THE PROCESSING AND IN VITRO DEGRADATION PROPERTIES OF GRAVITY SINTERED CALCIUM POLYPHOSPHATE POWDERS

by

Jeffrey Donald Wells

A thesis submitted in conformity with the requirements for the degree of Master of Applied Science Graduate Department of Metallurgy and Materials Science University of Toronto

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The Processing and In Vitro Degradation Properties of
Gravity Sintered Calcium Polyphosphate Powders

Jeffrey Donald Wells, Degree of Master of Applied Science, University of Toronto,
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In the current study, calcium polyphosphate (CPP) has been produced in porous
disk form (~4mm diameter x ~2mm thick) through the gravity sintering of particles of two
different size ranges (106 - 150μm and 150 - 250μm) to function as a biodegradable
scaffold for bone ingrowth. These disk samples were characterized in the “as-made” state
and after incubation of up to 30 days in two different buffered solutions (pH 7.4 and pH
4.0). Degradation of the disk samples was characterized physically, through diametral
compression analysis, and chemically, for the release of calcium and phosphate
degradation products.

Powders were sintered at temperatures near the melting temperature and it was
observed that variations in sintering temperature as minor as ±2°C could have deleterious
effects on densification. The behaviour of disk samples in the initial short-term
degradation studies suggested a two-step degradation process whereby intergranular
amorphous regions degrade initially, releasing degradation products at a high rate,
followed by the slowly degrading crystal grains. The loss of intergranular adhesion due to
this degradation promoted intergranular crack propagation resulting in an observed rapid
decrease in strength. Evidence of precipitation in was observed in the degradation study.
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In closing, I would like to dedicate this thesis to my family, my dad Don, my mom Janice, and my sister Kim who have supported me through more than two decades of my academic career. Their love and encouragement have always been my inspiration to achieve my goals.
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1. INTRODUCTION

1.1 Bone Substitute Materials

1.1.1 Autografts and Allografts

Bone is the primary load-bearing tissue in the body. Destruction or disease of bone can be debilitating and may require a replacement to substitute for bone lost as a result of; i) severely traumatized bone tissue; ii) the resection of diseased or tumourous bone tissue, and; iii) correction of congenital defects or bone augmentation due to adverse bone remodeling (e.g. resorbed crestal bone in mandibular or maxillary ridge). Bone, or a suitable substitute thereof, may also be required in particulate form for use as an adjunct to the placement of implants used in dentistry (dental implants) and orthopaedics (joint replacements) \(^1\).

Currently, the most extensively used substitutes for bone replacement applications are autografts and allografts, although their use presents a number of potential problems. Allografts are prepared from donated tissue and introduce risks associated with intraspecies tissue transplants, such as tissue rejection due to an extensive immune response, or disease transmission from the donated tissue to the host \(^2\). The supply of allograft tissue is also subject to availability by the location of medical centres equipped with “bone banks”. Access to these facilities by surgeons in remote areas may
be limited, as bone banks are commonly located in densely populated areas with a high number of potential tissue donors.

A number of problems exist which are inherent to autografting of bone tissue. Primarily, the retrieval of bone used in autografting requires a secondary surgery. This surgery subjects the patient to unnecessary trauma, increasing the risk of infection, fractures, and thrombosis, and also causes greater blood loss and post-operative pain. Autogenous tissue may also be subject to availability if it is limited in supply or not of satisfactory quality, for example in children or in patients suffering from osteoporosis.

1.1.2 The Need for a Synthetic Bone Substitute

Considering the possible complications and inconveniences of autografting and allografting bone, there is a demand in the orthopaedic and dental communities for a synthetic bone substitute material. Primarily this material, and any potential degradation or corrosion products of the material, must be biocompatible. Biocompatibility is a broad term which describes the suitable response of both the host and the implanted material during the period of implantation. Bearing this in mind, the primary considerations when investigating materials for applications as a synthetic bone substitute are twofold; i) it is pivotal that the implant not induce any toxicological or chronic immune response from the host, either locally or systemically, and; ii) it is also important that the material possess mechanical properties, such as strength, fatigue resistance, and fracture toughness, which
make it suitable for load-bearing applications. These are basic criteria which insure acceptance of the material by the host, while not interfering with significant physiological functions, such as the healing process and bone regeneration, or compromising the integrity of the load-bearing skeletal structure that includes the implanted material.

There are also secondary, or preferred, properties of a synthetic bone substitute material, which must be considered in the design of these biomaterials, that may improve the performance of these materials in vivo. For bone-interfacing applications two desired properties are; i) that the material be osteoconductive, and; ii) that the material be biodegradable and bioresorbable at a rate suitable for bone-ingrowth. An osteoconductive material is one whose surface orchestrates the pattern of bone growth, which is important in bone substitution for two main reasons. Firstly, an osteoconductive material will allow for a high rate of bone ingrowth (i.e. for porous-structured implants), which will help anchor the implant quickly and augment the mechanical properties of the implant through reinforcement with bone. Also, the intimate contact between an osteoconductive material and bone will increase the mechanical bond at the interface, allowing for the necessary transfer of stresses to the developing bone tissue.

A synthetic bone substitute which is osteoconductive may be advantageous for rapid bone ingrowth during the initial stages of implantation, whereas over the long-term, biodegradability and bioresorbability may be beneficial. Ideally, it is desired that the section of bone requiring augmentation ultimately be replaced by new bone, suggesting
that the role of a synthetic bone substitute may be a temporary one. By utilizing a biodegradable material which is resorbed by the host at a rate suitable for bone-ingrowth, the implant will gradually be replaced by bone, allowing a transfer of stress from the implant to the new tissue. The progressive transfer of stress to bone is favorable, and may reduce the total time required for ingrowth and remodeling.

1.1.3 Materials for Synthetic Bone Substitute Applications

Ceramics and polymers have been the most widely investigated materials as synthetic bone substitute materials. There have been limited studies in the past investigating titanium fibre implants for scaffolding applications, but metals are generally considered unsuitable for segmental replacement because of the potential deleterious effects of their corrosion products. Accordingly, this review will focus on ceramics and polymers utilized for synthetic bone substitutes, and specifically, ceramics and polymers which display biodegradability.

1.1.3.1 Calcium Orthophosphates and Bioactive Glasses

The application of ceramics for bone interfacing applications has generally been limited to the calcium phosphate ceramics (CaP) and bioactive silicate-based glasses (BG). A major concern with ceramic materials for load-bearing applications, however, is their poor fracture resistance and low fracture toughness properties. This imposes a limiting factor on the possible applications of ceramic materials for synthetic bone substitutes.
There are a number of advantages of ceramics which are; i) increased corrosion resistance; ii) potential control over degradation rates, and; iii) biochemical similarity to bone tissue (in the cases of CaP and BG).

The first studies involving calcium phosphate based materials were undertaken by Albee early in this century based on the premise that the release of calcium ions from these materials was beneficial to osteogenesis. Although Albee’s conclusions in these pioneering studies were encouraging, it wasn’t until the late 1960’s and early 1970’s that the first prosthetics produced from calcium phosphates were produced. Currently the literature surrounding calcium phosphates, and in particular hydroxyapatite (HA) and tricalcium phosphate (TCP), is extensive.

The literature on HA and TCP can be considered under two main topic areas; i) studies investigating various structural forms (i.e. monolithic, porous, particulate), and; ii) investigations involving different stoichiometric combinations of calcium and phosphate. These studies can be divided further into two groups investigating; i) biological response (in vitro or in vivo), and; ii) material response. The reader is referred to a number of publications covering all aspects of this diverse topic for further study. The present review will be limited to more recent studies focusing on HA and TCP as they pertain to structure and biodegradability, which is the focus of the current investigation of calcium polyphosphate ceramics.
As shown by Driessens \(^{11}\), only two phases of calcium phosphate are stable (insoluble) in an aqueous environment at room temperature depending on pH. Hydroxyapatite, \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\), the calcium phosphate most similar to the mineral phase of bone, is stable above pH 4.2, while at lower pH dicalcium phosphate dihydrate, \(\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}\), is the stable phase. Therefore, at the physiological pH 7.4, HA is stable while all other phases of \(\text{CaP}\) are considered soluble to varying degrees (Table 1.1).

Tricalcium phosphate (TCP, \(\text{Ca}_3(\text{PO}_4)_2\)) is considered a highly soluble \(\text{CaP}\) and dissolves at a rate which is considered practical for a bioresorbable implant. In a closed aqueous system (i.e. there is no flow of material in or out of the system), all unstable \(\text{CaP}\) will be converted to the stable phase at a rate proportional to their solubility \(^{11}\). Since solid state chemical reactions cannot occur

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th>Ca : P</th>
<th>Solubility Product (pH 7.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>monocalcium phosphate monohydrate</td>
<td>(\text{Ca}(\text{H}_2\text{PO}_4)_2\text{H}_2\text{O})</td>
<td>0.5</td>
</tr>
<tr>
<td>dicalcium phosphate dihydrate</td>
<td>(\text{CaHPO}_4 \cdot 2\text{H}_2\text{O})</td>
<td>1.0</td>
</tr>
<tr>
<td>octacalcium phosphate pentahydrate</td>
<td>(\text{Ca}_5\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O})</td>
<td>1.33</td>
</tr>
<tr>
<td>(\beta)-tricalcium phosphate</td>
<td>(\text{Ca}_3(\text{PO}_4)_2)</td>
<td>1.5</td>
</tr>
<tr>
<td>hydroxyapatite</td>
<td>(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2)</td>
<td>1.67</td>
</tr>
</tbody>
</table>

Table 1.1 - The chemical composition and solubilities of common calcium orthophosphates \(^{14}\).
at low temperatures, it is assumed that any chemical changes of CaP ceramics will occur at the material/aqueous interface and not within the bulk of the ceramic. In an aqueous environment, an equilibrium will be reached between the calcium phosphate and the liquid medium ultimately producing the most stable phase at the material's surface. For instance, Newesely $^{12}$ described the change of (TCP) to HA in an aqueous environment at room temperature and pH 7.4 as follows:

$$4Ca_3(PO_4)_2(aq) \xrightarrow{H^+} Ca_{10}(PO_4)_6(OH)_2(s) + 2Ca^{2+}(aq) + 4OH^-(aq)$$

Equation 1.1 - The chemical equation which describes the formation of hydroxyapatite from tricalcium phosphate in an aqueous medium at pH 7.4.

According to this equation, TCP will dissolve at the surface into the surrounding medium and HA will precipitate to produce stable solid phase deposits at the material surface, assuming a saturated solution of TCP exists to initiate the reaction and that a supersaturated solution of HA exists to initiate precipitation. The formation of stable HA may not necessarily occur in vivo as physiological processes intervene. Natural metabolic processes remove degradation products from the implantation site, and a natural CaP containing mineral phase is produced in the region of the implant. This makes detection of precipitated HA difficult to quantify. The extent of crystallization of the stable phase is dependent upon the degradability of the ceramic used, and the action of the host tissue upon the implant and its degradation products $^{13}$. 
As noted by Jarcho\textsuperscript{9}, there are a number of physical and chemical factors which govern degradability of CaP materials. He summarized these factors under three main topics; i) surface area (i.e. porous vs. dense materials of the same chemical structure); ii) chemistry or stoichiometry (CaP materials with different stoichiometric ratios of Ca:P, but the same physical structure, will dissolve at different rates, for example TCP will dissolve more quickly than HA with the same physical structure), and; iii) structure (microporosity (<5\mu m) may increase the rate of degradation by releasing small crystallized particles). It is important to include at this time two additional factors which also affect degradability; iv) the presence of lattice defect dopants, and; v) the morphology of the material (i.e. crystalline vs. amorphous). The biological contribution to the rate of degradation, either through phagocytosis or enzymatic catalysis, is difficult to determine but also plays a significant role in breaking down implanted materials.

Many investigators have fabricated biphasic, biodegradable ceramic implants incorporating TCP and HA in varying ratios. It is generally accepted that higher TCP content within a biphasic ceramic contributes to a greater rate of degradation\textsuperscript{15, 16}. There is a discrepancy over how the presence of TCP affects other significant properties such as osseointegration. It has been hypothesized that the rate of release of calcium ions from TCP may inhibit ossification at the surface of the implant by interfering with cell differentiation\textsuperscript{17}, or that materials degrading at a high rate may release large numbers of crystalline microscopic particles, thereby inducing an extended inflammatory response that may physically interfere with ossification\textsuperscript{18}. More recent investigations have
determined that CaP ceramics require some biosoluble TCP phase for improved osseointegration, and that ceramics formed as either pure HA or TCP perform poorly. It has been suggested that physiological apatite is comprised of many phases and ceramics with Ca:P < 1.67 better resemble the natural bone mineral phase, or that a certain amount or range of extracellular calcium and phosphate may facilitate mineralization of collagen. Processing HA at temperatures higher than ~900°C inevitably produces other CaP phases (TCP, CaO) which, considering the previous argument, help account for the successes reported for what has been referred to as “pure HA”, which having been commercially purchased and/or processed at high temperatures may contain significant amounts of other more soluble phases. An optimum range for TCP:HA has not been determined for osseointegration, and a clearer understanding of the physiologic response to TCP degradation products is required to achieve this objective.

Investigators have shown that osteoconductivity may be as much a function of surface morphology (on the order of 1µm) and porous structure (on the order of 100µm) as chemical composition. The rationale for use of segmental replacements is to provide a structure which acts as a scaffold to guide bone during its regeneration while also providing sufficient load-bearing support. There have been a number of investigations aimed at determining the factors influencing osteoconductivity of open-pored structures, most focusing on the effect of pore size. The generally accepted range of pore size for a satisfactory rate of bone ingrowth as determined for a number of
different materials is \(50 - 500 \mu m^3\). One important factor which must be considered when using porous structures for bone ingrowth is the dynamic nature of skeletal tissue. As stated by Jarcho \(^9\), bone will grow where it is required as governed by applied stresses and not where it is "guided". Further a biodegradable porous scaffold which resorbs at a rate similar to the ingrowth rate of bone will perform better than an inert scaffold since the former would allow the gradual transfer of stress from the implant to bone. This observation may be taken one step further to suggest that it is a combination of material resorption and the degradation of mechanical properties which is important for an optimal rate of bone ingrowth. This distinction is important because materials degrade by a number of different mechanisms, for example through surface dissolution and erosion or bulk hydrolysis, and the mechanism(s) will reflect how the mechanical properties change as the implant degrades.

The compressive and tensile properties of calcium phosphate ceramics, as with all porous materials, are related exponentially to the porosity of the ceramic. As described earlier, voids may be introduced intentionally into the ceramic as macropores on the order of \(100 \mu m\) in size to allow bone ingrowth or unintentionally as micropores on the order of \(1 \mu m\) in size due to incomplete sintering of particles or during crystallization of amorphous materials. dePutter et al. \(^{25}\) experimentally determined relationships between the compressive and tensile strengths of these ceramics and total volume of voids. The empirically derived equations are as follows:
\[ \sigma_{\text{comp}}(V_p) = 700\exp(-5V_p) \quad [\text{MPa}] \]

\[ \sigma_{\text{tensile}}(V_m) = 220\exp(-20V_m) \quad [\text{MPa}] \]

where: \( V_p = \text{total volume of pores} \) \((0 < V_p < 0.5)\)
\( V_m = \text{total volume of micropores} \) \((0 < V_m < 0.05)\)

Equation 1.2 - The empirically derived equations for compressive and tensile strength of CaP ceramics with micro- and macroporosities \(^{25}\).

As these equations demonstrate, the presence of voids greatly decreases the strength of CaP ceramics. At the defined maximum porosity levels for macroporosity (50 v\%, \( \sigma_{\text{comp}} = 57\text{Mpa} \)) and microporosity (5 v\%, \( \sigma_{\text{tensile}} = 81\text{MPa} \)), a CaP ceramic will theoretically only be suitable as a replacement for trabecular bone (\( \sigma_{\text{comp}} \sim \sigma_{\text{tensile}} = 5 - 10\text{Mpa} \)) \(^{26}\). In calculating these figures, significant factors such as loss of strength through degradation or fatigue were not considered. The lack of both tensile strength and other mechanical properties of these materials has therefore restricted their use at the present time to non-loaded skeletal applications \(^9,27\).

Bioactive glasses were developed in the early 1970's, for the same purpose as the CaP based ceramics, namely as bone interfacing biomaterials displaying a degree of reactivity with surrounding tissue. Bioactive glasses are formed using a number of different oxides but usually contain large percentages of SiO\(_2\), Na\(_2\)O, and CaO, with a smaller amount of P\(_2\)O\(_5\). Other minor additives (<1 wt%), may include Al\(_2\)O\(_3\) \(^{28,29}\), B\(_2\)O\(_3\),
Bioactive glasses are based upon a tetrahedral silica (SiO₄⁻) network which include other network formers such as B₂O₃ and P₂O₅ combined with network modifiers such as Na₂O, K₂O, and CaO. These network modifiers disrupt the tetrahedral silica structure, and alter the morphological and chemical properties of the bioactive glass. Each plays a role in the reactivity of the material with surrounding tissue and only certain compositions will form a useful glass which possesses the proper reactivity.

Bioactive glass has been determined to react with bone in vivo through a complex set of reactions at the implant surface. Hench et al. have been largely responsible for the development of bioactive glasses and have suggested the following mechanism for the evolution of a biologically-produced surface layer of HA. There is an initial rapid dissolution of alkali ions from the glass (i.e. Na⁺ or K⁺) which are exchanged for H⁺ or H₃O⁺ from the surrounding body fluids. The hydroxyl groups react with the SiO₂ leading to the formation of silanol (SiOOH) which is quickly reversed through condensation and repolymerization to produce (Si-O)n and water, resulting in a SiO₂ rich layer at the surface. Ca²⁺ and PO₄³⁻ ions then diffuse through the SiO₂ layer and form a CaO/P₂O₅ surface layer. These initial steps occur within minutes and continue to produce thicker surface layers as the alkali ion exchange continues through the SiO₂ and CaO/P₂O₅ layers. Eventually, perhaps within 1 - 6 weeks, the outer layer is crystallized by the addition of CO₃²⁻, F⁻, and OH⁻ ions while at the same time, mineralized bone substance is produced in the interface region. It has been suggested that the presence of P₂O₅ in bioactive glass is
not required for the formation of an apatite layer, although the glass must contain a significant proportion of silica for this to occur^36.

Bioactive glasses, like other ceramics, do not possess mechanical properties which are ideal for load-bearing hard tissue prosthetics. Investigators have reported compressive strengths of approximately 1 GPa\(^{31,37}\), which suggests the possible use of bioactive glass for cortical bone replacement assuming compressive loading. This strength is greater than that reported for HA, but both materials possess equally low fracture toughness\(^ {38}\). Therefore, bioactive glasses suffer the same disadvantages as the CaP ceramics for load-bearing applications due to their limited mechanical properties. However, much more is known about the interaction of bioactive glass with tissue \textit{in vivo}, which is valuable information for developing a better understanding of material/bone tissue interactions.

1.1.3.2 Polymers

The use of organic-based biodegradable polymers for bone interfacing applications emerged in the early 1970's\(^ {39}\). The majority of studies investigating polymers for fracture fixation systems and synthetic bone scaffolding, as possible alternatives to metals, ceramics, and autografting, have involved biodegradable polymers. The motivation for using biodegradable polymers has been a combination of their degradability, the ability to manipulate the materials' mechanical properties through processing and composites, and the relative ease and cost of fabrication. In comparison to metals they are lighter,
have a lower modulus of elasticity ($E_{\text{polymer}} < 1\text{GPa}$, $E_{\text{bone}} = 6 - 20\text{GPa}$, $E_{\text{metal}} = 100 - 200\text{GPa}$), and, assuming safe and complete resorption of the polymer, a secondary surgery is not required to remove the implant. Polymers are also tough in comparison to ceramics making them preferred candidates for load-bearing applications. The main disadvantage of biodegradable polymers has often been the non-biocompatibility of degradation products with the surrounding host tissue.

Poly(lactic acid) (PLA, $(\text{OCHCH}_3\text{CO})_n$) and poly(glycolic acid) (PGA, $(\text{OCH}_2\text{CO})_n$) are produced from monomers of cyclic dimers of their respective acids (Appendix A). The synthesis of these polymers through a ring-opening polymerization reaction over certain catalysts produces a high molecular weight, relatively monodispersed polymer. PLA can be produced in two isonomic forms, poly-D-lactic acid (PDLA) and poly-L-lactic acid (PLLA). The D isonomer is amorphous and hydrolyzes much more quickly than the L form, which is highly crystalline. The chemical similarity of these polymers permits simple copolymerizations. Poly($\alpha$-hydroxy acids) are hydrophilic which makes them susceptible to bulk degradation, and copolymerization is often utilized to combine these polymers in an attempt to control degradation rates. The most common combinations are PLLA with PDLA, and PLLA with PGA. These materials have been the primary subjects of degradable polymers research since the 1960's. The available literature on PLA and PGA in various forms and chemical compositions is extensive. A number of books whose focus is upon PLA an PGA are available. A brief review of
the more recent literature on PLA and PGA that is considered particularly relevant to the study undertaken follows.

PLA and PGA have proven quite useful for short term, soft-tissue applications, such as degradable sutures, but their effective use for longer term bone interfacing applications is debatable. The principal concern is that healthy, calcified tissue does not grow directly in contact with the surface of implanted PLA and PGA \(^{42,43}\), which may suggest that these materials are not osteoconductive. In some cases this has been attributed to the development of a "dense fibrous capsule" surrounding the implant and perhaps due to a response to eroded particulate matter observed in the region of the implant \(^{44,45,46,47}\). This fibrous encapsulation of these polymers raises questions about the suitability of these materials for bone-interfacing applications, and for bone substitutes in particular. The absence of mineralized tissue directly adjacent to the implant reduces the mechanical stability of the implant, which in turn could be detrimental to healing through the effect of significant movement of the implant relative to bone \(^{48}\). Also, the effect of fibrous encapsulation and possible inflammatory reactions on the rate of degradation is unknown. It is assumed that the former may retard degradation by effectively separating the implant from the surrounding tissue and thereby decreasing the passage of water, enzymes, and phagocytic cells to the material, while also inhibiting the expulsion of degradation products. Fundamentally, the \textit{in vivo} rate of degradation is dependent upon a number of factors as described by Anderson \(^{49}\) in Table 1.2. The most general factors are material composition (chemical and morphological), implant size and
shape, and host species/implantation site. Evidence of remnants of crystalline PLLA (the slowest degrading of PLA and PGA polymers) has been reported in human implantation studies up to 5.7 years post-implantation.

An important consideration in design of materials for implant use is the extent and duration of the inflammatory response related to their implantation. Degradable systems are dynamic and the implant is in a continuous state of change which becomes an important factor in assessing biocompatibility. Therefore, not only is the initial host response to the material important, but also the chronic response to its degradation.

| Water permeability and solubility (hydrophilicity/hydrophobicity) |
| Chemical structure (nature of hydrolytically unstable bonds) |
| Mechanism of hydrolysis (noncatalytic, autocatalytic, enzymatic) |
| Additives (acidic, basic, monomers, drugs) |
| Morphology (amorphous, crystalline) |
| Device dimensions (size, shape, surface area to volume ratio) |
| Glass transition temperature (glassy, rubbery) |
| Molecular weight and molecular weight distribution |
| Physico-chemical factors (ion exchange, ionic strength, pH) |
| Sterilization |
| Site of implantation |

Table 1.2 - Factors which affect the hydrolytic degradation behaviour of biodegradable polymers.
products. Chronic inflammation inhibits the healing process. Therefore, it is important to consider both the chemical (pH, toxicity) and physical (particulates) effects on the host response for the duration of implantation. PLA and PGA are hydrophilic and degrade by bulk hydrolysis to form acidic monomers and highly crystalline, slowly degrading particles. Particulate remnants are usually visible and can be found years after implantation encapsulated in surrounding tissue or within the cytoplasm of multinucleated cells. The response to the acidic monomer produced during degradation is more difficult to quantify and appears to be related to implant size, host species, and chemical composition of the material. Studies involving rabbits show no inflammatory response, as do small implants, such as fixation pins, in humans. It also appears that implants containing higher proportions of crystalline PLLA have a longer acute inflammatory response and display reduced degradation rates presumably due to the presence of the crystal particles described previously. However, considering the variety of different compositions, polymer suppliers and methods used in material and implant fabrication, one cannot condone or condemn poly(α-hydroxy acids) for use in hard tissue interfacing. A tentative conclusion would suggest that PLA and PGA are suitable for small implant applications, such as fixation pins, but not for larger devices such as segmental replacements or fracture fixation plates.
Other Polymers

The extensive literature involving PLA and PGA provides a good base for the current research on novel degradable polymer systems. The possible disadvantages and concerns of these polymers have been identified and, based on the information derived from *in vivo* studies of PLA and PGA, these materials can serve as useful standards for comparing the performance of new polymers. There are a number of different biodegradable polymers reported in the literature with applications centering around drug delivery systems. Many are quite new and are either proprietary or in various stages of characterization. Two such polymers with interesting properties which have been applied to orthopaedic applications are hydrogels and polyphosphazenes. Other polymers such as poly(ortho esters) (POE) \(^6^0\), polycaprolactone (PCL) \(^6^1\), polycarbonates \(^6^2\), organoapatites \(^6^3\)-\(^6^5\), polydioxanone (PDS) \(^6^6\), poly(ethylene glycol) (PEG) \(^6^7\), poly(anhydrides-co-imides) \(^6^8\), \(^6^9\), and polyurethanes \(^7^0\) have also been investigated for orthopaedic applications, but have found more extensive use in controlled drug delivery applications. The chemical structures of these polymers are listed in Appendix A.

Hydrogels

A hydrogel is a polymer which is soluble in water and expands when infiltrated by water due to extensive cross-linking. Poly(2-hydroxyethyl methacrylate) [p(HEMA)] is a non-biodegradable hydrogel which has been utilized for research in drug delivery \(^7^1\), and microencapsulation of cells \(^7^2\), but more recently its swelling characteristics have been
investigated as an anchoring mechanism for intraosseal implants. \textsuperscript{73} \textit{p}(HEMA), in an unaltered form, is nonosteoconductive due to observed fibrous capsule formation which occurs on implantation in bone. However, Smetana et al. showed recently that a small percentage of denatured calf skin collagen (1\% w/v) introduced prior to the polymerization steps makes the polymer biodegradable, and osteoconductive when implanted intraosseally in dogs and pigs. \textsuperscript{74} Further research into degradation mechanisms of these polymers is being pursued by the investigators.

\textbf{Polyphosphazenes}

Polyphosphazenes are high molecular weight polymers with an alternating double-bonded phosphorus and nitrogen backbone which hydrolyzes to produce ammonia, phosphate, and other products derived from the side groups. The root polymer contains two chlorine molecules on the phosphorus atom and can be combined with any number of reactants through a substitution reaction, usually evolving a salt or an acid, to produce a variety of characteristics. This extremely versatile polymer literally has a limitless number of possible pendant groups available to it to alter degradation rates and biocompatibility. For example, Laurencin et al. \textsuperscript{75,76} produced different combinations of polymers based on poly[ (imidazolyl)(methylphenoxy) phosphazene] and poly[(ethyl glycinato) (methylphenoxy) phosphazene] for an \textit{in vitro} investigation of osteoblast cell response. They determined that an increase in imidazolyl reduced cell attachment but increased the polymer degradation rate compared to ethyl glycinato, which increased both cell adhesion and degradation rate.
1.1.4 Calcium Polyphosphates

Calcium phosphate materials appear to be the most chemically biocompatible synthetic materials for hard tissue interfacing. They are completely nontoxic and in most cases express excellent osteoconductivity. However, they are limited by their mechanical properties and unsuitable degradation rates. They have low fracture toughness and are stiff, and usually are not completely resorbable due to the presence of insoluble HA. Devices produced from organic polymers have other problems when used as bone-interfacing biomaterials. The biodegradable polymers often do not possess the mechanical properties required for high load-bearing applications and their degradation products may induce an inflammatory response in the surrounding tissue.

If the ratio of calcium to phosphorus is decreased during the preparation of CaP ceramics, the basic structure of the material may become more polymeric in form. A common example is the condensed phosphates. Condensed phosphates are polymers of phosphate in which phosphorus atoms share common oxygen atoms and are not exclusive to salts involving calcium. The simplest condensed phosphate is the dimer pyrophosphoric acid (H₄P₂O₇) and includes anything greater in size. The structure of condensed phosphates is usually depicted as a series of phosphate tetrahedra (PO₄³⁻) with four oxygen atoms at the vertices. Any common vertex within a condensed phosphate is a shared oxygen atom which represents a centre for hydrolysis (Figure 1.1). For example, short chain calcium polyphosphate usually undergoes hydrolysis at the terminating phosphate group, but hydrolysis also occurs at random points along larger chains.
Another form of cleavage occurs spontaneously by the release of cyclic calcium
tripolyphosphate, but this form of degradation does not involve reaction with a water
molecule and is therefore not hydrolysis.

Condensed phosphates can be divided into three main categories: a) linear
polyphosphates; b) ring structure metaphosphates and; c) three dimensional or cage-
structure ultraphosphates (Table 1.3). Linear polyphosphates are of interest in the
current study for possible synthetic bone substitute applications. In early literature, the
linear polyphosphates were often synonymous with metaphosphates. This is not
necessarily an erroneous distinction as very high weight metaphosphate rings and long
chain linear polyphosphates are very similar in chemical composition (Table 1.3). These
materials are most commonly found as a salt with any of the alkali metals, the most
common of which are calcium, sodium, and potassium. Linear polyphosphates may also
be categorized by chain length. Short chain polyphosphates (2 < n < 10) are very
soluble compared to longer chain polyphosphates and form highly crystallized salts.
Oligophosphates (10 < n < 50) are usually amorphous and found in a glassy, gummy, or
oily state. The long chain polyphosphates (n > 50) may be highly crystalline and have
limited solubility. The chain length of polyphosphates depends upon the method of
fabrication and the alkali metal salt within the complex. The value of n for
polyphosphates can range from 50 - 2 x 10^5 units and calcium polyphosphate may reach
lengths up to n = 10^4, though high temperature melt processing may produce chains of
Figure 1.1 - Three processes of calcium polyphosphate degradation: A) Terminating phosphate hydrolysis; B) Long-chain mid-point hydrolysis; C) Spontaneous release of tripolyphosphate without hydrolysis with water.
approximately \( n = 400 \) units in length. Linear polyphosphates are produced through a condensation reaction and, therefore, any water present will shift the balance of the reaction and produce shorter polymers.

Fibres of long chain calcium and sodium polyphosphates were first introduced as a biodegradable replacement for asbestos. Consequently, much of the research in biomaterials involving these materials deals with the linear polyphosphates in fibre form. These fibres have primarily been utilized in a reinforcing capacity to increase stiffness, strength and fracture resistance of the matrix polymer in composites for degradable fracture fixation devices (\( \sigma_{\text{CPPfibre}} \sim 1 \text{GPa (diameter dependent)} \)). Calcium polyphosphate (CPP), \([\text{Ca(PO}_3\text{)}_2]_n\) (commonly abbreviated \(\text{Ca(PO}_3\text{)}_2\)), is of particular interest in such bone interfacing applications because its hydrolytic degradation product is calcium orthophosphate, a non-toxic salt which may be a potential building block for physiological hydroxyapatite.

| Linear Polyphosphates | \( \text{P}_n\text{O}_{3n+1}^{(n+2)^-} \)\(|\text{At large } n, \text{ approaches metaphosphate}\) | \( \text{chains} \) |
|------------------------|---------------------------------|-----------------|
| Metaphosphates         | \( \text{P}_n\text{O}_{3n}^{n^-} \) | \( \text{rings} \) |
| Ultraphosphates        | \( \text{P}_n\text{O}_{3n+m}^{(n+2m)^-} \) \( (1 \leq m \leq n/2) \) | \( \text{cages, sheets, 3D structures} \) |

*Table 1.3 - The three groups of condensed phosphates.*
Calcium polyphosphate is most often formed through a reaction of calcium oxide or salts and phosphorus pentoxide or salts at high temperatures. Depending on which reactants are used, the secondary products of the reaction are usually H₂O and CO₂. In 1976 Abe et al. 92 developed the first CPP ceramics for biomaterial applications produced from the melt of calcium phosphate monobasic monohydrate powder, Ca(H₂PO₄)₂H₂O. After initial studies in bone bonding 93, they continued to research CPP through the next decade. They reported varying reagents from which they produced CPP and similar ceramics including 55CaO-45P₂O₅ (in mole%) 92, CaCO₃ - H₃PO₄ 94, and H₃PO₄ - CaCO₃ - Ca(H₂PO₄)₂H₂O 95. Their reports showed no variation in physical properties due to variations in formation. Others have also used Ca(H₂PO₄)₂H₂O in the formation of CPP 96, 97. To produce the glass, reagents are brought to temperatures in the range of 980 - 1250°C for a period of 1 - 2hrs. At these temperatures, phosphates are mildly volatile, so heating beyond two hours may alter the Ca:P ratio rendering a non-stoichiometric ceramic. The glass is formed by quickly cooling the melt on graphite or in water or a crystallized form may be produced through controlled cooling rates and/or crystal seeding of the melt. More recently Nelson et al. 98 produced a porous CPP at temperatures <800°C through chemical means.

As with other CaP ceramics, any trace impurities in CPP tend to disrupt the growth of crystals. Titanium and boron have been identified as having such an effect on CPP 80, 81. However it has also been reported that certain additives can enhance the ceramic’s physical and chemical properties. Trace impurities of zinc, aluminum,
molybdenum, iron, nickel, and silver existing at levels of 250 - 1000 ppm show enhanced crystal formation\textsuperscript{80, 81}, whereas the addition of small amounts of Fe\textsubscript{2}O\textsubscript{3} increases the strength of CPP fibres\textsuperscript{82, 85}.

The Monsanto Company, through a project headed by Edward Griffith, first introduced CPP and other alkali-substituted polyphosphates in fibre form\textsuperscript{80, 81}. They produced acicular crystal fibres intended as a biodegradable mineral fibre substitute for asbestos through mechanical means from crystallized glass solids. Fibers may also be drawn from the melt. The diameter of melt-drawn fibers is inversely proportional to draw speed and directly proportional to melt viscosity\textsuperscript{85}. Although this unique application for asbestos replacement never materialized, many biomaterials scientists took advantage of these fibres as reinforcements for use in totally degradable, load-bearing composite implants, such as fracture fixation plates. Foregoers in the field\textsuperscript{86-90, 99} hypothesized combining the high strength and modulus of the fibres with the toughness of the polymer matrix to create an ideal, completely bioresorbable composite implant. By slowly degrading over the course of fracture healing, the implants would theoretically transfer stress to the bone. This stress transfer would aid in healing and tissue remodeling, and also eliminate the need for a second surgery to remove the implant, as is required with conventional metal devices. However, the problems encountered were twofold. Primarily there was a problem with interfacial bonding of the fibre to the matrix polymer, which led to the problem of wicking and premature degradation of the fibres. The result was rapid loss of reinforcement and low initial mechanical properties. Some
research attempted to correct these problems by, for example, coating the fibres \(^8\), but most techniques showed little or no improvement. Recently Guo et al. \(^8\) approached the problem of rapidly degrading fibres through the addition of different ceramic dopants (TiO\(_2\) and Fe\(_2\)O\(_3\)) in the production of melt-drawn CPP fibres. It was found that small amounts of these additives, 1 mole% and 5 mole% respectively, decreased the degradation rate of amorphous CPP by approximately 80%.

Two Japanese groups led by Abe and Watanabe, have extensively studied the crystallization characteristics of calcium polyphosphates \(^1\)\(^0\) - \(^1\)\(^5\). Watanabe's group first reported the appearance of voids under specific crystallization and composition conditions arising from differences in density between the CMP glass and the resulting ceramic \(^1\)\(^4\). A mixture of 52CaO - 47P\(_2\)O\(_5\) - 1 Al\(_2\)O\(_3\) (mole%) displayed a pattern of voids resembling the cross-sectional rings of a tree emanating from surface nucleated crystallization sites. These rings were encountered only in the composition ranges of CaO:P\(_2\)O\(_5\) = 1.0 - 1.2 (mole ratio) and only between the temperatures of 600 - 660\(^\circ\)C. Beyond 660\(^\circ\)C the crystallization of the glass is rapid and the voids cannot diffuse, leaving a dispersed pattern. Below 600\(^\circ\)C, the crystallization speed is low and the voids may accumulate to form large defects in the ceramic. Calculations showed that the volume of such voids is equal to that which is expected from the density difference of the glass and ceramic. A more recent study by the group attempted to eliminate the production of voids which greatly affect mechanical properties of the ceramic \(^1\)\(^5\). They hypothesized that by adding greater quantities of Al\(_2\)O\(_3\), up to 10mol%, the formation of
the less dense AlPO₄ copolymerized with phosphate decreased the density of the resulting ceramic to that of the original glass. The ceramic, effectively void free, had a three-point bending strength of 144 MPa, a 2.5 fold increase over ceramics containing only 3mol% \( \text{Al}_2\text{O}_3 \) where failure-causing voids of up to 1mm in diameter were found.

Fukui et al. first reported implanting a calcium phosphate ceramic with a similar composition to CPP in 1977 \(^9^3\) (55 mol% CaO, 45 mol% \( \text{P}_2\text{O}_5 \)). They implanted fine crystalline rods (1 x 3mm) into the femur of rats. After follow-up periods of 2, 4, and 6 weeks they discovered mature bone in direct contact with the rods at 4 weeks. They suggested that the ceramic was an excellent material for a synthetic bone substitute. They also performed many other investigations of the crystallization properties of CPP and obtained patents for a fibrous form CPP for asbestos analogues, but appear to have abandoned their research, as did the Monsanto Company, due to possible legal pressures arising from questionable results from inhalation studies involving rats \(^7^8\). More recently Nelson et al. \(^9^8\) produced porous CPP (10 - 200\( \mu \)m diameter pores) through chemical means for implantation into dog mandibles. They report better host response and a higher rate of new bone ingrowth over control autografts and other studies involving coralline HA.
2. OBJECTIVES

The search for a suitable biodegradable synthetic segmental bone substitute has, until recently, focussed on calcium orthophosphate ceramics and poly(α-hydroxy) acids. As the literature has shown, these materials are limited in their mechanical and/or biological performance and new materials should be sought for bone substitute applications. Calcium polyphosphate (CPP) shows potential as a material for biodegradable synthetic bone substitute implant applications. The biocompatibility of CPP has been exploited in the past as a potential material for a possible biodegradable alternative to asbestos, and as the reinforcing component in totally biodegradable composite fracture fixation devices. Research involving CPP for bone substitute applications, however, has been limited. Information on the processing behaviour of CPP is negligible or proprietary and focuses mainly on fibre fabrication techniques. Similarly, the degradation properties of long-chain, monolithic form CPP has not been adequately characterized despite applications which exploit its biodegradability.

The current study is an initial investigation of the use of CPP for bone substitute applications, a primary objective of which is the processing and characterization of structures produced from CPP which are suitable for bone ingrowth, namely, a form containing interconnecting channels of appropriate size and possessing strength suitable for load-bearing applications. Since CPP is being utilized largely for its biodegradability,
it is also necessary to *characterize the degradation properties of these materials both physically and chemically*, the second primary objective here. This includes determining i) the loss of strength, and; ii) the release of degradation products over time.
3. POWDER AND SINTERED STRUCTURE PROCESSING AND CHARACTERIZATION

3.1 Processing

3.1.1 Powder Processing

The fabrication of calcium phosphate-based ceramics is well documented in the literature \[106, 107\]. As Table 3.1 shows, the definitive characteristic of the calcium phosphate ceramics is the molar ratio of calcium to phosphorous. For the purpose of producing calcium polyphosphate, \( \text{Ca}(\text{PO}_4)_2 \), an initial ratio of 0.5, Ca:P, is required. In this study, calcium phosphate monobasic monohydrate, \( \text{Ca}(\text{H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O} \) (J.T. Baker, Phillipsburg N.J.), was chosen as the precursor powder. This material was selected as it contains the required Ca:P ratio eliminating errors which may occur in measuring exact amounts of other forms of calcium and phosphorus. Modest errors in measuring two materials may result in a biphasic material, which could potentially be detrimental to the physical and degradation characteristics of the final product. The production of CPP powder occurs in three principal steps: calcination, glass formation and milling.

The initial step in producing CPP powders for use in this study was calcining of the precursor powder. Approximately 70g of the precursor powder was heated in a 125cc platinum crucible at 500°C for 10hr in air (Thermolyne Type 1500 furnace, Omega model #CN6081-K temperature controller, Omega K-type thermocouple). This induced a
<table>
<thead>
<tr>
<th>CaP</th>
<th>Abbreviation</th>
<th>Chemical Formula</th>
<th>Ca : P</th>
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<tbody>
<tr>
<td>monocalcium phosphate monobasic</td>
<td>MCPM</td>
<td>Ca(H₂PO₄)₂·H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>dicalcium phosphate dihydrate</td>
<td>DCPD</td>
<td>CaHPO₄·2H₂O</td>
<td>1.0</td>
</tr>
<tr>
<td>octacalcium phosphate pentahydrate</td>
<td>OCP</td>
<td>Ca₈H₂(PO₄)₆·5H₂O</td>
<td>1.33</td>
</tr>
<tr>
<td>tricalcium phosphate</td>
<td>TCP</td>
<td>Ca₄(PO₄)₂</td>
<td>1.5</td>
</tr>
<tr>
<td>hydroxyapatite</td>
<td>HA</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>1.67</td>
</tr>
<tr>
<td>calcium polyphosphate</td>
<td>CPP</td>
<td>Ca(PO₃)₂</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3.1 - Common calcium orthophosphates, abbreviations, and molar ratios of constituents.

condensation reaction during which water was liberated as governed by the following equation:

\[
\frac{n}{\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}} \xrightarrow{500^\circ\text{C}} \left[\text{Ca}(\text{PO}_3)_2\right]_n + 3n\text{H}_2\text{O}
\]

Equation 3.1 - The chemical equation which describes the condensation reaction of calcium phosphate monobasic monohydrate to calcium polyphosphate.

The resulting powder and crucible were transferred to a tube furnace where the powder was melted at 1100°C to produce an amorphous glass. The CPP was held in the molten state for 1 hour to induce chain lengthening. The molten CPP was poured directly into distilled water which, due to rapid cooling, resulted in the formation of an amorphous frit. Previous attempts at cooling in air induced partial crystallization of the
glass, likely due to a slow cooling rate and the introduction of nucleation sites through contact with airborne dust particles. The frit was immediately dried in 100% ethanol and stored overnight at 85°C in air (Fisher Isotemp 500 Series). It was then relocated to a vacuum desiccator for indefinite storage.

The powder was produced from the resulting frit by ball milling using a stainless steel mortar and balls (Retsch Mixer Mill Model MM2). The powder was added to the mortar in small quantities (~2.5g) along with two balls. The frit is pulverized for 10s intervals and the powder was screened (stainless steel, W.S. Tyler Co., St. Catharines, Ontario) immediately to remove -50mesh (<300μm diameter) particles. The larger particles were returned to the remaining frit and repulverized until all powder passed through the 50mesh screen. This method was designed to maximize the yield of the powder size ranges required for this investigation. The powder was further screened and divided into two size ranges, 150-250μm (-60/+100 mesh) and 106-150μm (-100/+140 mesh) designated as coarse powder and fine powder respectively.

3.1.2 Porous Sintered Structure Processing

Porous CPP samples were prepared from powder in two different size ranges, 106-150μm and 150-250μm. These size ranges were chosen so that, upon gravity sintering, pores would be formed within the preferred size range of 50-500μm for bone
Two size ranges were chosen so that other properties such as strength, processibility and degradation rate could be compared.

Sintered samples were prepared by a modified Engel process\textsuperscript{108} which involved sintering the powder in vertically arranged platinum crucibles as described in Figure 3.1. These crucibles consist of a tube supported by an "end cap" which is removable to facilitate sample extraction. End caps were firmly secured with masking tape to their respective tubes to prevent powder from entering the space between the tube end and the cap. A measured amount of powder, approximately 0.3g, of a desired size was added to each of the crucibles using a small wax paper funnel. The powder-filled crucibles were secured with double sided tape to a vibrating platform (Grobet, model #21.816), and vibrated for approximately 5s to settle the powder. A duration of 5s was chosen to settle the powders to a high density while minimizing powder separation, where the smallest powders would fall to the bottom of the crucible. The volume of powder in the crucible was determined by measuring the depth to the top of the powder using a thin metal rod. A green state density was then estimated using the mass and apparent volume of powder.

Final porous structures were produced by sintering samples in air in a muffle furnace (Thermolyne Type 48000). A sintering temperature was chosen for each powder size range based on the extent of densification. To determine the ideal temperature, a series of sintering temperatures was chosen and the point at which there was not extensive densification, termed "oversintering", was selected. These were determined to
be 970°C for the coarse powders and 965°C for the fine powders. Samples were heated at a rate of 10°C/min to the sintering temperature, held for 2h and furnace cooled. This yielded a crystalline, porous CPP rod, approximately 15mm in length and 4mm in diameter. Disk samples were prepared by cutting the sintered rods across the diameter in 2mm lengths using a diamond wafering blade with 0g load at 2500rpm (Buehler Isomet Plus model Precision Saw). The disks were cleaned twice hydrosonically in de-ionized
water for 15 min periods and dried in 100% ethanol. Disk samples were stored overnight at 85°C and then transferred to a vacuum desiccator.

Sample dimensions and mass were measured prior to in vitro degradation studies (Section 4). The average of five diameters and the average of two thicknesses, measured using digital vernier calipers, were determined. The mass was determined and a density calculated for each individual sample. The average density of all samples was used as a characteristic density for the sample group. Disks were stored in cell culture dishes in a vacuum desiccator prior to use. Each sample was designated by its position in the dish and samples from the same batch were given specific groups of rows (i.e. disks from batch A were placed in rows A,B, and C as shown in Figure 3.2). Samples for degradation

Figure 3.2 - The arrangement of samples for identification within 96 well cell culture trays.
studies were selected by column, guaranteeing that each degradation condition was equally represented with disks prepared from different batches of sintered rods. Individual samples were designated by tray number and position. For example, the disk in tray 2 at position D7 was called 2D7. Tray sets were separated by powder size ranges.

3.2 Characterization of Powders and Porous Structures

3.2.1 Chemical Characterizations

3.2.1.1 Mass Ratio

During calcination, calcium phosphate monobasic loses three water molecules to produce calcium polyphosphate. By weighing the Ca(H$_2$PO$_4$)$_2$H$_2$O powder before and after calcining (Sartorius, Model B120S-0KR), an estimate of the amount of water lost was determined. The theoretical calculation is shown by the following equation:

\[
\frac{\text{Ca}(\text{PO}_3)_2}{\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}} \times \frac{198.02 \text{ g/mol}}{252.07 \text{ g/mol}} = 78.6\%
\]

Equation 3.2 - The theoretical mass ratio of calcium polyphosphate to calcium phosphate monobasic monohydrate following calcining.
3.2.1.2 Neutron Activation Analysis

Neutron Activation Analysis (NAA) was performed at the SlowPoke Reactor Facility at the University of Toronto. Samples of mass ~0.3g from each stage of processing were irradiated at a neutron flux of $1 \times 10^{11}$ n cm$^{-2}$s$^{-1}$ (2kW) for 180s in the Slowpoke-2 Reactor (Atomic Energy of Canada Companies). After a 120s transfer delay, a gamma flux count over 200s was performed at a distance of 3.75cm from the gamma counter. Peaks representing the decay of Ca and P were integrated and mass fractions were determined using custom computer software. The mass fractions of phosphorus to calcium were determined and the ratio compared to that of theoretical CPP:

$$\frac{Ca}{2P} = \frac{40.078g/mol}{2 \times 30.974g/mol} = 0.647$$

Equation 3.3 - The theoretical mass ratio of phosphorus to calcium in calcium polyphosphate.

3.2.1.3 X-ray Diffraction Crystallography

Samples of CPP at all stages of processing were analyzed by x-ray diffraction to assess their crystal structure. This analysis was used to ensure that the initial powder was amorphous and that the sintered porous structures were crystallized. The x-ray spectrum for the crystallized CPP was produced with a Rigaku diffractometer using a Cu
- Kα radiation source. Diffraction spectra were compared to those for commercially available CPP fibres (Monsanto Company, Monsanto, Ca).

3.2.2 Morphological/Physical Characterizations

3.2.2.1 Scanning Electron Microscopy

All Scanning Electron Microscopy (SEM) work was performed using the Hitachi Model S2500 Scanning Electron Microscope at the Department for Biomaterials in the Faculty of Dentistry at the University of Toronto. A monolayer of each powder size was produced by applying powder to double-sided tape on an SEM sample holder. Excess powder was removed with a stream of air and samples were made electrically conductive by coating with a 3.0nm thick layer of platinum. For each sample, a series of nine overlapping pictures was taken at magnifications of 100x for coarse powders and 150x for fine powders. Particle sizes were characterized using a digitizer (Kurta Series II Model 1000 Digitizer) interfaced with a Macintosh Plus computer running custom data acquisition software (Chris Pereira, 1996). SEM images of individual particles were arranged on a two dimensional XY planar arrangement as shown in Figure 3.3. The longest dimension of each particle was oriented along the Y axis and the widest point perpendicular to this was positioned on the X axis. Four diameters were measured each on the Y, X, XY and the X(-Y) axes. The average of the four diameters was taken as the characteristic diameter and the quotient of the Y and X axis measurements was taken as
the aspect ratio. Histograms and skewness of the distributions were calculated using Statview 4.1 software (Abacus Concepts Inc. Berkley Ca.).

Figure 3.3 - The orientation of particles for determining characteristic diameter.

3.2.2.2 Diametral Compression Strength

CPP is a brittle, ceramic material; this limits the mechanical testing methods available to evaluate its properties. The diametral compression test is appropriate to measure the tensile strength of these materials and was selected for a number of reasons.
Primarily it is a test which converts compressive force into a tensile stress perpendicular to its loaded diameter. This takes advantage of the relatively higher compressive strength of brittle materials compared to properties in tension. Also, by using this method, test requirements were such that small diameter samples, similar to those produced here, could be evaluated.

Tests were carried out in a specially made test jig comprised of two flat stainless steel platens and a positioning shoe as shown in Figure 3.4. Testing conditions were at room temperature in air using an Instron uniaxial servo-hydraulic materials tester model #8501. The purpose of the shoe was to position each sample on its circumferential face in the centre of the platens until a setting load of approximately 0.5kg was applied, at which point the positioning shoe was removed. A cross-head speed of 0.5mm/min was selected as suggested by Thomas in similar tests of hydroxyapatite. Samples were loaded until fracture and data was collected using the LabView software package (National Instruments) with a custom data acquisition procedure run on a Macintosh IIfx computer. Fracture was typified by a sharp decrease in the load applied to the test specimen (Figure 3.5).

In some cases samples were discarded and the results could not be used in calculations. Due to the brittle and porous nature of these samples, flattening of the contact points during testing was inevitable; in the weakest samples excessive flattening
Figure 3.4 - Diametral compression analysis test jig arrangement with positioning shoe.
occurred. If the width of the flattened ends exceeded approximately 20% (0.8mm) of the diameter, the maximum tensile stress across the loaded plane of the sample falls below the theoretically calculated stress, and the test was considered ineffectual and the results ignored 111. The width of the flattened regions could not be measured precisely due to the apparatus positioning, so the width was estimated and questionable samples discarded. The fracture pattern of disks which experienced extensive flattening often occurred as a fracture line which intersected the loaded diameter at an angle (Figure 3.6), suggesting shear failure 109. In some cases failure did not occur in either tension or shear before excessive crushing of the contact points exceeded the approximate width limit and these samples were also discarded. An example plot of load versus displacement for a sample which underwent excessive crushing is shown in Figure 3.7.

![Figure 3.5 - An example plot of load versus displacement showing a sharp decrease in load typifying fracture for the diametral compression test.](image-url)
Figure 3.6 - Shear failure in diametral compression analysis with excessive crushing at contact points.

Figure 3.7 - An example plot of load versus displacement of a disk sample which underwent excessive crushing at the platen contact points.
Porosity of the sintered CPP structures was characterized by two methods aside from the gross density method based on dimensions and mass referred to in Section 3.2. The pore size distribution was measured by mercury intrusion using the Filling Apparatus for an Autoscan-60 Porosimeter (Model 6 Filling Apparatus, Quantachrome Corp., Syosset, New York). Pressures ranging from approximately 1 - 24 psia were applied to intrude mercury into pores ranging from approximately 5 - 180 µm in size. The data was analyzed using the Autoscan Data Reduction System (PORO2PC Software Version 1.0, Quantachrome Corp.) to produce a distribution plot of pore size frequency.

Porosity was also characterized as a void percentage of sintered structure volume. Sintered samples of each powder size were imbedded in poly(methyl methacrylate) under vacuum, and cross-sections were cut with a slow speed diamond saw (Buehler). The samples were polished successively with 180 grit, 240 grit, 400 grit, and 1200 grit silicon carbide paper and made electrically conductive by coating with 2nm of platinum. Backscatter electron images were taken of random sections at 12, 3, 6, and 9 o'clock positions on the cross-section. Micrographs of backscatter images were digitized and porosity was determined as the percentage of micrograph area occupied by void space.
3.3 Results

3.3.1 Chemical Characterizations

The results of the chemical characterizations for the as-made powders are summarized in Table 3.2 and suggest that the powders produced are calcium polyphosphate. The mass of the calcined powders was 78.7% ± 0.03% (n=14) of the mass of the precursor powder. This agrees closely with the theoretical value of 78.6% (Equation 3.1) calculated for Ca(PO₃)₂ of infinite chain length produced from Ca(H₂PO₄)₂·H₂O.

As described in section 3.2, calcined powders were melted and an amorphous frit was produced by pouring the molten CPP into distilled water. The high viscosity of the molten CPP, which quickly increased due to rapid cooling while pouring at room temperature, left a large amount of solidified CPP in the melting crucible. Because of this, the mass of the melted powders could not be taken and NAA was used to determine the calcium to phosphorus ratio of the CPP at all steps of production. NAA of the as-made powders (n = 10) determined that Ca:P(mass) = 0.655 ± 0.022 which is equivalent to Ca:P(molar) = 0.506 ± 0.017. The results of the mass comparison and of NAA are summarized in Table 3.2.
<table>
<thead>
<tr>
<th></th>
<th>Measured Experimentally</th>
<th>Theoretically Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-calcined mass ratio (CPP:Ca(H₂PO₄)·H₂O)</td>
<td>78.7% ± 0.03%</td>
<td>78.6%</td>
</tr>
<tr>
<td>As-made powder Ca:P as determined by NAA</td>
<td>0.655 ± 0.022 (mass)</td>
<td>0.647</td>
</tr>
<tr>
<td></td>
<td>0.506 ± 0.017 (molar)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table 3.2 - A summary of results of the chemical analyses of as-made powders.

3.3.2 Morphological/Physical Characterization

3.3.2.1 SEM Characterization

**Powder**

SEM images of both coarse and fine powder samples are shown in Figure 3.8. As shown in the micrographs, the powder particles are angular and granular with irregular shape. Figures 3.9 b) and d) show the distribution of aspect ratios for both powder size ranges. The average aspect ratios for the fine and coarse powders were 1.6 ± 0.5 and 1.7 ± 0.8 respectively, confirming that the dimensions of the particles were not distinctly acicular. A small percentage of particles appeared to be somewhat acicular, although this was a random occurrence. Figures 3.9 a) and c) show the distribution of characteristic diameters for each powder size range. The average particle size for the coarse powder size range (150-250μm) was 199μm ± 53μm, and for the fine powder size range (106-150μm) the average particle size was 119μm ± 25μm.
Figure 3.8 - SEM images of a) 106 - 150μm size range powders, and; b) 150 - 250μm size range powders.
Figure 3.9 - a) Particle size distribution for 106-150μm particle size range; b) Aspect ratio distribution for 106-150μm particle size range; c) Particle size distribution for 150-250μm size range; d) Aspect ratio distribution for 150-250 particle size range.

Although the average particle size for each size range was near the median value for each size range, both distributions were skewed slightly toward the larger particle sizes. The fine particle range (106 - 150μm) and coarse particle range (150-250μm) had
skewness values of 0.37 and 0.91 respectively, compared to a zero value for a perfectly normal distribution.

Porous Structures

At low magnification (Figure 3.10 (a) and (b)) the presence of macropores was highly evident in cross-sections for both the coarse and fine powder sintered samples. The coarse powder structure appeared less dense with a high concentration of macropores, whereas the fine powder structure appeared to contain a dispersed range of pore sizes within a more densely sintered form. There was no apparent density gradient visible from the periphery to the centre of disks in the micrographs of cross-sectional planes, and the distribution of pores was relatively uniform, although random non-uniformities, such as regions of high or low density were observed.

The formation of sinter necks was examined at higher magnification. Figures 3.11 (a) and (b) show typical sinter necks for both the fine powder and coarse powder sintered structures. The sintered particles showed extensive necking and continuity of form across sinter neck regions (i.e. a smooth transition between particles with no variance of crystal grain structures). The surfaces of the particles displayed a crystallized structure with prominent grain boundaries and visible evidence of microporosity within these grain boundaries. The particles appeared to lose their angularity upon sintering and crystallization, and acquired a more rounded form, with rounded crests and a lack of sharp
angles. Interconnecting macropores were highly evident and were on the order of 100μm in size for samples of both powder size ranges.

The crystal grains observed on the surface of particles and at sinter neck regions were approximately 1 - 10μm in size independent of the particle size range of the sintered sample (Figures 3.12 (a) and (b)). Within the grain boundaries a large quantity of microvoids (< 1μm) was also observed.

3.3.2.2 Size and Density

The average measurements for thickness, diameter, mass, and density of disk samples for coarse and fine powders are summarized in Table 3.3. The difference in sample thickness for the two powder sizes is due to the cutting technique and is not attributed to the sintering procedure. The fine powder, however, experienced greater densification upon sintering which resulted in smaller diameter samples. The average sintered density, measured by size and mass methods (Section 3.1.2), was 55.5% ± 5.3% for coarse powder samples and 67.3% ± 4.2% for fine powder samples, which was an average increase of 2.3% (p = 0.0058) and 16.6% (p < 0.0001) over green state densities respectively.
Figure 3.10 - Low magnification micrographs (30x) of cross-sections of sintered disks of powder size range a) 106-150 µm, and b) 150-250 µm.
Figure 3.11 - Micrographs showing the smooth transition across sinter necks between particles from powder size ranges of: a) 106-150μm and b) 150-250μm.
Figure 3.12 - Micrographs showing the crystal grain structure on the surface of particles and the presence of intercrystalline microvoids within sintered structures of a) 106-150µm and b) 150-250µm powder size ranges.
3.3.2.3 Porosity Measurements

The pore size distribution as determined by mercury intrusion for both coarse and fine powder sintered samples is shown in Figure 3.13. The average pore size for the 150-250μm powder sample was in the range of 80-95μm and followed an approximately normal distribution with a majority of the macroporosity within the 50-120μm size range. The 106-150μm powder samples showed a more dispersed pore size distribution weighted toward the smaller pore sizes. The average pore size was within the 70-100μm range with the majority of macroporosity within the 40-140μm size range.

Figure 3.13 - The pore size distributions for sintered structures for both powder size ranges. The units for frequency are arbitrary and the plots are not to scale.
The back scatter image analysis of the sintered 106 - 150µm size range powders verified the density of disk samples as determined by weight and volume techniques (ref. Section 3.1.2). The density of these samples as measured by back scatter image analysis was 66.7% as compared to 67.3% ± 4.2%. Back scatter images of the coarse powder size range (150 - 250µm) revealed that these samples contained a high volume of microvoids within the individual particles. These samples appeared much more porous within the particle bulk compared to the fine powder samples and had densities of 56.5% (microvoids inclusive) and 58.2% (microvoids exclusive) in comparison to the measured density of 55.5% ± 5.3%. The exclusion of microvoids was performed with caution and only microvoids within the particle bulk and those which could be justified as definite microvoids were excluded. This method provided a minimum estimation of the density of the intergranular microvoids at 1.7% although the actual contribution of these voids is expected to be somewhat higher. The back scatter images for both particle size ranges are displayed in Figure 3.14

3.3.2.4 Diametral Compression Strength

The greater density of sintered fine powder structures resulted in a fourfold increase in diametral compression strength over the coarse powder samples (Table 3.3). The average strength of fine powder sintered samples (n=16) was 24.1MPa ± 6.8MPa compared to 5.9MPa ± 1.6MPa for samples produced from coarse powder (n=20).
One batch of samples was discarded from the fine powder samples for the degradation tests due to unsuitable failures (ref. Section 4.3.2).

Three distinct characteristics of the load / displacement curve were observed during the fracture of sample disks using the diametral compression method (Figure 3.15). The initial loading of the disk in the early stages of the test caused minor fluctuations in load as particles protruding from the loaded section of the periphery were fractured. The second region of the curve showed a stable increase in load once the surfaces of the platens had made sufficient contact with the perimeter of the disk. As the load was increased through this region, two small plateaus were often observed which represented minor crushing of the contact points of the disk by the platens. The third section of the curve was characterized by a sharp decrease in load, signifying fracture of the sample.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Fine Powder (106-150(\mu)m)</th>
<th>Coarse Powder (150-250(\mu)m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Thickness (mm)</td>
<td>1.9 ± 0.2</td>
<td>2.1± 0.1</td>
</tr>
<tr>
<td>Average Diameter (mm)</td>
<td>3.8 ± 0.2</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Average Mass (mg)</td>
<td>40.2 ± 5.0</td>
<td>43.4 ± 5.3</td>
</tr>
<tr>
<td>Average Density (%theoretical)</td>
<td>67.3 ± 4.2</td>
<td>55.5 ± 5.3</td>
</tr>
<tr>
<td>Average Green Density (%theoretical)</td>
<td>50.7 ± 0.6</td>
<td>53.2 ± 0.8</td>
</tr>
<tr>
<td>Diametral Strength (MPa)</td>
<td>24.1 ± 6.8</td>
<td>5.9 ± 1.6</td>
</tr>
</tbody>
</table>

Table 3.3 - Summary of measurements for as-made sintered disk samples for fine and coarse powder size ranges.
Figure 3.14 - Back scatter electron images of gravity sintered disk cross sections. a) 106 - 150μm, and; b) 150 - 250μm particles size ranges.
Figure 3.15 - A plot of a loading curve for a sample disk displaying the three typical characteristics of fracture: a) Load fluctuation as protruding particles are fractured; b) Two plateaus in load as minor crushing occurs at the points of contact; c) A sharp fracture point with a significant decrease in load.
3.4 Discussion

The objective of the processing section of the current study was to produce porous scaffolds for use as bone substitute implants. These porous scaffolds were made by sintering CPP powders of appropriate size and shape. Two powder size ranges were used (106-150μm and 150-250μm). These resulted in structures with pores that were considered suitable in terms of pore size and distribution (i.e. three dimensional interconnected pores) for bone ingrowth. The diametral compression strength of the samples produced from the two powders differed, however; the fine powder samples underwent greater densification and displayed a wider range of pore sizes and higher strengths as a result.

The disk samples produced for this investigation were fabricated from a single batch of CPP powder, and displayed satisfactory consistency of sintering and physical properties. However, significant variation in sintering behaviour, and hence physical properties, occurred among powder batches, or often even within single powder batches. The extent of densification was difficult to control accurately, and the sintering characteristics of both the coarse and fine powders displayed surprising sensitivity to sintering temperature. It was apparent when determining suitable soaking temperatures for the two powder size ranges (Section 3.3) that a very narrow range of temperatures yielded samples with suitable density for the purposes of this investigation (%ρ = 55 -
65%). It was observed that minor adjustments to sintering temperature, often as small as ±2°C, could have significant and potentially deleterious effects on sintering behaviour.

Consideration of sintering theory may provide an explanation to these variable sintering properties of CPP powders. It was assumed early in the study that CPP, although possessing ceramic-like properties due to the ionic cross-linking effect of calcium, may behave differently from most ceramics during the sintering process due to its polymeric structure involving a backbone of covalently bonded phosphate (PO$_4^{3-}$) tetrahedra. Within the literature, there is limited information describing the sintering properties of polymers. This is not surprising since polymer processing temperatures are usually low in comparison to ceramic processing temperatures, and allow alternative methods of forming solid and porous structures, such as melt forming and various foaming techniques. A series of papers by Spector, Sauer et al. at Clemson University describe using a sintered “compaction of granules of high density polyethylene”$^{112-116}$ and polysulfone$^{117}$ for the investigation of bone ingrowth properties into porous polymers. However these investigators used commercially-acquired proprietary materials for their studies and did not discuss processing characteristics.

Therefore, to better comprehend the sintering characteristics of the CPP powders used in this study, the theoretical equation describing the initial stage sintering properties of particles as a ratio of sinter neck diameter $X$ and particle diameter $D$, termed sinter neck ratio, was considered$^{118}$. 
Equation 3.4 - The model for isothermal neck growth during initial stage sintering.

\[
\left( \frac{X}{D} \right)^n = \frac{Bt}{D^m}
\]

where \( B \) is a function of different material properties, and \( n \) and \( m \) are constants, all of which are dependent on the mass transport mechanism involved, and \( t \) is time. In the current study involving CPP, in which the molecules are polymeric, it is assumed that the dominant sintering mechanism is mass transport by viscous flow where \( B = 3\gamma/2\eta, n = 2, \) and \( m = 1, \) and where \( \gamma \) is the surface energy and \( \eta \) is the viscosity. Thus:

\[
\left( \frac{X}{D} \right) = \left( \frac{3\gamma t}{2\eta D} \right)^{\frac{1}{2}}
\]

Equation 3.5 - The revised model for isothermal neck growth during initial stage sintering for a viscous flow transport mechanism.

Viscous flow mass transport was assumed to be the primary sintering mechanism in this system for two reasons. Primarily, polymeric and amorphous materials such as CPP are generally considered to sinter by a viscous transport mechanism \(^{118}\). Also, the determined sintering temperatures were much higher in comparison to melt temperature \( (T_m) \) than for conventional ceramics \(^{118}\), and were within the low end of the melting temperature range reported for CPP (950 - 985°C) \(^{79,96,100}\). This may suggest that
softening or partial liquification of the CPP particles occurred in order to achieve sintering.

The melt temperature \((T_m)\) of a polymer is directly related to the molecular weight of the polymer \(^{119}\). As observed for most polymers, dispersion of the molecular weight distribution will produce a melting range as opposed to a defined melt temperature, and may provide a rational explanation of the observed high sensitivity of CPP sintering characteristics to temperature. With the current method for producing CPP powders, molecular weight likely varies from batch to batch considering the conditions used for forming CPP (i.e. melting in air as opposed to a controlled atmosphere). Factors such as moisture and airborne contaminants may be significant in controlling molecular weight and the formation of metaphosphate ring structures in the CPP materials. The assumed inconsistency of molecular weight and chain versus ring structure of CPP molecules may consequently produce inconsistent melting temperature ranges and therefore variable sintering characteristics.

Ideally, correlating molecular weight to melting and sintering temperature could provide a method for precisely controlling densification. However, the molecular weight of CPP cannot be determined directly by traditional methods such as gel-permeation chromatography, light scattering, and titration techniques because of its insolubility in all solvents \(^77\). As an alternative approach to directly measuring the molecular weight of CPP, sodium may be substituted for calcium in CPP through a reaction with sodium
ethylenediamine tetraacetic acid (EDTA) \(^{78,120}\). Sodium polyphosphate is water soluble and molecular weight of the altered CPP may then be determined. However it was beyond the scope of this study to investigate this approach.

The melt temperature range of CPP powder is an important factor for determining sintering conditions and may be identified through thermal transition curves obtained by thermal analyses such as differential thermal analysis (DTA) and differential scanning calorimetry (DSC). However, correlating the sintering temperature and melt temperature using DTA/DSC may not be sufficient to accurately describe sintering consistency, as such methods do not account for viscosity, a property directly related to molecular weight, which is a primary determinant of viscous mass transport behaviour.

As described in Equation 3.5, viscosity is a significant parameter in the sinter neck ratio model and is inversely proportional to the sinter neck ratio. The melt viscosity, \( \eta \), of a polymer is directly related to the weight average molecular weight, \( M_w \), raised to a power, \( A^{119} \):

\[
\log \eta = A \log M_w + B
\]

or

\[
\eta = M_w^A + B'
\]

**Equation 3.6 - A general equation describing the dependence of the melt viscosity (\( \eta \)) of a polymer on weight average molecular weight (\( M_w \)).**
The value of $A$ is dependent on molecular weight, and falls in the range of 1 - 2 at low
molecular weights (commonly closer to 1) and changes to 3 - 4 at higher molecular weights
(a value of 3.4 is generally accepted for most polymers). The value of $M_w$ at which the
transition occurs is termed the \textit{transition molecular weight} and is dependent on the
specific polymer. At the transition molecular weight polymer entanglements critically
impede single chain diffusion. At chain lengths greater than the transition $M_w$, polymer
chains diffuse as groups, greatly increasing the viscosity of the polymer melt. At low
molecular weights (i.e. below the transition $M_w$) the molecular weight has little effect and
the sinter neck ratio is inversely proportional approximately to the square root of
molecular weight ($X/D \propto M_w^{1/2}$). Above the transition molecular weight the effect
becomes significant and the sinter neck ratio is proportional nearly to the square of
molecular weight ($X/D \propto M_w^2$).

The correlation of viscosity to molecular weight, and how this affects the sinter
neck ratio, presents an uncertainty in controlling the sintering properties of CPP. As
reported by Griffith, the molecular weight of CPP produced by melt techniques is
commonly $< 400$ and is influenced by the presence of moisture and contamination\textsuperscript{78}. In
the current study the variability of molecular weight may play an important role in
determining the sintering behaviour of the CPP powders. This is a topic recommended
for further investigation in order to attain better control over densification and the
associated properties such as strength and porosity. Although the importance of
molecular weight cannot be over-emphasized, other parameters, such as time, and material properties, including particle size and shape, particle size distribution, and contamination, also play significant roles in determining the sintering behaviour of CPP powders.

The particle size range of powders had a significant effect on sintering characteristics. The physical differences between the two powder size ranges investigated were observed primarily in the sintered densities and diametral strengths (Table 3.3). The particle size distributions shown in Figure 3.7 are very close to symmetrical with mean particle sizes falling near the medians of each size range. The width of the particle size distributions, however, as determined by the image analysis method of Section 3.2.2.1, was greater than the apparent range limits for the particle diameter as determined by screening, and can largely be accounted for by the irregular shape of particles produced by pulverizing the CPP glass frit. Particles with significantly high aspect ratios will have large and small diameters perpendicular to each other.

In different orientations, each irregularly shaped particle will produce a unique cross-sectional shape with a particular sieve diameter. It is assumed that a percentage of these irregularly shaped particles will achieve the proper orientation to fall through respective screens within the screening time, and that the rest will not, resulting in the observed widened particle size distributions as determined by the digitization method.
As the viscous sintering model indicates, the sinter neck ratio is inversely proportional to the square root of particle diameter. The non-uniform shape of particles in the current investigation may, in effect, have increased the variability of sintering characteristics, thereby decreasing the predictability of the extent of densification of CPP powder due to the range of particle diameters that occur. The size and shape of particles influence the packing density and the number of contact points for neck formation in the unsintered “green” compact. The randomness of particle size and shape results in inconsistent packing densities, and consequently differences in sintering characteristics from region to region, creating localized high and low density regions within the structure, as were observed in the current study. As shown in Figure 3.9, the particle size distributions for the powder sieve diameter ranges of 106-150\(\mu\)m and 150-250\(\mu\)m, span \(\sim 70-180\mu\)m and \(\sim 100-375\mu\)m respectively when measured by image analysis. This represents a greater than 100% increase in width of each particle size distribution. Taking into consideration the effect of particle diameter \(D\) in the sintering model described by Equation 3.5, this theoretically increases the variability of the sinter neck ratio, \(X/D\), by a factor of \(\sim 1.6\) for the 106-150\(\mu\)m powder size range, and \(\sim 1.7\) for the 150-250\(\mu\)m powder size range. While these calculated factors are based on theory and cannot be proven experimentally, they do demonstrate the sensitivity of sintering properties to particle size and shape as predicted by the viscous sintering model.

Since the randomness of particle shape cannot be controlled under the current method of powder production, other approaches to limit the variation of the particle size
distributions need to be considered. The simplest solution would be to decrease the initial particle size range by using more closely spaced screens for powder sizing. This technique, however, decreases the yield of powder within a desired size range for a particular batch of powder. Alternate approaches to producing CPP powders of more consistent particle sizes and shapes are worth exploring. Ideally, for the purpose of consistency and predictability of processability and sintered form properties, spherical particles of CPP of uniform size may be utilized. The production of spherical particles, although not possible through traditional ceramic milling techniques, may be accomplished through methods borrowed from other fields. The relatively low melting point of CPP may be advantageous, for example, in the application of certain powder metallurgy processes (e.g. atomization) for producing spherical particles.

During the course of this study, the inner walls of the sintering tubes exhibited extensive pitting. Early on, this was considered to be a result of mechanical attrition by the CPP powders during forceful extraction of the sintered rod structures from the Pt tubes after sintering. It was observed that, after an extended period and a high number of sintering runs, the sintering behaviour of CPP became less consistent. Platinum was selected as the crucible material due to its chemical and high temperature inertness in order to avoid contamination of the sintered samples. Liquid phase phosphates, however, have been described as universal solvents by Griffith, which have been known to affect platinum and gold. Although the samples only approach the liquid transition, it is possible that some platinum contamination occurred which may have affected the CPP.
Other possible sources of contamination are from the stainless steel mortar and balls used during pulverization and the stainless steel container used when pouring the melt form CPP into water to produce the frit. Both containers were made from stainless steel of different compositions. At high temperatures, powders and melts were exposed to platinum crucibles as well as to airborne contaminants. Contaminants of interest are metal oxides that may affect the chemical structure or crystal structure of polyphosphates\textsuperscript{77, 78, 120}. Tetrahedral oxides, such as Al\textsubscript{2}O\textsubscript{3} may combine with backbone polyphosphates, as shown by Wantanabe et al.\textsuperscript{104, 105} to alter density. Others may affect crystallization, such as Fe\textsubscript{2}O\textsubscript{3}, to disrupt crystal growth\textsuperscript{80, 81}. In the current investigation, steps were taken to avoid contamination although evidence of contamination, such as the observed correlation between the physical pitting of the sintering tubes and the inconsistency of sintering characteristics, presents a possible inconsistency in the processing of sintered structures which may be investigated further.

A notable observation in the morphological characterization of the sintered CPP powders was the similarity between the pore size distributions for the 106 - 150\textmu m and 150 - 250\textmu m powder size ranges, in contrast to the variation of other properties such as density and strength. The minor increase in density of the 150 - 250\textmu m powders from the green form to the sintered form (+2.3%, \(p = 0.0058\) as compared to +16.6%, \(p < 0.0001\) for 106 - 150\textmu m powder size range) did not appear to correspond to the observed extent of neck formation. This may have been due to the higher quantity of microvoids in the coarse powder sintered samples as observed by comparing back scatter electron
micrographs of sintered fine and coarse powder samples (Figure 3.14). Similar behaviour was observed by Godest et al. in the sintering of fibres used as a two-dimensional model for spherical particles. They observed typical sinter neck behaviour as predicted by Equation 3.5 for temperatures between 940°C and 960°C and for fibre diameters from 95μm to 155μm. The relation between \( \frac{X}{D} \) and \( D^a \) was relatively consistent, and the average value of \( a \) determined was 0.499 ± 0.105 (theoretically \( a = 0.5 \), Equation 3.5).

However, the data deviated at higher temperatures and the value of \( a \) increased to ~1 at temperatures of 970°C and 975°C. This was verified by a series of data showing that the sinter neck ratio approached a maximum value within the temperature range of 955°C to 960°C over the entire range of fibre sizes, but decreased at higher temperatures at a rate directly related to fibre diameter. The change of the value of \( a \) was accompanied by an apparent increase in the volume of microvoids, particularly in the largest diameter fibres. This observed increase in the quantity of microvoids may be due possibly to the accumulation of microvoids in the larger fibres. As the voids increase in size their diffusivity decreases and the largest voids become trapped effectively countering densification (i.e. causing lower sinter neck ratio values).

The relative insensitivity of the sinter neck ratio to soaking time, due to the direct relationship between these variables, is well documented in the literature.

Nonetheless, the effect of time was investigated as a controlling parameter for sintering density for the CPP powders studied. A series of 150-250μm powder size range samples were sintered at 970°C for 1, 2, 5, and 10 hours. Similar sintering behaviour was observed
among the samples for these different time periods, and it was concluded that a driving force equilibrium was achieved at short soaking times and two hours was selected as the sintering time. This time period was considered optimal for achieving the required densification without significant volatilization of phosphates at the operating temperatures used (Section 1.1.4), and taking the heat up lag-time associated with the muffle furnace into consideration (Appendix B).
4. **IN VITRO DEGRADATION STUDIES**

4.1 Degradation Conditions

Disk samples, prepared as described in Section 3.1.2, were subjected to degradation studies, and evaluated for loss of calcium and phosphorus and loss of strength. Two degradation media were selected. 0.1M tris-buffered solution at pH 7.4 was utilized to represent normal physiological pH, and 0.05M potassium hydrogen phthalate buffer at pH 4.0 (VWR Scientific, West Chester Pennsylvania) was selected to represent pH local to osteoclasts in regions of bone resorption, as expected during the remodeling process in vivo.

Four degradation periods were selected: 1d, 5d, 10d, and 30d. Single disk samples were placed in 15ml of medium in sealed polystyrene vials (Becton Dickinson). Sample sizes of n = 20 were used for each condition (powder size, degradation period, medium). Twenty samples for each powder size were kept for “as prepared” (t = 0) characterization. Sample sets were designated by their degradation conditions; for example, the small powder specimens incubated in pH7.4 buffered solution for 10 days were identified as “106-150/10d/7.4”.

Samples were incubated at 37°C (Fisher Isotemp Incubator) and continuously agitated using an aliquot mixer (Thermolyne model #M26126). At the end of the
degradation period disk samples were removed, rinsed in deionized water and dried using 100% ethanol. Specimens were returned to their designated positions in cell culture trays for diametral compression studies. Aliquots were drawn from the degradation medium for analysis of calcium and phosphorus content.

4.2 Characterization Methods

4.2.1 SEM Analysis

Samples were examined after degradation and diametral compression analysis using scanning electron microscopy. Samples for SEM investigation were selected from a single batch of disks of each powder size range which had been incubated in pH 7.4 buffered solution and closely represented the average strength for each powder size range. The fractured surface of each specimen was examined, and micrographs were taken at magnifications of 500x and 1.5Kx of five randomly selected sinter necks separated by approximately one particle from the plane of fracture. The micrographs were surveyed for qualitative evidence of erosion. Increased crack initiation due to degradation was estimated by a scoring system for the presence of secondary cracks in the fractured specimens. The micrographs of sinter necks taken at 1.5Kx were given a value of 1 (positive) if any number of secondary cracks were found, and a value of 0 (negative) if none were found. This gave a maximum possible score of 5 for each sample. Due to the subjectivity of defining a "crack" in a material such as CPP with large intergranular
spacing, the average score per sample was based on an average of scores determined by ten randomly selected colleagues.

4.2.2 Diametral Compression Testing

After aging in their respective buffered solutions, sintered samples were rinsed in distilled water, dried with absolute ethanol, and analyzed for diametral compression strength using the methodology described in section 3.2.2.2.

4.2.3 Chemical Degradation Analysis

4.2.3.1 Phosphorus Determination

The technique for determining the concentration of dissolved phosphates in degradation medium is based upon a method reported by Fiske and Subbarow in 1925.\textsuperscript{123} In this analysis, ammonium molybdate combines with phosphate to form phosphomolybdic acid. This acid is reduced by the addition of 1,2,4-aminonaphtholsulphonic acid to produce a blue coloured solution, the intensity of which is proportional to the amount of phosphate (phosphorus) present. A series of standard phosphate solutions was prepared by diluting from 20\(\mu\)gP/ml stock (0.08787g KH\textsubscript{2}PO\textsubscript{4} per litre of tris-buffered or potassium hydrogen phthalate buffer). The colour intensity of the standards was measured and a calibration curve plotted of the spectrophotometer absorbance reading versus the concentration of phosphorus.
A single 5ml aliquot was extracted from each of the 15ml vials of degradation medium following the removal of the sample disk. A high proportion of the degradation product was expected to be in oligomeric form \textsuperscript{123}. The colourimetric method is effective only with orthophosphates, however, and dimers must be digested enzymatically prior to analysis. To digest the dimers, the 5ml aliquots were incubated in the presence of pyrophosphatase (PP\textsubscript{i}ase) according to the method described by Xu et al. \textsuperscript{124}. As reported, for the digestion of low concentrations of pyrophosphate, 0.2 units of PP\textsubscript{i}ase enzyme per 300\mu l of aliquot incubated at 37\degree C for one hour is sufficient to digest dimers present in solution. To verify this, four samples of sample set 106-150/5d/7.4 received no enzyme and four others received twice the amount of suggested PP\textsubscript{i}ase. After the allotted incubation time, the samples were analyzed.

Following incubation, 1ml of 2.5% ammonium molybdate in 5N sulphuric acid was measured and added to each aliquot to initiate the production of phosphomolybdic acid. To each sample, 50\mu l of aminonaphtholsulphonic acid reagent (0.25% 1,2,4-aminonaphtholsulphonic acid, 0.5% sodium sulphite, 14.63% sodium bisulphite in deionized water) were added to initiate the reduction of the phosphomolybdic acid, producing the blue colour. Samples were mixed on a vortex mixer (Fisher Scientific Genie 2 #12-812) and allowed to stand for at least 20 minutes. Although Fiske and Subbarow reported complete colour production within a maximum of 8 minutes in the presence of many retarding agents, a precautionary extended reaction time was allotted due to the presence of at least one known retardant, NaCl \textsuperscript{122}, used in the buffered solutions.
Three 2ml aliquots were drawn from each sample solution and tested individually in a spectrophotometer (Bausch and Lomb Spectronic 21). A linear expression for the absorbance of the calibration standards, with slope $k$, was determined at a wavelength of 625nm. The average of three absorbance readings ($Ab$) for each sample was compared to the calibration expression and the concentration of phosphorus in the degradation medium was determined in grams of P per millilitre of medium. The total amount of soluble phosphorus in the form of phosphate was then compared to the amount of phosphorus available in each sample (based on Equation 4.1 as derived in the Appendix C) and an average for each sample set was determined. The average percentage of available phosphorus degraded was plotted against time of degradation.

$$\% \text{ of Available } P = \frac{3(\text{Ab} \times k)}{2M_w[P] \times M_{Ca(PO_4)_2} \times m_{sample}} \times 100$$

Equation 4.1 - Equation for determining the percentage of phosphorus available from a particular sample.

As reported by Xu et al. $^{125}$, the activity of pyrophosphatase is greatly diminished in solution where $pH < 7.0$ or $pH > 8.0$. As a result, the $pH$ of the individual aliquots containing potassium hydrogenphthalate buffered medium were adjusted accordingly for analysis using potassium hydroxide preceding incubation with PP$i$ase
enzyme. The objective was to alter the pH of each 5ml aliquot to within the 7.0-8.0 range (ideally 7.4 to match tris-buffered samples) while limiting the increase in volume. To accomplish this, 70μl of 5M KOH were added to each aliquot. After vortexing, the pH was measured (usually falling between 6.2 & 6.8) and a smaller amount of 1M KOH was added, between 2 & 20μl, to more accurately adjust the pH to ~7.4. Samples reading higher than 8.0 were discarded. The resulting increase in volume was determined to be ≤ 2.0% and was considered insignificant compared with volume fluctuations due to evaporation and losses during analysis.

4.2.3.2 Calcium Determination

The method for determining the concentration of calcium in the buffered media is similar to that described above for phosphates. In this method cresolphthalein complexone (CPC) forms a complex with Ca²⁺ to produce a violet colour with a maximum absorbance at 575nm. The standard calcium solutions used to produce the calibration curves were diluted from 50μgCa/ml stock (0.1838g CaCl₂·H₂O per litre of tris-buffered or potassium hydrogen phthalate buffer). The colour intensity of the standards was measured and a calibration curve plotted of the spectrophotometer absorbance reading (Ab) and concentration of calcium.
A 2ml aliquot was extracted from each of the vials of degradation medium and treated with PPiase as described above. The addition of PPiase was not considered necessary for the liberation of Ca\(^{2+}\), but was performed for experimental control.

After a 1 hour incubation period, 6ml of CPC solution (0.01% CPC in 0.25 M sodium borate buffer at pH 8.5) were added to each aliquot and mixed on a vortex mixer; colour production was immediate. Three 2ml aliquots were drawn from each sample and tested at 575nm in a spectrophotometer. An average of the three readings was calculated for each sample, and the concentration of Ca\(^{2+}\) was determined in grams of calcium per millilitre of solution by comparison to the calibration curve. The total mass of dissolved calcium was determined for each sample and compared to the amount of calcium available for each sample disk (based on Equation 4.2 as derived in the Appendix). The calculated percentage of available calcium dissolved in the buffered solutions was plotted against time.

\[
\% \text{ of Available } Ca = \frac{7.5(Ab \times k)}{M_\text{[Ca]} \times M_\text{[Ca}(PO_4)_{2}]} \times m_{\text{sample}} \times 100
\]

Equation 4.2 - Equation for determining the amount of calcium dissolved in the incubation buffered-solution as a percentage of the total amount of calcium available from a particular sample.
4.3 Results

4.3.1 SEM Analysis

Two distinct characteristics of the incubated samples were observed by SEM over the periods of incubation. The more obvious difference in samples was the apparent erosion of the crystal grains. Figure 4.1 shows micrographs of samples at all periods of degradation for both the 106 - 150μm powder size range samples and the 150 - 250μm powder size range samples. The surfaces of the as-made samples appeared relatively smooth and the crystal grain boundaries were visible, as were the microvoids within the intergranular boundaries. As the duration of incubation increased, the crystal grains appeared to become more defined and the surfaces acquired a pebbly texture, due to apparent dissolution of the surfaces of the crystal grains. The greatest contrast can be seen when comparing the as-made micrographs with the micrographs of samples which were incubated for 30 days (ref. Appendix C). Also observed in the micrographs was the presence of small crystals which were visible on the surface of incubated samples. The density of these crystals increased over time, and may be a precipitation product of dissolved calcium phosphate from the degraded CPP.

Table 4.1 summarizes the average values for scores given in the investigation of secondary crack formation in fractured samples. There is a discernible increase in the number of cracks visible on the surface of sinter necks for both powder size ranges from
the as-made disks to the samples incubated for 30 days (p = 0.0019 for fine powders, p < 0.0001 for coarse powders).

<table>
<thead>
<tr>
<th>Powder Size Range</th>
<th>As-made</th>
<th>1 day</th>
<th>5 days</th>
<th>10 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>106-150μm</td>
<td>2.4 ± 0.5</td>
<td>3.1 ± 1.1</td>
<td>3.0 ± 1.0</td>
<td>2.8 ± 1.0</td>
<td>3.8 ± 0.9</td>
</tr>
<tr>
<td>150-250μm</td>
<td>1.2 ± 0.6</td>
<td>2.2 ± 1.0</td>
<td>2.5 ± 0.5</td>
<td>2.6 ± 1.0</td>
<td>2.9 ± 0.9</td>
</tr>
</tbody>
</table>

Table 4.1 - Average scores for the presence of secondary cracks in sinter necks after incubation in pH 7.4 tris-buffered solution.

4.3.2 Diametral Compression

The loss of diametral compression strength of samples over different periods of incubation is illustrated by the series of plots (Figure 4.2) of loading curves for samples from the same batch of disks. The representative batch is from the 106 - 150μm powder size range incubated in tris-buffered solution (pH 7.4), and was selected because it displayed strength near the average value of strength, at each time point, determined for each sample set in identical conditions.

In the early stages of degradation (1 to 5 days), the shape of the loading curve remained similar to the as-made curve. The curve was smooth with an abrupt fracture point, and limited crushing of the contact points occurred. The only marked difference was the reduction in fracture strength (Figure 4.2 (A) and (B)). Over longer periods of
Figure 4.1 - Micrographs (1.5Kx) showing the surfaces of sintered particles at different stages of degradation. a) - e) show samples incubated for 0, 1, 5, 10, and 30 days respectively for the 106-150μm powder size range. f) - j) show the same for the 150-250μm powder size range.
Figure 4.1 (cont’d)
Figure 4.1 (cont'd)
incubation (10 days), however, there was greater crushing of the contact points resulting in more fluctuation in load of the loading curve. The curve itself appeared flatter (reduced slope) and the fracture point, although still distinct, became less defined (Figure 4.2 (C)). At thirty days the samples were at their weakest point and extensive crushing of the contact points occurred. More severe fluctuations of the loading curve were observed as the contact points crushed at lower loads. The point of fracture was still observed in the plotted data, although fracture of the sample itself was not always evident upon visual inspection. In many cases, the sample disk had to be removed from the Instron machine after the suspected failure point, and the two halves of the disk had to be separated physically in order to verify proper tensile fracture. With uneventful fractures such as these, the disk samples continued to crush at the contact points because the two halves of the disk did not separate after fracture, resulting in a rounded, undefined fracture point in the loading curve. Figure 4.2 (D) illustrates a loading curve for a disk of average strength, after 30 days of incubation, which experienced minor crushing after fracture.

One batch of samples, totaling 22 disks, was removed from the 106 - 150μm powder size range sample set. In the diametral compression strength analysis of incubated samples, 17 of 18 samples were discarded due to excessive crushing or improper failure, which constituted 65% of all discarded samples for that size range. Since discarded samples could not be utilized in average diametral strength calculations, it was decided that the entire set be removed. It was determined that this batch had an
average density of 54.1% and was not sintered sufficiently, resulting in disks with much lower strengths.

In total 9 disks (5.7%) were discarded from the 106 - 150μm powder size range sample set and 27 (15.9%) were discarded from the 150 - 250μm size range (Table 4.2). Figure 3.7 shows a typical loading curve for a sample disk which underwent extensive crushing and was discarded from testing. There was no apparent trend in the number of discarded samples due to the period of incubation, although in total, both powder size ranges had twice as many disks removed from the analysis for the pH 4.0 buffered solution as for the pH 7.4 buffered solution.

<table>
<thead>
<tr>
<th></th>
<th>106-150μm pH7.4</th>
<th>106-150μm pH4.0</th>
<th>150-250μm pH7.4</th>
<th>150-250μm pH4.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-made</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 day</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>5 days</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>10 days</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>30 days</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total (% of total set)</td>
<td>3 (1.9%)</td>
<td>6 (3.8%)</td>
<td>9 (5.3%)</td>
<td>18 (10.6%)</td>
</tr>
</tbody>
</table>

Table 4.2 - A summary of the number of disks discarded from diametral compression strength analysis.
The average strength of each sample size in the two buffered solutions is plotted at each period of incubation (Figure 4.3). As the graph shows, there is significant difference in strength between the two powder size ranges \((p < 0.0001)\), however there is a significant difference within the 106-150\(\mu\)m powder size range between the samples incubated in pH 4.0 and pH 7.4 buffered solutions \((p = 0.0252)\), but not for the 150-250\(\mu\)m powder size range \((p = 0.7709)\). All sample sets showed no significant difference between the strength at 5 days and 10 days, although coarse powder samples in pH 7.4 buffered solution showed no significant difference at 1, 5, and 10 days due to a slight, uncharacteristic increase in strength at 10 days.

The strength of samples sets for both powder size ranges and both incubation solutions was further calculated as a percentage of as-made strength (Figure 4.4). In this form, the data showed no significant difference between pH 4.0 and pH 7.4 for either the fine powder samples \((p = 0.1534)\) or the coarse powder samples \((p = 0.8350)\). Overall, there was no significant difference between the two particle size ranges in the percentage of as-made strength remaining at each time period \((p = 0.2761)\). As stated before, there was a significant decrease in strength between time periods for the two powder size ranges in each buffered solution, except for the coarse powders in pH 7.4 solution which again showed no notable difference between 1, 5, and 10 days of incubation.

The entire data set of Figure 4.4, including the coarse and fine powder samples in both incubation buffered solutions, is summarized in Figure 4.5 as a single plot. The data
was plotted in this form due to the lack of statistical difference between the two particle size ranges and incubation solutions as determined above. In this form the graph summarizes the percentage of strength lost over time in incubation; the data is summarized in Table 4.3. The loss of strength is statistically different over time ($p < 0.0001$) for all cases except between 5 and 10 days ($p = 0.4743$).

<table>
<thead>
<tr>
<th>Incubation Period (days)</th>
<th>Percentage of As-made Strength Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76.6 ± 22.9</td>
</tr>
<tr>
<td>5</td>
<td>58.2 ± 17.7</td>
</tr>
<tr>
<td>10</td>
<td>55.6 ± 17.1</td>
</tr>
<tr>
<td>30</td>
<td>35.7 ± 12.0</td>
</tr>
</tbody>
</table>

Table 4.3 - Strength remaining as a percentage of as-made strength at each period of incubation
Figure 4.2 - Typical loading curves for disk samples (106 - 150μm powder size range) after incubation in tris-buffered solution for: (A) 1 day, (B) 5 days.
Figure 4.2 (cont’d) - (C) 10 days; (D) 30 days.
Figure 4.3 - The average diametral compression strength of disk samples for both powder size ranges and in both buffered solutions at different periods of incubation.
Figure 4.4 - The diametral compression strength of disk samples as a percentage of as-made strength for both powder size ranges and in both buffered solutions at different periods of incubation.
Figure 4.5 - The average strength remaining, as a percentage of as-made average strength, for disk samples of both powder size ranges and both buffered solutions.
4.3.3 Chemical Degradation

Statistical analysis of the accumulation of calcium and phosphorus in the incubation media over time indicates a significant interaction of the variables of incubation; pH, particle size range, and time. Consequently, the data cannot be analyzed in a similar manner to the strength degradation data, and must be considered on a time-point-to-time-point basis in order to determine trends. The accumulation of calcium and phosphate in the buffered solutions was plotted as a percentage of each component available in individual disk samples (Figures 4.6 - 4.9).

The quantity of phosphate in tris-buffered solution (pH 7.4) exhibits a trend similar to the loss of strength over time (Figure 4.6). The initial loss of phosphate from disk samples occurs rapidly up to 5 days and decreases from this point up to 30 days, and the quantity of phosphate in solution increases significantly at each time period (p < 0.0001). The quantity of calcium in solution is similar to the amount of phosphate in the tris-buffered medium (Figure 4.7). Although the values display more scatter, the general trend is preserved and produces a close to 1:2 molar release of calcium and phosphate.

The quantity of dissolved phosphate, and to a lesser extent calcium, in the potassium hydrogen phthalate buffer (pH 4.0) displays an unusual decrease between 5 and 10 days of incubation (Figure 4.8). Up to 5 days there is an initially high rate of release of phosphate and calcium as observed for the pH 7.4 samples, however there is an apparent divergence of the amount of phosphate released. Between 5 and 10 days the
level of phosphate and calcium in solution decrease significantly and the amount of dissolved phosphate approaches the amount of dissolved calcium (Figure 4.9). Up to 30 days of incubation the divergence of the quantity of the two components in solution occurs once again.
Figure 4.6 - A plot of the amount of phosphorus detected, as a percentage of phosphorus available in disk samples, in tris-buffered solution (pH 7.4) for both powder size ranges at different periods of incubation.
Figure 4.7 - A plot of the amount of calcium detected, as a percentage of calcium available in disk samples, in tris-buffered solution (pH 7.4) for both powder size ranges at different periods of incubation.
Figure 4.8 - A plot of the amount of phosphorus detected, as a percentage of phosphorus available in disk samples, in potassium hydrogen phthalate buffered solution (pH 4.0) for both powder size ranges at different periods of incubation.
Figure 4.9 - Plot of the amount of calcium detected, as a percentage of calcium available in disk samples, in potassium hydrogen phthalate buffered solution (pH 4.0) for both powder size ranges at different periods of incubation.
4.4 Discussion

Porous disk samples produced by gravity sintering CPP powder were incubated in buffered solutions of pH 4.0 and pH 7.4 to determine the rate and characteristics of hydrolytic degradation. The diametral compression strength of these porous disks decreased rapidly up to 5 days of incubation and continued to decrease at a lesser rate up to 30 days. The accumulation of calcium and phosphorus released from disk samples incubated in tris-buffered solution occurred in a manner similar to the loss of strength, increasing rapidly up to 5 days and continuing gradually up to 30 days. However in pH 4.0 buffered solution, the quantity of phosphate and calcium in solution dropped abruptly between 5 and 10 days, and increased once again up to 30 days of incubation.

The primary concern when conducting the measurements of calcium and phosphorus dissolved in the incubation solutions was the error introduced by “drift” of the absorbance readings of the spectrophotometer. Prior to each set of samples, the spectrophotometer was zeroed with a blank sample and a calibration curve was then plotted using known concentrations of the element being analyzed (including a zero concentration). However it was realized that the spectrophotometer reading of the zero concentration standard could shift (± 5) over short periods of time (i.e. within measuring 5 - 10 samples). Phosphate absorbance readings were often high on the scale (i.e. in the 100’s of units) and therefore this was not detrimental to the measurements taken for phosphate. However, due to the colourimetric method used, the absorbance readings for calcium measurements were often low (i.e. < 40) and this drift sometimes counted for
>50% of the calcium absorbance readings. This was offset slightly by the large sample set used (n = 20) and by frequently re-zeroing the spectrophotometer. However large error bars were incorporated into the plots of calcium to account for the spectrophotometer drift. The method used here is suitable to determine the quantities of dissolved phosphate in aliquots, however different methods should be investigated to attain more accurate results for the measurement of dissolved calcium.

The most interesting observation made concerning the mechanical properties of the gravity sintered disks when examining the results of the degradation studies was the substantial loss of diametral compression strength of the disk samples in comparison to the negligible dissolution of the calcium and phosphate degradation products. More specifically, after 30 days of incubation in the buffered solutions, the disk samples retained only 33% of their as-made strength, but lost < 1% of their mass. This suggests that the small amount of degraded calcium and phosphate lost during incubation of the disk samples occurs in regions which have a great effect on the diametral compression strength of these samples. This phenomenon was considered by examining sample morphology and degradation theory.

SEM analysis of the fractured as-made disks showed that crack propagation occurred predominantly within the intergranular boundaries with some transgranular crack propagation (i.e. propagation through grains). The presence of the intergranular microvoids in the sintered CPP powders was most likely a principal contributor to the
 initiation of intergranular cracks by introducing stress risers between crystal grains of the disk samples. In addition, SEM analysis showed an increase in the number of secondary cracks in sinter necks of incubated samples within the region of the primary cracks (ref. Section 4.3.1). This suggests that a separation or weakening of adhesion of the granular interface occurs during incubation which may facilitate crack initiation resulting in lower strength.

The decreased intergranular adhesion of these samples can be explained by a combination of the morphology of the disk samples and the degradation characteristics of CPP. The increased intergranular failure of fractured samples over time of incubation suggests a preferential degradation of CPP samples at the surface of the crystal grains and, more importantly, within the interface regions of the crystal grains. The dissolution of the surface of crystal grains was observed by SEM analysis (ref. Figure 4.1); however dissolution of the intergranular regions was more difficult to observe directly.

Surface area is a primary factor which governs the rate of degradation of materials (Table 1.2). This may have been a significant factor in degradation of the intergranular regions due to the additional surface area introduced between the crystal grains by the presence of the microvoids. By directly exposing the intergranular regions to the incubation media, the microvoids provided for immediate degradation and dissolution of regions within the bulk of the sintered samples. This immediate loss of intergranular material may have been a primary factor in decreasing intergranular adhesion, resulting in
the rapid loss in diametral compression strength observed in the early periods of incubation.

The rapid rate of intergranular degradation may have been further enhanced by the presence of intergranular amorphous regions. X-ray diffraction analysis of gravity sintered CPP disk samples did confirm that these materials were highly crystalline. However, it is likely that minor regions of intergranular amorphous material existed as a result of disorder in regions of transition between crystal grains \(^{118,119}\). Tupy et al. have observed that amorphous CPP degrades at a significantly higher rate than crystalline CPP (~30x) \(^{123}\). This may have had a significant effect on the degradation of the intergranular regions depending on the quantity of intergranular amorphous material and the accessibility of the incubation solution to these regions by the microvoids. Further study of the morphology of gravity sintered CPP (i.e. quantity and location of microvoids and amorphous material) is required to further comprehend the effects of these factors on crack initiation and propagation, and the loss of strength.

The initial rapid decrease of strength observed in the early stages of incubation occurred simultaneously with the rapid increase of dissolved calcium and phosphate in the incubation solutions. This behaviour corresponds to the rapid degradation of amorphous regions in the disk samples as discussed above, and suggests the possibility of a two stage degradation process. Initially the intergranular amorphous regions are degraded resulting in the high rate of release of the degradation products and the rapid loss
of strength. At later periods of incubation when the small amount of amorphous material has likely been fully degraded the rate of degradation decreases as the degradation of crystallized regions predominates. This process has been observed for degradable polymers such PLLA $^{44,45,50}$ and usually results in the release of highly crystalline microscopic particles which degrade slowly in the surrounding medium.

Using degradation protocols similar to that employed here, Tupy et al. $^{123}$ found that amorphous CPP degrades ~30 times faster than crystalline CPP, with amorphous CPP degrading at a rapid rate of ~9% of mass per day compared to ~0.3% of mass per day for crystallized CPP. These results support the notion of the two stage degradation process as outlined above. However, relating these results to the current study in terms of the independent rates of degradation of the amorphous and crystalline CPP is difficult, due to the fact that the quantity of amorphous material in the gravity sintered disks is not known, nor are the parameters required to accurately determine a degradation model (i.e. surface area, concentration factors within the porous structure etc.). Nevertheless, if it is assumed that the particulate form crystalline CPP used by Tupy had a similar morphology to the disks produced here (i.e. intergranular amorphous regions and microvoids) then a comparison of the characteristics of degradation of the crystalline CPP can be made over similar periods of incubation.

Figure 4.10 shows a plot comparing the percentage of phosphate released from crystalline CPP in tris-buffered solution for up to 5 days of incubation for each study.
Although the samples are not in the same form (i.e. gravity sintered vs. particulate) a similar trend of phosphate release is observed for both studies. The increased surface area of the particulate form CPP in Tupy's study is most likely responsible for the higher concentration of phosphate in solution, although other unknown dissimilarities in methods between these two studies may have affected the rate of degradation. One significant variation in method, although not related directly to the incubation of samples, was the time of digestion of phosphate oligomers in aliquots with \( PP_{\text{iasc}} \). Tupy's data was determined after 24 hours of digestion compared to 1 hour in the current investigation. One hour of digestion was selected here, as suggested by Xu et al.\textsuperscript{124}, to completely hydrolyze the phosphate dimers in solution. It is possible that during the extended incubation with \( PP_{\text{iasc}} \) in Tupy's studies that oligomers in solution greater in size than dimers were digested, resulting in an overall increase in dissolved \( PO_4^{3-} \) in solution. A more controlled comparison of these two forms of CPP is required to determine the effects of surface area and sintering on the rate of degradation of CPP.
Figure 4.10 - A comparison of the quantity of phosphate released from crystalline CPP in tris-buffered solution over time for the current study and that conducted by Tupy et al. 123.

The sealed vials used in the degradation studies represent a closed system (i.e. no material flows in or out of the system). Therefore the solubility of degradation products and the possibility of the evolution of precipitates were the primary considerations in this section of the investigation. Since the chemical analyses of calcium and phosphate were dependent on those components remaining in solution, it was imperative that the concentration of degradation products remained low enough not to induce precipitation of different calcium orthophosphate phases. Monocalcium orthophosphate monobasic (MCPM), the expected principal degradation product 78,120, has a solubility of 1.8g/100ml (in water at 20°C). Taking this into consideration, 15ml of buffered solution was used for
incubation studies which represented approximately 6 times the amount of solution required to completely dissolve an average size disk sample.

Contrary to the preventative steps taken, however, evidence of precipitation of calcium phosphate from the incubation solutions was observed. This was first considered as an explanation for the divergence and peculiar behaviour of the dissolved calcium and phosphate in the pH 4.0 incubation medium (Figures 4.8 and 4.9). At pH 4.0, the least soluble phase of calcium orthophosphate is dicalcium phosphate dihydrate (DCPD, Ca(HPO₄)·2H₂O) which may be produced from the degradation products of CPP in essentially a single step:

\[
[Ca(PO₃)₂]_n + 4H₂O \rightarrow [Ca(PO₃)₂]_{n-1} + Ca(HPO₄)·2H₂O + H₃PO₄
\]

**Equation 4.3 - The production of dicalcium phosphate dihydrate from the hydrolytic degradation products of CPP at pH 4.0.**

This reaction is based partially on the assumption and may involve an intermediate hydrolysis reaction of dissolved monocalcium phosphate monohydrate (MCPM, Ca(H₂PO₄)·H₂O) with water to produce the dihydrate form, but this is dependent on concentration, temperature, and pH and the exact reaction in the conditions used in this study are too specific to be found in the current literature. The conversion of CPP to DCPD also liberates a phosphoric acid molecule in a molar ratio of 1:1 with the
production of DCPD. The hypothesis of an increase in concentration of phosphoric acid due to this type of reaction was supported by the observed divergence of dissolved phosphate from calcium in solution up to 5 days and again from 10 to 30 days. This does not, however, account for the drop in calcium and phosphate between 5 and 10 days (Figure 4.8). This may have been in some way related to an alternate reaction as a result of the build-up of phosphoric acid in solution (e.g. the production of pyrophosphate in solution 77).

In order to discount human or equipment error for the peculiar behaviour of the degradation products in pH 4.0 buffered solution, the quantities of dissolved phosphate were measured a second time with more stringent precautions. The second set of readings were taken in random order in a single afternoon and the spectrophotometer was zeroed at shorter intervals (every four to six samples) to reduce the effects of the drift in absorbance readings as discussed previously. The results for both tests were similar, indicating that something besides experimental method was responsible for this unexpected behaviour. One variable of concern within the procedure was the readjustment of pH from 4.0 to within the range of 7 - 8 to account for the activity of PP_{i_{ase}} (ref. Section 4.2.3.1). Although PP_{i_{ase}} activity reaches a maximum at pH of 7 - 8, there is still substantial variation of activity within this range 124,125 which introduces variability in the rate of digestion of phosphate between aliquots. A more consistent method of adjusting the pH of aliquots, or perhaps employing methods which avoid this
technique need to be investigated for more accurate measurements of phosphates outside of the pH 7 - 8 range.

At pH 7.4 the chemical route to stable hydroxyapatite (HA, \( Ca_{10}(PO_4)_6(OH)_2 \)) from the degradation products of CPP requires four reaction steps. At each step high concentrations of the intermediate phase are required to drive the reaction to produce the next less soluble phase. The production of hydroxyapatite occurs as follows:

\[
\text{i) } [Ca(PO_3)_2]_n \xrightarrow{H_2O} Ca(H_2PO_4)_2 \cdot H_2O \quad 78 \\
\text{ii) } Ca(H_2PO_4)_2 \cdot H_2O \xrightarrow{H_2O} CaHPO_4 \cdot 2H_2O + H_3PO_4 \quad 77 \\
\text{iii) } 8CaHPO_4 \cdot 2H_2O \xrightarrow{H_2O} Ca_8H_2(PO_4)_6 \cdot 5H_2O + 2H_3PO_4 + 11H_2O \quad 77 \\
\text{iv) } 5Ca_8H_2(PO_4)_6 \cdot 5H_2O \xrightarrow{H_2O} 4Ca_{10}(PO_4)_6(OH)_2 + 6H_3PO_4 + 17H_2O \quad 77 \\
\]

Equation 4.4 - The four steps of precipitation of hydroxyapatite from the degradation of CPP at pH 7.4.

Each step of this conversion requires a high concentration of the product on the left side of the equation in order to drive the reaction to create the next less soluble phase. It may be assumed that the first step of this reaction was never surpassed for two reasons. Primarily the solubility of MCPP is too high and, due to the relatively large volume of solution, a sufficient concentration is not reached to drive the second step reaction. Also,
the data shows no divergence of phosphate and calcium, as in the pH 4.0 incubation study, which would be evident in the second, third, and fourth steps by the production of H$_3$PO$_4$.

However, contrary to what the data and theory might suggest, there was physical evidence of precipitation on the surface of incubated samples. The series of pictures of incubated samples show an increase over time in the density of small particles, possibly crystallites, on the surface of the CPP. Originally these particles were considered to be dust or debris encountered during preparation of the samples for SEM analysis. However, it was realized that the density of these particles increased over time in a consistent manner, suggesting that these were a product of some type of precipitation reaction during incubation. This does not necessarily imply that these crystallites are HA, but may be a precipitate produced from the salts in the incubation solution. Furthermore, if the particles are some form of precipitated calcium orthophosphate, this may have occurred due to high concentrations localized within the porous and microporous structures of the samples due to low rates of diffusion to the surface. A further analysis of the crystallites is required to identify the chemical composition.
5. SUMMARY, CONCLUSIONS, AND FINAL RECOMMENDATIONS

1. Autografting and allografting are currently the most widely used procedures for augmentation and replacement of skeletal tissue. These methods introduce possible complications such as secondary surgery, disease transfer, and often are subject to availability. This has prompted researchers to investigate synthetic bone substitutes for these purposes. The current study investigated gravity sintered calcium polyphosphate as a biodegradable porous synthetic bone substitute.

2. Porous disk-shaped samples were produced by gravity sintering CPP powder of two different size ranges, 106 - 150\(\mu\)m and 150 - 250\(\mu\)m. These samples were 55 - 66\% full density with pores of suitable size for bone ingrowth. The diametral compression strengths of these disks were 5.9 \(\pm\) 1.6 MPa and 24.1 \(\pm\) 6.8 MPa for the coarse powder samples (150 - 250\(\mu\)m) and fine powder samples (106 - 150\(\mu\)m) respectively.

3. Significant variation in densification occurred among powder batches, resulting in inconsistent physical properties. The extent of densification was difficult to control accurately and powders displayed surprising sensitivity to temperature fluctuations. This was believed to be a result of a combination of powder characteristics such as particle size distribution, chemical structure, and possible trace element contamination.

4. Initial short-term degradation studies of up to 30 days were carried out at 37\(^\circ\)C in pH 7.4 and pH 4.0 buffered solutions. Degradation was characterized physically, by determining the loss of diametral compression strength over time, and chemically, by measuring the accumulation of calcium and phosphate in incubation solutions over time. Approximately 45\% of strength was lost after 5 days of incubation followed by a more gradual loss of strength up to 30 days where \(\sim\) 35\% of the as-made strength remained. Similar behaviour was observed for the accumulation of phosphate and calcium in solution. These degradation products were released rapidly up to 1 - 5 days and more gradually afterward.

5. This behaviour suggests that a two step degradation process may occur in crystalline CPP. This process includes the rapid degradation of intergranular amorphous material followed by the long-term degradation of the crystal grains. The loss of intergranular material resulted in the rapid release of degradation products and reduced intergranular adhesion. This promoted intergranular crack propagation and the observed rapid loss of strength.
6. The quantity of dissolved phosphate in the pH 4.0 incubation solutions diverged from the quantity of dissolved calcium up to 5 days. Between 5 and 10 days the amount of soluble phosphate decreased significantly and diverged again up to 30 days. This behaviour was surprising and may have been the result of the precipitation of an insoluble orthophosphate. At pH 4.0 dicalcium phosphate dihydrate (DCPD) is the most stable phase of calcium phosphate and may be produced from the degradation products of CPP in a single reaction step.

Based on the research presented here, gravity sintered porous calcium polyphosphate possesses properties which make it a suitable bone substitute scaffold material for nonload-bearing or low load-bearing applications. This largely takes into consideration the relatively low strength of these highly porous samples which makes them unsuitable for high load-bearing applications such as cortical bone replacement, but also considers the excellent pore size distribution and microtexture of these samples which are suitable for good bone ingrowth. This also suggests that crystalline calcium polyphosphate, not necessarily in sintered form but perhaps in other forms such as particulates, may be a suitable candidate as an adjunct to orthopaedic and and dental implants. These conclusions, however, are based on the properties of these materials as determined by in vitro methods. Further study with biological systems is required to determine the biocompatibility of these materials for bone substitute applications.

Following are a number of recommendations for further study of calcium polyphosphate based on the results of the studies presented here.

1. To attain better control over sintering properties, a study relating the molecular weight, melting temperature and sintering temperature of these materials is required. This is a primary concern for consistency of density and mechanical properties of these materials.

2. Although steps were taken to avoid trace element contamination in this study, an investigation into the effects of potential contaminants due to the processing protocol and trace elements inherent in the precursor powder is required for more consistency in the properties of the resulting calcium polyphosphate.

3. Alternative methods for powder production may be investigated for more consistent powder size distribution and shape.

4. An alternative, more sensitive method for determining calcium levels in incubation solutions is required for further in vitro investigations.
5. An *in vivo* investigation is required to determine the biocompatibility and osteoconductivity of calcium polyphosphate. These may be used to determine bone ingrowth and *in vivo* degradation rates which may further define possible applications for these materials.

6. Further study into the production of potential precipitates at both pH 4.0 and pH 7.4, and how these precipitates may possibly occur and affect the host response *in vivo* is required.
6. REFERENCES


35. Hench LL. 7th *CIMTEC World Ceramic Congress, (Montecantini Term, Italy)* 1990.


121. Godest CA, Wells JD, Pilliar RM. *Unpublished data*.


123. Tupy J, Guo W, Grynpas MD, Pilliar RM. *Unpublished data*.


7. Appendices
Appendix A: Chemical Formulas for Common Biodegradable Polymers
Poly(lactic acid)

Poly(glycolic acid)

Poly(ortho ester)

Poly(ε-caprolactone)

Polydioxanone
Polyphosphazene

\[
\begin{array}{c}
\text{Polyphosphazene} \\
\begin{array}{c}
\left[ \begin{array}{c}
N \\
R \\
R \\
n
\end{array} \right]
\end{array}
\end{array}
\]

Poly(DTH carbonate)

\[
\begin{array}{c}
\text{Poly(DTH carbonate)} \\
\begin{array}{c}
\left[ \begin{array}{c}
\text{O} \\
\text{CH}_2-\text{CH}_2-\text{C}-\text{NH}-\text{CH}-\text{CH}_2-\text{C}=\text{O} \\
\text{O} \\
\text{C}_6\text{H}_{13}
\end{array} \right]
\end{array}
\end{array}
\]
Appendix B: Furnace Calibrations
Appendix C: Scanning Electron Micrographs of As-Made and 30 day Samples
106 - 150μm, as-made
106 - 150μm, 30 days
150 - 250μm, as-made
Appendix D: Statistical Data
ANOVA tables for the accumulation of calcium and phosphorus in incubation solutions as a percentage of each element available from disk samples.

### ANOVA Table for % Degraded
**Split By: Element**
**Cell: calcium**

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>3</td>
<td>1.634</td>
<td>.545</td>
<td>441.533</td>
<td>&lt;.0001</td>
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<tr>
<td>Powder Size</td>
<td>1</td>
<td>.191</td>
<td>.191</td>
<td>154.483</td>
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</tr>
<tr>
<td>Days * Powder Size</td>
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<td>.039</td>
<td>.013</td>
<td>10.500</td>
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<tr>
<td>pH</td>
<td>1</td>
<td>.107</td>
<td>.107</td>
<td>86.629</td>
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</tr>
<tr>
<td>Days * pH</td>
<td>3</td>
<td>.081</td>
<td>.027</td>
<td>21.966</td>
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<tr>
<td>Powder Size * pH</td>
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<td>3.309E-4</td>
<td>3.309E-4</td>
<td>.268</td>
<td>.6050</td>
</tr>
<tr>
<td>Days * Powder Size * pH</td>
<td>3</td>
<td>.671</td>
<td>.224</td>
<td>181.304</td>
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</tr>
<tr>
<td>Residual</td>
<td>291</td>
<td>.359</td>
<td>.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### ANOVA Table for % Degraded
**Split By: Element**
**Cell: phosphorus**

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<th>F-Value</th>
<th>P-Value</th>
</tr>
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<tbody>
<tr>
<td>Days</td>
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<td>5.410</td>
<td>1.803</td>
<td>1062.107</td>
<td>&lt;.0001</td>
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<tr>
<td>Powder Size</td>
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<td>.197</td>
<td>.197</td>
<td>115.966</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Days * Powder Size</td>
<td>3</td>
<td>.157</td>
<td>.052</td>
<td>30.904</td>
<td>&lt;.0001</td>
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<tr>
<td>pH</td>
<td>1</td>
<td>2.632</td>
<td>2.632</td>
<td>1550.040</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Days * pH</td>
<td>3</td>
<td>1.684</td>
<td>561</td>
<td>330.576</td>
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</tr>
<tr>
<td>Powder Size * pH</td>
<td>1</td>
<td>.115</td>
<td>.115</td>
<td>67.576</td>
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</tr>
<tr>
<td>Days * Powder Size * pH</td>
<td>3</td>
<td>.187</td>
<td>.062</td>
<td>36.804</td>
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<tr>
<td>Residual</td>
<td>207</td>
<td>.351</td>
<td>.002</td>
<td></td>
<td></td>
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</tbody>
</table>

These analyses of variance indicate a significant interaction between all variables, and therefore does not allow for an analysis of the effects of individual variables.
ANOVA table for the strength remaining in disk samples as a percentage of the "as-made" strength.

<table>
<thead>
<tr>
<th></th>
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<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>days</td>
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<tr>
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<td>.125</td>
<td>.125</td>
<td>2.915</td>
<td>.0887</td>
</tr>
<tr>
<td>days * pH</td>
<td>4</td>
<td>.110</td>
<td>.027</td>
<td>.639</td>
<td>.6349</td>
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<tr>
<td>powder size</td>
<td>1</td>
<td>.026</td>
<td>.026</td>
<td>.607</td>
<td>.4367</td>
</tr>
<tr>
<td>days * powder size</td>
<td>4</td>
<td>.068</td>
<td>.017</td>
<td>.394</td>
<td>.8129</td>
</tr>
<tr>
<td>pH * powder size</td>
<td>1</td>
<td>.067</td>
<td>.067</td>
<td>1.558</td>
<td>.2129</td>
</tr>
<tr>
<td>days * pH * powder size</td>
<td>4</td>
<td>.226</td>
<td>.056</td>
<td>1.317</td>
<td>.2636</td>
</tr>
<tr>
<td>Residual</td>
<td>315</td>
<td>13.495</td>
<td>.043</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This analysis of variance indicates that the variable of time ("days") significantly affects the amount of strength remaining in disk samples, however pH and powder size do not.

**Fisher's PLSD for strength %**

**Effect: days**

**Significance Level: 5 %**

<table>
<thead>
<tr>
<th></th>
<th>Mean Diff.</th>
<th>Crit. Diff</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>zero days, one day</td>
<td>.234</td>
<td>.069</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>zero days, five days</td>
<td>.418</td>
<td>.068</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>zero days, ten days</td>
<td>.444</td>
<td>.072</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>zero days, thirty days</td>
<td>.643</td>
<td>.069</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>one day, five days</td>
<td>.184</td>
<td>.069</td>
<td>&lt;.0001</td>
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<tr>
<td>one day, ten days</td>
<td>.210</td>
<td>.072</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>one day, thirty days</td>
<td>.408</td>
<td>.070</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>five days, ten days</td>
<td>.026</td>
<td>.072</td>
<td>.4748</td>
</tr>
<tr>
<td>five days, thirty days</td>
<td>.225</td>
<td>.070</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>ten days, thirty days</td>
<td>.199</td>
<td>.073</td>
<td>&lt;.0001</td>
</tr>
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</table>