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Submitted in partial fulfilment of the requirements for the Degree of
Clinical Doctorate in Dentistry (Paediatric Dentistry)
Eastman Dental Institute
University College London

Submitted by:

MOHD RIDZUAN MOHD RAZI

DDS (Malaysia) MFDS RCS (Edinburgh), MPaedDent RCS (England)

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Contents

THESIS

EFFECTS OF POLYACRYLIC ACID ON BRUSHITE BONE CEMENT SETTING, MECHANICAL PROPERTIES, DEGRADATION AND CHLORHEXIDINE RELEASE

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DECLARATION OF ORIGINALITY

I hereby declare that the work presented in this thesis was carried out by me myself. Information derived from the published and unpublished work of others has been acknowledged in the text and the relevant references are included in this thesis.

Mohd Ridzuan Mohd Razi
Eastman Dental Institute, University College London
September 2012

ABSTRACT

In the field of Paediatric Dentistry, brushite cement has potential as an endodontic medicament and bone substitute material. Clinical applications however are limited due to their inherent properties, such as rapid setting time and poor mechanical properties. Antimicrobial e.g. chlorhexidine (CHX) could be incorporated into the cement for localised drug release. ***Aim and objectives:*** The aim of this study was to assess if partial replacement of citric acid (CA) by polyacrylic acid (PAA) can improve the properties of conventional brushite cements. The objectives were to assess the effects of varying PAA and CHX concentrations in brushite cements on their setting kinetics, final composition, microstructure of the cement, mechanical properties, degradation and CHX release profile. ***Materials and Methods****:* The cements consisted of equimolar β-tricalcium phosphate and monocalcium phosphate monohydrate (β-TCP/MCPM) and 6 or 11% (w/w) CHX. The liquid phase consisted of aqueous 800 mM CA and PAA solution at different ratios. Compositions with no CHX and/or PAA were used as control cements. Setting kinetics and final composition were determined using FTIR and Raman spectroscopy respectively. Brushite microstructure was examined using scanning electron microscopy. The cements were tested for microhardness and biaxial flexural strength (BFS). CHX release was quantified with UV spectroscopy and degradation by mass loss. ***Results***: The setting times for compositions with PAA were delayed by up to 12 hours. FTIR indicated formation of dicalcium citrate and polyacrylate complexes could delay brushite formation. High CHX content inhibited the acid retarding effects and complex formation. Raman mapping demonstrated discrete regions of brushite and CHX in all set cements. Microscopically, PAA addition resulted in denser and less porous structure. The BFS ranged from 5.8 ± 1.3 MPa to 11.1 ± 1.2 MPa. CHX incorporation resulted in reduced BFS and modulus whilst PAA addition increased it. The average mass change was significantly different between compositions with and with no chlorhexidine; 12% and 0.2% respectively at the end of study period. The daily degradation rate ranged from 0.1 ± 0.03 wt% to 0.6 ± 0.15 wt%. PAA presence reduced CHX release from more than 90% to less than 20% over 4 weeks. ***Conclusion***: PAA substantially slowed the setting reaction and chlorhexidine release characteristics, altered the final brushite crystal microstructure, increased mechanical properties but did not affect the degradation kinetics.

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ABBREVIATIONS

β – TCP	beta – tricalcium phosphate
ζ	reaction extent
ε	molar extinction coefficient.
υ	Poisson's ratio
µm	micrometre
σ	stress
dP/dω	load versus central displacement
Δm(%)	percentage mass change
a	radius of support ring
A	absorbance
AAPD	American Association of Paediatric Dentistry
ACP	amorphous calcium phosphate
A_0	initial absorbance
A_c	corrected absorbance
A_f	absorbance at final time
A_t	absorbance at time *t*
BFS	biaxial flexural strength
BSPD	British Society of Paediatric Dentistry
CA	citric acid
CaO	calcium oxide
CHX	chlorhexidine
CI	confidence interval
CPC	calcium phosphate cements
d	diagonal
DCPD	dicalcium phosphate dihydrate

DF	dilution factor
E	biaxial flexural modulus
F	force in kilograms - force
FeO	ferrous ions
FTIR	fourier transform infrared spectroscopy
GPa	gigapascals
gf	gram - force
h	depth of indentation in micrometres
HERS	Hertwigs's epithelial root sheath
IADT	International Association of Dental Traumatology
kgf	kilogram - force
kN	kilonewtons
kV	kilovolt
m_0	mass at initial
m_t	mass at time *t*
MCPA	monocalcium phosphate anhydrous
MCPM	monocalcium phosphate monohydrate
mg	milligrams
mM	millimoles
mm	millimetre
ml	millilitre
MPa	megapascals
MTA	mineral trioxide aggregate
nm	nanometre
PAA	polyacrylic acid
PDL	periodontal ligament
PHA	Precipitated hydroxyapatite
PLR	powder to liquid ratio

ppm	part per million
SD	standard deviation
SEM	scanning electron microscope
r	radius
UK	United Kingdom
VHN	Vickers Hardness Number
wt%	weight percentage
w/w	weight over weight

CHAPTER 1

INTRODUCTION

1.0 INTRODUCTION

1.1 Statement of the Problem

Traumatic dental injuries occur with frequency in preschool, school- age children, and young adults. They comprise 5% of the injuries sustained to the body (Andreasen et al., 2007). The injuries may result from falls, fights, road traffic accidents, sports and non – accidental injuries (Martin et al., 1990; Onetto et al., 1994; Perez et al., 1991; Stockwell, 1988). The reported prevalence of traumatic dental injuries varies depending on the population studied (Al-Majed et al., 2001; Alonge et al., 2001; Altay and Güngör, 2001; Bastone et al., 2000; Cunha et al., 2001).

In children, the management of non – vital immature teeth and premature tooth loss as a result of traumatic dental injuries pose significant challenges to clinicians. Although there are available clinical guidelines (Andersson et al., 2012; DiAngelis et al., 2012) to help the clinician in managing these cases, current dental materials and clinical approaches do have limitations which may affect long term outcomes and prognosis of the affected tooth/area.

An immature tooth refers to a tooth which is not fully formed, especially the root end / apex. An intact and vital pulp (neurovascular bundle) is necessary for the development and maturation of the tooth root. Therefore, if vitality is lost, the maturation process will cease, leaving the tooth with a wide root canal, thin canal walls and an open apex. Root canal treatment is complicated by the lack of apical closure against which to condense and contain a root filling. Classically these are managed by creating an apical barrier at the root end against which the root filling can be condensed. Currently there are two methods of creating the barrier ; either physiologically with non – setting calcium hydroxide or artificially with mineral trioxide aggregate (MTA) cement (Vaidyanathan et al., 2010). Although these techniques have been shown to be successful, there is emerging evidence on the limitations and disadvantages of both materials (Andreasen et al., 2002; Moore et al., 2011; Rosenberg et al., 2007). Therefore, development of new techniques is needed to overcome the problems associated with these materials.

Another challenging issue is alveolar bone resorption as a sequalae to premature tooth loss. Studies have shown that this process can be very rapid (Rodd et al., 2007); resulting in loss of bone height and volume. This greatly complicates subsequent prosthetic replacement of the tooth. In adults dental implants are considered the best solution for management of tooth loss (Henry et al., 1996). In children however, these are not recommended as the bone is yet to mature. The time between tooth loss and sufficient maturity for implant placement (i.e. when growth has ceased) could be years. Increasing bone loss in this period further complicates implant placement and ultimate treatment outcome (Day et al., 2008). New methods to reduce alveolar bone resorption at an early stage would therefore be beneficial.

Brushite cements have a real potential in both described clinical conditions. An important benefit is their ability to act as a reservoir of calcium and phosphate ions for remineralisation of the hard tissue.

Several studies have also shown that brushite cements can simultaneously act as localised drug reservoirs (Ginebra et al., 2006). There are lots of studies looking at brushite cements as bone substitutes (Kurashina et al., 1997; Sanzana et al., 2008; Theiss et al., 2005). However, as far as we aware, there is no study exploring the potential use of brushite cement as endodontic material.

There are however, several issues which may limit their application. These include short setting times, poor mechanical properties (Ishikawa and Asaoka, 1995), rapid drug release (Bohner et al., 1997a) from the cement, poorly controlled degradation and limited adhesion to the bone/dentine. Thus, the development of modified brushite cements as possible alternative root filling materials and as potential alveolar bone substitutes will offer the clinician alternatives in managing above mentioned problems.

CHAPTER 2

LITERATURE REVIEW

2.0 LITERATURE REVIEW

2.1 Introduction

Traumatic dental injuries are defined as any injuries to the teeth and/or tooth – supporting structures (periodontal ligaments and alveolar bone). Generally, dental injury can be classified into hard tissue injuries and luxation injuries (Andreasen et al., 2007; WHO, 1978). Table 2.1 describes different types of traumatic dental injuries.

In children there are two peak ages during which this could happen; 18 months to 3 years old and between 10 to 12 years old. Greater incidence of dental injuries in toddlers mainly results from falls as the child begins to walk and run with developing motor coordination (Cunha et al., 2001; Oikarinen and Kassila, 1987). In younger children it may be due to vigorous play (Andreasen and Ravn, 1972). Boys experience more dental injuries than girls (Andreasen et al., 2007; Bastone et al., 2000; Chadwick et al., 2006).

When a tooth is traumatised, sequelaes from the injured pulp and periodontal ligaments may affect the long – term prognosis of the affected tooth. Hence, the immediate and long – term management of dental trauma pose significant challenge to the clinicians.

2.2 Sequealae of dental trauma

Dental trauma represents a significant threat to dental health in young children as their dentition is still developing. There are various possible complications that could arise from traumatic dental injuries. These include impaired dental aesthetics, pulpal complications, arrested root development, root resorption, ankylosis and tooth loss. The outcome of dental trauma depends on type and severity of injuries, stage of tooth development, immediate and long-term treatment and healing potential of the affected tissue. In children however, loss of vitality, arrested root development and root resorption are the most debilitating outcomes which have a large impact to both patients and clinicians.

Table 2.1: Classification and definition of traumatic injuries to teeth (Andreasen et al., 2007; WHO, 1978).

Type of Dental Trauma	Definition
Hard Tissue Injuries	
Enamel infraction	An incomplete fracture (crack) of the enamel without loss of tooth substance.
Uncomplicated crown fracture	A fracture with loss of enamel with / without dentine, but not involving the pulp.
Complicated crown fracture	A fracture involving enamel and dentine with pulpal involvement.
Crown – root fracture	A fracture involving enamel, dentine and cementum with / without pulpal involvement.
Root fracture	A fracture involving dentine, cementum and the pulp
Luxation Injuries	
Concussion	An injury to the tooth – supporting structures without abnormal loosening or displacement of the tooth, but an increase reaction to percussion.
Subluxation	An injury to the tooth – supporting structures with abnormal loosening but no displacement of the tooth
Extrusion	Partial displacement of the tooth out of its socket.
Lateral luxation	Displacement of the tooth in a direction other than axially. This is accompanied by comminution or fracture of the alveolar socket.
Intrusion	Displacement of the tooth into the alveolar bone. This is accompanied by comminution or fracture of the alveolar socket.
Avulsion	Complete displacement of the tooth out of its socket.

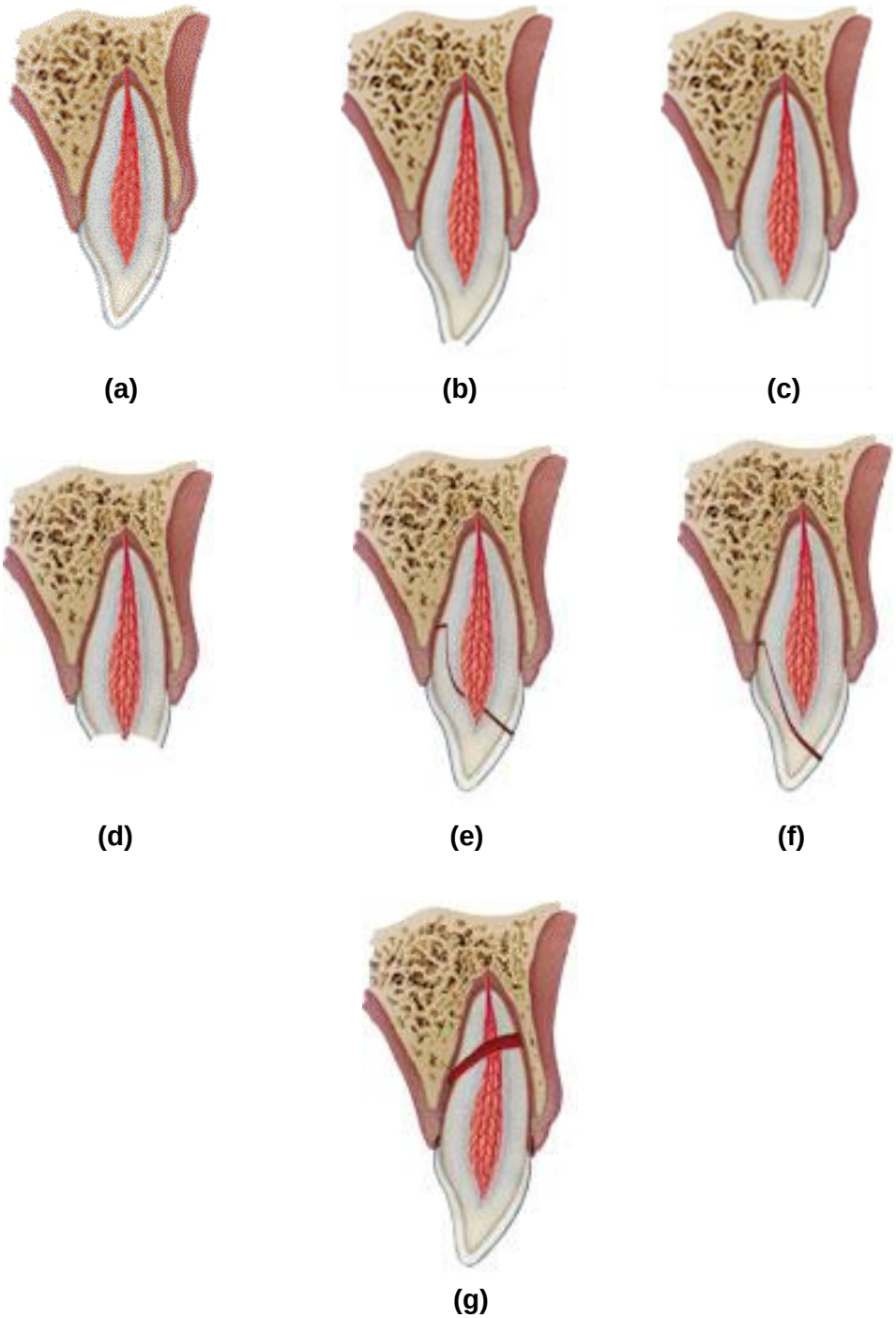

Figure 2.1: Illustrations of different types of hard tissue injuries resulting from dental trauma (with permission to reproduce from John Wiley and Sons – see Appendix 5).

(illustrations adapted from (DiAngelis et al., 2012)). (a) infraction, (b) uncomplicated crown fracture – enamel only, (c) uncomplicated crown fracture involving both enamel and dentine, (d) complicated crown fracture, (e) crown – root fracture with pulpal involvement, (f) crown – root fracture without pulpal involvement, (g) root fracture.

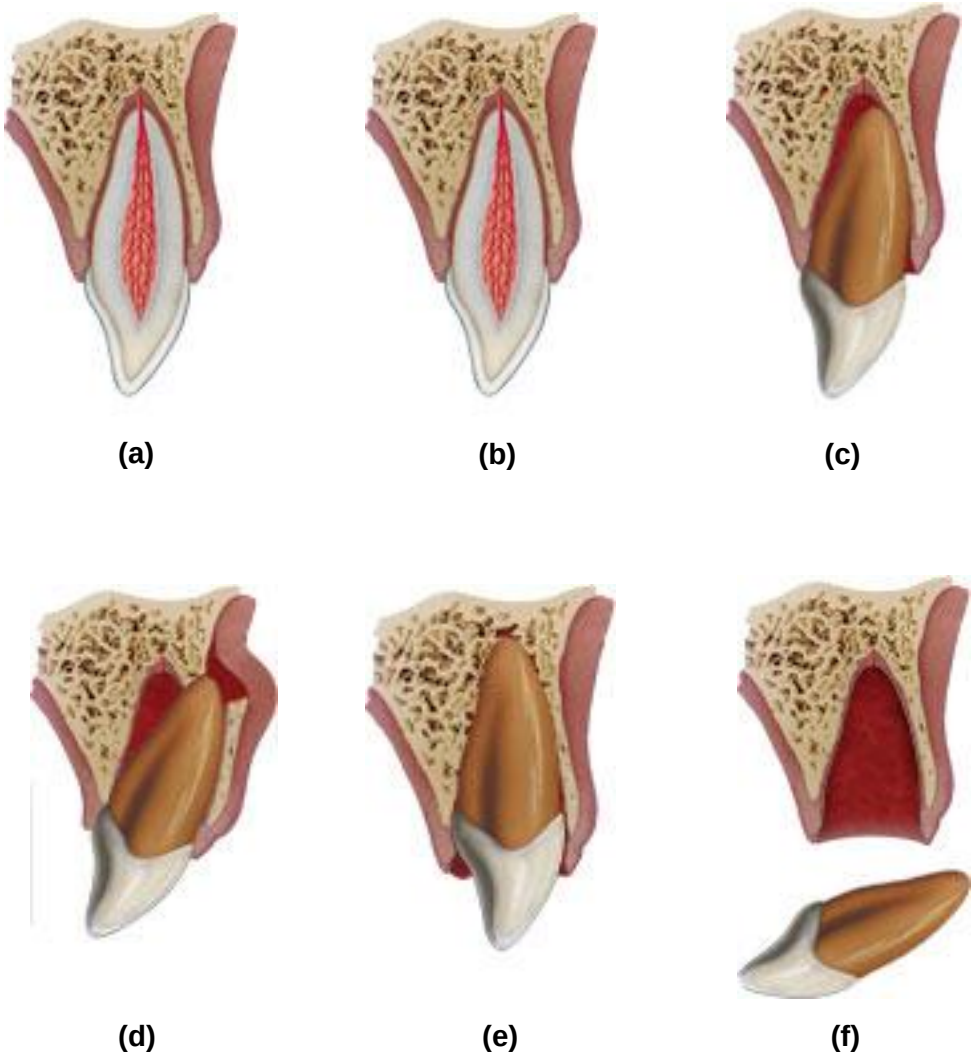

Figure 2.2: Illustration of different types of luxation injuries resulting from dental trauma (with permission to reproduce from John Wiley and Sons – see Appendix 5 and Appendix 6).

(illustrations adapted from (Andersson et al., 2012; DiAngelis et al., 2012) (a) concussion, (b) subluxation, (c) extrusion, (d) lateral luxation (e) intrusion, (f) alvulsion.

2.2.1 Loss of vitality

Epidemiological studies showed that 1 – 6% of teeth with crown fractures and 15 – 59% of teeth with luxation injuries developed pulpal necrosis (Andreasen et al., 2007). Loss of pulp vitality resulting from dental injuries can be either due to contamination of pulp with bacteria and its by-products or crushing and/or laceration of pulp from luxation injuries. Bacteria can immediately contaminate the pulp when there is complicated crown fracture (pulpal exposure) or through the dentinal tubules in uncomplicated crown fracture. When the crown of a tooth is fractured and involves the dentine, the dentinal tubules are exposed to the oral environment.

In children, the diameter of these tubules are wider compared to adults (Bath - Balogh and Fehrenbach, 2011) and thus will allow rapid progression of bacteria towards the pulp complex resulting in irritation of the pulp. If the exposed dentine be sealed off immediately with restorations, it will only elicit insignificant changes in the pulp complex which may resolve. However, if the deeply exposed dentine is left unprotected, the communication between oral environments and penetration of bacteria may result in inflammation of the underlying pulp and subsequently loss of pulp vitality (DiAngelis et al., 2012).

Crushing and/or laceration of the neurovascular bundle at the apical foramen could result in loss of blood supply to the dental pulp and subsequently lead to ischemia and necrosis. Although there is potential for revascularisation of the dental pulp, this process depends on the factors discussed above. Loss of pulp vitality in immature teeth can have severe detrimental effects on its long term survival as they are prone to root fracture.

2.2.2 Arrested root development

Root development is still taking place although the teeth have erupted into the mouth and generally it takes up to 3 years for the root to completely form (Bath - Balogh and Fehrenbach, 2011). As such, for the upper incisors root formation is estimated to complete at about 10 years old. As aforementioned, the peak incidences of traumatic dental injuries occur before root formation is complete and may result in devitalising of the pulp. Vital pulp is fundamental for

continuation of root development without which the root development will arrest. Maturation of the root involves a complex interaction between Hertwigs's epithelial root sheath (HERS) and the dentinogenesis process (Bath - Balogh and Fehrenbach, 2011). These processes result in an increase in root length and thickened canal walls. Cvek (1992) classified root development into 5 different stages based on radiographic presentation (see Figure 2.3).

Therefore, if the pulp vitality diminishes, the maturation process will cease leaving the tooth with a short root, wide root canal, fragile and thin canal wall and open apex. Management of immature teeth pose significant challenges to the clinician as root canal treatment is complicated by the lack of apical closure against which to condense and contain a root filling (Vaidyanathan et al., 2010).

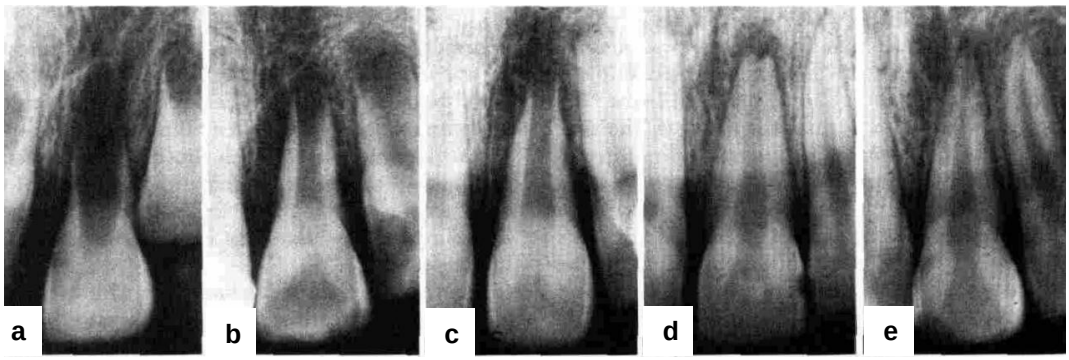

Figure 2.3: Radiographic classification on stages of root development (with permission to reproduce from John Wiley and Sons – see Appendix 7).

Adapted from (Cvek, 1992). **(a)** Stage1 – wide and divergent apical opening and root length less than half of estimated final length, **(b)** Stage 2 - half of the estimated root length has form, **(c)** Stage 3 – 2/3 of estimated root length has form, **(d)** Stage 4 – wide apical canal with nearly complete root length, **(e)** stage 5 – close apical foramen and complete root formation.

2.2.3 Replacement resorption

Root resorption may result as complication of severe luxation injuries to the teeth. There are three types of root resorption: surface resorption, inflammatory resorption and replacement resorption (Trope, 2002). Replacement resorption is an expected phenomenon following severe tooth injuries or non- physiological extra - oral storage following avulsion (Andersson et al., 2012; Andreasen, 1981; DiAngelis et al., 2012). This is an irreversible process which leads to resorption of the root structure by osteoclasts and followed by subsequent osteoblast infiltration of the area for osseous healing (Trope, 2002). The development of replacement resorption depends on both the degree of damage to the periodontium at the time of injury, and the viability of the periodontal ligament cells remaining on the root surface. It has been reported that if less than 20% of the root surface is involved, a transient ankylosis may occur. The areas affected by transient ankylosis may later be resorbed due to functional stimuli of the tooth. However, if the damaged surface is large (>4 mm^2), a permanent ankylosis is created, thus the tooth becomes subjected to the bone remodelling process (Andreasen and Kristerson, 1981).

Apart from the severity of dental trauma, replacement resorption rate is also dependent upon age and basal metabolic rate. In young children, replacement resorption causes significant problems to the developing dentition and occlusion. As the rate of bone turnover in children is very rapid compared to adults, the progression of replacement resorption can lead to premature tooth loss. It has been estimated that the affected tooth will be lost within 1 – 5 years (Finucane and Kinirons, 2003). Apart from tooth loss, as a consequence of ankylosis, there is also a lack of vertical growth of the alveolar bone resulting in progressive infraocclusion (Malmgren and Malmgren, 2002), thus affecting the aesthetic of the dentition.

2.2.4 Premature tooth loss

Although it is best to maintain the traumatised tooth for as long as possible, the long - term prognosis should be taken into account during treatment planning. Tooth loss resulting from dental trauma can be an immediate or delayed outcome. Immediate tooth loss should be considered if the injuries are severe

and the tooth is unrestorable (DiAngelis et al., 2012). Patient medical history or condition of avulsed tooth may contraindicate reimplantation of the avulsed tooth (Andersson et al., 2012).

Where reimplantation of avulsed teeth is carried out, it has been reported that it has a poor long – term survival rate; 73 – 96% were lost prematurely (Andreasen and Andreasen, 2007). A tooth with non - viable periodontal fibres when replanted will result in ankylosis and latter replacement resorption. Other complications from traumatised teeth e.g. persistent infection, cervical root fracture during and after treatment, have resulted in extraction of the affected tooth.

In children, missing upper teeth have been shown to lead to impaired aesthetics, occlusal problems and possible speech issues (Weinberg, 1968) and their self – esteem (Shaw et al., 1991). Other implications include loss of space and most importantly alveolar bone resorption due to remodelling of the bone.

2.2.5 Alveolar bone resorption

Alveolar bone resorption following tooth loss (e.g. trauma, caries or periodontal disease) is an expected outcome resulting in a decrease of alveolar bone volume, height, width and a change in bone morphology (Atwood and Coy, 1971; Botticelli et al., 2004; Lekovic et al., 1997). Even the loss of a single tooth can result in a pronounced change in the buccal profile and a marked decrease in the dimension of the marginal portion of the edentulous ridge (Schropp et al., 2003). Although the degree of bone loss varies among individuals and anatomical sites, it is well accepted that as much as 40% of the alveolar bone height and 60% of the alveolar bone width may be lost in the first six months following extraction (Schropp et al., 2003).

In children, undesirable marked alveolar bone loss will complicate treatment planning and the final treatment outcome. Dental implant placement is contra - indicated as the bone is yet to mature. Hence, if no measures are taken to preserve or regenerate the alveolar bone after tooth loss, future implant placement will be a problem, due to insufficient bone volume. Alveolar bone augmentation may be necessary to facilitate correct placement and aesthetics of

a prosthetic crown (Day et al., 2008). Therefore, an ideal outcome in young children would be preserving the alveolar bone and soft tissue as long as possible to improve the restorative options that can be offered in early adulthood after growth has ceased (Day et al., 2008).

2.3 Management of the traumatised tooth

Currently there are available clinical guidelines to help clinicians to manage traumatic dental injuries. (AAPD, 2011; Albadri et al., 1997; Andersson et al., 2012; DiAngelis et al., 2012; Gregg and Boyd, 1998; Malmgren et al., 2012; Vaidyanathan et al., 2010). These guidelines are produced by various professional bodies e.g. International Association of Dental Traumatology (IADT), British Society of Paediatric Dentistry (BSPD) and American Association of Paediatric Dentistry (AAPD). The availabilities of such guidance highlight the importance of prompt and appropriate immediate and follow up treatment to improve the successful outcome of dental trauma management. Although there are some disagreements between these guidelines, the consensus is that every effort should be made to protect and preserve pulp vitality and to return the injured teeth to acceptable function and appearance.

2.3.1 Management of crown fracture

The management of crown fracture depends on the extent of structure involved. The restorative treatment options range from no treatment to complex procedures. When the crown fracture involves the dentine, the tooth should be restored as soon as possible to prevent bacteria ingression to the pulp. This could be achieved simply by restoring the tooth with tooth coloured restoration. However, when the fracture surface is too close to the pulp or the pulp is exposed then pulp therapy; pulp capping, Cvek's pulpotomy or coronal pulpotomy must be carried out to protect and preserve the pulp vitality.

The objective of pulp therapy is to promote hard tissue formation over the traumatic exposure and thus isolating the dental pulp from oral environment. Currently there are two available materials that could be used by the clinicians to achieve this; calcium hydroxide and mineral trioxide aggregate (MTA).

Calcium hydroxide is widely used in dentistry and has been shown to be effective in management of traumatic pulpal exposure (Cvek, 1978; Ravn, 1982; Winter, 1977). It has antibacterial properties and the ability to induce hard tissue formation owing to its high pH (Forsten and Soderling, 1984). When calcium hydroxide is applied over the vital pulp, it causes a localised and superficial tissue necrosis (Faraco and Holland, 2001), approximately 1- 1.5 mm in depth. This leads to a cascade of events resulting in hard tissue formation. The necrotic tissue generates a low – grade irritation which is not strong enough to destroy the pulp but sufficient to trigger defensive reactions within the pulp. The underlying tissues react to this irritation by producing collagen that is subsequently mineralised. This is later followed by differentiation of dentine, resulting in the formation of the hard tissue barrier (Watts and Paterson, 1981).

Mineral trioxide aggregate (MTA) is a biocompatible (Camilleri et al., 2004; Keiser et al., 2000), hydrophilic cement, that sets in the presence of moisture (including blood). It also has a pH level which is comparable to calcium hydroxide (Torabinejad et al., 1995). Mineral trioxide aggregate powder consists of tricalcium silicate, tricalcium aluminate, tricalcium oxide, tetracalcium aluminoferrite and silica oxide (Camilleri et al., 2005; Torabinejad et al., 1995). Hydration of this powder with water/saline produced a colloidal gel that hardens.

Mineral trioxide aggregate cement has been shown to stimulate reparative dentine formation when applied on the vital pulp (Andelin et al., 2003; Ford et al., 1996; Tziafas et al., 2002). Although, MTA has a similar action to calcium hydroxide in promoting the dentine bridge (Faraco and Holland, 2001; Tziafas et al., 2002) it provides a better protective barrier against microleakage, thus reducing chances for re-contamination of the pulp with bacteria.

2.3.2 Management of immature teeth with non- vital pulp

The endodontic management of immature teeth with non – vital pulp is often difficult due to its wide and infected canal. Therefore, the aims of managing such cases are to eliminate infection, promote periapical healing and formation of an apical barrier to allow placement and containment of the root filling material. Currently there are two methods of creating an apical barrier; physiologically with non- setting calcium hydroxide or artificially with mineral trioxide aggregate

cement (Vaidyanathan et al., 2010). This method is also widely known as apexification.

The physiological approach requires multiple visits to redress the canal with non – setting calcium hydroxide. Although there are disagreements on how frequent the dressing should be changed (Abbott, 1998; Chosack et al., 1997), regular replacement has a number of advantages; allows clinical assessment of barrier formation and may increase speed of bridge formation (Kinirons et al., 2001; Mackie et al., 1988). The success rate of this approach has been reported to range from 74 – 100% (Sheehy and Roberts, 1997). The calcific barrier is expected to form within 5 to 20 months after initiation of treatment. However, if the size of opening of the root end is more than 2 mm, the barrier would take a much longer time to form. The reaction of periapical tissues to calcium hydroxide is similar to pulpal tissue (Holland et al., 1977). Histologically the hard tissue barrier is composed of cementum, dentine and bone which derived from connective tissue at the apices (Dylewski, 1971).

A hard apical barrier can alternatively be produced using MTA (Torabinejad and Chivian, 1999). This technique only requires two visits where the cement is inserted into the root canal to form a 4 – 5 mm apical plug and which then carefully condensed. The canal must be thoroughly cleaned and adequately disinfected prior to mineral trioxide aggregate placement as its removal at a later date can be challenging or even impossible. Depending on future treatment planned, the remainder of the canal is either completely obturated with MTA (Bogen and Kuttler, 2009; Pinar Erdem and Sepet, 2008) or backfilled with gutta – percha (Vaidyanathan et al., 2010). When compared to calcium hydroxide, mineral trioxide aggregate has been shown to be clinically superior in terms of periapical healing and providing an apical barrier (El-Meligy and Avery, 2006; Pradhan et al., 2006).

2.3.3 Management of replacement resorption

Replacement resorption is an irreversible and progressive phenomenon. As previously described, this process is very rapid in children and most likely will result in tooth loss. In order to maintain the alveolar bone dimension, a decoronation procedure can be performed; the crown of the affected tooth is

removed, leaving the root in the alveolar bone (Filippi et al., 2001; Sapir and Shapira, 2008). This procedure involves a full mucoperiosteal flap and decoronation of the crown just beneath the cervico-enamel junction, approximately 1mm under the crestal bone margins. All root canal filling or medication must be removed to allow complete bone healing. Bleeding is initiated to encourage blood clot to form within the canal. This is important as the blood clot is organised from the surrounding tissues (Malmgren, 2000). The flap is then coronally repositioned and sutured to allow primary wound healing process. The decoronated root will be gradually subjected to bone turnover. This technique potentially allows for complete preservation of the width and height of the alveolar process with additional vertical bone apposition; ideal bone volume for future implant placement. However it is experimental and outcomes are not known at present. It is also difficult for patients.

2.3.4 Management of alveolar bone preservation.

Alveolar bone preservation or regeneration after tooth loss can be achieved with immediate bone filling of the extraction socket. Generally bone filling materials can either be biological materials or synthetic materials. An ideal bone graft material should mimic the iliac bone crestal bone which is commonly used (Meyer et al., 2009).

The bone graft materials should have osteogenesis, osteoconductive, and osteoinductive properties (Lindhe et al., 2008; Meyer et al., 2009). Osteogenesis occurs when viable osteoblasts and precursor osteoblasts are transplanted with the grafting material into the defects, where they may establish centres of bone formation. Osteoinduction involves new bone formation by the differentiation of local uncommitted connective tissue cells into bone – forming cells under the influence of one or more inducing agents. Osteoconduction, however, occurs when non – vital implant material serves as a scaffold for the ingrowth of precursor osteoblasts into the defect. This process is usually followed by a gradual resorption of the implant material.

When considering immediate bone filling, clinicians can advocate either biological bone graft material or synthetic/biomaterial bone substitute. The biological bone grafts include autografts, xenografts and allograft. Autografts

refer to bone harvested and transferred from one position to another within the same individual, thus eliminating the risk of disease transmission or non-histocompatibility (Lindhe et al., 2008). The harvested bone can be obtained either from intraoral or extraoral donor sites (Sandor et al., 2010). Intraoral donor sites are tuberosity, ramus, symphysis, tori, zygomatic arches, edentulous ridge and retromolar area. Extraorally, the common sites for bone harvesting are iliac crest, tibia, calvarium and ribs. The graft comprises both cortical and cancellous bone including the marrow. Autograft is superior to other materials in lieu of their properties. The graft may retain viable cells which could promote bone healing through osteogenesis and osteoconduction at the recipient site, where new viable bone can be deposited as the bone graft is subjected to normal bone turnover (Lindhe et al., 2008).

Xenograft refers to a graft from a donor of another species. It includes only the inorganic portion of the bone such as hydroxyapatite, calcium carbonates and calcium phosphates. An example of a xenogeneic graft is the deproteinized bovine bone mineral e.g., Bio-Oss®. It is a biocompatible bone derivative (Artzi et al., 2000) and has been shown to be osteoconductive (Araújo and Lindhe, 2009; Camelo et al., 1998). In dentistry, xenograft bone substitute has been used in managing ridge defects (Norton et al., 2003) and extraction socket preservation (Carmagnola et al., 2003). However, complete alveolar bone preservation with this graft has yet to be successful (Fickl et al., 2008b; Nevins et al., 2006).

Allografts are grafts transferred between members of the same species but different genotype. It is mostly harvested from a diseased – free donor or cadaver. In general, there are three types of allografts; frozen, freeze – dried and demineralised freeze – dried. The presence of bone morphogenic proteins in allografts can induce host osteoprogenitor cells to differentiate and proliferate into osteoblasts (Becker et al., 1995).

Alloplasts or synthetic biomaterial bone substitutes have been used to manage alveolar bone augmentation or preservation. The materials act as a scaffold (Meyer et al., 2009) for new bone to regenerates. Examples of alloplastic materials include synthetic hydroxyapatite, bioactive glass, tricalcium phosphate ceramics, polymethylmethacrylate and osteoactive polymers. Alloplasts are commonly used when a moderate to large amount of graft is needed and the

donor site is restricted by the amount of bone volume. Alloplasts grafts should preferably be osteoconductive, non – immunogenic and may be resorbable.

2.4 Problems with current techniques for managing trauma

2.4.1 Calcium Hydroxide

Although calcium hydroxide can have a successful outcome in the management of crown fracture and inducing apical barrier in immature teeth, emerging evidence shows that it has some drawbacks. As aforementioned, the aim of treatment in complicated crown fracture is to create a reparative dentine bridge to stop communication of the pulp with the oral environment. The ideal reparative dentine should be a thick, continuous and atubular structure formed across the exposure to provide protection against penetration of noxious agents (Heys et al., 1981; Mjor, 1985). However, studies have shown that the formation of such a bridge is inconsistent and unpredictable with reported presence of thin, non – continuous and tunnel defects in the structure (Cox et al., 1996; Heys et al., 1981). Should microleakage at the tooth -restoration interphase occur, such defects would not able to provide a hermetic seal to the underlying pulp against bacterial progression to the pulp. Therefore, this might affect the long term prognosis of the tooth.

Another drawback of calcium hydroxide as a pulp capping medicament is that the remnants of necrotic tissue should be removed once a dentine bridge has formed. The necrotic remnant can serve as nutrients for bacteria that may have gained access through microleakage between restoration and the tooth structure. This will require more clinical appointments and impose a significant financial burden to the health system. Furthermore, in young children the co-operation required for dental procedures may be limited because of their anxiety and thus may complicate treatment delivery.

When calcium hydroxide is used as a medicament to induce physiological calcific barrier at the root end, available evidence shows that calcium hydroxide may react with the dentinal root wall and weaken the root structure. Studies have demonstrated that prolonged dressing of the immature tooth with calcium hydroxide resulted in significant reduction of resistance to fracture (Andreasen et

al., 2002; Doyon et al., 2005; Rosenberg et al., 2007; Twati et al., 2009). It was postulated that due to its strong alkalinity, calcium hydroxide may denature the protein and proteoglycans of the dentine (Andreasen et al., 2002). These structures serve as bonding agents between the collagen network and hydroxyapatite crystals, thus such alteration results in collapse of the dentine structure (Rosenberg et al., 2007). The frequency of cervical root fracture closely related to stage of root development; ranged from 77% in stage 1 to 2% in stage 5 of root development (Cvek, 1992) (see Figure 2.3). Therefore, teeth in young children are more susceptible to cervical root fracture following calcium hydroxide treatment.

In addition to cervical root fracture, apexification with calcium hydroxide has the disadvantages of increased treatment time and frequent dental visits to change the intracanal medicament. This can lead to re-infection of the root canal if the coronal seal is compromised and cause delays in completing final restoration. In addition, multiple visits for the apexification procedure can impose a financial burden to both patient/parents and health provider. It has been demonstrated that the estimated total cost for treating a paediatric patient with of traumatic dental injury was £856 (Wong and Kolokotsa, 2004).

2.4.2 Mineral trioxide aggregate

Although mineral trioxide aggregate is a suitable and superior alternative to calcium hydroxide as a pulp capping material and in an producing artificial apical barrier, it causes intrinsic gray discolouration to the tooth (Bogen and Kuttler, 2009; Karabucak et al., 2005; Parirokh and Torabinejad, 2010). This is a well recognised limitation of MTA which can impair the aesthetic outcome of the treatment. The gray discolouration is attributed to the reduction of ferrous ions (FeO); black compound into the dentinal tubules that might increase over time (Asgary et al., 2005).

In order to overcome this problem, a white MTA was developed without compromising the physical and therapeutic properties of the original MTA (Cardoso-Silva et al., 2011; Stefopoulos et al., 2008). The main difference between the gray and white MTA is the lack of iron ions in the latter (Song et al., 2006). However, still there are reported cases of tooth discolouration associated

with white MTA (Belobrov and Parashos, 2011; Kvinnsland et al., 2010; Moore et al., 2011).

Another potential problem with MTA is removal after placement and curing. It has been shown that MTA cannot completely be removed from the root canal (Boutsioukis et al., 2008), thus if a decoronation procedure is indicated, blood coagulum could not be introduced into the canal. MTA will be left *in situ* while the rest of the root is subjected to bone turnover. As MTA is not resorbable, it will reside within alveolar bone and may create difficulty in future implant placement.

2.4.3 Alveolar bone loss preservation and grafting technique

Autogenous bone grafts are the gold standard but require a donor site thus a second surgery is necessary to harvest the graft (Sandor et al., 2010).. It has associated morbidity to the patient. To subject a young child with multiple surgical sites (donor and recipient) will likely require pharmacological behaviour management i.e. general anaesthesia for these procedures to be successful (Davies et., 2008). General anaesthesia is a safe procedure but the risks associated with general anaesthesia are real.

Although allografts are pre – treated by freezing, radiation or chemicals in order to suppress foreign body reactions, the risks of antigenicity could not be ruled out (Lindhe et al., 2008). On the other hand, alveolar bone preservation with xenografts material e.g. Bio-Oss® particles has limited success as the grafts are mainly surrounded by connective tissues rather than bone formation (Becker et al., 1998; Carmagnola et al., 2003).

Table 2.2: Advantages and disadvantages of calcium hydroxide and mineral trioxide aggregate in management of dental trauma.

Calcium Hydroxide	Mineral Trioxide Aggregate
Advantages	
• Antibacterial properties. • Ability to stimulate hard tissue formation (dentine bridge and apical barrier). • Ability to arrest root resorption process.	• Shorter course of treatment • Reduces risk of crown root fractures. • Biocompatible. • Antibacterial properties. • Sets in presence of moisture.
Disadvantages	
• Unpredictable dentinal bridge quality. • Lengthy treatment times for apical barrier formation. • Requires multiple visits for apexification. • Denatures collagen and resulted in weak dentine structures. • Higher incident of cervical root fractures.	• Cost. • Not easy to place. • Tooth discolouration even with white MTA. • Difficult to remove once cement is set. • Long setting time (~ 4 hours). • Brittle material.

2.5 Brushite cement as a possible endodontic or bone graft material

Despite many advantages of both calcium hydroxide and MTA as endodontic medicaments, both materials have drawbacks as described above. Therefore, there is a need to search and develop a new material which will offer better properties to improve treatment outcome in managing sequelae of traumatic dental injuries.

Calcium phosphate cement has a great potential as both a dental material and resorbable bone graft (Brown and Chow, 1985). Conventional calcium phosphate cement however does have some drawbacks which limit its application. Therefore, a modified - brushite cement could be developed to be utilised as both an endodontic medicament and bone filling material. For endodontic medicament, modified brushite cement could be used as a substitute to calcium hydroxide or MTA in direct pulp capping, forming synthetic apical plug in non – vital immature tooth and as an obturation material.

In the field of synthetic bone filling materials, calcium phosphate cements have been of great interest since their discovery in 1980's. Brushite cements, one of the end products of calcium phosphate cement have the potential as drug carrier and bone filling materials. Brushite cement have been used in maxillofacial and craniofacial reconstruction (Gómez et al., 2005), treatment of fracture defects e.g., distal radius (Hidaka et al., 2002), treatment of surgically or traumatically created osseous defects, filling of cystic lesions and treatment of spinal fractures and vertebroplasty (Turner et al., 2008). They can also act as calcium and phosphate ion reservoirs for remineralisation of hard tissues. Therefore, this biomaterial may provide solutions to many of the problems associated with autologous, allografts and xenografts.

In the field of paediatric dentistry, the combination of their self – setting nature, moldability, injectability, biocompatibility of antibacterial releasing cements suggests a great possibility of brushite in regenerative therapy. Furthermore, injectability could offer immediate filling of the extraction socket without the need of additional surgical procedure which is an advantage in children. Hence, the development of brushite cements as a potential bone substitute will offer

clinicians alternatives and the opportunity of preserving the alveolar bone dimension.

2.6 Brushite Cement

2.6.1 Calcium orthophosphate

Brushite cements ($CaHPO_4.2H_2O$) (also known as dicalcium phosphate dihydrate) are an end product of calcium orthophosphate cements. Calcium orthophosphates have potential in the field of bone grafting mainly due to their chemical similarity to the mineral component hydroxyapatite (inorganic component of mammalian bones and teeth). In addition, this compound is known to be biocompatible, biodegradable and osteoconductive. Calcium orthophosphate cements however, have poor mechanical properties i.e. low fracture toughness, ductility and fatigue resistance which is a major setback for applications in load–bearing areas (Bohner, 2000; Dorozhkin, 2008).

Calcium orthophosphate compounds are derived from orthophosphoric acid which consists of four main elements: calcium, phosphorus, oxygen and hydrogen. The atomic architecture is built up around a network of orthophosphate groups $(PO_4)^{3-}$ (Dorozhkin, 2008). Most calcium orthophosphates are soluble in water but insoluble in alkaline solutions. All of them are easily soluble in acids. Table 2 summarizes names and chemical formulae of various calcium orthophosphates (Bohner, 2000). Generally, there are two forms of calcium phosphate that are used as bone substitute; calcium phosphate ceramics and calcium phosphate cements.

Table 2.3: Different types of calcium orthophosphate. Adapted from (Bohner, 2000) (with permission to reproduce from Elsevier – see Appendix 8).

Compound	Chemical Formula
Compounds which can be precipitated at room temperature in aqueous systems	
Monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$
Dicalcium phosphate dihydrate (DCPD)	$CaHPO_4 \cdot 2H_2O$
Dicalcium phosphate anhydrous (DCPA)	$CaHPO_4$
Octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(HPO_4)_4 \cdot 5H_2O$
Precipitated hydroxyapatite	$Ca_{10}(PO_4)_6(OH)_2$
Amorphous calcium phosphate (ACP)	-
Compounds obtained at high temperature	
Monocalcium phosphate anhydrous (MCPA)	$Ca(H_2PO_4)_2$
α-Tricalcium phosphate (α-TCP)	α- $Ca_3(PO_4)_2$
β-Tricalcium phosphate (β-TCP)	β- $Ca_3(PO_4)_2$
Sintered hydroxyapatite (SHA)	$Ca_{10}(PO_4)_6(OH)_2$
Oxyapetite (OHA)	$Ca_{10}(PO_4)_6O$
Tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$

2.6.2 Calcium Phosphate ceramics

Calcium phosphate ceramics for bone substitution are available as granules or blocks (Bohner, 2000). They can be obtained by a sintering process. Sintering is a method for making objects from powder, by heating the material in a sintering furnace below its melting point until its particles adhere to each other. Calcium phosphate ceramics can be inert or bioactive. Inert bioceramics exert only a minor fibrous reaction from the host. As for bioactive formulations there is direct biochemical and biological interaction with the adjacent bone tissue and therefore may promotes osseointegration (Oguchi et al., 1995). Hydroxyapatite is one of the most extensively studied bioactive ceramics.

The limited ability of hydroxyapatite to degrade fully and subsequently be replaced by surrounding tissues (Bohner, 2000) poses a major problem in the regenerative therapy of alveolar bone. Furthermore, due to its poor mechanical properties, hydroxyapatite has limited application as a bone grafting material in load – bearing areas (Akao et al., 1981). For these reasons there has been in recent years increased interest in the development of more degradable brushite – forming cement formulations.

2.6.3 Calcium Phosphate Cements

The concept of apatite calcium phosphate cements (CPCs) was first introduced by LeGeros in 1982 and later in 1986 when the first patent on self – setting CPC was obtained (Brown and Chow, 1986). The calcium phosphate cement discovery was an important breakthrough because the material was mouldable and therefore could be adapted to the bone cavity, presenting a good fixation and the optimum tissue – biomaterial contact necessary to stimulate bone ingrowth. These features give CPCs better handling characteristics than prefabricated calcium orthophosphate granules or blocks.

In general, CPCs are produced by a combination of single or several calcium orthophosphates (powder phase) with an aqueous solution (liquid phase). Upon mixing, calcium phosphate dissolves and precipitates into a less soluble compound. During the setting process, the calcium phosphate crystals grow and become entangled, thus providing a mechanical stiffness to the cements

(Bohner, 2000). This setting mechanism differentiates calcium orthophosphate cements from traditional bone substitute preparations (Bohner et al., 2000).

Despite the large number of formulations, CPCs can only have three different end products; apatite (PHA), brushite (DCPD) and amorphous calcium phosphate (ACP). However a study has shown that ACP is rapidly converted into PHA (Bohner, 2000). Therefore, the CPC formulations can be categorized into apatite CPC and brushite CPC. The final setting product is important in determining solubility and therefore, *in vivo* bioresorbability.

2.6.3.1 Brushite Cements

Set brushite cement is composed of dicalcium phosphate dihydrate. This cement is prepared by mixing water with powder consisting of an acidic calcium phosphate and a basic calcium phosphate. Several compositions have been proposed for brushite cement production e.g. β-TCP + MCPM (Mirtchi et al., 1989b) and TetCP + MCPM + CaO (Constantz et al., 1998). The result of this mixture is a mouldable paste that eventually solidifies with an exothermic reaction forming a hard material. All brushite cements are obtained through acid – base reactions and can only precipitate in acidic environments (pH lower than 6). For example, β-TCP and MCPM mixtures have a pH value close to 4 during setting which later slowly changes towards the equilibrium pH of 5.9 (Bohner et al., 1997b).

Commonly used acidic phosphate sources for precipitation of brushite cement are monocalcium phosphate monohydrate (MCPM) and monocalcium phosphate anhydrous (MCPA) (Nurit et al., 2002). However MCPM is much more preferred than MCPA in view of the presence of the water molecule which facilitates the setting reaction. MCPM contributes to one of two molecules required by dicalcium phosphate to precipitate to form brushite. MCPM is the most acidic compound among the calcium orthophosphates. In addition, MCPM also has high solubility which renders this compound by itself not suitable to be used as bone substitute (Bohner, 2000).

Tricalcium phosphate (TCP) is the most common source of basic calcium for brushite cement precipitation (Tamimi et al., 2012). There are two forms of TCP;

α – TCP and β – TCP. Both TCP have calcium to phosphate ratios of 1.5 and similar chemical compositions but the crystallographic structures are different (Bohner, 2000). Although both compounds have been successfully used to produce brushite cements, β – TCP is much more preferred. This is attributed to the sintering process of TCP where lesser energy is needed to produce β – TCP than its counterpart.

2.7 Properties of brushite cement

2.7.1 Brushite Cement Setting Reaction

Early brushite cements had rapid setting reactions which made them difficult to work with. The setting reaction consists of a cascade of events (see Figure 2.4). The setting time of brushite cement is dependent on the solubility of the basic phase (e.g. β – TCP). The higher the solubility of the basic phase, the faster the setting time will be (Bohner, 2000). The original composition of brushite cement set within 30 seconds (Mirtchi et al., 1989b). The reaction time however, can be increased to a workable length. This can be achieved either by altering powder to liquid ratio or incorporating setting retardant into the composition (Mirtchi et al., 1989a).

The amount of water present in the composition will have an effect on the setting time. Low powder to liquid ratio (i.e. high water availability) has been shown to extend the working time of the setting brushite cements (Mirtchi et al., 1989a). The reverse effect is to be expected if a high powder to liquid ratio (i.e. limited water available) is used as this will favour rapid precipitation of the crystal. The drawback of a low powder to liquid ratio is that the end product will have a higher porosity and therefore lower strength (Bohner et al., 2005; Hofmann et al., 2009).

Incorporating setting retardants into the composition will not only increase the setting time, it will also lead to precipitation of smaller crystals and thus an improvement of the mechanical properties of the cements. Examples of setting retardants used are sulphuric acid, sodium pyrophosphate and sodium citrate solution (Bohner et al., 1996). When citric acid is used as retardant, the citrate ions in the β – TCP/MCPM system will interact with the β – TCP particles, thus

limiting their dissolution and subsequently inhibit their setting (Alkhraisat et al., 2008).

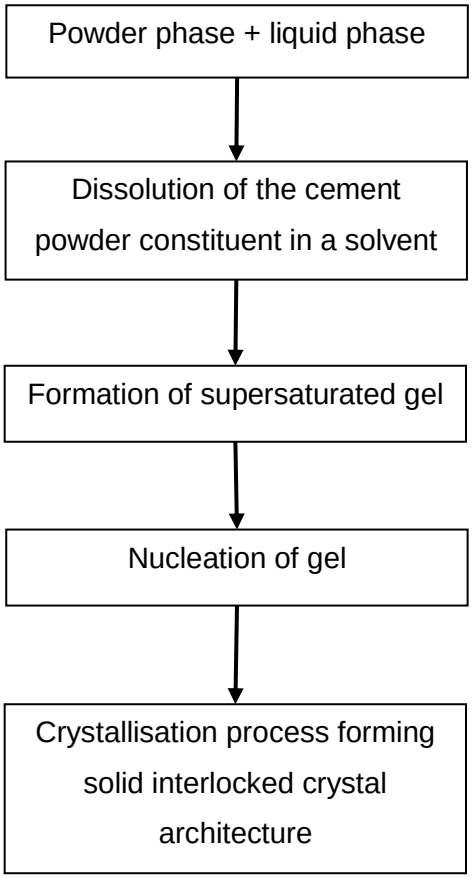

Figure 2.4: Brushite cement setting reaction cascade.

2.7.2 Mechanical Properties of Brushite Cements

Ideally, biomaterials for dental applications and bone regeneration therapy should have mechanical properties comparable to teeth and bone. When it was first developed, brushite only had a diametral strength of approximately 1 MPa (Mirtchi et al., 1989b). It has been shown that the compressive and diametral tensile strength of brushite cement inversely correlated to its porosity; the higher the porosity the poorer the mechanical performance (Hofmann et al., 2009).

High powder to liquid ratios will result in porosity reduction but at the expense of speeding up the setting reaction. In addition, the cement cannot be properly mixed and difficult to handle. In order to overcome this, introduction of additives such as citric acid are necessary not only to allow better cement mixing (Barralet et al., 2004) but also to act as a setting retardant. Setting retardants inhibit crystallisation and produce smaller brushite crystals and subsequently better cement compaction (Hofmann et al., 2009). In addition, delayed cement setting has been associated with higher compressive strength (Nurit et al., 2002). Although setting retardants have been shown to improve the mechanical performances of brushite cement, there is an upper optimal concentration beyond which strength reduction can occur (Hofmann et al., 2006).

The particle size of the calcium orthophosphate powder also has been shown to influence the mechanical properties. Hofmann et al. (2009) reported that at a combination of small particles and citric acid concentration of 800 mM, the powder to liquid ratio could be raised as high as 4 g ml^{-1}, producing low porosity cement with compressive strength of 52 MPa.

2.7.3 Degradation

The degradation process of brushite cement has been studied both *in vitro* and *in vivo* conditions. It has been shown that this cement can be degraded through cellular activity, dissolution, reprecipitated to hydroxyapatite or disintegration to smaller particles (Frayssinet et al., 1998; Grover et al., 2003; Theiss et al., 2005). During the first few weeks after implantation of brushite cements, rapid resorption could be observed (Apelt et al., 2004). This process is mediated by

simple dissolution and cellular activity. The macrophages rather than osteoclasts have been identified to regulate the early resorption activity (Constantz et al., 1998). This is in contrast to the *in vitro* findings where osteoclasts can penetrate and demineralise brushite cement (Xia et al., 2006). Rapid degradation of brushite could lead to immature bone deposition (Bohner, 2000).

Following *in vitro* incubation of brushite cement in aqueous media, depending on conditions, the cement may be stable, dissolve, hydrolyse to hydroxyapatite or may disintegrate (Constantz et al., 1998; Grover et al., 2003). In order for dissolution to occur, the aqueous medium must be undersaturated with calcium and HPO_4^{2-} ions. However, if the saturation of aqueous media favours hydroxyapatite formation, then hydrolysis of brushite cement to hydroxyapatite may take place. In addition, this conversion may result in pH drops due to dissolution of brushite cement could be expected due to its high solubility (Grover et al., 2003). The particles released from cement disintegration of cement could have an adverse effect on osteoblast function, viability, proliferation and subsequently affect bone deposition (Pioletti et al., 2000).

2.7.4 Brushite cement as a drug delivery system

Many studies have been carried out looking at the potential of brushite cements as a drug delivery system. Successful outcomes have been achieved when antibiotics (Bohner et al., 1997a; Bohner et al., 2000; Young et al., 2008), anticancer drugs (Otsuka et al., 1994a), anti – inflammatory medications (Otsuka et al., 1994b) incorporated with brushite cement. An advantage of brushite cement is that it has a porous microstructure and as such has the ability to release different chemicals introduced into it (Ginebra et al., 2006).

In general, a potential drug carrier must have the ability to incorporate the drug, to retain it a specific target site, and to deliver it progressively over time to the surrounding tissue at therapeutic dose (Arkfeld and Rubenstein, 2005) with predictable kinetics (Ginebra et al., 2006). Additional advantages are provided if the material is injectable and biodegradable (Ginebra et al., 2006).

Drug can be incorporated into the cement either in their liquid or powder phase. During the setting process, dissolution of calcium phosphate from the powder

phase takes place, followed by precipitation of crystallization of cements. It was postulated that the drug is dispersed within the matrix formed by the set cement (Ginebra et al., 2006), however microscale inhomogeneities have been noted with Raman Mapping (Young et al., 2008).

The drug release from brushite cement has been shown to be very rapid. This problem could be addressed by modifying the porosity of the cements or incorporation of polymers into the composition (Bohner et al., 1997a).

2.8 Chlorhexidine releasing, polyacrylic acid – modified brushite cement for dental applications

2.8.1 Chlorhexidine

In dentistry, chlorhexidine (CHX) has been used widely as a chemotherapeutic agent (Addy, 1986; Emilson, 1994; Gomes et al., 2001; Lang and Brecx, 1986). Apart from dental indications, chlorhexidine has also been shown to successfully reduce nosocomial infection in patients that require mechanical assisted ventilation (DeRiso et al., 1996; Houston et al., 2002; Koeman et al., 2006).

Chlorhexidine is a cationic bisbiguanide with broad antimicrobial activity, low toxicity and strong affinity for binding to skin, mucous membranes, teeth and pellicles. No bacterial resistance or overgrowth of potentially opportunistic organism or any other adverse changes in the oral microbial ecosystem have been reported with long term use of chlorhexidine (Briner et al., 1986). It also has been shown to have substantivity property which prolongs its therapeutic effect for several hours (White et al., 1997).

Chlorhexidine has a wide spectrum of activity against both gram-positive and negative bacteria, yeasts, dermatophytes and some viruses (Emilson, 1977). It has both bacteriostatic and bactericidal activities at low and high concentration respectively (Hugo and Longworth, 1964). The mode of action of chlorhexidine is focused in the reduction of membrane formation and modification of the bacterial cell wall. The phosphate and carboxyl group of bacterial cell is characteristically negatively charged. The positively charged chlorhexidine molecule is rapidly attracted to the bacterial cell surface, resulting in disruption

of bacterial cell membrane integrity (Hugo and Longworth, 1964). As a consequence permeability of the bacterial cell membrane increases and leakage of low – molecular – weight components, such as potassium ions occurs.

Chlorhexidine can be incorporated into brushite cements and implanted to a specific site for localised drug effects. Such potential sites include alveolar socket defects associated with periodontitis, post – extraction socket preservation and artificial apical barrier. However, rapid chlorhexidine release rates from this complex should be expected (Young et al., 2008) due to porosity of brushite cement (Ginebra et al., 2006), thus limiting the desired antimicrobial effectiveness. Therefore, modification of the cement is required to prolong the therapeutic effect of the system. This problem could be addressed by modifying the porosity of the cements or incorporation of polymers into the composition (Bohner et al., 1997a).

2.8.2 Polyacrylic acid

Polymers have been extensively studied for biomedical applications. Generally, polymers can be classified into natural (e.g. collagen, gelatine and cellulose) and synthetic (e.g. polyester and polyacrylic acid). Both types of polymer have been added to calcium phosphate cements with the aim to improve and overcome the intrinsic limitations of such inorganic cements (Perez et al., 2012). Such improvements include prolonged setting time, better cohesion and increase toughness and strength of calcium phosphate cement (Perez et al., 2012).

Polyacrylic acid is a synthetic polymer which comprises chains of monomer known as acrylic acids. It is in the liquid phase of glass ionomer cements; dental filling and luting materials which are used widely in dentistry. The advantages of glass ionomer cements include adhesion to the tooth structure without the need for coupling agents or etching technique that is required with dental composite placement. The adhesion is provided by interaction of the carboxylic group of the polyacrylic acid with hydroxyapatite of enamel and dentine (Wilson et al., 1967).

Incorporation of polyacrylic acid has considerable effects on the mechanical properties of calcium phosphate cements. Compressive strength of polyacrylic acid modified calcium phosphate cements increased substantially compared to cements without polyacryclic acid (Dos Santos et al., 2003). The improvement can be due to reduction in porosity (Dos Santos et al., 2003), improved ductility (Chen et al., 2008) and/or cross – linking between the polymer chains and cements structure. The molecular weight of the polyacrylic acid is also likely to influence the strength; the longer the chains the higher the strength due to lower mobility of the structure (Majekodunmi et al., 2003).

Apart from improving the mechanical properties, polyacrylic acid has been found to be able to regulate drug release from calcium phosphate cements. An *in – vitro* study by Bohner et al (2000) showed that antibiotic (gentamycin sulphate) release from brushite cement was successfully controlled over a period of a seven days at a constant release rate compared to without.

Hence, polyacrylic acid offers possible beneficial effects to overcome problems with conventional brushite cements; fast setting time, poor mechanical properties, rapid drug release and difficult to control degradation.

2.9 Hypothesis

As discussed previously, conventional brushite cement has limited clinical applications due to its inherent properties. Therefore, the hypothesis of this study is that partial replacement of citric acid with polyacrylic acids in the cement composition will:-

i) Improved working / setting time for application of the cement
ii) Improved mechanical properties
iii) Regulated chlorhexidine release from the cement
iv) Controllable degradation.

2.10 Aim

The aim of this study is to assess if any of these can be overcome through partial replacement of citric acid (used as a setting retardant) with polyacrylic acid to make the formulations more suitable for the applications discussed above.

The objectives are to assess the effects of varying polyacrylic acid and chlorhexidine concentrations in a brushite cement on its:-

i. Setting reaction kinetics and final composition
ii. Mechanical properties
iii. Microstructure
iv. Chlorhexidine release rate
v. Degradation

CHAPTER 3

MATERIALS AND METHODS

3.0 MATERIALS AND METHODS

3.1 Background

In order to test the hypothesis, this study investigated the properties of brushite cement modified by incorporating polyacrylic acid and chlorhexidine. The formulations studied consisted of equimolar monocalcium phosphate monohydrate (MCPM) and β-tricalcium phosphate (β-TCP) powders. Cements were prepared by addition of an aqueous solution (3:1 powder to liquid ratio by weight).The effects of two ratios of citric acid/polyacrylic acid and incorporation of chlorhexidine were studied. The citric acid weight was varied to allow partial replacement with polyacrylic acid. Figure 3.2 illustrates the experimental design and properties being investigated.

3.2 Materials

Monocalcium phosphate monohydrate (median particle size 62 micron) and β-TCP with median particle size of 7 micron were purchased from Rhodia, Birmingham, UK. The particle size was determined by scanning electron microscopy (SEM). Commercially available polyacrylic acid solution – Fuji IX (GC America Ins) and chlorhexidine diacetate salt hydrate from Sigma-Aldrich, UK were used as supplied. Fuji IX was sent to the School of Pharmacy, University College London for molecular weight determination. Analysis showed that the molecular weight of polyacrylic acid in Fuji IX was ~ 2100 g/mol. Distilled water was used as a solvent for citric acid crystals (Fisher, Longborough, UK) and as medium in the mechanical, degradation and drug release studies.

3.3 Cement and disc preparation

In the preparation of the cements, different compositions were manually mixed until a homogenous consistency was established. The fluid pastes were obtained by mixing powder : liquid in a weight ratio of 3:1. Powders contained equimolar MCPM and β-TCP (1 and 1.23g respectively) and chlorhexidine (0.2 or 0.4g). Liquid phases consisted of 800mM aqueous citric acid : polyacrylic acid

liquid in a 1:1 or 3:1 weight ratio. In control formulations, drug and/or polyacrylic acid were excluded but powder : liquid were maintained at 3:1. For identification, formulation are coded as C***x***P***y*** where x and y are approximate weight percentages of chlorhexidine (0, 6 or 11 wt%) and polyacrylic solution (0, 6 and 12 wt.%) in the whole formulation (see Table 3.1).

10 mm diameter and 1 mm depth discs were prepared for the experiments using metal washer rings as moulds. Acetate sheets were used to seal the surfaces of the setting cements. All formulations were allowed to set at room temperature for at least 24 hours before testing. The set cement discs were used in Raman, microstructure, mechanical, degradation and drug release studies (see Figure 3.1).

Figure 3.1: Examples of set cements used in the current study.

Table 3.1: Formulation codes and composition of each cement

Formulation Code	MCPM powder (g)	β-TCP powder (g)	CHX powder (g)	800mM citric acid solution (g)	Fuji IX liquid (PAA solution) (g)
C0 P0	1.00	1.23	0.00	0.74	0.00
C0 P6	1.00	1.23	0.00	0.55	0.19
C0 P12	1.00	1.23	0.00	0.37	0.37
C6 P0	1.00	1.23	0.20	0.80	0.00
C6 P6	1.00	1.23	0.20	0.60	0.20
C6 P12	1.00	1.23	0.20	0.40	0.40
C11 P0	1.00	1.23	0.40	0.88	0.00
C11 P6	1.00	1.23	0.40	0.66	0.22
C11 P12	1.00	1.23	0.40	0.44	0.44

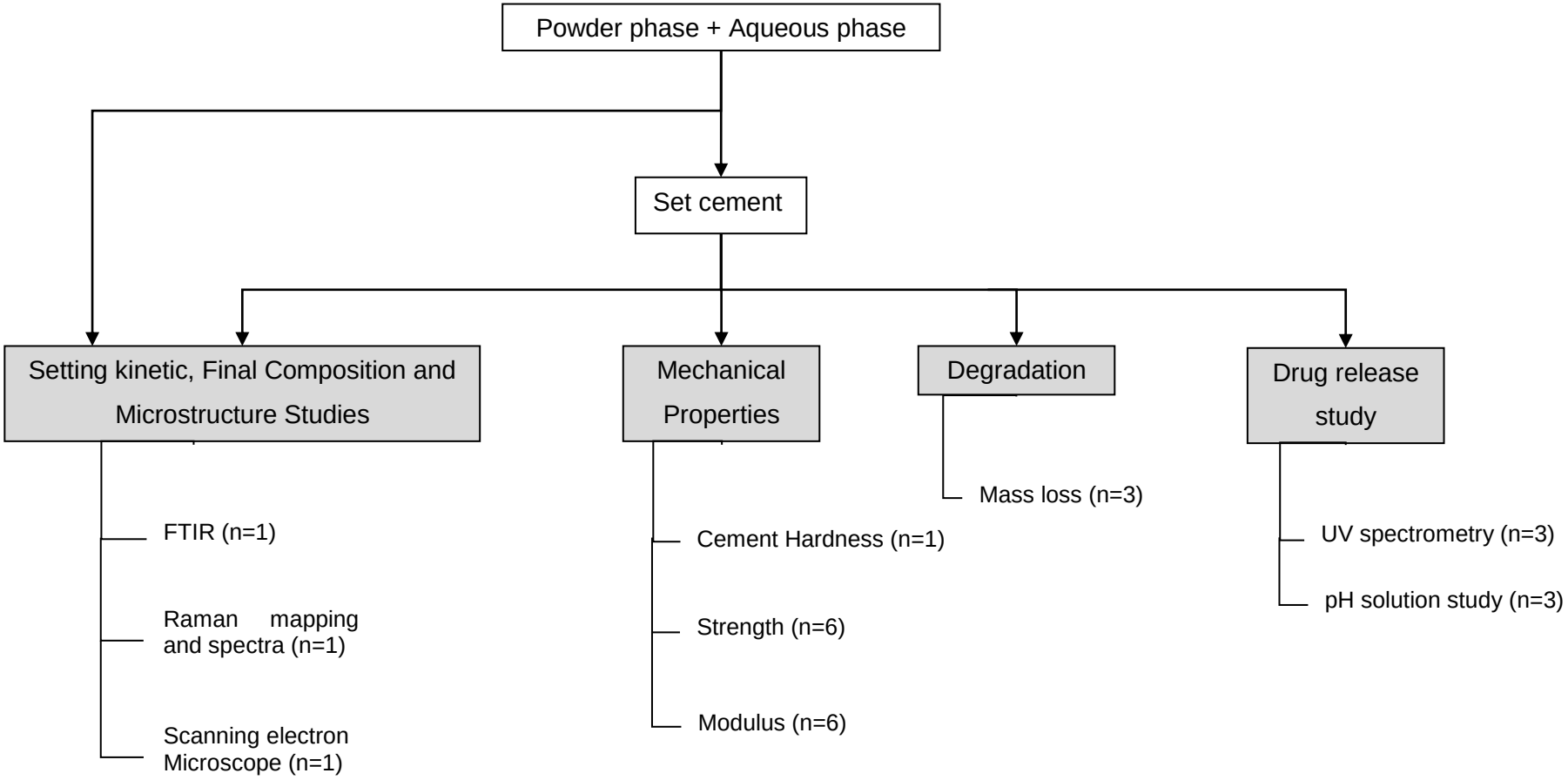

Figure 3.2: Flowchart of experimental designs

3.4 Study on setting kinetic, final composition and microstructure

3.4.1 Setting reaction kinetic

Fourier Transform Infrared (FTIR) spectroscopy is a type of infrared spectroscopy. This technique is based on absorption spectroscopy which involves absorption of electromagnetic radiation by sample over a range of wavelengths. The radiation from FTIR source covers the whole range of infrared frequencies (2×10^{-4} to 1×10^{-6} m).

An infrared spectrum is obtained by passing the infrared radiation through a sample. This radiation is absorbed at particular energy and causes vibration of atoms of a molecule. The energy at which the peak in the absorption spectrum appears correspond to the frequency of a vibration of a part of molecule. The vibration can affect the whole molecule, individual bonds or functional group within the molecule. The vibration can involve either a change in bond length (stretching) or change in bond angle (bending). In addition, some bonds can stretch in-phase (symmetrical stretching) or out – of – phase (asymmetrical stretching).

The frequency of vibration of the bond is directly proportional to the strength of the bond. The bonds of different groups within molecules will vibrate at different frequencies. The stronger the bond between atoms or molecules the higher the vibration frequency. As examples, double bonds are stronger than single bonds between like pairs of atoms, therefore they vibrate at higher frequencies.

In this study, FTIR was used to study the reaction kinetics of all compositions. This technique provided average spectra of the reactants and products versus time. Final set time could be determined when spectral changes became negligible.

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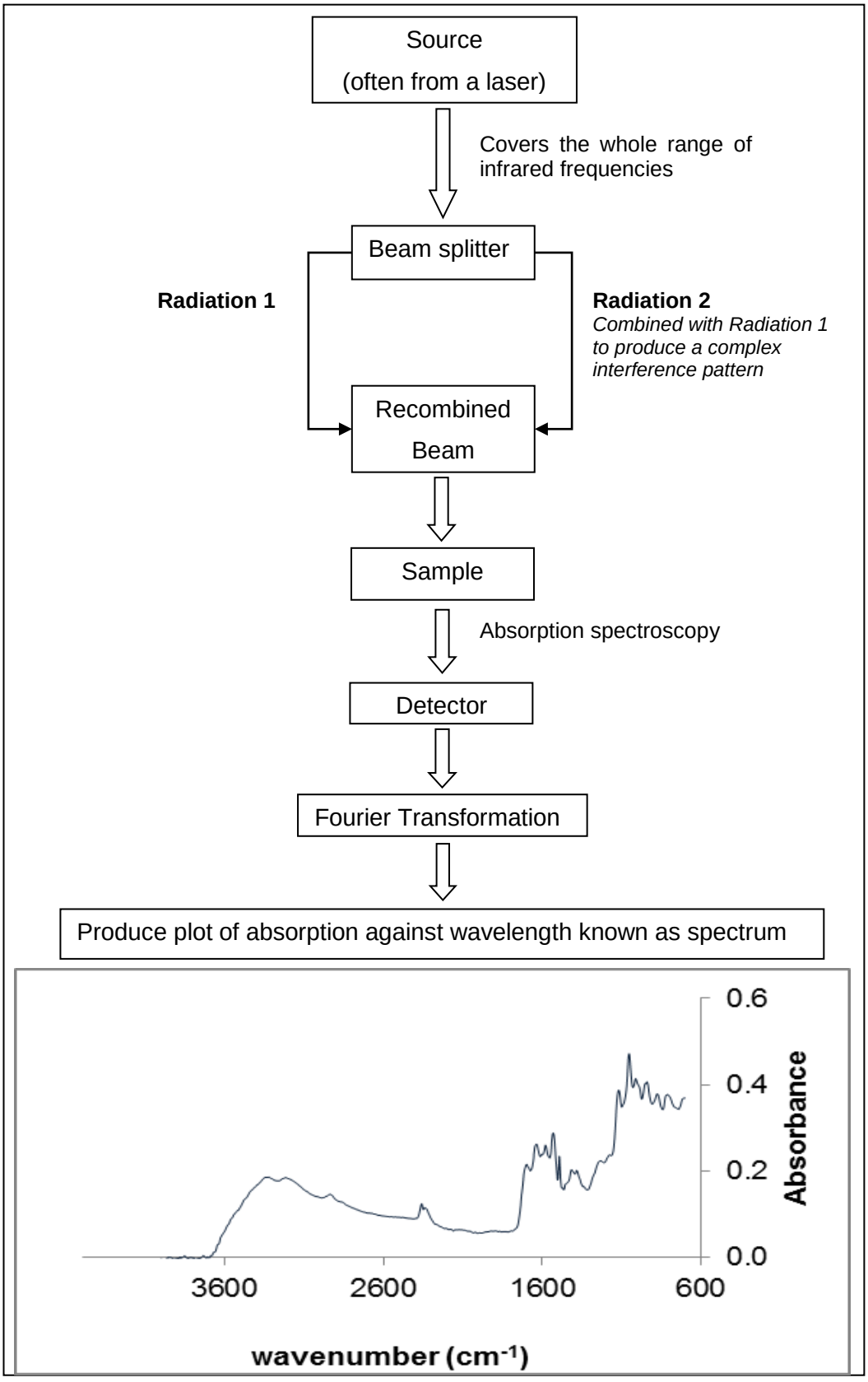


Figure3.3: Schematic illustration of spectrum production from sample by FTIR.

3.4.1.1 Experimental design for setting reaction kinetic

A Perkin Elmer Series 2000 FTIR spectrometer (Beaconsfield, UK) was used to assess real-time cement setting reactions. The background was scanned first without any sample on the ATR diamond piece so that the ratio of light intensity through the instrument with and without the sample could be calculated by the computer.

Reference spectra for the reactants were obtained. Approximately 0.35g of each formulation paste was then placed on the diamond of a golden gate ATR attachment (Specac) temperature controlled at 37°C. Chemical reactions of different formulations of chlorhexidine containing brushite cement were monitored and the end products were identified by comparison of spectra with those of pure compounds.

Cement spectra between 500 and 4000 cm^{-1} with a resolution of 8 cm^{-1} were obtained every 6.4s from within 30s from the start of cement mixing using Timebase software (Perkin Elmer). Each scan was initially set to last for 2 hours. However, it was noted that for cements containing polyacrylic acid, the setting reactions has not completed within 2 hours, thus scan time was extended up to 12 hours.

To confirm what processes were occurring during set, difference spectra were calculated by subtracting the first spectrum from that at later time. Such difference spectra are characteristic of chemical reactions occurring (Hofmann et al., 2006; Young et al., 2004).

The absorbance changes of the composition showed the formation of dicalcium phosphate and complexation between polyacrylic acid and brushite. Therefore, absorbance at 1052 cm^{-1} was used to quantify reaction rates because it shows substantial increase upon dicalcium phosphate formation in hydrated, anhydrous and complexed form (Hofmann et al., 2006). An apparent fractional reaction extent, ζ,, (Grover et al., 2005) was defined and calculated using:

$$\zeta = \frac{A_t - A_0}{A_f - A_0} \quad (1)$$

A_0, A_t and A_f are absorbance at 1052cm^{-1} initially, at time t and finally. A_0 and A_f were calculated by linear extrapolation of early absorbance versus time, or late absorbance versus inverse time, to zero (Hofmann et al., 2006). Furthermore, absorbance versus time at 1340 cm^{-1} is provided to demonstrate relative levels of polyacrylate - brushite complex formation.

3.4.2 Final composition study

In this study, the final composition of the set cement was determined with Raman spectroscopy. Spectroscopy refers to study of the interaction between matter and radiated energy. Generally, when a monochromatic light i.e. radiation of single wavelength interact with a sample, the light may be reflected, absorbed or scattered. The basis of Raman Spectroscopy is detecting scattered radiation of different wavelength from a sample to determine or identify the molecular structure. In this study, the source of radiation was generated from a laser. The laser produces a stream of photons to interact with the sample.

After interaction with a molecule, the scattered radiation will either have the same frequency / energy as the incident radiation or there is energy exchanged between photon and molecules during collision. The scattering process without a change of frequency is called Rayleigh scattering. On the other hand, when there are changes in the frequency, this phenomenon is known as Raman scattering. Raman scattering can further divides into Stokes' and anti – Stokes' radiation.

Radiation scattered with a frequency lower than of the incident radiation is referred to as Stokes' radiation, while that at higher frequency is called anti – Stokes'. Therefore, it is the change in the wavelength of the scattered photon due to either energy gained or lost during interaction between photon and sample will provides characteristic chemical and structural information.

Raman mapping is a technique for generating detailed chemical images based on a sample's Raman spectra. Several spectra of an area are acquired from all over the field of view. These spectra can then use to generate images showing the location and amount of different species.

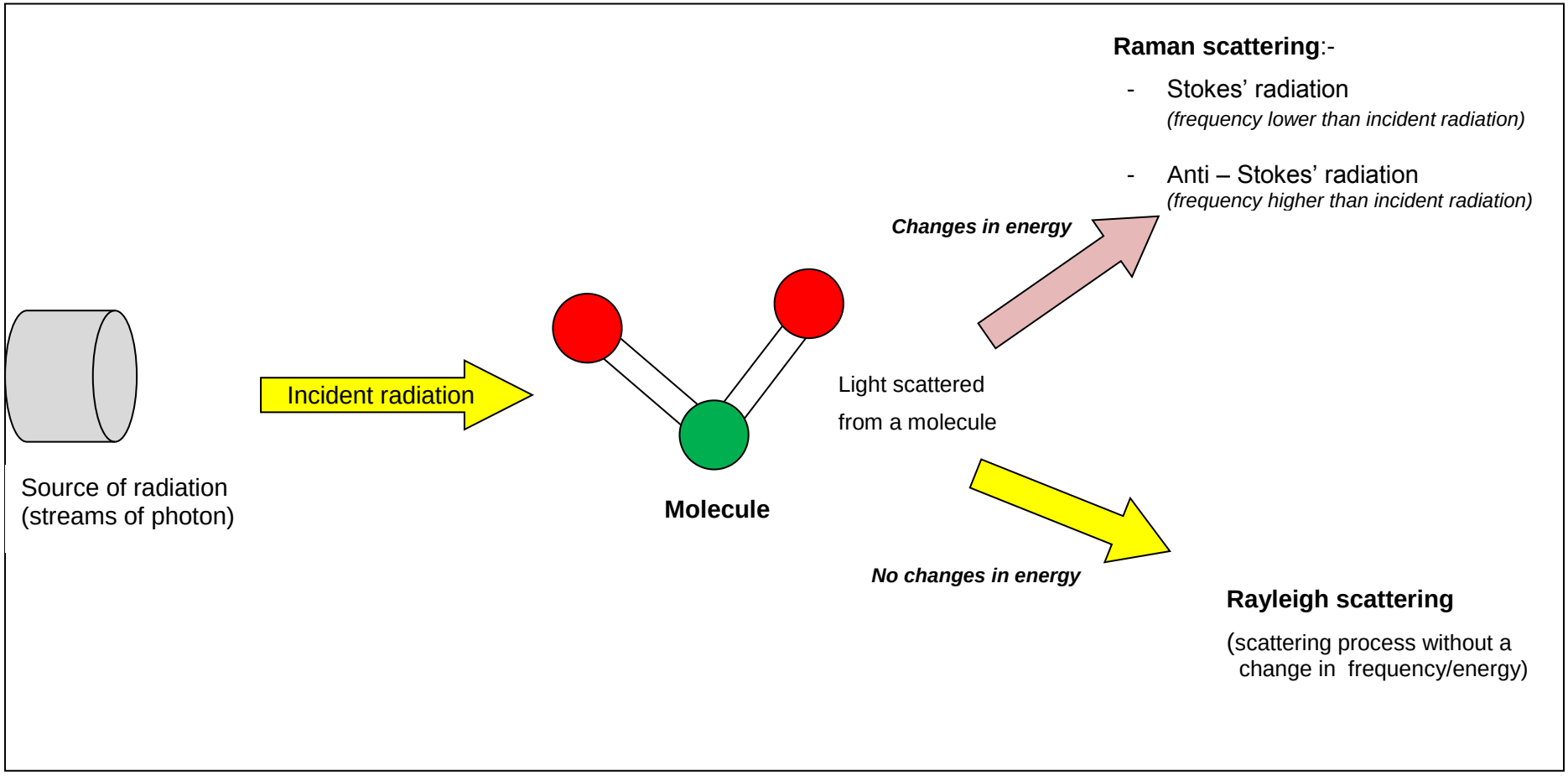


Figure 3.4: Theory behind Raman Spectroscopy. Incident radiation refers to radiation hitting a specific surface. The source of photon in this study derived from laser.

3.4.2.1 Experimental design for final composition study

Raman mapping studies and average spectra of sample discs were obtained using a LabRam spectrometer (Horiba JobinYvon, Stanmore, UK). Material bulk composition was examined by fracturing a dry sample and investigating the centre of the fracture surface. In addition, reference spectra for brushite and reactants used for cement preparation were also obtained to allow comparison between pure compounds and final product.

The instrument was equipped with a 633 nm laser and grating set at 1800. A X50 magnification objective and wavenumber range of 700 to 1650 cm^{-1} was used. Spectra were obtained every 2 µm across an area 50 x 50 µm for each specimen. Scan number and time were varied to ensure sufficiently noise free spectra. LabRam software (Horiba JobinYvon, Stanmore, UK) was used to generate average spectra of the cement samples and compositional maps of the material bulk.

3.4.3 Microstructure study of set cement

In this study, the effects of the polyacrylic acid and chlorhexidine on the microstructure of brushite cement were investigated using a scanning electron microscope (SEM). SEM uses a beam of highly energetic electron to examine objects on the micron scale. A stream of electrons is formed in high vacuum (by electron guns). This stream is accelerated towards the specimen (with a positive electrical potential) while is confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam. The sample is irradiated by the beam and interactions occur inside the irradiated sample, affecting the electron beam. These interactions and effects are detected and transformed into an image.

3.4.3.1 Experimental design for microstructure study of set cement

The morphology of each cement was examined with a JEOL 5410 LV (JEOL, UK) scanning electron microscopy at 20 kV. The cements were snapped in order to obtain cross-sectional fracture. These cements were then placed on a

aluminium stubs and sputtered by gold-palladium under vacuum (Polaron E5000, Quorum Technology, UK). High resolution images of the bulk of the cements were taken at x500, x1000, x1500, x2000 and x5000 magnifications.

3.5 Mechanical Property Studies

3.5.1 Hardness study

The hardness test evaluates the material's ability to resist plastic deformation from a standard source (indenter). It provides an indication of the resistance of the material to scratching or abrasion. There are two types of hardness tests; macro and microhardness depending on the load test used. Macrohardness study refers to experiment using larger test load (more than 1 kilogram – force (kgf). On the other hand, microhardness test utilised load test between 1 and 1000 grams – force (gf). In this study, the load test weighted 64.7 gf, therefore it is a microhardness test study.

This test is performed by pressing an indenter of specified geometry into the test surface using controlled test force. In this study the indenter geometry is in the shape of pyramid. This results in impression or indentation on the cement surface. The depth of the impression will depend upon the hardness of material.

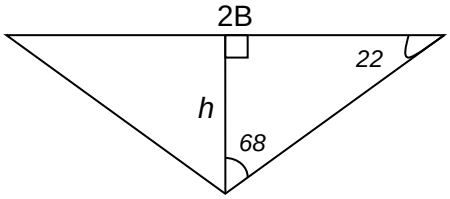
3.5.1.1 Experimental design for hardness study

The cements were examined using Wallace indentation hardness tester (H.M Wallace, Croydon, England – serial number 067851/1), with a load cell of 64.7g. Indentations were performed at 1 hour and 24 hours after mixing. The duration of each load application was 15 seconds. Seven applications were carried out for each composition. The depth of indentation was then recorded. For each sample an average of hardness measurement, expressed as the Vickers Hardness Number (VHN), was calculated using the following equation (Fuentes et al., 2003):

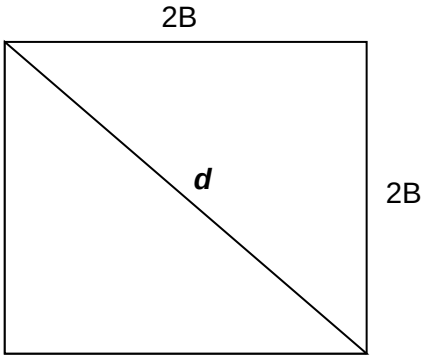
$$VHN = \frac{1.854F}{d^2} \qquad (2)$$

Where *VHN* is Vickers Hardness number, *F* is load and *d* is mean diagonal of indentation in millimetres. This formula is derived from the geometry of the indenter and area of indentation (see Figure 3.5).

Figure 3.5: Geometry of the Wallace hardness machine indenter (adapted from Wallace indentation hardness tester instruction manual)



(a) Cross section view



(b) Top view

Figure 3.6: (a) Cross section view of indenter and (b) top views of indentation of the cement surface. *h* is the depth of indentation and *d* is the diagonal of indentation.

VHN is expressed as:

$$VHN = \frac{F}{A} \tag{3}$$

where *F* is kilograms - force and *A* is area of indentation. Based on the geometry of the indenter (see Figure 3.5 and Figure 3.6), the area of indentation can be calculated from the following formula:

$$A = \frac{d^2}{2\sin 68^\circ} \tag{4}$$

$$\therefore A \approx \frac{d^2}{1.8544} \tag{5}$$

Where *d* is diagonal of indentation which can be calculated from Pythagorean Theorem:

$$d = 2\sqrt{B^2 + B^2} \tag{6}$$

$$\therefore d = 2 \times \sqrt{2}\, B \tag{7}$$

Where *B* is half the length of side of the cross section of the indenter (see Figure 3.6). *B* is calculated from the following equation.

$$B = \frac{h}{\tan 22^\circ} \tag{8}$$

Where *h* is depth of indentation by the Wallace hardness machine indenter in millimetres.

Therefore, VHN can be expressed as;

$$VHN = \frac{F}{A} \approx \frac{1.854F}{d^2} \tag{9}$$

The calculated VHN unit was then converted to GPa.

3.5.2 Biaxial flexural strength and modulus

In the literature, mainly the types of test used to evaluate calcium phosphate cement hardness were compressive test and diametral tensile test. Diametral tensile test is an alternative to direct tensile strength test and has been advocated to gauge strength of brittle materials such as brushite cement. In both test methods, the samples cements were casted in the form of cylindrical shape (Bohner et al., 1996; Mirtchi et al., 1989b; Ratier et al., 2001; Tamimi et al., 2008a).

In this study, however, the cement's strength was determined by biaxial flexural strength testing method. Biaxial flexural strength is defined as the maximum stress experienced in the material at failure when subjected to bending load. The rationales of using this type of test were based on possible applications of this cement a as dental material; which can be subjected to flexural stresses during mastication, the nature of brittle set cement and easy to produce disc - shaped testing samples (Chung et al., 2004; Morell, 1998).

The test is known as biaxial flexural test due to the set up of the experiment. The disc – shaped cement is supported near its periphery by a continuous ring shaped structure and later loaded by coaxially located ball. Hence, the support and ball are in the same axis during the test. In this study, given that the disc – shape is circularly symmetrical, the stress field is equibiaxial in the central region in which it is a maximum. In addition, this method will minimise the effect of the test specimen edge preparation compared to uniaxial test because the generated stresses are lowest at the test specimen edges (Morell, 1998).

Biaxial flexural modulus is a measurement of stiffness or elasticity of a material. It is a ratio of stress to strain which describes how much a material will stretch when put under a given stress. Hence, it is a resistance to elastic deformation.

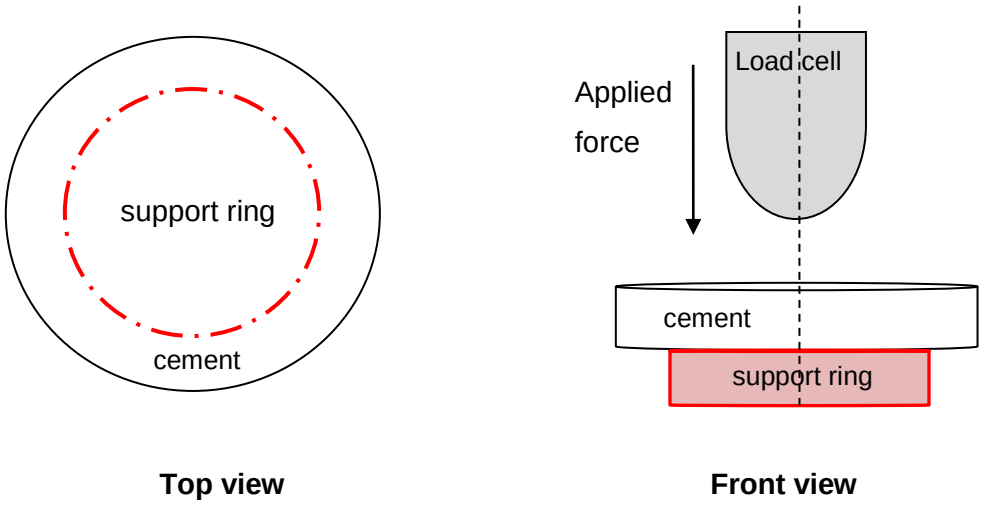


Figure 3.7: Top and front views of biaxial flexural strength experiment set up. The applied force and support are in the same axis.

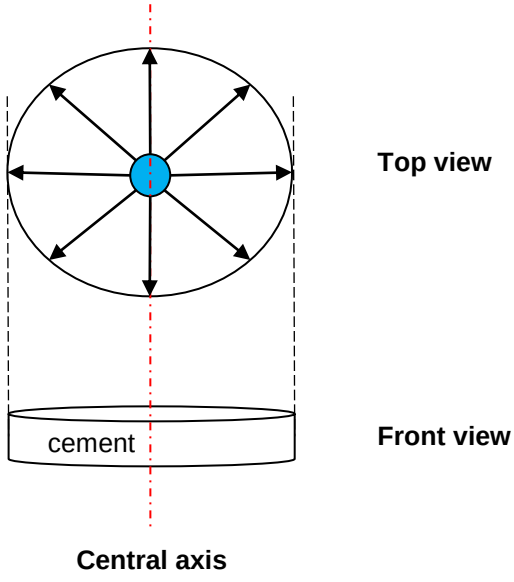


Figure 3.8: Stress field of disc - shaped cement under biaxial flexural test method is equibiaxial in the central region in which it is a maximum. Blue area demonstrates the maximum stress region. The stresses are equally distributed along its radial direction in which the stresses generated at the edge of the specimen are the lowest.

3.5.2.1 Experimental design for biaxial flexural strength and modulus

Six discs for each cement composition were hydrated in distilled water for 24 hours at 37°C. Sample thickness, *t,* was measured at three different points and averaged. The mechanical strength of the cements were determined using an Instron Model 4505 Universal Testing Machine compressive tester provided with a load cell of 1kN, as illustrated in Figure 3.9. The crosshead speed was fixed at 1 mm/min.

The hydrated disc specimen was placed on a knife edge ring support (radius a = 4 mm) and then loaded by a spherical tip (Börger et al., 2002) in an Instron mechanical testing jig. The maximum load (kN) at fracture, P and load versus central displacement gradient, *dP/dω* were determined. These were used to calculate strength, σ and biaxial flexural modulus, via (Timonshenko SP, 1964):

$$\sigma = \frac{P}{t^2}\left[(1+\upsilon)\left(0.485\ln\left(\frac{a}{t}\right)+0.52\right)+0.48\right] \quad (10)$$

and

$$E = \left[\frac{dP}{d\omega}\right]\left[\frac{0.5024a^2}{t^3}\right] \quad (11)$$

υ is Poisson's ratio and was taken as 0.3 (Akinmade and Nicholson, 1995; Charrière et al., 2001).

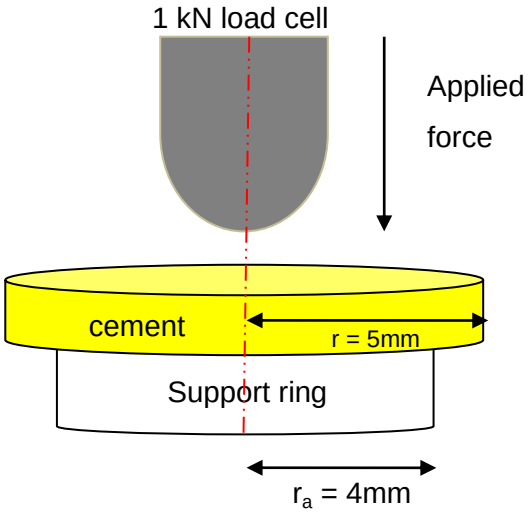


Figure 3.9: Schematic diagram of biaxial flexural strength test

3.6 Degradation, pH solution and Drug release studies

3.6.1 Degradation study

Fully set discs of each composition (n= 3) were weighed and immersed in 10 ml of distilled water (at 37 ºC) within sterilin tubes. At time points of 1, 2, 4, 8, 24, 72, 168, 336 and 672 hours the specimens were removed, weighed and replaced in fresh distilled water. Percentage mass change was calculated using:

$$\Delta m(\%) = 100\frac{m_t - m_0}{m_o} \quad (12)$$

where m_0 and m_t are sample mass initially and at time t.

The storage solutions were kept aside for pH analysis and UV analysis to determine chlorhexidine release kinetics.

3.6.2 pH solution study

The solutions obtained from chlorhexidine release study were all examined with Jenway 3340 pH/Ion Meters (BDH, Poole, Dorset, UK) to determine the pH of solution at different time point.

3.6.3 Drug release study

3.6.3.1 Chlorhexidine release profile

Ultraviolet (UV) spectroscopy is an important quantitative tool in analytical chemistry and was used, to quantify chlorhexidine release in this study. The UV spectrometer used operates on the double beam principle, with one beam passing through the sample and the other passing through the reference cell containing distilled water.

Cells for UV spectroscopy are made of quartz, glass or plastic. The standard UV cells are about 1 cm square and 3 cm high. The faces that are placed in the

beam are polished, and the cell is constructed so as to give a path length of exactly 1 cm.

After passing through the cells, the transmitted light will hit the detector, which will measure the ratio of the intensity of the reference beam (I_0) to the sample beam (I). UV spectroscopy follows the Beer – Lambart Law:

$$A = \log_{10}\left(\frac{I_0}{I}\right) = \varepsilon c l \qquad (13)$$

Where *A*, is absorbance, *c* is concentration of the compound, *l* is path length of the cell and ε is molar extinction coefficient which is constant. This constant is a measure of how strongly the compound absorbs at specific wavelength. Since the standardised UV cell has the same path length, therefore the equation could be simplified to:

$$A = \varepsilon c \qquad (14)$$

In general if the molar extinction coefficient for a compound is known at a given wavelength, measuring the absorbance at this wavelength permits the determination of the concentration of the sample.

3.6.3.2 Experimental design for chlorhexidine release profile

To quantify chlorhexidine release, UV spectra of each storage solution from mass loss studies was obtained between 200 - 400 nm using a Unicam UV 500 spectrometer (Thermo Spectronic, UK). The value ratio that an UV spectrometer can tolerate is either 10 ($\log_{10}$ 10 = 1) or 100 ($\log_{10}$ 100 = 2). In this study, the UV spectrometer maximum absorbance is 1. Hence, if absorbance exceeded 1 at 255nm, solutions were diluted with distilled water, the dilution factor recorded and new spectra generated.

A chlorhexidine calibration curve was obtained by plotting the absorbance at 255 nm against the concentration for standard chlorhexidine solutions containing 1, 5, 10 and 20 ppm of drug. For pure chlorhexidine solutions, a Beer Lambert law extinction coefficient of 45.9 cm^3/mg at 255 nm was found. This was used to

calculate chlorhexidine concentrations in storage solutions and converted to cumulative percentage at a given time from each specimen.

3.7 Error analysis

95% confidence interval was calculated using

$$CI = \frac{2SD}{\sqrt{n}} \tag{15}$$

SD is the standard deviation and n the number of samples (six for mechanical data and three for mass loss and chlorhexidine release). Results were considered significantly different when the CI error bars did not overlap.

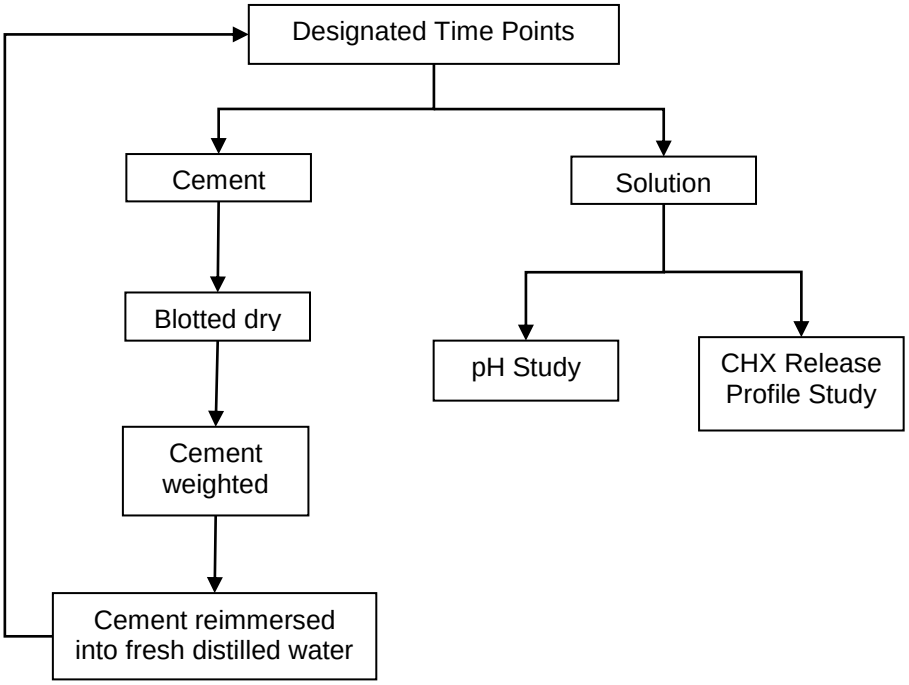


Figure 3.10: Experimental flowchart for degradation, pH and chlorhexidine release studies.

CHAPTER 4

Results I

Setting kinetic, Final Composition and Microstructure

4.0 RESULTS I – SETTING KINETIC, FINAL COMPOSITION AND MICROSTRUCTURE

4.1 Setting Kinetic

4.1.1 Reference spectrum for reactants

Figure 4.1 – Figure 4.6 illustrate the FTIR reference spectra for reactants used in the experiments. Figure 4.1 shows the reference spectrum for monocalcium phosphate monohydrate (MCPM). The spectrum consists of strong P – O stretch peaks at 1075 and 955 cm^{-1} and moderate peaks at 850 cm^{-1} (Hofmann et al., 2006; Xu et al., 1999).

Figure 4.2 illustrates the reference spectrum for β – tricalcium phosphate (β–TCP). A broad stretch at 1000 cm^{-1} can be observed in the spectrum. Figure 4.3 shows the reference spectrum for chlorhexidine (CHX). There were peaks around 1500 cm^{-1} due to benzene ring C = C, and N = C and N – H stretching (Harwood et al., 1999; Young et al., 2008).

Water spectrum is exhibited in Figure 4.4. The sharp peaks at 1640 cm^{-1} and broad peak at 3300 cm^{-1} both result from O – H stretching (Hofmann et al., 2006; Xu et al., 1999; Young et al., 2004). Figure 4.5 shows the reference spectrum of the aqueous phase of citric acid. Peaks at 1640 and 3300 cm^{-1} were due to water O - H stretching. The peaks at 1420 and 1532 cm^{-1} correspond with symmetric and asymmetric COO^- stretching (Hofmann et al., 2006). In addition there were moderate peaks at 1720, 1016 and 1096 cm^{-1} that correspond with C = O and C – O stretching (Young et al., 2004).

Figure 4.6 shows the spectrum of polyacrylic acid in the aqueous phase. 3340 and 1640 cm^{-1} peaks were correspondent to O – H stretching. There was also a weak peak at 1450 cm^{-1} which can be allocated to an O – H bend. The sharp peak at 1720 cm^{-1} was consistent with a C = O stretch (Young et al., 2004). In addition, there were peaks at 1092 and 1240 cm^{-1} which were likely due in part to the C – O bond (Harwood et al., 1999; Young et al., 2004).

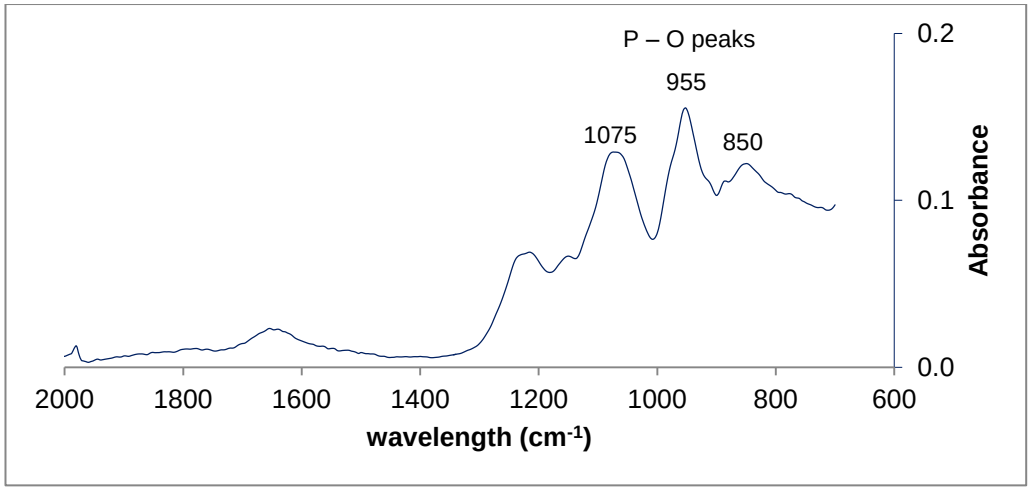


Figure 4.1: Reference spectrum of MCPM (powder phase).

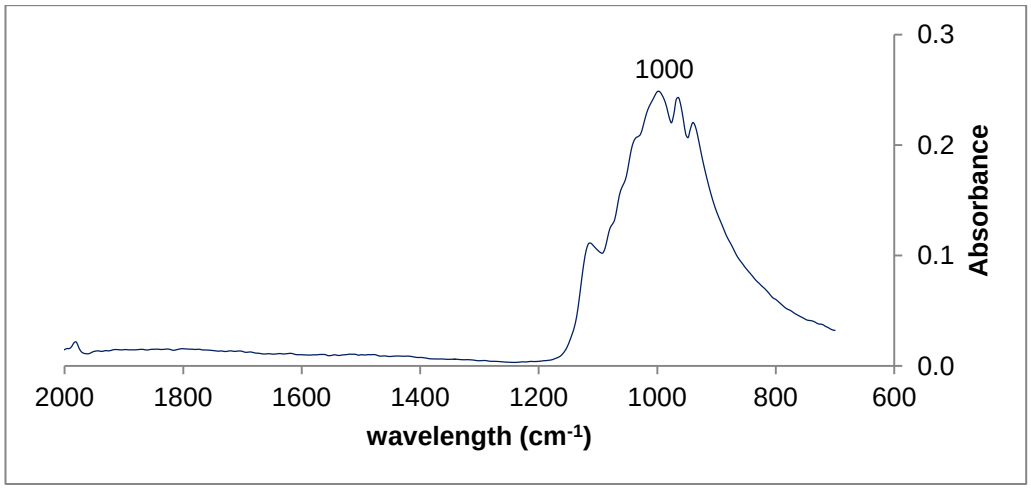


Figure 4.2: Reference spectrum of β - TCP (powder phase).

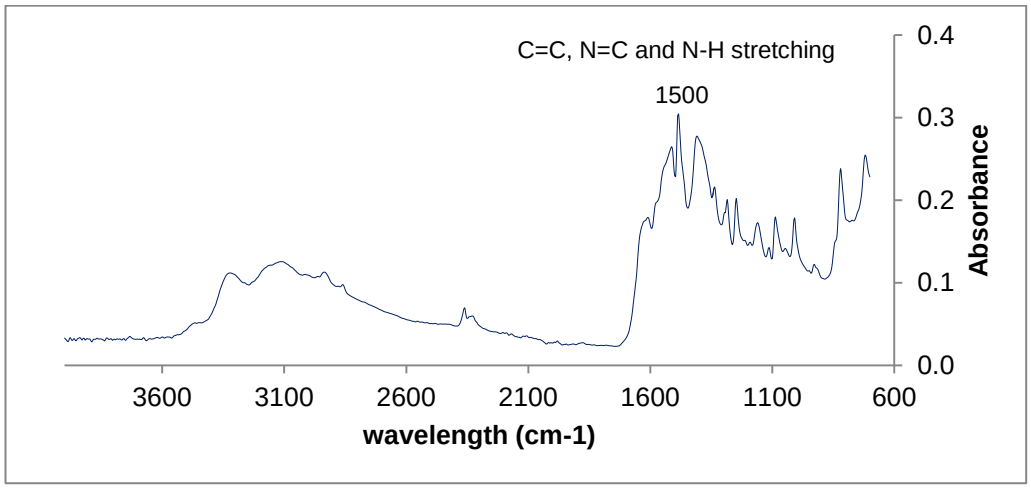


Figure 4.3: Reference spectrum of CHX (powder phase).

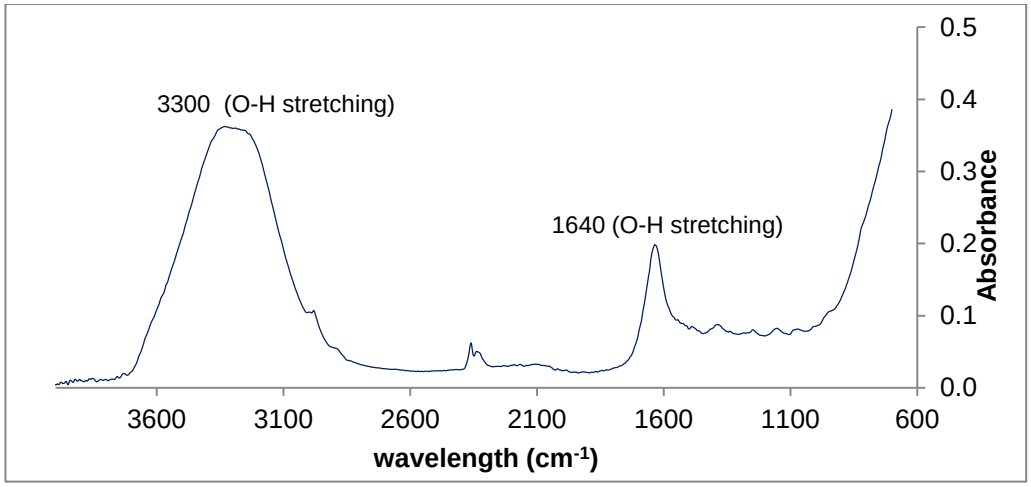


Figure 4.4: Reference spectrum of water.

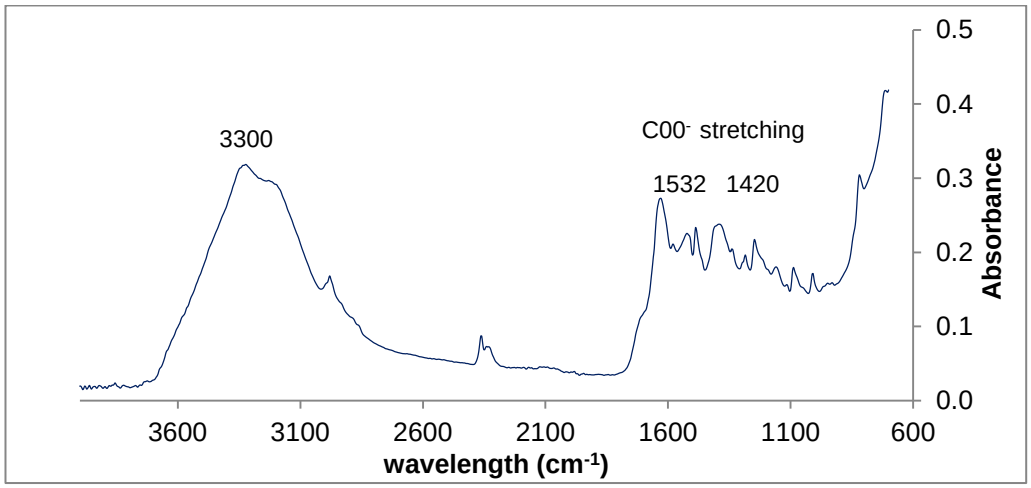


Figure 4.5: Reference spectrum of citric acid (aqueous phase).

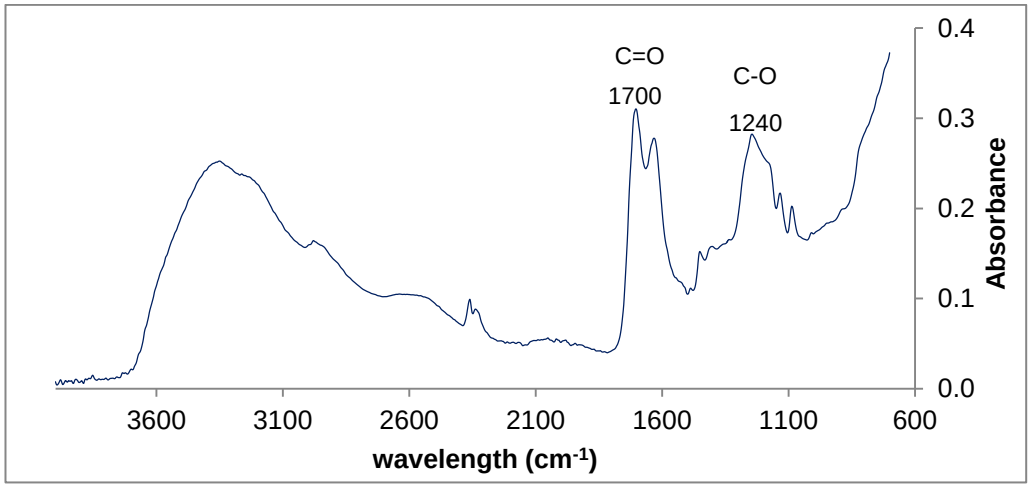


Figure 4.6: Reference spectrum for polyacrylic acid (aqueous phase).

4.1.2 Setting reaction

Figure 4.7 – Figure 4.15 illustrate the absorbance spectra of each composition at different times.

4.1.2.1 Setting reaction of brushite cement

Reference spectra for brushite cement (C0 P0) with PLR of 3 g ml^{-1} and 800 mM citric acid at 37°C is given in Figure 4.7. Peak changes due to an intermediate phase (1600, 1440 and 940 cm^{-1}) were noted initially but were then slowly replaced upon formation of the final product. The final product had sharp P - O peaks at 1116, 1050, 980, and 868 cm^{-1} in addition to 3 sharp (1652, 3540 and 3480 cm^{-1}) and 2 broad (3280 and 3172 cm^{-1}) peaks due to O - H stretching. These O - H and P - O peaks were consistent with dicalcium phosphate dihydrate (DCPD, brushite) formation (Hofmann et al., 2006; Xu et al., 1999).

4.1.2.2 Polyacrylic acid effects on setting reaction

Polyacrylic acid addition caused significant changes in the brushite cement setting kinetics. The sharpened brushite O - H peaks (e.g. at 1644 cm^{-1}) were not observed at any time. Additionally, only two of the sharp, strong P - O peaks (1120 and 1052 cm^{-1}) developed (see 4.8 and Figure 4.9). Addition of polyacrylic acid substantially delayed the setting rates. Both compositions with low (C0 P6) and high (C0 P12) polyacrylic acid were still showing absorbance changes at 1200 seconds. Indeed the P - O peaks did not reach their maximum values until 12 hours. As can be seen, after the initial period, the broad peaks at 1640 and 920 cm^{-1} reached maximum values, but were then slowly replaced by 1052 and 1120 cm^{-1} peaks.

4.1.2.3 Chlorhexidine effects on setting reaction

The absorbance spectra for the cement with low (C6 P0) chlorhexidine are given in Figure 4.10. All peaks consistent with brushite formation were observed. There were relatively no changes in absorbance from 1200 to 7200 seconds which suggested the cement had fully set and the chemical reaction was complete. Time – dependent spectra suggest that low chlorhexidine has a

limited effect on the setting reaction rate. When compared to reference spectra, additional peaks around 1550 cm^{-1} were present. These peaks correspond to chlorhexidine peaks.

Cement with high chlorhexidine (C11 P0) exhibited accelerated setting reaction. The strong P - O peaks and O - H peaks could be observed as early as 600s (see Figure 4.13) compared to C6 P0 and C0 P0 at 1200s. All corresponding peaks to brushite cement were formed with chlorhexidine 11% (w/w).

4.1.2.4 Combination effects of chlorhexidine and polyacrylic acid on setting reaction

The time - dependent absorbance spectra for cements with low chlorhexidine and varied polyacrylic acid concentration; 6% (w/w) and 12% (w/w) are illustrated in Figure 4.11 and Figure 4.12. The peaks around 1500 cm^{-1} were due to benzene rings C = C (aromatic hydrocarbon), and C = N and N - H stretching in chlorhexidine. Changes with time were similar to those observed in Figures 4.8 and 4.9 with polyacrylic acid i.e. P - O peaks at 1052 and 1120 cm^{-1} only and gradual change in absorbance at ~1350 cm^{-1}. They may indicate stable formation of calcium - polyacrylate complex. These complexes were present in all compositions with polyacrylic acid. The calcium - polyacrylate complex peaks gradually reached a maximum value at 43200 seconds.

The absorbance spectra for compositions with high chlorhexidine and varied polyacrylic acid are given in Figure 4.14 and Figure 4.15. For C11 P6, all of the P - O (1126, 1054, 984 and 868 cm^{-1}) and O - H (3482, 3536, 3280, 3160 and 1648 cm^{-1}) peaks which correspond to brushite could be observed. However, when the concentration of polyacrylic acid increased to 12% (w/w) 2 sharp P - O peaks (1052 and 1124 cm^{-1}) with additional weak peaks of 984 and 820 cm^{-1} were noted. There was a strong peak at 1520 cm^{-1} associated with chlorhexidine spectrum. By increasing the chlorhexidine concentration from 6% (w/w) to 11% (w/w), the formation of the calcium – polyacrylate complex was not as observable as to compositions with low chlorhexidine concentration.

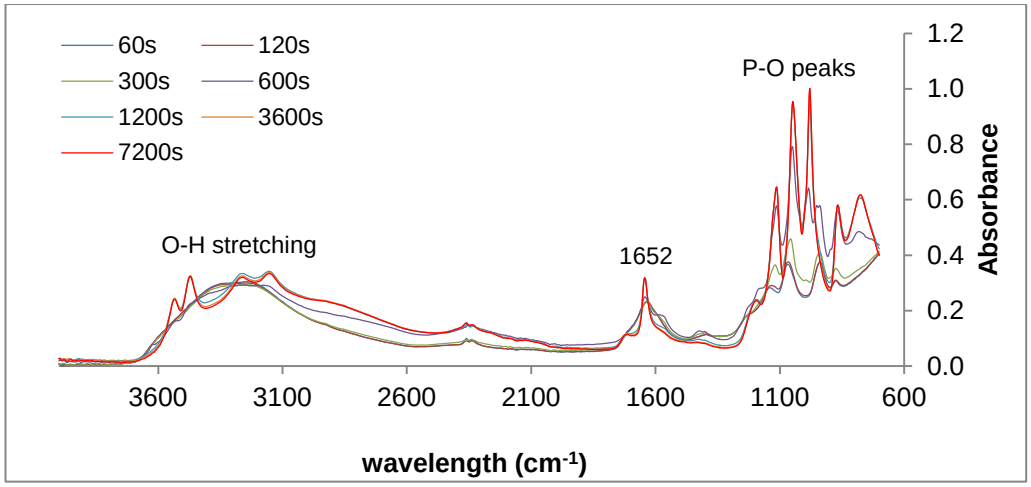


Figure 4.7: Reference spectra of brushite cement (C0 P0) at PLR of 3 g ml^{-1} at 37ºC, prepared with citric acid concentration of 800 mM.

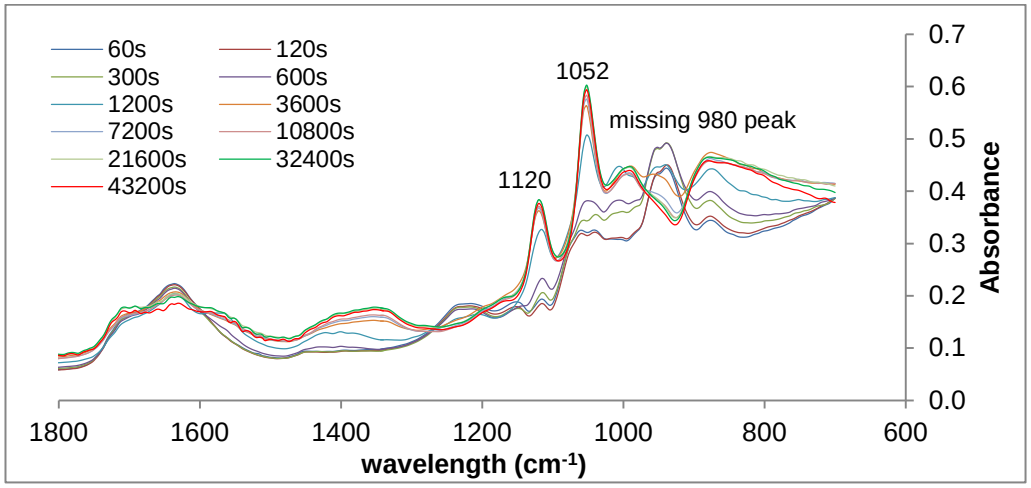


Figure 4.8: Time – dependent FTIR spectra of setting cement with low PAA (C0 P6) with time at 37ºC.

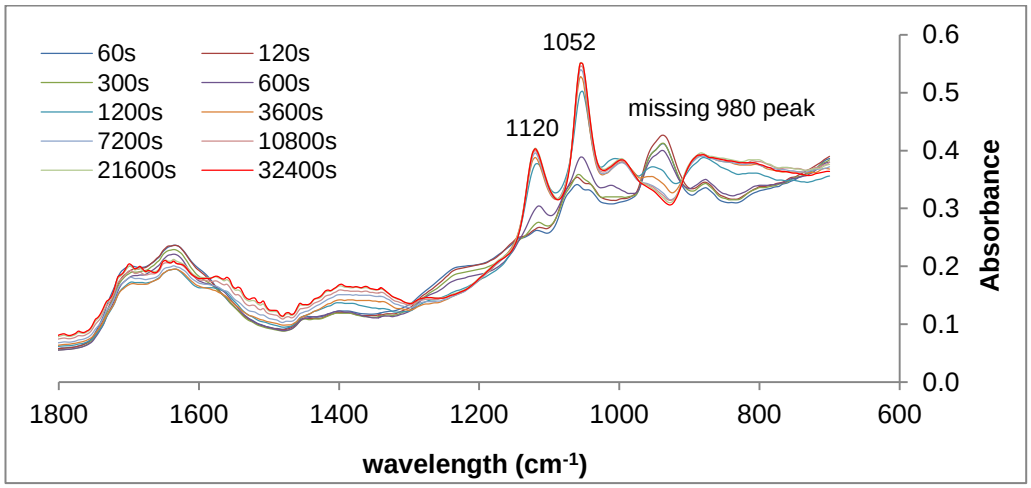


Figure 4.9: Time – dependent FTIR spectra of setting cement with high PAA (C0 P12) with time at 37ºC.

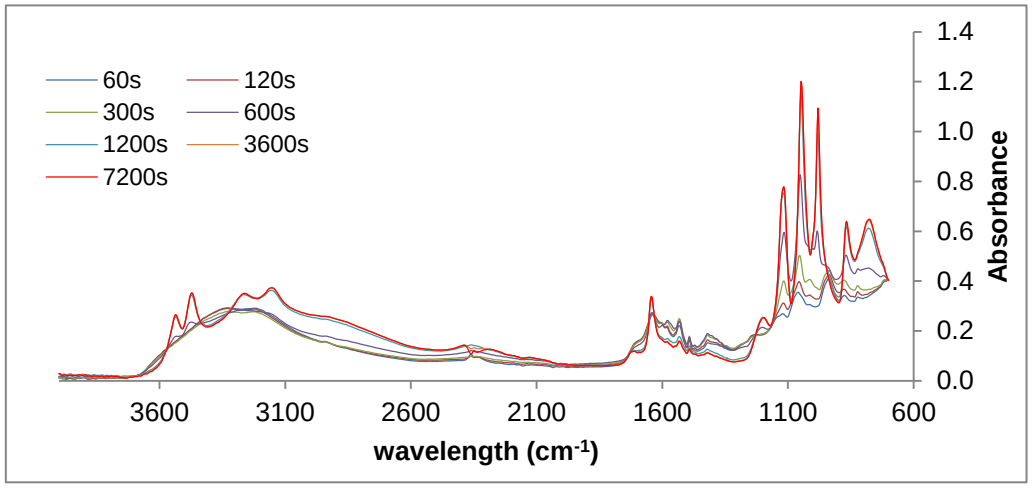


Figure 4.10: Time – dependent FTIR spectra of setting cement with low CHX (C6 P0) with time at 37ºC.

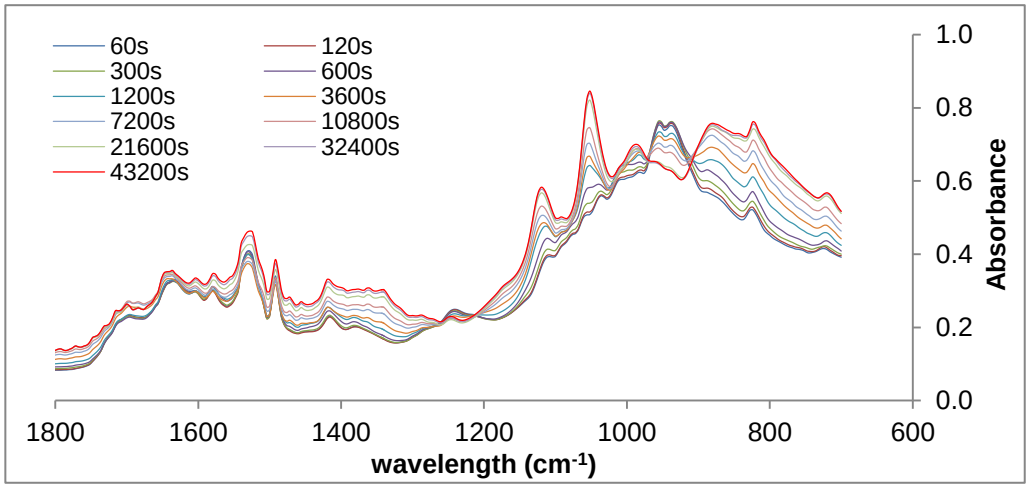


Figure 4.11: Time – dependent FTIR spectra of setting cement with low CHX and low PAA (C6 P6) with time at 37ºC.

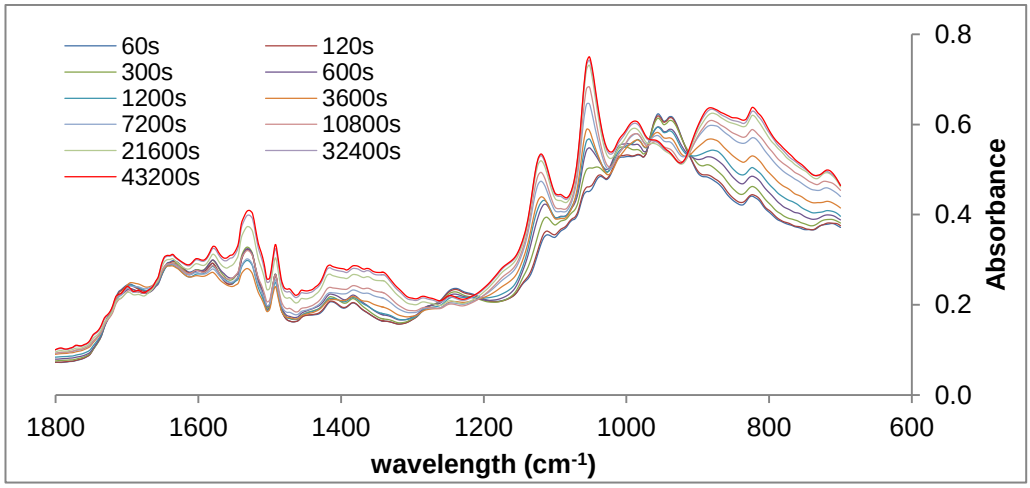


Figure 4.12: Time – dependent FTIR spectra of setting cement with low CHX and high PAA (C6 P12) with time at 37ºC.

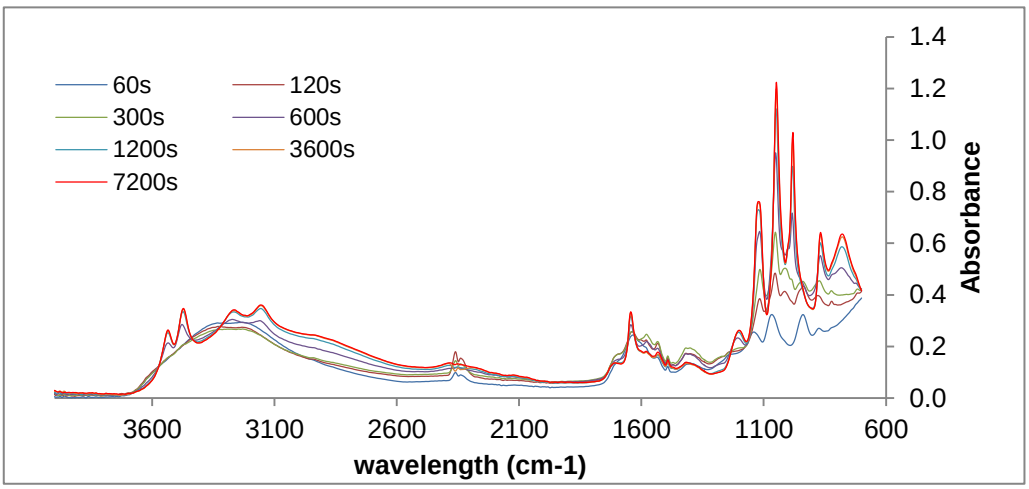


Figure 4.13: Time – dependent FTIR spectra of setting cement with high CHX (11 P0) with time at 37ºC.

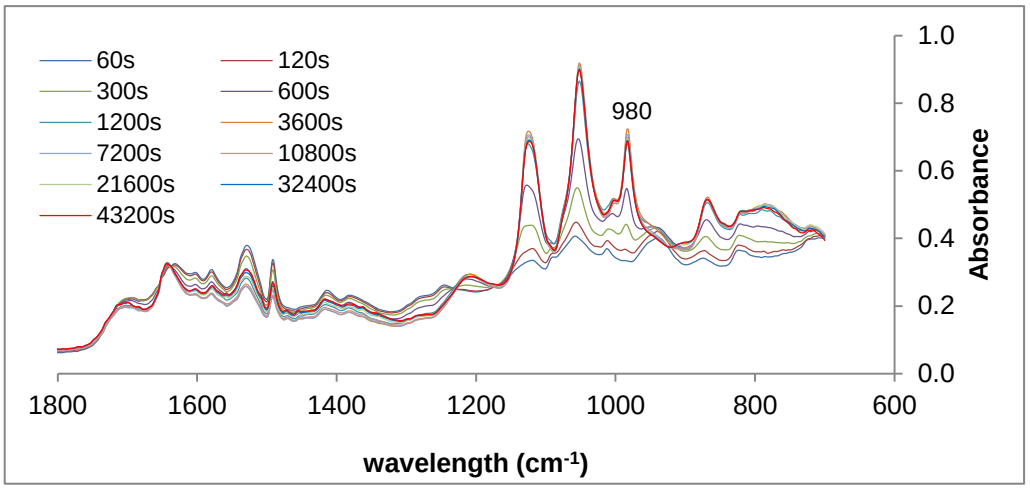


Figure 4.14: Time – dependent FTIR spectra of setting cement with high CHX and low PAA (C11 P6) with time at 37ºC

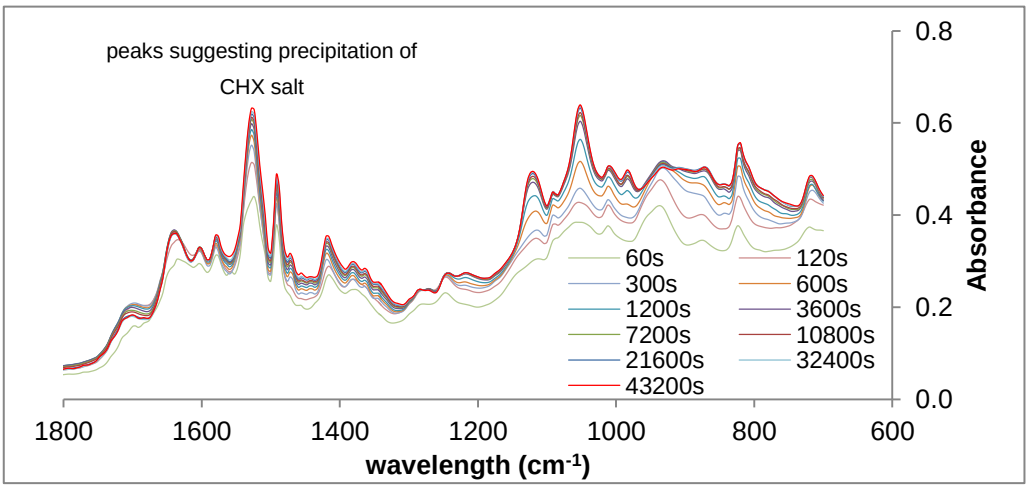


Figure 4.15: Time – dependent FTIR spectra of setting cement with high CHX and high PAA (C11 P12) with time at 37ºC

4.1.3 Difference spectra

The following figures (Figure 4.16 – Figure 4.24) represent difference spectra for each composition. Difference spectra, obtained by subtracting one spectrum from another at a different time i.e. absorbance changes, are characteristic of the reaction taking place.

4.1.3.1 Absorbance changes of brushite cement

Figure 4.16 shows the difference spectra of compositions with no chlorhexidine or polyacrylic acid (C0 P0). The difference spectra suggested that there were two processes occurring before final product formation. The first process occurred up to 600s. This process involves the formation of dicalcium peaks (1110 and 1052 cm^{-1}) and concomitant intermediate complex (dicalcium citrate). The intermediate complex peaks could be observed at 1440, 1576 and 960 cm^{-1}; comparable in shape and position to the peaks in the calcium citrate spectra (Hofmann et al., 2006). During this period, water was yet to be bound into the final product.

After the initial period, at 1200s, the dicalcium phosphate peaks reached their maximum values. This was followed by the second process which indicates dissolution of the intermediate complex. This process could be observed with development of troughs in the region of 1440, 1576 and 880 cm^{-1}. The dissolution was accompanied by rapid formation of strong peak at 980 cm^{-1} to form the final product which is brushite. This has several sharpened O – H peaks indicative of water complexed with brushite.

4.1.3.2 Polyacrylic acid effects on absorbance changes

Figure 4.17 shows difference spectra of cement with low polyacrylic acid (C0 P6). The setting reaction also involves two processes. The same first process as in brushite could be observed at initial period. However, in contrast to brushite, at 600s, only weak peaks of dicalcium phosphate and dicalcium citrate complex could be noted (see Figure 4.17) which suggests a delay in the setting reaction. At 1200s, rapid formation of both compounds could be observed.

Instead of dissolution of dicalcium citrate, the second process involved formation of stable dicalcium polyacrylate. This is represented by formation of a broad shoulder at the region of 1340 and 1520 cm^{-1} at 3600s. In addition, there were also two broad troughs centred around 1240 and 1700 cm^{-1}. The broad shoulder can be assigned to symmetric and asymmetric stretching vibrations of the acrylate COO^-. This chemical group would be formed upon neutralisation of a polyacrylic acid, COOH group (Young et al., 2004). This process would result in loss of C = O and C - O stretch peaks around 1700 and 1240 cm^{-1} respectively and account for the troughs in the difference spectra.

Compared to brushite cement formation, this stable complex did not dissolve upon formation of final product. Furthermore, 980 cm^{-1} and O - H peaks could not be observed during the setting reaction.

Figure 4.18 shows the difference spectra of compositions with high polyacrylic acid (C0 P12). The same processes as in C0 P6 could be observed. However dicalcium citrate complex could not be observed. This results from a higher concentration of polyacrylic acid in addition to lower citric acid levels.

4.1.3.3 Chlorhexidine effects on absorbance changes

The difference spectra of low and high chlorhexidine are demonstrated in Figure 4.19 and 4.22. Both compositions exhibit the same difference spectra profiles as brushite cement. The dicalcium phosphate peaks, 980 cm^{-1} and O-H peaks also could be observed in the end-product of these compositions. Therefore, incorporation of chlorhexidine does not interfere with formation of brushite cement.

The setting time however, was accelerated by the presence of chlorhexidine in the composition. This was more pronounced in compositions with high chlorhexidine concentration (see Figure 4.22). With C11 P0, O - H peaks could be detected as early as 600s compared to C0 P0 and C6 P0. The rapid setting process could be explained by interactions between chlorhexidine and citric ions inhibiting the calcium chelating effect of citric acid.

4.1.3.4 Combination effect of chlorhexidine and polyacrylic acid on absorbance changes

The difference spectra of compositions with low chlorhexidine; C6 P6 (see Figure 4.20) and C6 P12 (see Figure 4.21) were similar to C0 P6 and C0 P12. Both dicalcium phosphate and dicalcium polyacrylate characteristic spectra could be observed. In addition to this, at early times there were troughs noted in the 1500 cm^{-1} region which suggests dissolution of chlorhexidine. These troughs however, were replaced by weak peaks which suggest formation of dicalcium polyacrylate complex at later time. O - H peaks and 980 cm^{-1} peak were missing from the final product spectra.

Different absorbance changes were exhibited in the compositions with high chlorhexidine; C11 P6 and C11 P12. With C11 P6 (see Figure 4.23), at 600s, there were moderate peaks of dicalcium phosphate and 980 cm^{-1}. In addition, broad troughs developed at regions between 1240 and 1700 cm^{-1}. This would suggest dissolution of chlorhexidine and polyacrylic acid during the setting reaction, which allows the formation of the 980 cm^{-1} peak. The high availability of chlorhexidine in the composition resulted in interaction of chlorhexidine with both polyacrylic acid and citric acid thus preventing the formation of dicalcium citrate and dicalcium polyacrylate complexes.

Between 600s and 1200s there was rapid formation of dicalcium phosphate and 980 cm^{-1} peaks. Furthermore, O - H peaks could be observed as early as 300s. These features suggest the end product was brushite cement.

Figure 4.24 shows difference spectra of composition with high chlorhexidine and high polyacrylic acid (C11 P12). In addition to the dicalcium peaks, the final spectra consist of weak peak of 980 cm^{-1} and an extra peak at 1010 cm^{-1}. The 1010 cm^{-1} is most likely to correspond to P - O stretch of dicalcium citrate complex (Hofmann et al., 2006).

The strong peaks at region of 1500 cm^{-1} correspond to chlorhexidine spectra. The same strong peaks could not be observed in other compositions. This suggests precipitation of chlorhexidine salt on the cement surface. In contrast to C11 P6, water was not bound into the cement during its precipitation.

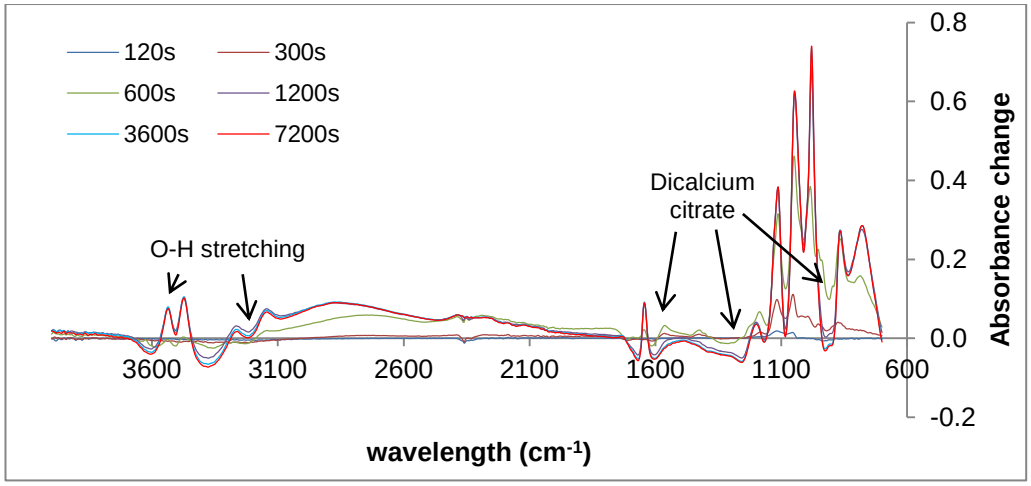


Figure 4.16: Effect of time on difference spectra of brushite cement – C0 P0 (calculated by subtraction of spectra at initial) for cements at 37 °C.

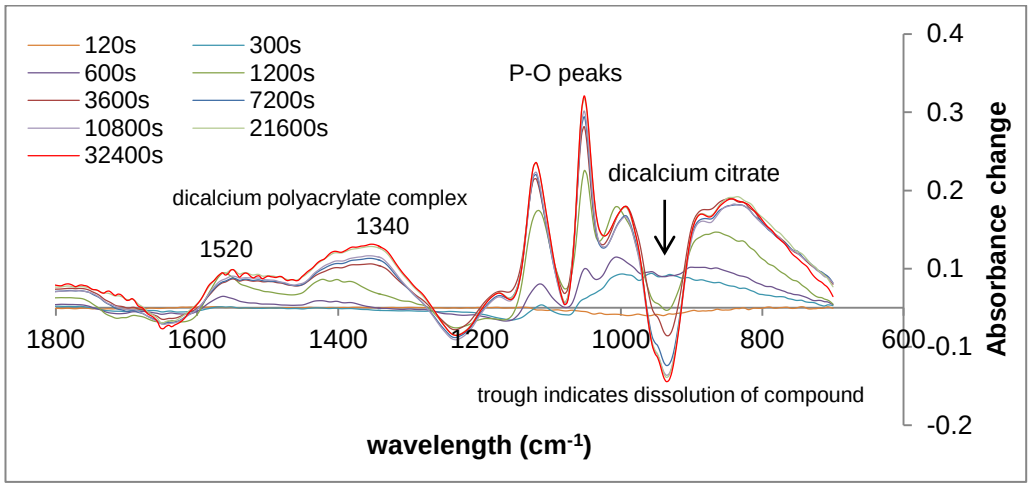


Figure 4.17: Effect of time on difference spectra of cement with low PAA – C0 P6 (calculated by subtraction of spectra at initial) for cements at 37 °C.

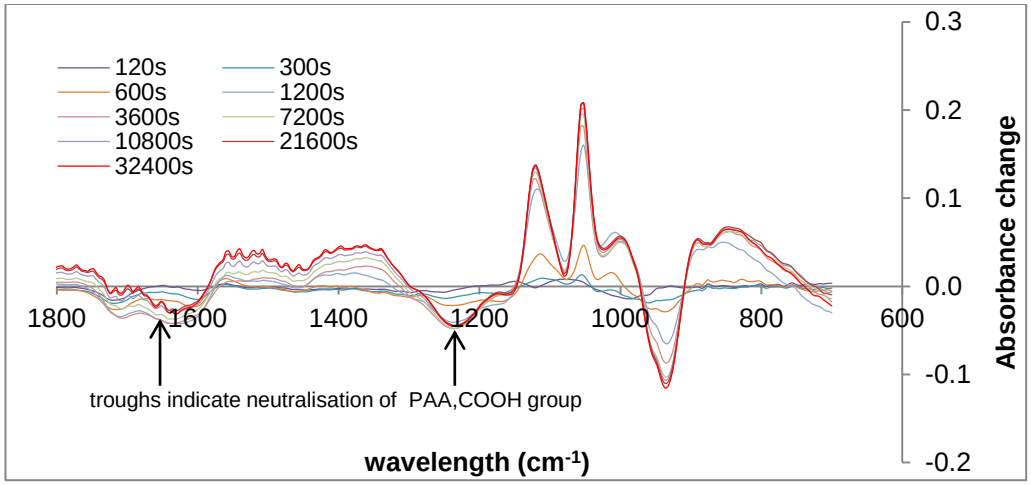


Figure 4.18: Effect of time on difference spectra of cement with high PAA – C0 P12 (calculated by subtraction of spectra at initial) for cements at 37 °C.

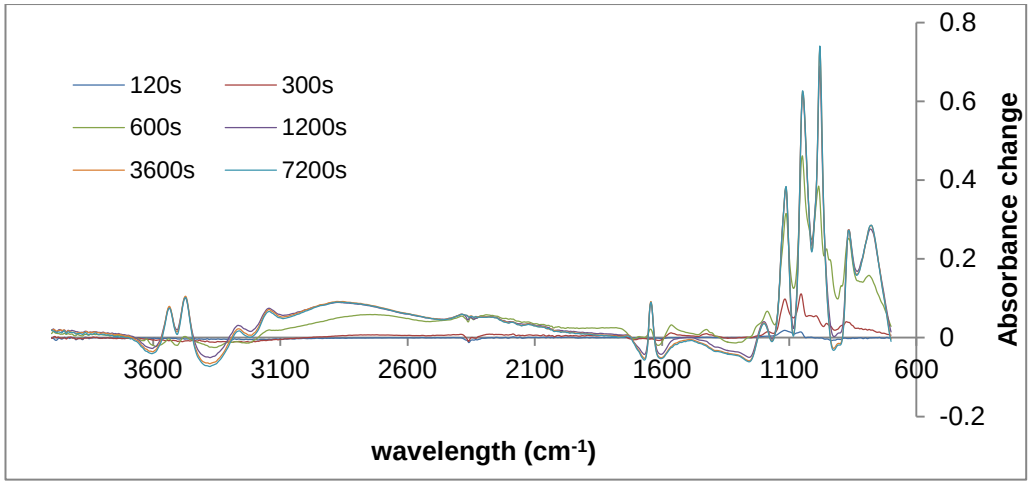


Figure 4.19: Effect of time on difference spectra of cement with low CHX – C6 P0 (calculated by subtraction of spectra at initial) for cements at 37 °C.

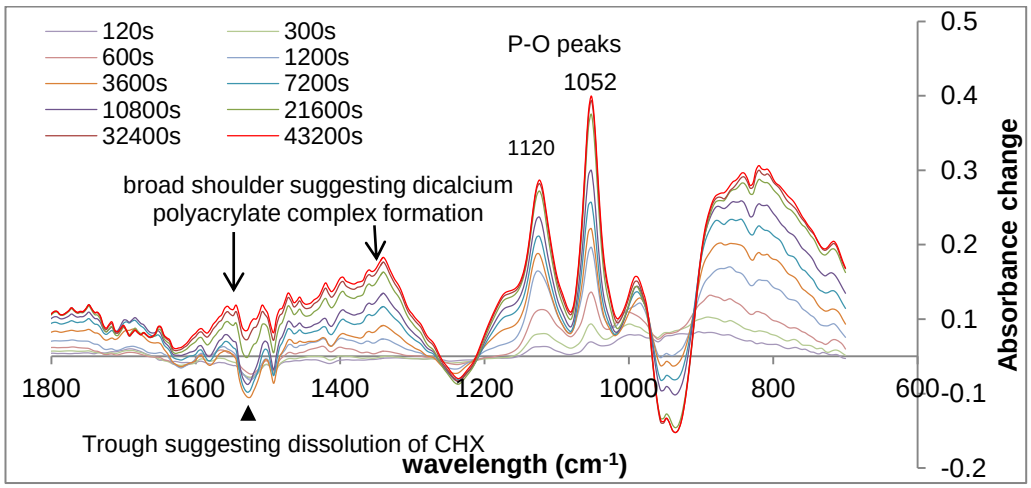


Figure 4.20: Effect of time on difference spectra of cement with low CHX and low PAA – C6 P6 (calculated by subtraction of spectra at initial) for cements at 37 °C.

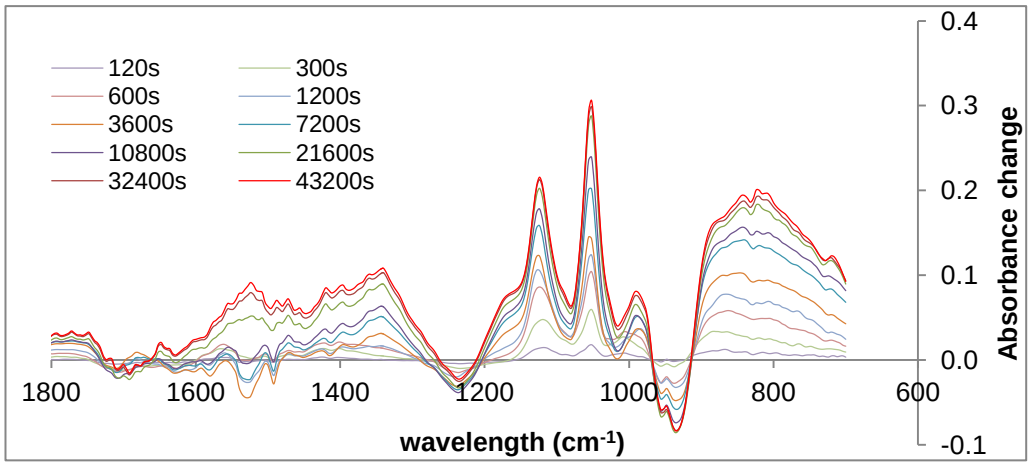


Figure 4.21: Effect of time on difference spectra of cement with low CHX and high PAA – C6 P12 (calculated by subtraction of spectra at initial) for cements at 37 °C.

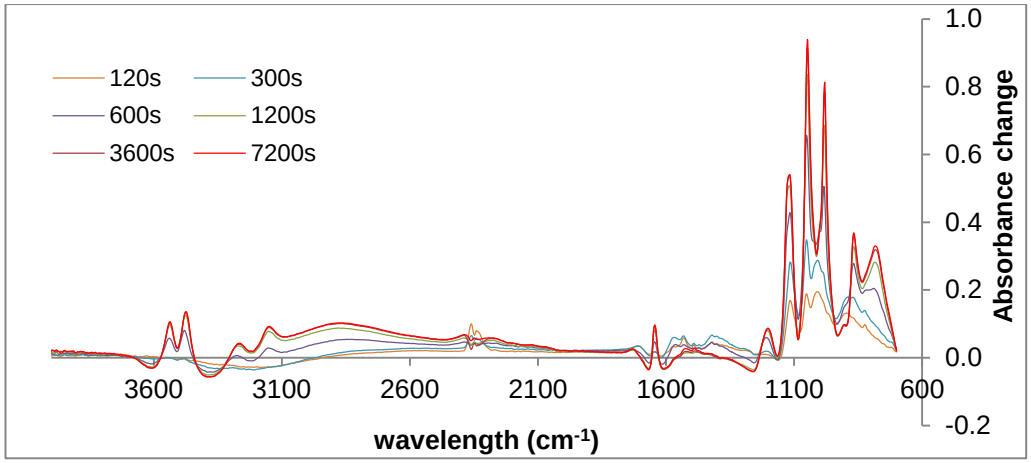


Figure 4.22: Effect of time on difference spectra of cement with high CHX – C11 P0 (calculated by subtraction of spectra at initial) for cements at 37 °C.

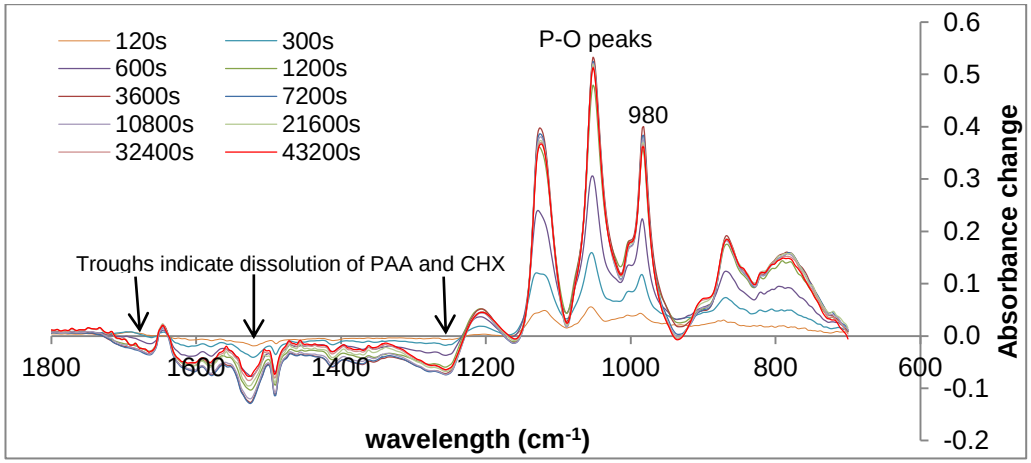


Figure 4.23: Effect of time on difference spectra of cement with high CHX and low PAA – C11 P6 (calculated by subtraction of spectra at initial) for cements at 37 °C.

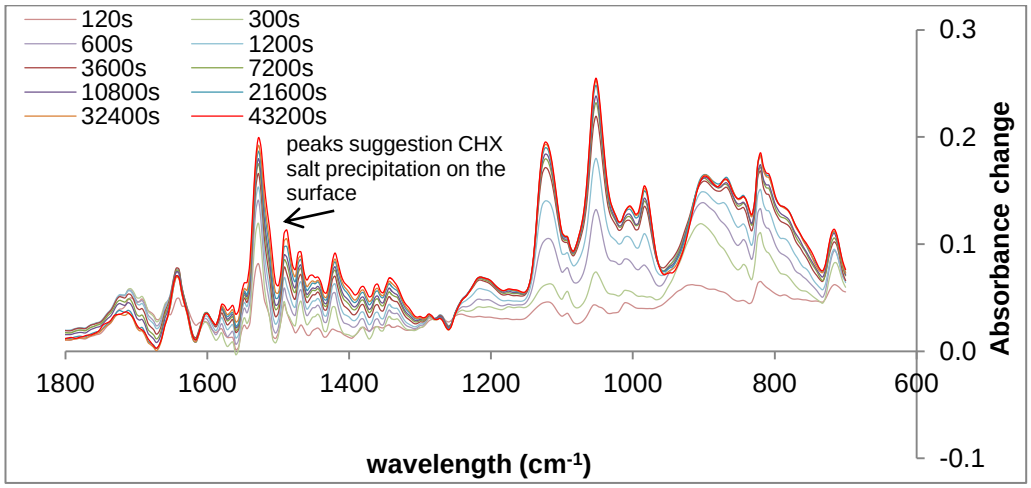


Figure 4.24: Effect of time on difference spectra of cement with high CHX and high PAA – C11 P12 (calculated by subtraction of spectra at initial) for cements at 37°C.

4.1.4 Reaction extent

Reaction extent refers to how far the setting reaction has gone towards completion, with a value of 1.0 indicating the setting process has completed. The following equation can be used to describe in detail how the reactions vary with time:

$$\zeta, = (A_t\text{-}A_0)/(A_f\text{-}A_0) \quad (16)$$

Where, A is the FTIR absorbance at a wavenumber where significant change with time is observed. The subscripts, t, 0 and f indicate values at any time t, initially and finally respectively.

Based on absorbance spectra and absorbance changes from all compositions, it was noted that the absorbance at 1052 cm^{-1} (corresponding to the P – O stretch peak) shows the largest increase with formation of both brushite and the dicalcium polyacrylate complex. Therefore the reaction extent from this specific wavelength is calculated using the above mentioned equation.

For a simple reaction i.e. brushite formation in composition without polyacrylic acid, reaction extent will be independent of the wave number chosen to examine the reaction. However, the presence of polyacrylic acid results in more complex setting processes, therefore reaction extent versus time at several wave numbers needs to be examined i.e. 1520 and 1340 cm^{-1}. The 1340 cm^{-1} peak corresponds to formation of dicalcium polyacrylate complex.

4.1.4.1 Chlorhexidine effects on the reaction extent

Figures 4.25 – 4.27 provide the reaction extent from the 1052 cm^{-1} peak for all samples. With zero (Figure 4.25) and low chlorhexidine (6% w/w) (Figure 4.26) there was a delay in the setting reaction noted initially. With 11% (w/w) chlorhexidine (Figure 4.27) however, no such delay was detected. The high concentration of chlorhexidine prevented the delay in setting that provides the working time of the cement. It suggests interaction between this drug and the citric acid, thus the brushite crystal precipitates without the inhibitory effect of the citrate ion.

4.1.4.2 Polyacrylic acid effects on reaction extent

Generally, increasing polyacrylic acid concentration results in declined ζ_{1052} and a subsequently prolonged setting reaction. With no polyacrylic acid, ζ_{1052} was within 90% of its maximum value of 1.0 within 1000s irrespective of chlorhexidine content. The same however could not be observed in C0 P6 and C0 P12 where both compositions reached a reaction extent of 1.0 (approximately) at 15000s (4.2h). The plotted reaction extents for both compositions overlapped at a later time (1500s and onwards) which suggests that differences in polyacrylic acid concentrations only affected the earlier reaction kinetics.

4.1.4.3 Combination effect of both CHX and PAA on reaction extent

The combined effects of chlorhexidine and polyacrylic acid on brushite cements setting kinetic are complicated. When compared to controls, at initial time points (1000s), all samples with both reactants had values of ζ_{1052} far less than 1. The reaction extent demonstrated that the formation of dicalcium phosphate was substantially slowed.

For all formulations with low chlorhexidine concentrations, 6% (w/w) (see Figure 4.26), the setting reaction was complicated by a second slow complexation reaction between polyacrylic acid and dicalcium phosphates to form a stable dicalcium polyacrylate complex, which takes 12 hours to complete. This substance is more dominant in C6 P12 as there are more polyacidic groups available to bind with calcium phosphate. The formation of such a complex could be observed from reaction extent of 1340 cm^{-1}.

For a given chlorhexidine concentration, the plotted reaction extent versus time overlaps at short time. Chlorhexidine, being basic, may complex with both polyacrylic and citric acid. This can reduce the ability of these acids (negatively charged) to chelate with calcium ions. With higher concentrations of chlorhexidine, more of the acid will be bound. This will reduce the ability of the acid to slow the setting process. When there is a higher concentration of polyacrylic acid and lower level of chlorhexidine the second setting process

becomes more dominant as there are more polyacidic groups available to bind with the calcium phosphates (see Figure 4.27).

The reaction extent after 10000s calculated using absorbance at 1340 cm^{-1} could be made to overlap for all compositions containing both chlorhexidine and polyacrylic acid by varying the value used for A_0 (see Figure 4.28). This suggests that varying neither polyacrylic acid nor chlorhexidine can speed up the rate at which the second process reaches equilibrium. For all samples this second process is 90 % complete at 30000 s (8.3 hours).

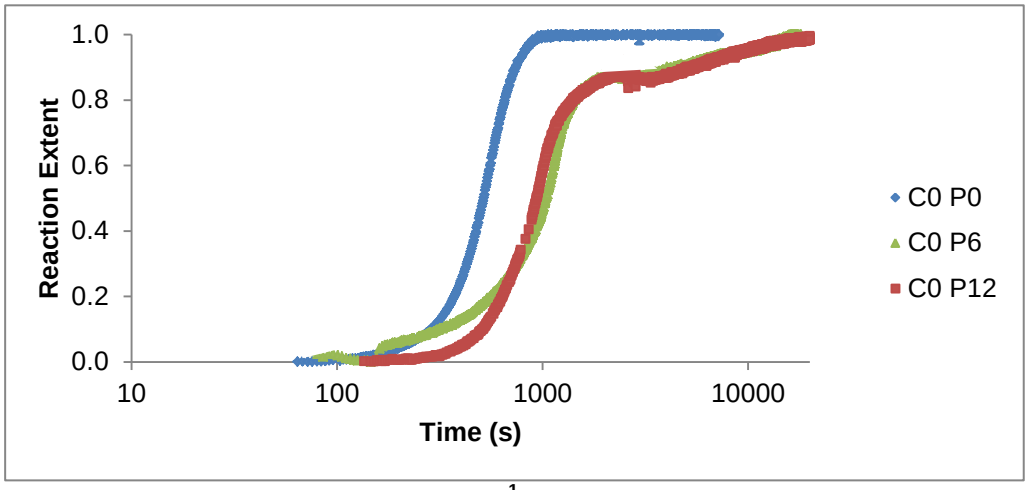


Figure 4.25: Reaction extent (1052 cm^{-1}) as a function time for samples without CHX. Time is is log_{10} scale

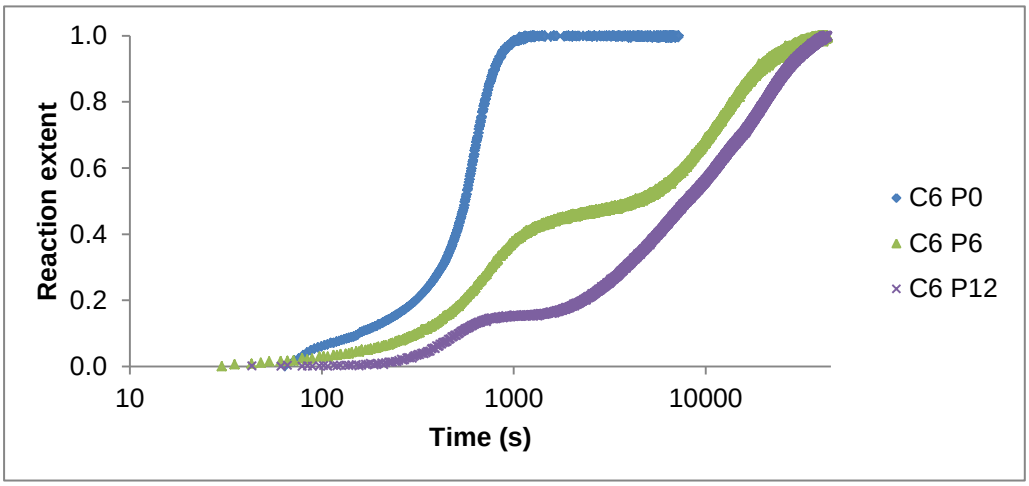


Figure 4.26: Reaction extent (1052 cm^{-1}) as a function time for samples with low CHX. Time is in log_{10} scale.

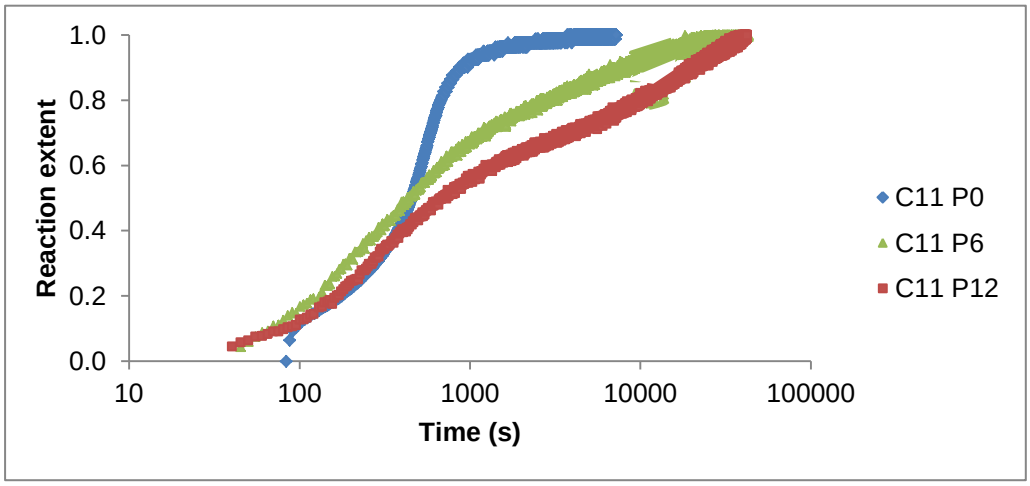


Figure 4.27: Reaction extent (1052 cm^{-1}) as a function time for samples with high CHX. Time is in log_{10} scale.

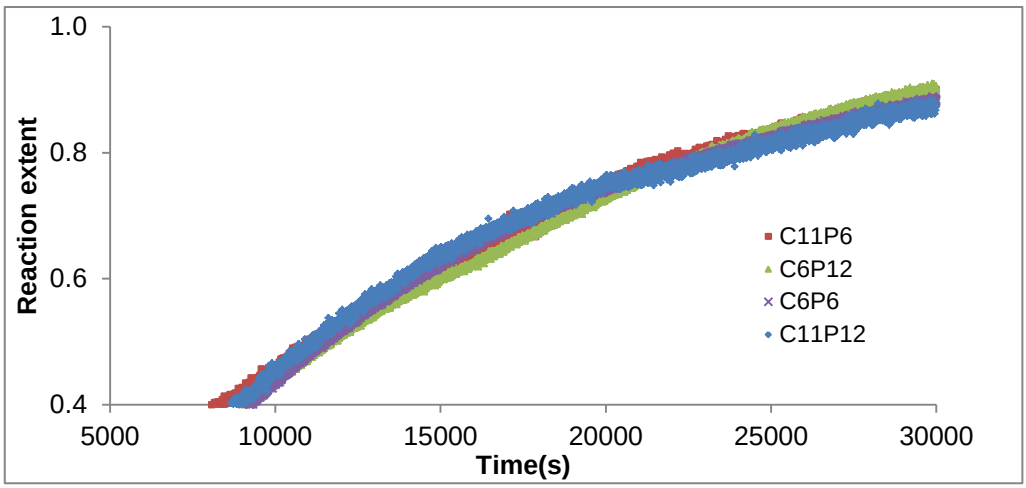


Figure 4.28: Reaction extent (1340 cm-1) as a function time for samples with both CHX and PAA.

4.2 Final Composition

4.2.1 Raman reference spectra

Figure 4.29 shows the Raman reference spectra and peak assignments for the reactants used in the experiment. The spectrum of polyacrylic acid in the aqueous phase was associated with noticeable noise. This spectrum had broad peaks centred in the region of 1200 and 1400 cm^{-1}.

In addition, a reference spectrum for brushite was also obtained to allow comparison between the samples and the control. As can be seen in Figure 4.29, set brushite cement exhibited strong and moderate peaks at 980 and 872 cm^{-1} respectively. The sharp peak at 980 cm^{-1} could be allocated to the P – O stretching and moderate peak at 872 cm^{-1} is in concur to P – O(H) stretching (Xu et al., 1999).

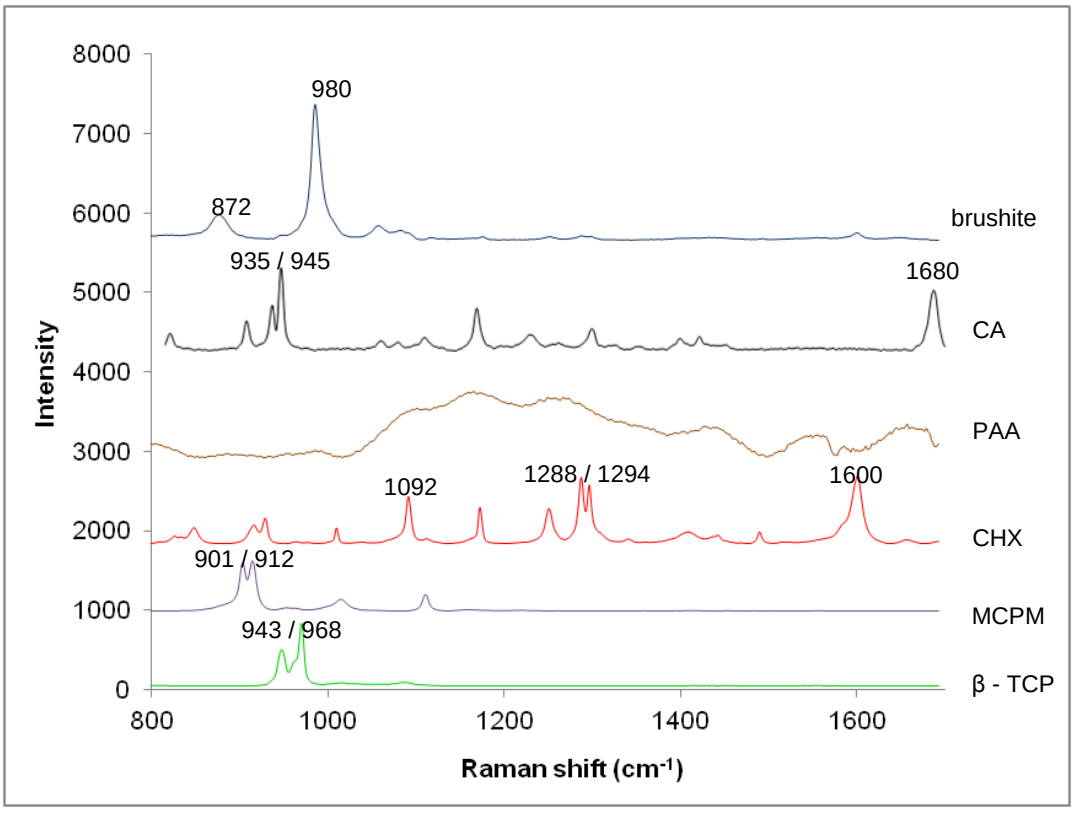


Figure 4.29: Raman reference spectra of brushite, CA, PAA, CHX, MCPM and β – TCP.

4.2.2 Final composition mapping and spectra

The composition mappings and average Raman spectra where obtained from the bulk of fractured dry samples which were left to set for 24 hours at room temperature.

4.2.2.1 Polyacrylic acid effect on cement composition

Figure 4.30a and Figure 4.31a demonstrate the compositional mapping of the set cements with 6% (w/w) (C0 P6) and 12% (w/w) (C0 P12) polyacrylic acid respectively. The fracture surfaces of both set cements could be assigned to pure brushite (blue) and non – reacted β - TCP particles (green).

The average spectra in Figure 4.30b and 4.31b had strong peaks which are consistent with brushite formation. Weak peaks due to non – reacted β – TCP could also be observed. The broad peak around 890 cm^{-1} region in both spectra could be assigned to P – O(H) stretching. The polymer spectrum however, was too weak for detection.

4.2.2.2 Chlorhexidine effect on cement composition

Figure 4.32a and Figure4.33a show the fracture surface mapping of C6 P0 and C11 P0. In both compositional mappings obtained, there were regions that could be assigned to brushite (blue) or pure chlorhexidine (red) and mixtures of the two components (mauve). Chlorhexidine distribution was evenly dispersed throughout both compositions. The composition with higher concentrations of chlorhexidine exhibited more pronounced and intense red areas throughout the cement fracture surface compared to C6 P0.

The average spectra C6 P0 and C11 P0 are shown in Figure 4.32b and Figure 4.33b respectively. The average spectra were consistent to reference spectra of chlorhexidine and brushite cement.

4.2.2.3 Combination effect of polyacrylic acid and chlorhexidine on cement composition

The mapping of the fracture surface of cements containing both polyacrylic acid and chlorhexidine are exhibited in Figure 4.34a, 4.35a, 4.36a and 4.37a. These mappings show discrete regions of chlorhexidine separate from dicalcium phosphate brushite and chlorhexidine regions. In addition, there was a low level of non - reacted β - TCP particles detected within the samples.

The average spectra for all compositions (Figure 4.34b, 4.35b, 4.36b and 4.37b) were dominated by a strong peak around 980 cm^{-1} regions indicative of brushite formation. Peaks due to non – reacted β - TCP could also be observed in all set cements except in C11 P12. Moreover, strong chlorhexidine peaks could be detected in all compositions.

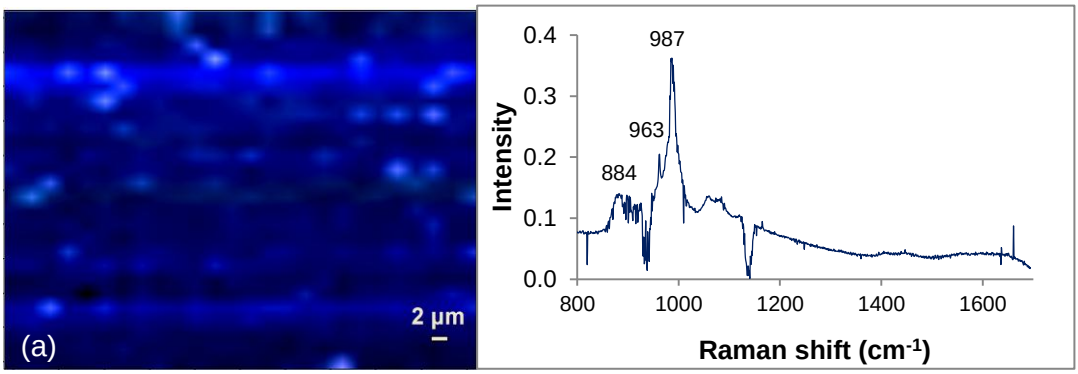


Figure 4.30: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with low PAA (C0 P6) determined via Raman. Blue area represent brushite, green area refers to non – reacted β - TCP (b) average spectra of the corresponding area.

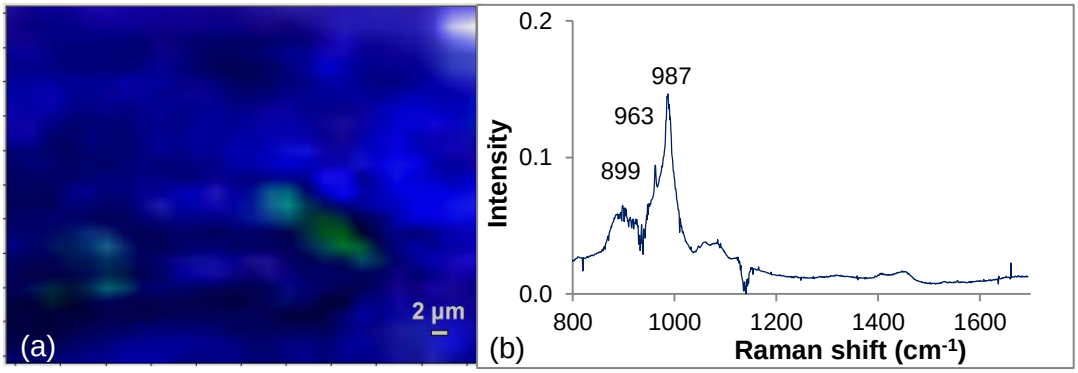


Figure 4.31: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with high PAA (C0 P12) determined via Raman. Blue area represent brushite, green area refers to non – reacted β - TCP (b) average spectra of the corresponding area

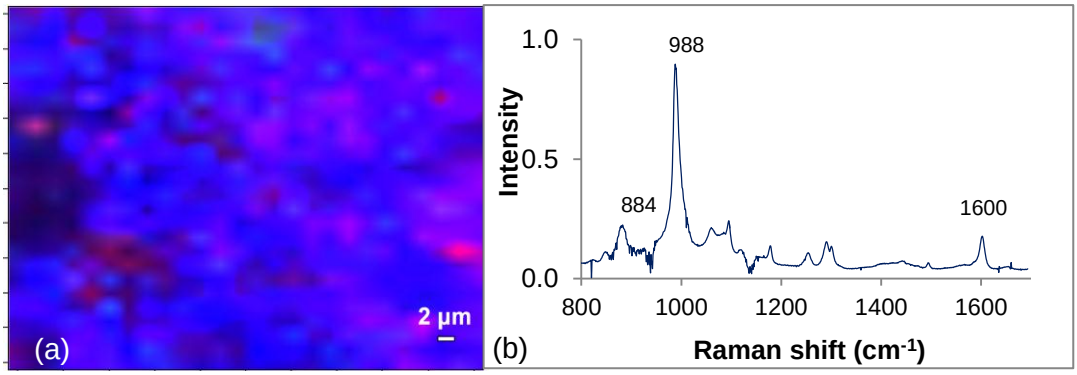


Figure 4.32: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with low CHX (C6 P0) determined via Raman. Blue areas represent brushite, red regions refer to CHX and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area.

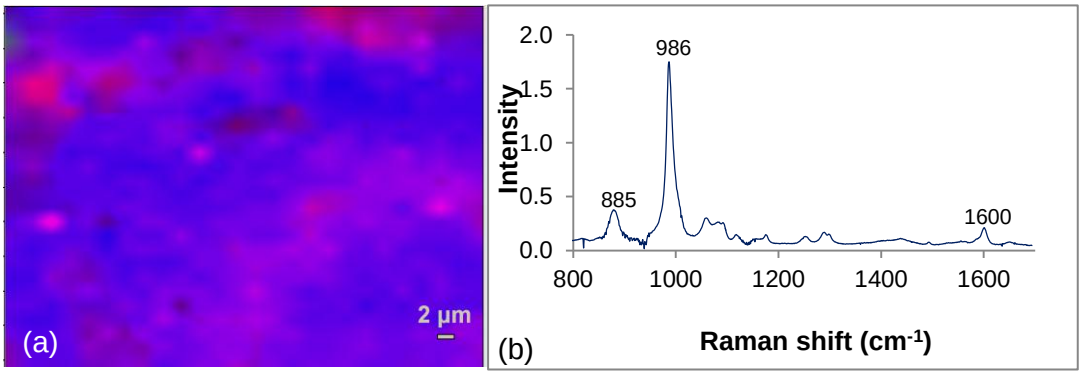


Figure 4.33: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with high CHX (C11 P0) determined via Raman. Blue areas represent brushite, red regions refer to CHX and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area.

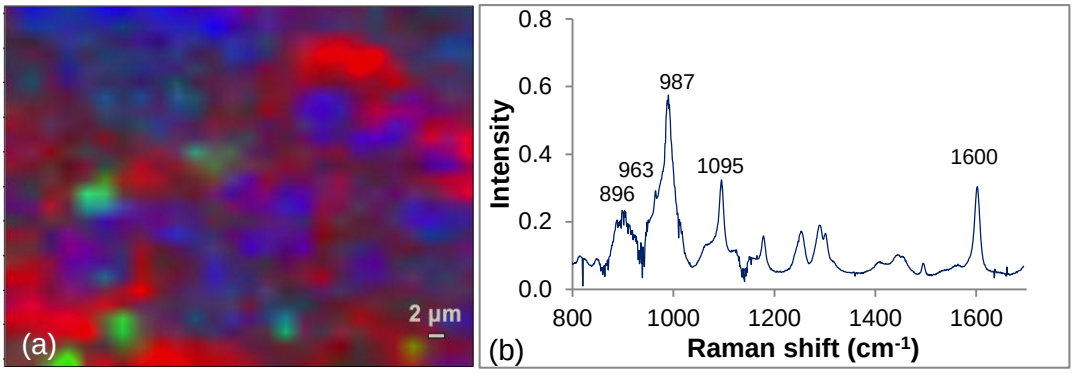


Figure 4.34: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with low CHX and low PAA (C6 P6) determined via Raman. Blue areas represent brushite, red regions refer to CHX, green areas demonstrated non – reacted β – TCP particles and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area.

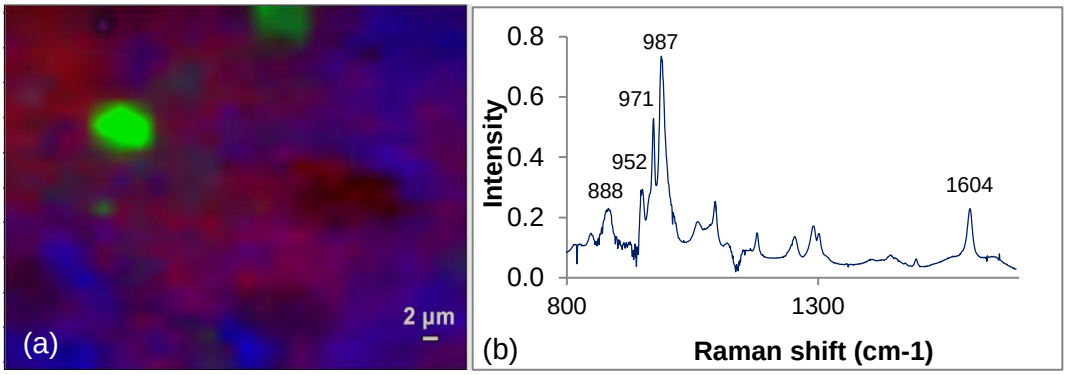


Figure 4.35: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with low CHX and high PAA (C6 P12) determined via Raman. Blue areas represent brushite, red regions refer to CHX, green areas demonstrated non – reacted β – TCP particles and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area.

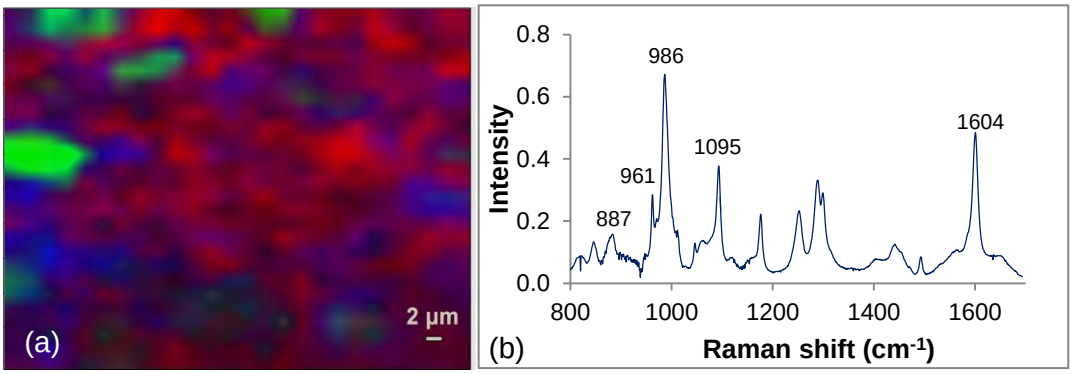


Figure 4.36: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with high CHX and low PAA (C11 P6) determined via Raman. Blue areas represent brushite, red regions refer to CHX, green areas demonstrated non – reacted β – TCP particles and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area.

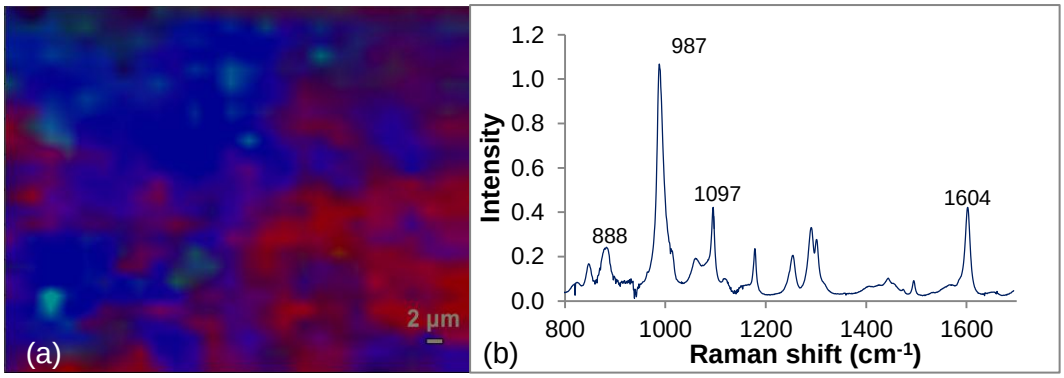


Figure 4.37: (a) Compositional mapping (50 µm x 50 µm) of fracture surface of cement with high CHX and high PAA (C11 P12) determined via Raman. Blue areas represent brushite, red regions refer to CHX, green areas demonstrated non – reacted β – TCP particles and mauve areas indicate mixture of CHX and brushite (b) average spectra of the corresponding area

4.3 Microstructure study of the set cement

The fracture surface microstructure of the cements was observed under scanning electron microscope (SEM) at different magnifications (x500, x1000, x1500, x2000, and x5000).

4.3.1 Microstructure of brushite

Figure 4.38a and 4.38b shows the morphology of the fractured surface of brushite cement (C0 P0) at x500 and x5000 magnifications respectively. At low magnifications, the surface appeared non – homogeneous with areas of porosity. At higher magnifications, brushite crystals composed of needle – like crystals and continuous thin platelets with large voids were observed.

4.3.2 Polyacrylic acid effects on cement microstructure

Generally, the crystalline structure could be consistently observed in compositions without polyacrylic acid. These crystalline structures however, became less apparent when polyacrylic acid was incorporated into the composition. In compositions with zero chlorhexidine (C0 P6 and C0 P12), the surface looked more homogenous and relatively less porous compared to brushite cement. There were also strands of polymer that could not be observed in any other compositions (see Figure 4.39 and Figure 4.40). Although at low magnification, the surfaces of these compositions did not differ, at higher magnification the crystals size, arrangement and thickness were not similar. In contrast to C0 P6, C0 P12 crystals were uniform in size, loosely arranged, thin and not inter-connected as C0 P6.

4.3.3Chlorhexidine effects on cement microstructure

At low magnifications (x500), the structure of brushite cements with low and high chlorhexidine concentrations (C6 P0 and C11 P0) were not readily distinguishable. At higher magnifications (x5000) however the images indicate different crystal configurations when chlorhexidine was added to the

compositions. C6 P0 and C11 P0 (see Figure 4.41b and Figure 4.42b) exhibited larger size crystals and less connected structures; a mixture of rod and sheath–like arrangement compared to the brushite appearance.

4.3.4 Combination effect of polyacrylic acid and chlorhexidine on cement microstructure

The fracture surfaces of cements with chlorhexidine and polyacrylic acid in all scales exhibited more uniform, homogenous and dense appearance compared to brushite cements. Furthermore, it was noted that the degrees of porosity of these cements was relatively low and porosities were small (see Figure 4.43a, Figure 4.44a, Figure 4.45a and Figure 4.46a). Distinctive morphological features could be observed when these cements were examined at a higher magnification.

At high magnifications (x5000), the fracture surface of cements with low chlorhexidine and low polyacrylic acid (C6 P6) exhibited thick and interconnected structures that varied in size (see Figure 4.43b). In comparison to this, C11 P6 had a loosely arranged architecture and not as connected as C6 P6. The crystals had flat leaf - like appearance (see Figure 4.45b). When polyacrylic acid concentration increased to 12% (w/w), the SEM images exhibited a much more uniform, layered sheath – like arrangement with a lack of crystalline structures (see Figure 4.44b and Figure 4.46b) regardless of the concentration of chlorhexidine.

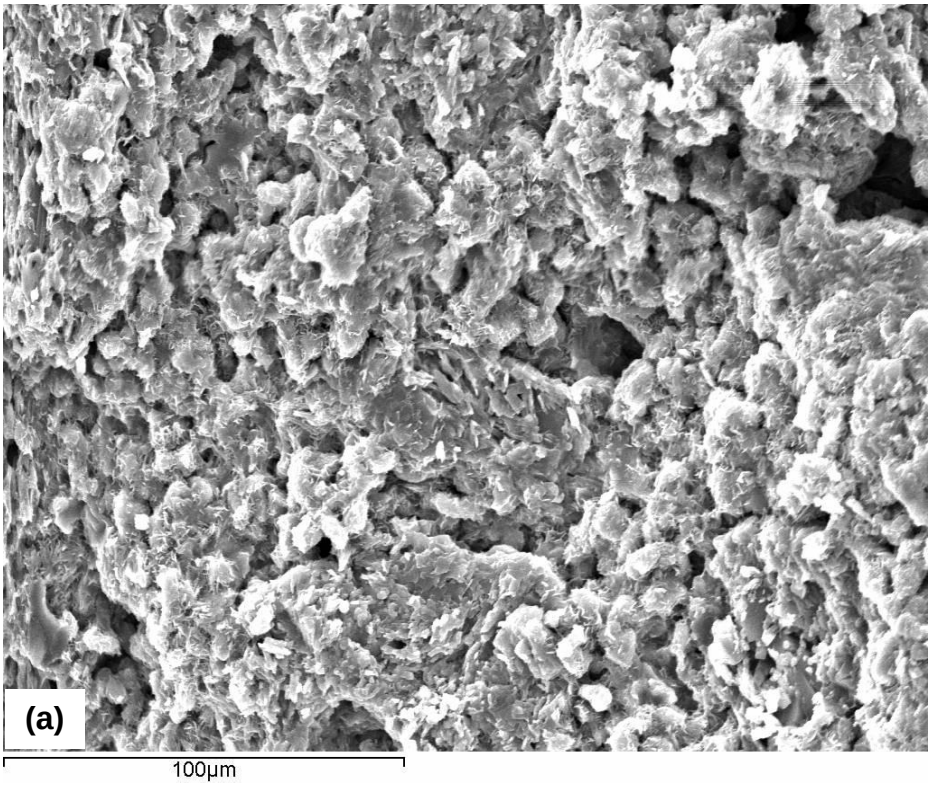


Figure 4.38: SEM images of fracture surface brushite cement (C0 P0) at a) x500 and b) x5000 magnification

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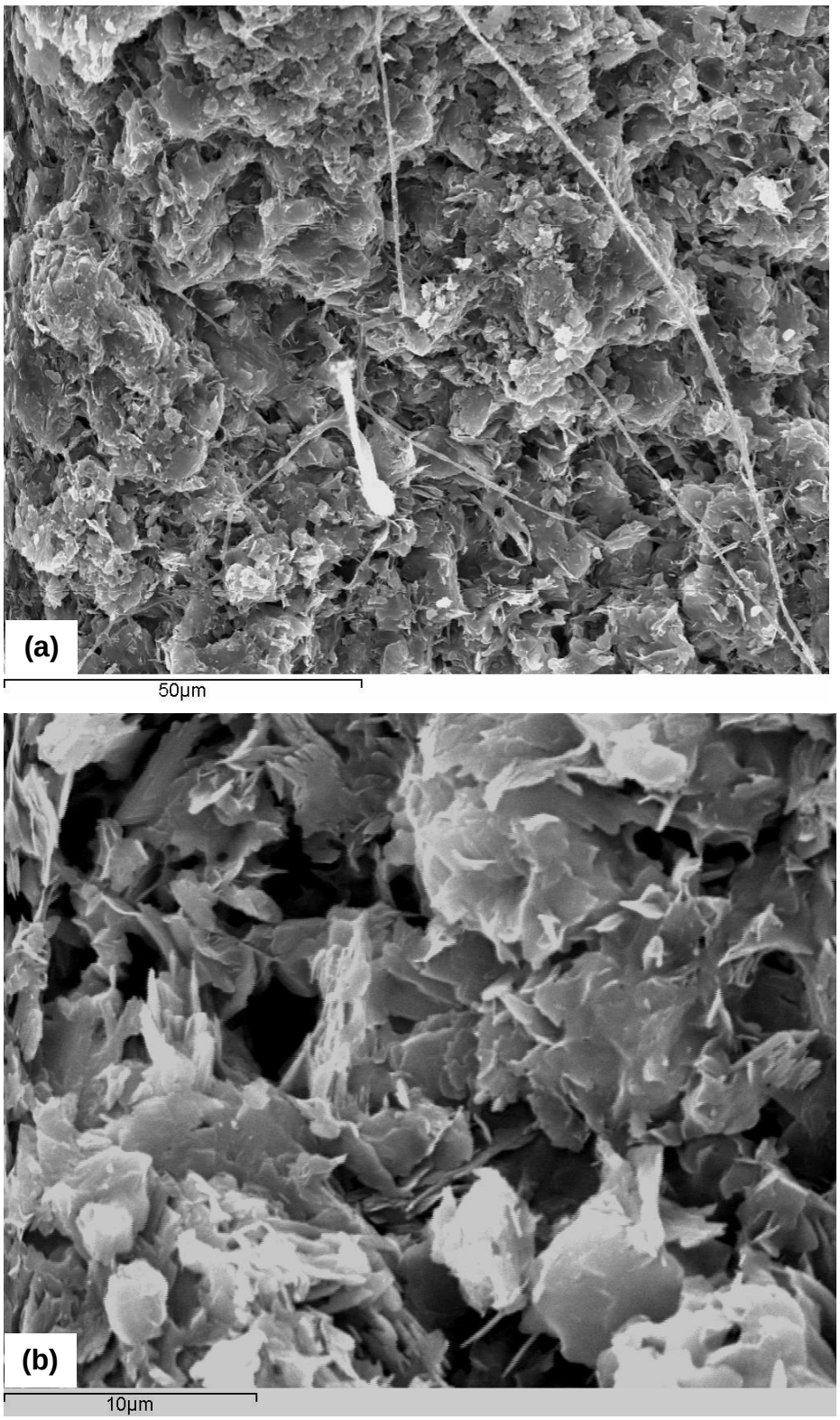
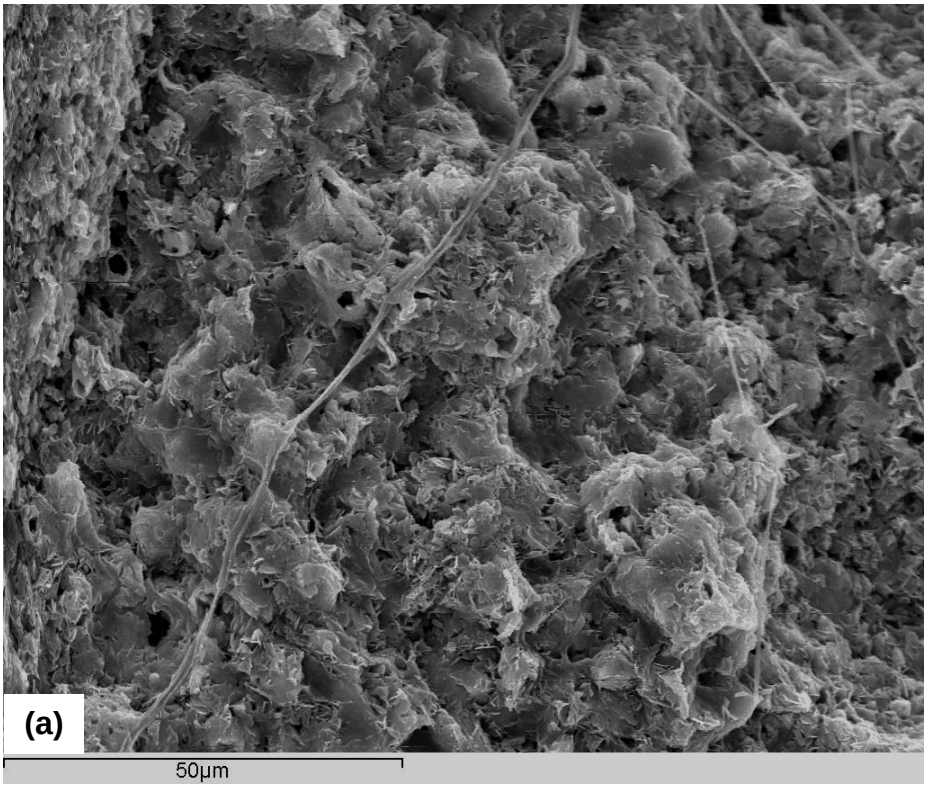


Figure 4.39: SEM images of fracture surface cement with low PAA (C0 P6) at a) x1000 and b) x5000 magnification.



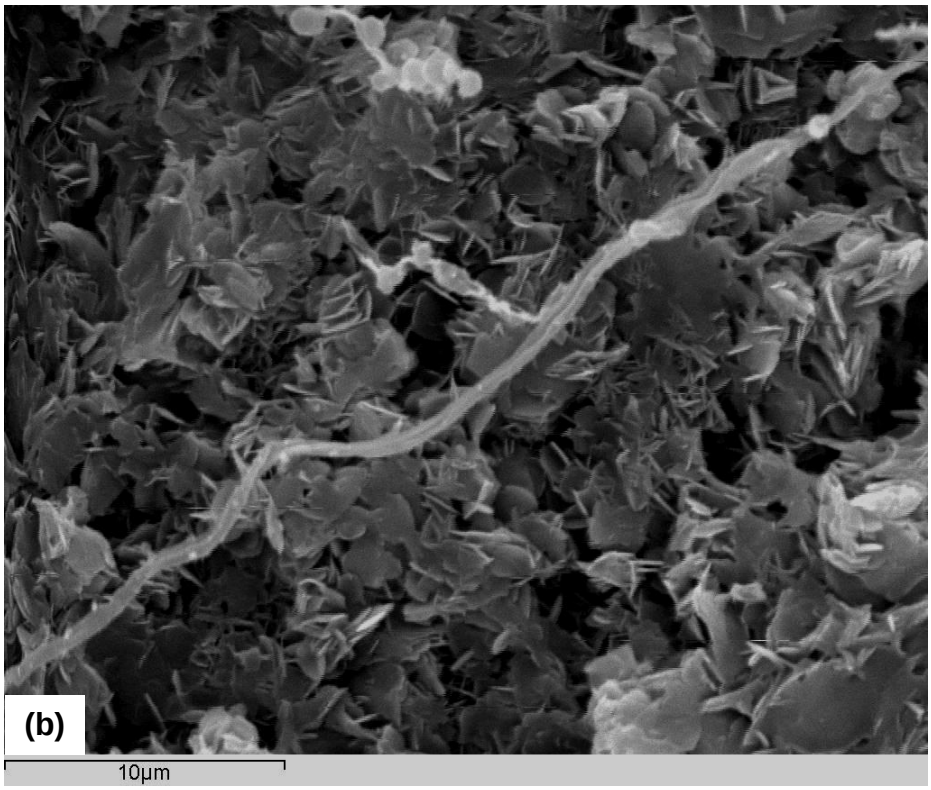


Figure 4.40: SEM images of fracture surface of cement without CHX and high PAA (C0 P12) at a) x1000 and b) x5000 magnification.

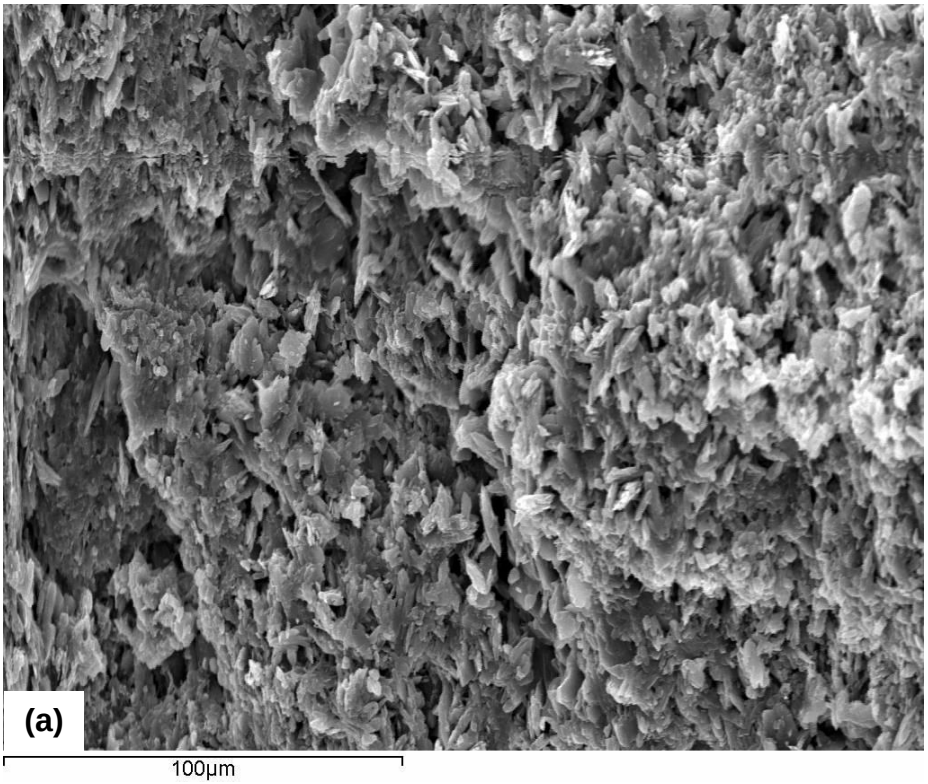


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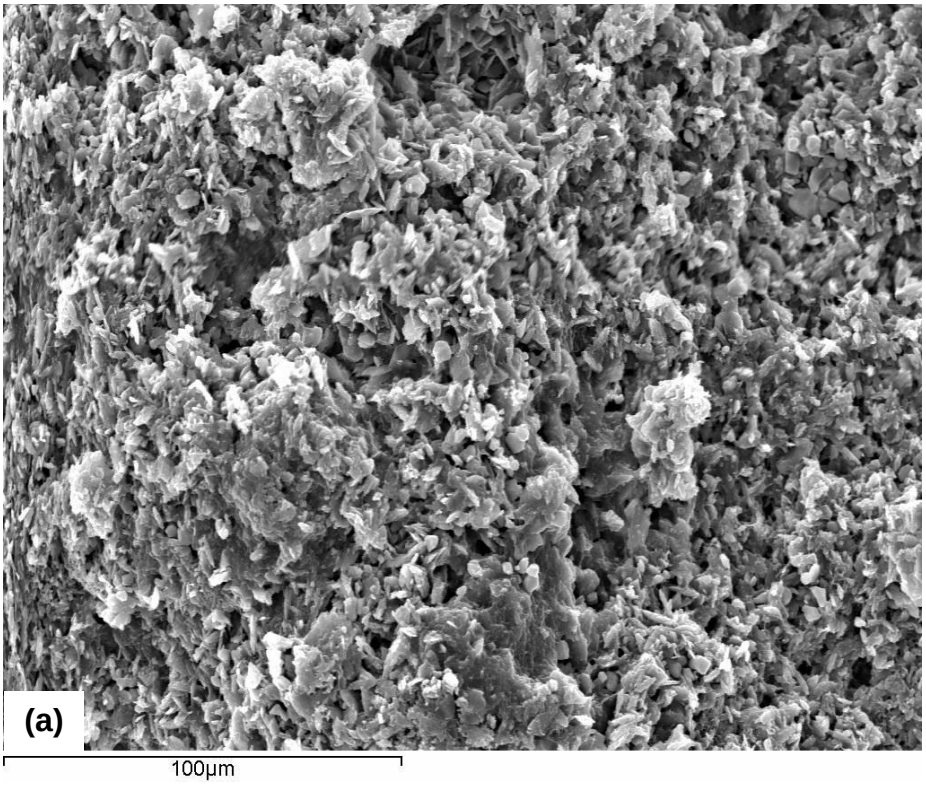
Figure 4.41: SEM images of fracture surface cement with low CHX (C6 P0) at a) x500 and b) x5000 magnification.



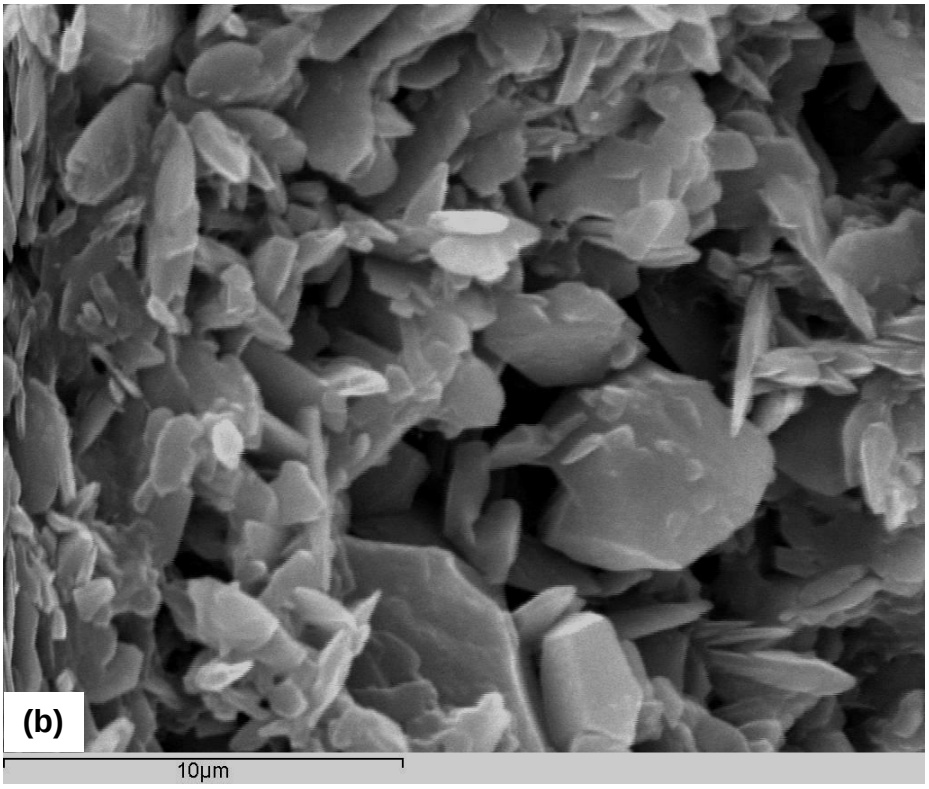


Figure 4.42: SEM images of fracture surface cement with high CHX (C11 P0) at a) x500and b) x5000 magnification.

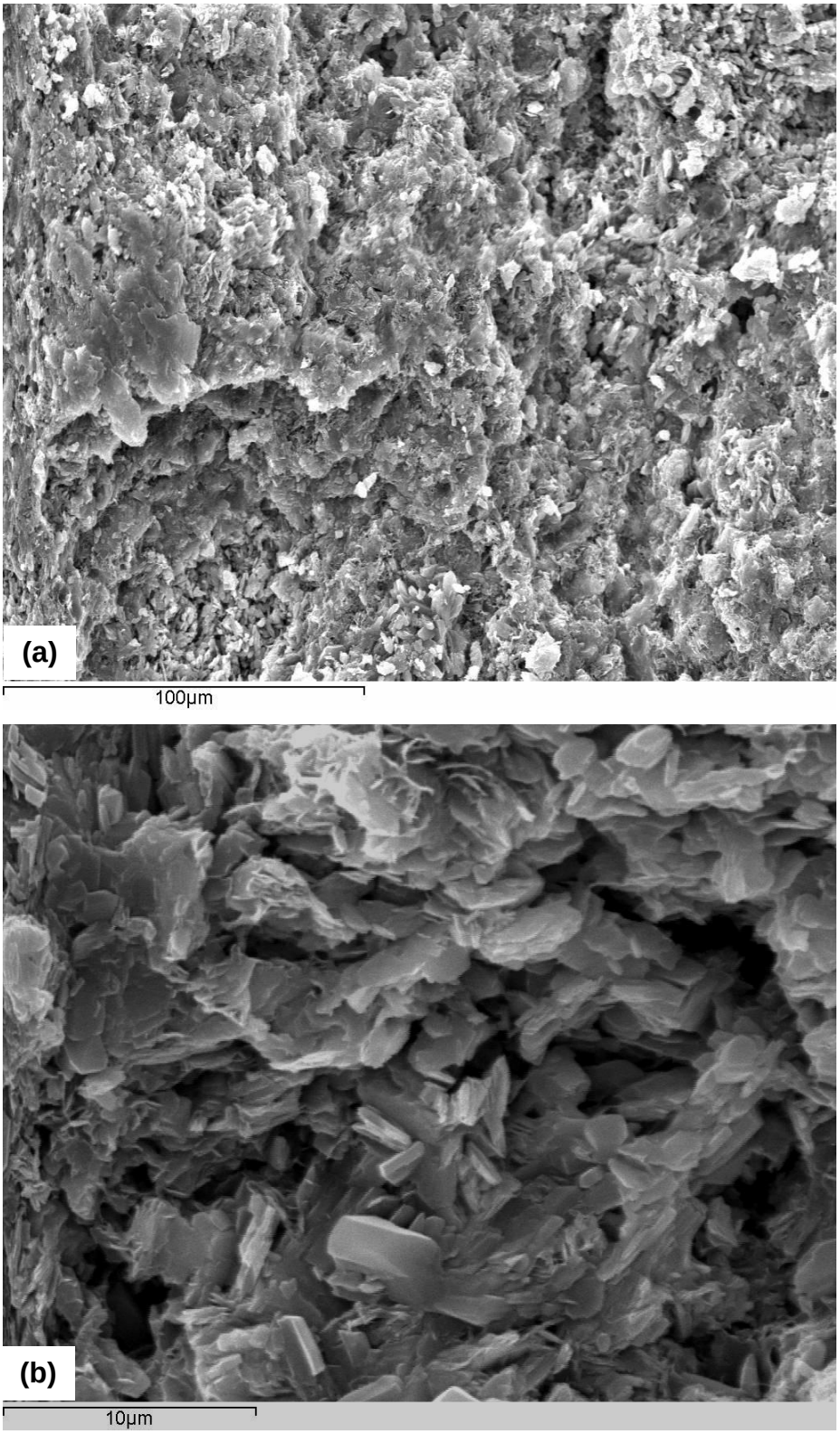


Figure 4.43: SEM images of fracture surface cement with low CHX and low PAA (C6 P6) at a) x500 and b) x5000 magnification.

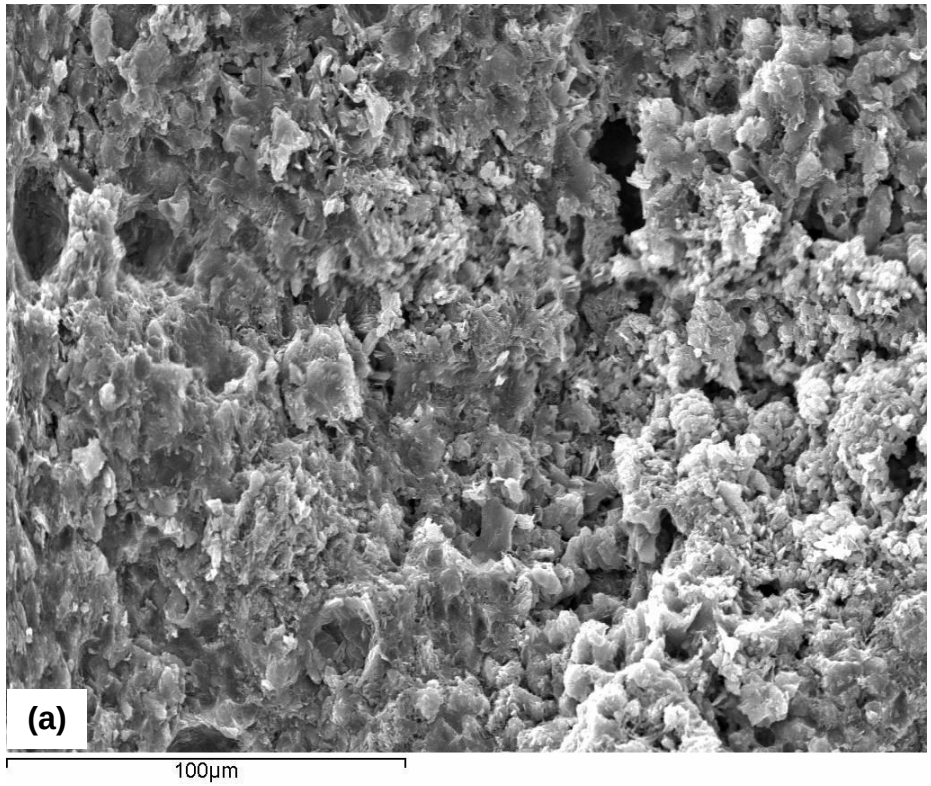


Figure 4.44: SEM images of fracture surface cement with low CHX and high PAA (C6 P12) at a) x500 b) x5000 magnification.

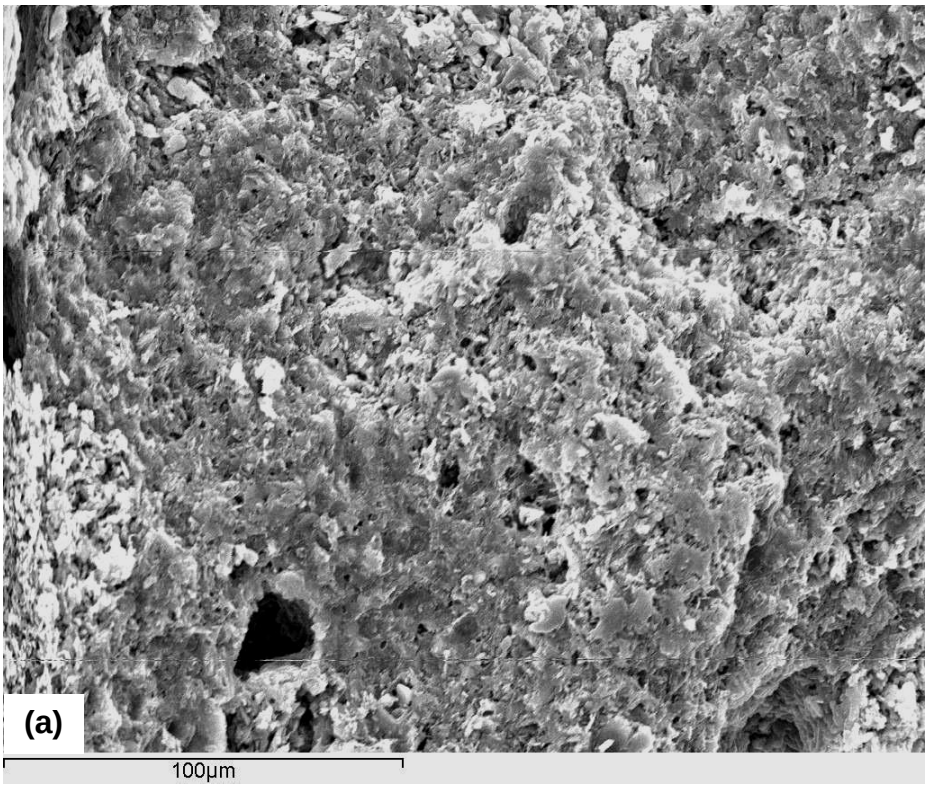
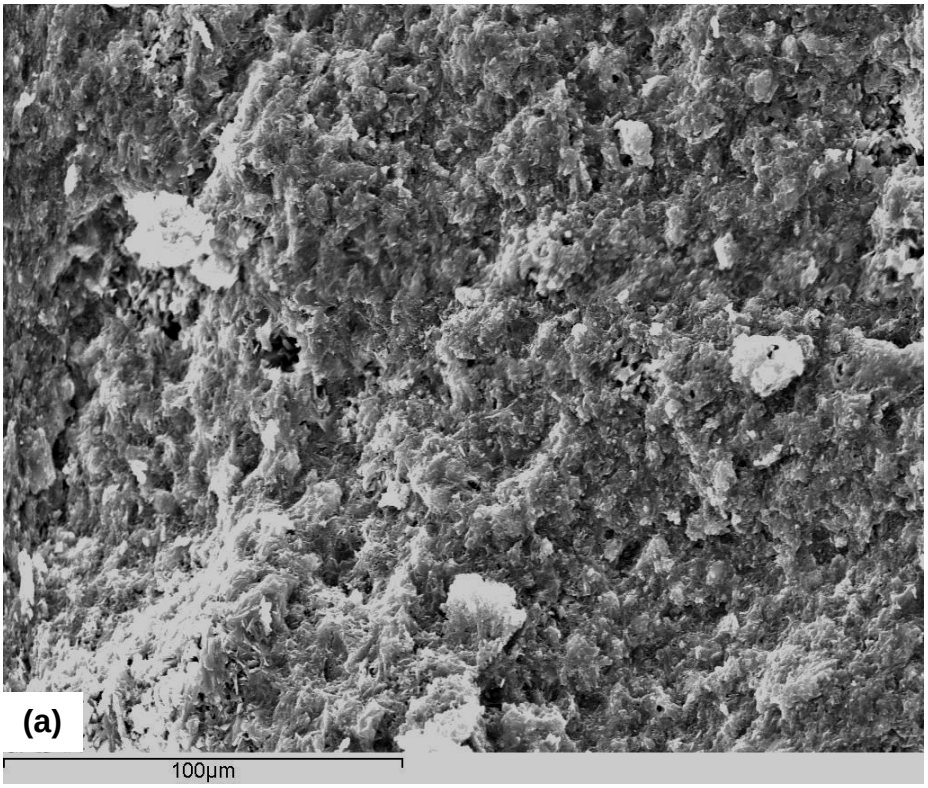


Figure 4.45: SEM images of fracture surface cement with high CHX and low PAA (C11 P6) at a) x500 and b) x5000 magnification.



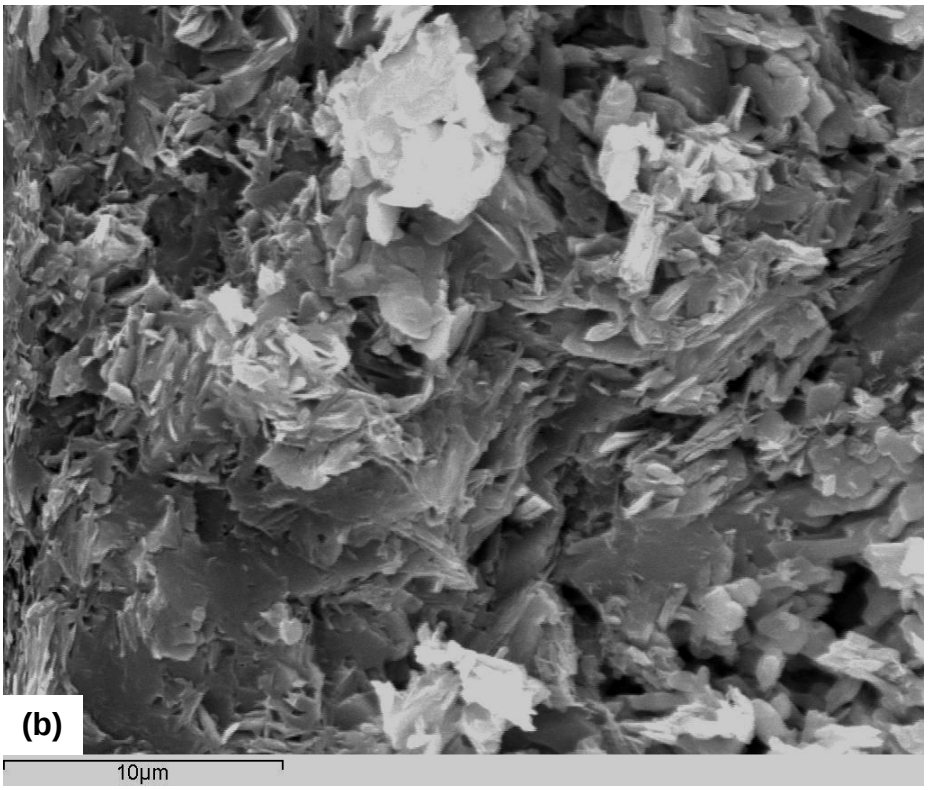


Figure 4.46: SEM images of fracture surface cement with high CHX and high PAA (C11 P12) at a) x500 and b) x5000 magnification.

CHAPTER 5

Result II

Mechanical Properties Studies

5.0 RESULTS II - MECHANICAL PROPERTIES STUDIES

5.1 Hardness

5.1.1 Effect of time

The hardness of cement surfaces was determined at 1 and 24 hours after mixing. The mean and 95% confidence intervals are presented in Table 5.1. These show that at 1 hour, hardness values ranged from 12.8 ± 2.2 to 25.2 ± 2.0 GPa. After 24 hours there was a significant increase in all hardness values. These ranged from 28.2 ± 4.7 to 73.1 ± 3.2 GPa.

Table 5.1: Surface hardness (GPa) of each composition at 1 at 24 hours after mixing.

Cement Code	At 1 hour	At 24 hours
	GPa (95% CI)	GPa (95% CI)
C0 P0	20.2 (2.5)	33.1 (3.2)
C6 P0	22.2 (2.9)	64.1 (4.1)
C11 P0	25.2 (2.0)	45.6 (1.8)
C0 P6	17.4 (2.5)	28.2 (4.7)
C6 P6	17.2 (2.3)	73.1 (3.2)
C11 P6	12.8 (2.2)	69.3 (3.0)
C0 P12	23.1 (3.5)	44.9 (3.8)
C6 P12	24.7 (3.9)	37.2 (2.3)
C11 P12	24.1 (3.0)	30.6 (3.2)

5.1.2 Results at 1 hour

5.1 shows that at 1 hour after mixing, the characteristic of surface hardness was not significantly influenced by the chlorhexidine concentration. Also at zero and low chlorhexidine concentration, varying the polyacrylic acid concentration did not significantly affect the cement hardness. C11 P6 however, had significantly lower hardness compared with C11 P0 and C11 P12.

5.1.3 Results at 24 hours

shows hardness of each of the cement composition at 24 hours after mixing. As can be seen, there was a significant increase of cement hardness for compositions with no polyacrylic acid (P0) as the chlorhexidine concentration was raised to 6% (w/w). Beyond 6% (w/w) chlorhexidine however, the hardness declined.

Cement hardness for C6 P6 and C11 P6 were significantly higher compared to C0 P6. The hardness for compositions with high polyacrylic acid declined steadily as the chlorhexidine concentration increased.

With no chlorhexidine cements with high and low polyacrylic acid had the highest and lowest hardness respectively. With low chlorhexidine the opposite was observed.

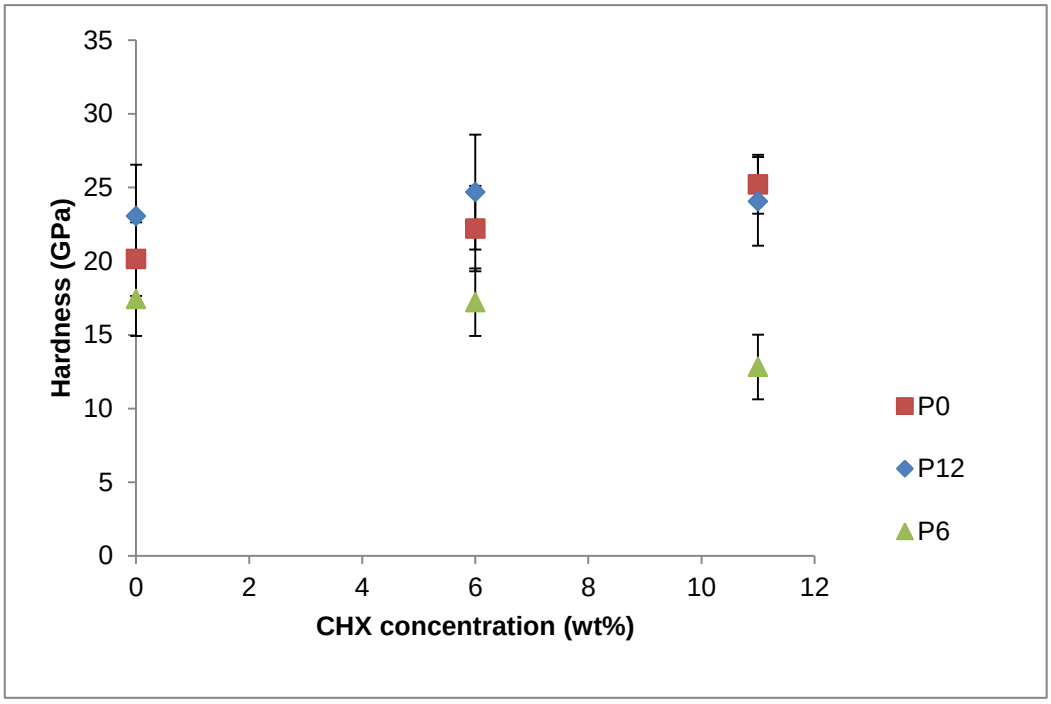


Figure 5.1: Hardness of brushite cements as a function of concentration of CHX and PAA at 1 hour after mixing. . Errors bars represent 95% confidence interval.

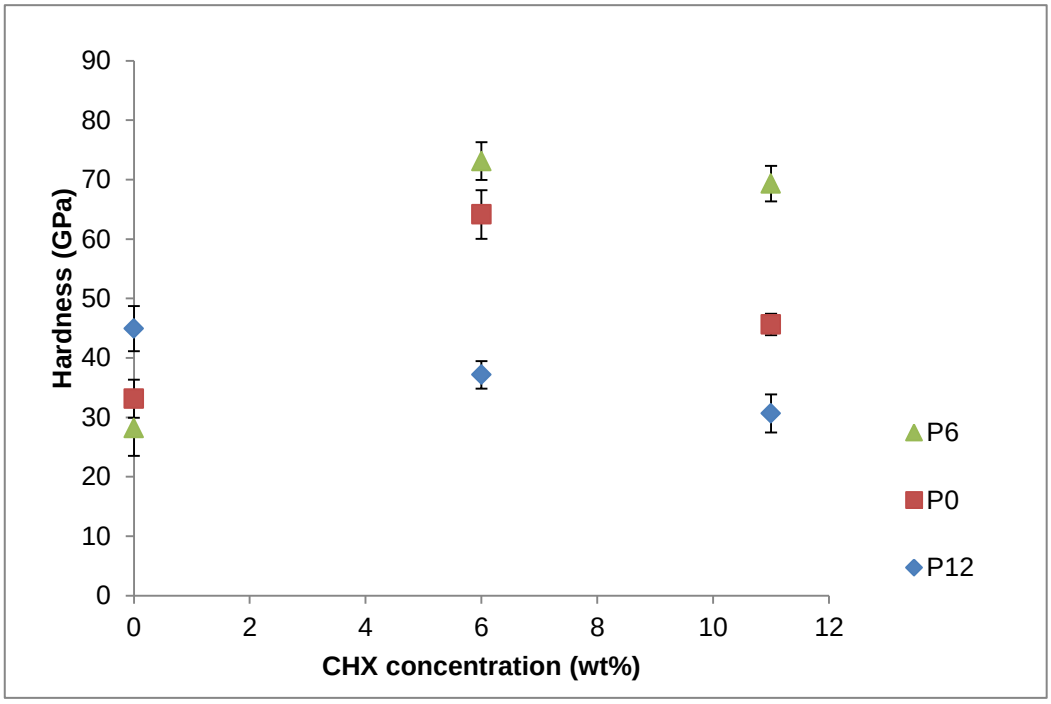


Figure 1: Surface hardness of brushite cements as a function of concentration of CHX and PAA at 24 hours after mixing. Errors bars represent 95% confidence interval.

5.2 Biaxial flexural strength

The mean and 95% confidence intervals of biaxial flexural strength are presented in Table 5.2. These showed that C0 P6 had the highest flexural strength (11.1 ± 1.2 MPa) and C11 P0 had the lowest strength (5.8 ± 1.3 MPa).

With zero or low polyacrylic acid there was a slight reduction in strength with addition of chlorhexidine into the composition. With high polyacrylic acid the opposite trend was observed (see Figure 5.3).

Table 5.2: Biaxial flexural strength (MPa) and Modulus (GPa) of each composition.

Cement Code	Biaxial flexural strength	Biaxial flexural Modulus
	MPa (95% CI)	GPa (95% CI)
C0 P0	7.9 (0.8)	1.1 (0.4)
C6 P0	7.2 (1.4)	0.6 (0.2)
C11 P0	5.8 (1.3)	0.5 (0.1)
C0 P6	11.1 (1.2)	1.5 (0.1)
C6 P6	10.8 (1.7)	1.1 (0.3)
C11 P6	9.6 (1.0)	0.7 (0.1)
C0 P12	8.7 (0.9)	1.4 (0.3)
C6 P12	9.5 (1.4)	1.0 (0.1)
C11 P12	9.3 (0.2)	0.8 (0.2)

The incorporation of 6% (w/w) polyacrylic acid into the composition resulted in an increase of flexural strength regardless of chlorhexidine concentration. With further polyacrylic acid addition, flexural strength declined - particularly when the chlorhexidine concentration was low. These results suggest an upper optimal limit for polyacrylic acid concentration. When chlorhexidine concentration is high this limit may be raised.

5.3 Biaxial flexural Modulus

Biaxial flexural modulus is a measurement of the stiffness of an elastic material and was determined from the maximum gradient in stress – strain curves obtained during flexural strength testing. The data in Table 5.2 showed that C0 P6 had the highest modulus of 1.5 ± 0.1 GPa and C11 P0 exhibited the lowest (0.5 ± 0.1 GPa).

The effect of chlorhexidine and polyacrylic acid on modulus is shown in 5.4. There was a systematic linear reduction of modulus of all cements when chlorhexidine concentration was increased. At 6% (w/w) chlorhexidine, when polyacrylic acid incorporated there were significant increase in modulus, but there were no significant difference between 6% (w/w) and 12% (w/w).

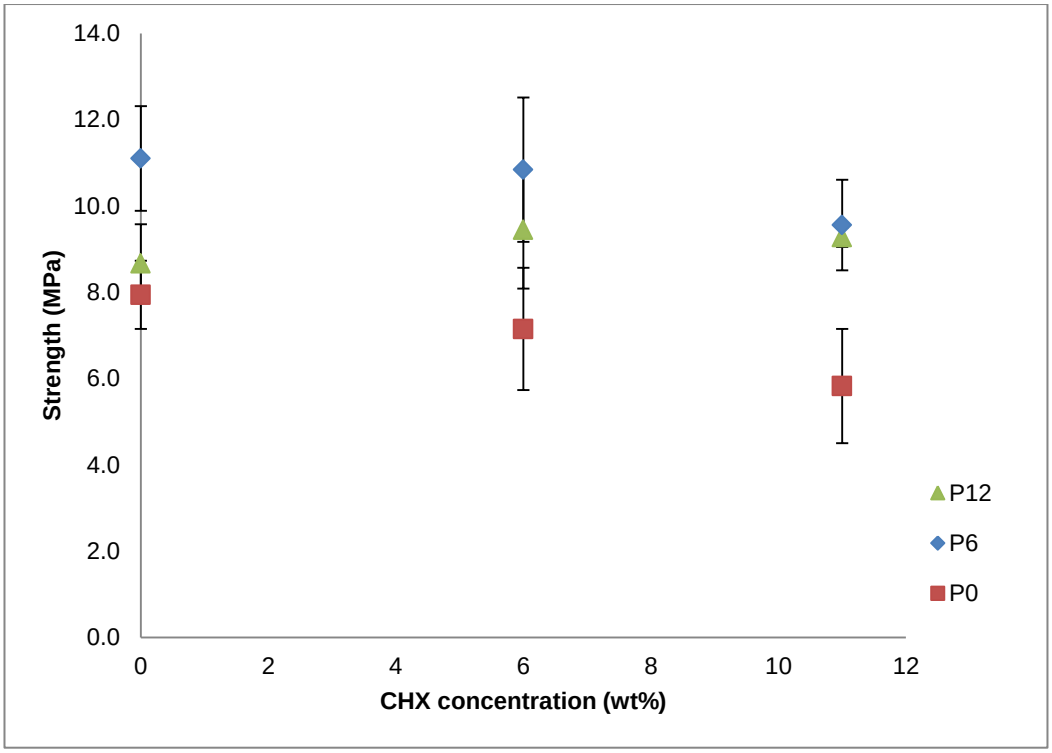


Figure 5.3: Biaxial flexural strength of brushite cements as a function of concentration of CHX and PAA. None overlapping 95% CI interval error bars indicate significantly different results.

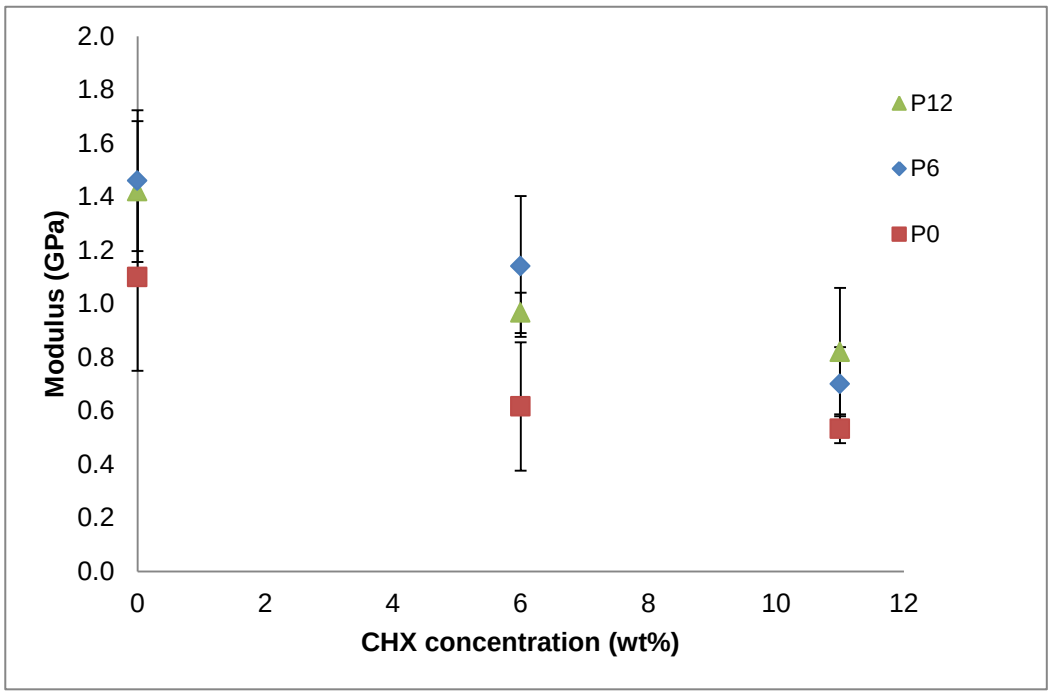


Figure 5.4: Relationship between concentration of CHX and PAA on biaxial flexural Modulus.

CHAPTER 6

Result III

Degradation, pH and Drug Release Studies

6.0 RESULTS III – DEGRADATION, pH SOLUTION AND DRUG RELEASE STUDIES

6.1 Degradation study

6.1.1 Mass change

shows the percentage of mass change versus time for all cement compositions. There was an initial increase in mass during the first 24 hours up to 7% (with an average of 4%) for all samples due to water filling the pores in the cement structure. The plotted graph shows no significant change between cement compositions until 336 hours (2 weeks). The average mass change however, was significantly different between compositions with and with no chlorhexidine; 12% and 0.2% respectively at the end of study period.

6.1.2 Degradation rate

To estimate degradation rate a linear trend line was fitted for each composition with early time points excluded due to mass gain. The gradient of the trend line gives an indication of cement degradation rate. 6.2 shows percentage mass loss per day after 24 hours. This indicated a daily degradation rate range from 0.1 ± 0.03 wt% to 0.6 ± 0.15 wt%. With zero chlorhexidine, the cement degradation was minimal. However, when chlorhexidine was incorporated into the compositions the rate of degradation was significantly increased. There was no significant difference between low and high chlorhexidine concentrations on cement degradation. Polyacrylic acid did not appear to significantly affect the rate of degradation.

6.1.3 Water sorption

The intercept obtained from the linear trend line of mass change against time gives an estimate of water sorption. 6.3 shows the percentage of maximum mass increase obtained by each composition. These ranged from 2.2 ± 0.6 wt% to 7.8 ± 1.4 wt%. With low PAA, raising chlorhexidine concentration resulted in an increased early mass gain. However there was an upper optimal

limit of 6% (w/w) of chlorhexidine after which the percentage significantly dropped. At any given chlorhexidine concentration, varying the polyacrylic acid concentration did not significantly affect water sorption.

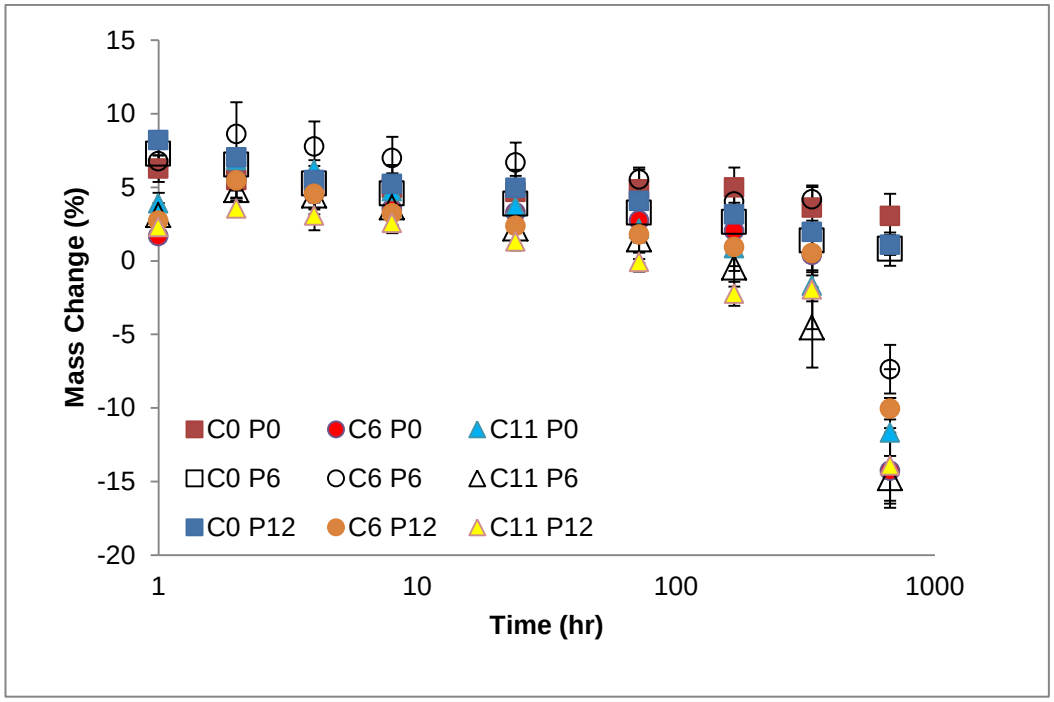


Figure 6.1: Mass change vs $\log_{10}$ of time for all cement compositions. None overlapping errors bars indicates significantly different results ($p < 0.05$).

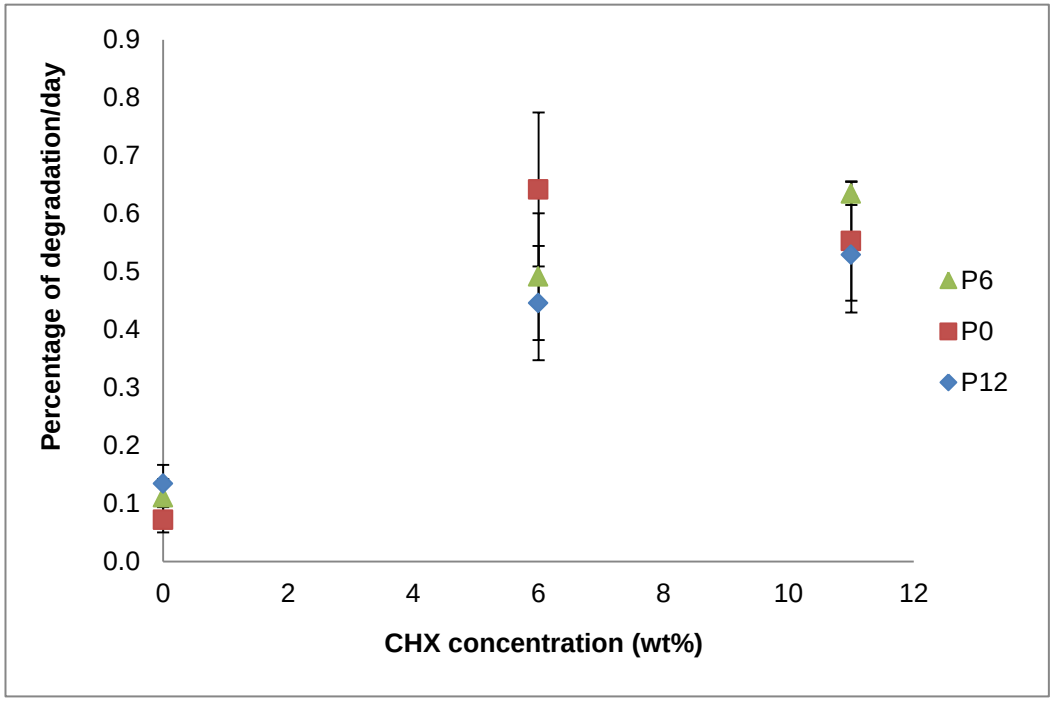


Figure 6.2: Average percentage of degradation rate per day of each cement composition. Errors bars indicate 95% confidence intervals.

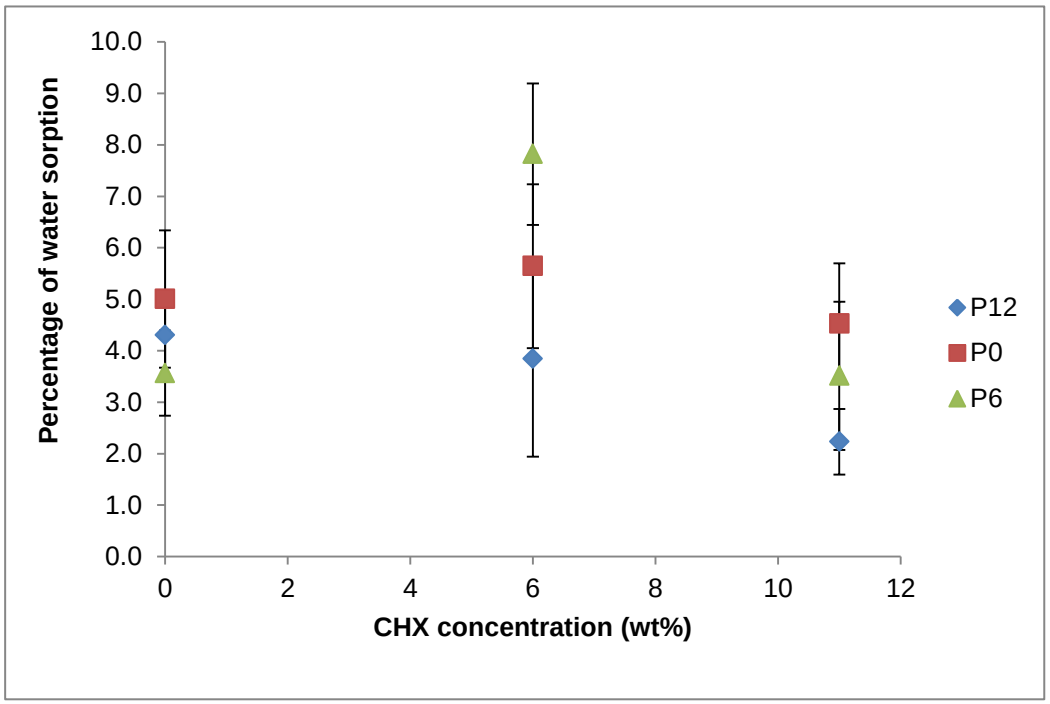


Figure 6.3: Extrapolated maximum water sorption for each cement composition. Errors bars indicate 95% confidence intervals.

6.2 pH solution Study

Figure 6.4 shows the changes in the pH levels of the distilled water with time in the presence of each composition. Generally, the pH levels were slightly acidic. The pH values ranged from 5.8 to 7.2.

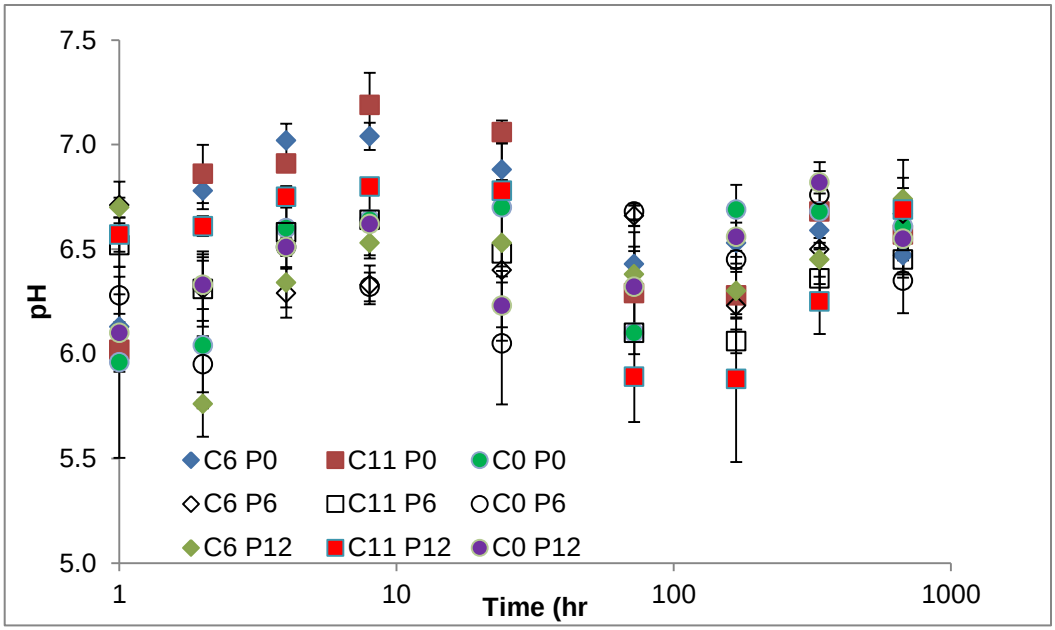


Figure 6.4: pH level of each composition solution at different time points. Time in $\log_{10}$ scale.

6.3 Chlorhexidine release profile

6.3.1 Calibration Curve of Chlorhexidine

Chlorhexidine diacetate has a peak absorbance at 230 and 255 nm. The calibration curves were generated by plotting the absorbance at 230 and 255 nm against the concentration of CHX in aqueous solutions.

Table 6.1: The absorbance of various concentrations of CHX at 230 and 255 nm

Concentration of CHX (ppm)	Absorption at 230 nm	Absorption at 255 nm
1.03	0.035	0.036
5.15	0.232	0.227
10.3	0.450	0.444
20.6	0.980	0.962

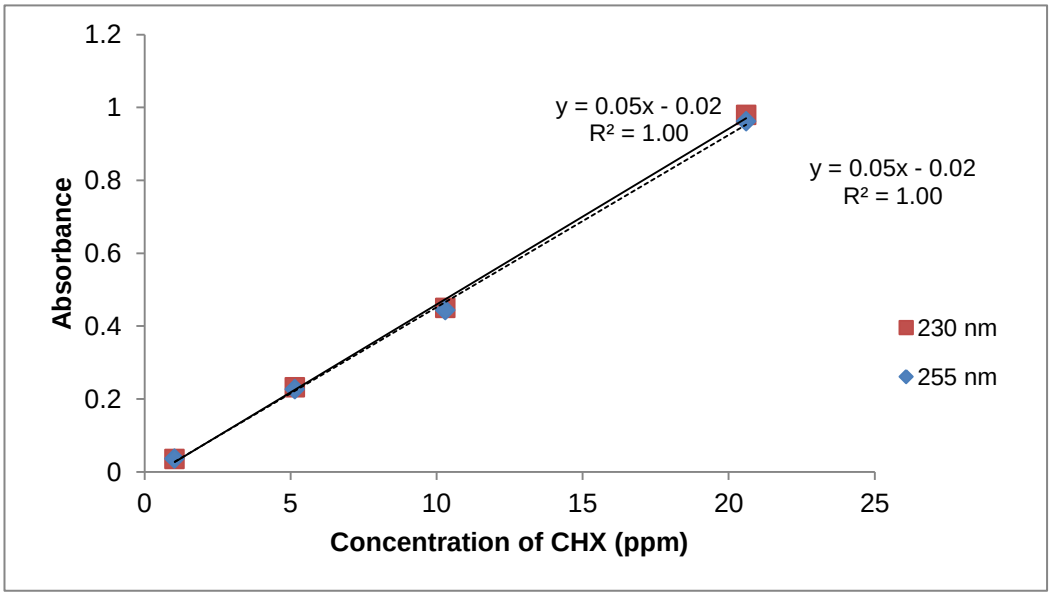


Figure 6.5: Calibration curve of CHX at 230 and 255 nm.

From the calibration curves of CHX, the following equations are developed:-

At 235 nm: $y = 0.0467x$; $R^2 = 0.997$

At 255 nm: $y = 0.0459x$; $R^2 = 0.997$

R^2 is the Pearson product moment correlation coefficient and represents the level of deviation of data points from the straight line. As in the above cases, it is very close to one i.e. a straight line describes the results very well.

With the establishment of these equations, the concentration of chlorhexidine in the storage solution can be deduced (see Appendix 1).

6.3.2 Calculation of Chlorhexidine release

The sample storage solutions at different time points were diluted in order to obtain absorbance at 255 nm always below 1 before the UV analysis. The cumulative percentages in CHX release from the cements were obtained. The calculation procedure for average percentage release in the samples at 1h time point at 255 nm is shown in Appendix (see A1).

The corrected Absorbance (A_c) was calculated using Absorbance (A) multiplied by dilution factor (DF). For C6 P0, the dilution factor was 10 (1 part sample solution : 9 part distilled water). The percentage of chlorhexidine released was then calculated by summing the cumulative mass of chlorhexidine (mg) at each time point and dividing by the total mass in the sample.

6.6 shows the cumulative percentage of chlorhexidine release with respect to time. Control formulations containing 800 mM citric acid and no polyacrylic acid exhibit about 30 – 50% chlorhexidine release over 24 hours. By 4 weeks more than 90% of the drug was released from the controls. This is expected with a diffusion controlled process through a porous cement. With cements containing polyacrylic acid, the drug release was substantially affected with less than 20% of the chlorhexidine being released over the same periods of time. At 4 weeks, the drug release from samples with polyacrylic acid was levelling off. This indicates incorporation of polyacrylic acids in the formulation has successfully slowed the drug release.

The sample with 12% (w/w) of polyacrylic acid and 11% (w/w) of chlorhexidine exhibited the highest drug release (slightly more than 20%) compared to other cements containing polyacrylic acid.

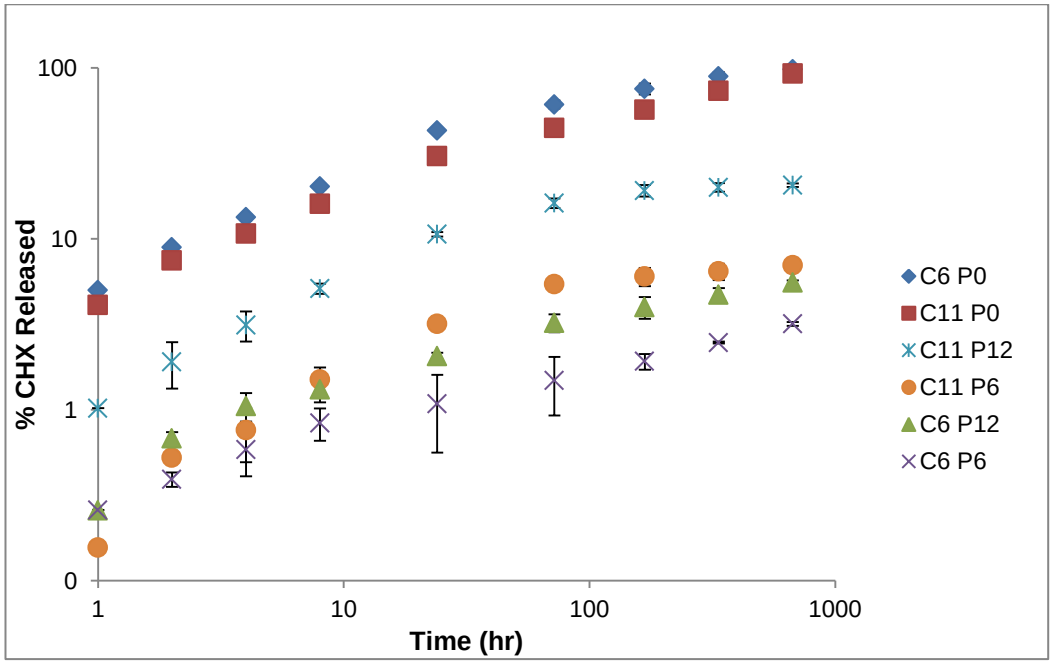


Figure 6.6: Average percentage total drug release versus $\log_{10}$ time from cements containing different concentrations of CHX and PAA. Both time and percentage of CHX released axes are in $\log_{10}$ scale.

CHAPTER 7

DISCUSSION

7.0 DISCUSSION

Brushite cements or dicalcium phosphate dihydrate are of clinical interest because of their biodegradability under physiological conditions compared to precipitated hydroxyapatite. In addition, this cement is composed of resorbable calcium phosphate compounds which are suitable for hard tissue regeneration. Unlike ceramic- based materials, brushite cement is mouldable therefore can be easily placed and packed into bone defects or a cavity without prior shaping. In dentistry, such materials could serve as remineralising dental cements and resorbable bone substitutes in alveolar bone preservation and augmentation.

Currently, limiting factors for their extensive clinical applications are mainly short setting times, low mechanical strength, rapid drug release and difficult to control degradation. This study was carried out to quantify the effects of partially substituting citric acid for polyacrylic acid and varying chlorhexidine levels on setting time and setting reaction, final composition, microstructure, mechanical properties, degradation and release kinetics of chlorhexidine from brushite cement.

7.1 Setting Kinetics, Final Composition and Microstructure

7.1.1 Setting Kinetics

Brushite cement is precipitated through acid - base reactions between several calcium orthophosphate combinations. This reaction occurs in the presence of an aqueous phase. Brushite however will only precipitate at a low pH, (approximately 4.2) otherwise the end product of this chemical reaction is hydroxyapatite cement (Mirtchi et al., 1989b). In MCPM and β–TCP system, upon mixing the powder phase with aqueous phase, the initial setting reaction is characterised by dissolution of MCPM, loss of hydrogen ions and precipitation of brushite crystals. This event results in a rapid and pronounced decrease in pH which is subsequently followed by dissolution of β–TCP and uptake of hydrogen ions to precipitate more brushite crystal (Mirtchi et al., 1989b).

The setting reaction of the conventional brushite cement is very rapid; approximately 30 seconds (Mirtchi et al., 1991; Mirtchi et al., 1989a) which gives

limited time for manipulation and placement of the cement in clinical environment. This setting time however, can be readily controlled by manipulating powder to liquid ratios (Mirtchi et al., 1989a) and/or using a setting retardant such as citric acid (Barralet et al., 2004), glycolic acid (Marino et al., 2007) or tartaric acid (Alkhraisat et al., 2008).

In this study the powder to liquid ratio (PLR) was kept to 3 g ml^{-1} for all compositions. Although this high PLR could accelerate the setting reaction by favouring a faster crystal precipitation, incorporation of citric acid can counteract this effect to prolong the setting time. Citric acid acts by decreasing the initial cement viscosity such that the cement mix was is a freely flowing liquid (Barralet et al., 2004), thus allowing a workable cement paste to be formed. It has also been suggested in the MCPM and β–TCP systems that the citric acid can interact with β–TCP particles thus limiting their dissolution to precipitate to brushite crystals (Alkhraisat et al., 2008). Therefore citric acid has the capability to chelate the calcium ions and cause delays in precipitation of brushite crystals.

FTIR studies previously (Hofmann et al., 2006) have demonstrated the formation of an intermediate complex due to interaction between citrate ions (negatively charged) and calcium ions (positively charged). This intermediate complex is known as dicalcium citrate complex. It is relatively short lived and later will dissolve to allow precipitation of brushite crystal formation. Dicalcium citrate complex is however difficult to detect unless the citric acid concentration is high (>1000 mM).

In contrast to an earlier study by Hoffmann et al. (2006), in the current study, although only 800 mM citric acid was used, the FTIR analysis could readily detect the intermediate complex in composition with no polyacrylic acid (C0 P0, C6 P0 and C11 P0) at an early time (see Figure 4.16, Figure 4.19 and Figure 4.22). This is possibly attributed to the lower PLR used in this study (3 g ml^{-1}). In this study, the dicalcium citrate complex had peaks at 1532 and 1420 cm^{-1} which are consistent with the formation of CO_2^- symmetric and asymmetric stretching peaks (Hofmann et al., 2006). The appearance of these peaks at the same time as other sharp peaks at 1110, 1050 and 1010 cm^{-1}, but none initially at 980, 1640 or 3300 cm^{-1}, suggests formation of this intermediate complex. At a later time point however, the 980 cm^{-1} peak achieved its maximum value with a subsequent trough centred at 908 cm^{-1} which indicates dissolution of the

intermediate complex to precipitate brushite crystals. In addition, O – H peaks can be observed which indicate water was bound into the composition. An identical final FTIR absorbance and difference spectrum irrespective of time suggests that any reactive intermediates are quickly converted to the final products. The peaks are consistent in both position and relative intensity with that of pure brushite cement (Hofmann et al., 2006; Xu et al., 1999). Therefore, citric acid delayed the setting time but did not prevent the formation of brushite cement.

The setting time was significantly prolonged when polyacrylic acid was incorporated as a partial replacement of the aqueous phase. The setting time was delayed for up to 12 hours. This is possibly attributed to the ability of polyacrylic acid to interact with calcium ions thus affect the primary nucleation and interfering with the precipitation of brushite cement (Ginebra et al., 2006). Therefore it can be postulated that polyacrylic acid has the same mechanism as citric acid in regulating the setting time. This study suggests that the effect of polyacrylic acid on setting time is much more potent compared to citric acid.

Partial substitution of citric acid with polyacrylic acid not only delays the setting time but also affects later time reaction kinetics. FTIR analysis showed that dicalcium - polyacrylate complex is stable and does not dissolve over time unlike dicalcium citrate complex. The stability of such a complex is probably attributed to both the larger molecular size of polyacrylic acid and the larger number of possible ionic interactions with a single polyacrylic acid molecule compared to a citric acid molecule. In addition, the final spectra of C0 P6 and C0 P12 did not demonstrate O – H and 980 cm^{-1} peaks, thus suggesting the spectrum of the complex is more similar to that of monetite than brushite. The monetite – like spectra however could not be observed in compositions with high chlorhexidine and low polyacrylic acid (C11 P6) (see Figure 4.23). This is most likely attributed to the higher availability of chlorhexidine to interact with both polyacrylic acid and citric acid, thus allowing precipitation of the brushite crystal. Brushite formation was marked by formation of strong 980 cm^{-1} and sharpened O – H peaks in the final spectra.

When chlorhexidine was incorporated into the compositions (C6 P0 and C11 P0), the setting rates of these cements in this study were comparable with those observed previously (Sapir and Shapira, 2008). The final spectra of both

compositions were comparable to brushite reference spectra. Therefore, chlorhexidine does not interfere with the chemical reaction to form the brushite crystals.

Chlorhexidine concentrations greater than 6% (w/w) in previous work (Young et al., 2008) and 11% (w/w) in this new study, appeared to reduce the inhibition period of citric acid. This is most likely due to positive chlorhexidine ions interacting with negative citrate ions. Therefore, such interaction resulted in less availability of citric acid to inhibit the brushite precipitation.

When both chlorhexidine and polyacrylic acid were incorporated into the composition, the reaction kinetics varied depending on the concentration of both reactants. With low chlorhexidine, the formation of both dicalcium phosphate and dicalcium polyacrylate could be observed. When chlorhexidine concentration is low, both citric and polyacrylic acid will compete to form complexes with the dicalcium phosphate. Citric acid, however, is likely to attach more quickly due to its smaller size and faster diffusion. These dicalcium complexes could not be observed in C11 P6. This is possibly attributed to higher availability of chlorhexidine to interact with both polyacrylic acid and citric acid, thus allowing brushite cement to precipitate in C11 P6.

A high intensity of chlorhexidine peaks could be observed in C11 P12 which could not be observed in other compositions. This finding suggests that the chlorhexidine salt is precipitated on the cement surface and thus comes in contact with the flat ATR - FTIR diamond.

In this current study, difference spectra indicating absorbance changes were very significant at 1052 and 1340 cm^{-1}. The 1052 cm^{-1} peak indicates formation of dicalcium phosphate while 1340 cm^{-1} demonstrates the formation of dicalcium polyacrylate. Therefore these peaks were further investigated for reaction extent. Reaction extent refers to how far the setting reaction has gone towards completion, with a value of 1.0 indicating the setting process has completed. Reaction extent analysis demonstrated that partial substitution of citric acid with a polyacrylic acid solution did not significantly alter the early delay time period (see Figure 4.25, Figure 4.26 and Figure 4.27). It did, however, substantially slow the reaction at later times.

The reaction rate of dicalcium phosphate is accelerated by incorporation of chlorhexidine but significantly delayed when polyacrylic acid is incorporated into the composition. The acceleration of reaction rate is proportional to chlorhexidine concentration. This advantageous initial delay during the setting process (which will allow time for cement manipulation and placement in the clinical setting) could not be observed with C11 P0 (see Figure 4.27). The formation of dicalcium polyacrylate complex however is independent upon chlorhexidine and polyacrylic acid concentrations.

7.1.2 Final chemistry of the cements

FTIR and Raman spectroscopy are complimentary techniques. Both are caused by vibration of molecules. Raman mapping and spectra suggest that neither addition of chlorhexidine nor polyacrylic acid prevents final formation of a brushite – like structure. All compositions had a strong peak at 980 cm^{-1} comparable with that observed for brushite (Xu et al., 1999). As this is slightly different from a Raman monetite spectrum this anhydrous dicalcium phosphate can be tentatively ruled out as a product. As brushite was not observed by FTIR this suggests that a dicalcium phosphate - polyacrylate complex has a similar Raman spectrum to brushite. The polyacrylate is likely occupying the coordination sites generally occupied by water in the brushite crystal structure.

All compositions with polyacrylic acid exhibited low level of non – reacted particles of β–TCP. Polyacrylic acid probably prevents dissolution of β–TCP and its precipitation as dicalcium phosphate. Furthermore, polyacrylic acid resulted in discrete regions of dicalcium phosphate and chlorhexidine within the cement.

7.1.3 Scanning electron microscope (SEM)

The fracture surface of brushite cement (C0 P0) showed the characteristics of small crystals (needle – like appearance) (see Figure 4.38). This is an expected finding as citric acid is known to decrease the crystal growth rate of brushite, thus resulting in much smaller, thinner and rounded crystals (Bohner et al., 1996). Moreover, smaller crystal size previously resulted in reduced porosity of the cement microstructure.

In this study, SEM images of C6 P0 and C11 P0 exhibited larger crystal sizes and less interconnected structure when compared to the control cement (see Figure 4.41 and Figure 4.42). This is attributed to the absence of the inhibitory effect of brushite cement precipitation from citric acid (Tamimi et al., 2008a). As previously discussed, interaction between chlorhexidine and citric acid during the setting process results in a rapid setting time. This subsequently results in larger crystal size and a higher degree of cement porosity.

Strands of polymer could be observed in both compositions with no chlorhexidine (C0 P6 and C0 P12). These strands however could not be observed in any other compositions probably due to interactions between chlorhexidine (positively charged) to carboxylic groups of polyacrylic acid (negatively charged). Dissolution of chlorhexidine at an earlier time in compositions with both polyacrylic acid and chlorhexidine might explain the absence of the strands in these compositions.

Generally addition of polyacrylic acid into the compositions resulted in denser, more homogenous and less porous structures compared to compositions without. This is possibly due to polyacrylic acid causing a delay in the setting reaction and affecting the crystal precipitation (Bohner et al., 1996). Furthermore, polyacrylic acid is a polymer and is expected to fill the gaps between the intercrystalline structures. At higher magnifications, a distinctive crystalline structure could be observed especially in composition with the low chlorhexidine concentration which can be attributed to the dicalcium polyacrylate complexes (see Figure 4.43 and Figure 4.44).

C11 P6 exhibited leaf – like structures and flat crystals (see Figure 4.45) which could not be observed in any other compositions with both chlorhexidine and polyacrylic acid. In addition the crystal arrangement was less dense. FTIR study demonstrated spectra consistent with brushite cement with this composition (see Figure 4.14). Therefore, these crystals are most likely to be brushite crystals rather than dicalcium polyacrylate complex.

When viewed under the microscope at both high and low magnifications, porosities on the fracture surface could be seen for all formulations. Although qualitative assessment from SEM images in this study demonstrated that compositions with polyacrylic acid have less porosity, a definitive conclusion

could not be made solely from this finding. Other quantitative method such as mercury porosimetry (Grover et al., 2003; Matějíček et al., 2006) or porosity measurement with Archimedes principles (Matějíček et al., 2006) could be carried out to accurately measure the porosity.

7.2 Mechanical properties of brushite cement

7.2.1 Hardness

The hardness test determines the material's ability to resist plastic deformation from a standard source. The hardness value is an empirical value. In this study, the cement surface hardness was determined at 2 different time points. This was carried to evaluate the effect of time on cement hardness. At 1 hour after mixing, apart from C11 P6, the difference between cement hardness was not significant. This could be explained by the setting kinetics at the early time. FTIR study indicates that partial replacement of citric acid with polyacrylic acid did not significantly alter the early delay time period thus allowing precipitation of dicalcium phosphate cement. It is likely that precipitation of this complex at an early time provides surface characteristic to this cement.

At 24 hours after mixing however, there was a significant difference between the cement hardness. This is expected as the setting reaction has completed, therefore there was an increase in cement hardness for all compositions. There is an upper optimal limit of 6% (w/w) of chlorhexidine after which cement hardness declines (see Figure 5.2). Varying the polyacrylic acid concentration also appeared to affect the surface hardness.

The FTIR study for compositions without polyacrylic acid showed that although the setting reaction was completed within 1 hour, there was a marked difference between the 1 and 24 hours hardness values. This could be possibly explained from the reaction rate of the composition at different temperatures. The FTIR experiment was carried out at 37 °C, whereas the cement for the hardness test was left to set at room temperature (~ 23 °C).The reaction rate is double for every 10 °C increase in temperature. Therefore the setting reaction of the composition at room temperature is much slower than the composition undergoing FTIR experiment.

7.2.2 Biaxial flexural strength and modulus

The biaxial flexural strength of the samples was obtained after 24 hours setting and incubation of the samples in distilled water. This is to mimic and more closely represent the clinical environment. It is well established that hydration of the samples affects the mechanical properties of the cements (Charrière et al., 2001; Pittet and Lemaître, 2000), therefore dry strength alone may be misleading with regards to clinical performance (Barralet et al., 2004).

The original cement formulation had poor mechanical properties and several approaches have been previously explored to address this concern (Mirtchi et al., 1989a; Mirtchi et al., 1989b). In the literature, strength data for brushite cements are varied. Comparing reported strength values is complicated by variations in testing methods and condition of the samples. Therefore, caution must be exercised when comparing strength values reported in the literature because of this. Strengths have been reported to range from 1 to 52 MPa for compressive strength (Gbureck et al., 2005; Hofmann et al., 2009; Pittet and Lemaître, 2000) and from 0.7 to 4.5 MPa for diametral tensile strength (Bohner et al., 1996).

Previous studies have concluded that the strength of brushite cements is inversely correlated to its porosity (Hofmann et al., 2009); the higher the porosity of the cement the lower its mechanical performance. The reduction of porosity size of the set cement could be achieved by compaction of the setting cement (Barralet et al., 2004), addition of a setting regulator e.g. citric acid (Bohner et al., 1996), higher powder to liquid ratio and smaller particle size of the calcium orthophosphate powder phase (Hofmann et al., 2009).

In this study, the increase of biaxial flexural strength and modulus up to 11.1 ± 1.2 MPa and 1.5 ± 0.1 GPa was achieved by incorporating polyacrylic acid into the composition compared to control. This is attributed to complexation of the polyacrylic acid with dicalcium phosphate crystal structures (Majekodunmi et al., 2003) and pore filling by the polymer. This is demonstrated by the SEM findings where compositions with polyacrylic acid exhibited more homogenous, denser and relatively less porous structures when compared to those without. It is therefore expected that denser aggregates will increase the ultimate strength of

the cement (Mirtchi et al., 1989b). The biaxial flexural strength in the current study however, is lower compared to strength of compacted, dry β- TCP cement (24 MPa) with similar test methods (Meganck et al., 2005).

The decrease in biaxial flexural strength and modulus of compositions with chlorhexidine (C6 P0 and C11 P0) compared to controls is also attributed to the degree of porosity. As described above, SEM studies showed that by increasing chlorhexidine concentration, the cement structure becomes less interconnected and the crystal structures become larger (see Figure 4.41 and Figure 4.42). Moreover, accelerated setting time resulted in less time to pack the cement thus producing a cement with a high degree of porosity. In addition, high drug release upon 24 hours water immersion prior to strength testing will also increase porosity and thereby reduce strength.

7.3 Degradation and Chlorhexidine Release

7.3.1 Degradation

In contrast to previous studies (Mirtchi et al., 1989b; Young et al., 2008) there was an initial increase in mass for all cement compositions due to water sorption. In this study samples were left for longer at room temperature and atmospheric humidity before placement in water. This was to ensure that the brushite setting reaction was complete. Therefore, this is likely to have enabled greater dehydration of the cement.

After the early water sorption period, mass loss was independent of drug content as expected from earlier work (Young et al., 2008). At a later time however, it was noted that chlorhexidine incorporation significantly affected the dissolution kinetics of the cement compared to compositions with no chlorhexidine (see Figure 6.1). Polyacrylic acid complexation with brushite however does not affect the dissolution kinetics of the cement.

In this study, the total weight loss at the end of study period suggests dissolution of both chlorhexidine and brushite components from the cement composite. Distilled water which is undersaturated with calcium and phosphate ions may create gradient for dissolution of brushite components. In addition the dynamic

protocol - replacement of aqueous medium with fresh distilled water at a designated time point favours dissolution of the cement.

7.3.2 Chlorhexidine Release Kinetics

In order to assess and understand the chlorhexidine release profile in this study, it is necessary to characterise the kinetics of the chlorhexidine release. There are various factors involved in determining a drugs release kinetics from its carrier. These factors include interaction and bonding between drugs and the matrix which holds it, microstructure of the carrier and degradation of the matrix (Ginebra et al., 2006). Although brushite is resorbable, the rate of matrix degradation is much lower than the rate of drug release from the system. Hence, in this study, it is justifiable to presume that the drug release is mainly regulated by a diffusion – controlled processes rather than degradation of the cement (Ginebra et al., 2006).

In the current study, chlorhexidine release from formulations without polyacrylic acid (C6 P0 and C11 P0) was comparable with previous work (Young et al., 2008) and as expected for diffusion controlled drug release from a porous cement (Bohner et al., 1997a; Bohner et al., 2000; Peppas, 1983). At the initial stage, chlorhexidine release from brushite cement is in concordance with Fick's equation for diffusion from a thin disc of thickness;

$$\frac{M}{M_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(\frac{-(2n+1)^2 \pi^2 Dt}{4l^2}\right) \qquad (17)$$

Where t is the time after placement in water, D is the diffusion coefficient, M is the cumulative mass of drug released at time t and M_∞ is the mass of drug in the sample, l known thickness of the disc.

At early time, this equation is proportional to the square root of time because at short time, when *Dt* is small, the equation can be reduced to;

$$M/M_{\infty} = 2\sqrt{Dt/\pi l^2} \quad (18)$$

Fick's law indicates that the fraction of drug released from the sample is expected to be independent of its initial concentration in the brushite cement i.e. no matter what weight percentage of drug is present in the sample, the same percentage of that drug content is released with time.

During the first few hours of incubation, chlorhexidine would be easily released into the surrounding medium. This is attributed to the effect of diffusion release through the porous cement (square – root kinetic time) observed during the first 12 hours. During the initial burst nearly 50% of the chlorhexidine was released over 24 hours. Subsequently over 90% of the loaded dose of chlorhexidine was released in about 4 weeks in both C6 P0 and C11 P0 (see Figure 6.6). Diffusion - controlled release gives faster drug release at early times. This may be beneficial in establishing an effective drug level in the surrounding environment if drug diffusion away from the device site is restricted or in acute infection.

7.3.2.1 Interaction and bond between drugs and the cement matrix

Polyacrylic acid, when incorporated into the composition, affected the release profile substantially. Setting kinetic studies demonstrated that incorporation of polyacrylic acid resulted in chemical interaction between brushite and chlorhexidine. Therefore, chlorhexidine release from the drug carrier did not follow a pure diffusion release compared to C6 P0 and C11 P0. Chlorhexidine release from compositions with polyacrylic acid is likely to be attributed to two mechanisms: diffusion of free / unbound chlorhexidine through the porous cement (according to diffusion - controlled kinetic) and dissociation of chlorhexidine from the polyacrylate – chlorhexidine complex (zero order kinetic) (Bohner et al., 2000). In this context, zero order kinetic refers to drug release from the carrier that is independent of time or the amount of drug present.

These complex release mechanisms may be as a result of chlorhexidine interaction with the cement matrix forming a chlorhexidine – polyacrylic complex

(chemically bound) or entrapped within dicalcium polyacrylate complex (physically bound). Loss of chlorhexidine absorbance in FTIR spectra at early times in combination with the effects of chlorhexidine levels on cement setting kinetics both provide evidence for the chemical interaction. Competition between chlorhexidine and brushite for complexation with polyacrylic acid would explain the complex dependence of chlorhexidine release upon both cement composition and time (Bommareddy et al., 2006).

Although the chlorhexidine release from C11 P12 was significantly lower than in controls (C6 P0 and C11 P0), compared to other compositions with polyacrylic acid it released the most (see Figure 6.6). Based on data from the FTIR study, it was likely that chlorhexidine salt were precipitating on the cement surface may be an explanation to the higher drug release (see Figure 4.15).

7.3.2.2 Microstructure of the carrier

Previous studies have shown that drug release could also be controlled by reducing the porosity (Bohner et al., 1997a; Hofmann et al., 2009; Tamimi et al., 2008b). In this study, the SEM images demonstrated the relative reduction in porosity and denser structure of compositions with polyacrylic acid. Therefore it is very likely that the substantial reduction of the initial burst and overall chlorhexidine release over the study period is attributed to low porosity system compared to C6 P0 and C11 P0. Furthermore, a low porosity cement system decreases the volume of release media which can enter the matrix to allow dissolution of the drug (Hofmann et al., 2009).

7.3.2.3 Degradation of the matrix

Around 80% of chlorhexidine load in the cements with polyacrylic acid was not released during the release study; this fraction however is thought to be released slowly over larger time periods or upon degradation of the cement structure. It should be noted that brushite cements degrade more rapidly *in vivo* (Constantz et al., 1998; Frayssinet et al., 1998; Grover et al., 2003) than *in vitro* due to the action of cells and enzymes. Later time release of chlorhexidine is therefore likely also to be faster *in vivo* as the surrounding brushite matrix is lost.

CHAPTER 8

CONCLUSIONS

8.0 CONCLUSION

This thesis is mainly focused on assessing the effect of varying the concentration of polyacrylic acid and chlorhexidine on a brushite cement. The following conclusions can be drawn from this study:

8.1 Setting Kinetics, Final Composition and Microstructure

8.1.1 Setting time and reaction kinetics

It has been demonstrated in this thesis that the setting reaction and final product were affected by both chlorhexidine and polyacrylic acid concentrations. High chlorhexidine concentration results in acceleration of the setting reaction. Polyacrylic acid on the other hand results in delayed setting reaction. FTIR study demonstrated complexation between polyacrylic acid and calcium ions and formation of structures with similarities to dicalcium polyacrylate. There were also some changes consistent with a brushite – type structure but with no water – binding. In addition, compositions with high chlorhexidine also suggested complexation between polyacrylic acid and chlorhexidine.

8.1.2 Final composition of the cement

Raman microscopy was used to analyse final composition of the cements. Although the final FTIR spectra of cements containing polyacrylic acid were not generally entirely consistent with brushite formation Raman spectra were. It is probable therefore that the final complex formed has similarities in structure to brushite but without water binding and calcium polyacrylate. Dicalcium phosphate/polyacrylate complex formation is consistent with all spectroscopic results.

8.1.3 Microstructure of the cement

The microstructure of the cement can be affected by incorporation of chlorhexidine and polyacrylic acid. The crystals become larger and less interconnected with chlorhexidine. Polyacrylic acid however results in a more dense and homogenous architecture with relatively less porosity.

8.2 Mechanical Properties

8.2.1 Hardness study

The hardness of the cements surface increased significantly with time.

8.2.2 Mechanical properties of the cement

The mechanical properties are significantly increased with polyacrylic incorporation. There is however an upper optimal limit of polyacrylic acid (6% w/w) for biaxial flexural strength and modulus. Chlorhexidine results in strength reduction and this is proportional to chlorhexidine concentration.

8.3 Degradation, pH and Drug Release

8.3.1 Degradation

Incorporation of chlorhexidine into the compositions resulted in more significant degradation of cement compositions when compared to the control at a later time point. On the other hand, polyacrylic acid does not affect degradation of the cement.

8.3.2 pH solution study

Incorporation of both chlorhexidine and polyacrylic acid did not significantly affect the pH values of the solutions obtained from degradation and drug release studies.

8.3.3 Chlorhexidine release study

Polyacrylic acid can be successfully used to regulate chlorhexidine release from brushite cement.

CHAPTER 9

FUTURE RESEARCH

9.0 FUTURE RESEARCH

9.1 Determination of cement porosities

The cement porosity has a significant impact on the mechanical properties of the cement. In addition, drug release from the composite cement has been shown to be regulated better with a less porous cement (Hofmann et al., 2009). In this study, we did not undertake a quantitative analysis of cement porosity. SEM images will only provide a qualitative assessment of the cement microstructure and shown relative reduction in porosity. Therefore further studies need to be carried out to understand the impact of polyacrylic acid on the microstructure and drug release regulation.

Either mercury porosimetry technique or Archimedean porosimetry experiment could be used to determine the porosity of a material. Mercury porosimetry involves measuring the volume of mercury that has intruded into the pores of the specimen at a gradually increasing pressure. The data obtained from this experiment could also give information on distribution of pore size (Grover et al., 2003; Matějíček et al., 2006). On the other hand, Archimedes porosimetry is based on water being incorporated into the composition. The weight of the specimen changes as the pores are filled with water. The weight gain is proportional to the material porosity volume (Matějíček et al., 2006).

9.2 Determination of the antibacterial activity of chlorhexidine releasing polyacrylic acid- modified brushite cement

This study has shown that polyacrylic acid is capable of chlorhexidine release over a period of time when compared to a control. In addition, FTIR study also indicated an interaction between chlorhexidine and polyacrylic acid especially with composition high chlorhexidine concentration. Therefore, it is important to know whether the concentration of chlorhexidine released from modified brushite cement achieves the acceptable minimum inhibitory concentration required to exert an antibacterial activity. It is also important to understand whether the interaction between chlorhexidine and polyacrylic acid has any effect on the antibacterial activity of chlorhexidine incorporated in the cement.

9.3 Investigation of modified brushite cement on adhesion to dentine and bone

The carboxylic group in polyacrylic acid has the capability to chemically bond to hydroxyapatite. Therefore it is interesting to know the whether the presence of polyacrylic acid in the composition will improve the bonding capability of brushite cement to bone and dentine. The potential chemical bonding between polyacrylic acid modified brushite and the surrounding dentine will provide a hermetic seal to eliminate microleakage and subsequent bacterial penetration to the underlying pulp or periradicular area.

9.4 Comparison to mineral trioxide aggregate (MTA) as endodontic medicament

The investigated cement in this study should be compared to MTA in term of mechanical properties, biocompatibility and clinical performance as endodontic medicament. Mechanical property comparison between different materials could only be done if the method of testing and geometry of testing sample are standardised (Morell, 1998). Therefore further study should be carried in order to assess the mechanical property of both materials with MTA is considered as gold standard.

Although MTA has been successfully used as pulp capping material and as artificial apical barrier, the discolouration associated with MTA may limits its application. Therefore the investigated antibacterial releasing, modified brushite cement may have the potential to substitute MTA. Further research involving animal model (*in vivo studies*) looking at pulpal and periradicular tissues response to the cement should be design to provide enough data for material development.

9.5 *in vivo* assessment of brushite cement as socket preservation/alveolar bone regeneration

Extraction socket and alveolar bone preservation in children is important to ensure sufficient bone is available for future dental implant placement. Following tooth loss, this antibacterial releasing, polyacrylic acid brushite cement has a great potential to be used as possible bone substitute and therefore its application should be examined *in vivo.*

Previous animal model studies (Boix et al., 2006; Fickl et al., 2008a), have carried out to evaluate the effectiveness of bone substitute materials for alveolar bone preservation. The same experimental methods could be implemented to assess and compare cellular responses to brushite cement before clinical human trials could be carried out.

9.6 Cement injectability

Setting brushite cement is mouldable and therefore can conform to the recipient area for better adaptation. Development of injectable chlorhexidine - releasing brushite cement for extraction socket preservation and as an obturation material offers a quick and easy placement of this material. For young children especially, such an approach will be an advantage in view of their limited co-operation for lengthy and complicated clinical procedures.

Brushite cement injectability however, is limited due to potential powder liquid separation during injection process. Modification of the cement is required to improve the ability of the material to be extruded without separation. Incorporation of polyacrylic acid in the aqueous phase may improve viscosity of mixing liquid and subsequently the cement injectability (Barralet et al., 2004). Therefore further study needs to be carried out to determine the injectability of the compositions used in this study.

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APPENDICES

APPENDICES

Appendix 1: Calculation of CHX concentration release from cement

Table A1: Calculation of chlorhexidine concentration release from Sample 1 (C6 P0)

	Sample 1a	**Sample 1b**	**Sample 1c**
Absorbance (A)	0.268	0.316	0.259
Corrected Absorbance (A_c) $A_c = A \times DF$	2.68	3.16	2.59
Concentration of CHX (ppm) CHX (ppm) = A_c / E *Where;* *A_c – Corrected absorbance* *E – Slope calibration curve of CHX at 235 nm (0.0467)*	57.39	67.67	55.46
Concentration of CHX (mg)	0.57	0.68	0.55
Initial mass of CHX (mg) *M_0 x fraction of CHX* *Where;* *M_0 – Initial mass of cement* *Calculation of CHX fraction =* *0.2 g / (1+1.23+0.2+0.8) g*	11.05	12.82	11.69
Percentage of CHX release (%) *% = concentration / Initial mass of CHX*	5.19	5.29	4.74

Appendix 2: Average percentage of mass change for degradation.

Table A2: Average percentage of mass change for degradation study

Time (hr)	Average Percentage of Mass Changes (95% CI)								
	C0 P0	C0 P6	C0 P12	C6 P0	C6 P6	C6 P12	C11 P0	C11 P6	C11 P12
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	6.3 (0.9)	7.3 (0.5)	8.2 (0.6)	1.6 (0.3)	6.8 (0.4)	2.7 (1.2)	3.9 (0.7)	3.1 (0.6)	2.3 (0.2)
2	5.5 (1.2)	6.5 (0.1)	7.0 (0.5)	5.5 (0.8)	8.6 (2.2)	5.5 (1.2)	6.8 (0.3)	4.8 (0.8)	3.6 (0.5)
4	5.2 (1.6)	5.2 (0.5)	5.5 (0.6)	4.5 (0.6)	7.8 (1.7)	4.5 (1.4)	6.2 (0.3)	4.4 (0.9)	3.1 (1.0)
8	5.0 (1.6)	4.6 (0.6)	5.2 (0.7)	3.5 (1.1)	7.0 (1.5)	3.2 (0.9)	4.7 (0.2)	3.6 (0.8)	2.6 (0.7)
24	4.7 (1.5)	3.9 (0.8)	5.0 (0.8)	3.2 (1.1)	6.7 (1.3)	2.4 (1.4)	3.7 (0.6)	2.2 (1.3)	1.3 (0.6)
72	4.9 (1.4)	3.3 (0.8)	4.1 (0.8)	2.7 (1.1)	5.5 (0.8)	1.8 (1.7)	2.2 (0.9)	1.5 (1.6)	-0.1 (0.7)
168	5.0 (1.4)	2.6 (0.9)	3.2 (0.8)	2.0 (1.1)	4.0 (0.8)	0.9 (1.6)	0.9 (1.2)	-0.5 (1.3)	-2.2 (0.8)
336	3.6 (1.5)	1.4 (1.4)	2.0 (0.3)	0.4 (1.2)	4.2 (0.8)	0.5 (1.5)	-1.7 (1.1)	-4.5 (2.7)	-1.9 (2.7)
672	3.0 (1.5)	0.8 (1.1)	1.1 (0.7)	-14.3 (2.5)	-7.4 (1.7)	-10.1 (0.7)	- 11.7 (2.4)	-14.8 (1.5)	-13.9 (2.6)

Appendix 3: Average pH value of solutions obtained from degradation and CHX release study

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Table A3: Average pH value of solutions at different time points

Time (hr)	Average Percentage of Mass Changes (95% CI)								
	C0 P0	C0 P6	C0 P12	C6 P0	C6 P6	C6 P12	C11 P0	C11 P6	C11 P12
1	5.96 (0.5)	6.28 (0.1)	6.10 (0.2)	6.13 (0.0)	6.7 (0.1)	6.70 (0.0)	6.02 (0.0)	6.52 (0.1)	6.57 (0.1)
2	6.04 (0.4)	5.95 (0.1)	6.33 (0.1)	6.78 (0.1)	6.31 (0.2)	5.76 (0.0)	6.86 (0.1)	6.31 (0.2)	6.61 (0.0)
4	6.60 (0.2)	6.51 (0.0)	6.51 (0.3)	7.02 (0.1)	6.29 (0.1)	6.34 (0.0)	6.91 (0.0)	6.58 (0.0)	6.75 (0.1)
8	6.64 (0.0)	6.32 (0.1)	6.62 (0.2)	7.04 (0.1)	6.33 (0.1)	6.53 (0.1)	7.19 (0.2)	6.64 (0.0)	6.80 (0.0)
24	6.48 (0.3)	6.05 (0.3)	6.23 (0.2)	6.88 (0.1)	6.40).3)	6.53 (0.0)	7.06 (0.1)	6.48 (0.1)	6.78 (0.1)
72	6.10 (0.0)	6.68 (0.0)	6.32 (0.2)	6.43 (0.2)	6.66 (0.0)	6.38 (0.1)	6.29 (0.3)	6.10 (0.0)	5.89 (0.2)
168	6.69 (0.1)	6.45 (0.0)	6.56 (0.1)	6.53 (0.1)	6.23 (0.1)	6.30 (0.1)	6.28 (0.1)	6.06 (0.1)	5.88 (0.4)
336	6.68 (0.1)	6.76 (0.2)	6.82 (0.1)	6.59 (0.1)	6.50 (0.2)	6.45 (0.1)	6.68 (0.0)	6.36 (0.1)	6.25 (0.2)
672	6.61 (0.2)	6.35 (0.2)	6.55 (0.2)	6.47 (0.0)	6.67 (0.1)	6.74 (0.1)	6.57 (0.1)	6.45 (0.1)	6.69 (0.2)

Appendix 4: Average percentages of chlorhexidine release (absorbance at 255 nm)

Table 1: Average percentage of CHX release (absorbance at 255 nm)

Time (hr)	Average Percentages of chlorhexidine release (95% CI)					
	C6 P0	C6 P6	C6 P12	C11 P0	C11 P6	C11 P12
1	5.0 (0.3)	0.3 (0.0)	0.3 (0.1)	4.1 (0.5)	0.2 (0.0)	1.0 (0.6)
2	8.9 (0.6)	0.4 (0.2)	0.7 (0.2)	7.5 (0.7)	0.5 (0.3)	0.5 (0.6)
4	13.4 (0.9)	0.6 (0.2)	1.1 (0.2)	10.8 (0.6)	0.8 (0.3)	0.8 (0.3)
8	20.3 (0.8)	0.8 (0.5)	1.3 (0.1)	16.1 (0.4)	1.5 (0.1)	1.5 (0.3)
24	43.0 (3.3)	1.1 (0.6)	2.1 (0.4)	30.5 (2.5)	3.2 (0.3)	3.2 (1.0)
72	61.1 (5.5)	1.5 (0.2)	3.2 (0.6)	44.5 (2.5)	5.4 (0.7)	5.4 (1.5)
168	75.6 (4.9)	1.9 (0.0)	4.0 (0.5)	57.1 (2.5)	6.0 (0.7)	6.0 (1.1)
336	894 (2.6)	2.5 (0.1)	4.7 (0.2)	73.6 (2.5)	6.5 (0.1)	6.5 (0.5)
672	98.0 (2.9)	3.2 (0.1)	5.6 (0.1)	92.6 (1.6)	7.0 (0.2)	7.0 (0.3)

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Appendix 9: Abstract submitted for EAPD 2012

Abstract submitted for 11th European Academy of Paediatric Dentistry (EAPD) Congress, 2012, Strasbourg, France (24 – 26 May 2012)

Antibacterial Release from Polyacrylic acid modified -Brushite Cements

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In children, alveolar bone loss as sequalae to premature teeth loss in trauma may complicates implant placement later. Brushite cement has a potential use as bone filling material and a carrier for antimicrobial agent which offers answer to this problem ***Aims:*** The aims of this study were to assess how partial replacement of citric by polyacrylic acid (PAA) affects setting kinetics, chlorhexidine release, degradation, microstructure and mechanical properties of brushite cement. ***Materials and Methods:*** The cements consisted of equimolar β-tricalcium phosphate and mono-calcium phosphate monohydrate (β-TCP/MCPM) and 6 or 11wt% chlorhexidine. The liquid phase consisted of aqueous 800mM citric acid and PAA solutions at different ratio. Setting kinetics and final composition were determined using ATR-FTIR and Raman respectively. Chlorhexidine release was determined with UV spectroscopy and degradation by mass loss. Brushite microstructure and mechanical properties were assessed using SEM and an Instron™. ***Results:*** ATR-FTIR indicated formation of polyacrylate salts in addition to brushite but the former was inhibited by high chlorhexidine content. Raman spectra indicated almost complete transformation of β-TCP/MCPM to brushite in all samples. PAA addition reduced 4 week chlorhexidine release to less than 20%. A mass loss of ~15% for all formulations over 4 weeks was noted irrespective of PAA level. Microscopically, powder-like structures were observed in samples with PAA in comparison to the needle-like structure without. PAA addition increased both strength and modulus. ***Conclusion:*** PAA substantially slows setting and chlorhexidine release kinetics, alters final brushite crystal microstructure, increases strength/modulus but does not affect the degradation kinetics.

CLINICAL AUDIT

An Audit on Management of Infraoccluded Primary Molars in Children

Submitted in partial fulfilment of the requirements for the Degree of
Clinical Doctorate in Dentistry (Paediatric Dentistry)
Eastman Dental Institute
University College London

Submitted by:

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DDS (Malaysia), MFDSRCS (Edinburgh), MPaedDent RCS (England)

2012

CONTENTS

SUMMARY

The aims of this audit were to assess the management of infraoccluded primary molar teeth in children, develop clinical protocols and to assess the quality of reporting of case notes.

This audit was designed to appraise both literature and clinical case notes on the management of infraocclusion. A search on Medline and retrospective analysis on patients' case notes referred to the Joint Orthodontic – Paediatric Dentistry Clinic (between 2001 and 2011) was carried out. Two different sets of data collection forms were used to extract information from articles and case notes.

6 longitudinal studies and 17 patients' records were included in this audit. Information on demographic features, clinical and radiographic findings and quality of data collection and reporting were assessed. The determining factor on managing infraoccluded primary teeth were the presence or absence of successor and degree of severity of infraocclusion. There was poor reporting of information and baseline recording in the case notes.

Clinical protocol / pathway and introduction of a pro forma to collect and document information are to be implemented for when a patient diagnosed with infraocclusion to improve treatment outcome and patient care. All cases would be benefit from multidisciplinary care involving both paediatric dentist and orthodontist.

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LIST OF ABBREVIATIONS

DPT	dental panoramic tomogram
EDH	Eastman Dental Hospital
GDP	general dental practitioner
LLD	lower left first primary molar
LLE	lower left second primary molar
LRD	lower right first primary molar
LRE	lower right second primary molar
LL4	lower left first premolar
LL5	lower left second premolar
LR4	lower right first premolar
LR5	lower right second premolar
ULD	upper left first primary molar
ULE	upper left second primary molar
URD	upper right first primary molar
URE	upper right second primary molar
UL4	upper left first premolar
UL5	upper left second premolar
UR4	upper right first premolar
UR5	upper right second premolar

1.0 BACKGROUND AND REVIEW OF THE LITERATURE

1.1 Introduction

Primary molars can be found with their occlusal surfaces inferior to the occlusal plane of the adjacent teeth. The affected tooth remains static while eruption of the adjacent teeth continues with vertical growth. This condition is known as infraocclusion. In the literature, there are various terms that have been used to describe this condition. These include submergence, secondary retention, , and arrested eruption. For the purpose of this audit, the term ‘infraocclusion’ will be used throughout the report. Clinically, infraocclusion of primary molars can vary from slight / mild degree to complete submergence of the tooth within the bone.

There is evidence available to link infraocclusion with other dental anomalies. A reciprocal association has been established between infraocclusion of primary molars with missing aplasia of second premolars, small size of upper lateral incisors, palatally impacted permanent maxillary canine and enamel hypoplasia (Baccetti, 1998; Bjerklin et al., 1992; Brown, 1981). The existence of these associations is clinically relevant, as early diagnosis of one anomaly may indicate an increased risk for others.

1.2 Prevalence

The prevalence of infraocclusion of primary molars varies among different populations. It has been reported to range from 0.6% to 24.8% (Andlaw, 1977; Darling and Levers, 1973; Koyoumdjisky-Kaye and Steigman, 1982b; Kurol, 1981; Kurol, 1984). An epidemiological study in Israeli children found that the frequency of infraocclusion varied among different ethnic groups (Koyoumdjisky-Kaye and Steigman, 1982a). Other studies showed that there is a higher incidence among Caucasians and Hispanics than blacks and Orientals (Albers, 1986).

1.3 Aetiology

The developing dentition of a child is characterised by constant movement of teeth in a vertical direction correlated to the growth and development of the face and the alveolar processes. The general concept is that the infraoccluded primary molar has formerly been fully erupted and in occlusion but for some reason the tooth is no longer capable of maintaining its position adjacent to other teeth in the developing dentition.

Currently, there is no consensus on the aetiology of infraocclusion. However, genetic predisposition is a well recognised factor with many children with infraocclusion in the primary dentition having siblings with the same condition (Kurol, 1981; Kurol, 1984; Winter et al., 1997). Excessive masticatory pressure, local trauma and infection (Adamson, 1952), disturbance of local metabolism (Biederman, 1962; Dixon, 1963), deficient eruptive force (Dixon, 1963), abnormal facial morphology and tongue pressure (Kurol and Thilander, 1984b) have also been suggested as possible aetiological factors of infraocclusion with ankylosis most commonly implicated.

Ankylosis is a well established and frequent association with infraocclusion of deciduous molars (Darling and Levers, 1973; Kurol and Magnusson, 1984), however the underlying factors and exact mechanism for the initiation of such process is unknown (Kurol and Thilander, 1984). Ankylosis occurs when cementum and dentine are not separated from the surrounding alveolar bone by the periodontal ligament and subjected to dynamic bone turnover. It has been postulated that ankylosis can occur as a result of a disturbance in the interaction between normal resorption and hard tissue repair which takes place in primary molars during eruption of successor teeth. Although this hypothesis might be valid, ankylosis is also found in primary teeth without successors.

1.4 Clinical and Radiographic Presentation

Infraocclusion is primarily diagnosed by clinical presentation and it can affect one to multiple numbers of primary molars at the same time. Generally, mandibular primary molars are affected more than 10 times as often as maxillary

primary molars (Kurol, 1981). The lower primary second molar is the most commonly affected by infraocclusion (Biederman, 1962; Dixon, 1963). There is no site predilection noted but infraocclusion may be unilateral or bilateral and can concurrently affect both upper and lower arches. The presentation of infraocclusion is ten times more common in the primary dentition than the permanent dentition (Dixon, 1963).

Infraocclusion of primary molars can be classified as slight, moderate or severe based on the relation (Ekim and Hatibovic-Kofman, 2001) of the involved tooth to the occlusal plane and to the adjacent teeth. Slight infraocclusion refers to when the entire occlusal surface is located approximately 1 mm below the occlusal plane of the neighbouring teeth. Moderate infraocclusion is defined as when the occlusal surface of the tooth is within the interproximal contact and severe infraocclusion is judged to be present when the occlusal surface of the deciduous molar is below the interproximal gingival tissue of one or both adjacent tooth surface.

Ankylosis can be assessed by percussion of the affected tooth. A high-pitched tone or metallic sound on percussion indicates at least 20% of the root surface has direct contact and fused to the surrounding bone. In addition, fusion between roots and the surrounding alveolar bone resulted in loss of physiological mobility (Biederman, 1962).

Radiographically, there may be obliteration of the periodontal membrane space or lack of definition of lamina dura which suggest ankylosis of the affected tooth.

1.5 Sequalae of infraocclusion of primary molar

Many potential sequelae are possible following infraocclusion, including delayed exfoliation (Kurol and Thilander, 1984b), impaction and / or delayed eruption of the permanent successor, lateral open bite (Becker and Karnel-R'em, 1992b), malocclusion (Becker et al., 1992) tipping of adjacent teeth (Becker and Karnel-R'em, 1992a), over – eruption of the opposing teeth (Kurol and Thilander, 1984b), caries and abscess formation (Teague et al., 1999b) of infraoccluded tooth.

1.6 Management

When considering treatment options for infraoccluded primary molars, the most influrential factor is the presence or absence of the permanent successor (Kurol and Koch, 1985; Kurol and Thilander, 1984a).

1.6.1 Successor present

Where permanent successors are present, most of the infraoccluded primary molar will exfoliate spontaneously. It has been recommended to await normal exfoliation, although it might be slightly delayed with continuous observation of the occlusal development. Degree of infraocclusion is not related to amount of delay (Kurol and Thilander, 1984a).

Unless there are definite indications for extraction e.g. fast progression of infraoccluded teeth or the affected teeth do not exfoliated within the accepted delay time of six months (Ekim and Hatibovic-Kofman, 2001), the primary molar should be left *in situ*. The need for a space maintainer and balancing / compensating extraction should be judge case by case (Rock, 2002).

In the literature, there are conflicting opinions whether infraoccluded teeth with permanent successor present should be restored or not (Kurol and Thilander, 1984b; Teague et al., 1999a). The aims of restoring these teeth are to maintain the occlusion and to prevent tipping of adjacent teeth. When the patient presented with mild to moderate infraocclusion without tipping of the adjacent teeth or overeruption of the opposing teeth should have the occlusal surface restored to prevent such consequences (Teague et al., 1999a). This can be achieved by using preformed metal crowns, direct composite restoration of the crown or even porcelain onlays.

1.6.2 Agenesis of successor

When the primary molar is infraoccluded and the successor is missing, root resorption is slow and spontaneous exfoliation is less likely. The present of such a condition will commonly cause disturbance in the occlusion due to insufficient alveolar bone support. It has been shown that recovery of bone only occurs in cases where a successor is present (Kurol and Olson, 1991).

The treatment options depend on patient's age, the condition and prognosis of primary molar, occlusion and patient and parents' preference. In young child, unless there is severe hypodontia, generalised spacing in the arch the ideal option is to extract the tooth and close the space (Lindqvist, 1980).

Early treatment is indicated with a severely affected deciduous molar and where there is evidence of possible future crowding in the arch. Tipping of the first permanent molar is common association with severely infraoccluded primary molar (Winter et al., 1997). This results in impaction of the primary molar and complicates the process of removal. In these circumstances, orthodontic up righting of the tipped permanent molar might be needed to facilitate removal of affected primary molar.

In selected cases, retaining the primary molar is also an option. However, the prognosis of the infraoccluded tooth is uncertain due to variable degree of root resorption and progression of infraocclusion (Lindqvist, 1980). If latter removal becomes necessary due to progression of infraocclusion, occlusion disturbance such as tipping of neighbouring teeth, severe overeruption, progressive root resorption or caries, the options are to either accept the space or space closure. Space closure can be achieved orthodontically or with fixed / removable prosthesis until definite replacement with implant.

As conclusion, management of infraoccluded primary molar is challenging. Currently there is yet a general consensus on management of the infraocclusion. A multidiscipline approach involving orthodontist and paediatric dentist is highly recommended in view of complexity of the case and to ensure best treatment outcome. The patients should be followed up and specific criteria for success outcome should be tailored individually.

2.0 AIMS AND STANDARDS

This clinical audit was undertaken to review both the literature and patient clinical records on the management of infraoccluded primary molars.

Therefore, the aims of this audit were:

i. To review the available literature on infraoccluded primary molars
ii. To review patients being managed for infraoccluded primary molars at Eastman Dental Hospital (EDH).
iii. To assess the quality of data collection during history taking, clinical examination, radiographic reporting and record keeping in EDH.
iv. To develop a clinical protocol for the management of infraoccluded primary molar teeth in children and adolescents based on the above.

The following standards should be met:

i. 100% clinical notes should have:
    - Family history of any associated dental anomalies.
    - Clinical classification of infraocclusion.
    - Report on the presence of the developing successor, root resorption and/or ankylosis.
    - Future treatment plan.
ii. In cases where no active treatment was indicated at that visit, records should be obtained as a baseline to monitor any progression of infraocclusion. These could be photos or study models.
iii. All cases should have been followed up or discharged to appropriate care.

3.0 MATERIALS AND METHODS

3.1 Literature Review

A search was done on Medline for the following search terms: ‘infraoccluded’, ‘infraocclusion’, ‘submerged’, ‘submergence’, ‘secondary retention’, ‘ankylosis’, ‘disinclusion’, ‘impacted’, ‘reimpaction’, ‘reinclusion’, ‘arrested eruption’ and ‘incomplete eruption’. The articles included in this audit were only limited to prospective or longitudinal studies in children (age 16 years and below at initiation of studies). A data collection form was developed and used to extract needed information from the articles (see Appendix 1).

3.2 Patient Notes Review

Over the last 10 years (between 2001 and 2011) a number of cases of infraoccluded teeth have been seen on the joint Orthodontic – Paediatric Dentistry Clinic. These cases were usually referred to the joint clinic for multidisciplinary input on the long – term management. The cohort of cases for this audit study was obtained retrospectively from the clinic lists and these notes were provided by the medical records department.

A data collection form was used to extract data from the clinical notes (see Appendix 2). The form was designed based on the information extracted from articles included in this audit. Information gathered included patients demographic, clinical presentation, reporting of radiograph, method of observation, treatment approach and treatment outcome. The overall quality of reporting of the clinical and radiographic findings was assessed. Quality was determined by the presence or absence of information.

4.0 RESULTS

4.1 Literature review on management of infraoccluded primary molars

4.1.1 Results of the search

A total of six longitudinal articles on management of infraoccluded primary molars met the criteria and were included in this audit (see Table 1). A total of 217 subjects were reported in these studies. The periods of observation were only reported in three articles (Kurol and Koch, 1985; Kurol and Magnusson, 1984; Kurol and Thilander, 1984a; Kurol and Thilander, 1984b), and ranged from 1 to 5 years.

4.1.2 Demographic and clinical features

When looking at all of the included studies, a total of 463 primary molars were found to be infraoccluded in 217 subjects. Out of these, 195 (42%) teeth had a permanent successor present and 268 (58%) had missing developing premolars. The lower second primary molar was the commonest tooth affected. Table 2 summarises the demographic and clinical features of all the studies, split by the presence or absence of a successor tooth. The primary maxillary molar to mandibular ratio for agenesis and presence successor group was 1 to 8.6 and 1 to 4.6 respectively.

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Table 2: List of articles included in the audit.

No.	Author(s)	Title of Article	Journal	Year/Volume/Page
1	Bodil Rune and Karl-Victor Sarnäs	Root resorption and submergence in retained deciduous second molars. *A mixed-longitudinal study of 77 children with developmental absence of second premolars*	European Journal of Orthodontics	1984/6/123 - 131
2	Jüri Kurol and Goran Koch	The effect of extraction of infraoccluded deciduous molars: A longitudinal study.	American Journal of Orthodontics	1985/87/46-55
3	Jüri Kurol and Birgit Thilander	Infraocclusion of primary molars with aplasia of the permanent successor.	The Angle Orthodontist	1984/54/283 - 294
4	Krister Bjerklin, Midea Al-Najjar, Henrick Kårestedt, Anders Andren	Agenesis of mandibular second premolars with retained primary molars. A longitudinal radiographic study of 99 subjects from 12 years of age to adulthood.	European Journal of Orthodontics	2008/30/254 - 261
5	Jüri Kurol and Birgit Thilander	Infraocclusion of primary molars and the effect on occlusal development, a longitudinal study	European Journal of Orthodontics	1984/6/277 – 293
6	Kirsten Ith-Hansen and Inger Kjær	Persistence of deciduous molars in subjects with agenesis of the second premolars	European Journal of Orthodontics	2000/22/239 - 243

Table 3: Demographic and clinical feature comparison of subjects with infraoccluded primary molars depending on presentation of successor premolar.

Demographic and clinical features	Successor	
	Agenesis n (%)	Present n (%)
Number of subjects	206 (74)	71 (26)
Gender		
Male	82 (40)	38 (54)
Female	124 (60)	33 (46)
Male to female ratio	*1 : 1.5*	*1.2 : 1*
Mean age at presentation (years)	12.0 ± 0.6	9.7 ± 0.3
Number of teeth affected		
Maxilla	28 (10)	35 (18)
Mandible	240 (90)	160 (82)
Maxilla to mandible ratio	*1 : 8.6*	*1 : 4.6*
Type of teeth affected		
Upper first primary molar	2 (1)	11 (6)
Upper second primary molar	26 (10)	24 (12)
Lower first primary molar	2 (1)	68 (35)
Lower second primary molar	238 (89)	92 (47)
First to second primary molar ratio	*1 : 66*	*1 : 1.5*

4.1.2.1 Ankylosis and tipping of adjacent teeth

i) *Agenesis successor group*

Ankylosis of the affected teeth was assessed clinically (mobility assessment and percussion test). All teeth were described to be ankylosed. Only 12 adjacent teeth were tipped.

ii) Successor present group

Ankylosis was clinically assessed in only 2 studies (Ith-Hansen and Kjaer, 2000; Kurol and Koch, 1985) involving 46 infraoccluded primary molar which all were ankylosed. One study made the assumption that infraoccluded teeth (123 teeth) were ankylosed (Rune and Sarnas, 1984) and one study did not record whether or not teeth were ankylosed (Bjerklin et al., 2008).

Tipping of adjacent teeth was only reported in one study (Kurol and Koch, 1985). In this study, tipping occurred when the affected primary molar had an infraocclusion of 4 mm or more. It was found that only 3 adjacent teeth were tipped.

4.1.3 Management

i) Agenesis successor group

When the permanent successor was absent, none of the primary teeth had exfoliated physiologically at the end of study period. 24 teeth (9%) however, were lost either due to caries, progressive root resorption or progression of infraocclusion (see Table 3).

Only 1 study (Ith-Hansen and Kjaer, 2000) attempted compensatory composite onlay to maintain occlusal contact with opposing tooth. This study showed that at reassessment appointment (15 years later), none of the opposing teeth were over-erupted and tipping of adjacent teeth were minimal.

ii) Successor present group

When the permanent successors were present, most of the infraoccluded teeth exfoliated spontaneously (97%). Only 3% were extracted either due to progression of infraocclusion or severe displacement of the developing premolar (see Table 3).

Infraocclusion progression was only noted in 24 out of 195 teeth (12%). The eruptions of the successors were reported within normal range regardless degree of severity of infraocclusion.

Table 4: Fate of infraoccluded primary molars

Fate of infraoccluded primary molars	Successor	
	Agenesis n (%)	**Present n (%)**
Retained	244 (91)	0(0)
Loss		
• **Physiological exfoliation**	0 (0)	189 (97)
• **Extraction**		
- Caries	5 (1.9)	0 (0)
- Severe progression of infraocclusion	7 (2.6)	2 (1)
- Replacement with implant	2 (0.7)	0 (0)
- Replacement with autotransplantation	2 (0.7)	0 (0)
- Severe displacement of successor	0 (0)	4 (2)
• **Progression of root resorption**	8 (3.0)	0 (0)
TOTAL	**268 (100)**	**195 (100)**

4.1.4 Base line records and observation methods for both groups

All subjects in these studies had radiographs in order to determine the status of developing successor. The types of radiographic exposure include dental panoramic tomogram or standardised intra – oral periapical radiographs. 83 subjects (38%) had dental cast made and 47% had intraoral photos taken as baseline records and as a tool to monitor the progression of infraocclusion

4.2 Clinical Note Audit

4.2.1 Demographic and clinical features

A total of 17 infraoccluded primary molars cases were seen in the Orthodontic – Paediatric Dentistry Joint Clinic in Eastman Dental Hospital from 2001 to 2011. These notes were retrospectively audited.

The overall age range of patients with infraoccluded primary molar teeth attending the joint clinic was 6 to 12 years old with a mean age of 9.8 ± 2.9 years. The ratio of male to female patients was 1 to 1.8. Table 4 and Table 5 show comparison between demographic and clinical features depending on the presence of permanent successor. 2 subjects had multiple infraoccluded primary molar with a mixture of both agenesis and present of developing premolars. The ethnicity distribution of patients is displayed in Figure 1.

It was found that 43 primary teeth were infraoccluded in 17 patients which ranged from 1 to 7 primary molar teeth with average of 2.5 teeth per patient. The ratio of maxillary primary molar to mandibular primary molar was 1 to 1.9. Irrespective of dental arches, the occurrence of infraocclusion was approximately twice more common in second primary molar compared to first primary molar but no site predilection was noted.

21 teeth (49%) had severe infraocclusion, 11 (26%) were moderate and 3 (7%) were slight. 19% of the infraoccluded teeth had no information of the severity in the notes.

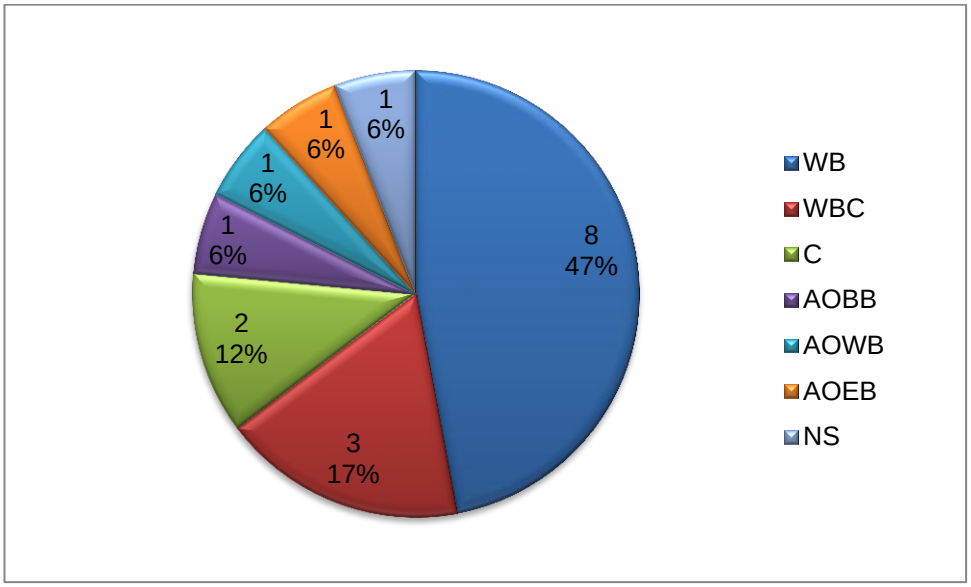
Only 1 patient was documented to have associated dental anomalies (peg shaped both upper lateral incisors). 3 notes clearly had information on the condition of adjacent teeth; 2 reported tipping of neighbouring teeth. A total of 6 teeth (14%) which were all second primary molar had missing successor.

Table 5: Demographic features of patients with infraoccluded primary molar seen in Joint Orthodontic – Paediatric Dentistry Clinic depending on presentation of successor premolar.

Demographic features	Successor		
	Agenesis n(%)	Present n(%)	Both (agenesis and present) n(%)
Number of subjects ($n_{subjets}$ = 17)	2 (12)	13 (76)	2 (12)
Gender			
Male	2 (100)	3 (23)	1 (50)
Female	0 (0)	10 (77)	1 (50)
Male to female ratio	*1 : 0*	*1 : 3.3*	*1 : 1*
Mean age (years)	11.2 ± 4.2	9.4 ± 2.7	11.4 ± 0.0

Table 6: Number and type of infraoccluded primary molars teeth seen in Joint Orthodontic – Paediatric Dentistry Clinic depending on presentation of successor premolar.

Clinical features	Successor (n_{teeth} = 43)	
	Agenesis Number of teeth (%)	Present Number of teeth (%)
Number of teeth affected		
Maxilla	2 (33)	13 (35)
Mandible	4 (67)	24 (65)
Maxilla to mandible ratio	*1 : 2*	*1 : 1.8*
Type of teeth affected		
Upper first primary molar	0 (0)	5 (13)
Upper second primary molar	2 (33)	8 (22)
Lower first primary molar	0 (0)	8 (22)
Lower second primary molar	4 (67)	16 (43)
First to second primary molar ratio	*0 : 6*	*1 : 1.8*



Legend

WB ***:*** *White British*

WBC ***:****White and Black Caribbean*

C ***:****Caribbean*

AOBB ***:****Any other Black background.*

AOWB ***:*** *Any other White background*

AOEB ***:*** *Any other ethnic background*

NS ***:****Not stated*

Figure 1: Ethnic distribution of patient with infraoccluded primary molar referred to joint clinic

4.2.1.1 Ankylosis and tipping of adjacent teeth

The status of ankylosis of the affected primary molar was unknown as none of the clinical notes documented it. Overall only 3 case notes recorded tipping of adjacent tooth (see Table 6).

Table 7: Tipping condition of adjacent teeth of infraoccluded primary molar according to the status of developing successor.

Tipping of adjacent tooth	Successor	
	Agenesis Number of tooth	**Presence** Number of tooth
• Yes	1	1
• No	1	-

4.2.2 Current practice at Eastman Dental Hospital

Majority of the patients, 71% were first seen in Paediatric Dental Clinic before referred to joint Orthodontic – Paediatric Dentistry Clinic.

4.2.3 Management and outcome

The following table (see Table 7) demonstrates the management of infraoccluded of primary molars seen in Joint Orthodontic – Paediatric Dentistry Clinic.

Table 8: Management of infraoccluded primary molar teeth seen in Joint Orthodontic - Paediatric Dentistry Clinic.

Management	Successor (n_{teeth} = 43)	
	Agenesis Number of teeth (%)	**Present** Number of teeth (%)
No active treatment	-	8 (22)
Extraction		
Local anaesthesia	3 (50)	5 (14)
General anaesthesia	-	23 (62)
Planned for extraction		1 (2)
Restoration	3 (50)	-
TOTAL	**6 (100)**	**37 (100)**

Out of 23 teeth removed under general anaesthesia, 5 teeth were surgically removed and the rest had forceps extractions.

4.2.4 Base line records and observation methods for both groups

All cases had radiographic assessment with dental panoramic tomogram (DPT). 3 cases had additional radiographic exposure (2 had long – cone periapical and 1 had bitewings). Only 1 case had study models of dentition made.

4.3 Quality of data collection and recording

Standard 1: 100% clinical notes should have:-

1a) Family history of dental anomalies

The notes were assessed for the quality of history taking, clinical and radiographic collection and recording. For history taking, all patients had their medical status assessed but only 2 patients (12%) had information on whether they had family history of dental anomalies or not. Only 1 clinical note (6%) had data of any other associated dental anomalies presented in the dentition.

1b) Clinical classification of infraocclusion

81% of infraoccluded molar were classified into degree of severity of presentation (slight / mild, moderate or severe). 3 clinical notes (18%) had the condition of adjacent teeth recorded.

1c) Radiographic reports on presence of developing successor, root resorption and ankylosis

The information of the presence of successor(s) was recorded in all clinical notes. However none of the notes had reported on presence or absence of ankylosis of the affected tooth. The root resorption of the infraoccluded molar was only reported in 3 notes (18%). 94% (16 cases) had no indices taken for baseline records to monitor any progression of infraocclusion.

1d) Future treatment plan

No clear outline of treatment plan was documented in any of patient notes.

Standard 2: Records should be obtained as a baseline to monitor any progression of infraocclusion. These could be photos or study models.

1 patient (6%) had cast made as record to monitor the progression of infraocclusion. No other method of observation e.g. clinical photograph or measurement of depression from occlusal plane was carried out.

Standard 3: All cases should have been followed up or discharged to appropriate care

8 patients (47%) had an ongoing review in either joint clinic, Paediatric Dentistry Clinic or Orthodontic Clinic. 4 (24%) had orthodontic treatment, 3 patients (18%) were discharged. Of these 2 patients were discharged to the referring clinicians (external referral) and 1 patient requested no further treatment and follow up. 1 patient (6%) had no further appointment arranged and 1 (6%) did not attended follow up appointment.

5.0 DISCUSSION

This audit encompasses a combination of review on published articles on the management of infraoccluded primary molar teeth and retrospective analysis of similar cases seen in Eastman Dental Hospital (EDH). Therefore, it has the advantage of observing and analysing the management of infraocclusion teeth from both sources. Currently there was no appropriate or standardised pathway on the management of infraocclusion in EDH. Apart from management, information on demographic features, clinical and radiographic findings were gathered for comparison and analysis.

In total 6 prospective studies and 17 cases which were seen in joint Orthodontic – Paediatric Dentistry Clinic were included in this audit. Data from each source were extracted using designated form (see Appendix 1 and 2). Review of the literatures provided common themes and pivotal information deemed necessary for management of such cases. From these findings, form to be used to gather similar information from case notes were designed and piloted.

Only a small number of infraoccluded cases were able to be identified from the joint clinic's patient list. At the moment, apart from this particular list, there was no centralised data which registers or identified patients with infraocclusion. Patients were only to be seen in this joint clinic when referral was made for multidisciplinary input. Therefore, it was very plausible that the number of cases included in this audit to be underreported and not a true representative of total number of patients who were actually treated for such condition from 2001 to 2011. Centralisation of the records would enable greater ease in patient selection should future audit involving infraocclusion be planned.

Overall, it was apparent that there was lack of standardisation in information collection and reporting in all aspects except on ethnicity and medical history in the case notes. A good reporting on this information was contributed by the use of designated pro forma. A pro forma is an effective tool to facilitate data collection as well as improved documentation and reporting important information (Davenport et al., 1995). This is to ensure a high quality of record keeping and improves the completeness of the recording of the assessment of

patient which has implications for clinical practice and future audit (Wallace et al., 1994).

Many case reports (Cozza et al., 2004; Dewhurst et al., 1997; Winter et al., 1997) and epidemiological data (Kurol, 1981; Kurol, 1984) had showed genetic predisposition and familial tendencies of infraocclusion between family members. It was very unfortunate however, this information was poorly reported in both case notes and articles involved in this audit. Although the questions related to family history on infraocclusion and any other dental anomalies might had been asked by the clinician during the initial visits, the failure to document the findings resulted in loss of valuable information in establishing any pattern of inheritance.

When comparing demographic features, the figures showed that the male to female ratio for both group (literatures and EDH's patients) was almost similar, with female had almost twice infraocclusion to male. The mean age was 11 year old in literature compared to younger population seen in EDH. It was not possible to establish any correlation between ethnicity and infraocclusion due to low number of cases available for the audit.

Majority of the cases referred to joint clinic had severe degree of infraocclusion. Although the prevalence of severe infraocclusion in the population were reported to be low (Winter et al., 1997), the finding of this audit would be contributed to small number of patient referred to the joint clinic. It was possible that the cases with lesser severity were not referred to the multidiscipline clinic as the treating clinicians were confident making treatment plan for the patients.

Tipping of adjacent first permanent molar was a common occurrence when the primary molar teeth had severe infraocclusion (Winter et al., 1997). However, similar finding could not be extracted from cases treated in EDH as insufficient information reported on such condition. Only 3 case notes (18%) had information of tipping of first permanent molar.

When the management of infraoccluded primary teeth was evaluated, only deductive conclusion could be drawn from the articles as only two studies had active interventions and the rest were case – control. The treatment plan would be either to extract or monitor the affected teeth. The determining factors were

the presence or absence of successor tooth, degree of severity and progression of infraocclusion.

In Eastman Dental Hospital, the majority of infraoccluded primary molar were extracted even if the permanent successor present. The reasons behind the decision were not documented in the case notes, however it can be postulated that this decision was based on the severity of the infraocclusion.

Baseline records are important to monitor the progression of infraocclusion and to appraise treatment outcome. These records provide objective comparison during review and follow up appointment rather than subjective clinical assessment. When case notes were assessed, apart from radiograph assessment, there was no information on other forms of records was taken by the clinician except in 1 case. In all cases, DPTs were taken as adjunct to clinical findings and as a screening tool to assess the developing dentition. This radiographic view however, could not be used as baseline record due to lack of standardisation in positioning the subjects relative to the radiation source thus resulting dissimilar films (Becker and Karnel-R'em, 1992a).

The audit showed that there were no predetermined success criteria in both articles and case notes included in this audit. There was also no consensus on follow up periods. This problem could be avoided if there was a standardised guideline on the management of infraocclusion of primary molar. The guideline should determine the success criteria and reasonable follow up period should be for each condition (present and agenesis of successor).

One of the significant finding of this audit was the poor reporting quality of the case notes. The absence of relevant information on family history, clinical and radiographic findings, decision making process and treatment outcomes resulted in inadequate data to measure treatment success. These valuable data were also important for research and audit purposes.

The current practice on management of infraoccluded primary molar in Eastman Dental Hospital did not achieve the standard set for this audit. It was apparent that there were lacking in all aspects in management of such cases from record keeping, baseline recording and follow up appointment. Therefore, an action

should be taken to improve the inadequacy in both management of infraocclusion and data collection and reporting.

5.1 Action Plan

A suggested protocol would be having a system that would allow all patients who are presented with infraocclusion to be referred to the joint orthodontic – paediatric dentistry clinic for assessment. This would allow multidisciplinary input and planning of short and long-term treatment with appropriate follow up in the department or shared care with the general dental practitioner.

A suggested pathway on the management of new cases with infraocclusion of primary teeth is outline in Figure 2. The patients should be assessed using a designated newly developed pro forma (see Figure 3) to ensure high quality of data collection and documentation. This pro forma will be available in the clinic and to be used when patient presented with infraocclusion identified.

Suggestion of treatment pathway

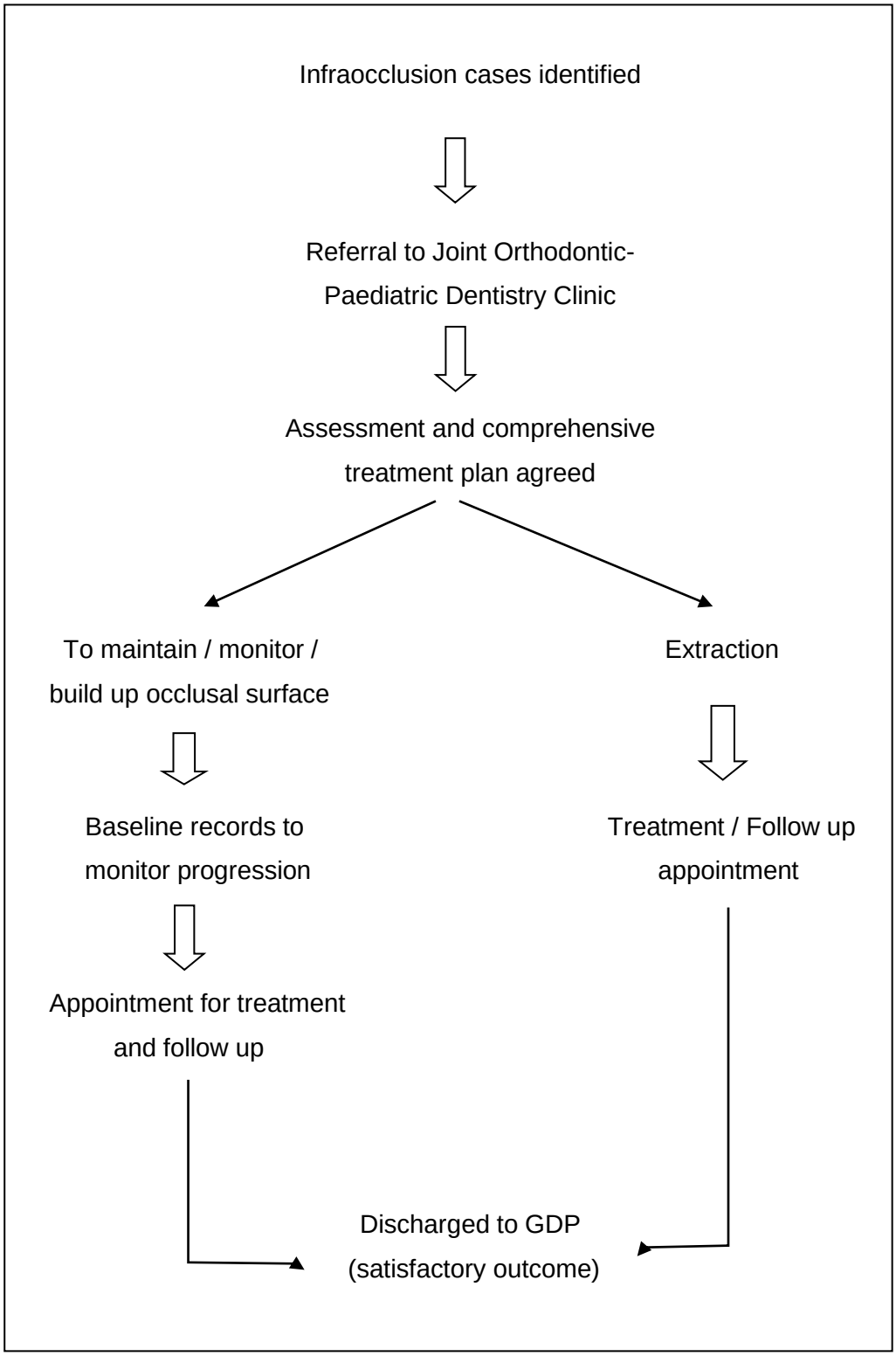


Figure 2: Flowchart to illustrate suggested pathway for management of infraoccluded primary molar in Eastman Dental Hospital.

Suggestion of Clinical Pro forma

Infraocclusion Clinical Pro forma

Patient's Sticker

Demographic Data

- Gender: Male / Female
- Age of first presentation: _____
- Family history of dental anomalies : Yes / No

  If yes: _____

Clinical Presentation

- Referred by: Orthodontic / Paediatric Dentistry / Other_____
- No of teeth affected: _____
- Type of teeth and stage of infraocclusion

	Maxilla				Mandible			
Types of teeth	URE	URD	ULD	ULE	LLE	LLD	LRD	LRE
Stage of infraocclusion								

*Stage of infraocclusion → **S**: Slight **M**: Moderate **Se**: Severe*

- Tipping of adjacent teeth: Yes / No
- Other dental anomalies: Yes / No

  If yes: _____
- Evidence of ankylosis: Yes / No

Continue

Radiograph

- Radiograph taken: DPT/ Periapical / Other _____
- Root resorption: Yes / No

  *If yes:*_____
- Premolar Successor:

	Maxilla				**Mandible**			
Types of teeth	UR5	UR4	UL4	UL5	LL5	LL4	LR4	LR5
Presence **(P)** / Agenesis **(A)**								

Records and Treatment Plan

- Methods of observation:
  - ☐ Clinical pictures
  - ☐ Study model
  - ☐ Sequential (standardised) radiograph
  - ☐ Other _____
- Treatment Plan:

  _____

  _____

  _____

  _____

  _____

  _____

Figure 3: Pro forma for history, clinical and radiographic finding of infraoccluded primary molar cases

6.0 CONCLUSION

Appraisal of the literatures showed that the presence or absence of successor, and degree of severity of infraoccluded tooth determines the treatment. In Eastman Dental Hospital, a joint Orthodontic – Paediatric Dentistry Clinic offers multidisciplinary approach of such cases to provide a comprehensive care for the patients. Although this service was available, there was no standardised clinical pathway or protocol in managing paediatric patients with infraoccluded primary molar. Essential information was noted absence from the case notes. A new clinical protocol and infraocclusion pro forma is required to improve data collection, reporting and improve patients care.

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APPENDICES

Appendix 1: Data extraction form for the articles

Article Detail

- Article No:
- Article Title:
- Journal:
- Year/Volume/Page:

Demographic Data:

- No of subjects:
- Age/ Mean age:
- Gender: _____ Male _____ Female
- Ethnicity:
- Family history of dental anomaly:
- Medical history:
- Period of observation:

Clinical Presentation

- No of teeth affected:
- Type of teeth affected:

Maxilla				Mandible			
URE	URD	ULD	ULE	LLE	LLD	LRD	LRE

- Successor premolar:

Maxilla				Mandible			
UR5	UR4	UL4	UL5	LL5	LL4	LR4	LR5

- Tipping of adjacent teeth: Yes / No

Continue

Radiographic and observation:

- Type of radiograph taken:
    - DPT ☐
    - Standardised periapical ☐
    - Other ☐ _____
- Root resorption : Yes / No
- Ankylosis: Yes / No
- Methods of observation:
    - Study model ☐
    - Clinical photograph ☐
    - Other ☐ _____

Approach and outcome

- Approach:

  _____

- Outcome:

  _____

Figure 4: Data extraction form for the articles

Appendix 2: Data extraction form for the clinical notes

Patient's Sticker

Demographic Data

- Gender: Male / Female
- Age of first presentation: _____
- Ethnicity:

White
- ☐ British
- ☐ Irish
- ☐ Any other White Background

Black or Black British
- ☐ Caribbean
- ☐ African
- ☐ Any other black background

Asian or Asian British
- ☐ Indian
- ☐ Pakistani
- ☐ Bangladeshi
- ☐ Any other Asian background

Other Ethnic Group
- ☐ Chinese
- ☐ Any other ethnic group

Mixed
- ☐ White and Black Caribbean
- ☐ White and Black African
- ☐ White and Asian
- ☐ Any other mixed background

☐ Not Stated

- Family history of dental anomalies : Yes / No / Not recorded

  If yes: _____
- Medical history: Yes / No

  If yes: _____

Continue

Clinical Presentation

- First seen in: Orthodontic / Paediatric Dentistry
- No of teeth affected:

	Maxilla				Mandible			
Type of teeth	URE	URD	ULD	ULE	LLE	LLD	LRD	LRE
Stage of infraocclusion								
Successor								

Stage of infraocclusion → ***S****: Slight* ***M****: Moderate*
Se*: Severe* ***NR:*** *Not recorded*

Successor → ***P****: Present* ***A****: Agenesis* ***NR:*** *Not recorded*

- Tipping of adjacent teeth: Yes / No / Not recorded
- Other dental anomalies: Yes / No / Not recorded

  If yes: _____
- Evidence of Ankylosis: Yes / No / Not recorded
- Radiograph taken: DPT/ Periapical / Other _____
- Root resorption: Yes / No / Not recorded
- Methods of observation:
  - ☐ Clinical pictures
  - ☐ Study model
  - ☐ Sequential (standardised) radiograph
  - ☐ Other _____

Treatment and Outcome

- Treatment:

  _____

- Outcome and follow up:

  _____

Figure 5: Data extraction form for the clinical notes

Appendix 3: Audit registration form

Received by Clinical Governance: ☐

Eastman Dental Hospital Clinical Audit - Project Registration Form

Project Title: An Audit on Management of Infraoccluded Primary Molar in Children		
Start Date: 1 March 2011	**Expected End Date:** 1 March 2012	**Department:** Paediatric Dentistry
Project Lead: Mohd Ridzuan Mohd Razi	**E-mail:**mohd.razi.09@ucl.ac.uk	**Phone:** 02079151022
☐ **Approved by Departmental Audit Lead (_____)**	☐ **Approved by Departmental Clinical Lead (_____)**	
Other Personnel Involved (please include contact details)**:** Dr Paul Ashley (p.ashley@eastman.ucl.ac.uk)		
Reasons for audit: (*e.g.* It is thought that practise can be improved; Response to feedback/complaint; validation against recently published guidelines; Recent changes in law, etc.) ▪ To evaluate the efficacy of current practice		
Aims and objectives with an overview of the project: ▪ To review both the literature including case reports on the management of infraoccluded teeth and cases managed at the Eastman Dental Hospital. ▪ To develop a clinical protocol for the management of infraoccluded teeth in children and adolescent.		
Standards: This is what your audit will measure your current activity against (e.g. 90% of patients are seen within 10 minutes of their appointment time; Failure of an implant is defined as…etc.) ▪ All cases of infraocclusion should be referred to multidiscipline team for definitive treatment planning. ▪ All cases of infraocclusion should be monitored for treatment progress.		
Materials and method for measuring against standards (include sample size & timescale): (*e.g.* A questionnaire to be completed prospectively by the clinician/patient; Patient interview) ▪ Proforma (see protocol), ▪ Timescale : one year		
Interdepartmental audit:	☐ **No**	☑ **Yes,** specify departments**:** Orthodontic, Hypodontia
Multi-professional audit:	☑ **No**	☐ **Yes,** specify professions**:**
Type of audit project:	☐ **Structure**	☑ **Process** ☐ **Outcome**
Scope of audit project: (tick one or more)	☐ **National**	☐ **Regional** ☑ **Local**
Does this project link with research?	☑ **No**	☐ **Yes,** give details**:**
Does this project link with the clinical audit priorities in the annual report?	☑ **No** If No, please give good reason for audit:	☐ **Yes**, please specify: Currently, there is no robust evidence on management of infraoccluded teeth in children

Audit Office support required: (*e.g.* project design, database creation, Formic)	☑	**No**	☐	**Yes,** please specify**:**

Figure 6: Audit registration form