zn-HAp.

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Pseudomorphism is also observed in tandem with the compositional modification on the *B*-site. For example, φ increases when larger As^{5+} replaces P^{5+} in $[\text{Ca}_{10}][(\text{P}_{1-x}\text{As}_x\text{O}_4)_6][(\text{OH})_2]$ [208], but this trend reverses at high As content indicative of a phase transformation to a triclinic pseudomorph isostructural with $[\text{Ca}_{10}][(\text{AsO}_4)_6][\text{F}_2]$ [38].

(iv) Oxygen Nonstoichiometry

Oxygen super- and sub-stoichiometry within the framework lead to partial or complete replacement of BO_4 tetrahedra by BO_5 or BO_3 polyhedra, without change in the cation subnet. The partial replacement of PO_4^{3-} by CO_3^{2-} can be achieved via the introduction of framework counter ions and/or tunnel modifications [mechanisms (2-7), (2-8) and (2-9)], but no reports of carbonate-substituted-HAps (CHAps) in *M* or *A* pseudomorphism has been reported. The metaprisim twist angles for 13 CHAps are collated in Table 6-7. Typically $22^\circ < \varphi < 24^\circ$ for CHAps depending on mode of incorporation (type A, B or mixed), but no simple correlation can be made due to the limited data available, and the cooperative effects of A-B-type substitution or framework counter ions is not readily systematized [Figure 6-5 (b)]. For example $\varphi = 25.7^\circ$ in a Na-CHAp $[\text{Ca}_{9.13}\text{Na}_{0.87}][(\text{PO}_4)_{5.05}(\text{CO}_3)_{0.95}][(\text{CO}_3)_{0.86}(\text{OH})_{0.28}]$ single crystal [88]. In some cases, quantification of HPO_4^{2-} , protons or H_2O is difficult due to incomplete compositional knowledge.. Oxidation or reduction of the *B*-site element can also initiate conversion of the tetrahedra. A particularly clear illustration is the reduction of hexagonal $\text{P6}_3/m$ mimetite $[\text{Pb}_{10}][(\text{As}^{5+}\text{O}_4)_6][\text{Cl}_2]$ to finnemanite $[\text{Pb}_{10}][(\text{As}^{3+}\text{O}_3)_6][\text{Cl}_2]$ [209], and the triclinic *P* 1 $[\text{La}_{10}][(\text{GeO}_4)_5(\text{GeO}_5)_1][\text{O}_2]$ 2A pseudomorph which is the oxidized variant of $[\text{La}_{9.33}][(\text{GeO}_4)_6][\text{O}_2]$ [20].

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Figure 6-4. Largest metaprisism twist angle ($\phi_{\max}$) versus framework to tunnel ionic radius ratio (F:T) in this work and various apatite collated for comparison. (a) In general a larger F:T would increase $\phi_{\max}$ and transformation to lower symmetry pseudomorph is expected when $\phi_{\max} > 27^\circ$. (b) The incorporation of carbonate does not alter ϕ significantly, but (c) show sensitivity to tunnel contents.

Figure 6-5. Structure drawings of $P6_3/m$ $[\text{Ca}_4^{\text{F}}\text{Ca}_2^{\text{T}}](\text{PO}_4)_6(\text{OH})_2$ hydroxyapatite. (a) An [001] projection of the zeolitic $[\text{Ca}_4^{\text{F}}(\text{PO}_4)_6]^{10-}$ framework that is constructed from $\text{Ca}^{\text{F}}\text{O}_6$ metaprisms (grey polyhedra) and PO_4 tetrahedra (green polyhedra). The one-dimensional channels are occupied by Ca^{T} (green cation) and OH (yellow oxy-anion) that combine as $[\text{Ca}_2^{\text{T}}(\text{OH})_2]^{10+}$ counter ions. (b) The [110] projection shows the alternation of twist along [001] between successive metaprisms and emphasizes the isolation of the PO_4 tetrahedra. (c) A clinographic projection of apatite illustrating the spatial relationship between the framework polyhedra and channel ions.

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The remaining three mechanisms of apatite modification are not significant to HAp, at least to our current understanding.

(v) Polymorphism

Polymorphic framework transformations can be driven by temperature and/or pressure without compositional modification. To date, there have been limited studies in this regard. Comodi *et al* [210] studied the compressibility of single crystal fluorapatite (FAp) and found that the structure remained stable up to 7 GPa with no change in symmetry.

(vi) Cation Ordering

Lower symmetry pseudomorphs ($P6_3$, $P3$, $P6$) arising from cation ordering is usually observed in the chemically complex A-site substitutions. In these cases, $\varphi < 25^\circ$ and the $A_4^F(BO_4)_6$ framework distortion is minimal, with only long range ordering validated. For example in $P6$ $[La_2Na_2Ba_6][(PO_4)_6][F_2]$ [211], and $P3$ $[Sr_{5.5}Ba_{0.24}Ca_{0.12}Na_{1.96}La_2][(PO_4)_6][(OH)_2]$ [212].

(vii) Polysomatism

Polysomatism is presently restricted to compositions with lead (Pb) and bismuth (Bi) as major components and where rotational twinning of $A_2^FA_3^TB_3O_{18}X_\delta$ modules is observed in ordered and disordered sequences [23]. The stabilization of these structures may require the presence of stereochemically active lone pair electrons from Pb^{2+} .

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Table 6-7. Largest metaprisism twist angle ($\varphi_{\max}$) and framework to tunnel ionic radius ratio (F:T) of apatite-2S pseudomorphs with $S = H, M, T$ and A .

Apatite crystal chemical formula	pseudomorph	space group	$\varphi_{\max}$	F:T	reference
<i><u>This work</u></i>					
[Ca ₁₀][(PO ₄) ₆][(CO ₃) _{0.13} (OH) _{1.74}] (600 °C)	2H	P6 ₃ /m	25.1	1.04	Chapter 6
[Ca ₁₀][(PO ₄) ₆][(CO ₃) _{0.06} (OH) _{1.88}] (700 °C)	2H	P6 ₃ /m	24.1	1.02	
[Ca _{9.77} Fe _{0.1} □ _{0.13}][(PO ₄) _{5.80} (CO ₃) _{0.20}][(CO ₃) _{0.09} (OH) _{1.82}]	2H	P6 ₃ /m	25.6	1.04	Chapter 3
[Ca _{9.80} Fe _{0.20}][(PO ₄) _{5.95}][(CO ₃) _{0.15} (OH) _{1.71}]	2M	P2 ₁ /m	30.6	1.04	
[Ca _{9.49} Fe _{0.51}][(PO ₄) _{6.00}][O _{0.26} OH _{1.48}]	2M	P2 ₁ /m	29.4	1.09	
[Ca _{9.9} Zn _{0.1}][(PO ₄) _{5.92} (CO ₃) _{0.08}][(CO ₃) _{0.1} (OH) _{1.8}]	2H	P6 ₃ /m	23.0	1.04	Chapter 4
[Ca _{9.8} Zn _{0.2}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.7	1.04	
[Ca _{9.7} Zn _{0.3}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.3	1.04	
[Ca _{9.6} Zn _{0.4}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.1	1.05	
[Ca _{9.5} Zn _{0.5}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.0	1.05	
[Ca _{9.9} Mg _{0.1}][(PO ₄) ₆][(OH) ₂] (600 °C)	2H	P6 ₃ /m	24.6	1.03	
[Ca _{9.8} Mg _{0.2}][(PO ₄) ₆][(OH) ₂] (600 °C)	2H	P6 ₃ /m	24.8	1.03	
[Ca _{9.7} Mg _{0.3}][(PO ₄) ₆][(OH) ₂] (600 °C)	2H	P6 ₃ /m	24.2	1.03	
[Ca _{9.6} Mg _{0.4}][(PO ₄) ₆][O _{0.06} (OH) _{1.88}] (600 °C)	2H	P6 ₃ /m	24.3	1.04	
[Ca _{9.9} Mg _{0.1}][(PO ₄) ₆][(OH) ₂] (700 °C)	2H	P6 ₃ /m	24.1	1.03	
[Ca _{9.8} Mg _{0.2}][(PO ₄) ₆][(OH) ₂] (700 °C)	2H	P6 ₃ /m	24.0	1.03	
[Ca _{9.7} Mg _{0.3}][(PO ₄) ₆][(OH) ₂] (700 °C)	2H	P6 ₃ /m	23.6	1.03	
[Ca _{9.6} Mg _{0.4}][(PO ₄) ₆][O _{0.03} (OH) _{1.92}] (700 °C)	2H	P6 ₃ /m	23.7	1.04	
<i><u>Ca^F/Ca^T-site substitutions</u></i>					
[Ca _{9.8} Fe ²⁺ _{0.2}][(PO ₄) ₆][(OH) ₂]	2M	P2 ₁ /m	22.9	1.03	[147]
[Ca _{9.74} Co ²⁺ _{0.26}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.6	1.04	[190]
[Ca _{9.27} Co ²⁺ _{0.73}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.4	1.06	
[Ca _{8.83} Mn ²⁺ _{1.0} Fe ²⁺ _{0.1} Na ⁺ _{0.07}][(PO ₄) ₆][F _{1.48} (OH) _{0.52}]	2H	P6 ₃ /m	22.6	1.07	[213]
[Ca _{2.88} Mn ²⁺ _{1.12}] ^F [Ca ²⁺ _{5.76} Mn ²⁺ _{0.24}] ^T [(PO ₄) ₆][F ₂]	2H	P6 ₃ /m	25.3	1.04	[191]
[Ca _{9.95} Sr ²⁺ _{0.05}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	22.3	1.04	[214]
[Ca _{9.65} Sr ²⁺ _{0.35}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	22.3	1.04	
[Ca _{9.41} Sr ²⁺ _{0.59}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	22.5	1.04	
[Ca _{9.8} Cd ²⁺ _{0.2}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.48	1.03	[194]
[Ca _{8.77} Cd ²⁺ _{1.23}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	23.61	1.03	
[Ca _{7.65} Cd ²⁺ _{2.35}][(PO ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	24.15	1.03	
<i><u>B-site substitutions</u></i>					
[Ca ₁₀][(P _{0.89} As _{0.11} O ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	24.55	1.04	[208]
[Ca ₁₀][(P _{0.76} As _{0.24} O ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	25.39	1.04	
[Ca ₁₀][(P _{0.57} As _{0.43} O ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	25.47	1.05	
[Ca ₁₀][(P _{0.34} As _{0.66} O ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	25.96	1.05	
[Ca ₁₀][(P _{0.20} As _{0.80} O ₄) ₆][(OH) ₂]	2H	P6 ₃ /m	25.53	1.06	
[Ca ₁₀][(P _{0.9} V _{0.1} O ₄) ₆][F ₂]	2H	P6 ₃ /m	22.1	1.05	[38]
[Ca ₁₀][(P _{0.5} V _{0.5} O ₄) ₆][F ₂]	2H	P6 ₃ /m	23.3	1.06	
[Ca ₁₀][(P _{0.1} V _{0.9} O ₄) ₆][F ₂]	2H	P6 ₃ /m	23.9	1.07	

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Carbonate apatites

$[\text{Ca}_{10}][(\text{PO}_4)_{6.00}][(\text{CO}_3)_{0.50}(\text{OH})_{1.00}]$	2T	P3	23.3	1.07	[148]
$[\text{Ca}_{9.75}][(\text{PO}_4)_{5.5}(\text{CO}_3)_{0.5}](\text{CO}_3)$	2H	P6	23.1	1.09	[179]
$[\text{Ca}_{10}][(\text{PO}_4)_{6.00}][(\text{CO}_3)_{0.14}(\text{OH})_{1.72}]$	2H	P6 ₃ /m	22.7	1.08	[123]
$[\text{Ca}_{10}][(\text{PO}_4)_{5.51}(\text{CO}_3)_{0.735}][(\text{CO}_3)_{0.69}(\text{OH})_{0.62}]$	2H	P6 ₃ /m	23.4	1.07	[123]
$[\text{Ca}_{10}][(\text{PO}_4)_{5.83}(\text{CO}_3)_{0.17}][(\text{OH})_{2.00}]$	2H	P6 ₃ /m	23.2	1.03	[123]
$[\text{Ca}_{9.30}(\text{NH}_4)_{0.10}][(\text{PO}_4)_{4.95}(\text{CO}_3)_{1.05}][(\text{OH})_{1.65}(\text{H}_2\text{O})_{0.45}]$	2H	P6 ₃ /m	23.1	1.03	[122]
$[\text{Ca}_{9.13}\text{Na}_{0.87}][(\text{PO}_4)_{5.05}(\text{CO}_3)_{0.95}][(\text{CO}_3)_{0.86}(\text{OH})_{0.28}]$	2H	P6 ₃ /m	25.7	1.08	[88]
$[\text{Ca}_{9.85}\text{Na}_{0.1}\square_{0.05}][(\text{PO}_4)_{5.8}(\text{CO}_3)_{0.2}][(\text{CO}_3)_{0.2}\text{F}_{1.8}]$	2H	P6 ₃ /m	23.6	1.05	[215]
$[\text{Ca}_{7.8}\text{Na}_{1.3}][(\text{PO}_4)_{4.3}(\text{CO}_3)_{1.7}][\text{O}_{1.3}(\text{OH})_{0.74}]$	2H	P6 ₃ /m	21.5	1.01	[188]
$[\text{Ca}_{9.0}\text{Na}_{0.5}][(\text{PO}_4)_{4.5}(\text{CO}_3)_{1.5}][(\text{OH})_2]$	2H	P6 ₃ /m	23.2	1.03	[216]
$[\text{Ca}_{8.40}\text{K}_{0.34}][(\text{HPO}_4)_{2.02}(\text{PO}_4)_{4.88}(\text{CO}_3)_{1.55}][(\text{OH})_2]$	2H	P6 ₃ /m	22.1	1.01	[217]

Tunnel nonstoichiometries

$[\text{Ca}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$	2M	P2 ₁ /b	24.2	1.04	[202]
$[\text{Ca}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$	2M	P2 ₁ /b	24.6	1.04	[203]
$[\text{Ca}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$	2H	P6 ₃ /m	24.8	1.04	[146]
$[\text{Ca}_{10}][(\text{PO}_4)_6][(\text{OH})_2]$	2H	P6 ₃ /m	25.3	1.04	[218]
$[\text{Ca}_{9.7}][(\text{PO}_4)_{5.7}][(\text{OH})_{0.6}(\text{H}_2\text{O})_{1.9}]$	2H	P6 ₃ /m	24.4	1.09	[16]
$[\text{Ca}_{9.9}][(\text{PO}_4)_{5.98}][(\text{OH})_{1.84}]$	2M	P2 ₁ /b	24.7	1.04	[219]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{O}_{0.39}\text{OH}_{1.22}]$	2A	P1	28.0	1.05	[21]
$[\text{Ca}_{10}][(\text{PO}_4)_6]\text{O}_2$	2H	P6	22.7	1.03	[206]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{F}_{0.58}\text{Cl}_{0.94}\text{OH}_{0.48}]$	2M	P2 ₁ /b	21.3	0.99	[204]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{F}_{0.78}\text{Cl}_{0.66}\text{OH}_{0.56}]$	2H	P6 ₃ /m	22.1	1.12	[204]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{F}_{1.0}\text{Cl}_{0.8}\text{OH}_{0.2}]$	2H	P6 ₃ /m	22.1	1.13	[31]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{F}_1\text{Cl}_1]$	2H	P6 ₃ /m	22.3	0.99	[220]
$[\text{Ca}_{10}][(\text{PO}_4)_6]\text{F}_2$	2H	P6 ₃ /m	23.5	1.08	[221]
$[\text{Ca}_{10}][(\text{PO}_4)_6]\text{Cl}_2$	2M	P2 ₁ /b	18.5	0.94	[222]
$[\text{Ca}_{10}][(\text{PO}_4)_6]\text{Cl}_2$	2H	P6 ₃ /m	19.1	0.94	[19]
$[\text{Ca}_{10}][(\text{PO}_4)_6]\text{Br}_2$	2H	P6 ₃ /m	14.8	0.91	[223]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{Cu}_{0.72}\text{O}_{1.44}\text{OH}_{0.56}]$	2M	P6 ₃ /m	22.5	0.95	[224]
$[\text{Ca}_{10}][(\text{PO}_4)_6][\text{Cu}_{0.54}\text{O}_{1.72}\text{H}_y]$	2M	P6 ₃ /m	22.7	0.94	[193]

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6.2.5 Bond Length and Polyhedral Volume

Framework $[(Ca^F-O)_{ave}]$ and Ca^FO_6

The focus of these studies was the introduction into HAp of cations smaller than Ca^{2+} , with discrete ionic radii and valence $Fe^{3+} < Mg^{2+} < Zn^{2+}$ (Table 6-6), that lead to systematic adjustments of bond lengths and polyhedral volumes. The variation in the framework Ca^F-O and P-O bond length is indicative of the extent of structural modification. The average Ca-O bond length is 2.37 Å (from Table 6-6), but in Fe-, Zn- and Mg-HAps the average framework Ca-O bond length $[(Ca^F-O)_{ave}]$ is > 2.4 Å. Bond length dilation arises due to size differences or cooperative insertion of extra-stoichiometric species. In Fe-Aps, small amount of Fe ($x < 0.2$) increased the distance between the metaprismatic Ca^F and the framework oxygen, creating local stress. To obtain reasonable bond valence sums, a pseudomorphic transformation to monoclinic $P2_1/m$ $[(Ca^F-O)_{ave}]$ decreased from ~ 2.44 Å to ~ 2.41 Å is initiated at higher Fe loading ($x = 0.5$). On the other hand, simultaneous incorporation of water dilates the tunnel and offsets the effect caused by the insertion of smaller Zn^{2+} into the Ca^T site, but $(Ca^F-O)_{ave}$ gradually decreases at higher Zn loadings when the latter substitution becomes dominant. The $(Ca^F-O)_{ave}$ in Mg-HAps calcined at 600 °C increases when Mg^{2+} the Ca^F site, but the crystals are more nearly equilibrated after 700 °C calcination with $(Ca^F-O)_{ave} \sim 2.41$ Å (Figure 6-6).

The volume of the Ca^FO_6 metaprism contracts significantly with increasing Fe loading, consistent with $(Ca^F-O)_{ave}$ shortening in Fe-HAps. A gradual reduction in Ca^FO_6 polyhedral volume is observed in Zn-HAps, that is due to crystallographic water dilating the tunnel and alleviating the effect of smaller Zn^{2+} in place of Ca^T . It is noteworthy that carbonate incorporation in Zn-HAp ($x = 0.1$) lead to a significant reduction in the lattice parameters but the bond lengths and polyhedra volume remain in accord with the systematics. Mg^{2+} with bonding characteristics typical of alkali earths, prefer the less voluminous Ca^FO_6 in order to satisfy bond valence requirements. The Ca^FO_6 volume is

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invariant with Mg^{2+} loading, demonstrating the ability of the framework polyhedra to accommodate this substituent [Figure 6-7 (a) and (c)].

In general, the framework contracts to stabilize and maintain structural integrity in the presence of tunnel non-stoichiometry (A-type carbonate and water) and pseudomorphism, that is found in Fe- and Zn-HAps. But in Mg-HAp no significant structural distortion was instigated and thus the framework bonding and polyhedral remain largely invariant.

Framework [(P-O)_{ave} and PO₄]

The volume of the PO₄ tetrahedra vary slightly across all three series [$1.75 < \text{PO}_4 < 1.90 \text{ \AA}^3$] with the average P-O bond lengths as anticipated [$1.52 < (\text{P-O})_{\text{ave}} < 1.55 \text{ \AA}$] [Figure 6-8 (a) and (b)]. The P-O bonds generally resist change with an average of $1.54 \text{ \AA}$ (from Table 6-6), but the $(\text{P-O})_{\text{ave}}$ follows the systematics of Fe-HAp < Mg-HAp < Zn-HAp. This is because the framework is constructed by corner connected PO₄ tetrahedra and Ca^FO₆ metaprisms, where elongation in $(\text{Ca}^{\text{F}}\text{-O})_{\text{ave}}$ will inevitably compress the $(\text{P-O})_{\text{ave}}$.

Tunnel modifications [(Ca^T-O)_{ave} and Ca^TO₆X]

The dilation of Ca^TO₆X polyhedra in all three series is attributed to pseudomorphism and tunnel non-stoichiometry, both of which lead to dilation through A-type carbonate insertion and dehydroxylation [Figure 6-7 (b) and (d)]:

The lattice parameter *a*-edges are extended when the larger cations (ionic radii $\text{Fe}^{3+} < \text{Mg}^{2+} < \text{Zn}^{2+}$) replace Ca^{2+} , and a dilation of Ca^TO₆X polyhedra is also observed. Meanwhile, a smaller Ca^TO₆X along the channel is linked to a reduction in *c*-axis [Figure 6-8 (c)].

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Figure 6-6. Bond length changes with degree of crystallographic modifications. (a) The average framework Ca-O bond length $[(Ca-O)_{ave}^F]$ is shorter when a relatively larger cation ($Fe^{3+} < Mg^{2+} < Zn^{2+}$) is incorporated. The average P-O bond length $[(P-O)_{ave}]$ behaves in the opposite way where $(P-O)_{ave}$ of Fe-Aps is shorter compared. Note the three distinct PO_4 polyhedra in monoclinic $P2_1/m$ Fe-Ap ($x = 0.2$ and 0.5).

Figure 6-7. Polyhedral volume ($\text{\AA}^3$) changes against Fe, Zn and Mg loading (at%). Incorporation of Fe and Zn modifies the CaF_6 and $CaTO_6X$ polyhedra considerably, but invariant with Mg loading. Distinctive regions with increasing lattice parameter a is correlated with increasing ionic radius of the substituted cations, with an anomaly in Zn-HAp ($x = 0.1$) that is ascribed to crystallographically bound carbonate and water.

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Figure 6-8. PO₄, Ca^FO₆ and Ca^TO₆X polyhedral volume (Å³) versus unit cell volume (Å³) of all three apatite series. The trend is consistent with increasing ionic radius of substituted cations dilating the unit cell volume, with PO₄ being relatively rigid. The elongation in *c*-axis is associated with tunnel Ca^TO₆X.

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6.3 Summation

In this study, detailed investigation of the crystal chemistry of the Fe, Zn and Mg-Ap series have been made to correlate structure with functionality, particularly to biological and catalytic applications. Some matters remained to be addressed as follows:

- i) *Synthesis*: The strength and weaknesses of the present synthesis route are clear. A superior fabrication method is required to improve homogeneity and produce highly crystalline single phase materials. In this regard, hydrothermal approaches may prove beneficial [84]. In addition, carbonate inclusion and dehydroxylation must be avoided while attempting to extend solid solubility limits.
- ii) *Characterization*: Employment of multiple characterization techniques is essential due to the complex nature of the materials, and the sensitivity limits of each procedure providing complementary information of the fully crystallographic description, including the exact symmetry, partitioning preferences and solid solution limits. This study would have benefited by greater use of EXAFS (to confirm valence), XAFS (to study coordination), and NMR to investigate PO₄ modifications and the role of protons.
- iii) *Functionality*: Screening the excipient or catalytic properties of these novel materials should be expanded to encompass a wider range of potential applications.

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Conclusion

Crystallochemical adaptation can transform hydroxyapatite into a functional material suitable for a wide range of applications, with performance controlled by tailoring the type and extent of cation and anion incorporation. Pseudomorphic structures are frequently involved, and these are best analyzed through a combination of diffraction, microscopic and spectroscopic techniques. The introduction of ferric (Fe^{3+}) and magnesium (Mg^{2+}) enhance cell proliferation of HAp powders and porous materials, while reconnaissance studies show zinc (Zn^{2+}) and Mg^{2+} promote catalytic decomposition of 2-propanol. Nonetheless, fabrication of single phase products remains challenging, and the development of robust synthesis routes will be essential to exploit the crystallochemical-functionality relationship.

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The hypothesis of this study was that the introduction of small cations, specifically Fe^{3+} , Zn^{2+} and Mg^{2+} into hydroxyapatite will enhance bioactivity and biocompatibility and promote catalytic activity. The crystallographic modification of nano-powders and ceramic foams was validated by establishing solid solubility limits and determining the site preference of the incorporated species, and although characterization was challenging, monitoring the metaprism twist angles and bond valence sums were employed as a tool for confirming the quality of the crystallochemical analyses. Finally, the crystal chemistry was correlated with functionality to provide guidance for optimizing the performance of these materials for bone repair and as catalysts.

7.1 Principal Outcomes

Hydroxyapatites containing the first row transition metals (Fe^{3+} , Zn^{2+} and Sc^{3+}) and an alkali earth (Mg^{2+}) in hydroxyapatite were synthesized:

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Characterization and testing of these materials lead to the following outcomes:

- (i) *Significance of amorphous phases:* Soft chemical routes and low temperature treatments for preparing apatite nano-powders will, in some cases, lead to a persistent amorphous phase, the amount of which can be reliably estimated by PND. Transmission electron microscopy should be an important tool for examining HAp, but was conducted sparingly due to electron beam damage. In future work, *in situ* cooling should be considered to extend observation time. Although amorphous calcium phosphate (ACP) is essential in biological systems, synthetic polyphase materials may not show similar bio-response, nor are the implications of ACP in catalytic reactions yet understood.
- (ii) *Pseudomorphism:* The introduction of small cations requires the simultaneous creation of vacancies, and presence of extra-stoichiometric species (i.e. CO_3^{2-} and O^{2-}), that compensate for size discrepancies and allow charge balance, sometimes accompanied by pseudomorphic phase transformations ($2H \rightarrow 2M \rightarrow 2A$) (*Chapters 3 and 4*). This is in contrast with the ease of synthesizing fully stoichiometric apatites containing larger alkali earth, alkali and rare earth elements. Nonetheless, fabricating single phase small cation-substituted apatites, while avoiding framework and tunnel nonstoichiometry will invariably lead to better understanding of the crystal chemistry (*Chapter 5*), but avoiding tunnel oxidation or dehydroxylation is challenging due to the volatility of channel anions. Moreover, the interpretation of microanalysis and spectroscopy data, is often ambiguous due to the assumptions inherent in the quantification process. Finally, the nature of these crystallographic modifications with respect to biological and catalytic functionalities has not been fully explored, which requires much investigation. Polymorphic transformations (change in symmetry without change in composition) and polysomatism (change in the number of apatite modules in crystallographic repeat) were not observed in this study.

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- (iii) *Crystal chemistry diagnostics*: The metaprism twist angle (ϕ) can be employed as a tool for monitoring crystallochemical incorporation and metal partitioning (*Chapter 6*). The approach can ascertain the extent of solid solubility and extra-stoichiometries associated with the framework modification. This rationale proved invaluable for assessing apatite structures derived from Rietveld refinements of powder materials, that go beyond conventional bond length and bond angle analysis. The systematics and anomalies in ϕ for hydroxyapatite (*Chapter 6*) serve to emphasize the reliability of ϕ in crystal chemical diagnostics (e.g. detecting disequilibrium) for future development of apatite systems as biomaterials and catalysts.

7.2 Thesis Novelty

- (i) In *Chapter 3*, diffraction and spectroscopy were used to confirm a $2H$ $[\text{Ca}_{4-x}^{\text{F}}\text{Fe}_x][\text{Ca}_{6-y}^{\text{T}}]_y[(\text{PO}_4)_{6-y}(\text{CO}_3)_y][(\text{OH})_{2-x}(\text{CO}_3)_x] \rightarrow 2M$ $[\text{Ca}_{3.8}^{\text{F}}\text{Fe}_{0.2}][\text{Ca}_{6-x}^{\text{T}}\text{Fe}_x](\text{PO}_4)_6[(\text{OH})_{1.8-x}\text{O}_{0.2+x}]$ pseudomorphic transformation in Fe-HAp initiated by increasing iron content. The substitution in Fe-HAp take place over the framework Ca^{F} site (up to $x = 0.2$), beyond which Fe^{3+} preferentially displaces the tunnel Ca^{T} site to the solid solubility limit ($x = 0.5$). In addition, the chemical state and magnetism of iron showed that magnetic nanoparticles for targeted therapeutic applications are not feasible as the magnetic susceptibility is too small. However, the materials possess no cytotoxicity in standard biocompatibility evaluation.
- (ii) In *Chapter 4*, the synthesis procedure developed for Fe-HAp was extended to zinc-hydroxyapatite (Zn-HAp) $[\text{Ca}_4^{\text{F}}][\text{Ca}_{6-x}^{\text{T}}\text{Zn}_x](\text{PO}_4)_6[(\text{OH})_{2-y}\text{O}_{y/2}]$ to a solid solution limit ($x = 0.5$). Zinc incorporation destabilized the apatite structure by inhibiting crystal growth, as evidenced by significant amorphous content in X-ray and neutron diffraction. Although found to occupy the tunnel Ca^{T} site, a transformation to lower symmetry could not be substantiated

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unequivocally. The surface properties explored via TPD and in situ DRIFTS were associated with catalytic functionality by assessing the conversion and selectivity in decomposition of 2-propanol.

- (iii) In *Chapter 5*, magnesium was incorporated into HAp as $[\text{Ca}_{10-x}\text{Mg}_x][(\text{PO}_4)_6][(\text{OH})_{2-y}\text{O}_{y/2}]$ ($0 \leq x \leq 0.4$, $0 \leq y \leq 0.12$ Mg-HAp) and the first report of neutron diffraction for the series is described. Pseudomorphism was not observed as magnesium enters the framework Ca^{F} site, although chemical analysis found that non-stoichiometry is present. A novel porous Mg-HAp fabricated from nano-crystalline powders was devised by modifying the method of Phonthammachai *et al* [65]. Both the powders and foam were bioactive and an assessment of Mg-HAp on cell proliferation showed no cytotoxicity. This study provides insights to tailoring of biocompatible materials systematically to optimize the performance. The catalytic performance in decomposition of 2-propanol was found to favor production of propene.
- (iv) In *Chapter 6*, a comprehensive review of the crystal chemistry of transition metal bearing HAp is presented. The difficulties in fabricating single phase products and the respective challenges in obtaining reliable crystallographic data are discussed. The insertion of small cations exploits the structural flexibility of HAp through the mechanisms of inter tunnel ordering, framework distortion, cation ordering, and tunnel nonstoichiometry. The application of metaprism twist angle (φ) to validate crystallochemical modification proved reliable and consistent with compositional variation. Correlation of structure and functionality were also discussed.

Taken together, these outcomes confirm the central hypothesis proposed in *Chapter 1*, and substantiated in *Chapter 2*, that the incorporation of small

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cations in hydroxyapatite has the potential to deliver superior biomaterials and catalysts.

7.3 Future Research

The tailoring of hydroxyapatite, compositional and structurally, requires deep understanding of crystal chemistry, and in this thesis the primary concern was to explore the linkage between structural modification and functionality. Future work should be broadened substantially to provide a more complete understanding to underpin crystallographic engineering. Areas that require further investigation include:

- (i) Comprehensive characterization of materials prepared over a range of calcination times and temperatures to examine the hydroxyapatite-tricalcium phosphate stability region, improve crystallinity and optimize yield;
- (ii) The incorporation of more intricate “ambi-site” species (e.g. Ti^{4+} , Mn^{5+}) of different size and valence into hydroxyapatite, to extend our understanding of the crystal chemistry of transition metals in the HAp;
- (iii) Evaluation of the role and properties the amorphous phase to systematically manipulate the precursors and product. Single crystal growth of the materials would also be valuable to provide insight from high quality structural solutions;
- (iv) Systematic study on carbonate insertion and the effect of tunnel non-stoichiometry with respect to crystallographic modification so that the incorporation of transition metals is directly controlled;
- (v) Fabrication of a three-dimensionally porous ordered (3DOM) apatite scaffold [8] to enhance osteoblast cell proliferation for tissue engineering;
- (vi) Assessment of the durability, mechanical and physio-chemical properties of the small cations incorporated porous hydroxyapatite for functionalization as described in (v); and

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- (vii) Thermodynamic and chemical kinetic studies on the catalytic decomposition of 2-propanol to optimize the performance of these apatites for demonstration in large scale application.

In conclusion, this challenging and exciting research has provided greater insights for the systematic tailoring of hydroxyapatite systems which serves as a methodical foundation for the development of apatite-based ceramics as biomaterials and catalysts.

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Appendix

Figure A-1. Rietveld plots of Fe-HAp ($x = 0.2$) prepared by sintering under N₂ (600°C/2h) and spiked with CaF₂ (20 wt%) to extract amorphous content. The upper trace is an overlay of the experimental pattern (o) and the calculated intensity shown as a continuous line. The lower trace contains the intensity differences between the experimental and calculated patterns. Bottommost are the Bragg markers for Fe-HAp (upper) and CaF₂ (lower).