

EXTRACTABLES AND LEACHABLES FROM POLY- ϵ -CAPROLACTONE/ PHOSPHATE
BASED GLASS COMPOSITES

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ABSTRACT

In this study poly- ϵ -caprolactone and iron containing phosphate based glass composites have been investigated in terms of their degradability, structural changes and potential for fracture fixation devices. Any material being used within the body requires Regular acceptance. A key aspect of this is safety and a toxicological assessment of any extracted or leached species. As a crucial part of the project, PBG fibres were assessed for extractables and leachables. Weight loss was also investigated in order to assess the durability with increasing iron content. The following glass fibre compositions were studied: 50P45Ca5Na, 50P35Ca5Na5Fe and 50P30Ca5Na10Fe. They were then fabricated into composites, with PCL, by compression moulding and stored in pH4 and pH7 buffer, krebs solution, saline solution and water for 78 weeks at 37°C. The resulting composites, and their corresponding solutions, were assessed for extractables and leachables, weight change, pH shifts, porosity and pore size, thermal properties and structural changes.

It was demonstrated that the addition of 10%w/w iron into the PBG structure increased the durability by an order of magnitude due to the iron acting as a network former within the P-O-P backbone. The order of leaching was phosphate > sodium > calcium > iron. This was explained by the amount of the oxides originally present in the PBG structure, breakage of the phosphate backbone within the PBG, divalent calcium ions forming chelate cross links which decreases the dissolution rate compared to sodium and Fe-O-P bonds being more resistant to hydration than P-O-P bonds. The weight loss for 50P35Ca5Na10Fe was a third less than the other two PBGs and the weight loss for the equivalent composite was half of the non-iron containing composite. The leaching rate of the 10%w/w iron containing composite was approximately half that of the non-iron containing composite. Unpolymerised caprolactone was observed, alongside 2 oligomers, for the organic leachables from the PCL component of the composites. The density of the PCL/PBG composite was greater than for PCL alone. As iron was added to the PBG, within the composite, it further increased due to the Fe-O-P bonds within the PBG structure which have a dual nature of tetrahedral and octahedral coordination. Differential Scanning Calorimetry (DSC) showed a decrease in

crystallinity and glass transition (T_g) when PBG was added to PCL but an increase in melting point due to cross linked density of the PBG network. After storage in aqueous media the crystallinity and T_g increased which could partially be attributed to realignment of the polymer chains within the PCL under storage in aqueous media at 37°C.

The amount of iron in the glass structure extended the time for dissolution. Elemental analysis showed that the presence of iron within the composite reduced the leachables rate. Hence, the accumulation of the ions in a local area is lowered due to fewer ions being released over the same time period for iron containing PBG. The leachables were within acceptable toxicological limits and the amount of calcium present (within the PBG) is beneficial for the bone healing process. Hence, this material is suitable for use in a bone fixation device.

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LIST OF ABBREVIATIONS

6-HCA	6-hydroxycapronic acid
ASTM	American Society for Testing Materials
BHT	Butylated hydroxyl toluene
CDRH	Centre for Devices and Radiological Health
D	Dextrorotatory
DSC	Differential scanning calorimetry
DTA	Dynamic thermal analysis
Ea	Energy of activation
EC	European Commission
EU	European Union
FDA	Food and Drug Administration
FTIR	Fourier transfer infrared (spectroscopy)
GC	Gas chromatography
GPC	Gel permeation chromatography
GPa	Giga Pascals
HAP	Hydroxyapatite
HBSS	Hanks buffered saline solution
HMWPE	High molecular weight polyethylene
ICP-OES	Inductively coupled plasma/ optical emission spectroscopy
IPAC-RS	International Pharmaceutical Aerosol Consortium on Regulations and Science
ISO	International Standards Organisation

ITFG	Inhalation Technology Focus Group
kCal	Kilo calories
KJ	Kilo Joules
L	Levorotatory
LD ₅₀	Lethal Dose for 50% of the population
Mn	Number average molecular weight
MS	Mass spectrometry
Mw	Weight average molecular weight
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
OEL	Occupational exposure limit
OINPDS	Orally inhaled and nasal drug products
PBG	Phosphate based glass
PBS	Polybutylene succinate
PCL	Poly-ε-caprolactone
PDLLA	Poly-D-L-lactic acid
PDS	Polydioxanone
PEEK	Polyether ether ketone
PET	Polyethylene terephthalate
PG	Phosphate glass
PGA	Polyglycolic acid
PHB	Poly(3-hydroxybutyrate)
PLA	Polylactic acid
PLGA	Polylactide-co-glycolide
PP	Polypropylene
PPC	Polypropylene carbonate

ppm	Parts per million
PQRI	Product Quality Research Institute
PTFE	Polytetra fluoro ethylene
PU	Polyurethane
PVA	Polyvinyl alcohol
SASX	Small angle x-ray scattering
SEM	Scanning electron microscopy
SCOGS	Select Committee on Generally Regarded as Safe Substances
SCT	Safety concern threshold
SOP	Standard Operating Procedure
Std. dev	Standard deviation
$T_{1/2}$	Half life
T_c	Temperature of crystallisation
T_g	Glass transition temperature
T_m	Melting temperature
TGA	Thermogravimetric analysis
T_m	Melting point
TPS	Thermoplastic starch
UHMWPE	Ultra high molecular weight polyethylene
XRD	X-ray diffraction
μm	Microns

CHAPTER 1 – INTRODUCTION

1.1. Background

Bone augmentation has been conducted for many years and can be required for several reasons. These can range from development of benign bone tumours, osteoradionecrosis, congenital abnormalities or from an injury which has broken or shattered existing bone [1-8]. It is of interest in the dental field too for reasons such as tooth development defects and an injury to the face, etc. [9-11]. Although human bone is available, and can be grafted with successful results, it is not a freely available commodity [12, 13]). There can be potential problems such as contour irregularities, disease transmission, infection and introduction of foreign material [14-17].

In the case of broken bone, a fracture fixation device is used to stabilize the fracture, enable faster healing of the injured bone and return early mobility and full function of the injured extremity. Internal fracture treatment consists of closed reduction to restore the bone alignment. External stabilization maybe achieved with traction or external splinting by splints, or casts. The numerous devices used for internal fixation include: wires, pins and screws, plates, and intramedullary nails or rods. Staples and clamps are also used occasionally for osteotomy or fracture fixation [18].

The ultimate aim for a biomaterial is to restore the structural integrity of damaged bone and in order to accomplish this a comprehensive understanding of the material properties, device design and physiological requirements are required. The selected materials must incorporate chemical and mechanical demands of the biological environment for the appropriate functional outcome. Such demands include in vivo performance, practical manufacturing, marketing requirements, reasonable cost, ability to be fabricated and ease of surgical fabrication.

1.2 Biomaterials for Implantation

Historically metals have been the most popular material for implantation. Metallic materials, such as 316L stainless steel, cobalt chromium, nickel, titanium, and titanium alloys, demonstrate an acceptable combination of strength, ductility,

corrosion resistance, and biocompatibility. However, there are limitations of these materials. Metal implants, including pins, have the potential to shatter existing bone if the bone is already weakened or has too small a surface area to spread any stress during application. Metal implants are not easily formed, and can protrude if implanted close to the skin's surface. Due to metal being such a good thermal conductor it can heat up and cool quickly causing discomfort to the patient. The majority of metals are not suitable as implants due to their poor tolerance and even minimal dissolution products can cause toxicological problems within the body. Growth restrictions, pain and implant migration are all potential problems when using metals [26].

Polymers materials may also offer selected opportunities as implant materials [19, 22-24]. A wide variety of materials have been studied for implantation activities. These include polyurethane (PU), acrylics, polyether ketone and polypropylene (PP) with collective characteristics such as fatigue resistance, sterilizability, corrosion resistance, suitable integration with surrounding tissue, strength, flexibility and good fracture resistance [175, 179,197]. There can however be disadvantages of using polymer materials which include lower modulus, potential for distortion, wear, autoimmune disease and non- bioresorbability. Studies using high molecular weight polyethylene (HMWPE) and polytetrafluoroethane (PTFE) have been conducted which have shown wear and creep issues to form a mechanochemical deterioration, in vivo, that is analogous to fretting corrosion in metals [23].

Advantages of using biodegradable polymers negates the need to remove the fracture fixation device and temperature sensitivity should not be a problem. There have been a number of biodegradable polymers approved for safe internal use for surgical applications over the last 35 years. They include Dexon (polyglycolic acid, (PGA)), Vicryl (PGA/ polylactide (PLA)), PDS (polydioxanone) and Maxon (PGA/ Trimethylene carbonate (TMC)). The commonly used biodegradable polymers are created from L Lactide, D Lactide and Glycolide. There is over 20 years of successful clinical history with screws, pins, plates, membranes, anchors, dowls, rods, delivery devices and scaffolds [26]. However, there can be disadvantages of using such polymers which include degradation being too quick which can cause surrounding tissue reactions or degradation being too slow yielding little advantage over other materials, such as metal. Biodegradable polymers such as polyglycolide or lactide-

glycolide copolymer have been used in the form of rods in the internal fixation of a variety of fractures and osteotomies in patients. However, in one particular clinical study a foreign-body reaction occurred in 7.9% of patients, producing a fluctuant swelling at the implantation site after on average of 12 weeks. The reaction was believed to be from liquid remnants of the degrading implants and the local capacity of tissue to clear the polymeric debris [25].

To solve these types of issues scientists, physicians and engineers are continually investigating and developing new materials for replacement and repair.

1.3 Poly- ϵ -caprolactone and PBG as a Potential Biodegradable Fracture Fixation Material

Poly- ϵ -caprolactone (PCL) is a member of the aliphatic polyesters and is a biodegradable, bio-absorbable material. It is Food and Drug Administration (FDA) approved and due to its properties this makes it a good candidate material for fracture fixation and for controlled drug release [28-32]. In particular, it has an acceptable toxicological profile and is susceptible to hydrolytic and enzymatic chain cleavage leading to α -hydroxy acids generally metabolised in the human body [80]. The reason for pursuing PCL is due to the extended biodegradation time in comparison to other polyesters such as PLA and PGA, which have been popular biodegradable materials to date. Typically the degradation time for PCL is 2 years [28] but this can also be 3-4 years for complete loss of material [221]. PCL is of particular interest for non-loadbearing applications such as maxillofacial reconstruction due to its modulus and yield strength [35].

The tensile strength for bulk (>95% density) PCL can range from 10.5 to 16.1 MPa and modulus from 343.9 to 364.3 MPa. Tensile yield strength 8.2 to 10.1MPa [37]. The tensile strength of bone ranges from 149MPa for the radius to 3.7 MPa for the vertebrae in the lumbar region [38]. It is therefore, beneficial for PCL to be reinforced with materials such as PBG to improve its mechanical and physical properties [39-41]. A key advantage of adding PBG to PCL for a fracture fixation device is the increased osteoconductive nature due to the presence of calcium [20]. PBG is primarily composed of sodium (Na), calcium (Ca) and phosphorous (P).

however, several other elements such as aluminium (Al), silver (Ag), boron (B), cobalt (Co), chromium (Cr), iron (Fe), potassium (K), magnesium (Mg), silicon (Si), titanium (Ti), have been studied [75-79]. The presence of iron in PBG is known to stabilise the structure and decrease the degradation rate which will reduce the localised leachable ions within a given area [69, 409]. The addition of metal ions to the basic calcium phosphate glass structure can raise concerns about the toxicology and safety aspects of PBG. Hence, extractables and leachables are monitored for toxicological and safety purposes.

1.4 Extractables and Leachables

The definition of an extractable is [42]:

A species which can be extracted from a material under extreme conditions such as elevated temperature and using aggressive means over a given time. Hence, simulating the worst possible case conditions.

The definition of a leachable is:

A species which can be extracted from a material under normal environmental conditions over time.

Hence, an extractable is a species which is extracted from a material under ‘aggressive’ conditions in a reasonably short time period (e.g. 48 hours) and a leachable is a species observed over the ‘normal’ service life of a material. These parameters can be monitored to establish dissolution rates of the material and hence, the suitability of PCL/ PBG composite as a potential fracture fixation material. The degradation rate vs. bone formation is very important. As the biomaterial degrades, the rate at which the osteoblasts form, attach and proliferate is key to a successful fracture fixation operation. The amount and rate of leachables, from any material which is implanted into the body, also has to be monitored to ensure biocompatibility with no acute adverse reactions or toxicological issues. Hence, a toxicological assessment is vital for any fracture fixation biomaterial to determine safety thresholds [43-45, 266-284].

There have been many studies conducted on mechanical, chemical and cytotoxic aspects of PLA with PBG [46-52] and PGA/ PLA combinations [53- 59]. There have been a variety of studies conducted on the mechanical behaviour of PBG [60-63], PCL blends [64, 65] and PCL/ PBG composites [40, 463]. Leachables from PBG [68-71] and from the degradation products of PCL [51, 67] have been documented. However, there are limited studies on the leachables from PCL/ PBG composite materials [40, 72-74]. No papers have been found on extractables from PCL/ PBG or the leaching of PCL oligomers from such composites. Any ion release studies have been conducted over minimal time periods, the maximum being 5 weeks [40]. Hence, if such materials are to be used as fracture fixation devices for bone repair where the materials will be in the body for months, there is a need to investigate the extractables, leachables and structural changes, that may occur over such extended time periods.

1.5 Aims and Objectives

The objective of the thesis is to manufacture three novel PCL/ PBG composites and determine whether the composites studied are suitable fracture fixation materials for potential bone repair. Three different PCL/PBG composites will be fabricated by altering the amounts of iron present in the PBG formulations.

- i. $50\text{P}_2\text{O}_5\text{-}35\text{CaO-}5\text{Na}_2\text{O-}10\text{Fe}_2\text{O}_3$
- ii. $50\text{P}_2\text{O}_5\text{-}40\text{CaO-}5\text{Na}_2\text{O-}5\text{Fe}_2\text{O}_3$
- iii. $50\text{P}_2\text{O}_5\text{-}45\text{CaO-}5\text{Na}_2\text{O}$

The aim is to determine the effect of iron concentration on the dissolution rate of the composites, the leachables and extractables profile will be investigated and the resulting structure of the composites will be examined after storage in various aqueous leachables media.

The composites will be in the form of a sheet of 90%w/w compressed PCL and 10%w/w PBG fibres. Due to the biodegradable nature of the PCL and PBG, it is expected that as they degrade a porous material will emerge. Long term storage of such composites types (in aqueous media) has not been reported, to date, and an

understanding of how the material behaves over this time period is crucial for fracture fixation devices. The composites will be stored in aqueous media for 78 weeks. After storage the resulting solutions will be analysed using a variety of techniques. Similar analytical techniques, to those mentioned in the literature, are used to qualify and quantify these changes. Density will be measured using a piece of equipment based on air volume difference as opposed to an ethanol immersion liquid (as documented in the literature) due to it being non-destructive and a quick/ easy method. Inductively coupled plasma (ICP) will be used for determination of leached/ extracted ions from the glass (instead of ion chromatography as commonly used in the literature) due to established methods, accuracy and ease of use.

CHAPTER 2 – LITERATURE REVIEW

2.1 Introduction

This thesis investigates three composites of PCL/PBG biomaterial for potential fracture fixation. This chapter presents a detailed review of the literature, beginning with a review of current biomaterials, their uses, advantages, limitations and regulatory requirements. The following three sections then focus on PCL, PBG and the two materials combined as a composite.

PCL has been recognised as a biodegradable polymer since 1973 [81] and research continues to investigate the degradation rate to match the growth rate of bone [82-84]. PBG has been recognised since the 1800s and considered as a biomaterial due to its osteoconductive nature and chemical composition of calcium and phosphorous, found in hydroxyapatite (a major constituent of bone) [86-89]. By combining these two materials there is potential to alter the dissolution rate to be in line with bone regrowth and to limit the amount of degradation products in a localised area.

It is important to understand the structure, properties, characteristics and behaviour of these materials. The pharmaceutical industry enforces strict controls over materials being used as implants and drug products [43, 44]. Extractables profiles are required and leachables are monitored during Phase III stability studies or performed as accelerated testing from Phase I if deemed necessary. The types of species which leach or extract from the composites will be organic (from the polymer) and inorganic (from the glass), processing and manufacturing aids can also be monitored. Their quantities and rates of leaching/ extracting can affect the strength of a potential fracture fixation device and the actual end result of the bone re-growth. The types of species detected and their amounts need to be monitored to ensure there are no toxicological issues. Degradation rates and pathways can indicate whether a composite is a suitable candidate for implantation and understanding this can lead to a

more suitable material design [95]. Density and morphology changes, porosity and pore size, also contribute to the strength and potential for osteoblast adherence to a fracture fixation device [91, 92]. Thermal properties are important factors to monitor in order to understand physical, chemical and biological properties of a material which could impact residual cure and setting times, stability, shrinkage, expansion, effects of sterility methods and storage conditions. Thermal behaviour can intrinsically be linked to morphology and clinical performance and provides a valuable insight into oxidation and crystallisation behaviours [93, 94]. Investigations into potential structural changes, after storage of materials in aqueous media, will also enhance the Scientist's or Engineer's understanding of the materials in order to produce the most appropriate fracture fixation device material for the body [47].

2.2 Biomaterials

A biomaterial is a material that interacts with human tissue and body fluids to treat, improve or replace anatomical element(s) of the human body. Biomaterial devices used in orthopaedics are commonly called implants [27].

Key factors which are important for an implant material are shown below [97, 98]:

- i. Biologically compatible
- ii. Chemically compatible
- iii. Mechanically compatible
- iv. Non-toxic
- v. Non carcinogenic
- vi. Suitable strength and function
- vii. Excellent resistance to degradation (e.g. corrosion resistance for metals or resistance to biological degradation in polymers).
- viii. Acceptable load bearing, transmission and distribution, if required.
- ix. High wear resistance to minimise wear debris generation.
- x. Articulation if required (for example an artificial knee replacement)
- xi. Amenability to engineering design, manufacturing and sterilisation.

Numerous materials have been examined as potential biomaterials. They include biomimetic composites, composites with biological macromolecules, ceramics, ceramic polymers, metals, glass and polymers.

2.2.1 Biomimetic Composites or Composites with Biological Macromolecules

The advantages of using these types of materials is that the body is less likely to reject them as they develop specific interactions with living systems [99, 100]. The types of compound used for these materials include HAP, collagen, gelatine, bone Gla protein (osteocalcin) which may be used alone or mixed with a polymer [101-103]. There is also continued research into biomimetic nanocomposites for bone graft applications along with patent filings [104-106].

2.2.2 Ceramics and Bioglass

Ceramics (inorganic, non-metallic compounds) have been used as biomaterials due to their high strength, good biocompatibility, stability in the physiological environment, osteoconductivity, and low stress shielding [98, 107,108, 110-115]. Table 2-1 provides information on these types of materials and some of their uses.

Ceramics and Bioglass		
Ceramic	Use	Reference
Alumina	Wear surfaces, femoral head for hip replacement, drug delivery	[116-119]
Zirconia	Ball heads, orthopaedic repair, dental implants	[118, 120-123]
Calcium phosphate (hydroxyapatite)	Hard tissue replacement, ligament stabilisation	[124-126]

Table 2-1 Uses of alumina, zirconia and calcium phosphate as biomaterials

Bioglass was introduced in the early 1970s and different forms of these implant materials have been studied in animals and humans [128-132, 137]. It is essentially a ceramic with an elevated concentration of glass phase [127]. It is silica based with phosphate, calcium and sodium. The ratio of Ca/P is at least 5, as lower ratios do not bond to bone. More recently there has been evidence for use in the repair of

periodontal bone defects, ridge preservation and sinus augmentation [133,134]. Although they are biocompatible and exhibit bone bonding they require osteogenic growth factors as a possible coating to enhance this [135,136].

2.2.3 Metals

Metals such as stainless steels, titanium, titanium alloys, and cobalt chromium alloys are used for orthopaedic and dental surgery [138-140]. Type 316L stainless steel is commonly used in surgical procedures to replace biological tissue or to help stabilise a biological structure such as bone tissue to aid the healing process. Other metals can be added to stainless steel in order to make it more corrosion resistant. These include chromium, nickel, molybdenum and carbon [33]. The ferrite element is not incorporated into stainless steel because it can create magnetic properties. Titanium is less dense than stainless steel but has very good strength and is ideal for reconstructive surgery. The most common forms of titanium are made of aluminium and vanadium or the aluminium and niobium combination which are typically used as manufacturing rods and spinal clamps. However, there are problems with metal implants including stress shielding, bone weakening under load and difficulties in matching implants to corresponding bones. Table 2-2 shows some advantages and disadvantages of metal implants.

Metal Implants

Metal	Advantages	Disadvantages	Reference
Cobalt chromium	Higher corrosion resistance than iron based alloys, fatigue and cracking resistance	Can have low ductility and fatigue fracture if Nickel, carbon and nitrogen are not added.	[145, 147-149]
Stainless steel	Machinability, low impurities, high strength and ductility	Erosion due to inter-granular corrosion, pitting and fretting, *fatigue life and *biocompatibility. Can be limited to fracture plates, screws and hip nails due to corrosion when in highly stressed and O ₂ depleted region.	[144-146]
Titanium and Titanium alloy	High strength, low weight, low modulus of elasticity (~60GPa), limits the amount of stress on bone structures, excellent corrosion resistance and less prone to immune reaction. Endures a high strain during internal fixation.	Low fracture toughness, poor wear properties.	[33, 150]

*Compared to other alloys.

Table 2-2 Typical list of advantages and disadvantages of metals such as cobalt chromium, stainless steel, titanium and its alloys when used as metal implants

The demand for implant materials has attracted researchers to develop newer biomaterials or improve the performance of existing biomaterials in terms of better biocompatibility, corrosion resistance, lower density, lesser wear and osseointegration [141-143].

2.2.4 Polymers

There are numerous polymers available as implantation materials. Their advantages, include biocompatibility, stability, customisability, flexibility, mouldability and

favourable dissolution properties (in the case of biodegradable materials) [74, 151]. Their weaknesses, in some cases, are lower modulus and being non-bioresorbable. They can have a high risk of autoimmune disease, wear, distortion and inadequate mechanical properties. Hence, care in choosing the appropriate material must be taken. Sometimes polymers are blended with other materials such as other polymers, ceramics and biological macromolecules to give them superior properties. A list of polymers and some of their uses is given in Table 2-3.

Polymers

Application	Properties and Design Requirements	Polymer	Reference
Cardiovascular	fatigue resistance, lubricity, sterilizability, lack of thrombosis, emboli formation, lack of chronic inflammatory response	polyurethane	[179]
		Teflon	[186]
		silicones	[185]
Dental	stability and corrosion resistance, plasticity, strength and fatigue resistance, coating activity, good adhesion/ integration with tissue, low allergenicity	polyglycolic acid	[168]
		polylactic acid	[170]
		polyurethane	[175]
		polymethylmethacrylate	[182, 183]
Drug Delivery	appropriate drug release profile, compatibility with drug, biodegradability	polyanhydride	[159]
		polylactic acid	[191]
		polylactic/ glycolic acid	[192]
Ophthalmic	gel or film forming ability, hydrophilicity, oxygen permeability	acrylic	[154,155]
		silicone	[180]
		polyacrylamide gels	[184]
Orthopaedic	strength and resistance to mechanical restraints and fatigues, good integration with bones and muscles	Acrylic, nylon	[152,153], [156,158],
		polydioxanone	[160, 161]
		poly-ε-caprolactone	[162,163, 189]
		polyether ether ketone	[164,165]
		ultra high molecular weight polyethylene	[165]
		polyglycolic acid	[167, 169, 188]
		polylactic acid	[167,187,188]
		polyurethane	[174]
		polymethylmethacrylate	[190]
Sutures	good tensile strength, strength retention, flexibility, knot retention, low tissue drag	silk	[193]
		catgut	[194]
		Polylactic acid	[195]
		Polypropylene	[196]
		Nylon	[197]

Table 2-3 List of polymers used in the following areas: cardiovascular, dental, drug delivery, ophthalmic, orthopaedic and sutures with corresponding properties and design requirements

Polymers

Application	Properties and Design Requirements	Polymer	Reference
Stents	Flexibility, strength, good fracture resistance, corrosion resistant, fatigue resistant, visible using standard x-ray or MRI methodology, vascular compatible	Nylon	[157]
		polyurethane	[176]
Soft Tissue Engineering		Polyglycolic acid	[166]
		polylactic acid	[171]
		polypropylene	[172, 173]
		polyurethane	[177,178]
		silicone	[181]

Table 2-3 List of polymers used in the following areas: stents and soft tissue engineering with corresponding properties and design requirements

2.2.4.1 Definition and Categories of Polymers

Biodegradable polymers are capable of being decomposed by bacteria or other living organisms. Bioabsorbable polymers are able to be absorbed by living cells and organisms and resorbable polymers can break down and be assimilated into the body. [55, 198, 488]. Biodegradable polymers will in time chemically disintegrate into their basic units. This will result in CO₂ and H₂O for polyesters. Biodegradable polymers from renewable resources can be seen in Table 2-4.

Biodegradable Polymers from Renewable Sources		
Biodegradable Polymer	Comment	Reference
Cellulose	The world's most abundant natural, renewable biodegradable polymer. Can be chemically modified with phosphorylation to promote bone generation	[199,200]
Chitosan	A mucopolysaccharide of marine origin. In vivo tests show it is non-toxic and when sterilized it is pyrogen free.	[201]
Gelatine	A water soluble protein prepared from boiled hide and bone. Have been used as porous scaffolds.	[202,203]
Plant oil based polymers	Examples are soya bean oil, linseed oil and sunflower oil. They are often considered as potential 'green' alternatives to petrochemicals.	[204]
PLA and PGA	They are versatile and have been used as porous scaffolds. They comprise of a large family which have varying properties.	[205,206]
Poly(3-hydroxybutyrate) (PHB)	A biodegradable polyester which is accumulated by many microorganisms under unfavourable conditions. When PHB is combined with 3-hydroxyhexanoate the osteoblast attachment is significantly higher than that of the lactic acids.	[207]
Thermoplastic starch (TPS)	Corn starch can be blended with synthetic polymers to produce suitable scaffold biomaterials.	[208]
Table 2-4 List of biodegradable polymers obtained from renewable resources with their corresponding uses		

Biodegradable polymers from petroleum sources are illustrated in Table 2-5.

Biodegradable Polymers from Petroleum Sources

Biodegradable Polymer	Comment	Reference
Aliphatic polyesters	Important class of synthetic biodegradable polymers. Typically semi-crystalline, hydrophobic solids which can be strategically tailored to suit a variety of applications.	[209]
Poly(butylene succinate) (PBS)	This polymer is hydrophilic and ductile with good degradability and excellent processability. Studies have shown this to be an excellent biopolymer.	[210, 211]
Poly(vinyl alcohol), (PVA)	Often developed as hydrogels for controlled drug release.	[212]

Table 2-5 Aliphatic polyesters, PBS and PVA as biodegradable polymers from petroleum sources

By combining the biomaterials from Tables 2-3 to 2-5 the properties can be altered according to the required end use. This can involve molecular interaction studies to understand the chemistry of the blend in order to deliver bespoke materials [213-215]. For example, blends of PVA-MPEG-collagen have been produced which exhibit higher thermal stability than PVA alone [216]. Electrospinning technology has offered potential to control composition, structure and mechanical properties of biomaterials. The addition of PLGA to a polymer blend improves this process [217]. Some of these combinations and type of medical implant have been patented for the repair of damaged bone and/or cartilage [218]. The examples listed here are a snapshot of what can be achieved by blending various polymer materials together. When ceramics and glass are also added to the equation the potential for developing suitable biomaterials for tissue regeneration is vast.

2.2.4.2 Advantages and Limitations of Current Polymer Biomaterials for Implantation

There are many types of polymer biomaterial available for implantation. However, the properties of the materials can differ quite dramatically. Hence, they are either used for different purposes or they are combined with other materials to give them the desired functionality.

Poly(lactic acid) (PLA) and Poly(glycolic acid) (PGA) are both approved FDA polymer [91]. They have the advantages of acceptable degradation profiles, hydrolytic degradation properties, eliminated by natural pathways, ease of use, radiolucency, can have tailored mechanical properties and degradation rate, and resorbability. PLA has high strength and modulus and is derived from lactic acid which is a chiral molecule and can therefore exist as D and L isomers. The L isomer is the biological metabolite. It is more hydrophobic than PGA due to the additional methyl group and has a slower degradation rate [219]. Studies on Poly-D,L-lactic acid (PDLLA) and poly-(lactide-co-glycolide) (PLGA) have shown that they are suitable materials for cartilage tissue engineering and encourage chondrocyte adhesion, proliferation and gene expression [220]. However, the disadvantage includes a faster degradation rate than some other polymers such as PCL which can limit their uses [221].

Polyurethane has a wide range of mechanical properties. These include low viscosity, good abrasion resistance, good flexibility, good toughness, low shrinkage, good colourability, good impact resistance, hydrolytically stable. This material has many applications outside of the Pharmaceutical industry. These consist of automotive applications, use as a modeling material, coating applications, prototyping, foam, industrial applications, textile applications, adhesives, molds/dies/tools [225]. Unfortunately, as an implant material the surface is radically different from the bulk which can lead to high mobility and soft segments. Synthesis can lead to some low molecular fractions, which will impact on the mechanical properties of the artefact.

PTFE has a high resistance to chemical degradation and has low frictional properties but it is not biodegradable. Its disadvantages include a low wear resistance and it does not display typical thermoplastic flow behaviour, high cost low strength and low stiffness [226, 227].

UHMWPE has a relatively high wear resistance and can be made oxidation resistant however, it is not biodegradable [228].

PCL is also an FDA approved material but is not often used as a load bearing material due to its low tensile strength and it does not encourage chondrocyte adhesion (for cartilage repair) to the same degree as PDLLA or PLGA [91, 220]. It is however, highly compatible with osteoblasts making it very desirable as an implantation material. The degradation rate can also be made more appropriate depending on the route of manufacture [230]. The homopolymer has a degradation time of two years which is highly desirable if a scaffold material is required. Although there is limited information on PCL as a load bearing material and the majority of the literature focuses on non- load bearing uses.– Researchers are keen to investigate this area further to obtain the best possible solutions for structural bone replacements [221].

PCL has been investigated as a biomaterial and has shown promise in several areas [232]. It has been used for cranioplasty in humans [162] and its presence as the hydrophobic component of polyethylene oxide blends in PU has shown good bone healing ability when implanted into the external abdominal oblique muscle of Wajill sheep [231]. Poly(ϵ -caprolactone-co-iodo- ϵ -caprolactone) is chemically bound to iodine and its opacity to X-rays has been assessed. PCL has been used in conjunction with Pluronic (F68) as a contraceptive implant in rats and dogs over a 2 year study. No adverse effects have been observed [233]. Artificial nerve guides have been produced for peripheral nerve regeneration by blending PCL with a natural polymer for the external layer. The results have shown enhancement of the biocompatibility, biomimetics, a faster degradation rate and improved production costs [234]. A three dimensional scaffold has been produced in PCL for cartilage and a tricalcium phosphate reinforced PCL for the bone portion. The hybrid scaffolds were shown to provide sufficient support to new osteochondral tissue formation when implanted into the medial femoral condyl of adult New Zealand white rabbits [163].

2.3 Extractables and Leachables

Extractables can originate from the actual material formulation or from the processing aids used during manufacture. They could be from the base material, for example oligomers and polymers in the case of polymeric formulations, ions from a glass formulation or from specific additives used to improve material properties. There are

many additives available. Those generally used with polymers include UV stabilisers, colourants, antiblock agents, fillers and flame retardants [247]. For implant materials additives are kept to a minimum due to potential toxicological concerns. A list of acceptable additives can be found typically in EU Directives such as 2002/72/EC (additives relating to plastic materials and articles in direct food contact). The examination of extractables tells the investigator what “*could*” happen. It is a proactive approach. Leachables can be generated within the product formulation from primary packaging/ device contact over the shelf life of the product. Hence what ‘*could*’ happen. This type of study shows what ‘*does*’ happen and is often termed the reactive approach).

2.3.1 Requirements

Historically there have been product recalls due to leachables issues. Examples include Tylanol and Lipitor due to a nauseating mouldy odour, Glumetza blamed on treated wooden pallets, protein degradation caused by tungsten in pre-filled syringes [266]. The Pharmaceutical industry regulates extractables and leachables using various guidelines [267-271, 273-283]. It is therefore a heavily regulated area. Groups such as the Inhalation Technology Focus Group (ITFG), International Pharmaceutical Aerosol Consortium on Regulation and Science (IPAC-RS) have existed for over 15 years and they provided guidance for primary packaging and formulation interactions. Leachables from Pharmaceutical products or implantation devices can impair efficacy, safety and product compatibility and hence, require to be monitored during stability and end of shelf life testing [266]. The Food and Drug Administration (FDA) has provided “Guidance for Industry” and chapters of the United States, European and Japanese Pharmacopoeia address various aspects linked to leachables. Directives and the Code of Federal Regulations also play their part in ensuring that materials, components and medical devices are safe for patient use. In industry, Pharmaceutical/ Medical companies set their own internal standard operating procedures and documentation based on the actual product of manufacture and guidance provided for the countries of product launch.

A typical programme for an extractables and leachables study is described Figure 2-1. This would normally include an extractables study followed by a toxicological assessment of the species detected. It is advisable for the analyst or responsible person to contact the supplier or manufacturer of the device in order to establish what types of materials or processing aids have been used. This will give a better baseline for knowing which extraction techniques and analytical instruments to use. A system suitability test is often conducted (to establish recovery of extractables by spiking of control samples) followed by validation of a quantitative leachables method which will be used during leachables stability testing. The leachables detected, are normally cross checked with the extractables profile. This may help with identification particularly if something from the device has reacted with a product from the extractables profile. It is necessary to provide a toxicological evaluation of the leachables to Regulatory Authorities in order to prove the patient safety of a device before it can be commercialised.

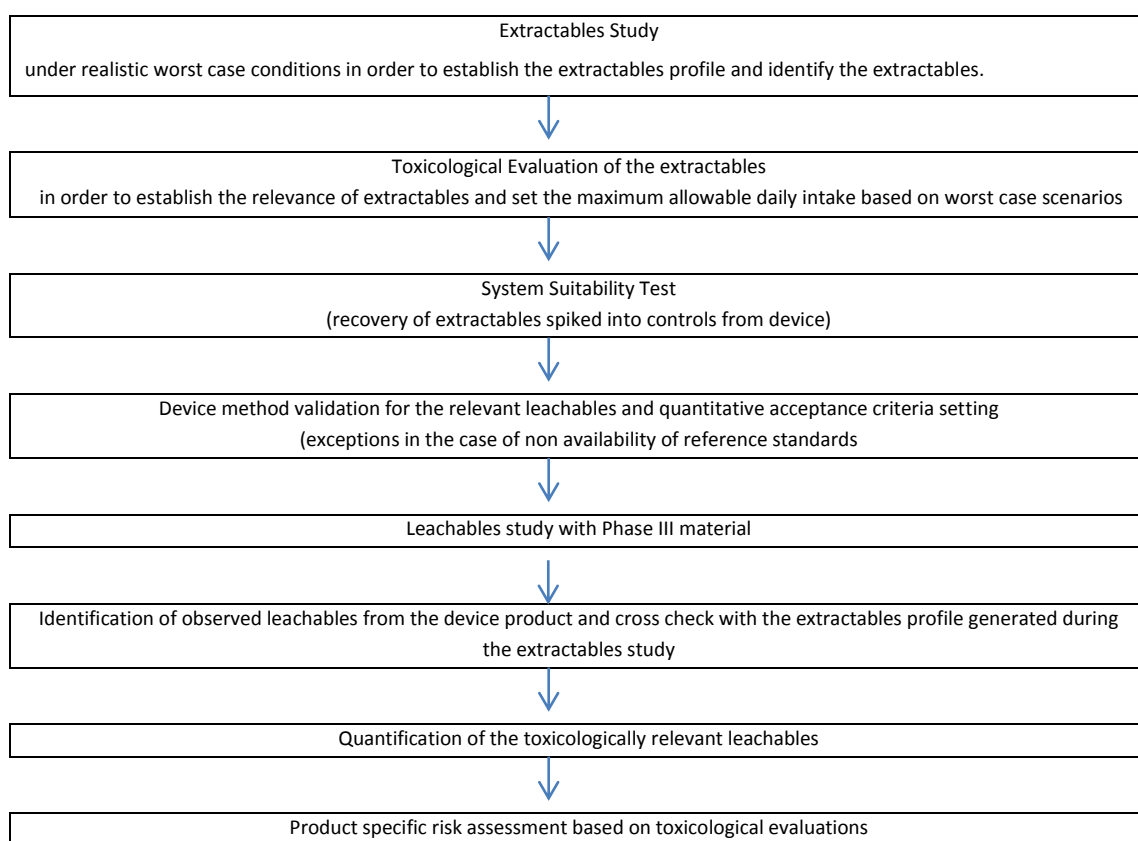


Figure 2-1 Flow diagram to represent a typical plan for an extractables and leachables study [85]

Typical leachables stability test conditions and timepoints can be seen in Table 2-6.

Conditions	Storage Time (months)						
	Initial	6	12	24	36	48	60
5°C	X		X	X	X	X	X
25°C/60%RH	X		X				
30°C/75%RH				X	X	X	X
40°C/75%RH		X					

Table 2-6 A typical study plan using 4 conditions and specified storage time points for a leachables study to the end of product shelf life [85]

The Product Quality Research Institute (PQRI) has set safety concern thresholds (SCT) for Orally Inhaled and Nasal Drug Products (OINDPs) in 2008 (SCT = 0.15µg/day), [272]. A SCT is a “*threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects*”. It is finalising a similar approach to parenteral and ophthalmic drug products. Currently these values are 150 µg/day for Class I, 5µg/day for Class II and 1.5 µg/day for Class III products.

Class I: Class of compounds which are not sensitisers, irritants, genotoxins or carcinogens.

Class II: Class of compounds which are known or expected to have sensitising or irritating properties, but do not have any indications or genotoxicity or carcinogenicity.

Class III: Class of compounds which are known is expected to be genotoxic or carcinogenic.

Extractables and leachables testing are required by the Centre for Devices and Radiological Health (CDRH) by the FDA for many medical devices. ISO-10993 guidance regulates medical devices [44] and a recent draft for comment detailing evaluation and testing was sent for review in 2013 by the FDA [284]. The scope of the document specifically covers all aspects of ISO-10993 and also associated American Society for Testing and Materials (ASTMs) in order to ensure that the device materials should not, either directly or through the release of their material constituents:

- i. Produce adverse local or systemic effects
- ii. Be carcinogenic

iii. Produce adverse reproductive and developmental effects.

Leached contaminants from implants can gain direct access to the patient's blood, unlike most pharmaceuticals, which are ingested orally and have to go through the digestive tract [248]. Testing generally includes: in vitro cytotoxicity; acute, sub chronic and chronic toxicity; irritation; sensitisation; haemocompatibility; implantation; genotoxicity; carcinogenicity; and effects on reproduction, including developmental effects.

An **LD50** is a standard measurement of acute toxicity that is stated in milligrams (mg) per kilogram (kg) of body weight. An **LD50** represents the individual dose required to kill 50 percent of a population of test animals. This is often used to calculate a safety threshold for extractables and leachables. The safety threshold is based on the exposure time and weight of a typical human male or female. The LD50 for calcium oxide (CAS: 1305-78-8) is not available however an occupational exposure limit (OEL) of 1mg/m³ over 8 hours has been documented [244]. For Na₂O (CAS: 1313-59-3) the LD50 for acute exposure is 1180mg/kg for the rat, 1572mg/kg for a mouse and 3147mg/kg for a rabbit [96]. The LD50 (rat) for Fe₂O₃ (CAS 1309-371) is 5,000 to 10,000mg/kg [229]. Calcium and iron oxides are generally regarded as safe according to the FDA Select Committee on Generally Regarded as Safe Substances (SCOGS). They come under heading number 1: There is no evidence in the available information on [substance] that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future.

For phosphorous pentoxide (CAS: 1314-56-3) the LD50 is 6293mg/m³ per 4 hours of exposure. A 0.2-0.4% phosphate solution (CAS: 7778-77-0) has an LD50 (rat) of >90mL/kg and LD50 (rabbit) >4650mg/kg [246].

2.3.2 Regulatory Approved Products

A list of properties of Regulatory approved PLA type and PCL products are shown in Table 2-7.

Polymer	Comparison of mechanical properties	Degradation and re-sorption process via hydrolysis	Molecular weight loss/ loss of mechanical properties (in months)	Mass loss (in month)	Area of Application	Products with regulatory approval	Reference
Poly(L-lactide)	+++	Bulk erosion	9-15	36-48	Orthopaedic surgery, oral and maxillofacial	FixSorb® System (screws, nails, pins) Neofix™ screws, nails pins)	[245]
Poly(L-lactide-co-D,L-lactide) 70/30	++	Bulk erosion	5-6	12-18	Oral and maxillofacial surgery, orthopaedic surgery	Resorpin™, Leadfix™, MacroSorb™ System (screws and plates, mesh, nails, pins)	[245]
Poly(D,L-lactide)	+	Bulk erosion	1-2	5-6	Orthopaedic surgery	Biofix™	[245]
Polycaprolactone	+	Bulk and surface erosion	9-12	24-36	Drug delivery	Capranor™	[245]
	+				Traceal splint	Kalydeco™	[263]
	+				Craniofacial, eyelids	Osteopore™, Osteomesh®, Osteoplug™	[264]
	+				Dermal filler for face and hands	Ellansé™	[265]

Table 2-7 Properties and areas application for regulatory approved PLA and PCL polymers

A Molecular weight and mass loss vary depending on factors such as chemical structure and composition: presence of ionic groups and of side group defects; configuration of the structure molecular weight and molecular weight distribution (polydispersity); presence of low molecular weight components (monomers, oligomers, solvents, softener's, drugs, growth factors, etc.) production and manufacturing procedures and their process parameters, implant design, sterilisation method, morphology (amorphous versus semi-crystalline, presence of microstructures and stress within the components), tempering, storage, implant site.

+++ good, ++ average, + poor.

2.4 PCL

2.4.1 Description and Synthesis

The cyclic monomer ϵ -caprolactone has been studied since the 1930s [312]. The chemical formula for PCL is $(-\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}-)_n$. Figure 2-2.

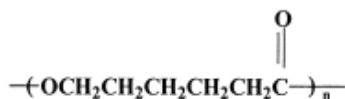


Figure 2-2 Structure of Poly- ϵ -caprolactone [230]

It is a bioabsorbable aliphatic poly(α -hydroxy acid [291] thermoplastic belonging to the aliphatic esters. It has a linear, hydrophobic and partially crystalline structure. Its hydrophobic (low wettability) nature is due to linear chains of methylene groups [292].

ϵ -caprolactone can be manufactured using a process which utilises a high strength oxidising agent to produce a high purity peracetic acid. Peracetic acid is used to oxidise cyclohexanone by a Bayer-Villager reaction. The unreacted cyclohexanone is separated by distillation and recycled to the oxidation stage. Acetic acid is also recycled to the oxidation stage [294], Figure 2-3. The polymerisation of ϵ -caprolactone can be achieved by using various catalysts through cationic, anionic or co-ordination mechanisms with or without active hydrogen compounds. The synthesis of ϵ -caprolactone, involving ring opening, can be seen in Figure 2-4 [293].

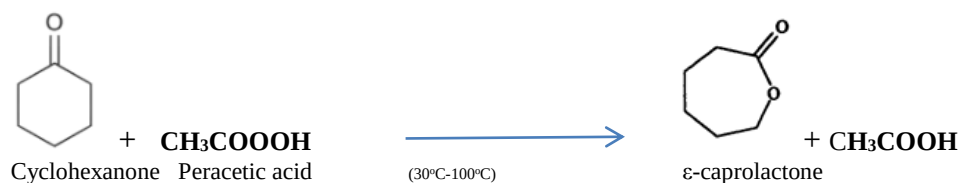
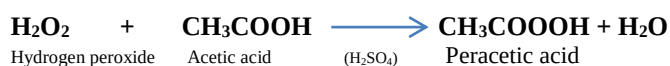


Figure 2-3 Synthesis of ϵ -caprolactone from cyclohexanone and peracetic acid [294]

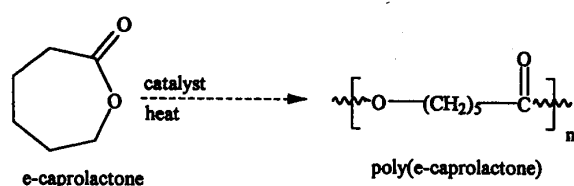


Figure 2-4 Ring opening synthesis of PCL[293]

In order to obtain high molecular weight PCL, the catalysts are often organometallic compounds such as those of tin, aluminium, zinc or rare earth metals. These catalysts, in large amounts, could pose a risk from an extractables and leachables perspective. Hence, it is important to monitor these in the end product. The degradation rates of polymers produced with such catalysts can also be too slow for bone reconstruction. Hence, by using an alternative catalyst system such as BF_3 with glycerol it can be prepared with a more appropriate degradation rate [230] suitable for cranio and maxillofacial treatment, with more rapid cell growth [295]. There have been many investigations into the synthesis of PCL. The list is extensive and not fully covered in this thesis, however, the various types of catalyst are tabulated because they influence certain synthesis parameters such as polymerisation rate, polydispersity, degree of monomer conversion, degradation rate and final molecular weight of PCL, Table 2-8.

Catalyst	Influence	Reference
Aluminium (III) and tin (II) complexes	59% to 98% conversion to PCL. Polydispersity Index (PDI) 1.17-1.37. Mn 6100-37400.	[296, 297]
Amidines	83 to 93% conversion to PCL. PDI 1.21-1.65. Reaction is fast and in a controlled manner. Unimolecular molecular weight distribution polyesters.	[298]
Aluminium alkoxides	PDI <1.2. Perfect control of molecular weight and the nature of the end groups which affect the degradation rate.	[299]
Diethyl zinc	Linear PCL's obtained with a defined molecular weight. The presence of macrocyclic products is not observed in some cases. Degree of conversion is 100% and PDI between 1.05 and 1.1	[35]
Escherichia coli whole cell biocatalyst	Metal free synthesis and low production cost. Monomer conversion >90%. PDI 1.17 to 1.47. Linear and cyclic monomers produced. Mw 2140g/mol.	[300]
Fervidobacterium nodosum	Polymerisation effective at 90°C. Approx. 100% conversion. PDI 1.34. Mw. 2340g/mol.	[301]
Perfluoroalkaneslphonates and perfluoroalkanesulfonimides	Mn 4800-13500. PDI 1.10 – 1.48.	[303]
Samarium (III) and Titanium (IV) complexes	PDI 1.26 to 1.72 with conversion up to 96% depending on solvent also used.	[304]
Zirconocene dihydrides or alkoxides	Degree of polymerisation >100% . PDI 1.05 and 1.1.	[306. 307]
Yttrium trisphenolate	Polymerisation effective at 100°C. Mw controlled by caprolactone/ 1,2-propanediol molar ratio. Conversion >99%. PDI 1.40-1.74 and Mn 14800 – 19300.	[308]

Table 2-8 Catalysts that can be used to synthesise PCL. The catalysts can control the molecular weight and end groups of the resulting polymer and hence, the degradation rate.

It has also been documented that low molecular weight alcohols can control the molecular weight of the polymer [221].

2.4.2 Properties and Characteristics

PCL is a semi crystalline polymer [221, 309] with a melting point of 58-65°C and a glass transition temperature of -60°C to -65°C. The homopolymer has a degradation time of over two years. Further properties of PCL can be seen in Table 2-9. The ranges provided are based on various molecular weights of PCL. The number average molecular weight of PCL may vary from 3,000 to 80,000 g/mol and can be graded according to the molecular weight. Where a single figure is seen, this is based on Mw 44,000 [311]. The melting point and glass transition temperature are dictated by the crystalline nature of the PCL which enables easy formability at relatively low temperatures. In the body, the semi crystalline structure of PCL results in high toughness, because the amorphous domains are in the rubbery state [324].

Properties of PCL

Description	Symbol	Property
Density (g cm ⁻³)	ρ	1.110 – 1.146
Glass transition temperature (°C)	T _g	-60 - -65
Melting temperature (°C)	T _m	58 – 65
Heat of fusion (Jg ⁻¹)	H _f	34
Degradation temperature (°C)	T _d	350
Tensile modulus (MPa)	E	210 – 440
Ultimate tensile strain (%)	E	300 – 1000
Tensile strength (M Pa)	σ	16.0 – 42.0
*Approximate degradation (months)	-	>24

*Depending on the starting molecular weight of the material the range can be 2-4 years.

Table 2-9 Physical and mechanical properties of PCL [221, 310, 311]

2.4.3 Biodegradation

It is essential to understand the PCL degradation process, products and rates, including kinetics for specific anatomical sites, in order to tailor materials for the intended use. PCL can be biodegraded by bacteria and fungi but it is not normally biodegradable in animal and human bodies because of the lack of suitable enzymes, unless the PCL has been modified. PCL is however, bioabsorbable [221]. This aspect is closer to pharmacology than to material science. A definition of the terms biodegradable and bio-resorbable are shown in Table 2-10.

Definition of Biodegradable and Bio-resorbable

Description	Definition
Biodegradable	Solid polymeric materials and devices which break down due to macromolecular degradation with dispersion in vivo but no proof for the elimination from the body (this definition excludes environmental, fungi or bacterial degradation). Biodegradable polymeric systems or devices can be attacked by biological elements so that the integrity of the system, and in some cases but not necessarily, of the macromolecules themselves, is affected and gives fragments or other degradation by-products. Such fragments can move away from their site of action but not necessarily from the body.
Bio-resorbable	Solid polymeric materials and devices which show bulk degradation and further resorb in vivo; i.e. polymers which are eliminated through natural pathways either because of simple filtration of degradation by-products or after their metabolization. Bioresorption is thus a concept which reflects total elimination of the initial foreign material and of bulk degradation by-products (low molecular weight compounds) with no residual side effects. The use of the word 'bioresorbable' assumes that elimination is shown conclusively.

Table 2-10 Definition of biodegradable and bio-resorbable [221]

Polyesters primarily undergo a bulk degradation, via an initial hydrolysis mechanism [219, 313] and the degradation rate of PCL is slower than other aliphatic polyesters (PGA>PDLLA>PLLA>PCL) due to the hydrophobic nature and high crystallinity of the polymer [314, 315]. Bulk erosion can cause a fracture fixation material to fail prematurely. A schematic diagram explaining the difference between surface erosion and bulk erosions is shown in Figure 2-5 and Table 2-11 provides a more detailed explanation.

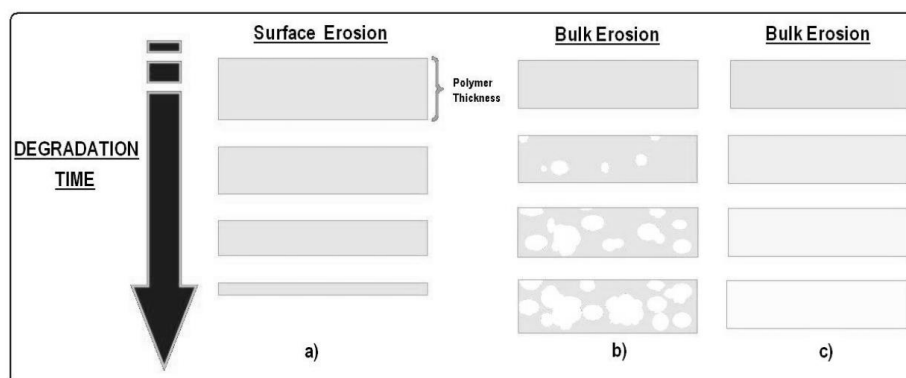


Figure 2-5 Schematic illustration of three types of erosion phenomenon (a) surface erosion, (b) bulk erosion with autocatalysis and (c) bulk erosion without autocatalysis [80]

Bulk degradation for PCL can occur due to autocatalysis. The sequence of degradation is shown in Figure 2-6. The amorphous regions tend to degrade preferentially and the polymer becomes more crystalline as the process continues the polymer loses its mechanical strength.

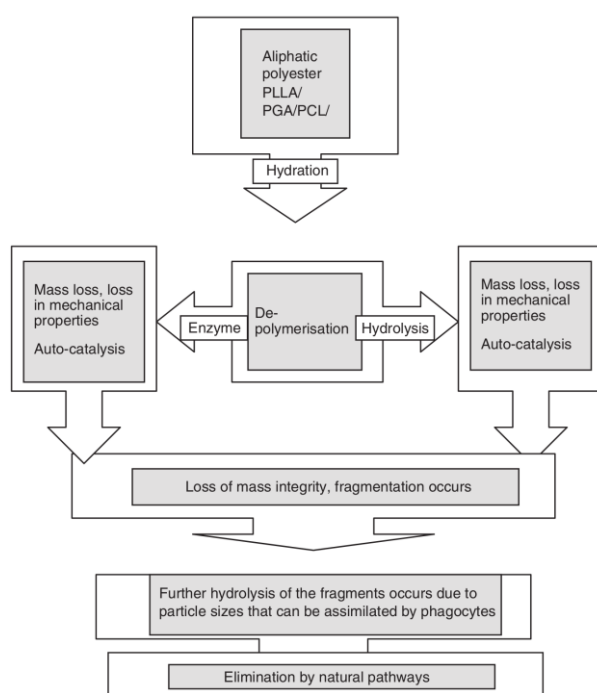


Figure 2-6 Flow diagram to illustrate the sequence of events which may occur during the enzymatic or hydrolytic degradation of aliphatic polyesters [219]

A definition of the terms surface and bulk erosion are given in Table 2-11.

Definition of Erosion Mechanisms

Description	Definition
Surface	Surface degradation or erosion involves the hydrolytic cleavage of the polymer backbone only at the surface [19]. This situation arises when the rate of hydrolytic chain scission and the production of oligomers and monomers, which diffuse into the surroundings, is faster than the rate of water intrusion into the polymer bulk. This typically results in thinning of the polymer over time without affecting the molecular weight of the internal bulk of the polymer, which would generally remain unchanged over the degradation period.
Bulk (non-auto catalysed)	Bulk degradation occurs when water penetrates the entire polymer bulk, causing hydrolysis throughout the entire polymer matrix (Fig. 3b). Random hydrolytic chain scission would take place and produce an overall reduction in molecular weight. If water molecules can diffuse into the polymer bulk, hydrolyse the chains enabling the monomers or oligomers to diffuse out, erosion will occur gradually and equilibrium for this diffusion–reaction phenomenon would be achieved.
Bulk (auto catalysed)	If this equilibrium is disturbed, the degradation mechanism could provoke internal autocatalysis, via the carboxyl and hydroxyl end group by-products. Whereas surface oligomers and carboxyl groups may freely diffuse into the surroundings (surface erosion situation), in the case of bulk degradation the internal concentration of autocatalysis products can produce an acidic gradient as the newly generated carboxyl end group formed during ester bond cleavage accumulate. This in turn accelerates the internal degradation compared to the surface, leaving an outer layer of higher molecular weight skin with a lower molecular weight, degraded, interior.

Table 2-11 Definitions of surface, bulk (non-catalysed) and bulk auto catalysed erosion mechanisms for aliphatic polyesters [221]

Potential breakdown products, leachables in particular, have already been discussed as a potential concern for implant materials. PCL degrades hydrolytically to form hydrolysis intermediates (oligomers and 6-hydroxy caproic acid via an intermediate ω -oxidation). During glycolysis, within the body, glucose is converted to 2 pyruvate molecules as part of the 1st stage of cellular respiration. During aerobic respiration Acetyl co-enzyme A is then produced. Biodegradation of the intermediates proceeds through β -oxidation with acetyl coenzyme A which further degrades through the citric acid cycle. [221]. The degradation of PCL and its copolymers involves similar mechanisms to that of PLA and proceeds in two stages [316]. The same kinetics are observed from *in-vitro* (water, pH 7.4) and *in-vivo* studies [317-319]. A two stage mechanism was proposed. The first stage of *in-vivo* degradation involves non enzymatic random hydrolytic ester scission and is believed to be auto catalysed by the generated carboxylic acid end groups. The duration of the degradation is determined

by the initial molecular weight of the polymer. It has been reported to last a minimum of 6 months when subcutaneously implanted in a rat model [320]. Biodegradable polyester degradation occurs by uptake of water followed by the hydrolysis of ester bonds [367]. A diffusion process, investigated by IR spectroscopic methods, suggested that water molecules at first diffuse into the free volume (micro voids) or are molecularly dispersed into the PCL matrix and then form hydrogen bonds with the C=O group of the polymer during the process of water diffusion [364,365]. Table 2-12 shows the IR structural vibrations associated with this process. The second stage (chain scission) begins with a deviation from the linear relationship between semi logarithmic plots of intrinsic viscosity and molecular weight versus time and involves loss of mechanical strength and weight. The role of hydroxyl free radicals in the degradation mechanism was examined because free radicals (in particular O and H₂O₂), from implant materials, have been documented to contribute to inflammatory reactions in-vivo [291]. Different methods (Gel Permeation Chromatography (GPC), Differential Scanning Calorimetry (DSC) and Scanning Electron Microscopy (SEM)) were employed to investigate the mechanism of degradation. The data indicated that the hydroxyl radical is likely to be a major factor in the degradation of this polymer. A proposed hydrolytic mechanism can be seen in Figure 2-7.



Figure 2-7 Proposed hydrolytic degradation mechanism for PCL involving the hydroxyl radical [291]

Biodegradable polyester degradation occurs by uptake of water followed by the hydrolysis of ester bonds [367]. A diffusion process suggested by Peng et al., [364, 365], using IR spectroscopic methods, suggested that water molecules at first diffuse into the free volume (micro voids) or are molecularly dispersed into the PCL matrix and then form hydrogen bonds with the C=O group of the polymer during the process of water diffusion. Table 2-12 shows the IR structural vibrations associated with this process.

Wavenumber (cm ⁻¹)	Structural Vibration	Comments	References
3203	-OH	Symmetric (OH) stretching vibration of H ₂ O fully hydrogen bonded with hydrophilic carbonyl group (-C=O) of PCL.	[364, 365]
3410	-OH	Antisymmetric (OH) stretching vibration of H ₂ O fully hydrogen bonded with hydrophilic carbonyl group (-C=O) of PCL.	
3593	-OH	Symmetric (OH), stretching vibration of H ₂ O partially hydrogen bonded with hydrophilic carbonyl group (-C=O) of PCL.	
3641	-OH	Antisymmetric (OH) stretching vibration of H ₂ O partially hydrogen bonded with hydrophilic carbonyl group (-C=O) of PCL.	

Table 2-12 IR spectral vibrations associated with PCL interactions with H₂O

2.4.3.1 Enzymatic Degradation

Enzymatic surface erosion has been documented [331, 325], however, in vivo studies have shown that PCL does not normally undergo non-enzymatic degradation which is substantially influenced by the crystallinity of the polymer. However, PCL can be modified so that surface erosion can occur. Pulkkinen *et al.*, wrote a paper [326] providing proof that in-vivo studies (rat model) show enzymatic surface erosion and biocompatibility when PCL is linked with 2,2-bis(oxazoline) for up to 12 weeks. In addition, the erosion rate of PCL-O can be controlled by the PCL block length i.e. polymers with shorter PCL blocks exhibit faster degradation rates. This behavior may provide new possibilities for pharmaceutical and biomedical applications.

Certain lipases (pseudomonas lipase) and esterases can also enhance the degradation of polycaprolactone (PCL) when compared with incubation in buffer only. The enzymatic hydrolysis of PCL occurs mainly at the polymer surface since it is difficult for a hydrophilic enzyme to diffuse into a hydrophobic polymer like PCL. The surface erosion mechanism of PCL demonstrated by lipase enzyme may be useful to deliver

bioactive agents by an enzymatically controlled process [327, 330]. A schematic diagram shows the point of attack, Figure 2-8.

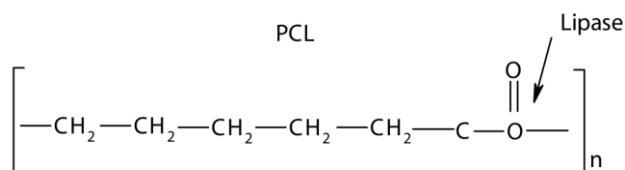


Figure 2-8 Lipases can be used to increase the enzymatic degradation of PCL. Schematic diagram of the point of lipase attack [327]

Sivalingam *et al.*, [328] stated that the enzymatic degradation of PCL is assumed to undergo a 4 step process and proved that the rate can be drastically reduced by blending with other polymers, Figure 2-9:

- i. Adsorption of enzyme on polymer substrate
- ii. Formation of transition complex between polymer and enzyme
- iii. Specific chain scission of the polymer
- iv. Interaction of the polymer enzyme and adsorbed complex and polymer

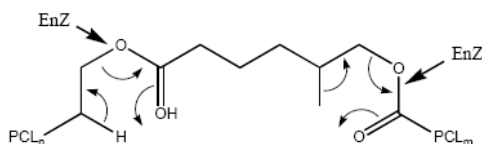


Figure 2-9 Chemical structure of PCL and the point of attack by the enzyme. The lipase cleaves the ester bonds in the main chain of PCL leading to specific products [328]

2.4.4 Thermal Degradation

An understanding of the thermal stability and thermal degradation behaviour is important for processing, application and thermal recycling [348]. Thermal degradation of PCL has been proposed to happen by three mechanisms and the resulting degradation products have been monitored by GC/MS, Raman and FTIR

spectroscopy. Investigations have been ongoing since the 1970s and it has been an area which has seen much debate.

2.4.4.1 Thermal Breakdown Products

Ouhadi *et al.*, [299] studied the bulk degradation of PCL at 120°C within an oxygen and argon atmosphere (for 40 days) and reported that PCL undergoes oxidative degradation in an O₂ atmosphere, but undergoes no degradation in an inert atmosphere. They stated that the hydroxyl end groups played a key role in the degradation process. Iwabushi *et al.*, [332] proposed a chain scission cationic mechanism for degradation. In 1997 Luderwald [333] performed studies which concurred with this theory using thermal and electron impact degradation reactions. A comprehensive list of breakdown products was produced. The proposed mechanism was cleavage of the ester bond and formation of ω-hydroxyl and ketone end groups, Figure 2-10a, cleavage of the O-CH₂ bond and formation of carboxyl and pentenyl end groups, Figure 2-10b. After electron impact and fragmentation of the pyrolysis products to carboxonium ions, an elimination of caprolactone via a ‘zip’ mechanism occurs, Figure 2-10c.

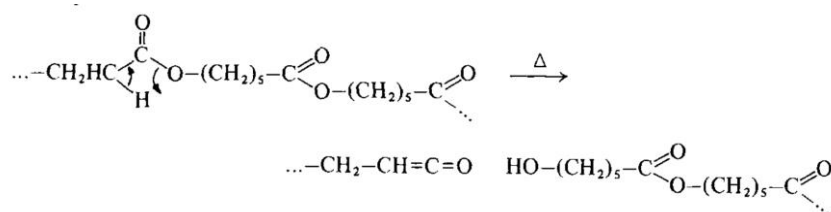
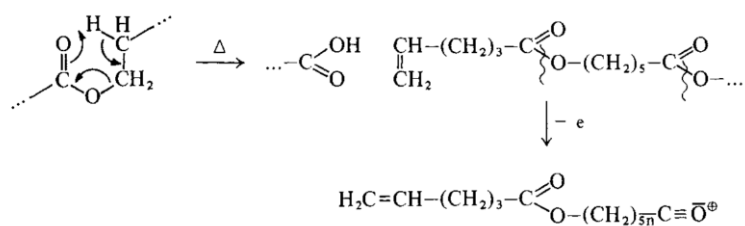


Figure 2-10a Cleavage of ester bond and formation of ω-hydroxyl and ketene endgroups [333]



m/e (n) 97 (0) 211 (1) 325 (2)

Figure 2-10b Cleavage of the O-CH₂ bond and formation of carboxyl and pentenyl endgroups [333]

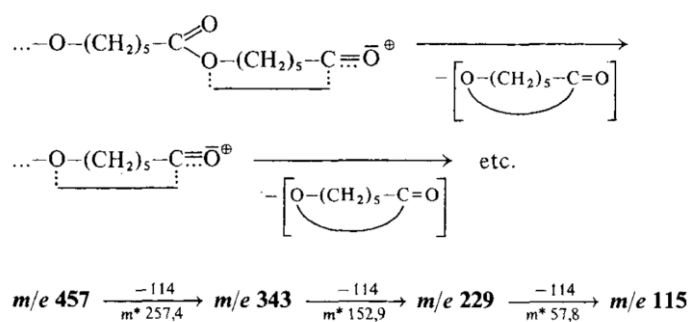


Figure 2-10c 'Zip' mechanism for caprolactone elimination reaction [333]

Further information on the fragments and their relative intensities, from the above process, are shown in Table 2-13.

Fragmentation Structure	
m/e (% rel. Intensity)	
41 (32)	C ₃ H ₅
55 (39)	C ₄ H ₇ , CH ₂ =CH-C≡O
69 (42)	C ₅ H ₉ , H ₂ C=CH-CH ₂ -C≡O
97 (36)	H ₂ C=CH-(CH ₂) ₃ -C≡O
114 (13)	O-(CH ₂) ₅ -CO
115 (100)	HO-(CH ₂) ₅ -C≡O
169 (3,4)	H ₂ C=CH-CO-O-(CH ₂) ₅ -C≡O
210 (2,8)	H ₂ C=CH-(CH ₂) ₃ -CO-O-(CH ₂) ₄ -CH=C=O
211 (2,6)	H ₂ C=CH-(CH ₂) ₃ -CO-O-(CH ₂) ₅ -C≡O
229 (12,4)	HO-(CH ₂) ₅ -CO-O-(CH ₂) ₅ -C≡O
247 (1,6)	H ₂ C=CH-(CH ₂) ₃ -CO-O-(CH ₂) ₅ -C $\begin{smallmatrix} \text{OH} \\ \text{OH} \end{smallmatrix}$
325 (3)	H ₂ C=CH-[(CH ₂) ₃ -CO-O-(CH ₂) ₂] ₂ -(CH ₂) ₃ -C≡O
343 (2,4)	H-[O-(CH ₂) ₅ -CO] ₂ -O-(CH ₂) ₅ -C≡O
438 (1,6)	H ₂ C=CH-[(CH ₂) ₃ -CO-O-(CH ₂) ₂] ₃ -(CH ₂) ₂ -CH=C=O
457 (1)	H-[O-(CH ₂) ₅ -CO] ₃ -O-(CH ₂) ₅ -C≡O
552 (1)	H ₂ C=CH-[(CH ₂) ₃ -CO-O-(CH ₂) ₂] ₄ -(CH ₂) ₂ -CH=C=O
570 (0,5)	H-[O-(CH ₂) ₅ -CO] ₄ -O-(CH ₂) ₄ -CH=C=O
571 (0,6)	H-[O-(CH ₂) ₅ -CO] ₄ -O-(CH ₂) ₅ -C≡O
666 (0,7)	H ₂ C=CH-[(CH ₂) ₃ -CO-O-(CH ₂) ₂] ₅ -(CH ₂) ₂ -CH=C=O
685 (0,5)	H-[O-(CH ₂) ₅ -CO] ₅ -O-(CH ₂) ₅ -C≡O
780 (0,5)	H ₂ C=CH-[(CH ₂) ₃ -CO-O-(CH ₂) ₂] ₆ -(CH ₂) ₂ -CH=C=O
799 (0,3)	H-[O-(CH ₂) ₅ -CO] ₆ -O-(CH ₂) ₅ -C≡O

Table 2-13 Proposed fragmentation pattern and intensities from thermal breakdown of PCL [333]

Persenaire *et al.* [334] also examined the fragmentation patterns of thermally degraded PCL, by TGA/FTIR, and saw evidence of CO₂ at m/z=44. In contrast, distinction between ε-caprolactone monomer and 5-hexenoic acid was more difficult as they showed similar molecular fragments (m/z=114). Fragments at m/z=96 were attributed to ketenic fragments, m/z=97 protonated ketenic derivatives, m/z=70 decarboxylated derivatives and other unclassified fragments at m/z= 84, 69, 55 and 42. Aoyagi *et al.*, [335] reported peaks at m/z= 28, 42, 55, 70, 84,114. Any relation to fragmentation patterns from leachables testing on PCL/PBG manufactured and studied in this thesis will be discussed further in Chapter 5.

Raman and IR spectroscopies have been utilised to determine the structure of the thermal breakdown products of PCL. He and Inoue [336] examined the crystallinity of the polymer and found that the results were comparable with other conventional techniques such as small angle x-ray scattering (SASX) [337], NMR [338] and density measurement [339]. The crystallinity was observed by a shift to the lower

wavelength for the carbonyl vibration, then an asymmetric band shape followed by a band intensity increase, Figure 2-11.

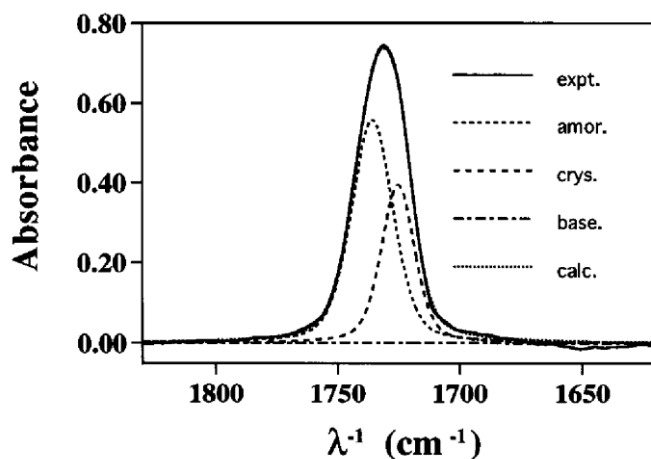


Figure 2-11 IR trace of PCL showing evidence of crystallinity by a shift to the lower wavelength for the carbonyl vibration [336]

A list of typical wavenumbers and corresponding structural vibrations (taken from FTIR and Raman data) can be seen in Table 2-14. This will act as a guide for discussion (section 5) to see if there are any structural changes, wavelength shifts or band broadening, when PCL is stored in aqueous media for 78 weeks.

Wavenumber (cm ⁻¹)	Structural Vibration	Comments	References
731	CH ₂	Rocking	[341]
912	C-COO	(vCC) crystalline domain	[342]
1107, 1045, 960	O-C	vibration	[341]
1163	γ-C-O -	stretch	[340]
1170, 1240	COO	ester vibration	[341]
1303-1281	CH ₂	(ωCH ₂)	[329, 342]
1365, 1418, 1470	CH ₂	CH ₂ band vibration	[341]
1470-1415	CH ₂	(δCH ₂)	[329, 342, 343]
1736	carbonyl	Sensitive to crystallinity	[336]
1720	-C=O	Crystalline domain, ester vibration	[341, 342]
1726	-C=O	(vC=O)	[344]
2865	CH ₂	Asymmetric vibration	[341]
2943	CH ₂	Symmetric vibration	[341]
3050-2850	CH-	Stretching region	[309]
3750-3250	C=O-	Stretching region, low wavenumber crystalline phase, high wavenumber amorphous phase.	[309]
3430	C=O-	Reorientation consistent with carbonyl absorption resulting from crystallinity	[309, 345]
3495	C=O- and H ₂ O	Carbonyl and bound water, amorphous PCL component	[309]
3500-3750	C=O- and H ₂ O	Carbonyl and bound water	[309]

Table 2-14 Typical FTIR and Raman absorption bands associated with structural vibrations of PCL

2.4.4.2 Degradation Mechanisms

The actual mechanisms for thermal degradation of PCL have been discussed in detail over the past few years. Aoyagi *et al.*, [335] proposed a one-step mechanism which involved an unzipping depolymerisation via elimination, Figure 2-12.

A parallel mechanism was proposed by several researchers [346-352]. Figure 2-13 shows the proposed random chain scission followed by specific end chain scission. It was suggested that pyrolysis provoked polymer chain cleavage via *cis*-elimination and the consecutive second step as an unzipping depolymerisation from the hydroxyl end of the polymer chains.

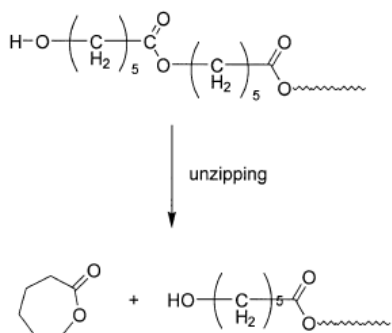


Figure 2-12 A one stage unzipping depolymerisation mechanism via elimination for the thermal decomposition of PCL [335]

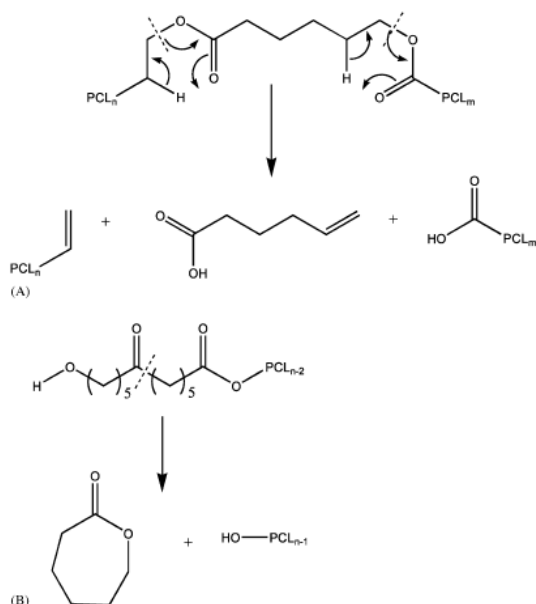


Figure 2-13 Proposed degradation mechanism for PCL: (A) random chain scission, (B) specific chain end scission [350]

The degradation of PCL was also investigated isothermally in sealed tubes in an N_2 atmosphere [353, 333]. They proposed that PCL degrades by a 3 stage mechanism (occurring at approximately 220°C , in N_2 and reduced pressure). Unger *et al.*, [354] performed studies also indicating that there could be a 3 stage mechanism. However, the mechanisms changed depending on the molecular weight ($M_n = 10\,000\text{g mol}^{-1}$ and

Mn = 80 000gmol⁻¹) of the PCL and the surrounding atmosphere. The proposed thermograms and mechanisms can be seen in Figures 2-14(a)-(c).

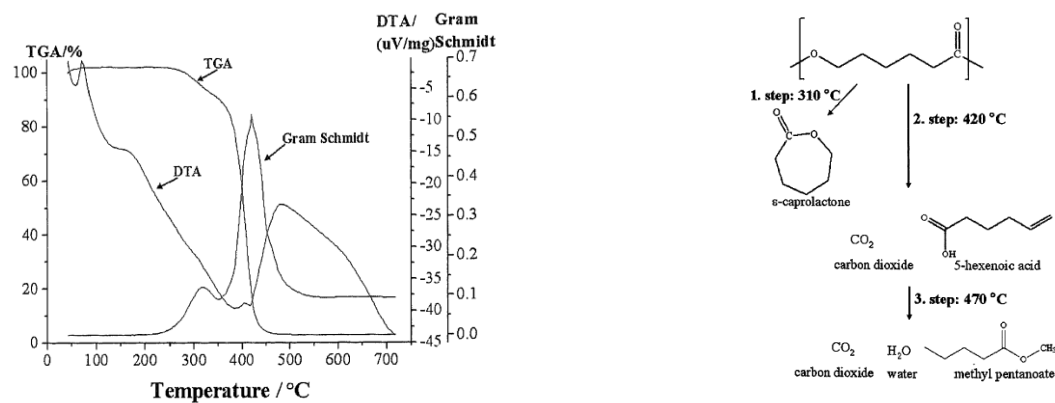


Figure 2-14(a) TGA, DTA and Gram Schmit Thermograms and proposed 3 stage degradation mechanism for PCL^{10K} in N₂ atmosphere [354]

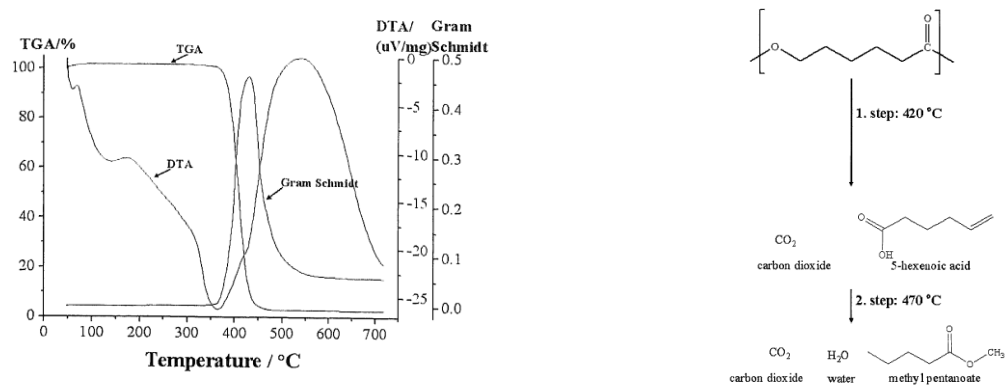


Figure 2-14(b) TGA, DTA and Gram Schmit Thermograms and proposed 2 stage degradation mechanism for PCL^{80K} in N₂ atmosphere [354]

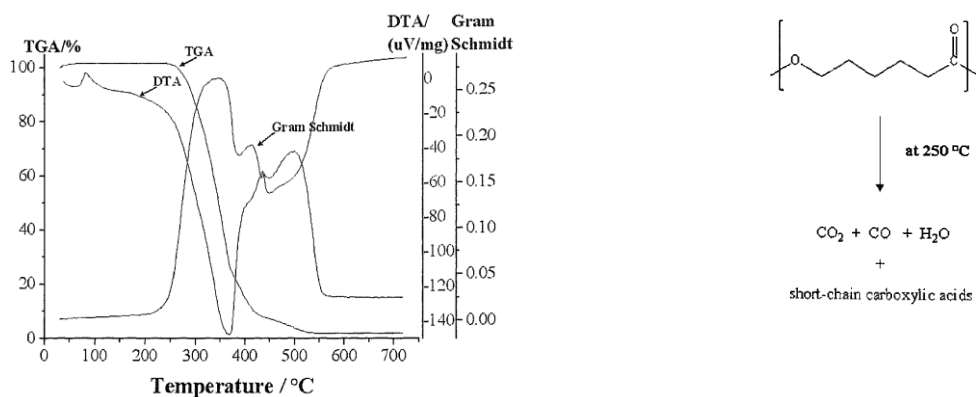


Figure 2-14(c) TGA, DTA and Gram Schmit Thermograms and proposed 1 stage degradation mechanism for PCL^{80K} in O₂ atmosphere [354]

Sivalingam *et al.* [350] investigated isothermal and non-isothermal degradation of PCL in a nitrogen flow. They concluded that there was distinctly different degradation behaviour depending on the heating type. Under isothermal heating the polymer degraded by pure unzipping of specific products from the hydroxyl end of the polymer chain. Under non-isothermal heating there was a simultaneous two stage degradation, random chain scission followed by specific chain end scission.

2.4.4.3 Activation Energies

The activation energies of PCL have been studied in order to obtain the kinetics, for degradation, which are believed to be first order. Activation energy is the minimum amount of energy required to activate atoms or molecules to a condition in which they can undergo a chemical transformation or physical transport [366]. A typical DSC trace for PCL can be seen in Figure 2-15, [348]. The endothermic peak at 67.26°C, without mass loss, is due to the melting of the crystalline phase of PCL (ΔH value 109.67Jg⁻¹) and the peak at between 380°C and 500°C corresponds to the PCL thermal decomposition (ΔH value 313.47Jg⁻¹).

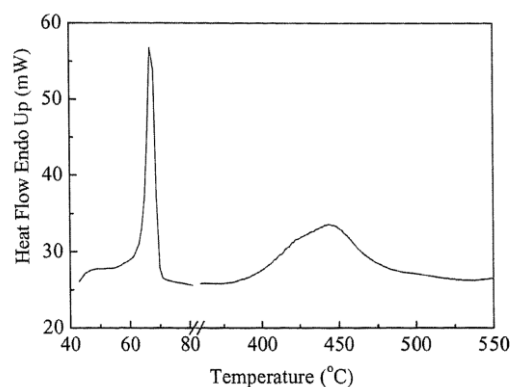


Figure 2-15 DSC Curve of PCL at 20°C min under N₂ Atmosphere. The endothermic peak at 67.26°C, without mass loss, is due to the melting of the crystalline phase of PCL and the peak at between 380°C and 500°C corresponds to the PCL thermal decomposition [348]

Freidman and Chang plots indicate a two stage degradation mechanism for non-isothermal heating when a 2.5 -3 fold shift in activation energy (from low to high temperature) occurs [351]. This suggested that the governing mechanism changes during degradation from random chain scission to specific chain end scission with increase in temperature. The activation energy for the random chain scission of PCL was 18.5kcal/mol and 55.5 kcal/mol for the specific chain scission. A summary of activation energies found in the literature can be seen in Table 2-15.

There are several methods for calculating the Energies of activation and this maybe where the differences in results occur. These include the Freidman [359] and Chang equations, Kissinger-Akahira-Sunrose (KAS), Kissinger [360], Flynn-Wall-Ozawa [361, 362] and Coats-Redfern methods [363], Appendix A. The advantage of the Kissinger and Flynn-Wall-Ozawa methods is that no previous knowledge of the reaction mechanism is required.

Energy of Activation for Random Chain Scission (KJ/mol)	Energy of Activation for Specific Chain Scission (KJ/mol)	Method	Reference
-	241±26	Kissinger	[355]
-	206 - 286	Flynn-Wall-Ozawa	
-	150.2 – 182.2 98.2 - 119.4 72.2 – 88.4 289.0 – 347.4 310.5 – 370.0 583.4 – 700.4	Coats-Redfern Method*	
63.6 – 90.2	114 – 158.1	KAS	[348]
70.0 – 95.9	118.9 – 163.1	Flynn-Wall-Ozawa**	
75.31	259.41	Freidman and Chang	[350]
-	101.67	Not specified	[357]
-	106	Not specified	[351]
77.40	232.21	Freidman and Chang	[416]
75.31	230	Not specified	[358]
74±4	-	Ozawa	[335]

* This depended on the melting mechanism, ** Different heating rates

Table 2-15 Summary of random chain scission and specific chain scission activation energies for PCL using various methods

2.4.4.4 Factors affecting PCL Degradation Rate

There has been much research on factors affecting the degradation rate of PCL. Catalysts have already been shown to influence the polymerisation rate, polydispersity, degree of monomer conversion, and final molecular weight of PCL, section 2.5.1. The rates of chain cleavage can also be affected by the degradation medium, pH, temperature, sample thickness and crystallinity of the PCL [321, 323, 370]. If the medium is more acidic or basic the degradation tends to be quicker, with basic conditions being more favoured [313]. The presence of ethanol, pentanol, 5% oleic acid, decamine and tributylamine increase the rate of chain cleavage [378]. The higher the temperature (increasing the energy of activation, E_a) and the thinner or smaller the sample size or less surface area the higher the degradation rate. If the PCL is less crystalline the degradation rate can also increase [293]. Surface modifications such as NaOH surface treatment can increase the degradation rate [371, 372]. This

occurs by an increase in concentration of the hydrolytic terminal groups (carboxyl and hydroxyl), [373-377]. The Processing conditions, the polymer form (whether filaments or thin film), scaffold structure, PCL fabrication methods (e.g. electrospinning, solvent casting, particulate leaching, foaming, hot pressing etc.) can all affect the degradation [322, 324]. Factors affecting the degradation kinetics, include chemical composition and configurational structure, processing history, molar mass (M_w), polydispersity (M_w/M_n), environmental conditions, stress and strain, morphology (e.g. porosity) and chain orientation, distribution of chemically reactive compounds within the matrix, additives [368, 369], presence of original monomers and overall hydrophilicity. The higher the molar mass the lower the degradation, the more hydrophilic the monomer, more hydrophilic acidic end groups, more reactive the hydrolytic group in the backbone, the greater the porosity and pore size the more accelerated is the polymer degradation [293,82]. Copolymerisation of ϵ -caprolactone with other polymers and addition of other species such as PBG has been shown to achieve a more desirable degradation rate and improved mechanical properties [379, 380-382].

2.4.4.5 Improvement of Physical and Mechanical Properties

The modulus of PCL alone is approximately 0.45GPa and the modulus of compact bone is 3-30GPa. The tensile modulus and tensile strength of PBG can range from 20-50GPa and 0.10-0.5GPa respectively [383, 221]. Table 2-16 shows historic examples of materials combined with PCL which can be tailored in order to achieve a fit for purpose material.

Description	Improved Property	Reference
PCL/ Chitin fibres	Tensile and flexural strength, Young's modulus	[384, 385]
Powder metallurgy methods	General mechanical properties	[386]
PCL/ carbon fibre	Increased tensile yield, bending strength and modulus	[387]
PCL/ smectite clays	Increased modulus	[388, 389]
PCL/PLA	Transparency and flexibility, fracture properties	[390-392]
PCL/ PLLA	Fracture toughness	[393]
PCL/PLA/ organo clay nanocomposites	Biodegradability	[394]
PCL/ starch	General mechanical properties	[395]
PCL/ polypyrrole	Electrical conductivities	[396]
PCL/ gelatine	Cell adhesion and biocompatibility	[255]
PCL/ chalcone	Fibroblast adhesion	[397]

Figure 2-16 Details of improved properties of PCL when combined with other materials as a composite

2.5 Phosphate Based Glass (PBG)

2.5.1 Introduction

Glasses in the P_2O_5 -CaO- Na_2O system have a chemical composition similar to that of the inorganic phase of bone, hydroxyapatite ($Ca_{10}[PO_4]_6OH_2$) [398, 399]. PBG have many unique properties and have excellent osteoconductivity [418]. From a biomedical perspective one of the most interesting is the ability to dissolve in aqueous media releasing bioactive and biocompatible ions [400, 414] however, the use of the PBG alone is restrictive due to their dissolution ability and brittle nature [401]. Therefore, by altering the chemical structure the dissolution rates can be altered, varying over several orders of magnitude. PBG have excellent mechanical strength and castability, formability, high thermal expansion co-efficients, low melting points [421] and good optical characteristics [422]. PBGs have been studied extensively and

other components have been added to increase their durability. A list of typical compositions can be seen in Table 2-17. Their current uses include; bacterial control devices, neural repair, oral healthcare, haemocompatibility and veterinary use [400, 342].

Composition	Reference
P ₂ O ₅ -CaO	[5, 403, 404]
P ₂ O ₅ -Na ₂ O	[405]
P ₂ O ₅ -CaO-Na ₂ O	[5, 73,74, 406-408]
P ₂ O ₅ -CaO-Fe ₂ O ₃	[410, 411]
P ₂ O ₅ -CaO-ZnO	[412]
P ₂ O ₅ -Na ₂ O- Fe ₂ O ₃	[413]
P ₂ O ₅ -CaO-Fe ₂ O ₃ -V ₂ O ₅	[410]
P ₂ O ₅ -CaO- Na ₂ O-Fe ₂ O ₃ -	[40, 68, 414]
P ₂ O ₅ -CaO-Na ₂ O-TiO ₂	[5, 402]
P ₂ O ₅ -CaO-Na ₂ O-K ₂ O	[415]
P ₂ O ₅ -CaO-Na ₂ O-AgO	[399]
P ₂ O ₅ -CaO-Na ₂ O-MgO	[416, 417]
P ₂ O ₅ -CaO-MgO-ZnO	[412]
P ₂ O ₅ -CaO-MgO--K ₂ O	[418]
P ₂ O ₅ -CaO-MgO-TiO ₂	[418]
P ₂ O ₅ -CaO-K ₂ O-MgO-SiO ₂	[416]

Table 2-17 Typical compositions of PBG which have been investigated for various medical purposes

2.5.2 Synthesis

There have been minor variations in the synthesis of PBG recorded in the literature. The primary method utilises appropriate mass fractions of the oxides, placing them in a Pt/10%Rh or Pt10%Au crucible, melting and cooling [40, 409]. Slight variations have been recorded in order to get a better annealing process (where heating and re-heating is performed to relieve stresses within the glass), [419]. Ahmed *et al.*, [420] have also, looked at comparing PBGs manufactured from different precursors (Group A: NaH₂PO₄ with CaO and P₂O₅, Group B: NaH₂PO₄ with Ca((H₂PO₄)₂). The results showed similar structural properties (glass transition temperatures and crystallinity) but there were significant differences in the physical properties (dissolution rates and density values).

2.5.3 Structure

PBGs are built via a network consisting of two bridging oxygen, one P=O double bond and one non bridging oxygen per tetrahedron. The bridging oxygen atoms, in the network form potential centres for hydrolysis, Figure 2-16 [400, 418].

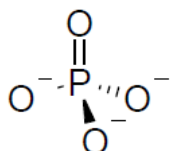


Figure 2-16 Illustration of the basic phosphate tetrahedral with two bridging oxygens, one P=O double bond and a non-bridging oxygen [400].

The tetrahedra are classified using the Qⁱ terminology originally devised for silicon glasses [425] but has been applied to phosphates [426], where i represents the number of bridging oxygens per tetrahedron, Figure 2-17.

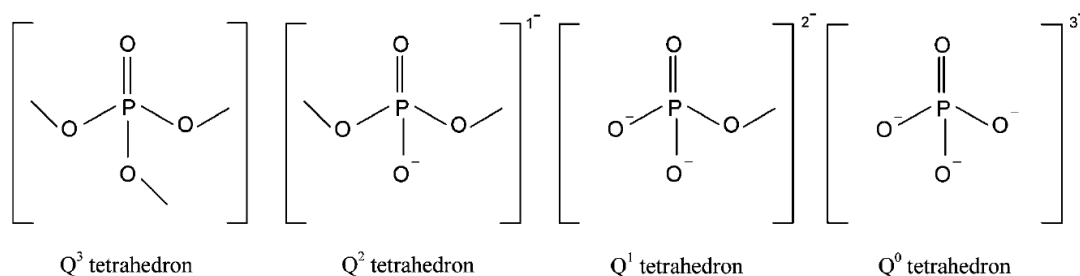
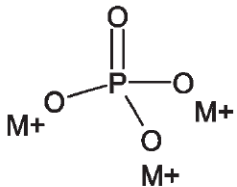
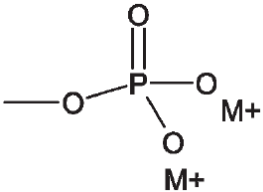
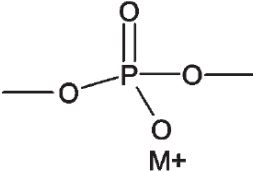
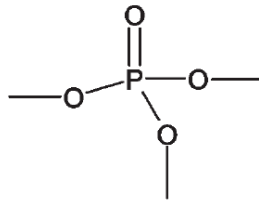


Figure 2-17 Nomenclature and representation of PO₄ with different polymerisations [70]

The prevalence of any particular Q species is dependent on the cation content of the glass composition. If a charge on an oxygen atom is balanced by a cation then it will not be possible for an oxygen bridge to form [427]. If phosphorus pentoxide, without any cation additions, is heated, then the Q³ species will be the only phosphate group present forming a highly cross-linked phosphate network. Vitreous PBGs can be

classified as 3D networks that are composed entirely of chains/ rings of PO_4 tetrahedra, which can be attached to a maximum of three neighbouring tetrahedra. The addition of modifying oxides will lead to the depolymerisation of the network resulting in the cleavage of the P-O-P bonds and creating non-bridging oxygen in the glass. A summary of this phenomenon for all 4 tetradron types is shown in Table 2-18.

Description	P ₂ O ₅ Network Type			
	Q ⁰	Q ¹	Q ²	Q ³
Structure				
Group Classification	P ₂ O ₅ + 3 M ₂ O	P ₂ O ₅ + 1 to 2 M ₂ O	P ₂ O ₅ + 1 M ₂ O	P ₂ O ₅ + < M ₂ O
Phosphate Type	Orthophosphate	Pyrophosphate	Metaphosphate	Ultraposphate
Additional Information	—	Theory of lower potential chain length. As this anion can only form one oxygen bridge it is termed a chain terminator, producing only phosphate dimers, or pyrophosphates, when it is the only species present.	Non branched phosphate chains are formed, theoretically of infinite chain length. Polymer-like structure.	Only phosphate present no cations. A highly cross-linked phosphate network is formed.

Where M is a metal oxide cation.

Table 2-18 Summary information on the tetrahedral structure of PBG for orthophosphate, pyrophosphate, metaphosphate and ultraposphate, including the group classification [400, 426, 428]

PBGs are referred to as binary, ternary and quaternary depending on how many elemental oxides (modifiers) are in the formula. The structure of PBG and PBG with additional modifiers can be seen in Figure 2-18(a) and (b).

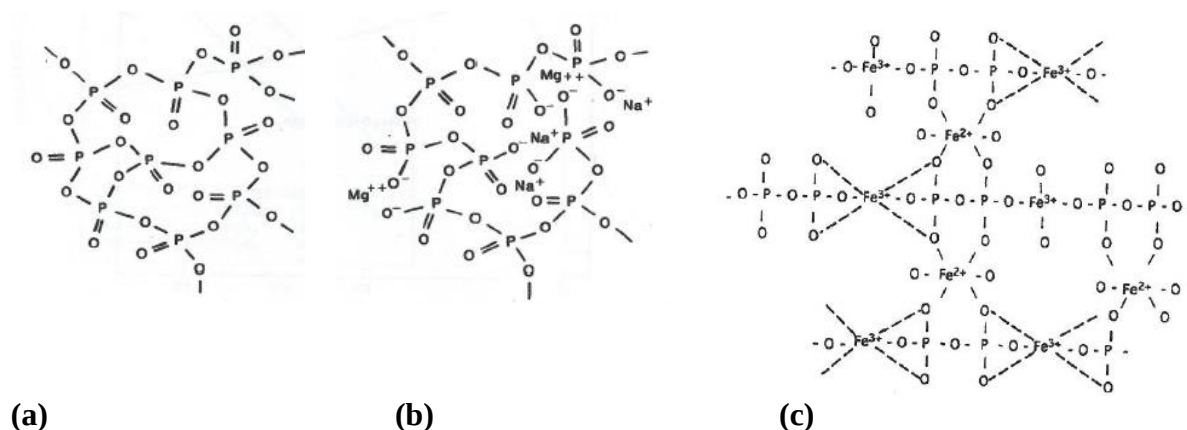


Figure 2-18 Structure of PBG (a) PBG with Na^+ and Mg^{2+} modifiers (b)[341] and an idealised PBG structure with Fe^{2+} and Fe^{3+} ions (c)[423]

Each phosphorous has an O^- to which a metal ion (modifier) can coordinate [424]. The effect of glass modifier on PBG dissolution has been reported in several studies [69, 400, 401, 407, 371]. The valency of the metal oxide also has a large effect on the properties of the glass. Replacing a monovalent oxide by a divalent alternative (forming chelate cross links) increases mechanical properties and decreases the dissolution rate [69]. Ca^{2+} and Mg^{2+} will coordinate to 2 of the O^- sites and Na^+ will only coordinate to 1. The addition of these modifiers cleave the P-O-P chains and create ionic cross linking between non bridging oxygens in the glass of two different chains [429]. Increasing the bond strength of this ionic link can also account for enhancement of glass durability [433].

Fe-O-P bonds are more resistant to hydration than P-O-P bonds [414] which can improve chemical durability (degradation rate) and biocompatibility [430]. These findings were also previously reported by Marasinghe *et al.*, and Ray *et al.*, [431, 432] who found that the addition of Fe_2O_3 produced glasses with greater durability (correlated to the P-O-P associated with Q^2 tetrahedra). The addition of 4-5% mol as the quaternary component, of PBG glass was sufficient for cell attachment and

proliferation [430]. Fe^{3+} can be present in both tetrahedral and octahedral coordination, thus acting as a network former within the P-O-P backbone [423]. It contains two Fe^{3+} ions and will require 6 sites but it can reduce to Fe^{2+} during heating or melting hence, taking up 4 sites [413].

2.5.4 Dissolution

2.5.4.1 Dissolution Mechanism

When PBGs degrade in solution they produce ions (termed leachables) which are dependent on the glass structure [69]. According to the general accepted theory of glass dissolution [435-437], PBGs dissolve in aqueous media in the following two interdependent steps which are similar to those of silicate glasses, Figure 2-19.

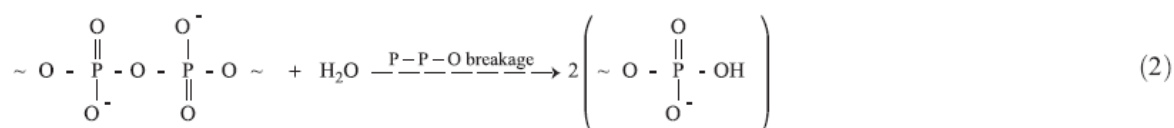
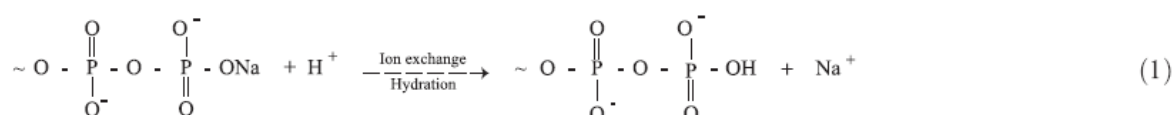


Figure 2-19 Dissolution mechanism of PBG [69].

(1) Hydration reaction: the glass exchanges its sodium ions with the hydrogen ions in water to carry out Na-H ion exchange reaction, resulting in the formation of a hydrated layer on the glass surface at the glass-water interface.

(2) Network breakage: under attack of hydrogen ions and water molecules, the P-O-P bonds in hydrated layer break up and result in the destruction of glass network and the release of chains of phosphates with different degree of polymerisation into solution.

Strauss and Treitler [438] studied hydrolysis of branched sodium polyphosphate solutions. They found a first-order dependence based on viscosity measurements. Ray [439] also mentioned first-order hydrolytic degradation with an activation energy of $104 \pm 8 \text{ kJ.mol}^{-1}$ determined by viscosity measurements in a temperature range of 25-90 °C.

Bunker *et al.*, [401] also investigated the dissolution mechanisms of glasses having the composition $(50-x)\text{M}_2\text{O} \cdot x\text{CaO} \cdot 50\text{P}_2\text{O}_5$. It was suggested that they dissolved uniformly due to acid or base catalysed hydration of the polymeric phosphate network. Acid/ base reactions could aid glass dissolution by disrupting the ionic interactions between chains. These reactions were said to control the surface pH of the glass. The hydrolysis reaction resulted in the cleavage of P-O-P bonds, and had the potential to totally disrupt the polymeric phosphate network to produce orthophosphate (PO_4^{3-}). Figure 2-20 illustrates the structure of the phosphates that have been observed during leachables testing, PO_4^{3-} is one of these.

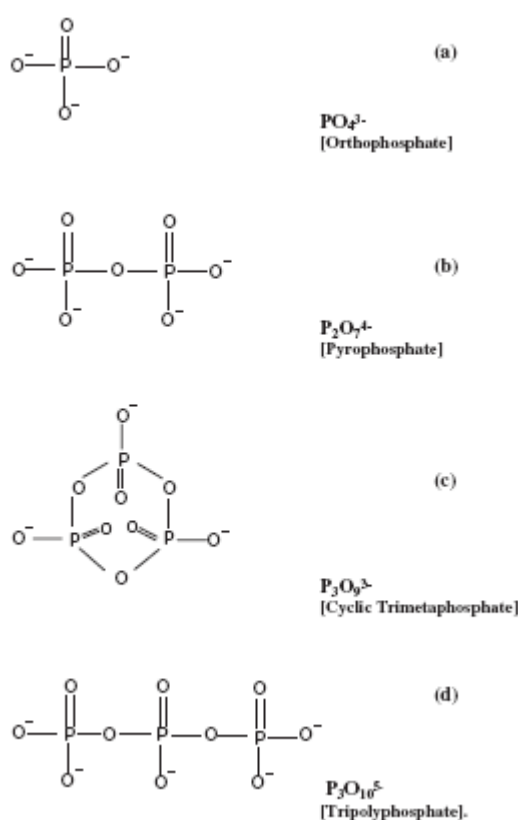


Figure 2-20 Structure of various PBG (PO_4^{3-} , $\text{P}_2\text{O}_7^{4-}$, $\text{P}_2\text{O}_9^{3-}$ and $\text{P}_3\text{O}_{10}^{5-}$) anions observed as leachables in aqueous solution [409]

The Hydration reactions involve the dissolution of intact chains. Bunker *et al.* [401] went on to say that the data they collated was more consistent with a dissolution mechanism involving simple hydration of the phosphate chains for compositions based on the above formula. During hydration reactions, totally hydrated chains

disentangle from partially hydrated chains still attached to the surface of the glass and float off into solution, resulting in uniform glass dissolution and linear kinetics. In acids, phosphate chains are protonated which disrupt ionic crosslinks between chains. Water is then able to penetrate the glass faster, resulting in rapid chain hydration and uniform dissolution. Clement *et al.*, [433] confirmed that PBG underwent a hydration mechanism at the surface of the glass when placed in simulated body fluid and totally hydrated phosphate chains disentangled and leached into the surrounding solution; simultaneously, a hydrated layer forms on the surface of the glass [69,151, 400, 401, 406]. This layer can be composed of calcium orthophosphate groups and the precipitation formation is determined by the characteristics of the surrounding medium and the solubility of the glass. This stage corresponds to the exchange of the sodium ions from the glass with hydrogen ions of the water. The velocity of formation and development of the hydrated layer depends on the diffusion rate of water molecules inside the glass. The second stage is a hydrolysis reaction corresponding to the cleavage of P-O-P links. The polymeric chains are hydrolysed by water molecules. The dissolution mechanism can be applied to bulk glass and fibres. Solubility measurements of PBGs show that there is compositional related non linearity in the dissolution of the glasses over time [88, 440]. Ahmed *et al.*, [409, 419] suggested that the proposed mode of degradation of ternary phosphate based glasses $45\text{P}_2\text{O}_5\text{-}55(\text{xCaO-Na}_2\text{O})$ was a hydrolysis reaction, with the small cyclic metaphosphates undergoing acid/ base catalysis. Anion release profiles showed a decrease for the PO_4 and linear polyphosphate P_2O_7 and P_3O_{10} species with increasing CaO content, Figure 2-24. This decrease is attributed to the cross-linking of the Ca^{2+} ions. In contrast, the cyclic P_3O_9 anion exhibited the highest amount of anionic release, which demonstrated similar trends to the cations. These release patterns suggest that the cyclic P_3O_9 species dominate the degradation rates. Hence, the proposed mode of degradation is a hydrolysis reaction, with the cyclic metaphosphate undergoing acid/base catalysis.

2.5.4.2 Factors Affecting PBG Dissolution

Factors affecting the dissolution rate of PBG are comprised of the chemical structure, manufacturing process, the storage medium, surrounding pH, temperature of the environment, the surface area to volume ratio and any heat treatments which can to alleviate internal stress.

The PBG composition has a significant impact on the dissolution rate of PBGs. The effect of various metal oxides has been studied. There was a trend observed for metal content and the more alkali the metal the glass contains, the lower the durability [401]. Durability being described as the resistance of glass to attack by a specified reagent [441]. In a static system, degradation rates go down with increasing di and tri-valent metal content [69] and a continuous flow has been shown to exhibit very different data.

In general, a lower dissolution rate is observed with an increase in CaO content [406, 419]. Increasing the concentration of cations within the glass was stated to reduce the dissolution rate by decreasing the velocity of water molecules inside the hydration layer [431]. However, the accumulation of Ca^{2+} ions and phosphate chains, in solution, have also been reported to slow down the dissolution in a static environment forming a protective layer [429]. Franks *et al.* [443] looked at quaternary based PBGs and substituted CaO and MgO. The CaO content was the most influential controlling factor. He discovered that the more CaO incorporated into the system the lower the solubility and more non-linear the solubility curve became. Bunker *et al.* [401] prepared glasses with the chemical composition $(50-X)\text{M}_2\text{O} \cdot X\text{CaO} \cdot 50\text{P}_2\text{O}_5$ (mol%) where M was either Li_2O or Na_2O . Solubilities were in the region

$3 \times 10^{-6} \text{ g cm}^{-2} \text{ min}^{-1}$ which mirrored values from Ahmed *et al.* [406]. Delahaye *et al.* [444] indicated a slower degradation rate compared to similar glass structures as Ahmed *et al.* [406]. This could be due to tests being performed at a constant pH3 and different precursors in preparing the glasses.

Five glasses containing HfO_2 and UO_2 were studied to see how these metal oxides affected the degradation rate of glasses containing phosphorous and iron [441]. The glasses were considered to be chemically durable (with dissolution rates of 10^{-9} to $10^{-8} \text{ g cm}^{-2} \text{ min}^{-1}$) which is similar to $40\text{Fe}_2\text{O}_3 \cdot 60\text{P}_2\text{O}_5$ (mol%).

Potassium was also studied and increasing amounts of this metal oxide can actually increase the degradation rate [415].

The addition of TiO_2 up to 5 mol% can decrease the degradation rate by one order of magnitude [5]. It was suggested this was due to high cross linked dense structure, evidence for this has been shown using glass transition (T_g) and density measurements.

Ion release data corresponds well with solubility and weight loss data and can give an indication of which ions will be released into solution first [448]. Bunker *et al.*, [401] showed that the durability decreases in the order $\text{Li} > \text{Na} > \text{K}$. Sodium ions have been shown to be released more quickly than calcium and magnesium ($\text{Na} > \text{Ca} > \text{Mg}$) [406, 429, 448]. Parsons *et al.*, [424] looked at several glasses containing iron, sodium and calcium and concluded that the solubility trend was $\text{Ca} > \text{Mg} > \text{Fe}$.

The decrease of Na^+ ion release with increasing Fe_2O_3 content has been attributed to two processes, the replacement of Na_2O with Fe_2O_3 (thus a reduced amount of Na^+ available) and increased durability of the glass with increasing Fe_2O_3 content. When samples contain the same amount of calcium but increasing Fe_2O_3 content calcium release reduces. It was stated that the release of iron is more complex than other cation species and it is dictated by the durability rather than the amount present in the original structure. Parsons *et al.* [413] showed that by adding iron to the glass structure the durability can be dramatically improved. 10 mol% Fe or more within the glass structure produced a constant density over 6 weeks in H_2O (37°C) and degraded from the surface and not the bulk. 5% Fe or less degraded completely within 24 hours and was believed to be due to some bulk degradation due to a reduced density. From 1-3 mol% Fe it was proposed that a viscous hydrated layer forms and that H_2O penetrates a significant distance into the glass. Highest degradation was seen for glasses containing 1% Fe_2O_3 . Ahmed *et al.* [430] saw an order of magnitude difference in degradation rate from 1 to 5 mol%. 10% Fe maintained 15 to 40% of the initial mass (after 6 weeks) and 15 to 20% Fe maintained 98% of the initial mass. The degradation rate was exponential with Fe addition. PBG can be synthesised to increase its durability by a factor of 2,000 when containing 20 mol% Fe in the structure (50P30Ca20Fe). Metcalfe *et al.* [411] examined 18 different structures of calcium PBG with iron. The amount of phosphorous ranged from 50 to 84 mol%, the

amount of calcium ranged from 8 to 48.7 mol% and the amount of iron ranged from 8 to 42 mol%. Further additions did not increase the stability of the composites. The results were similar to Kumar *et al.* [445] who reported dissolution rates in a pH7 buffered solution of $2\text{gm}^{-2}\text{day}^{-1}$ for a glass containing 5%wt iron oxide, reducing to $50\text{mgm}^{-2}\text{day}^{-1}$ when the iron oxide content increased to 22wt%. Abou Neel *et al.* [409] also reported that the addition of up to 5 mol% in the glass structure resulted in a significant reduction in degradation rate (by 2 orders of magnitude), which can be attributed to the more hydration resistant P-O-Fe bond [414].

It has been observed that there is often more of the cyclic trimetaphosphate ($\text{P}_3\text{O}_9^{3-}$) released than the orthophosphate PO_4^{3-} or linear $\text{P}_3\text{O}_{10}^{5-}$ due to higher amounts in the original glass structure [66, 409, 414]. Interestingly, no $\text{P}_2\text{O}_7^{4-}$ was detected in samples produced by Abou Neel *et al.*, [414] which contained iron and 50% P_2O_5 . However, small quantities (up to 18 ppm, in non-iron containing glasses) were observed in samples produced by Ahmed *et al.*, [408] which contained 50% to 55% P_2O_5 .

The storage media and surface area of the PBG to media volume ratio also has an impact on the degradation behaviour. The dissolution rate for iron containing glasses is slower in Hanks Buffered Saline Solution (HBSS) than deionised water due to the presence of ions in the medium [68,151]. The larger the surface area the more likely the degradation [69,429]. It has been reported that when bioactive glass ceramic materials are soaked in simulated body fluid at 37°C , a new calcium phosphate phase (similar to a calcium-deficient apatite crystal which forms in the living body), develops on their surfaces [446]. This can decrease the degradation rate. The rate was not reported as being dependent on the stirring speed of the solution and is a reaction based process. Several papers have documented changes in pH of solution after storage in deionised water. A summary can be seen in Table 2-19.

PBG	pH recorded	Comments	Reference
P:Ca:Na:Ga	7-8.6	-	[448]
P:Ca:Na:Mg	5.9	2 days: pH7, 7 days:pH7	[429]
P:Ca:Na:Ti	5.6-6.1	-	[449]
P:Ca:Na:Sr	4.0-4.7	-	
P:Ca:Na:Ti	6	10 days	[5]
P:Ca:Na:Ti	5	4 days	
P:Ca:Na	5.5	Seen in sample with higher Ca	[419]
P:Ca:Na	7	120 hrs	[399]
P:Ca:Na:Ag	5.5	-	
P:Ca:Na:Ag	6.5	In broth. After 72 hours.	
P:Ca:Na	3.5-4.0	-	[408]
P:Ca:Na:Fe	4	96 hrs	[414]
P:Ca:Na	6.4	From 6 to 7 then 6.4	[406]

Table 2-19 Typical pH changes of water after storage of various PBG structures.

It is clear from Table 2-19 that depending on the composition of the PBG and modifiers within the structure that the surrounding pH of the medium is affected. This in turn can alter the dissolution rate. There is a trend observed in that the higher the phosphate content, the lower the pH.

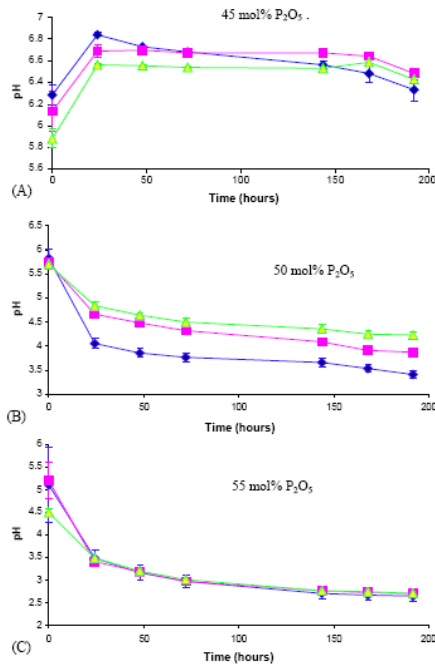


Figure 2-21: The pH profile changes depending on the amount of phosphate present suggested as due to the breakdown of higher phosphate compositions to release acidic HPO_4 and PO_4^{3-} ions into solution [406]

The lowering of pH in solution has been attributed to the increased dissolution profile [40] and breakdown of higher phosphate compositions to release acidic HPO_4 and more PO_4^{3-} ions into solution [406], Figure 2-21.

Bunker et al., [401] stated that below pH6.5, the PBG adsorbs an excess of protons, above this pH it adsorbs an excess of OH^- .

Hou *et al.* [447] noticed that the degradation rate accelerates for some PBGs when exposed to thermal convection of solution at high temperature and increased roughness of the surface after prolonged leaching.

Annealed fibres have shown low dissolution rates [69]. From composite degradation profiles, it can be seen that a non-heat treated fibre composite starts to degrade much earlier than a heat treated fibre composite[40]. Heat treating phosphate fibres alleviates stresses within the fibres, hence, chemical and physical degradation occurs much more slowly [450]. The heat treated fibre composite started to degrade at approximately 200 hours as compared to 48 hours for the non-heat treated samples. Although a plateau is observed in the degradation profiles, they do still continue to

degrade, which was probably due the resulting surface acidity around the fibres accelerating/ continuing the matrix degradation. Ternary PBGs containing copper were generated with pulling speeds of 400 cm⁻¹ and 1,200 cm⁻¹ [451]. The degradation rate was seen to be linear and observed to be greater for the fibres pulled at 1,200cm⁻¹. It was proposed that this was due to fibres pulled at higher speed having a lower diameter. With lower diameter there is an increase in degradation due to an increase in surface area to volume ratio.

There have been several studies on the weight loss and degradation behaviour of PBG alone and also PBGs combined with polymers. A summary of the results can be seen in Appendix B. It details literature results based on ternary PBGs of the composition P, Ca and Na with some glass structures also containing the elements silver (Ag), iron (Fe), gallium (Ga), magnesium (Mg), strontium (Sr) and titanium (Ti). The addition of iron stabilises PBG by at least an order of magnitude [68, 414]. Sr can increase the degradation rate by an order of magnitude due to expansion of the glass network caused by the larger ionic radius of Sr ions compared to other ions such as Ca [249]. Whilst the presence of Ti can again reduce the rate considerably (up to an order of magnitude with 5 mol%) due to smaller ionic radii and creating a polyhedral network between adjacent phosphate chains in the glass network [449].

2.5.4.3 Linear and Non Linear Degradation Behaviour for PBGs

Both linear and non-linear degradation behaviour has been observed for PBGs. Copper, gallium and strontium oxide doped PBGs have shown linear degradation behaviour [448, 449, 451]. A highly linear degradation rate would indicate that the material is suitable for sustained delivery of ions and biological macromolecules [449]. Ahmed *et al.* [419] also showed linear degradation behaviour of ternary PBGs. Abou Neel *et al.* [415] examined PBGs with the structures 50P20Ca(30-x)Na_xK, where x ranged from 0 to 0.3 in 0.05 mol increments. The glasses were stored for 8 hours at 37°C in deionised water. The degradation rates were linear. The PBG fibres produced (40P20Na16Ca24Mg) gave similar dissolution curves, reducing over 50 hours to 50% of the starting mass. In contrast to Bunker *et al.* [401] there was no t^{1/2}

dissolution stage followed by a linear phase [429]. For the first 50 hours the relationship was approximately linear and this corresponds to Rhinehart's 24 hour data [404]. After 50 hours, dissolution became markedly non-linear, in contrast to many of the reported approximations [69, 443, 444]. Linear and non-linear degradation behaviour has been observed for iron containing PBGs [411, 413, 414, 424]. The glass structures contained phosphorous, sodium and iron (1 to 20 mol%) exhibit some non-linear behaviour up to 160 hours. For glasses containing up to 5 mol% Fe_2O_3 after 96 hours the graphs of weight loss were not linear, they began to plateau. This was connected to the static environment, minimal surrounding water velocity, a phosphate layer on the PBG, causing an increase in the ion concentration in the surrounding solution [414].

2.5.5 Thermal Properties

The glass transition temperature (T_g), temperature for crystallinity (T_c) and melting temperature (T_m) values of PBG structures have been examined, however upon close examination of the literature this is not extensive. An explanation of the first two terms is provided below:

- i. The glass transition temperature is the reversible transition in amorphous materials (or in amorphous regions within semi crystalline materials) from a hard and relatively brittle state into a molten or rubber-like state [235]
- ii. Crystallinity is the degree of structural order in material.

Ahmed *et al.*, [406] showed that the T_g of PBG increased with increasing CaO content. It also increased with 45 to 50mol% P_2O_5 but decreased with 55 mol%. Metcalfe *et al.*, [411] discovered that by increasing the amount of Fe_2O_3 , in various compositions based on $\text{P}_2\text{O}_5\text{CaOFe}_2\text{O}_3$, the T_g was reduced significantly (due to high charge density of Fe). The glass could also contain two oxidation states of Fe. This is dependent on the furnace temperature during melting and the amount of Fe originally present. DTA thermograms have shown two melting points for glasses containing 10Fe (911 ± 1 and $887\pm 24^\circ\text{C}$), [236]. All the glasses showed a tendency to crystallise. Silver (at 3 mol%) and TiO_2 can increase the T_g of ternary glass composites [5, 399]. PBGs made from different precursors have been analysed, by Dynamic Thermal

Analysis (DTA), to see if there were any differences between the T_g, T_c and T_m [420]. The resulting traces showed no differences indicating that different precursors do not alter thermal properties. A summary of the thermal properties of various PBGs can be seen in Table 2-20.

Glass Structure	T _g (°C)	T _m (°C)	T _c (°C)	Reference
PCaNa	367.77-440.67	-		[406]
PCaNaFe	390-490			[430]
45P30Ca25Na	385	729 and 730	511 and 512	[121]
45P40Ca15Na	433	723 and 751	560 and 563	
45P50Ca5Na	502	748 and 925	612 and 613	
PCaNaFe	-	910-922 863-911		[236]

Table 2-20 Summary of thermal properties (T_g, T_m and T_c) for calcium, phosphate, iron and sodium containing PBG

It is evident that by altering the chemical structure the thermal properties can be controlled. The T_g varies from 368°C to 502°C which is a considerable difference and this clearly has an effect on the T_m and T_c. Detailed thermographs can be seen for iron containing PBGs in Figure 2-22.

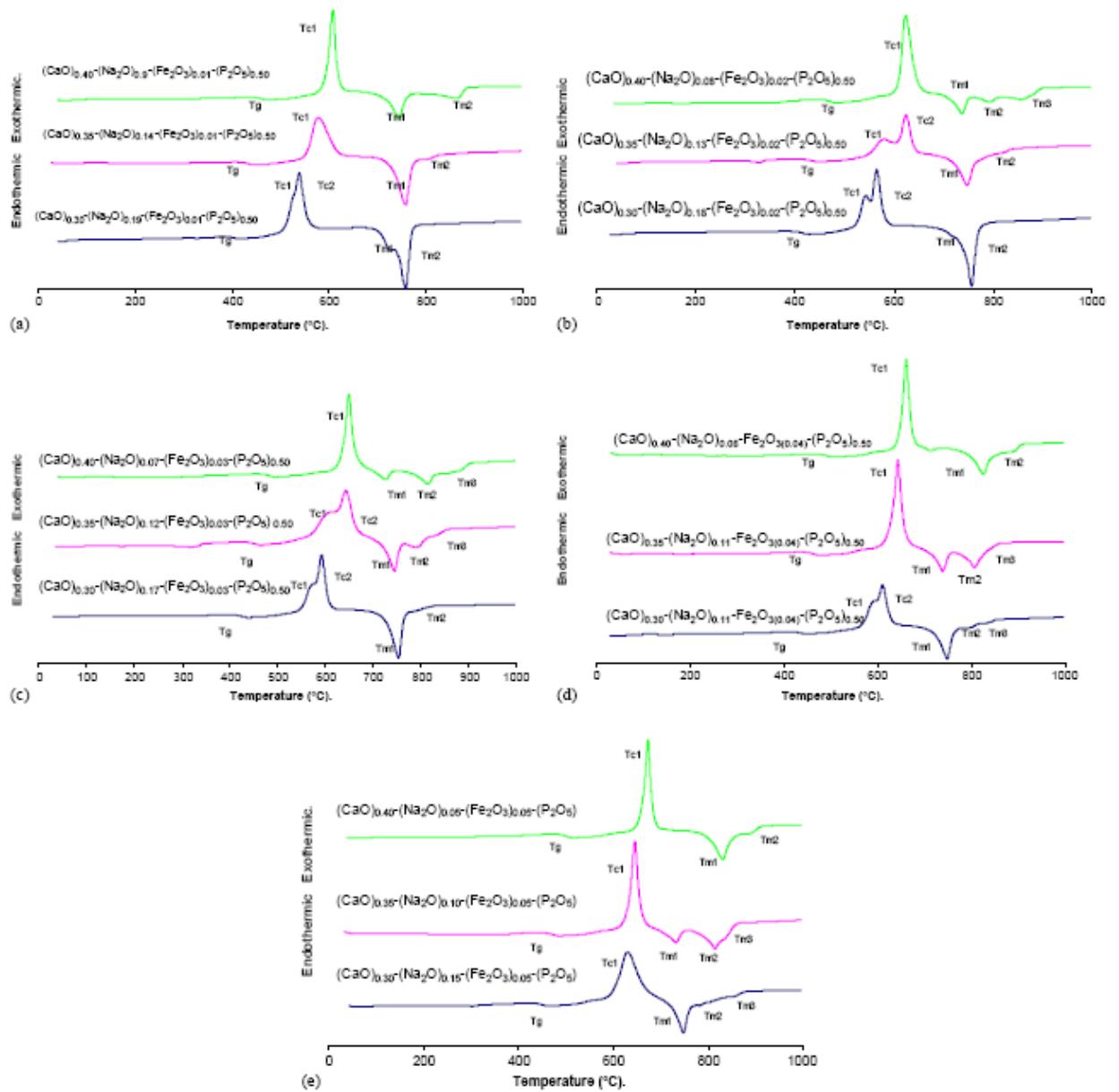


Figure 2-22 DTA traces for PBG containing (a) 1mol% Fe_2O_3 , (b) 2mol% Fe_2O_3 , (c) 3mol% Fe_2O_3 , (d) 4mol% Fe_2O_3 , (e) 5mol% Fe_2O_3 confirming changes in Tc, Tg and Tm with addition of Fe [406]

2.5.6 Characterisation

X-ray Diffraction (XRD) can complement thermal analysis when examining composites. Ahmed *et al.* [406,407] discovered two Tm peaks when performing thermal analysis on $\text{P}_x\text{Ca}_y\text{Na}_z$ glasses which indicated two crystalline phases, Table 2-21.

Glass Composition	Phase 1	Phase 2
P ₄₅ Ca ₃₀ Na ₂₅	Na ₄ Ca(PO ₃) ₃	
P ₄₅ Ca ₃₅ Na ₂₀	Na ₄ Ca(PO ₃) ₃	
P ₄₅ Ca ₄₀ Na ₁₅	NaCa(PO ₃) ₃	Ca ₂ P ₂ O ₇
P ₅₀ Ca ₃₀ Na ₂₀	NaCa(PO ₃) ₃	NaPO ₃
P ₅₀ Ca ₃₅ Na ₁₅	NaCa(PO ₃) ₃	
P ₅₀ Ca ₄₀ Na ₁₀	NaCa(PO ₃) ₃	Ca ₂ P ₂ O ₆
P ₅₅ Ca ₃₀ Na ₁₅	NaCa(PO ₃) ₃	
P ₅₅ Ca ₃₅ Na ₁₀	NaCa(PO ₃) ₃	Ca ₂ P ₂ O ₆
P ₅₅ Ca ₄₀ Na ₅	CaP ₂ O ₆	

Table 2-21 XRD results confirmed the existence of two phases identified in Na₂OCaOP₂O₅ glasses corresponding to two T_m peaks from thermal analysis [406, 407].

Ahmed *et al.* [419] also noticed more than one phase change when analysing PBG made from different precursors. However, XRD traces of Ag doped P₂O₅CaONa₂O glasses demonstrated the same phase for all three compositions investigated, NaCa(PO₃)₃, [399]. Ternary PBG containing TiO₂ (general composition: P₂O₅CaONa₂OTiO₂) showed one phase for the tertiary composite and 1 and 3 mol% TiO₂ (phase: NaCa(PO₃)₃). However, when 5 mol% of TiO₂ was added two phases were identified (phase: NaCa(PO₃)₃ and TiP₂O₇), [5].

The density of various PBGs has also been investigated but there is little information on this in the literature. Ahmed *et al.*, [419] showed that by increasing the amount of calcium the density increased, Figure 2-23. It was noted that there was a statistically significant difference between PBGs made with different precursors [420].

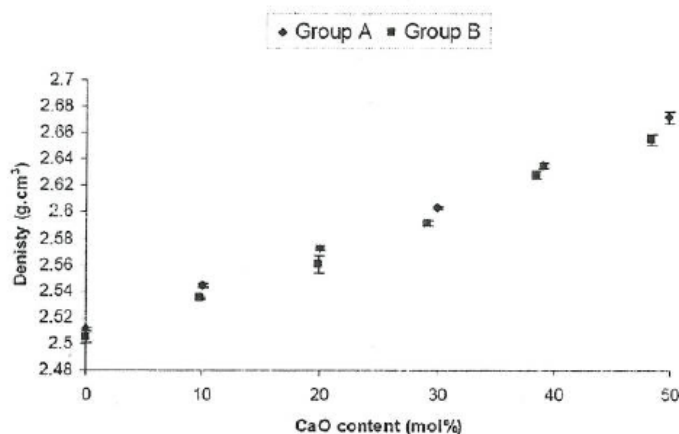


Figure 2-23 Density measurements of PBGs produced from different precursors [420], group A: NaH_2PO_4 , CaCO_3 & P_2O_5 , group B: NaH_2PO_4 & $\text{Ca}(\text{H}_2\text{PO}_4)_2$.

By increasing the amount of Fe_2O_3 the density also increases, Figure 2-24 [423], due to the formation of Fe-O-P bonds which have the dual nature of tetrahedral and octahedral co-ordination.

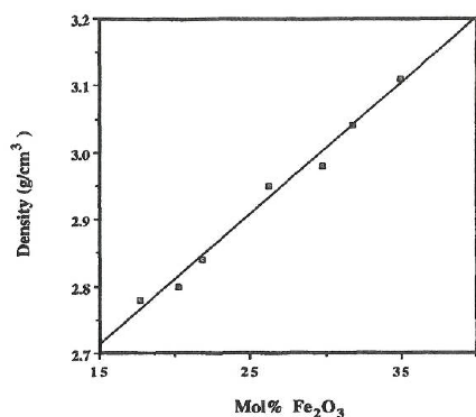


Figure 2-24 Increasing the amount of Fe_2O_3 in PBG increases the density due to the formation of Fe-O-P bonds [423]

Incorporation of potassium within PBG reduces the density. K^+ has a larger radius and is heavier than Na^+ but is less dense [415]. The literature papers reviewed all used the Archimedes principle using an alcohol, such as ethanol, or water to perform the measurements. No information was found on the density of PBG after storage in aqueous solution.

Fourier transfer infra-red (FTIR) studies have provided comprehensive information on structural aspects of PBG. Characteristic absorption frequencies for PBG are tabulated in Table 2-22. The spectra can show slight differences depending on the ions within the glass structure. For example if Fe_2O_3 is substituted by CeO_2 there is shift to lower frequency possibly due to the existence of covalent bonds between cerium and non-bridging oxygen's [237]. Spectra can be utilised to monitor tensile and compressive stresses [403]. This can be shown by a shift in frequency (near 1300 and 910 cm^{-1}) to higher wavenumber under tensile stress and lower wavenumber under compressive stress. Any changes in structure can also be detected by wavelength shifts, intensity differences and additional absent or presence of peaks [238, 239, 423]. Toloman *et al.*, [240] showed that the addition of more CaO leads to the breakdown of the ring type structures providing cross linking between non bridging oxygen of different chains and/ or groups, thus increasing the bond strength. Table 2-22 details wavenumbers associated with PBGs and can be used to aid identification of specific structural changes associated with a particular region of the PBG.

Wavenumber (cm-1)	Assignment
328	Bending of PO_4 units under a cation modifier
400-425	Bending vibration of P-O
478- 490	Bending harmonics of P-O-P
522	Overlapping vibrations involving iron polyhedral and $(\text{P}_2\text{O}_7)^{4-}$
~530	Bending harmonics of $\text{O}=\text{P}-\text{O}$
~550	Vibrations of Fe-O bonds in FeO_6 units
557-566	Bending vibrations of bridging phosphorous $\delta(\text{O}-\text{P}-\text{O})$
~630, ~640	Vibrations of Fe-O bonds in FeO_4 units
715-728	Stretching vibration of P-O-Ca bonding
~750	Stretching vibration of P-O-P
776-785	Symmetric stretching vibration of P-O-P in rings
780-940	Symmetric bending mode

Table 2-22 Typical frequencies and their assignments for the IR spectra of PBG [237, 240, 403, 410]

Wavenumber (cm ⁻¹)	Assignment
875-885	Assymetric stretching vibration of P-O-P groups linked with linear metaphosphate chain
~910, ~935	Asymmetric stretching vibration of P-O-P
924-948	Assymetric stretching of P-O-P groups linked with large metaphosphate rings
1002-1039	Assymetric stretching of P-O-P groups linked with small metaphosphate rings as cyclotriphosphate [P ₃ O ₉ ³⁻] or smaller groups
~1100, ~1030	Stretching vibration of P-O
~1090	Non bridging oxygen
1091-1123	Assymetric stretching of PO ₂ group, vas(PO ₂ ⁻) modes
1157-1174	Terminal phosphate groups
1230-1390	Asymmetric stretching mode
1278-1298	Assymetric stretching vibration of (P-O)
~1290	Stretching vibration of P=O
1386-1413	Symmetric stretching vibration of (P=O)

Table 2-22 Typical frequencies and their assignments for the IR spectra of PBG [237, 240, 403, 410]

Typical infra-red spectra of iron containing PBG can be seen in Figure 2-25. It can be seen that the triplets (800-1400cm⁻¹) decrease with increasing amounts of iron. The P=O bond breaks and the P-O-P bonds are replaced by P-O-Fe bonds. FeO₄ units appear with FeO₆ units. In the spectra below the CaO bonds are not directly evident.

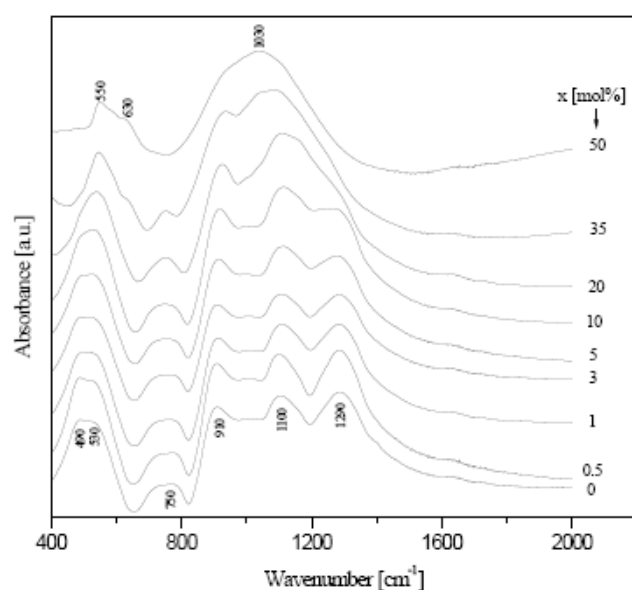


Figure 2-25 IR spectra of $x\text{Fe}_2\text{O}_3 \cdot (100-x)[\text{P}_2\text{O}_5 \cdot \text{CaO}]$ glasses. As the amount of iron increases the triplets ($800\text{--}1400\text{cm}^{-1}$) decrease the P=O bond breaks and the P-O-P bonds are replaced by P-O-Fe bonds [410]

2.5.7 In Vitro Behaviour

PBG has been shown to be completely soluble in an aqueous environment, releasing bioactive and biocompatible ions. They have been proposed as tissue engineering scaffolds for craniofacial skeletal muscle regeneration, where osteoblasts are seeded directly onto the fibres. Studies have shown that these cells have a preference in their initial attachment to fibres of certain composition and size, which in turn control the rate of degradation [414]. Cell adhesion studies performed Burling [243] reported that the least durable glass that allowed sustained cell adhesion contained 30 mol% Ca which possessed a dissolution rate of $1.7 \times 10^{-5} \text{ g cm}^{-2} \text{ h}^{-1}$. Whilst the least durable phosphate glass reported to allow osteoblasts to proliferate were Bitar's 40 mol.% CaO glass [242] and Parsons 20 mol.% Fe glass [413] which possessed a dissolution rate of $3 \times 10^{-6} \text{ g cm}^{-2} \text{ h}^{-1}$. Glasses with a dissolution rate above these two values do not sustain osteoblast cell adhesion or proliferation respectively.

The advantages of composite materials are that the certain key quality attributes of the materials can be combined to produce a material which is fit for the intended purpose.

In the case of potential bone scaffold materials this includes the high stiffness, strength, stability, customisability, flexibility and mouldability of a polymer with the added dissolution modification ability of a PBG.

The next section investigates composites of PCL and PBG including manufacturing, properties, behaviour and durability of such composites when stored in various aqueous media and when exposed to a thermal environment.

2.6 PCL and PBG Composites

2.6.1 Introduction

PCL/PBG composites are well tolerated in the body with minimal inflammation [453]. Combination of the two materials combines the advantageous processability of the polymers with the customisability of the phosphate glasses in order to meet the needs of their target application [74]. Table 2-23 references literature on PCL/PBG composites with intended end use.

Potential Use of PCL/PBG Composites	Reference
Cranial facial bone repair and bioactive root fillings	[72, 417]
Bone repair and tissue regeneration	[236, 453, 457]
Fracture fixation devices	[456]
Drug loaded samples	[73]

Table 2-23 Table summarising the potential use of PCL/PBG composites

There are many papers in the literature which focus on biomaterials with PBG [402, 458-462] but few primarily focusing on PCL/PBG composites. However, current research on this material does include, in vivo studies, degradation products and rates, morphology, mechanical properties and leachables studies [40, 72-74, 236, 417, 453, 456, 463].

This section will discuss composites of PCL and PBG. The literature shows that PCL/PBG composites have been manufactured by solvent extraction and thermal pressing techniques [40, 72-74]. This involves dissolving the PCL in chloroform or dimethyl carbonate, adding the glass, rotary evaporating and heat pressing. Surface treating the bioglass fibres with amine ended silane can initiate fibre surface polymerisation [464]. Onal *et al.* [417] examined in situ polymerised composite specimens. However, the work described in this thesis will look at PBG fibres which have been prepared in layers with PCL and melt compressed as this is a simple reasonably quick way of preparation, similarly to Mohammadi *et al.* [236], Khan *et al.* [456, 463] and Ahmed *et al.* [40].

2.6.2 Thermal Degradation

As far as the author is aware, there was no thermal information found in the literature relating to the combination of PCL/PBG containing iron. Papers mention the T_g, T_m and T_c of PBG containing calcium, iron, phosphorous and sodium used within a composite but no information on the actual resulting composite material [40, 236].

2.6.3 Hydrolytic Degradation

This section will focus on the effects of hydrolytic degradation of composites after storage in aqueous media and also the resulting media itself. In particular, extractables and leachables will be discussed as this is the main topic of this thesis

2.6.3.1 Leachables

The leachables from PCL/PBG have been monitored in vitro by ion chromatography [40, 72, 74, 236] or inductively coupled plasma spectrophotometry [73]. Literature tends to favour ion chromatography as this can be more discriminating for phosphate containing ions containing different valencies. No information has been found in the literature on extractables from PCL/PBG composites.

Ahmed *et al.* [40] reported the presence of higher phosphate species but this could not be quantified due to an absence of available commercial standards. To date, this has not been investigated further in current literature for PCL/PBG composites.

Table 2-30 shows the maximum and minimum amounts of ions recorded from the literature. The information is limited and the longest duration is for up to 28 days. It is clear that the presence of iron stabilises the leachables from the composite [72, 236]. From the limited data in Table 2-24 it appears that the ions released depends on the chemical composition of the glass and that the release of Ca , PO_4^{3-} , $\text{P}_2\text{O}_7^{4-}$, $\text{P}_5\text{O}_{10}^{5-}$ can increase by a factor of 10. The release of $\text{P}_3\text{O}_9^{3-}$ ions can have the potential to have a 100 fold increase. From the previous section there is evidence of two orders of magnitude decrease in leached Na^+ when up to 5%w/w Fe_2O_3 is incorporated in a PBG structure [414]. A later 14 day study (concluding PCL/ iron containing PBG composites as potential root canal obturation materials) showed that increasing the iron content from 1%w/w to 5% w/w decreased the amount of Na^+ leached. It decreased from approximately 290 ppm to 140 ppm [72] however, the initial amounts in the PBG were also lower initially (from 9 to 5 mol% respectively). It was clearer to see the decreased leaching of calcium as the quantity remained at 40 mol% as the Fe_2O_3 content was increased. The leached calcium ions decreased from 62 ppm to 36 ppm, indicating that by adding 5%w/w the leachables rate can potentially be halved.

Structure	Ion of Interest	Time	Highest Amount Detected (ppm)	Lowest Amount Detected (ppm)	Comment	Reference
$(\text{Na}_2\text{O})_{0.55-x}(\text{CaO})_x(\text{P}_2\text{O}_5)_{0.45}$	Na	600 hrs	400ppm	150ppm	24 mol% and 36mol % CaO composite respectively	[74]
	Ca	600hrs	150ppm	90ppm	24 mol% and 36mol % CaO composite respectively	
	PO_4	200hrs	300 ppm	22ppm	The 32 and 36mol% exhibited a tenfold increase in PO_4 release compared to 24 and 28 mol%.	
	P_2O_7	200hrs	40ppm	10ppm	24 mol% and 36mol % CaO composite respectively	
	P_3O_9	200hrs	180ppm	90ppm	36 mol% and 24mol % CaO composite respectively	
	P_5O_{10}	200hrs	110ppm	30ppm	24 mol% and 36mol % CaO composite respectively	
$0.45\text{P}_2\text{O}_5\text{-xCaO-(0.55-x)Na}_2\text{O}$	Ca	160hrs	0.325mM	0.15mM	Stored in PBS, composite contained 1%w/w Vancomycin drug. IPC used as analysis technique	[73]
	P	160hrs	1.0mM	0.2mM	Stores in PBS composite contained 1%w/w Vancomycin drug.	
50P ₂ O ₅ 50CaO	Ca	750hrs	1000ppm	300ppm	Higher volume fraction and heat treated glass, and low volume non-heat treated glass	[40]
	PO_4	750hrs	Values not reported	Values not reported	After 300hrs the release rate decayed. Non heat treated fibres released ions at slightly higher rate.	
	P_2O_7	750hrs	400ppm	100ppm	Heat treated fibres released higher quantities	
	P_3O_9	750hrs	1300ppm	300ppm	Heat treated fibres released higher quantities	
	P_5O_{10}	750hrs	Values not reported	Values not reported	Heat treated fibres released higher quantities. Quantities were relatively small for both types.	
50P ₂ O ₅ 40CaONa _x XFe ₂ O ₃	Na	336 hrs (14 days)	280ppm	140ppm	Composite stored in tooth canals. 1mol% Fe released higher quantities than the 5mol%.	[72]
	Ca	336 hrs	71ppm	36ppm	As above	
	Fe	336 hrs	N/A	N/A	This was not reported.	
	PO_4	336 hrs	512ppm	291ppm	Composite stored in tooth canals. 1mol% Fe released higher quantities than the 5mol%.	
	P_2O_7	336 hrs	88ppm	42ppm	As above	
	P_3O_9	336 hrs	165ppm	58ppm	As above	
	P_5O_{10}	336 hrs	26ppm	12ppm	As above	
	Ca	28 days	20ppm	-	The release values are less than other composites studied	
50P ₂ O ₅ 40CaO10Fe	Fe	28 days	10ppm	-		[236]
	PO_4	28 days	20ppm	-		

Table 2-24 Summary of leachables from PCL/PBG composites found in the literature. All samples stored in H₂O at 37°C unless otherwise stated.

The pH of PCL/PBG materials has been studied to observe how the degradation of the samples affects their local environment. If a composite is to be used as an implant material an adverse effect to surrounding tissues would be undesirable.

Placing PCL/ ternary PBG composites (P, Ca, Na, 80PCL:20PBG) into solution had a significant effect on the pH of the surrounding solution. Interestingly, the results from the study contradicted mass change and stiffness data. The biggest pH change was expected to be observed from high dissolution rate glasses. However, the changes were minimal. It was suggested that this was due to a buffering effect of the high calcium glass components. Also, as the calcium fraction of the composite increased, the pH of the surrounding solution accordingly became slightly acidic, with the 36 mol% composite sample solution dropping to just below pH6. This was due to a higher release of PO_4 , which consequently resulted in the pH becoming more acidic. The lowest calcium containing composite (24 mol%) remained mostly neutral throughout the study [74]. Ahmed *et al.*, [40] examined PCL/ binary calcium phosphate glass (60PCL:40PBG) composites and used several techniques plus pH data to suggest that the composite showed PBG dissolution was more or less complete after only 350 hours. The resulting acidity was due to phosphate ions in the solution, forming H_3PO_4 (phosphoric acid). Both of the studies just discussed saw fluctuation of increases and decreases in pH during storage eventually coming back to an approximately neutral solution (when the medium was changed at each time interval), indicating completion of PBG dissolution.

The addition of Fe_2O_3 to a PBG structure has already been discussed and evidence has shown that it can reduce the degradation rate. 5%w/w Fe_2O_3 containing PBG has shown a slight change in pH whereas, if the content is lower than this more significant pH changes have been observed (from pH5.7 to 4). The reason, provided, was again related to the presence of phosphorous oxides. Hydrated phosphorous chains dissociating into aqueous solution to form phosphoric acids [414]. Ahmed *et al.*, [52] concluded that a PLA/ PBG, of the same basic composition as a PCL/PBG composite studied in this thesis (50P:40Ca:5Na:5Fe), the PLA maintained neutral pH (as expected) and the composites dropped to approximately pH4 with a return to neutral solution upon complete degradation. Associated changes in pH have been studied over 56 days with 60PCL:40PBG composites containing phosphorous, calcium, silicon and iron (in de-ionised water at 37°C), [236]. The pH of neat PCL remained relatively constant however, the composites exhibited a decrease then increase in pH, as observed with

previous studies when the medium was replaced at each time interval. Overall, a slower rate of pH reduction was seen with addition of iron up to 10%w/w (50P40Ca10Fe), confirming the presence of Fe_2O_3 reducing PBG degradation.

2.6.3.2 PCL/PBG Weight Loss

The weight loss of PCL has been shown to be fairly consistent over time for storage in aqueous media, for up to 600 hours [74]. However, a slight increase has been observed due to water absorption (up to 50 hours). As a composite with binary PBG the weight loss is greater than for PCL alone, Figure 2-26. As the amount of CaO is increased the weight loss decreases showing that increased CaO content will increase durability, the 24 mol% calcium containing composite lost 20% of its weight whereas the 36 mol% composite lost approximately 4% of its weight. This is further evidence showing that the incorporation of PBG can help to control the degradation of PCL/PBG composites in order to produce a material which is fit for the intended purpose.

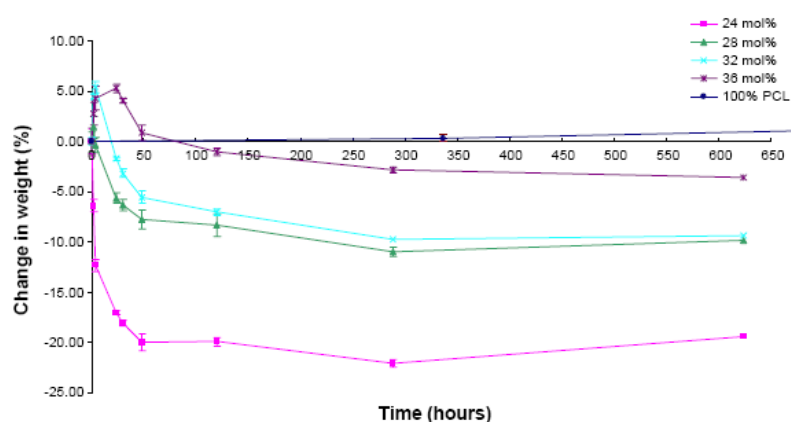


Figure 2-26 Profile of change in weight of PCL/PBG composites (based on Na:Ca) stored in H_2O at 37°C for 600 hours [74].

Appendix B provides a list of weight loss and solubility measurements taken for the literature. The weight loss for PCL and similar PCL/PBG (Na:Ca) composites containing 1% Vancomycin drug loading were also examined [73]. The samples were stored in phosphate buffer solution for 150 hours. The results showed slightly greater weight loss than those reported by Prabhaker *et al.*, [74]. It is proposed that this is due to the difference in the ratio of PCL to PBG in the structures. The PBG was the same composition for both studies

(45PxCa55-xNa) however, Prabhakar *et al.*, [74] used an 80:20 ratio of PCL/PBG and Kim *et al.*, [73] used a 50:50 ratio. Comparing the 150 hour timepoint, Table 2-25 shows a difference in the weight loss relative to the Ca composition for both studies.

Prabhakar <i>et al.</i> , [74]		Kim <i>et al.</i> , [73]	
mol%	Weight loss (%)	mol	Weight loss %
0	minimal	0	minimal
24	20	20	45
28	8	30	42
32	7	40	35
36	2	50	20

Table 2-25 Example of differences in degradation due to similar glass composition but different amount of PCL in the PCL/PBG structure [73,74]

The presence of iron can stabilise the PBG for up to 6 months, in H₂O at 37°C [463]. Further studies were performed which supported Ahmed's progressive studies [40, 399, 406-409, 419, 420, 430]. PLA and PBG with compositions similar to those studied in this thesis (50P40Ca9Na1Fe, 50P40Ca7Na3Fe, 50P40Ca5Na5Fe) were investigated. The experiment was held over a 6 week time period in deionised water at 37°C. The composites contained a 0.2 volume fraction of heat treated and non-heat treated PBG. The PLA exhibited no weight loss after 6 weeks which is similar to the findings for PCL [73,74]. The non-heat treated fibres exhibited 11% mass loss from 350 to 400 hours with a total weight loss of 14% after 1100 hours. The heat treated composite samples showed a weight loss for up to 200 hrs followed by a 10% mass loss after 1100 hours. This suggests that heat treating, which relieves stress within the PBG, can reduce overall composite weight loss by 4%.

Weight loss in simulated body fluid has shown a similar trend to deionised water however, to a lower extent due to the presence of ions [41].

As part of the structural analysis of composites, after being stored in aqueous media, density would be of interest because it is known that PCL undergoes bulk erosion and autocatalysis can occur. Density measurements can provide information on the extent of the bulk erosion and suitability of a material as a fracture fixation device. The density of PBG has been examined [236] however, there is no information on this parameter for PCL/PBG composites after storage in aqueous media.

2.6.3.3 Hydrolytic Degradation Rate and Mechanism

Studies indicate that the dissolution rate can be inversely proportional to the calcium fraction present within the glass [465, 400] so in glasses with low calcium fractions, their dissolution rate is more rapid. It was proposed that voids and micro cracks within the composite structure have an effect on degradation rate [74] and that surface area and stress was also a factor. L Onal *et al.* [417] stated that clearly, diffusion of water shapes the initial mass gain process. Since the curves indicate that moisture uptake by the pure PCL composite is negligible in contrast with the fibre reinforced specimens, this suggests that either the fibres are hygroscopic and / or that internal voids (including those at the fibre matrix interface) act as reservoirs. In glass alone SEM evidence shows pits and formation of precipitates on the fibre surface after 6 weeks. The hydrolytic degradation of PG fibre composites relies upon the interaction of water molecules with the molecular chains of both the polymer and the PBG. Different rates of hydrolysis will lead to the scission of macromolecular chains, particularly in the PBG fibres. The degradation period can therefore be summarised in three phases, which might be more or less coincident depending upon the solubility of the individual phases:

- i. moisture ingress via diffusion, manifested by mass gain.
- ii. Chemical interaction between water and macromolecular chains of degradable polymers.
- iii. Leaching of ions after scission of macromolecules, manifested by mass loss.

These studies showed that there was a clear transition from initial, diffusion dominated behaviour (where a moisture increase is observed) to a leaching effect, the changeover occurring at ~ 234 hours of exposure. There is a significant difference for the two fibre ratios examined. Although it follows that the initial moisture ingress is dominated by voids, rather than the saturation of the polymer alone, the void fraction rather than the fibre fraction, might be the main determinant of this event.

Mohammadi *et al.*, [236] agreed with this statement but focused on the stabilising effect of iron within the composite structure. He suggested that water uptake was associated with the glass fibre dissolution which created channels within the composites. This enabled H₂O penetration into newly formed porous structure at the fibre/ matrix interface. A plasticising effect [466] on the PCL then occurred alongside degradation of the glass fibres. Since the

solubility of 10%w/w iron containing PBG is very low (due to the iron acting as a network former within the P-O-P backbone and creating more hydration resistant bonds) it enables the composite to retain its mechanical properties for a much longer time period. The degradation rate is slower than PGA and PLA. It has also been demonstrated that PCL capsules remain intact when implanted for up to 24 months [467] and based on composites containing 10%w/w iron weight loss in H₂O, that the total degradation of the PBG maybe estimated to occur within 20 months (coinciding with PCL). This indicates that PCL with 10%w/w iron containing PBG is a strong contender for long term applications such as bone fixation devices.

2.6.4 Behaviour and Characterisation

2.6.4.1 Composite Structural Changes

There is minimal information available on structural changes of PCL/PBG composites in the literature. It was noted however, that the structure of the PCL/PBG composite changed after storage in phosphate buffer solution after 7 days [73], Figure 2-27.

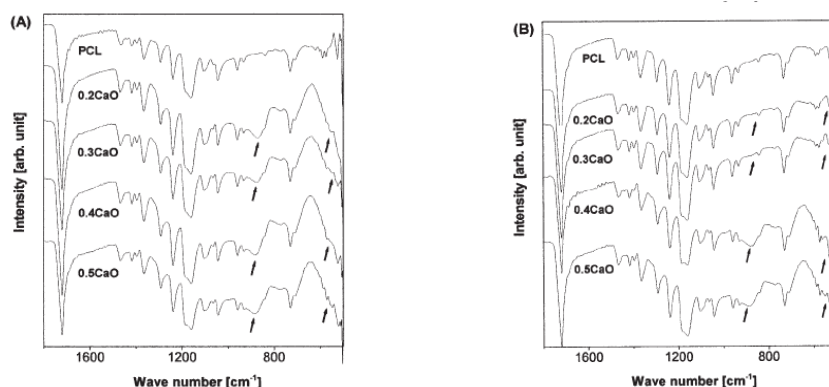


Figure 2-27 FTIR spectroscopy of PCL and PCL/PBG composites (Na:Ca) with different glass compositions before (a) and after (b) degradation for 7 days in phosphate buffered solution (PBS). Bands were attenuated for 0.2 and 0.3CaO composites after storage with an associated increase in molecular weight loss. [73]

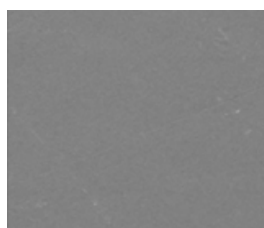
An approximate 50%PCL/ 50% w/w PBG composite (containing 45PxCa55-xNa) loaded with 1%w/w vanocymine drug was studied as a potential material for hard tissue engineering. Attenuated IR was used to examine the composites before and after storage in PBS. The

focus of the literature paper was on the change to the phosphate bands. The phosphate bands were present in the initial samples (irrelevant of CaO presence) however, after 7 days storage in PBS the bands for the lower amounts of CaO in the structure (0.2 and 0.3) exhibited attenuated bands and those with higher CaO (0.4 and 0.5) retained their initial intensities. An explanation for this could be due to the calcium ions creating ionic cross linking between non bridging oxygen's in the glass of two different chains and less hydrated phosphorous chains dissociating into aqueous solution.

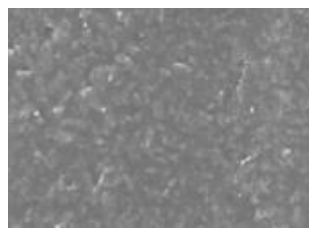
There is potential for structural changes of the PCL matrix due to interaction with PBG when processed at high temperatures. A study by Mohammadi et al., [41] examined the effect of heating PCL/PBG containing 10%w/w Fe_2O_3 using FTIR after heat treating at 25°C-60°C for 10 minutes, 100°C for 60 minutes and room temperature for 30 minutes. A 10%w/w SiO_2 PBG was also examined. The silicon containing composites showed a decrease in the carbonyl peak ($\text{C}=\text{O}$) at 1728 cm^{-1} and presence of a carboxylate peak at 1634 cm^{-1} . With accompanying data this indicates that Si containing glasses react with ester bonds due to high processing temperatures. This would be expected to result in a lower Mw PCL. This was not observed for PCL alone or the 10%w/w Fe_2O_3 PBG containing composite.

2.6.4.2 Morphology

The surface of PCL/PBG composites, before immersion in aqueous media, is relatively smooth Figure 2-33(a) and (b). However, after storage in H_2O for 25 days [74] surface pitting is observed (c). The surface pitting for a PCL/PBG composite with 1% drug loading (after 7 days storage in H_2O) was considerable [73]. This occurred over a quicker time interval due to the release of drug encapsulated within the PCL/PBG matrix. A transverse section of PCL/PBG (stored in H_2O) for 6 weeks) showed that the leaching of glass was almost complete [417]. Figure 2-28(e) and (f) show a 20 μm and 100 μm SEM image revealing a considerable amount of porosity.

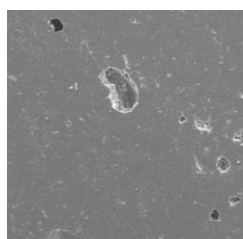


(a)

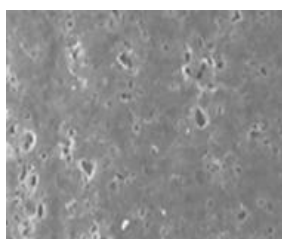


(b)

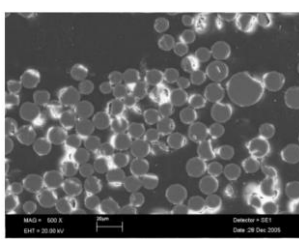
Figure 2-28 SEM images of (a) PCL/PBG and (b) PCL/PBG with 1% drug (Vancomycin) before storage in H₂O [73, 74].



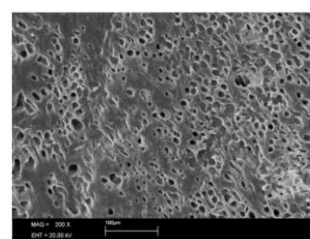
(c) scale 1mm -----



(d) scale



(e) scale 20um -----



(f) scale 100um -----

Figure 2-28 SEM images of (c) PCL/PBG and (d) PCL/PBG with 1% drug (Vancomycin) after storage in H₂O and phosphate buffer solution, 25 and 7 days respectively, [73, 74]. (e) and (f) traverse section exhibiting fibre loss [417].

Further studies by Ahmed *et al.*, [40] suggested that due to the difference in mass loss to mass fraction of fibres the volume fraction and surface area dominated over the intrinsic behaviour of the fibre within the polymer matrix. SEM images were taken of the composites investigated using non and heat treated fibres. The images show more detail than those from previous papers and confirms the dissolution of PBG from the PCL matrix, Figure 2-29(a) and (b). The length of the missing fibres within the matrix are greater than 100 μm long and the pore sizes are between 10 μm and 20 μm .

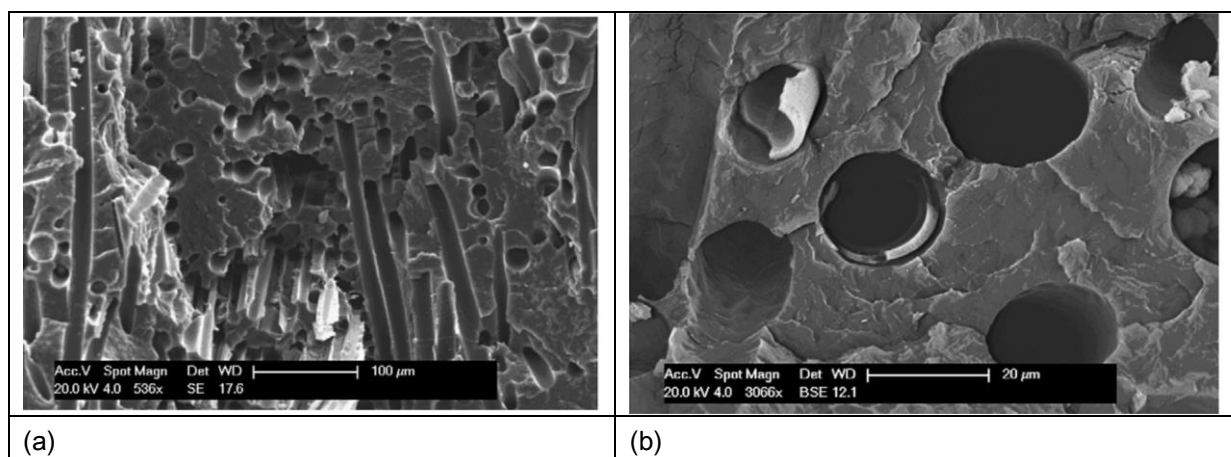


Figure 2-29 SEM images of PCL/PBG stored in water for 5 weeks. (a) non heat treated fibre composite, 100μm, (b), heat treated fibre composite, 20μm, [40].

The images looked similar to those of other PBG biomaterials. Images of PLA/ PBG can be seen in Figure 2-30.

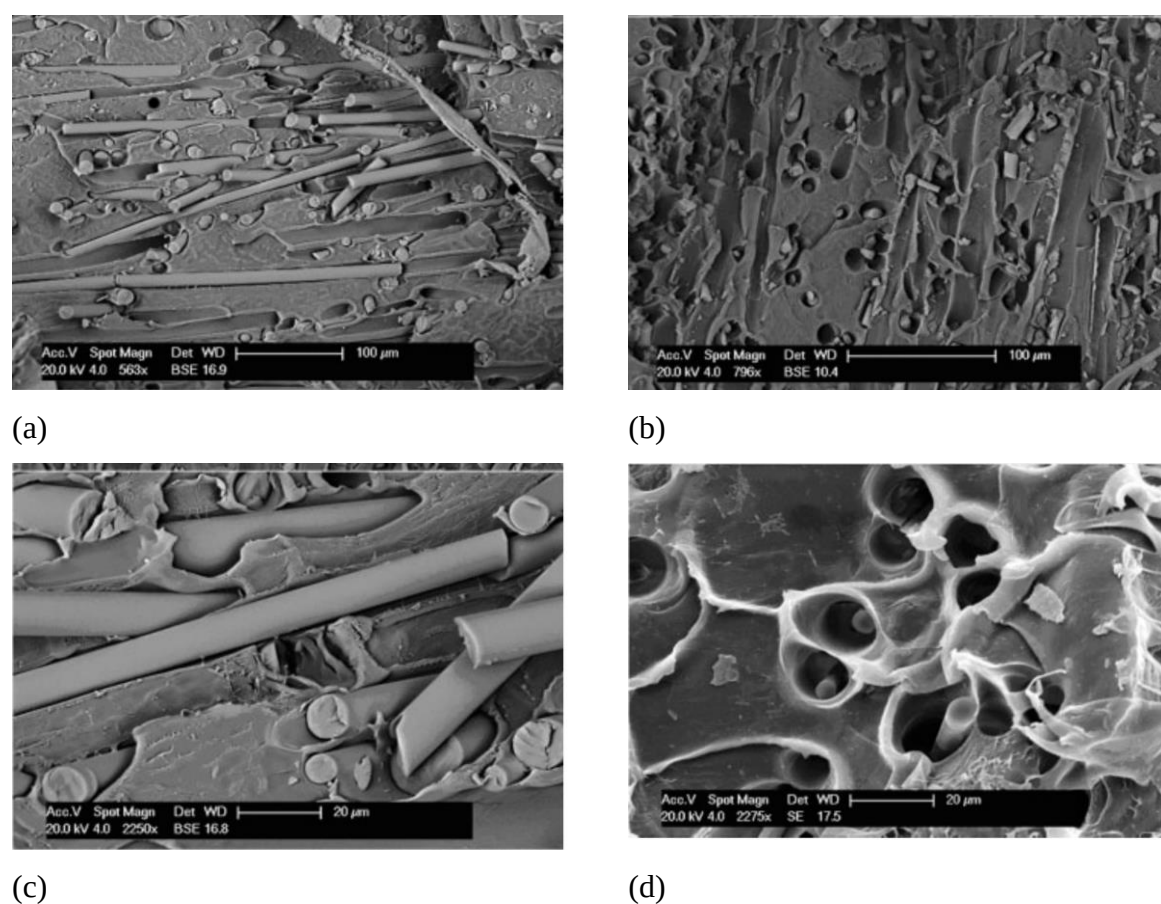


Figure 2-30 SEM images of PLA/ PBG composites after storage for 6 weeks in H₂O [52].

Mohammadi et al [236] managed to produce SEM images of cryogenically fractured PCL/PBG (50P40Ca10Fe) composites after storage in H₂O at 37°C for 56 days Figure 2-31.

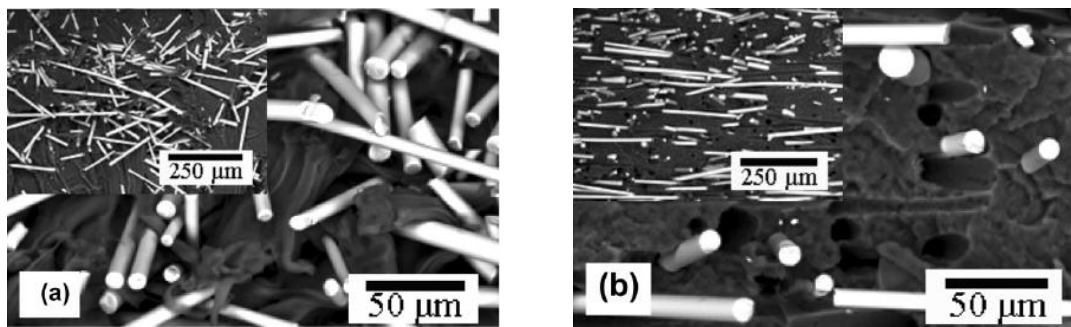


Figure 2-31 SEM image of cryogenically fractured PCL/PBG (50P:40Ca:Fe) composites stored in H₂O at 37°C for 56 days [236].

As part of the structural analysis of composites, after being stored in aqueous media, density would be of interest as this can be used to calculate porosity and pore volume [91]. Both of these factors are of importance when considering a scaffold material for hard tissue regeneration. The density of PBG has been examined [236] however, there is no information on this parameter for PCL/PBG composites after storage in aqueous media.

2. 7 Conclusion

From the literature reviewed for the purpose of this project, it can be concluded that totally resorbable composites for bone repair applications is required to avoid complications of rigid metal implant and removal surgery. Various biomaterials have been investigated as potential fracture fixation devices or scaffolds for bone support and regrowth. Many of these materials have limitations in relation to mechanical strength, dissolution rate or biocompatibility. Among the polymers developed so far PLA, PGA and PCL have been studied extensively for use in tissue regenerations [22, 97, 219, 221, 382, 468]. Due to the lower degradation rate of PCL compared to PLA and PGA attempts have been made to enhance/ control the degradation rate. PCL degrades via a hydrolytic process but it is a hydrophobic material, hence, the introduction of a hydrophilic component such a PBG can improve the rate of degradation [73]. PBG has attracted much attention in the hard tissue engineering field due to calcium phosphate ceramics having a similar ionic composition to that of hard tissue and

the dissolution of glass can be easily controlled by changing the composition [400, 88]. PBG can also improve biocompatibility. In-vitro tests have shown that PBG with a moderate dissolution rate can stimulate osteoblast and fibroblast cell responses [465]. A composite of PCL/PBG can improve flexibility and mouldability. PBG alone is highly brittle, lacks flexibility and can be available in limited sizes. In particular there is an interest in the addition of up to 10% Fe_2O_3 to the PBG structure as it has been shown to improve PBG durability due to being more resistant to hydration than P-O-P bonds and biocompatibility has also been shown to be favourable [414, 370]. There is a considerable amount of literature on PCL and PBG individually but little on the composite and even less on iron containing PCL/PBG. Potential gaps in knowledge include the leachables of these types of composites over a long term period and whether they would be suitable as fracture fixation devices.

CHAPTER 3 – MATERIALS AND METHODS

3.1 Introduction

Chapter 3 describes the materials and methods used to fabricate 3 novel compositions of PBG with PCL. Due to Fe_2O_3 being reported as having a significant influence on the degradation rate, 5%w/w and 10%w/w has been incorporated into two of the PBGs structures. Details of the techniques used to study these composites, after long term storage in aqueous media (for up to 78 weeks at 37°C) are described so that the results can be used to make an educated decision as to whether such composites can be used as scaffolds for hard tissue engineering.

3.2 Fabrication of PCL/PBG Composites

Three composites containing 90%w/w PCL and 10% w/w PBG were manufactured. The composition of the PBGs, within the composite, can be seen below:

- i. $50\text{P}_2\text{O}_5\text{-}35\text{CaO-}5\text{Na}_2\text{O-}10\text{Fe}_2\text{O}_3$
- ii. $50\text{P}_2\text{O}_5\text{-}40\text{CaO-}5\text{Na}_2\text{O-}5\text{Fe}_2\text{O}_3$
- iii. $50\text{P}_2\text{O}_5\text{-}45\text{CaO-}5\text{Na}_2\text{O}$

3.2.1 Rationale for Method

An extrusion technique was not employed as glass fibres can be difficult to handle due to their fine structures and there is potential for them to adhere in various orientations and break. Hence, the composites were manufactured using a compression moulder which enabled the build up of layers between glass fibre and polymer with some control over homogeneity. The method was similar to that of Callister [469] who demonstrated compression moulding using appropriate amounts of thoroughly mixed polymer and constituents placed between male and female mould cavities. Both mould cavities were heated, however, only one was mobile. The mould was closed then heat and pressure were applied, causing the plastic material to become viscous and conform to the mould shape.

3.2.2 Materials and Equipment

Materials and equipment used can be seen in Table 3-1.

Materials and Equipment		
Description	Batch/ Equipment Information	Additional Information
PBG	PBG	The PBG was made from oxides of Ca, Fe, Na, P at Nottingham University.
PCL	Solvay, Lot 2451	CAS 24980-41-4114 mpt 60°C Mn 42,500 Mw 65,000 Melt index 1.90g/ 10mins 80°C/ 0.3MPa
Flatbed compression moulder	Daniels UK	N/A
Hot stage microscopy	Mettler Toledo FT82HT	N/A

Table 3-1 Details of materials and equipment for PCL/ PBG composites

The thickness of the glass fibres was 0.061mm and the length varied in the region of 11mm to 16mm, Figure 3-1.

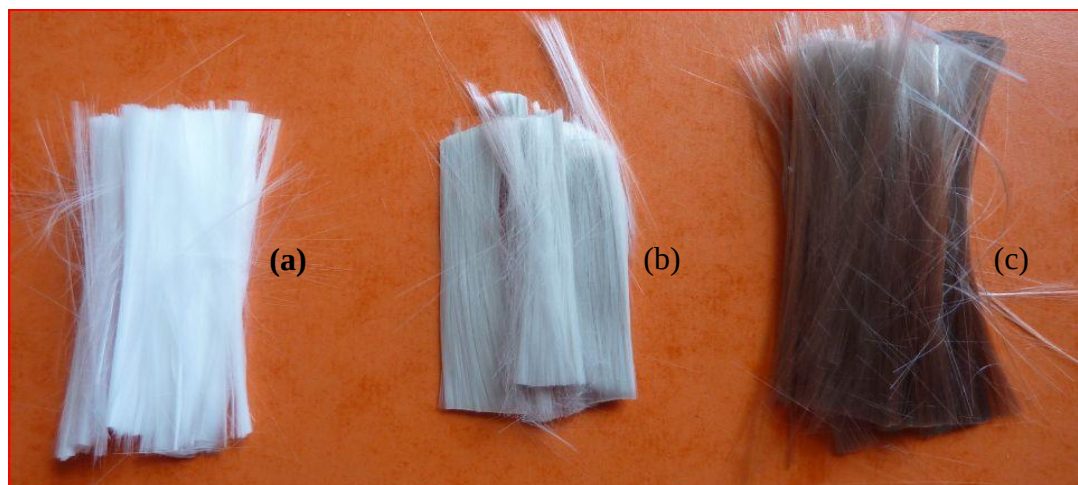


Figure 3-1 Photograph of the PBG fibres used for the compression moulded PCL/ PBG composites (a) 50P34Ca5Na, (b) 50P40Ca5Na5Fe, (c) 50P35Ca5Na10Fe (taken using Mondo Optima equipment)

3.2.3 Method

The composites produced comprised of 90%w/w PCL to 10%w/w PBG. 45%w/w of the polymer granules were placed in the compression moulder and the 10%w/w glass fibres were placed on top, the remainder 45%w/w polymer granules were added. It was noted that it took several hours to separate the glass fibres, from each of the 3 batches of PBG, due to a tendency for 'clumping' together. However, before compression moulding, a visually uniform distribution of glass fibres was achieved for the glass layer in the composites. The compression moulder lid was closed for 1 minute. 38 bar pressure and 100°C temperature was used. The resulting flat sheet composite of PCL/ PBG was cut into rectangles of dimensions approximately 1cm by 2cm. This equated to approximately 1g of material. The distribution of the fibres within the composites were examined using hot stage microscopy at room temperature, 60°C and at 90°C.

3.3 Leachables

3.3.1 Rationale for Method

Leachables testing was performed in aqueous media as this is the main constituent of body fluids. Five solutions were chosen to cover different levels and presence of salts similar to those found in simulated body fluids. Leachables from both the PBG material and the PCL polymer were of interest. However, to ensure no cross contamination from the actual storage container plastic vials were used for examining potential leaching of calcium, iron, phosphorous, sodium and glass vials for any organic leachates. Glass could leach sodium and plastic could leach oligomers and produce false results. Inductively coupled plasma (ICP) was chosen as one the analysis technique because it can detect calcium, iron, phosphorous and sodium in bioglass at micron levels in one analysis. The other technique was gas chromatography/ mass spectrometry (GC/MS). It can be used for quantitative and qualitative analysis and can determine the organic extractables and leachables from the polymer matrix of PCL. The GC can separate the various extracted/ leached components and the MS can identify and provide structural analysis of the organic species.

3.3.2 Materials and Equipment

Details of the Materials used for setting up the experiments can be seen in Table 3-2.

Materials for Experimental Set Up		
Description	Batch Information	Additional Information
Deionised and distilled water	-	Taken from GMP area within AstraZeneca -
Krebs solution	Produced by AstraZeneca (Ken McKechnie)	pH7.4 (see Table 3-3)
Potassium hydrogen phthalate buffer	Hanna Instruments H170004, Lot 241	pH4.01
Potassium hydrogen phosphate buffer	Hanna Instruments, Lot B146	pH7.4
Saline solution	Free Flex, Code 22-88-532E, Lot TB 7211 07	250ml NaCl 0.9%w/w Intravenous Infusion B.P
Plastic Tubes and screw caps	'Corning' 50ml plastic tubes with orange screw caps.	For inorganic extractables/ leachables analysis.
Glass vials and screw caps	Packard Bioscience.	For organic leachables analysis
Flint glass and plastic pipettes with pipette filler	VWR 14672-608, Magnacol 1ml, Brand® Z615927 respectively.	For organic and inorganic solution analysis

Table 3-2 Details of materials and equipment for leachables studies

The Krebs solution, Table 3-3 was made up to 1 litre with ultrapure water (Super-Q quality); the pH was adjusted to 7.2.

Composition of Krebs Solution

Description	Weight/ g	Concentration/ mmol
NaCl	6.87	116
KCl	0.33	4.4
MgSO ₄ .6H ₂ O	0.24	1.2
CaCl ₂ .2H ₂ O	0.37	2.5
Na ₂ SO ₄	0.16	1.1
KH ₂ PO ₄	0.21	1.5
NaHCO ₃	1.03	12.3
Glucose	2.00	11.1

Table 3-3 Composition of krebs solution

3.3.2.1 Materials and Equipment for ICP Testing for Presence of Calcium, Iron, Phosphorous and Sodium from PBG

Table 3-4 shows the ICP equipment and solutions used for ICP standards. The dilutions are described in section 3.2.3 (method).

ICP Equipment and Solutions for Standards

Description	Information
Calcium 1,000 ppm std.	In nitric acid, Fiseher Scientific, Code J/8010/05, Batch 0760980-
Iron 1,000ppm std.	In nitric acid, Fiseher Scientific, Code J/8030/05 Batch 0890428
Phosphorous 1,000ppm std.	Fiseher Scientific, Code J/8299/08, Batch 0800078
Sodium 1,000ppm std.	In nitric acid, Fiseher Scientific, Code J/8065/05/ Batch 0879562
Perkin Elmer ICP	Perkin Elmer 4300dv ICP-OES

Table 3-4 Details of ICP equipment and solutions for standards

3.2.2.2 Materials and Equipment for GC/MS Testing for Presence of Organics from PCL

Table 3-5 shows the materials and equipment used for GC/MS analysis.

GC/MS Equipment and Solutions for Standards	
Description	Information
Diethyl ether	Fischer Scientific, Code J/8010/05, Batch 0760980-
Polycaprolactone standard solution	Alfa Aesar epsilon caprolactone, 99%, A10299 or L05637, Lot 10152252
Glass extraction funnel and glass stopper	50ml volume
Glass scintillation vials and screw caps	Wheaton, code 11768819, VGA-870-050D
GC/MS	Agilent Technologies 6890 Gas GC and Associated Auto sampler, Find MS equipment.

Table 3-5 Details of materials and equipment for GC/MS analysis

3.3.3 Method

3.3.3.1 Sample Preparation and Storage in Aqueous Media

The 1cm by 2cm rectangles of PCL/ PBG composite material, produced in section 3.1.3, were cut into quarters in order to fit them into the vials for storage. The weight equated to approximately 1g of composite. They were placed in either a plastic tube (ICP samples) or a glass vial (GC/MS samples) with approximately 10mls of solution. The actual weight and amount of solution added was recorded. Controls containing PCL alone were also prepared in glass vials. This was due to potential for leachates from polymer vials causing false results in later tests. Five solutions were selected as the mediums into which the leachables would migrate. Five solutions were chosen to cover a different pH range, neutrality and presence of

salts similar to those found in body simulated fluids (Table 3-2). The samples were put into an incubator set to 37°C for up to 18 months. The samples were shaken once per week to agitate the solution. They were removed from the oven at 1 week, 2 weeks, 3 weeks, 1 month, 3 months, 6 months, 12 months and 18 months. Upon removal the PCL/ PBG composites were taken out of the tubes or vials and dried in an incubator at 37°C for 24 hours to remove residual water.

3.3.3.2 ICP for PBG Leachables

As stated in the method (section 3.2.3), plastic tubes were used for storage of the composites in aqueous media for ICP testing. Standards containing calcium, iron, phosphorous and sodium (1ppm, 10ppm and 100ppm) were prepared from solutions listed in Table 3-4 in order to perform quantitative analysis.

1ml of 1,000ppm ICP solution to a 1,000 ml flask = 1ppm standard

1ml of 1,000ppm ICP solution into a 100 ml flask = 10ppm standard

10mls of 1,000ppm ICP solution into a 100ml flask = 100ppm standard

The following wavelengths (already programmed in the IPC) were chosen for monitoring each element:

Ca: 317.933 nm

Fe: 238.204 nm

Na: 589.592 nm

P: 213.617

The analyses were carried out according to AstraZeneca Standard Operating Procedure LDMS_001_00007732 [470]. It was noted that after 6 months some of the samples contained particulates in the aqueous storage media. Hence, samples stored for 6 months and greater were centrifuged to reduce particulate presence and prevent blockage of the ICP sample tube. An Eppendorf (5702) bench top centrifuge was used on setting 5 for 10 minutes. There was also a degree of wavelength shift during some analysis. These samples were reprocessed at the correct wavelength.

3.3.3.3 GC/MS for PCL Leachables

As stated in section 3.2.3, glass vials were used for storage of the composites in aqueous media for GC/MS testing. GC/MS analysis was chosen for anionic analysis as the GC can separate the oligomeric species (from PCL) and the MS detector exhibits a plot of ion signal as a function of mass to charge ratio. The MS also has a library which can aid in identification of the fragmented ions. The method used was based on AstraZeneca SOP LDMS_001_00008267 [471]. However, the specific method parameters can be seen in Appendix C, which were developed to analyse leachables from oligomeric materials.

For analysis the GC method required the solutions to be in an organic solvent. Therefore, all samples underwent a solvent extraction into diethyl ether. Diethyl ether was chosen because it is a popular extraction solvent and better for extracting organic species from aqueous solutions than ethanol, methanol and acetone (which are more polar). This was performed by:

- i. Pouring the contents of the aqueous vial into an extraction flask followed by 10mls of diethyl ether.
- ii. The flask was shaken and the stopper opened to release gas build up.
- iii. This was repeated until there was no more gas build up.
- iv. The flask stopper was closed and the flask put in the upright position so that the layers separated.
- v. The diethyl ether is less dense than water. Hence, the top layer contained the organic species which was required for the GC/MS analysis, the bottom layer contained water.
- vi. The stopper was opened and the water layer was dispensed back into the original vial. The top layer was dispensed into a clean vial and the lid was replaced and labelled.
- vii. This procedure was repeated once more from (i) to ensure that all the organic species had been extracted from the aqueous layer. Diethyl ether is a volatile solvent hence, the extracted solutions were Diethyl ether is a volatile solvent hence, polytetrafluoroethane (PTFE) tape was wound round each vial lid until the

solution was dispensed via a pipette into a GC vial and then crimped just before analysis.

Two standards of caprolactone were made up of concentration ~ 147ppm and 2237ppm in diethyl ether and analysed alongside the samples in order to be able to quantify the amount of potential PCL leachables.

3.4 Extractables

3.4.1 Rationale for Method

Extractables testing was performed on PBG as an accelerated means of looking at the potential differences between the degradations of the 3 different PBG formulations. From the literature reviewed the PBG has been reported as being a significant factor in altering the degradation rate of a polyester/ PBG. Due to the limited availability of GC/MS equipment, at the time of testing, only extractables from the PBG were pursued, using ICP. Water, pH7 and pH9 solutions were chosen as the extraction media in order to see if pH had an effect on the rate and quantity of ions migrating into aqueous solutions. An elevated temperature can also be used. Since ultrasonication was used it was expected that there would be temperature increase as the duration of extraction increased. Transient bubbles collapsing are considered to be the main source of chemical and mechanical effects of ultrasonic energy. Each collapsing bubble can be considered as a micro-reactor in which temperatures of several thousand degrees and pressures higher than one thousand atmospheres are created instantaneously [250].

3.4.2 Materials and Equipment for Extractables Analysis

The materials and equipment used for the extractables method can be seen in Table 3-6.

Material and Equipment for Extractables Analysis

Description	Information
Deionised and distilled water	Taken from GMP area within AstraZeneca
pH4 Buffer solution	Hanna Instruments, pH4.01, Lot 241
pH9 Buffer solution	Mettler Toledo pH9.21, 20ml Reference 51 302 070
Plastic tubes and screw caps	'Corning' 50ml plastic tubes with orange screw caps
Ultrasonic bath	Decon F5 Minor

Table 3-6 Details of materials and equipment for extractables analysis

3.4.3 Method

1g of PCL/ PBG composite was cut into 4 parts and placed into a plastic ICP tube. 10mls of either H₂O, pH7 or pH9 solution was added. The weight and quantity of solution was recorded. The solutions were placed into an ultrasonic bath which was set to run for 1 hour, 2 hours , 4 hours, 8 hours and 16 hours. The resulting solutions were analysed using the ICP method described in section 3.2.3.2.

3.5 Weight Change

All the PCL/ PBG samples and PCL controls, put on storage for leachables testing, were weighed before immersion into their respective solutions. After the specified storage period these samples were removed and put into an incubator for 24 hours at 37°C before being re-weighed (Mettler Toledo AT246).

3.5 pH Measurements

3.5.1 Materials and Equipment

Materials and Equipment for pH measurements can be seen Table 3-7.

Material and Equipment for pH Analysis

Description	Information
pH7 Buffer solution	Mettler Toledo pH7.00, 20ml Reference 51 302 047
pH4 Buffer solution	Hanna Instruments, pH4.01, Lot 241
pH9 Buffer solution	Mettler Toledo pH9.21, 20ml Reference 51 302 070
pH Meter	Mettler Toledo M90

Table 3-7 Details of materials and equipment for pH analysis

3.5.2 Method

The remaining solutions, from the leachables testing (section 3.2.3.2), were examined for pH changes. These solutions originally contained PCL/ PBG composites. The pH meter was calibrated using pH 4.01, pH7.00 and pH 9.21 solutions, Table 3-7. AstraZeneca SOP [472] was followed. Each solution was examined separately and the pH recorded.

3.6 Density Measurements

3.6.1 Rationale for Method

The literature details density being calculated using ethanol as an immersion medium. For these studies analyses were based on a contained helium system which eliminated the use of solvents. It is a quantitative technique which is ideal for analysing the density of small PCL/ PBG samples. The sampling pan can take samples up to 1cm³. Its relative precision is a reproducibility to within $\pm 0.02\%$ of the nominal full scale volume and an accuracy to within 0.03% of reading, plus 0.03% of sample capacity.

3.6.2 Method

The density of the PCL, PCL/PBG control samples (from section 3.1) and PCL, PCL/ PBG samples after storage in various solutions (from section 3.2) were measured using a helium

gas displacement density meter (Micromeritics AccuPyc 1330 Pycnometer). The equipment was calibrated using a 1.0cm³ sample cup and steel calibration ball and samples analysed according to AstraZeneca SOP LDMS_001_00007375 [473].

3.7 Fibre Fraction

A combustion methods using a platinum crucible and an electric furnace were used to determine the fibre weight and volume fraction of the PBG within the PCL matrix according to a standard industry method (American Society for Testing and Materials).

3.7.1 Method

The standard test method for ignition loss of cured reinforced resins, ASTM 2584-94 [474] was used. The mass of the metal sample tray was measured to the nearest 1.0mg with (m_{t+c}), and without, (m_t), the sample. The samples were then placed into an electric furnace at 450°C for 1 hour, ensuring complete combustion of matrix. The mass of the tray and residue, (m_{t+r}), was measured after removal from the furnace. The fibre mass fraction (M_f), of the composites was calculated using Equation 1, from the mass residue (m_r) and composite (m_c) as shown:

$$M_f = m_r / m_c \times 100 \quad (\text{Equation 1})$$

The densities of the PCL matrix and PBG fibre reinforcement were used to calculate the fibre volume fraction of the composites using equation 2:

$$V_f = (m_r / \rho_f) / [(m_r / \rho_f) + [(m_c - m_r) / \rho_p]] \times 100 \quad (\text{Equation 2})$$

Key:

V_f : Volume fraction

ρ_f : Density of fibre

ρ_p Density of polymer

3.8 Thermal Analysis

3.8.1 Rationale for Method

PCL and PCL/ PBG samples, after storage in their respective solutions (Table 3-2), were analysed using DSC equipment (TA Instruments DSC Q1000 with DSC refrigeration Cooling system and 2920 DSC S/N 2920CE-5248) to monitor melting point, any glass transition states (T_g) and potential decomposition or crystallisation states. TGA was also conducted in order to observe potential mass change upon heating.

3.8.2 Methods

3.8.2.1 DSC Method

The use of the equipment was based on AstraZeneca SOP LDMS_001_00007643 [475]. The method involved heating the samples from -20 to 550°C at 10°C/min under nitrogen atmosphere and kept at 100°C for 5 minutes to eliminate the previous thermal history. The sample was then cooled to -80°C and re-heated to 550°C. The method can be seen below:

Method:

Data storage: off

Equilibrate at -80.00°C

Modulate +/- 1.00°C every 50 seconds

Isothermal for 5.00min

Data storage: on

Ramp 10.00°Cmin to 550°C

End of method

The main pressure regulators read 5psi on N₂ purge and 20psi on air cooling. The flow meters were set at:

Auto sampler = 1.5cm³/min

DSC cell purge = 35cm³/min

RCS = 125cm³/min

Between 2mg to 10mg samples of PCL or composites (controls) were cut and placed inside a clean metal DSC pan and covered with a suitable metal lid. The pans were handled with tweezers and not bare hands as any grease transferred to the pan could give rise to a thermal signal. No labelling of the sample pans was possible as the ink used could give rise to a thermal signal.

3.8.2.2 TGA Method

PCL and PCL/ PBG samples, after storage in their respective solutions, were analysed using TGA equipment (TA Instruments 2050 Thermogravimetric Analyser (TGA)) to monitor mass change on heating. AstraZeneca method LDMS_001_00007644 [476, 477] was followed. The glass alone was also examined however, due to being heated to 500°C the glass melted and destroyed the sample holder. Hence, no more PBG was analysed after this incident as it was considered to be too much of a destructive test.

3.9 IR Analysis

3.9.1 Rational for Method

Infrared and Raman spectroscopy are two of the most widely used techniques for the determination of molecular structure and for the identification of compounds. The two techniques are completely complimentary, and both are necessary for the vibrational analysis of a molecule [478]. These techniques have previously been utilised to investigate the structural changes for PCL and PCL/ PBG [73, 237, 240, 403, 410] and the literature has provided guidance on wavenumbers associated with potential structural changes within PCL/ PBG composites.

3.9.2 Equipment

The equipment used can be seen in Table 3-8.

Description
Perkin Elmer FTIR System 2000 spectrometer
Golden gate single reflection diamond ATR unit
Accessory base-plate to suit a Perkin –Elmer FT-IR System 2000 spectrometer.
Thumb nut
Ball Driver 3.0mm A/F
Allen key 2.0mm A/F
Calibrated torque wrench with a fitted snap adapter
1 conical shaped stainless steel head piece (part number 10502) for polymer pellets and beads
Lens cleaning tissues

Table 3-8 Details of FTIR equipment

3.9.3 Method

AstraZeneca standard operating procedure LDMS_001_00007304 [479] was followed for setting up the equipment. A resolution of 4cm^{-1} was chosen and 32 scans at 100 kHz was used due to this being appropriate for the baseline to noise resolution. A polystyrene reference standard was used for calibration. Once the equipment set up was complete, the samples were analysed by clamping the sample under the diamond tip of the ‘Golden Gate bridge’ and recording the spectrum from 400cm^{-1} to 4000cm^{-1} . The solid PCL/ PBG samples from all five aqueous solutions and all eight time points were analysed. Controls in the form of PCL alone and the three PCL/ PBG composites were also tested.

3.10 Scanning Electron Microscope (SEM)

3.10.1 Rational for Method

An SEM can reveal topological details of a surface with clarity and detail which cannot be obtained by any other means.

3.10.2 Equipment

The equipment used can be seen Table 3-9.

Description
Sputter coater VG Microtech Model SC7640
12.5mm metal sample holders
12mm carbon mounting discs
LEO Model 430 SEM (30keV electron beam)

Table 3-9 Details of SEM equipment

3.10.3 Method

The PCL/ PBG samples were placed on metal sample holders and sputter coated with gold/ palladium according to AstraZeneca method LDMS_001_00007217 [480]. Gloves and tweezers were used when handling the samples to ensure no grease was passed from the fingers onto the specimens (this could produce a poor image). The samples were transferred to the SEM and analysed using AstraZeneca method LDMS_001_00007136 [481]. PCL was also examined as a control.

CHAPTER 4– RESULTS

This chapter reports the results from experimentation described in Chapter 3, Materials and Methods. Three composites of PCL/ PBG were produced containing the following PBG compositions:

- i. $50\text{P}_2\text{O}_5\text{-}35\text{CaO-}5\text{Na}_2\text{O-}10\text{Fe}_2\text{O}_3$
- ii. $50\text{P}_2\text{O}_5\text{-}40\text{CaO-}5\text{Na}_2\text{O-}5\text{Fe}_2\text{O}_3$
- ii. $50\text{P}_2\text{O}_5\text{-}45\text{CaO-}5\text{Na}_2\text{O}$

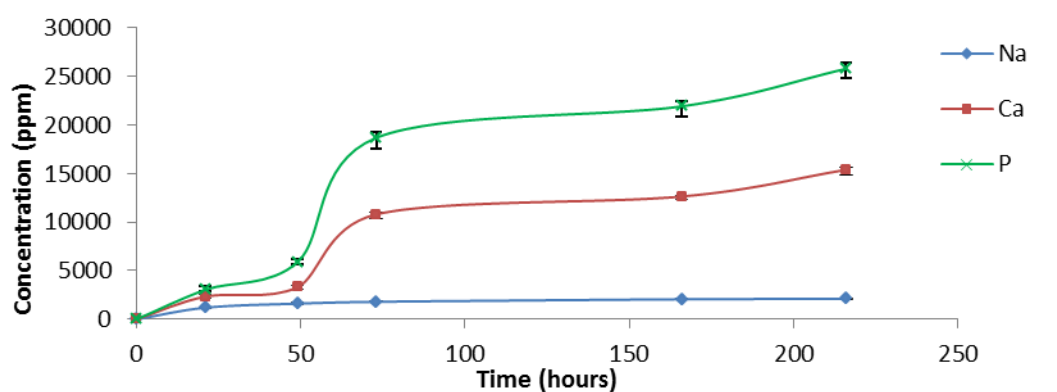
The PBGs will be referred to as 10Fe, 5Fe and 0Fe respectively throughout this thesis. The composites will be referred to as PCL/10Fe, PCL/5Fe and PCL/0Fe.

4.1 Leachables from PBG

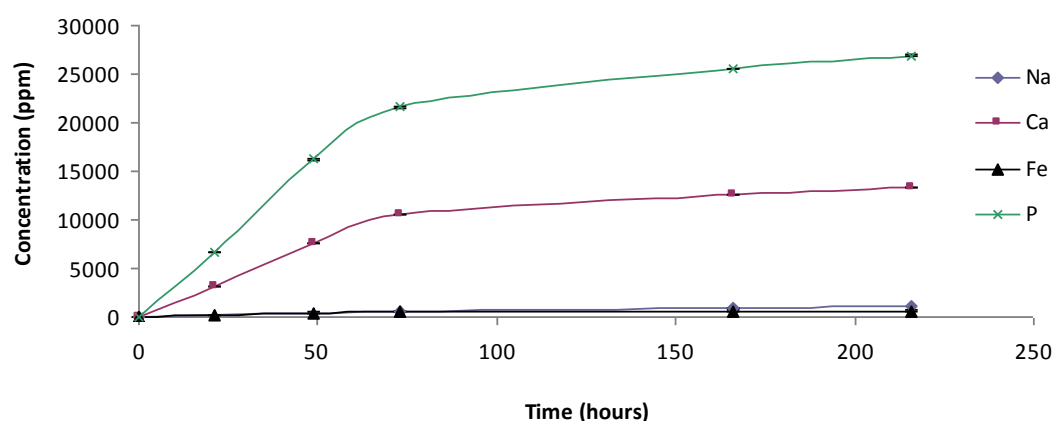
The studies were conducted in order to conclude whether any one of the materials studied was more durable compared to another before being fabricated as a composite with PCL. Durability being described as the resistance of PBG to attack by a specified reagent. The study was conducted over 216 hours for comparability with the current literature. The results can be seen in Figures 4-1 (a), (b) and (c). The results showed the following amount of leached species, greatest first for all three PBGs:

- i. 50P45Ca5Na: Phosphate > Calcium > Sodium.
- ii. 50P40Ca5Na5Fe: Phosphate > Calcium > Sodium > Iron.
- iii. 50P35Ca5Na10Fe: Phosphate > Calcium > Iron > Sodium.

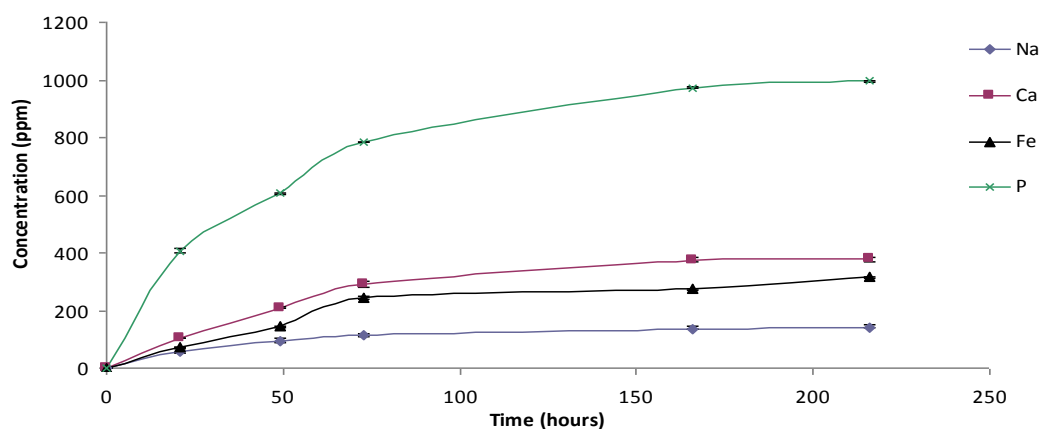
This was to be expected due to the quantities of these elements within each PBG formulation. It was however noted that the leachates are over an order of magnitude greater for the 0Fe and 5Fe PBG materials when compared to the 10Fe PBG.



(a)



(b)



(c)

Figures 4-1 (a) Sodium, calcium, iron and phosphorous leachables from (a) 0Fe PBG, (b) 5Fe PBG and (c) 10Fe after storage in deionised water at 37°C for 216 hours, n=1 with 6 replicate injections

Table 4-1 shows the weight loss from the glass before and after leachables testing (10.45% for 0Fe, 8.89% for 5Fe and 3.12% for 10Fe). The weight loss for the 10Fe PBG is a third less than the other two materials. These results also confirm that the 10Fe PBG material shows a slower dissolution rate than the 0Fe and 5Fe PBGs.

PBG	Initial Weight (g)	Final Weight (g)	Loss in Weight (g)	Loss in Weight (%)
0Fe	1.0421	0.9332	0.1089	10.45
5Fe	1.0368	0.9446	0.0922	8.89
10Fe	1.0664	1.0324	0.0340	3.12

Table 4-1 Weight loss for PBG after storage in water at 37°C for 216 hours

4.2 Fabrication of PCL/PBG Composites

The fabrication of the 3 PCL/PBG composites was performed using a compression moulding technique, section 3.2. The resulting materials were solid, smooth sheets of composite which could be easily cut into suitably sized samples (approximately 1cm x 1cm) for further analysis.

4.2.1 Fibre Volume and Mass Fractions

The results from mass fractions confirmed the approximate percentage of PCL (90%w/w) and PBG (10%w/w) within the composites (after manufacture). A residue on ignition technique, ASTM 2584-94, was used to determine the amount of inorganics within the organic matrix. Table 4-2(a) shows the amount of residue remaining after combustion of the PCL/PBG composites and Table 4-2(b) shows the resulting fibre mass and volume fractions (using equations 1 and 2 from Section 3.7.1). The analysis was performed in triplicate (n=3). The densities of the PCL and PBG were taken from Table 4-2.

PCL/ PBG Composite	Weight Crucible (g)	Weight Composite (g)	Weight Crucible & Composite (g)	Weight Crucible and Composite After Heating (g)	Weight of residue (g)
0Fe	26.6651	0.9184	27.5835	27.4877	0.0958
0Fe	27.4196	0.6053	28.0249	27.4776	0.0580
0Fe	43.6279	0.8569	44.4848	43.7295	0.1016
5Fe	26.7534	0.8522	27.6056	27.5043	0.1013
5Fe	26.3693	0.7063	27.0756	26.4414	0.0721
5Fe	27.2456	0.7023	27.9479	27.3108	0.0652
10Fe	27.5735	0.9205	28.4940	28.4083	0.0857
10Fe	27.3243	0.8714	28.1957	27.4580	0.1337
10Fe	37.7078	0.7352	38.4430	37.7973	0.0895

Table 4-2(a) Amount of residue remaining for three PCL/ PBG composites after combustion, n=3

PCL Composite	Density of PCL (g/cm ³)	Density of PBG (g/cm ³)	Fibre Mass Fraction (%)	Fibre Volume Fraction (%)
0Fe	1.14	2.64	10.43	4.67
0Fe			9.58	4.31
0Fe			11.86	5.40
5Fe	1.14	2.73	11.89	5.23
5Fe			10.21	4.45
5Fe			9.28	4.03
10Fe	1.14	2.86	9.31	3.87
10Fe			15.34	6.62
10Fe			12.17	5.15

Table 4-2(b) Fibre mass fraction and fibre volume fraction for three PCL/PBG composites, n=3

PCL Composite	Mean Fibre Mass Fraction (%) (n=3)	Standard Deviation	Fibre Volume Fraction (%) (n=3)	Standard Deviation
0Fe	10.62	± 1.15	4.72	± 0.56
5Fe	10.46	± 1.32	4.57	± 0.61
10Fe	12.27	± 3.02	5.21	± 1.38

Table 4-2(c) Mean and standard deviations for fibre mass fraction and fibre volume fraction for three PCL/PBG composites, n=3

The results from Tables 4-2 ((a) and (b)) are presented graphically in Figure 4-2.

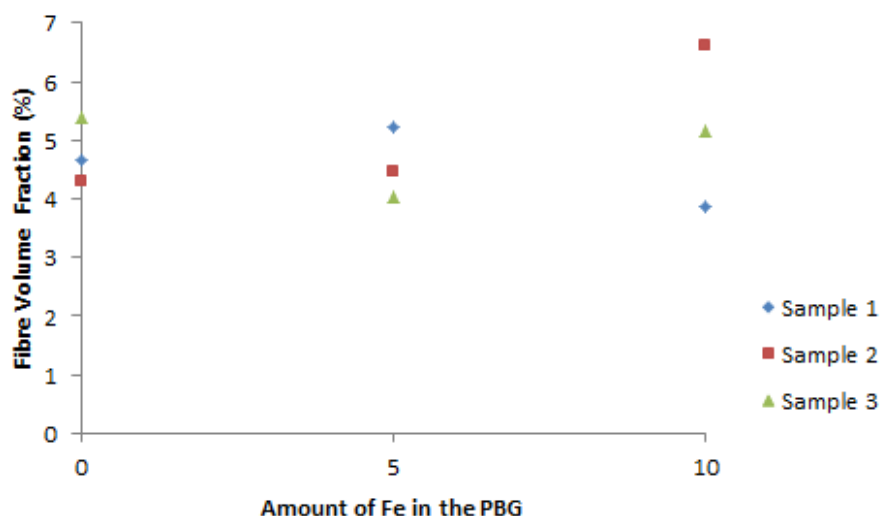


Figure 4-2 Spread of data for fibre volume fraction for three PCL/PBG composites, n=3

The results, from the residue on ignition, give a PBG mean fibre mass fraction of 10.62% and 10.46% for PCL composites containing 0Fe and 5Fe, respectively (Table 4-2(c), Figure 4-2 (a)). However, the composite containing 10Fe PBG has a mean fibre mass ratio of 12.27%. There is overlap in the data (Figure 4-2(a)) for the PCL/ 0Fe and PCL/ 5Fe composites however, the PCL/10Fe composite exhibited one higher value (15.34 %), thus increasing the spread of data. The mean volume fractions across the three composites show a tighter data set than those for the fibre mass fraction (Table 4-2 (c) and Figure 4-2 (b)) with a lower standard deviation. The PCL/10Fe sample could have exhibited a higher mass fraction of PBG than other samples due to a slightly higher amount of glass fibres being present within that particular area of the PCL matrix. The glass fibres were weighed and distributed as evenly as possible, with heat and compression being applied during composite fabrication however, it was observed (using hot stage microscopy that there was potential for some areas to contain a higher concentration of PBG fibres.

The three composite samples were examined and typical results can be seen in Figure 4-3.

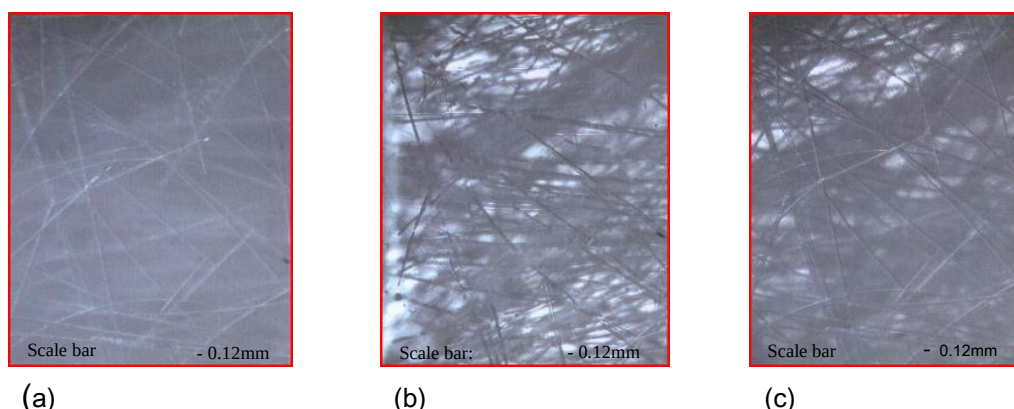


Figure 4-3 Hot stage microscopy photograph of the orientation of 10Fe fibres within a PCL/10Fe composite (a) initial, (b) 60°C, (c) 90°C

The average radius of the fibres is 0.061mm (std. dev .00016, n=6) The majority of the fibres have retained their original radius and length (from 11mm to 16mm).

The resulting ash was not examined further, after ignition because the residue should contain oxides of the PBG (namely, calcium, iron, phosphorous and sodium). The aim of the study was to determine the weight ratio of PBG to polymer.

4.3 Extractables from the PBG Component of the PCL/PBGs

As an accelerated study, the three PCL/ PBGs composites (0Fe, 5Fe and 10Fe) were examined for sodium, calcium, iron and phosphate extractables after ultrasonication for up to 48 hours, in acidic, neutral and alkaline solutions to see how the glass dissolution behaves in a pH shift environment.

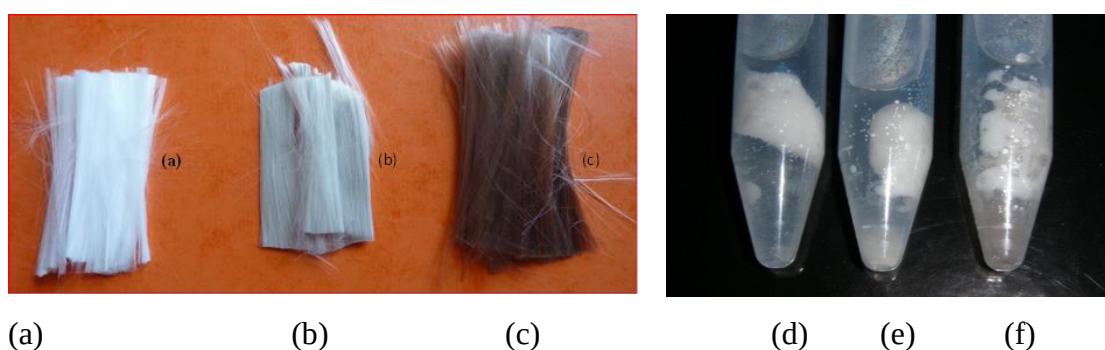


Figure 4-4 (a), (b), (c), Photograph of PBGs incorporated into PCL/PBG composites before extractables testing. and (d), (e), (f) corresponding PCL/ PBG composites after extractables testing via ultrasonication for 48 hours, n=1

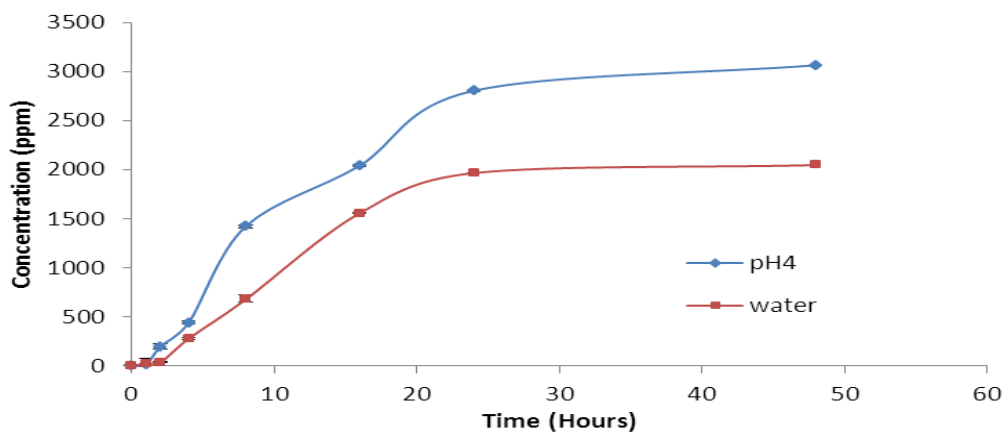
As the amount of Fe_2O_3 in the initial PBG samples increases so did the colour of the PBG, Figure 4-4 (a) to (f). It was noted that the grey colour of samples (b) and (c) (5Fe and 10Fe respectively) lost the grey intensity after ultrasonication for 48 hours (Figure 4-4 (e) and (f)). This is due to the dissolution of PBG and leaching into the surrounding solution. The PCL/PBG samples were originally introduced as 1g squares cut into 4 equal pieces. However, after ultrasonication the samples were observed to be moulded together due to heat and vibrational energy generated from the ultrasonic bath.

The propensity for sodium and phosphate to extract into an acidic, neutral or basic solution was not evident from the results and no solution appeared to be favoured, Figure 4-5 and Figure 4-8 (a to c). For calcium, pH9 showed a greater propensity for PCL/5Fe and PCL/10Fe composites, Figure 4-6 (a to c). Water extracted the highest amount of iron for both PCL/5Fe and PCL/10Fe composite materials, Figure 4-7 (a to c). However, due to only one sample being tested, per composite and aqueous medium, this comment is not conclusive and further samples would need to be tested in order to get a statistically relevant conclusion.

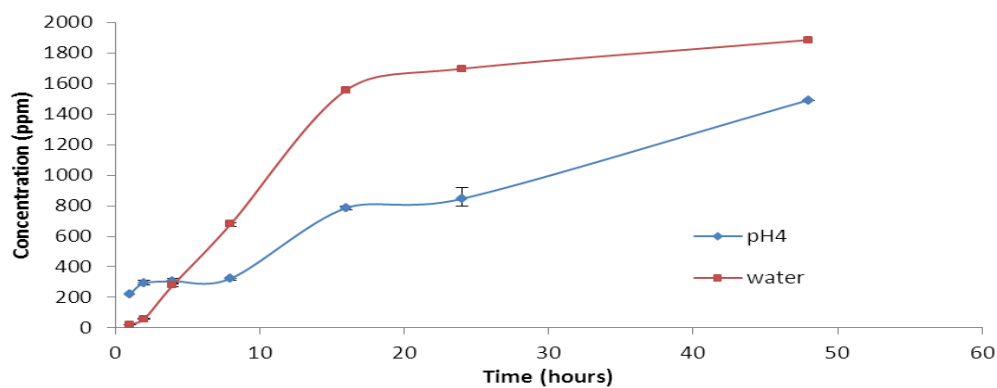
In general, the extraction rate is at its quickest from 0 to 15 hours. After this time point, the extraction curves began to plateau.

4.3.1 Extracted Sodium Ions from PCL/PBG Composites

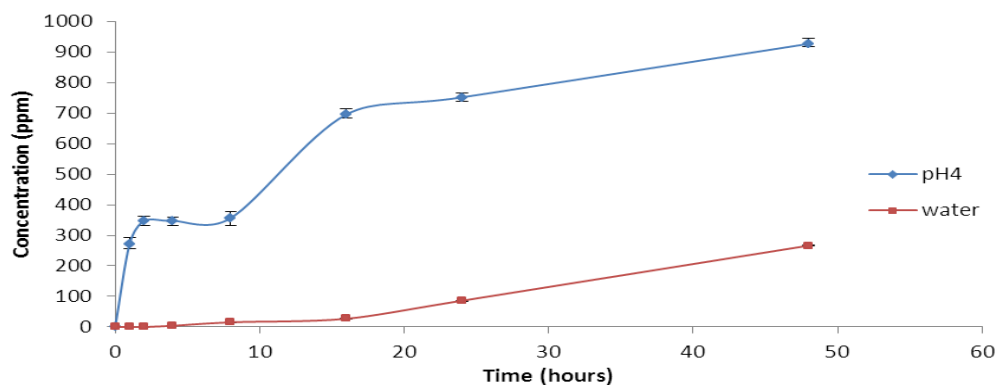
Due to saturation of sodium in pH9 buffer solution PCL/ PBG composites have only been reported after storage in water and pH4 buffer solution, Figure 4-5 (a) to (c).



(a)



(b)



(c)

Figure 4-5 Extracted sodium ions from (a) PCL/0Fe, (b) PCL/5Fe and (c) PCL/10Fe after storage at 37°C for 48 hours (n=1 with 6 replicate injections)

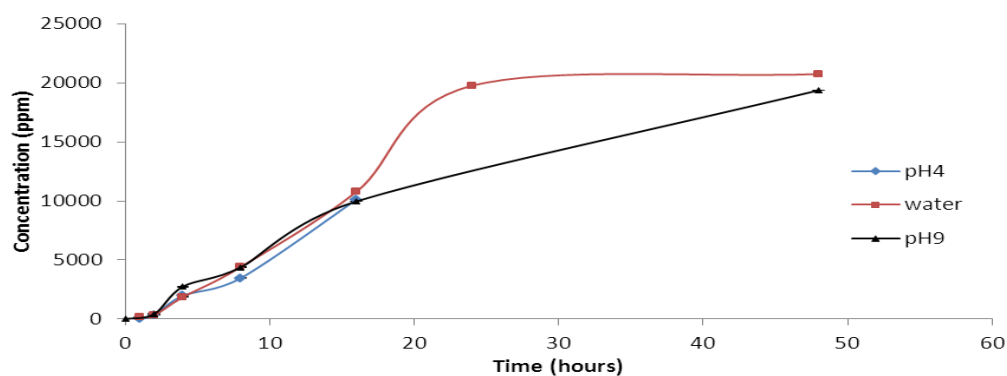
As the time increases the amount of extracted sodium ions increase. The actual amount of sodium in the glass formulation for each of the three composites is consistent (at 5%w/w). The results indicate that there is a trend of more sodium extracted from:

PCL/0Fe > PCL/5Fe > PCL/10Fe.

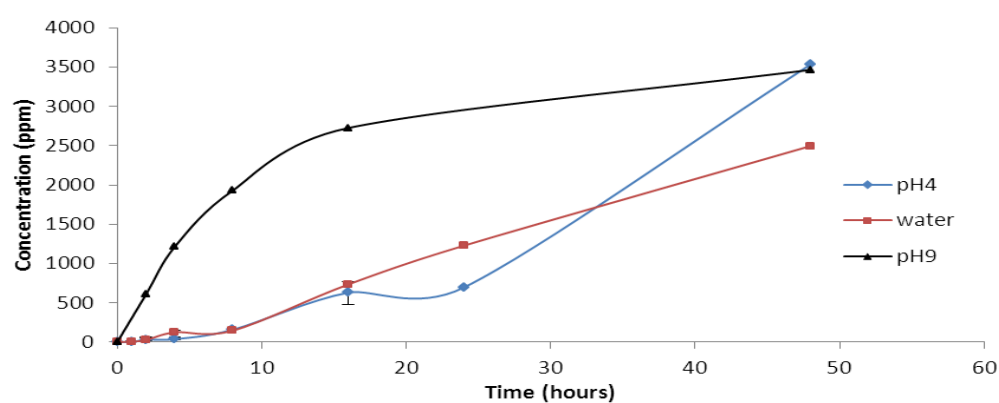
The PCL/ 10Fe material exhibited approximately a third less sodium ions extracted than the other two composites.

4.3.2 Extracted Calcium Ions from PCL/PBG Composites

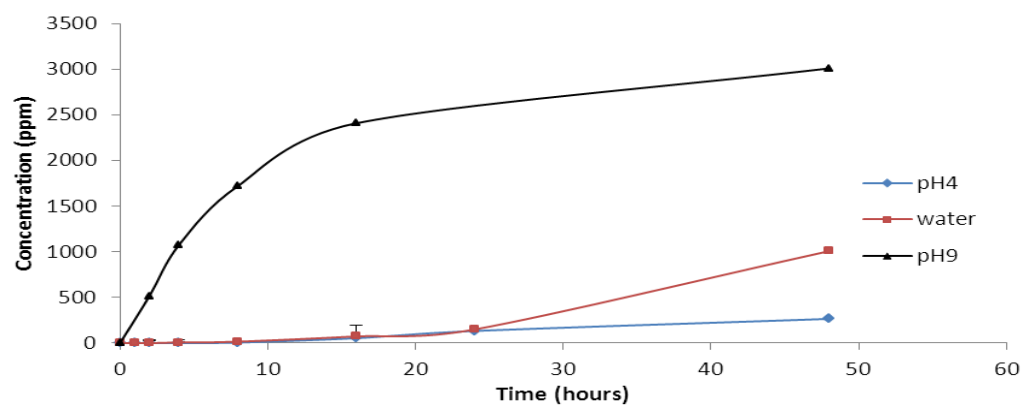
Extracted calcium ions can be seen in Figure 4-6 (a) to (c). A general trend is observed. As the amount of iron is increased within the PBG formulation. The PCL/0Fe composite showed significantly higher extracted calcium ions than the PCL/5Fe and PCL/10Fe composite materials by up to an order of magnitude.



(a)



(b)

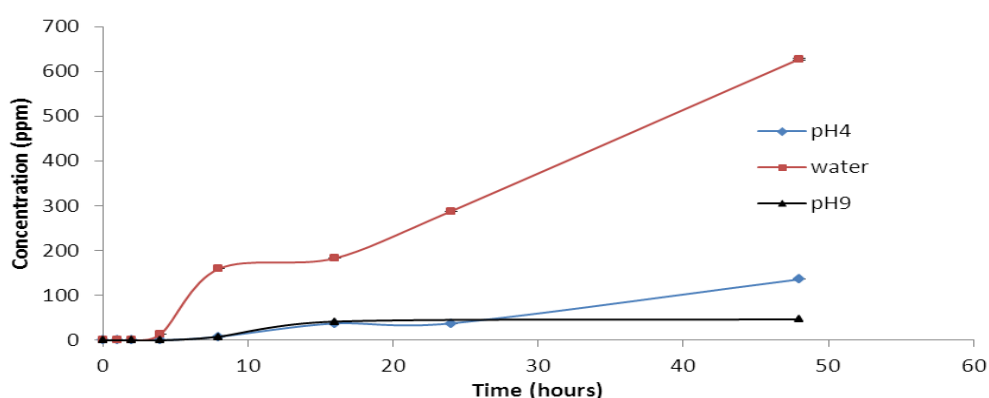


(c)

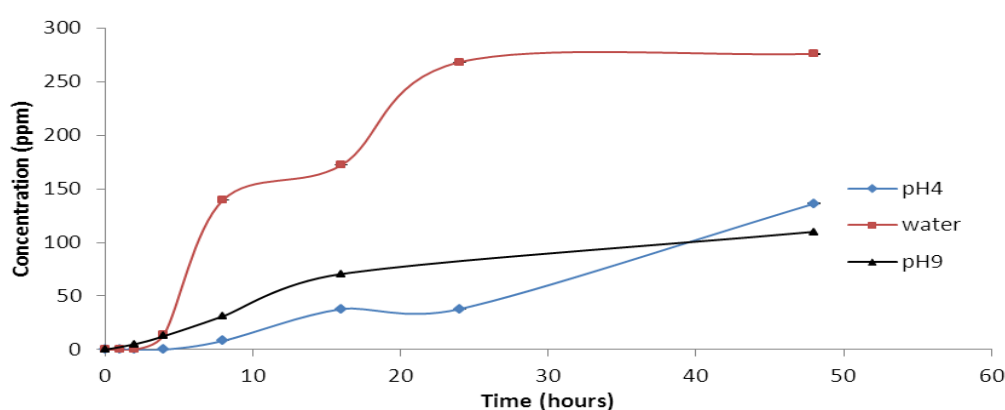
Figure 4-6 Extracted calcium ions from (a) PCL/0Fe, (b) PCL/5Fe, (c) PCL/10Fe after storage at 37°C for 48 hours, (n=1 with 6 replicate injections).

4.3.3 Extracted Iron Ions from PCL/PBG Composites

Extracted iron ions into pH4, water and pH9 can be seen in Figure 4-7 (a) to (b).



(a)



(b)

Figure 4-7 Extracted iron ions from (a) PCL/5Fe and (b) PCL/10Fe after storage at 37°C for 48 hours, (n=1 with 6 replicate injections)

No iron was extracted from PCL/0Fe. This was to be expected due to no iron being present in the composite formulation. Although there was 5%w/w more iron within the PCL/10Fe composite (compared to the PCL/5Fe material) the amount of extracted iron ions from PCL/10Fe was approximately half that of PCL/5Fe (~250ppm and ~600ppm respectively).

4.3.4 Extracted Phosphate Ions from PCL/PBG Composites

Extracted phosphate ions into pH4, water and pH9 can be seen in Figure 4-8 (a) to (c).

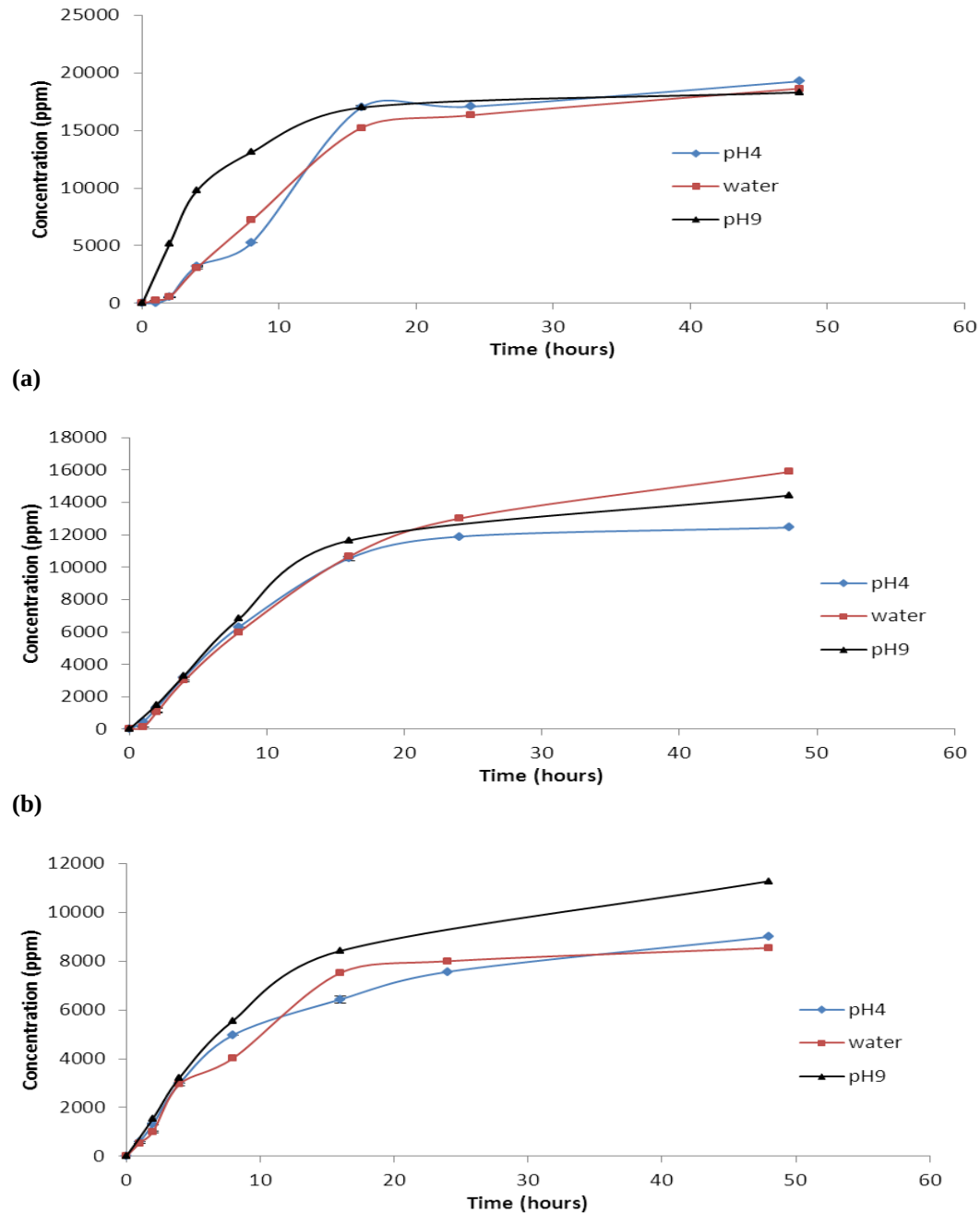


Figure 4-8 Extracted phosphate ions from (a) PCL/0Fe, (b) PCL/5Fe and (c) PCL/10Fe after storage at 37°C for 48 hours, (n=1 with 6 replicate injections)

The higher the amount of Fe present in the PCL/PGB composite the less phosphate was extracted. The quantity of extracted phosphate ions from the PCL/10Fe composite was approximately half of the PCL/0Fe material (~1000ppm and 2000ppm respectively). The PCL/5Fe produced results between these values (~1200ppm to ~1600ppm).

4.3.5 Estimation of Extracted Amounts

The estimated amount of ions extracted from the PCL/PBG composites is shown in Table 4-3. The actual weight loss of the composites were used for the calculation. However, it was assumed that the PCL/ PBG composites contained 10%w/w of PBG, Appendix D. From section 4-2 it has been shown that there is some variation in the fibre mass within the composites and the standard deviations (over 3 samples) were ± 1.15 for PCL/0Fe, ± 1.32 for PCL/ 5Fe and ± 3.02 for PCL/ 10Fe.

There are limitations in the data set for interpretation due to only one PCL/PBG composite being analysed for each solution type. Therefore, a full statistical evaluation cannot be conducted. However, due to the overall amount of samples tested, it can be seen that the presence of iron within the PBG has reduced the amount of extractables from the composites. It was noted that the extraction of each species (i.e. calcium, phosphorous, iron, sodium) from a particular PCL/PBG type (i.e. 0Fe, 5Fe, 10Fe) did not occur uniformly. A considerable amount of the PBG was extracted from the 0Fe material (phosphate: 87.6-91.9%, sodium 57.6-86.1%, calcium: 57.7-61.7%), 5Fe exhibited fewer extractables (phosphate 59.4-75.8%, sodium: 41.9-53.0%, calcium: 9.1-12.8% and iron 1.4-18.6%) and the least amount extracted was from the 10Fe material (phosphate: 40.8-53.8%, sodium 7.5-26%, calcium: (1.1-12.8% and iron:1.7-4.2%). This is consistent with the phosphate glass leachables data which showed that the addition of Fe increases the PBG durability, section 4.1. When PBG is within the PCL matrix, there is still evidence of increased glass durability by the addition of iron.

Although the graphs are plateauing, there is still PBG in all the composites studied due to the estimated % of species extracted. This indicates that the extraction time, of 48 hours, was not long enough for completed extraction. This would have to be extended for all three composites. PCL/0Fe is expected to have a shorter extraction time than the PCL/5Fe and PCL/10Fe the longest.

PCL / PBG Composite	Solution	Highest Actual Amount Extracted from Results of PCL/ PBG Composite (ppm)				Estimated % Extracted from PCL/ PBG Composite			
		Na	Ca	Fe	P	Na	Ca	Fe	P
0Fe	pH4	3,066	No data	NA	19,269	86.1	-	N/A	91.9
	Water	2,049	20,748	NA	18,648	57.6	61.7	N/A	89.0
	pH9	NA	19,376	NA	18,305	NA	57.7	N/A	87.6
5Fe	pH4	1,491	3,536	136	12,451	41.9	12.8	4.0	59.4
	Water	1,886	2,498	628	15,892	53.0	9.1	18.6	75.8
	pH9	NA	3,466	47	14,437	NA	12.6	1.4	68.9
10Fe	pH4	927	265	139	8,999	26.0	1.1	2.1	42.9
	Water	266	1,009	276	8,542	7.5	4.7	4.2	40.8
	pH9	NA	3,011	110	11,276	NA	12.8	1.7	53.8

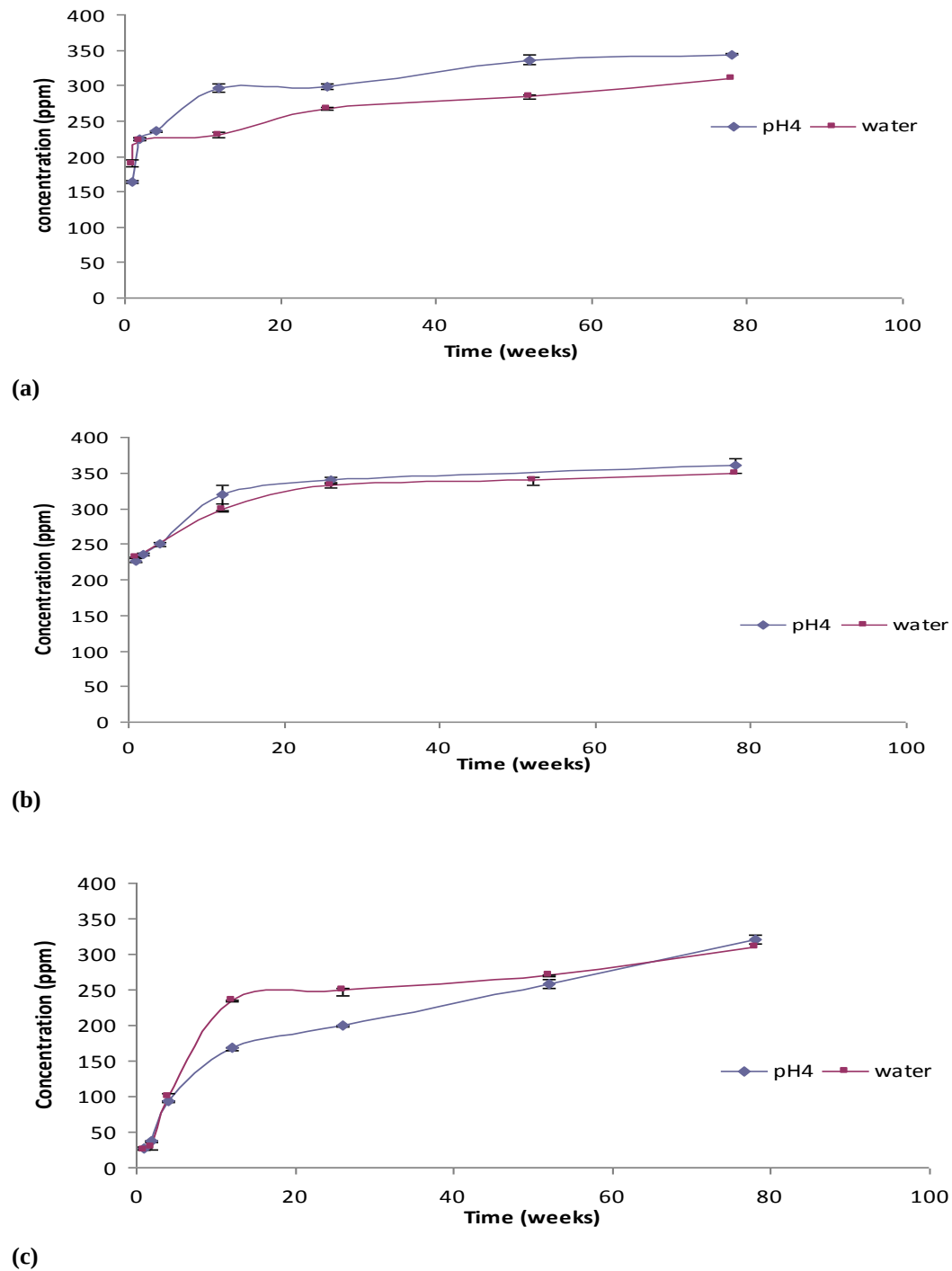
Table 4-3 Estimated Amount % of Ions Extracted from PCL/ 0Fe, PCL/ 5Fe and PCL/ 10Fe After Extraction in Acidic, Neutral and Alkaline Conditions after Ultrasonication for 48 Hours

4.4 Leachables from the PBG Component of the PCL/ PBGs

If a material is to be considered for implantation, leachables studies must be performed according to Regulatory guidance (ISO10993) to ensure patient safety. The ICP leachables results for the three PCL/PBG composites (after storage in pH7, pH4, water, saline and krebs solution for 78 weeks) are described in this section.

Sodium Leachables

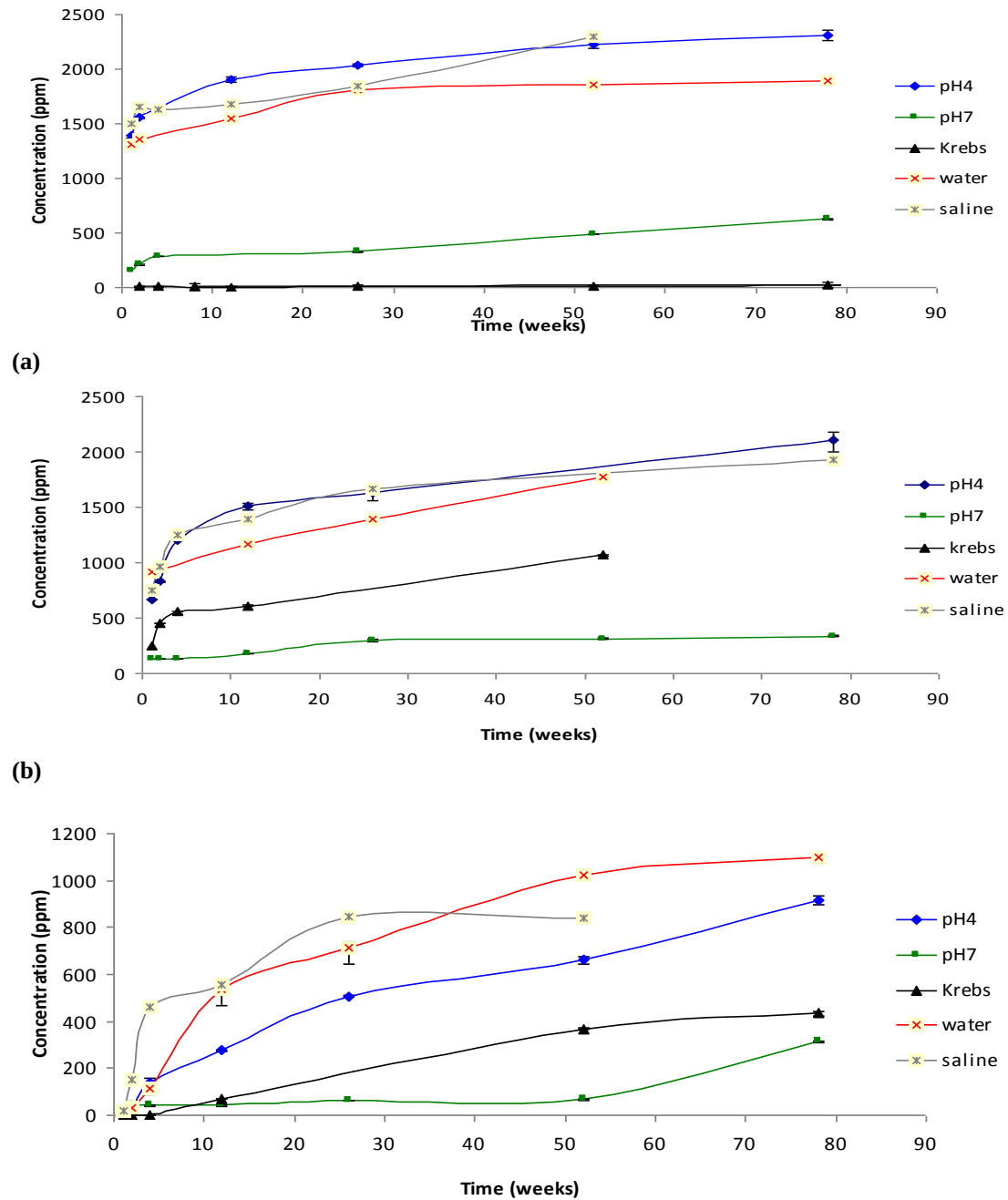
Sodium leachables were recorded for the PCL/PBG composites stored in pH4 and water (Figure 4-9 (a) to (c)). Results were not included for composites stored in pH7, krebs or saline solutions as they were already saturated with sodium.



(c)
Figure 4-9 Sodium leachables from (a) PCL/0Fe, (b) PCL/5Fe and (c) PCL/10Fe after storage in water and pH4 buffer at 37°C for 78 weeks, (n=1 with replicate injections)

Calcium Leachables

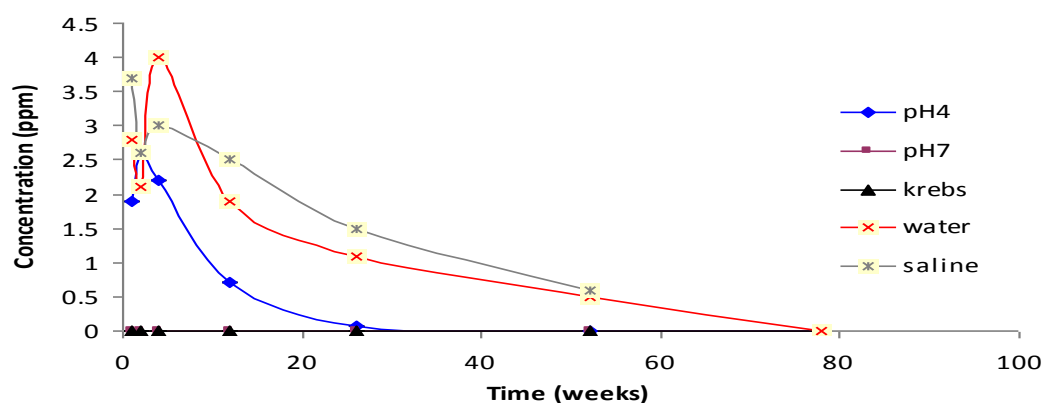
Calcium leachables were recorded for the PCL/PBG composites stored in pH4, pH7, krebs, saline and water, Figure 4-10 (a) to (c).



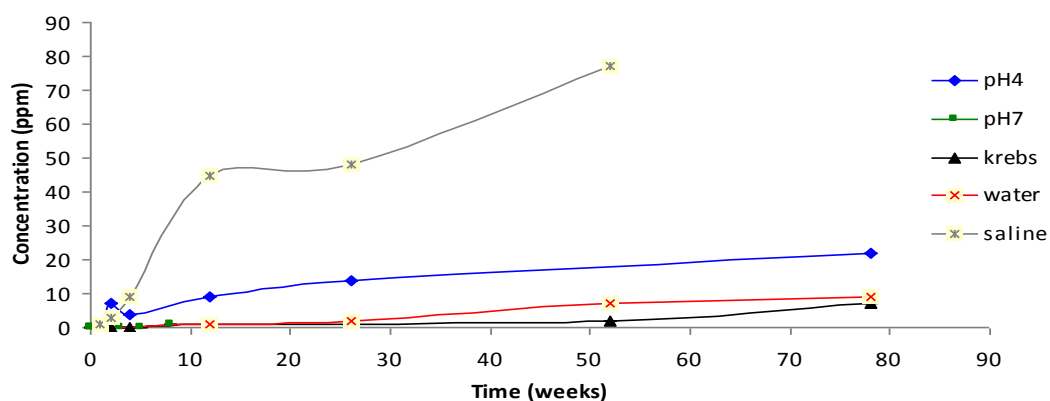
(c) Figure 4-10 Calcium leachables from (a) PCL/0Fe, (b) PCL5Fe and (c) PCL/10Fe after storage at 37°C for 78 weeks, (n=1 with 6 replicate injections)

Iron Leachables

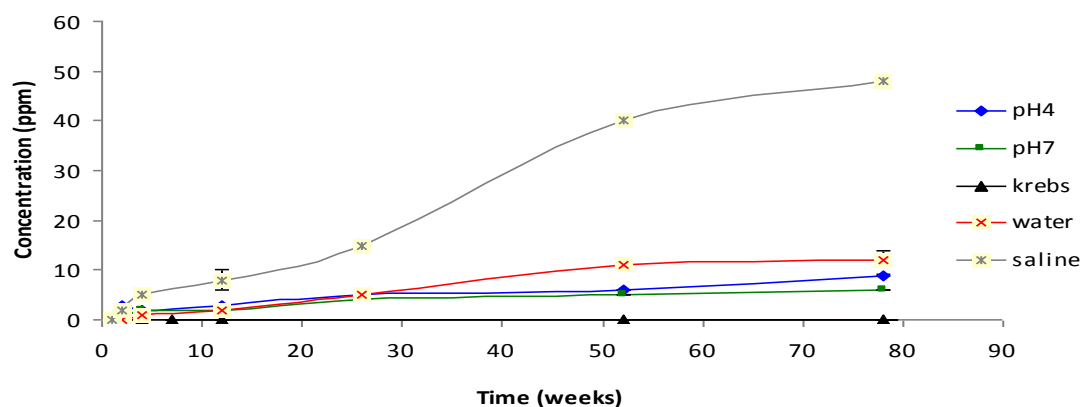
Iron leachables were recorded for the PCL/PGB composites stored in pH4, pH7, krebs, saline and water. The results can be seen in Figures 4-11 (a) to (c).



(a)



(b)



(c)

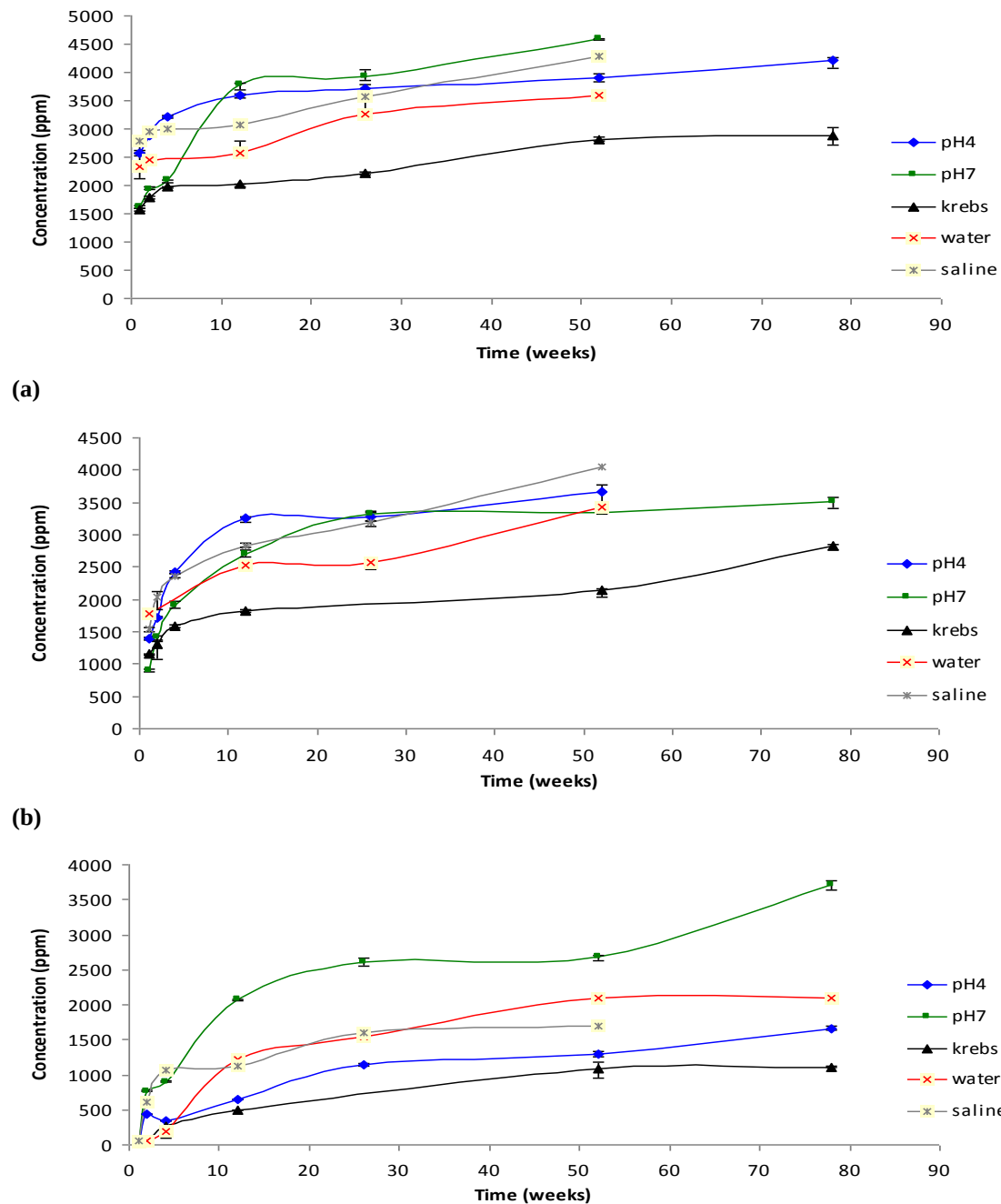
Figure 4-11 Iron leachables from (a) PCL/0Fe, (b) PCL/5Fe and (c) PCL/10Fe after storage at 37°C for 78 weeks, (n=1 with 6 replicate injections)

No iron leachables were expected to be seen from the PCL/0Fe composite because the material did not contain any iron within the structure. From the results no iron was detected in the krebs and pH7 solutions. However, it was detected at low levels within the pH4, saline and water sample (maximum 4.02ppm in water). This was

thought to be from contamination. An investigation root cause analysis was not conducted however it was suspected that this may have been picked up from contamination within the ICP laboratory.

Phosphate Leachables

Phosphate leachables were recorded for the PCL/PBG composites stored in pH4, pH7, krebs, saline and water. The results can be seen in Figure 4-12 (a) to (c).



(c)
Figure 4-12 Phosphate leachables from (a) PCL/0Fe, (b) PCL/5Fe and (c) PCL/10Fe after storage at 37°C for 78 weeks, (n=1 with 6 replicate injections)

Some data points were not reported due to the values being lower than expected and not fitting the expected increase in ion leaching. Whilst carrying out this experimentation some particulates were observed after ICP analysis. There is potential for ICP analysis tube blockage. The samples were centrifuged and re-analysed but the results were still low. A full root cause analysis was not conducted and the reason for the low results is unknown.

The detection of calcium, iron, sodium and phosphate ions confirms that the PBG is beginning to degrade, Figures 4-5 to 4-8. The leaching rate appears to be at its greatest, for the majority of ions, up to 12 weeks. The graphs display a slower rate followed by a plateauing effect up to 78 weeks.

The krebs solution appeared to leach the least amount of species for all composites tested. Water, saline, pH4 and pH7 did not appear to be discriminatory and no trend was observed. There are limitations in interpretation of the data due to only one sample being tested per composite type and storage solution. A statistical evaluation cannot be made. The leaching rate for sodium (for the samples tested) appeared to be slower for the PCL/10Fe composites up to approximately 12 weeks however, at 78 weeks the amount was similar for all 3 materials, Figure 4-5. There was a varying amount of calcium in the three composites. This ranged from 45%w/w for PCL/0Fe, 40%w/w for PCL/ 5Fe and 35%w/w for PCL/ 10Fe, Figure 4-6. Overall the amount of calcium leached from the PCL/10Fe was approximately half of that of the PCL/0Fe and PCL/5Fe composites. The amount of iron which leached from the PCL/10Fe material was also approximately half of that recorded for the PCL/5Fe composite, Figure 4-7. The leachables results for phosphates covers a larger range than from the other leachables species, Figure 4-8. Values were observed from 4,596 (pH7) - 2,877ppm (krebs) for PCL/0Fe, from 4,040ppm (saline) – 2,821ppm (krebs) for PCL/5Fe and 3,716ppm (pH4) – 1111ppm (krebs) for PCL/ 10Fe. Although the testing was not performed in triplicate there is an overall trend for the addition of iron stabilising the PBG component of the composite.

The next section examines the organic leachables from the PCL component of the composite.

4.5 Leachables from the PCL Component of the PCL/PBGs

GC/MS provides information on organics associated with the PCL material. Information is provided, in this section, for leachables species observed from the polymer, including unpolymerised caprolactone and PCL oligomers. Unfortunately, due to a poor extraction technique using diethyl ether and not dichloromethane (where PCL is soluble) [221], the amount of leachables detected is minimal and this is

therefore not a true representation of how much PCL degradation has occurred over the 78 week study time.

The results in this section are semi quantitative. Two caprolactone concentrations were run as standards and the peaks produced by each sample analysed were measured relative to this. Figure 4-13 shows an example of a 147ppm standard. The caprolactone peak can be seen at 7.44 minutes. An additional peak is seen at 13.97. This will be discussed in section (4.5.1)

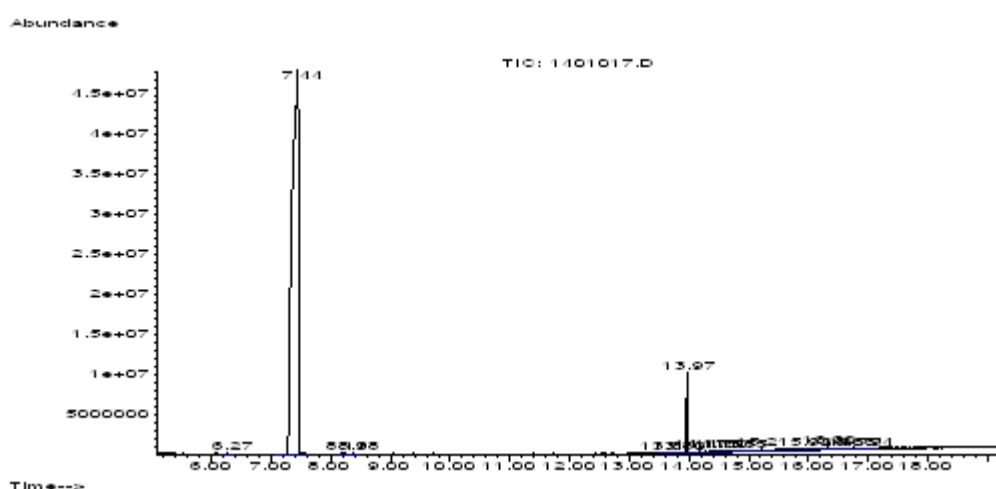


Figure 4-13 Caprolactone standard.

4.5.1 Identification of Leachables

Typically, the GC results showed up to 5 peaks which were attributed to caprolactone, phthalic anhydride, butylated hydroxyl toluene (BHT) and two oligomers. A typical chromatograph can be seen in Figure 4-14.

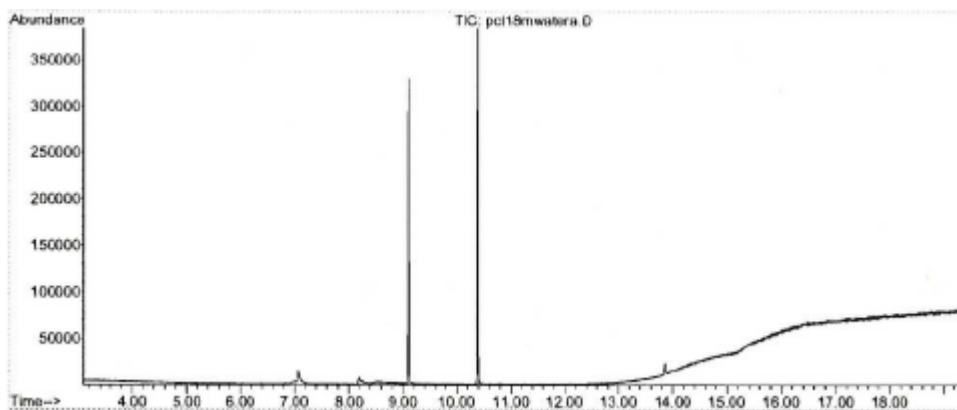


Figure 4-14 Gas chromatogram of PCL pellets after storage in water for up to 78 weeks

Table 4-4 details the elution time and ion m/z of these compounds. This has been taken from the online MS library and also from manually calculated structural fragmentation patterns. The peak (at 7.125 minutes) in Figure 4-14 is small (in the region 32 ppm). From the fragmentation pattern this peak could be potentially associated with unpolymerised caprolactone. Table 4-5 shows the fragmentation patterns for PCL associated with each peak (in Figure 4-14) which could be associated with various ions (m/z).

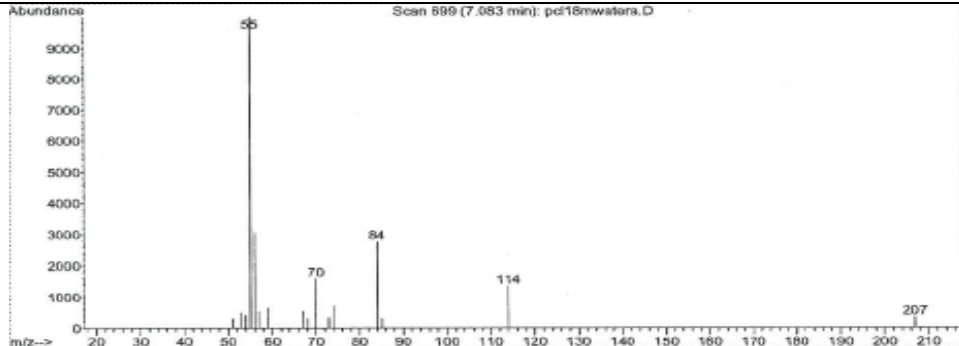
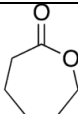
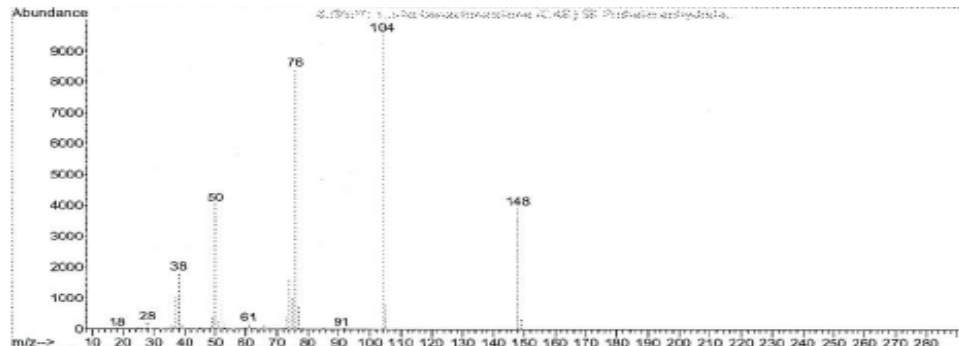
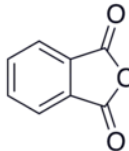
Elution Time (mins)	Ion Chromatograph (m/z)	Identification	Description
7.125	 Scan 599 (7.083 min): pc18mwstara.D		Caprolactone
8.175	 Scan 107 (8.175 min): pc18mwstara.D		Acetyl Anhydride

Table 4-4 GC details of ion fragmentation pattern and identification

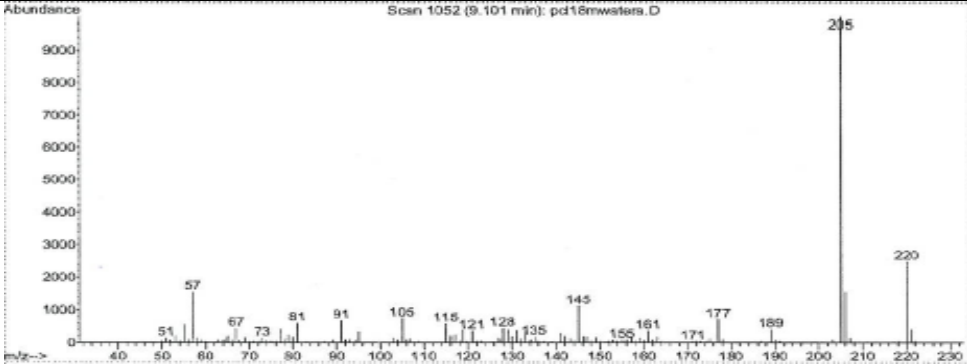
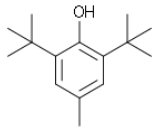
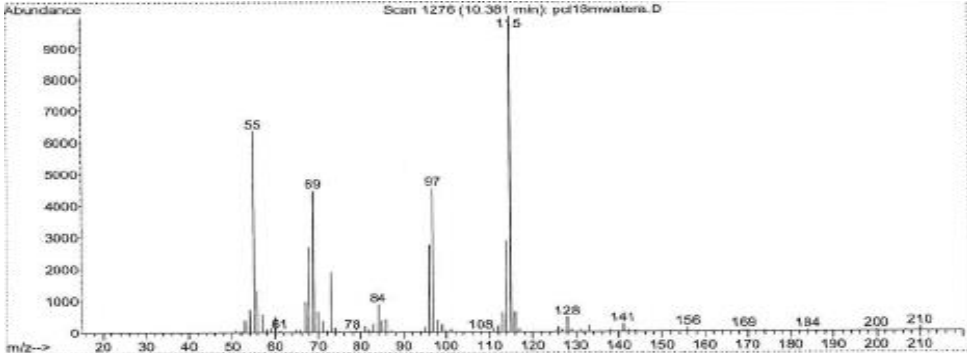
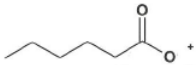
Elution Time (mins)	Ion Chromatograph (m/z)	Identification	Description
9.100			BHT
10.386			Oligomer

Table 4-4 GC details of ion fragmentation pattern and identification

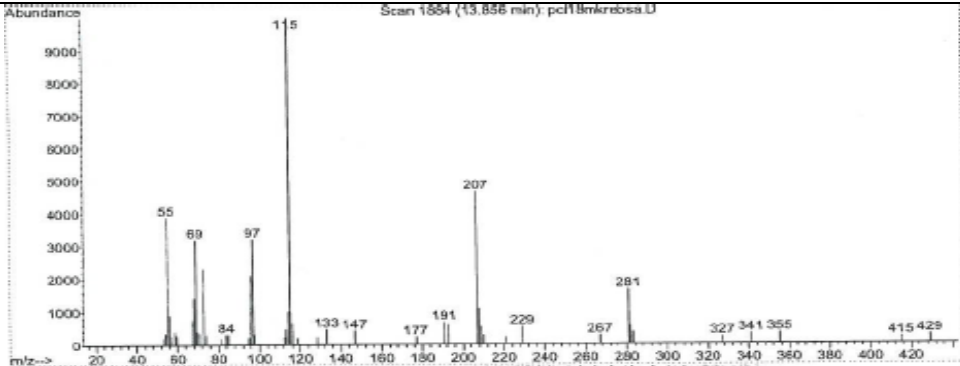
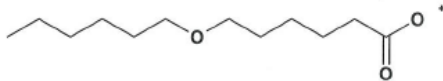
Elution Time (mins)	Ion Chromatograph (m/z)	Identification	Description
13.856			Oligomer

Table 4-4 GC details of ion fragmentation pattern and identification

Details of ions potentially associated with the fragmentation pattern of PCL are shown in Table 4-5.

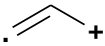
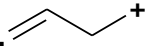
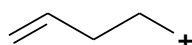
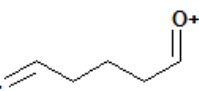
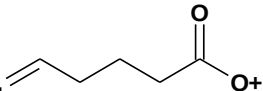
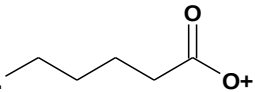
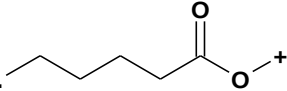
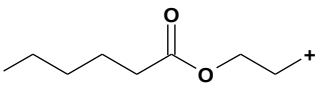
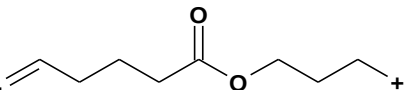
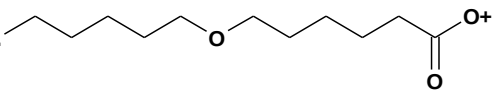
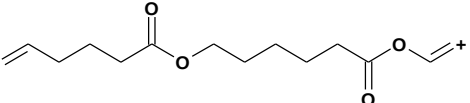
Structure	Ion (m/e)
	41
	55
	69
Background ion	73
	97
	113
	115
	128
Background ion	133
Background ion	141
Background ion	152
	156
Background ion	163
	169
Background ion	191
Background ion	207
	229
	253

Table 4-5 Structure of ions detected for PCL and PCL/PBG leached into either pH4, pH7, krebs, water or saline.

Structure	Ion
Background ion	267
Background ion	281
Background ion	315
Background ion	324
Background ion	341
Background ion	346
Background ion	355
Background ion	405

Table 4-5 Structure of ions detected for PCL and PCL/PBG leached into either pH4, pH7, krebs, water or saline.

The background ions were also present in the blank GC chromatograms.

There were 2 additional peaks observed, at low levels, in the GC Chromatograms for the krebs samples. The elution times were 5.335 mins and 9.821 mins. Figure 4-15 (a) and (b) shows the GC Chromatogram and corresponding ion fragmentation pattern. The fragmentation patterns for these compounds did not match any ions detailed in Table 4-5 which would associate them with PCL. They did not give a suitable match to any compounds within the online MS library. Hence, they were unidentifiable and the structure is unknown.

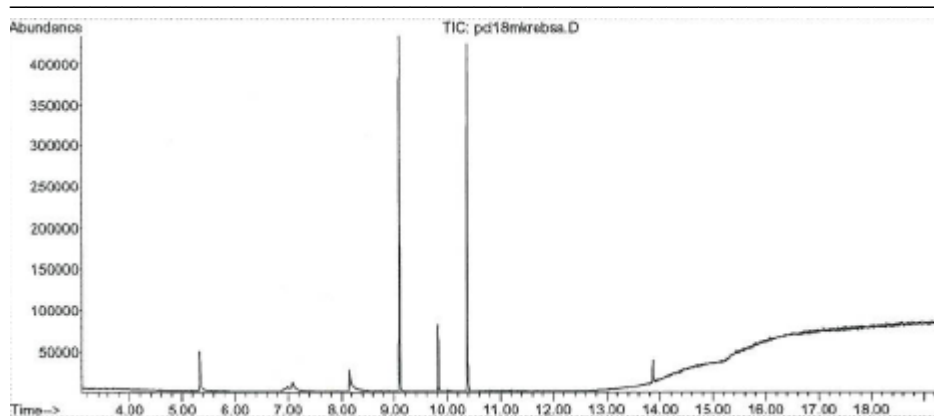


Figure 4-15 (a) Gas chromatogram of species detected in krebs solution after storage at 37°C for 78 weeks

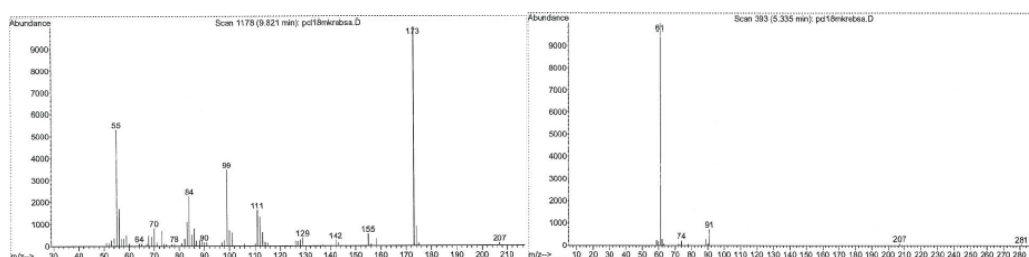


Figure 4-15 (b) Ion chromatographic information corresponding to peaks at 5.335 and 9.821 minutes

The leachables of interest, from the GC/MS results, are caprolactone and the 2 corresponding oligomers. Quantitative data for caprolactone has been reported. Semi-quantitative data for the oligomers has been calculated based on the areas for the gas chromatograms relative to the caprolactone standard.

4.5.2 Caprolactone Leachables

PCL and the three PCL/PBG composites (PCL/0Fe, PCL/5Fe, PCL/10Fe) were examined for caprolactone leachables, Figure 4-16 (a) to (d).

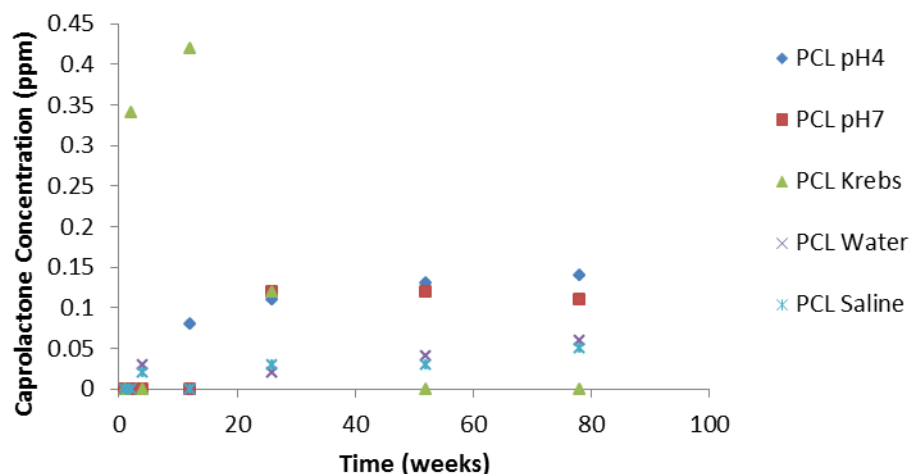
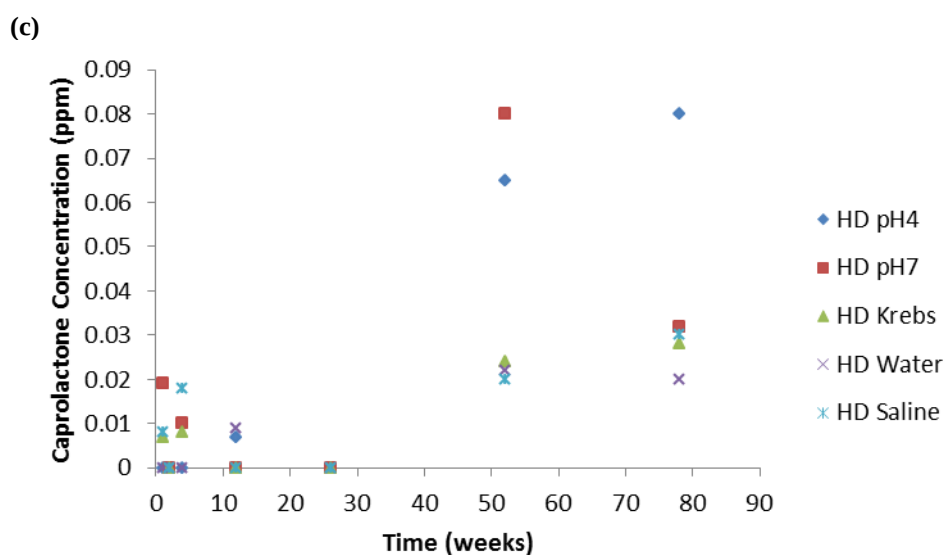
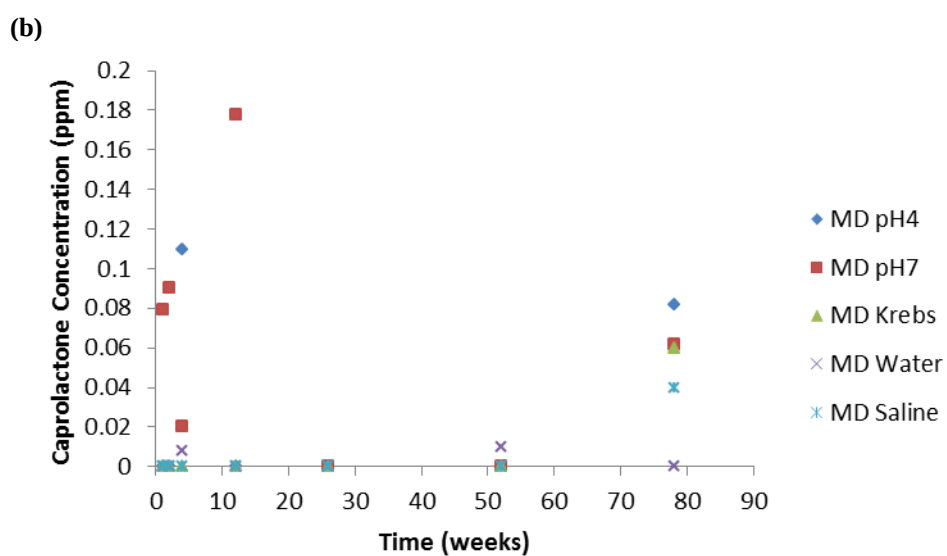
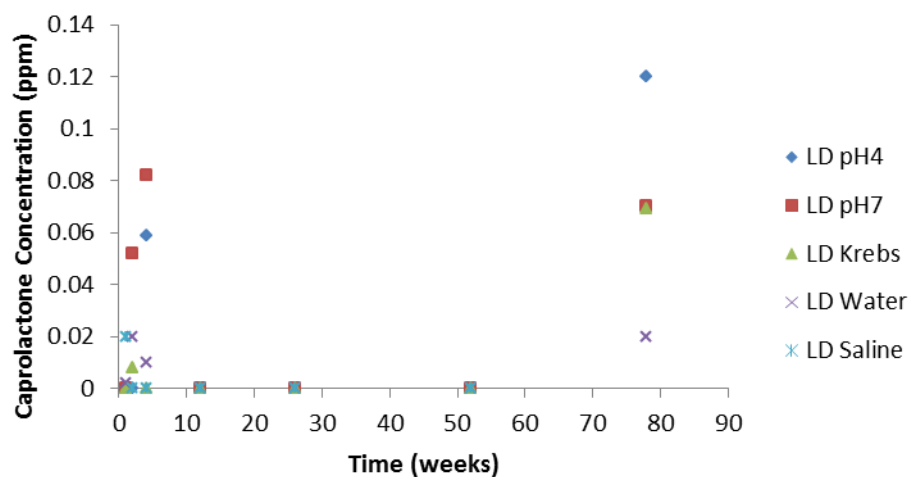


Figure 4-16 (a) Caprolactone leachables from PCL after storage at 37°C for 78 weeks



(d)
Figure 4-16 Caprolactone leachables from (b) PCL/0Fe, (c) PCL/5Fe and (d) PCL/10Fe after storage at 37°C for 78 weeks

Unpolymerised caprolactone monomer is present in all PCL and composite samples analysed. More caprolactone has leached from the PCL material compared to the PCL/PBG samples. This may be due to a larger amount of PCL being present (100% as opposed to 10% in the composite) and due to autocatalysis of the PCL material. The leachables from PCL/10Fe are less than any of the other 3 materials. However, it is difficult to confirm this statistically due to lack of replicates. Further studies would need to be conducted to evaluate whether this is confirmed and also the influence of the PBG upon the PCL component within the matrix.

4.5.3 Oligomeric Leachables

PCL and the three composites of PCL/PBG materials were examined for the following oligomeric leachables:

- _____ C
 $\text{H}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$ (m/z 115), Figures 4-17 (a) to (d).
- _____ C
 $\text{H}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$ (m/z 229), Figures 4-18 (a) to (d).

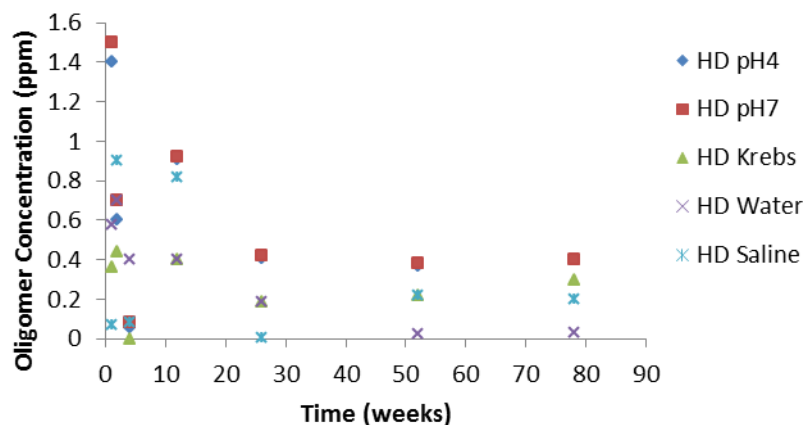
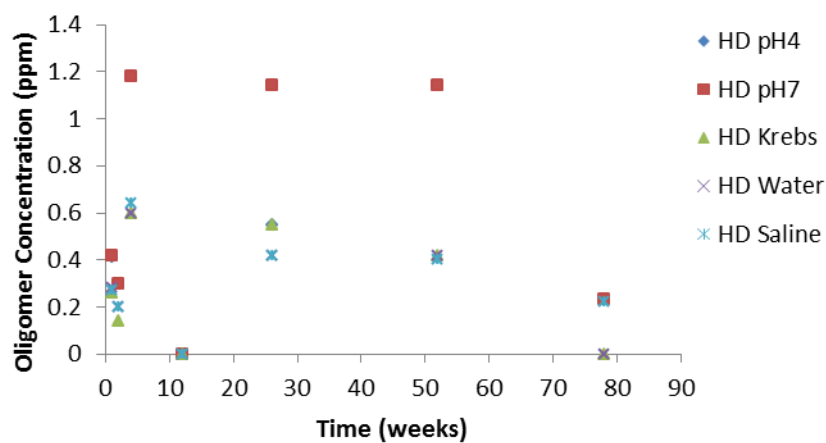
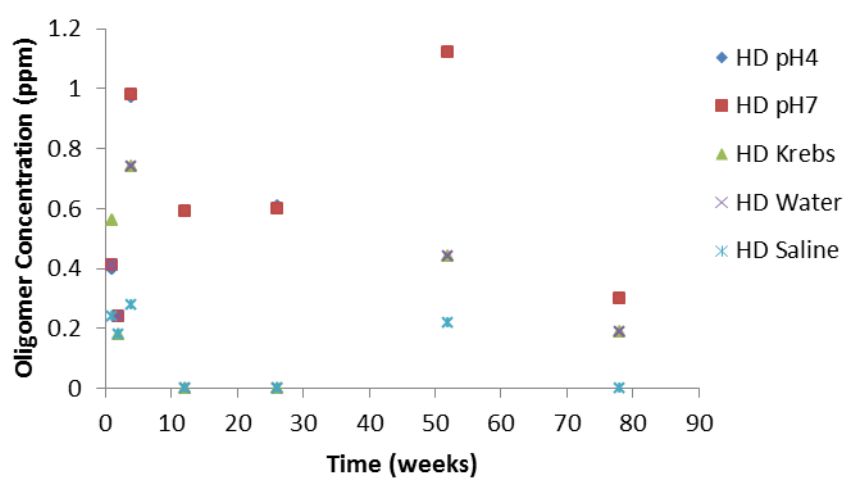


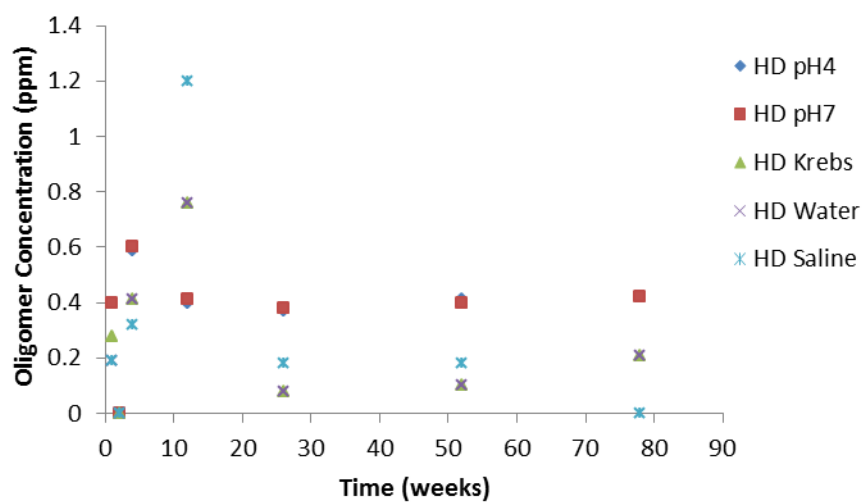
Figure 4-17 (a) Oligomeric leachables ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$) from PCL after storage at 37°C for 78 weeks



(b)

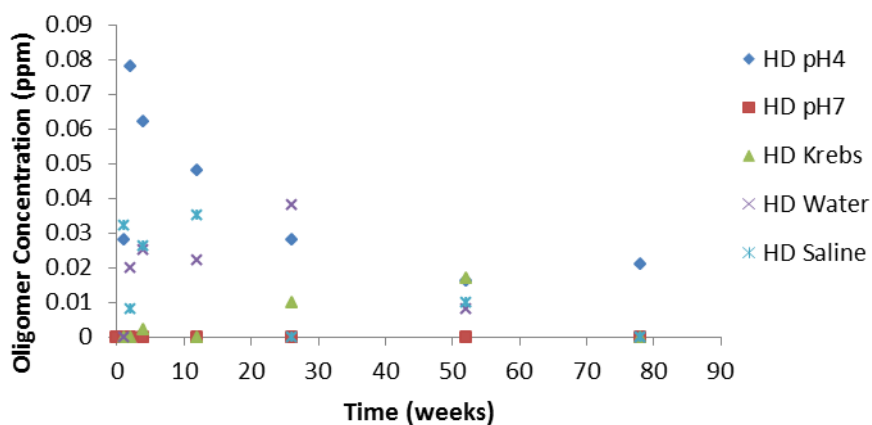


(c)

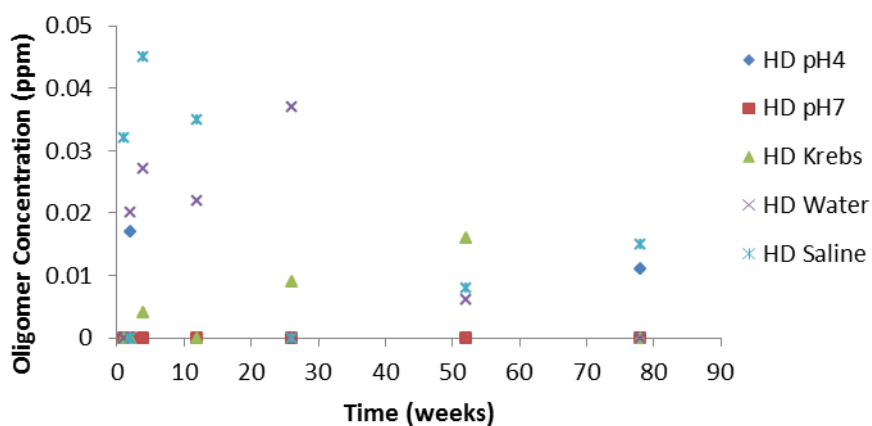


(d)

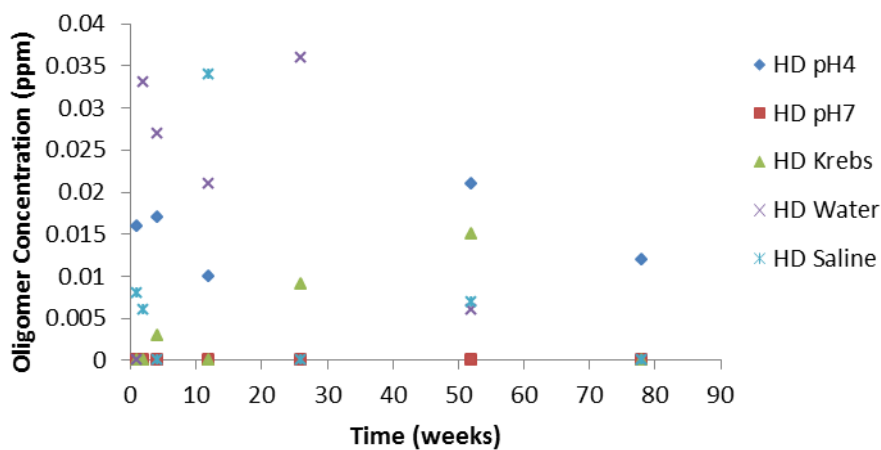
Figure 4-17 Oligomeric leachables ($\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$) from (b) PCL/0Fe, (c) PCL/5Fe and (d) PCL/10Fe after storage at 37°C for 78 weeks



(a)



(b)



(c)

Figure 4-18 Oligomeric leachables ($\text{CH}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$) from (a) PCL (b) PCL/0Fe and (c) PCL/5Fe after storage at 37°C for 78 weeks

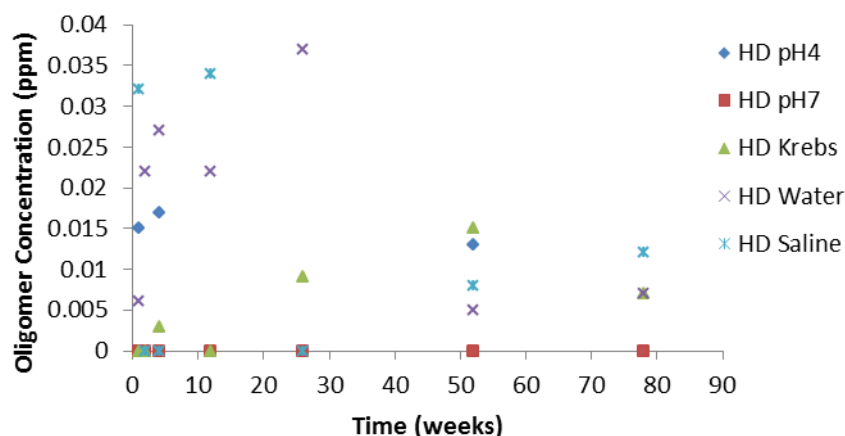


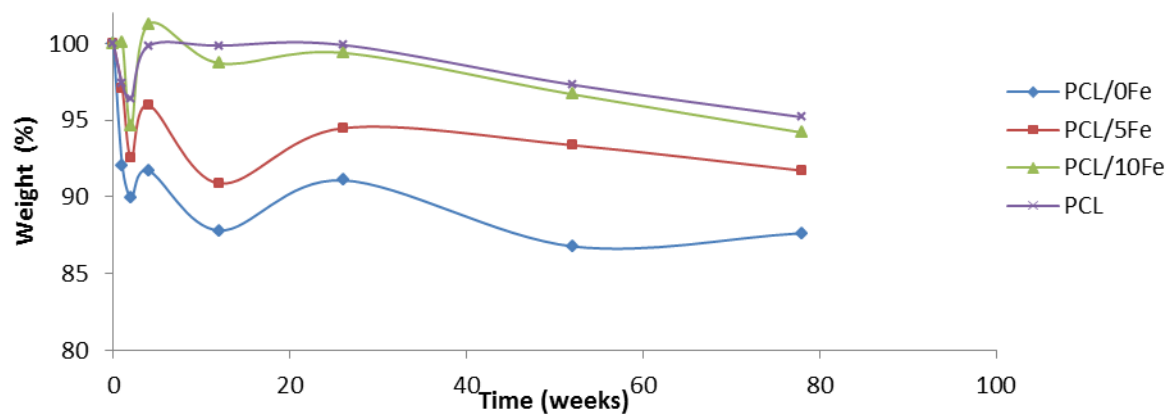
Figure 4-18 (d) Oligomeric leachables ($\text{CH}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$) from PCL/10Fe, after storage at 37°C for 78 weeks

$\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$ and $\text{CH}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$ oligomers were observed as leachables from PCL and the PCL/PBG composites. No aqueous solution appeared to be more discriminatory than the other, although for the latter oligomer no leachables were observed in the pH7 medium. It was expected that the amount of oligomers leaching over time would increase due to degradation of the PCL component, particularly as the glass is dissolving out of the composite structure and leaving behind a greater PCL surface area for the aqueous solution to penetrate. However, all composites showed similar results for the $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$ leachables. Leachables results for $\text{CH}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$ were an order of magnitude lower than the lower molecular weight oligomer.

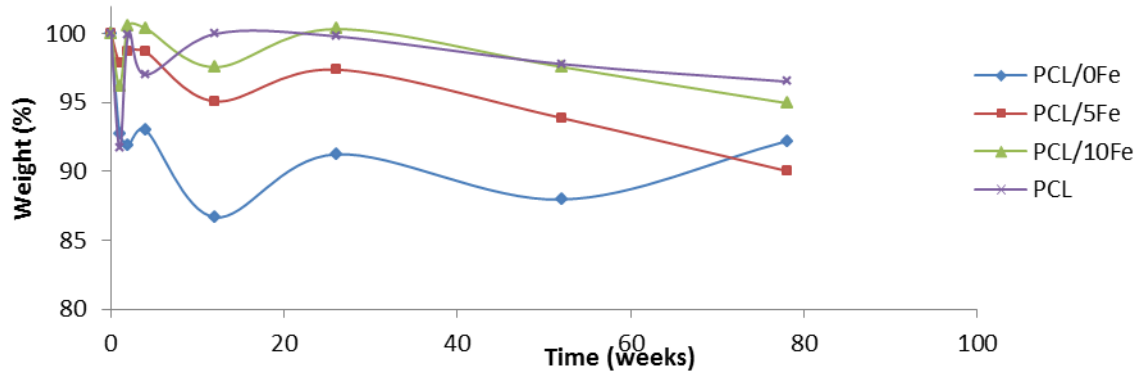
As discussed in the previous section there are likely to be issues with the experimental parameters. A more suitable solvent (DCM) should have been used and a minimum of 3 extractions into the organic solvent. Standards of the oligomers were also not available hence, the data was calculated against the caprolactone monomer solution. There could be slight differences in Rf factors when calculating the ppm concentration.

4.6 Weight Loss of PCL and PCL/PBG Composites Stored in Various Aqueous Solutions

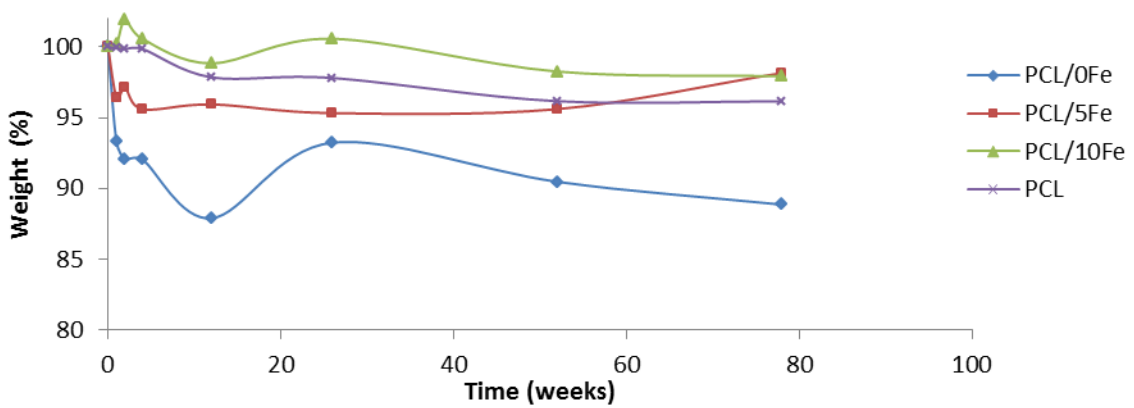
The resulting weight loss for PCL and three composites of PCL/PBG are shown graphically in Figure 4-19 (a) to (e). This is presented as % weight loss in order to compare data across each sample type and leachables medium.



(a)

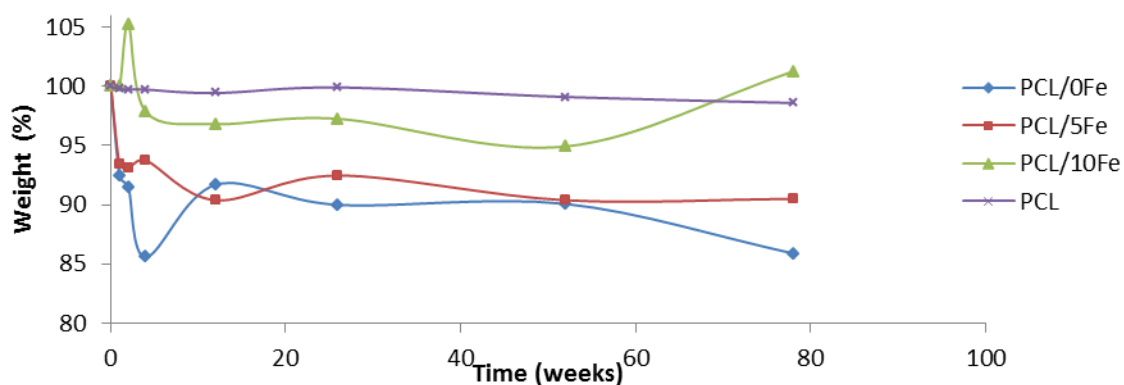


(b)

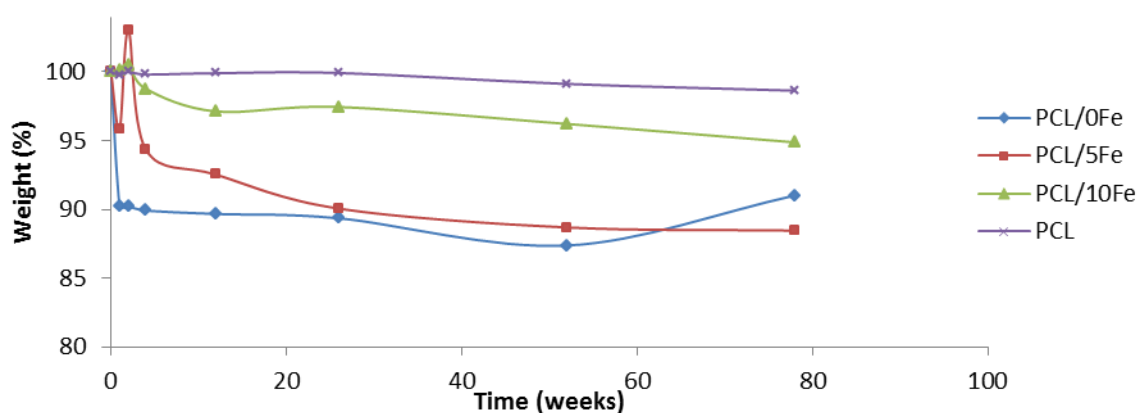


(c)

Figure 4-19 Graph of weight change for three PCL/PBG composites after storage in (a) pH4, (b) pH7 and (c) krebs solution for up to 78 weeks, n=1



(d)



(e)

Figure 4-19 Graph of weight change for three PCL/PBG composites after storage in (d) water and (e) saline for up to 78 weeks, n=1

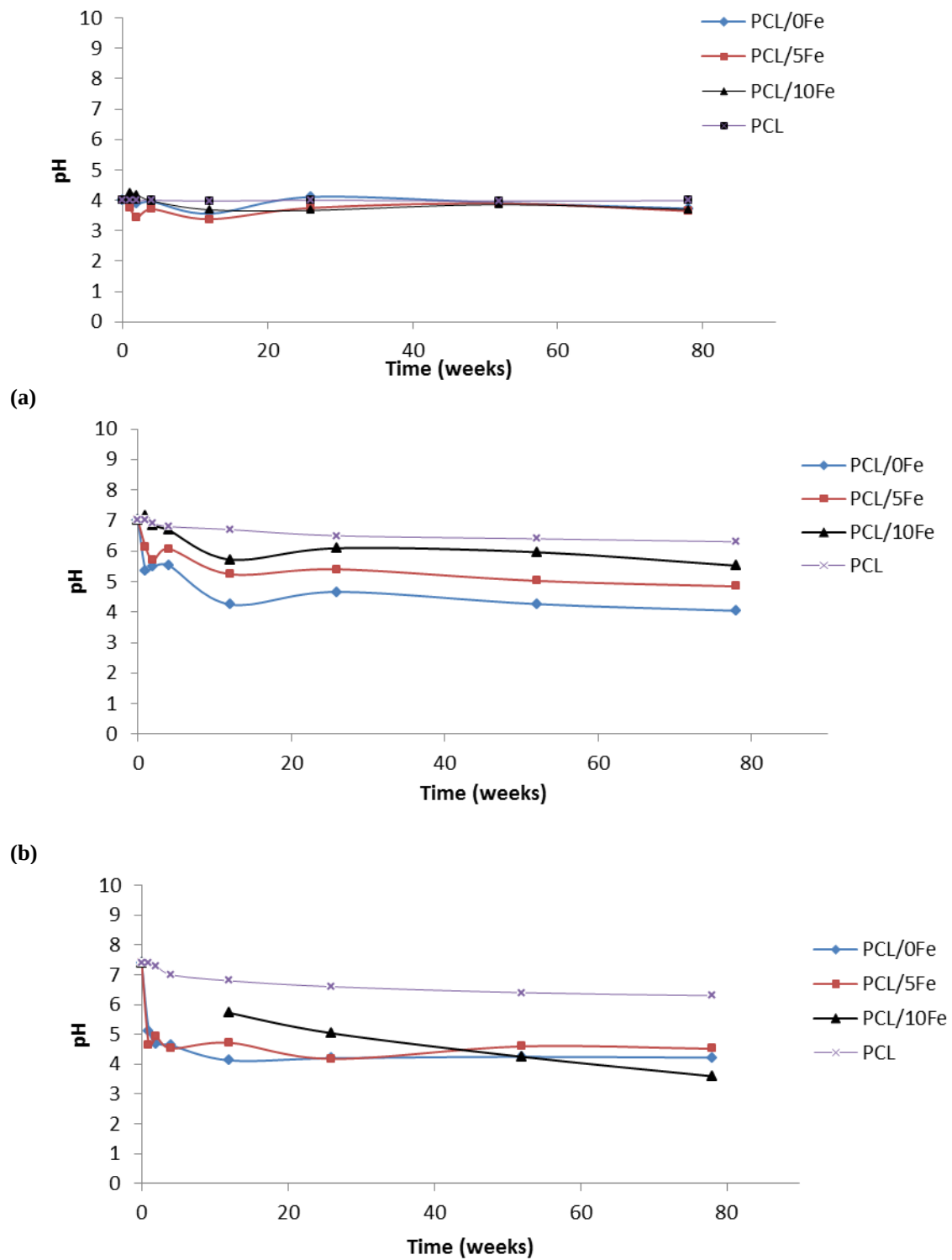
Figures 4-19 (a) to (e) show that there is a general trend of the greatest weight loss occurring for the PCL/0Fe composite material. This is followed by the PCL/5Fe composite, the PCL/10Fe composite and PCL exhibiting the least weight loss. There are some instances where the weight losses overlap for some samples and there are the occasional weight gains. PCL is prone to hydrolytic degradation at a lower rate than PLA and PGA however, the mechanism of hydrolysis is the same as PLA [485]. It proceeds via random ester cleavage and is a bulk process which is limited by sample hydrophobicity and crystallinity. The erosion rate depends on the particular specimen surface area/ volume ratio, porosity and hydrolysis depends on the temperature and pH of the solution. There is no solution which appears to contribute to the greatest weight loss but for PCL and PCL/0Fe show the greatest weight loss in an acidic environment (pH4) and for the non Fe containing composite the greatest weight loss is in the water solution which may have changed to acidic due PCL degrading to caproic acid.

There has been a weight gain for two of the composites, namely PCL/5Fe and PCL/10Fe up to 4 weeks storage. Since the solubility of the glasses can be relatively low, weight gain can be dominant at early time points (dependant on the PBG structure). Once the polymer matrix is saturated weight becomes predominant due to glass dissolution. This weight gain will be associated with slight dimensional changes (which were not measured here) and which would need to be considered during the development of a biomaterial implant. Two basic parameters that can characterise water uptake are equilibrium and kinetic uptake [486].

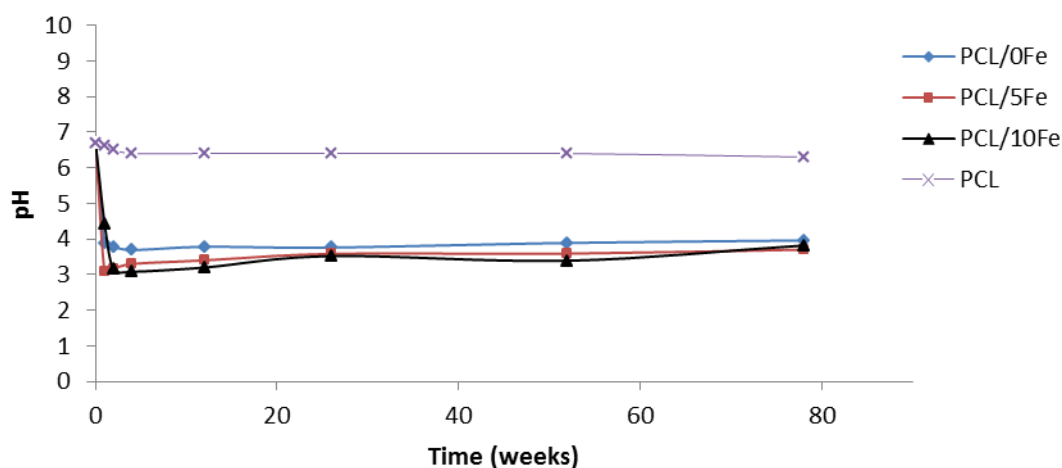
Gel permeation chromatography (GPC, or size exclusion chromatography (SEC))) would have provided more details on the degradation of the PCL component of the composite by addressing the molecular weight distribution. This, alongside the structure of a polymer, influences processing behaviour and physical properties of the finished product. Hence, understanding of the composite is essential for future novel synthesis, development and good material performance.

4.7 pH Changes to Aqueous Storage Mediums

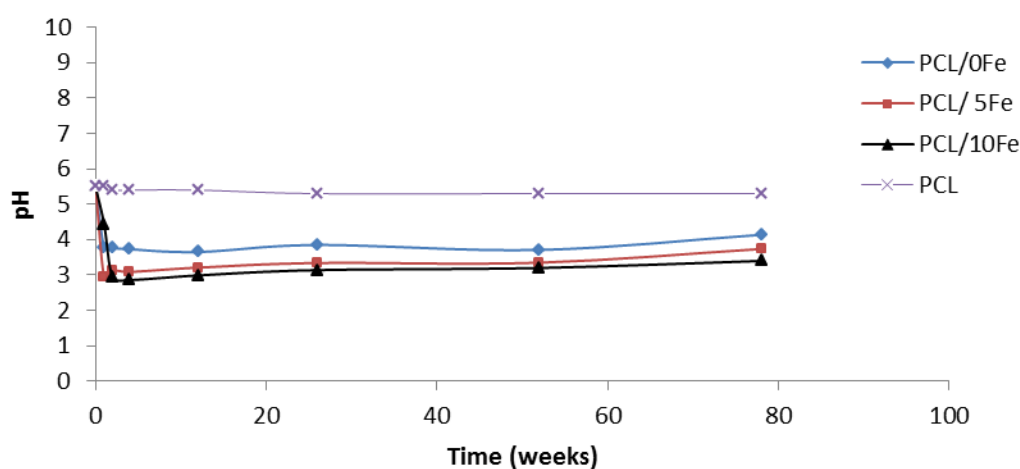
Within the literature it has been documented that storage of PBG in aqueous media causes a decrease in pH (Table 2-25). The increase in acidity is associated with the formation of phosphoric acid. The pH of aqueous storage mediums was analysed to get an understanding of the extent of acidic shifts occurring for the three composite materials, Figures 4-20 (a) to (e). PCL showed a minimal decrease in pH when stored in aqueous media. The degradation product of PCL is 6-hydroxycaproic acid which is insoluble in water. Conversely for the PCL/PBG composite there was a general tendency towards a resulting pH of ~4 after 78 weeks in aqueous media.



(c)
Figure 4-20 Graph of pH change for (a) pH4, (b) pH7 and (c) krebs solution After 78 weeks (at 37°C) due to storage of PCL/PGB composites, n=1 sample size with three measurements



(d)



(e)

Figure 4-20 Graph of pH Change for (d) water and (e) saline after 78 weeks (at 37°C) due to storage of PCL/PGB composites, n=1 sample size with three measurements

All PCL/PGB composites, stored in pH4 buffer, exhibited a similar profile of a minimal decrease in pH over the 78 week storage period. This varied from pH 4.24 (for PCL/10Fe over 2 weeks) to pH 3.38 (PCL/5Fe over 12 weeks).

PCL/PBG composite samples, stored in pH7, did not have a similar profile for varying iron content. PCL/10Fe showed a less acidic pH environment over the 78week time period than the other PCL/PBG composites. After 78 weeks the results were pH 4.04 (PCL/0Fe), 4.84 (PCL/5Fe) and 5.52 (PCL/10Fe) respectively.

There was a sudden decrease in pH from pH7.4 to pH5.11 and pH 4.63 after 1 week for PCL/0Fe and PCL/5Fe stored on krebs solution. An unusually high set of data was recorded

up to 12 weeks for PCL/10Fe (in the region of pH8-9). This data was excluded as no explanation was available for these results. After 78 weeks there was a continued decrease in pH for all samples, pH 4.22, 5.52 and 3.6 (PCL/0Fe, PCL5FE and PCL10Fe respectively).

After 1 weeks storage in water there was also a sudden decrease in pH observed, from 6.80 to 3.87, 3.08 and 4.43 (from PCL/0Fe, PCL/5Fe to PCL/10Fe respectively). This then remained fairly constant up to 78 weeks. For PCL/0Fe the pH was 3.96, for PCL/5Fe: pH3.70 and PCL/10Fe 3.82.

The pH of saline solution decreased from pH5.5 to pH 3.77 (PCL/0Fe), pH2.93 (PCL/5Fe) and pH4.44 (PCL/10Fe) after 1 week. As with the other samples this then remained fairly constant up to 78 weeks. At this time point the following readings were observed: pH4.14 (PCL/0Fe), pH3.74 (PCL/5Fe) and pH 3.4 (PCL/10Fe).

4.8 Visual Inspection, Density and Morphological Changes to Composites after Storage in Aqueous Media

4.8.1 Visual Inspection

The 0Fe fibres and PCL/0Fe composites were white in appearance. However, as the amount of iron was increased the degree of grey colour also increased for both of the fibres and composites. This decreased over the 78 weeks storage period, in aqueous media, resulting in visibly whiter samples. Figure 4-21 shows the PCL/10Fe samples after storage in water for up to 78 weeks.

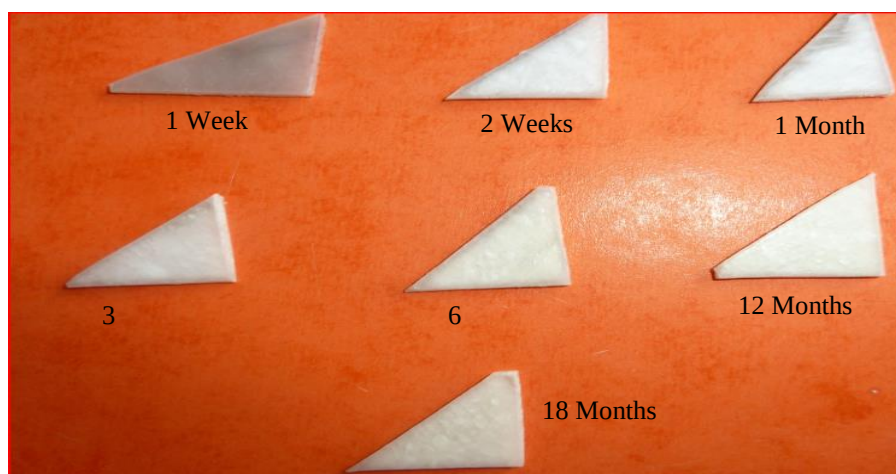


Figure 4-21 Photograph of PCL/10Fe after storage in water for up to 78 weeks at 37°C

The surface of the PCL/10Fe samples (at 52 weeks) appeared to be slightly raised. This could be due to water ingress resulting in composite swelling. Samples stored in pH4, pH7, Krebs and saline showed a grey edge with an inner white area for PCL/10Fe, Figure 4-21. This could be due to the migration of the PBG dissolving out of the composite. All samples were measured on the centre and at 3 points along the edge, however there was no indication that any area was significantly thinner or thicker than the other which could impact the strength of the material.



Figure 4-21 (b) Photographs of PCL/10Fe after storage in pH7 solution for up to 78 weeks at 37°C

4.8.2 Density, Porosity and Pore Volume of PCL/ PBG Composites

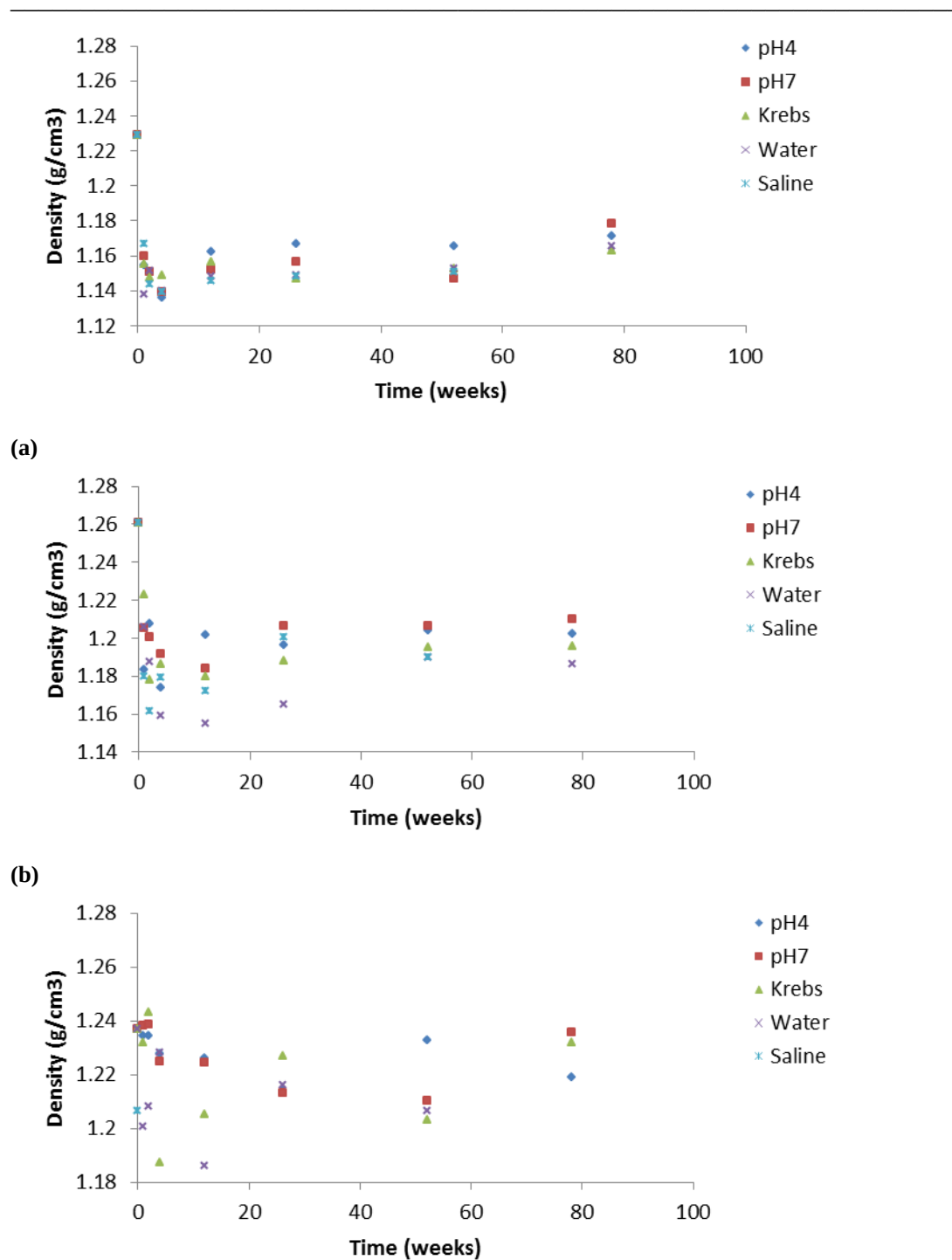
The initial density measurements of PCL, three PBG and the composites of PCL/PBG were measured. The results can be seen in Table 4-6.

Description (PCL, PBG and PCL/ PBG)	Density (g/cm ³)	Std. Dev.
PCL	1.1246	0.0064
0Fe	2.6423	0.0082
5Fe	2.7335	0.0096
10Fe	2.8562	0.0069
PCL/0Fe	1.2309	0.0188
PCL/5Fe	1.2606	0.0267
PCL/10Fe	1.2354	0.0110

Table 4-6 Initial density measurements for PCL, 0Fe, 5Fe and 10FE PBG fibres and PCL/0Fe, PCL/5Fe and PCL/10Fe composites. Based on n=3 samples.

From Table 4-6 it can be seen that PCL alone has the lowest density (1.1246gcm³). The density of the PBGs are more than double that of the PCL (2.6423 gcm³, 2.7335 gcm³ and 2.8562 gcm³). By adding increments of 5%w/w iron to the PBG structure the density increases in the order of 0.1g/cm³. A combination of the 10% w/w PBG with 90%w/w PCL increases the PCL density by a factor of 0.1g/cm³. The results show that the PCL/10Fe material is 0.02g/cm³ lower than the PCL/5Fe material. This could be due to the distribution of the fibres within the polymer matrix (section 4.2.1). Since the density of PBG is higher than PCL there could be more PBG in one particular area of the sample measured. The hot stage microscopy showed that there was potential for this to happen.

The density results for PCL/0Fe, PCL/5Fe and PCL/10Fe, stored in each aqueous solution, can be seen in Figure 4-22. All samples show a density less than the starting material at all time points.



(c)
Figure 4-22 Change in density over time for (a) PCL/ 0Fe, (b) PCL/5Fe and (c) PCL/10Fe in pH4, pH7, krebs, water and saline solution for 78 weeks. Number of replicates, n=5

Interpretation of the data is limited due to only one samples being analysed at each time point for each composite and storage solution therefore, it is not possible to conduct a statistical summary. There are instances where the density has risen slightly for some of the PCL/ PBG

composite material. Although only one sample was analysed over the 78 week period it has been shown that there is a standard deviation of (0.0188 (PCL/0Fe), 0.0267 (PCL/ 5Fe) and 0.0110 (PCL/ 10Fe) for the initial composite density measurements. The majority of the samples are within this tolerance.

The density measurements from Figures 4-22(a) to (c) were used to calculate the porosity and pore volume of the composite materials. The pore volume is the pore space and the porosity is a measure of voids/ empty space over the total volume of the material. (Table 4-7 (a) and (b)). The results are based on the equations in section 3.7. The initial density measurements of the PCL/PBG (Table 4-6) were used for the calculations. 4 weeks and 78 weeks are presented because 6 weeks (from the literature review) is a key time point for osteoblast growth and 78 weeks because the study was run for this length of time.

Time (weeks)	Solution	Porosity (%)		
		PCL/0Fe	PCL/5Fe	PCL/10Fe
4	pH4	7.61	6.85	0.76
	pH7	7.31	5.47	0.98
	Krebs	6.56	5.91	2.17
	Saline	7.35	6.44	2.74
	Water	7.25	8.05	2.01
78	pH4	4.72	4.60	1.46
	pH7	4.12	4.00	-
	Krebs	5.38	5.14	0.41
	Saline	6.45	5.48	2.50
	Water	5.19	5.87	0.41

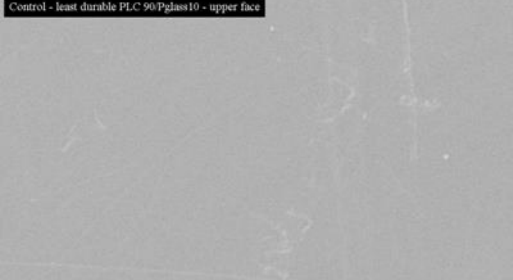
Table 4-7(a) Details of % porosity for 12 and 78 week samples of PCL/ PBG after 78 weeks storage in pH4, pH7, krebs, water and saline solution for 78 weeks

Time (weeks)	Solution	Pore volume(%)		
		PCL/0Fe	PCL/5Fe	PCL/10Fe
4	pH4	6.70	5.87	0.62
	pH7	6.42	4.59	0.08
	Krebs	6.56	5.91	2.07
	Saline	6.44	5.46	2.28
	Water	6.36	6.94	2.38
78	pH4	4.03	3.83	1.20
	pH7	3.50	3.04	-
	Krebs	4.62	4.30	0.33
	Saline	4.43	4.95	3.44
	Water	5.61	4.61	2.07

Table 4-7(b) Details of pore volume (%) for 12 and 78 week samples of PCL/ PBG after 78 weeks storage in pH4, pH7, krebs, water and saline solution for 78 weeks

4.8.3 Morphology of PCL/PBG Composites

PCL/0Fe Composites



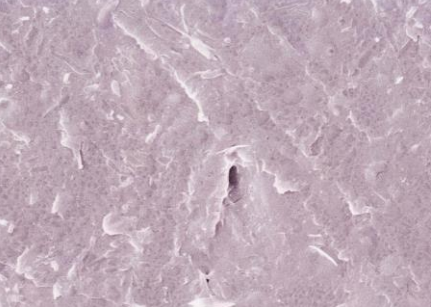
Control - least durable PLC 90P/glass10 - upper face

10µm

x500

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Date 23 May 2006 Time 11:00
File Name = 10000000000000

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EHT = 15.00 kV
Detector = SE1
Collector Bias = 300 V



User Name = ELAIN HARRIS
Date 24-Jul-2009 Time 16:43

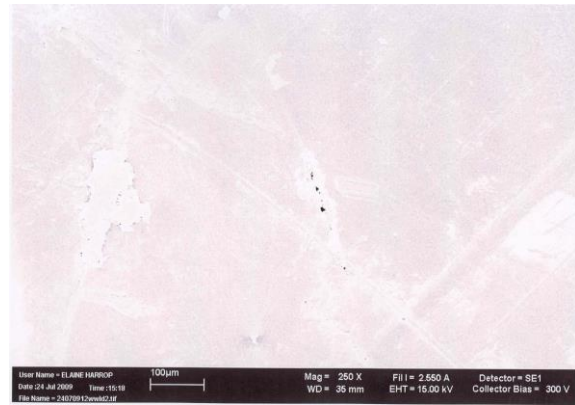
10µm

Mag = 2.50 K X
WD = 5 mm
Fill = 2.560 A
EHT = 15.00 kV
Detector = SE1
Collector Bias = 300 V

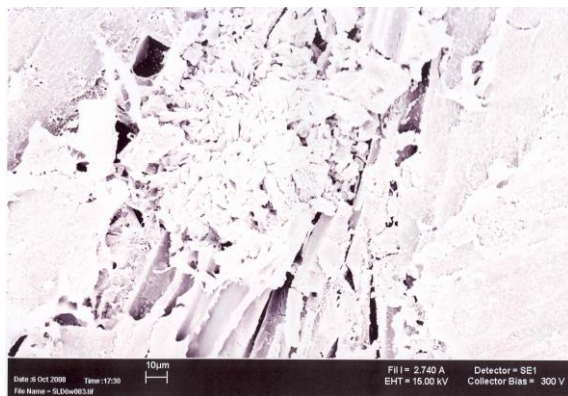
142



(c) 4 weeks (100µm scale bar)



(d) 12 weeks (100µm scale bar)



(e) 26 Weeks (10µm scale bar)



(f) 78 Weeks (10µm scale bar)

Figure 4-23 SEM images of PCL/0Fe Stored in water for up to 78 weeks at 37°C

From the SEM images the composites exhibit signs of degradation. The initial sample has a smooth surface and little topography is seen under the microscope. However, after a week it is evident that degradation is starting to occur. A pore is observed in the composite which is 1.7µm in diameter. This could potentially be where a PBG fibre was present. The general surface of the composite is also no longer smooth. After 4 weeks there is a clear cavity within the composite structure which is approximately 1.7µm in diameter and 183µm in length. This also appears to indicate dissolution of PBG from the PCL matrix. At 26 weeks there is further evidence of PBG dissolution within the structure. After 78 weeks, pores are visible which used to contain PBG fibres. The diameter of the pores observed, were in the region of 30µm.

The composites stored in water still retain some elasticity. However, the pH4 samples were very brittle and could be snapped easily in the hand. The SEM images for composites stored

in pH4 solution can be seen in Figure 4-24 (a) and (b). Fifty two weeks and 78 weeks are reported as up to this time point the composites looked similar to the water samples.

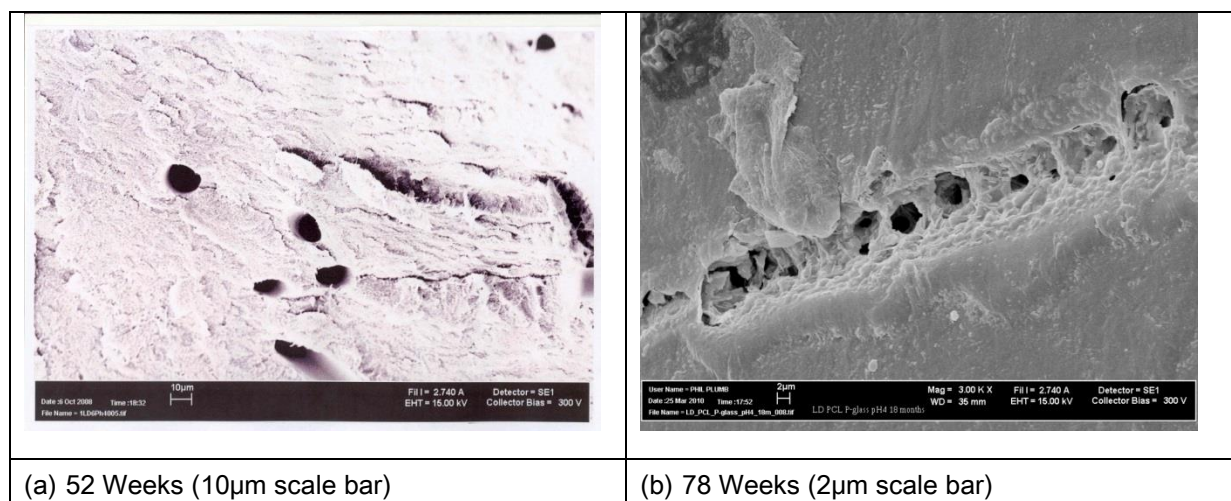


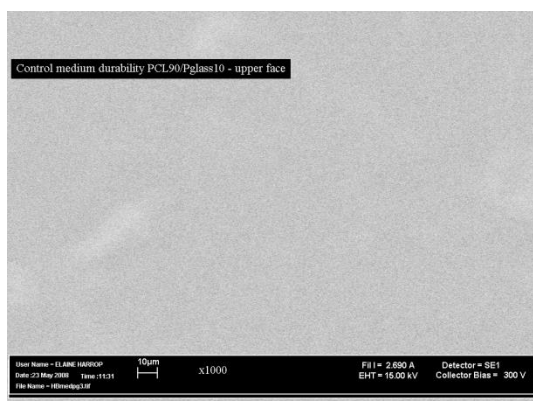
Figure 4-24 SEM images of PCL/0Fe stored in pH4 for up to 78 weeks at 37°C

The polymer matrix also appears to be degrading due to the surface rugosity (small-scale variations or amplitude in the matrix surface height). The above scales are 10 µm and 2µm. The pore size in the above samples range from approximately 1µm to 10µm from 52 weeks to 78 weeks.

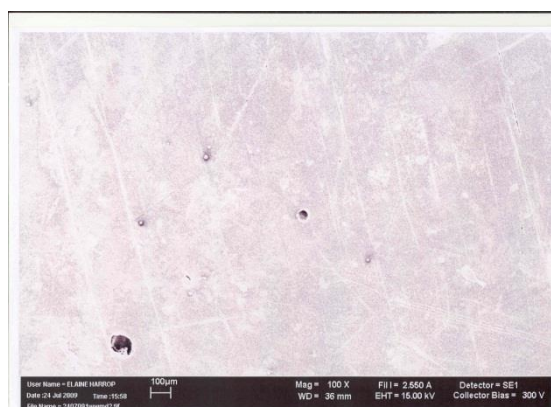
PCL/5Fe Composites

SEM images of the PCL/5Fe composite stored in water for up to 78 weeks can be seen in Figure 4-25

.



(a) Initial (10µm scale bar)



(b) 1 Week (100µm scale bar)



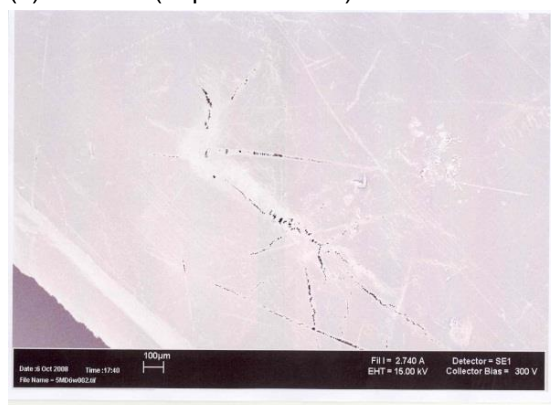
(c) 1 Week (10µm scale bar)



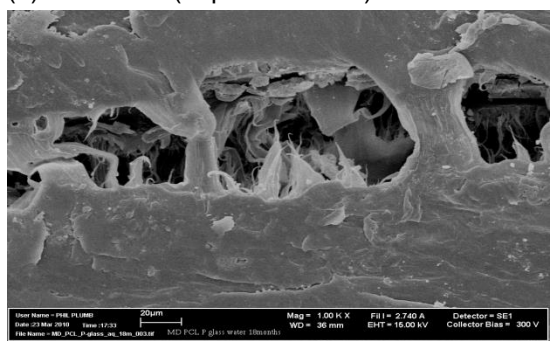
(d) 2 weeks (10µm scale bar)



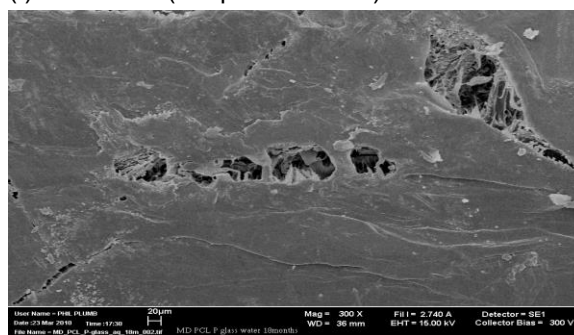
(e) 12 Weeks (10µm scale bar)



(f) 26 Weeks (100µm scale bar)



(g) 52 Weeks (20µm scale bar)

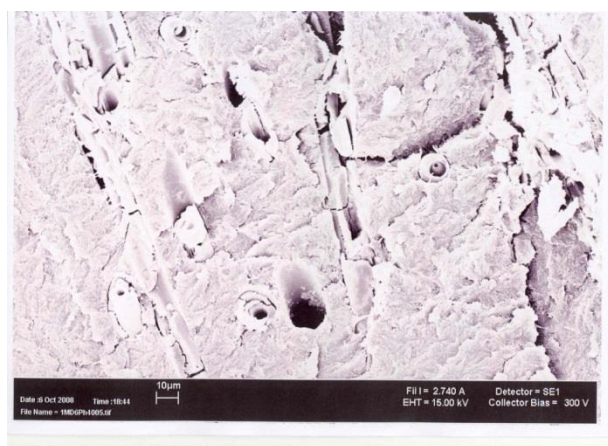


(h) 78 Weeks (20µm scale bar)

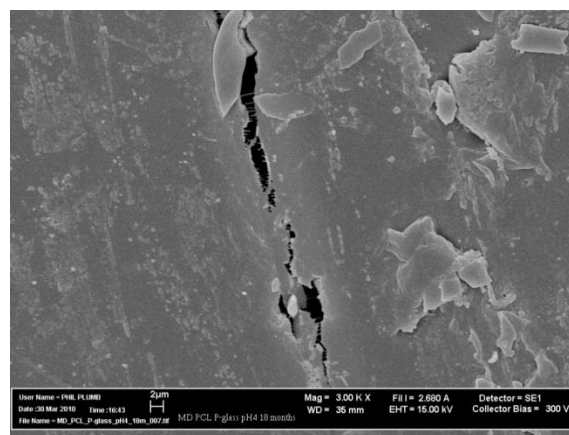
Figure 4-25 SEM images of PCL/5Fe stored in water for up to 78 weeks at 37°C

As with the PCL/0Fe material the initial PCL/5Fe samples had a smooth surface and little topography was seen under the microscope. After one week two well defined pores were observed in the composite which were between 50 and 100 μ m in diameter, Figures 4-26(b) and (c). Four other darker circular areas were also present, which could be due to PBG presence. The pores were larger than those found in the PCL/0Fe material and evidence of PBG fibres at the surface were more evident in the PCL/5Fe than the PCL/0Fe material. After 2 weeks there was a clear cavity within the composite structure which was approximately 30 μ m in diameter and at least 0.1mm in length. This cavity contains rod like structures which look like PBG fibres. The SEM image at 12 weeks shows a fracture in the polymer matrix with a defined cavity. Within this cavity there could still be presence of some PBG as there was a rod type structure inside. The cracks observed in the image for 26 weeks (f) shows possible drying artefacts from the vacuum used during SEM analysis. It is difficult to interpret the SEM scans at 52 and 78 weeks. The PCL appears to be stretched in some areas which could also be an artefact of the vacuum applied during analysis however, there was a region which may have contained PBG. Figure 7-26 (g) shows a cavity >120 μ m in length and 50 μ m in diameter. The surface of the PCL/5Fe shows some rugosity which would indicate that the PCL has started to degrade.

The SEM images for composites stored in pH4 solution can be seen in Figure 4-26 (a) and (b). Fifty two weeks and 78 weeks are reported as up to this time point the composites looked similar to the water samples.



(a) 52 Weeks (10 μ m scale bar)



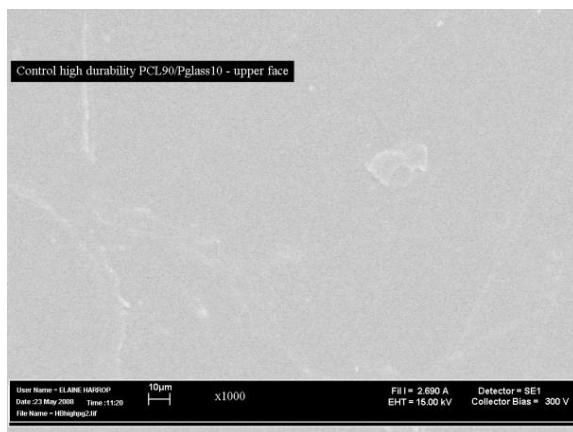
(b) 78 Weeks (2 μ m scale bar)

Figure 4-26 SEM images of PCL/5Fe stored in pH4 for up to 78 weeks at 37°C

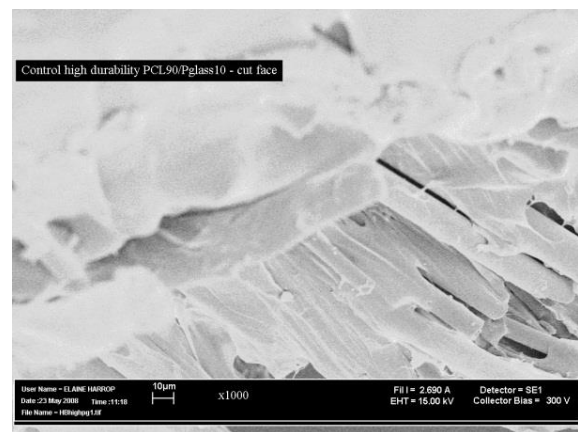
Figure 4-27 (a) shows pore sizes of up to 20 μ m. Some of the pores were defined but others appear to still contain glass fibres as rod type shapes could be seen within the pore cavities. This indicates that PBG has migrated away from the structure but there are still PBG fibres within the PCL matrix. This is confirmed by the leachables results (section 4.4) and weight loss (section 4.3) which shows that after 52 weeks there is still PBG within the polymer matrix. At 78 weeks (Figure 4-27 (b)) the polymer matrix exhibits a rupture, approximately 4 μ m in diameter and >30 μ m in length, a potential drying artefact of the SEM vacuum. The surface of the PCL/5Fe composite is no longer smooth indicating PCL degradation.

PCL/10Fe Composites

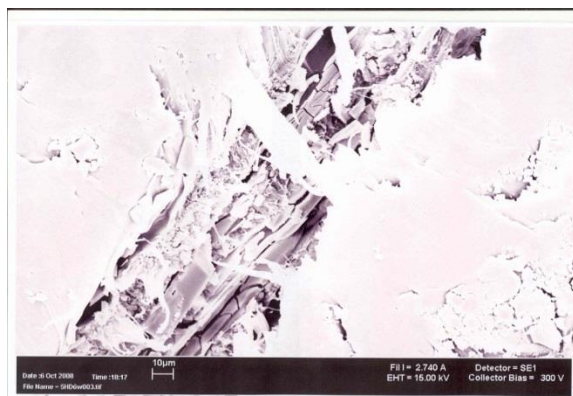
SEM images of the PCL/10Fe composite stored in water for up to 78 weeks can be seen in Figure 4-27.



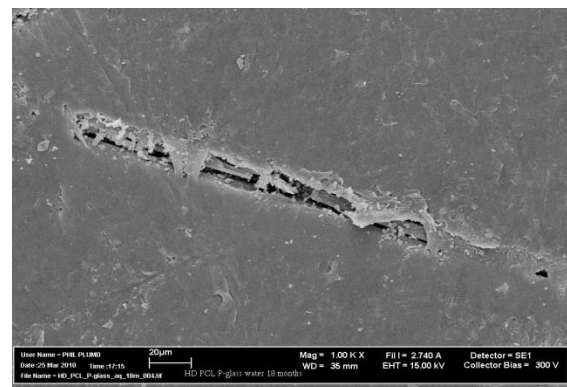
(a) Initial Top Surface (10 μ m scale bar)



(b) Initial Cut Surface (10 μ m scale bar)



(c) 26 Weeks (10 μ m scale bar), top surface



(d) 78 Weeks (20 μ m scale bar)

Figure 4-27 SEM pictures of PCL/10Fe Stored in water for up to 78 weeks

and could be snapped easily. The porosity appears to have weakened the structure of the composite.

4.9 Thermal Properties

The thermal properties of PCL and PCL/PBG composites were examined using DSC and TGA. A typical differential scanning calorimetry (DSC) thermograph can be seen in Figure 4-29. Two peaks were observed.

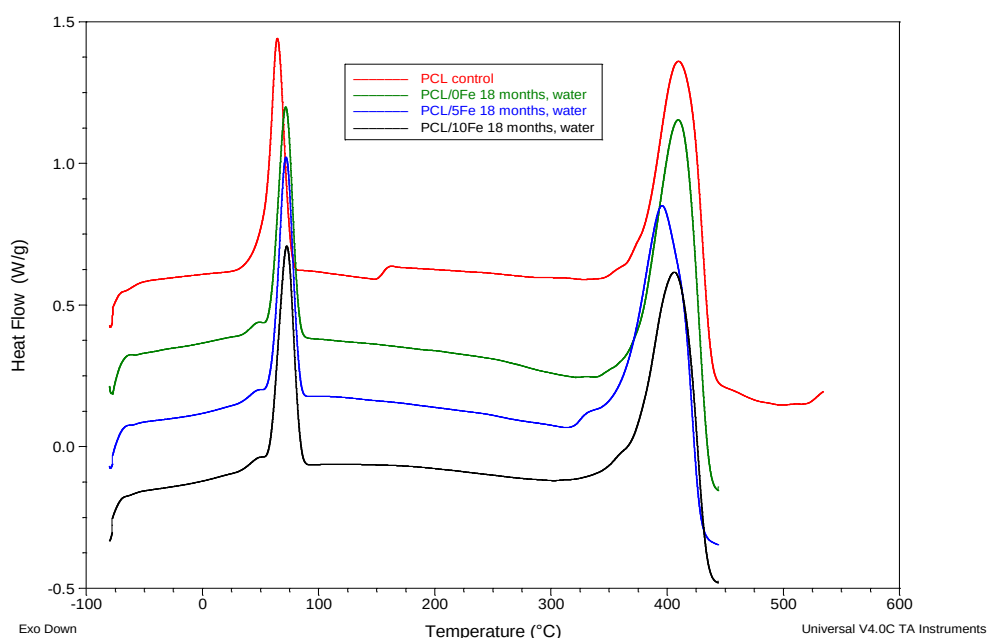
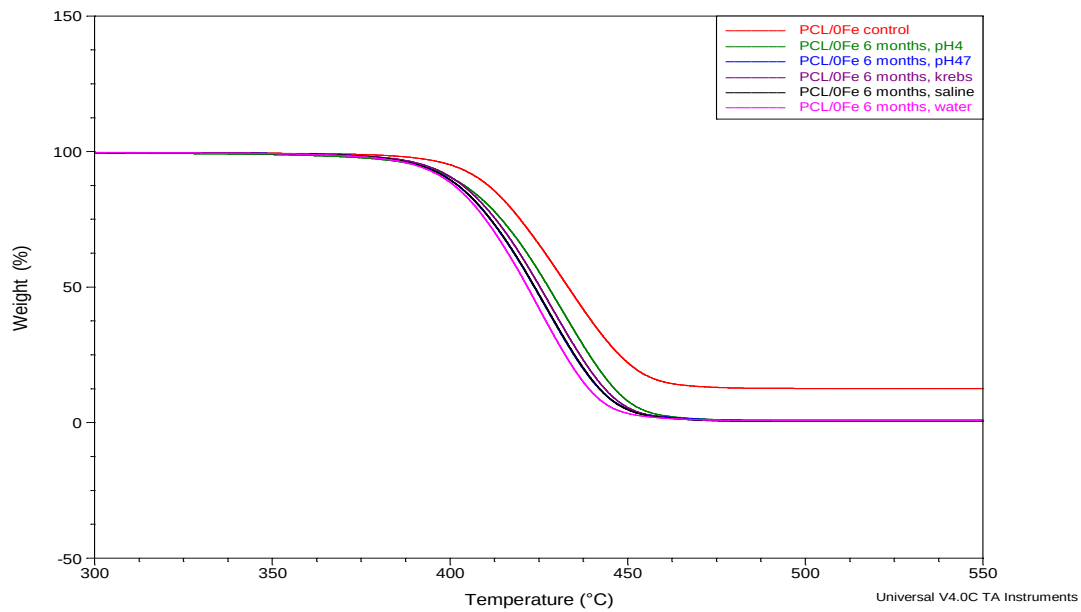
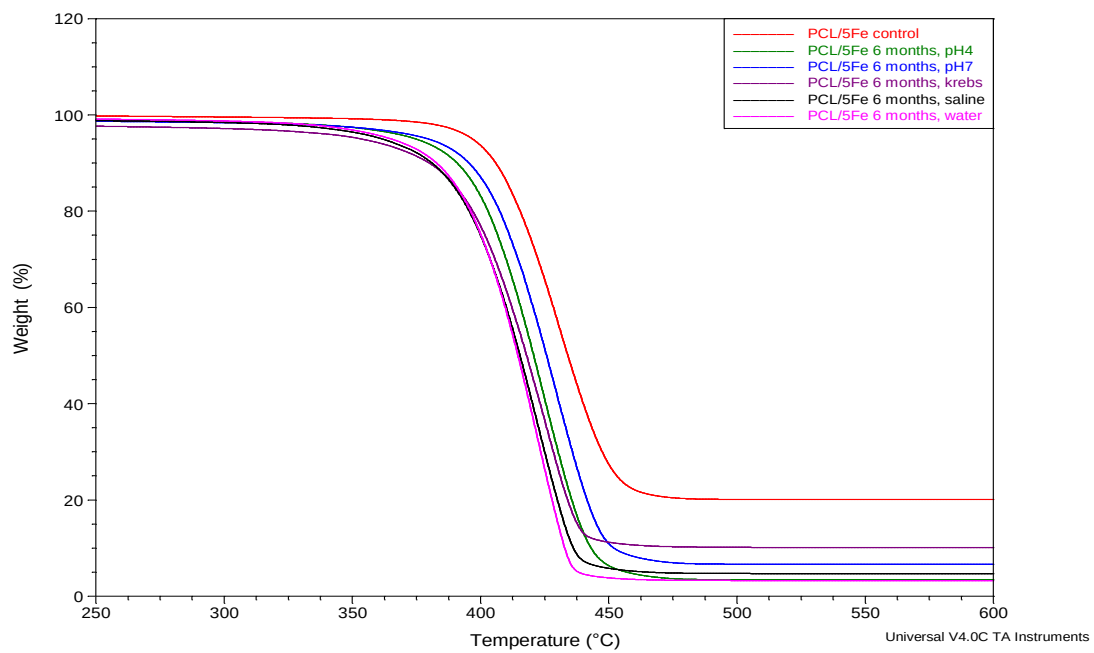


Figure 4-29 DSC thermograph of PCL and three PCL/PBG composites stored in water after 78 Weeks

The first peak is typically associated with the melting point of the crystalline phase of PCL and then second peak shows thermal degradation of the composite. This has been confirmed by Thermogravimetric analysis (TGA) which showed that there is a large decrease in weight after 400°C, Figure 4-30 (a) to (c). The addition of PBG caused an increased shift in the melting point of PCL, from 64.28°C to 70.36°C for the PCL/10Fe material, Figure 4-29. The PCL/5Fe and PCL/0Fe materials gave values of 69.04°C and 68.68°C respectively.



(a)



(b)

Figure 4-30 Weight loss (%) of (a) PCL/0Fe and (b) PCL/5Fe Composite in various aqueous storage mediums for 26 Weeks

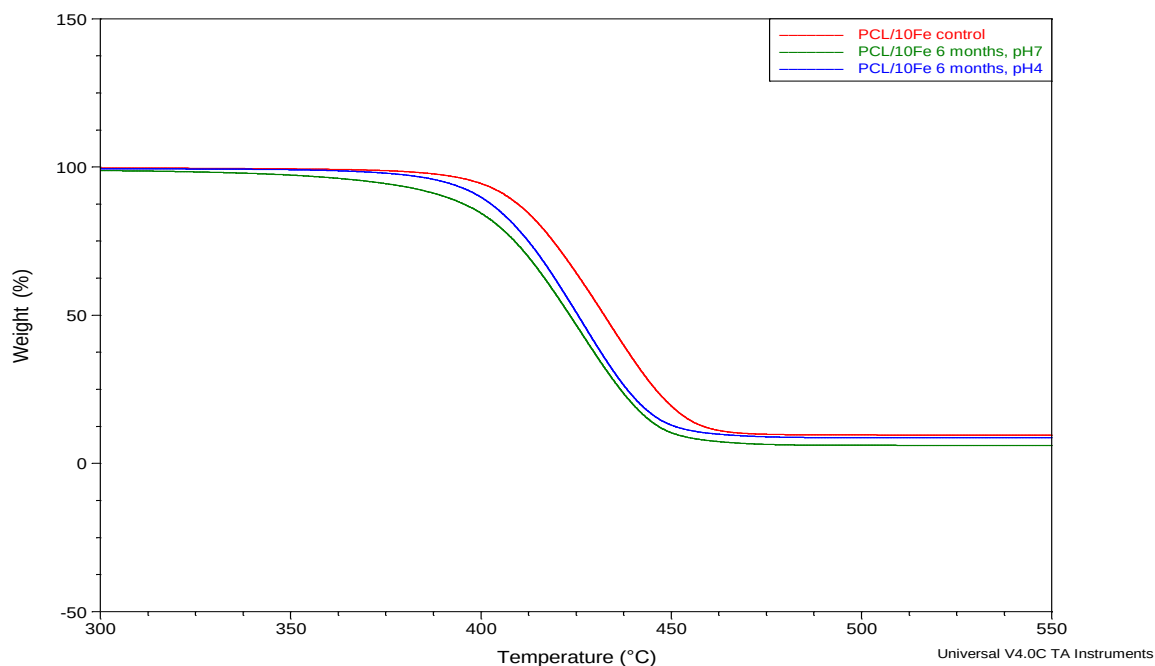


Figure 4-30 (c) Weight loss (%) of PCL/10Fe Composite in various aqueous storage mediums for 26 weeks

The results are documented up to 6 months. Due to presence of PBG within the samples the analysis pans were coated in glass. This could not be removed so further analysis was discouraged and therefore not performed. However, valuable data has been achieved for up to 6 months storage in aqueous media for all PCL/0Fe, PCL/5Fe and PCL/10Fe samples. All samples exhibited a higher weight loss during thermal decomposition after storage in the various aqueous media. The remaining weights are shown in Table 4-8. The data shows that after storage in aqueous media for up to 26 weeks the greatest weight loss is 0Fe > 5Fe > 10Fe this may be due to more glass being left in the 10Fe samples as the PCL burns off.

Description	Solution	Temperature where weight loss plateaus (°C)	Weight Remaining (%)
PCL/0Fe	Control sample	483.13	12.75
	pH4	475.26	1.125
	pH7	474.06	1.125
	Krebs	474.02	0.750
	Saline	476.29	0.750
	Water	469.48	1.125
PCL/5Fe	Control samples	481.83	20.250
	pH4	472.58	3.750
	pH7	471.78	7.125
	Krebs	463.34	10.500
	Saline	472.15	4.875
	Water	469.66	3.375
PCL/10Fe	Control samples	479.73	9.750
	pH4	477.14	6.375
	pH7	475.34	9.000

Table 4-8 Amount of composite remaining after TGA for PCL/0Fe, PCL/5Fe and PCL/10Fe and storage at 37°C in various media for up to 26 weeks

The melting temperature for PCL was 64.93°C, the degradation temperature 410.68°C and the Tg -61.9°C. Tg was calculated as the midpoint of the exothermic feature [an area of increasing heat flow (W/g)] during the heating cycle. The crystallinity is 56.49% using the following calculation [487]:

$$\text{Cr \%} = \Delta H_m (\text{J/g}) / \Delta H_m^0 (\text{J/g}) * 100\%$$

Where ΔH_m is the melting enthalpy for the composite samples, ΔH_m^0 is the melting enthalpy of the matrix when it infinitely crystallises. This is 139.5J/g for PCL.

The melting and degradation temperature, crystallinity and Tg for the PCL/PBG composites are detailed in Tables 4-9 to 4-11. The addition of PBG to PCL, as a composite, decreases the crystallinity and Tg, however, the melting and degradation temperature increases.

Description	Time Period	Solution	Melting Temp. (T _m [°C])	Degradation Temp (T _m [°C])	Glass Transition Temp. (T _g [°C])	Crystallinity (Cr[%])	ΔH (T _m) (J/g)	ΔH Degradation (J/g)
PCL/0Fe	N/A	N/A	69.04	414.91	-67.39	40.47	56.46	207.9
	1 week	pH4	66.08	410.35	-63.80	58.11	81.06	383.4
		pH7	65.79	407.88	-64.22	56.62	78.99	338.9
		krebs	66.08	410.35	-63.87	57.84	80.69	383.6
		saline	65.61	410.43	-63.11	60.52	84.43	376.9
		water	65.24	410.41	-63.78	58.18	81.16	346.3
	12 weeks	pH4	67.77	413.98	-61.19	67.25	93.82	431.4
		pH7	67.49	414.23	-63.75	58.28	81.30	387.2
		krebs	68.67	406.32	-63.26	59.99	83.68	382.3
		saline	67.79	412.72	-63.28	59.91	83.58	408.1
		water	68.24	414.25	-63.20	60.19	83.96	412.9
	26 weeks	pH4	65.56	386.52	-62.27	63.46	88.53	287.2
		pH7	64.86	386.95	-64.26	56.48	78.79	326.9
		krebs	65.98	387.23	-64.55	55.60	77.56	334.6
		saline	64.46	367.04	-64.59	55.35	77.21	376.6
		water	65.35	389.48	-64.15	56.86	79.32	373.5
	78 weeks	pH4	68.77	414.86	-61.85	64.91	90.55	260.4
		pH7	67.76	414.61	-67.31	45.82	63.92	204.7
		krebs	67.49	413.04	-65.31	52.82	73.68	284.9
		saline	68.72	411.97	-66.62	48.24	67.30	249.5
		water	67.79	410.60	-64.71	54.91	76.6	307.3

Table 4-9 Melting point, degradation temperature, glass transition temperature and crystallinity for PCL/0Fe composites after storage in aqueous media

Description	Time Period	Solution	Melting Temp. (T _m [°C])	Degradation Temp (T _m [°C])	Glass Transition Temp. (T _g [°C])	Crystallinity (Cr[%])	ΔH (T _m) (J/g)	ΔH Degradation (J/g)
PCL/5Fe	N/A	N/A	68.68	416.93	-69.61	40.34	56.28	187.3
	1 week	pH4	66.44	406.90	-65.46	52.28	72.93	372.6
		pH7	67.40	405.84	-65.45	52.32	72.98	358.1
		krebs	66.62	400.79	-66.43	48.89	68.20	391.9
		saline	67.70	413.78	-64.55	55.46	77.36	382.9
		water	68.34	412.98	-67.36	45.64	63.67	312.4
	12 weeks	pH4	68.24	410.33	-63.11	60.52	84.43	408.5
		pH7	67.84	407.70	-64.59	55.33	77.19	396.5
		krebs	66.97	397.56	-65.93	50.66	70.67	408.4
		saline	67.54	412.72	-64.81	54.57	76.12	400.3
		water	68.12	410.50	-65.73	51.35	71.64	361.5
	26 weeks	pH4	65.08	385.15	-61.68	65.53	91.41	396.1
		pH7	65.67	378.10	-65.77	51.21	71.44	368.0
		krebs	66.20	382.65	-65.16	53.35	74.43	424.9
		saline	64.30	367.04	-64.96	54.03	75.37	460.4
		water	64.86	379.67	-64.58	55.38	77.25	415.2
	78 weeks	pH4	68.51	412.45	-62.35	63.18	88.14	202.5
		pH7	68.23	392.38	-65.14	53.42	74.52	228.0
		krebs	65.65	409.19	-65.58	51.87	72.36	269.1
		saline	66.82	411.97	-68.56	41.45	57.82	224.1
		water	68.34	408.18	64.87	54.37	75.84	322.9

Table 4-10 Melting point, degradation temperature, glass transition temperature and crystallinity for PCL/5Fe composites after storage in aqueous media

Description	Time Period	Solution	Melting Temp. (T _m [°C])	Degradation Temp (T _m [°C])	Glass Transition Temp. (T _g [°C])	Crystallinity (Cr[%])	ΔH (T _m) (J/g)	ΔH Degradation (J/g)
PCL/10Fe	N/A	N/A	70.36	420.64	-69.89	39.76	55.46	112,5
	1 week	pH4	67.52	413.10	-65.94	50.61	70.60	278.6
		pH7	66.56	412.0	-66.79	47.63	66.45	224.6
		krebs	69.15	383.21	-66.59	48.33	67.42	322.2
		saline	68.57	412.40	-66.61	48.27	67.33	308.9
		water	68.53	414.50	-66.27	49.47	69.01	335.0
	12 weeks	pH4	66.08	411.42	-63.87	57.84	80.69	383.9
		pH7	67.49	414.19	-63.75	58.28	81.30	387.2
		krebs	66.45	407.10	-65.46	52.28	72.93	373.1
		saline	65.05	384.15	-61.66	65.56	91.40	395.9
		water	66.20	383.64	-65.16	53.35	74.43	424.7
	26 weeks	pH4	64.74	389.96	-62.08	64.11	89.44	468.7
		pH7	64.70	387.91	-66.12	49.97	69.71	333.6
		krebs	65.25	385.96	-66.41	48.97	68.31	350.8
		saline	65.17	389.12	-66.41	48.97	68.30	440.9
		water	62.78	379.47	-65.92	50.68	70.70	405.8
	78 weeks	pH4	69.13	412.22	-61.80	65.10	90.81	270.1
		pH7	67.43	411.44	-65.31	52.82	73.69	261.4
		krebs	66.12	409.21	-63.50	42.41	59.16	240.2
		saline	66.54	408.95	-68.28	42.41	59.17	260.4
		water	67.41	406.95	-64.51	55.60	77.56	291.6

Table 4-11 Melting point, degradation temperature, glass transition temperature and crystallinity for PCL1/0Fe composites after storage in aqueous media

There are limitations in the interpretation of the results. It is not possible to conduct statistical analysis due to only one sample analysed per composite, timepoint and aqueous solution. However, the data does show that over the 78 week time period the Tg increases and the amorphous content decreases in certain regions, after storage in aqueous media. The comparison is made to the original PCL/PBG composites before storage in aqueous media, Tables 4-9 to 4-11 and Figure 4-31(a) to (c).

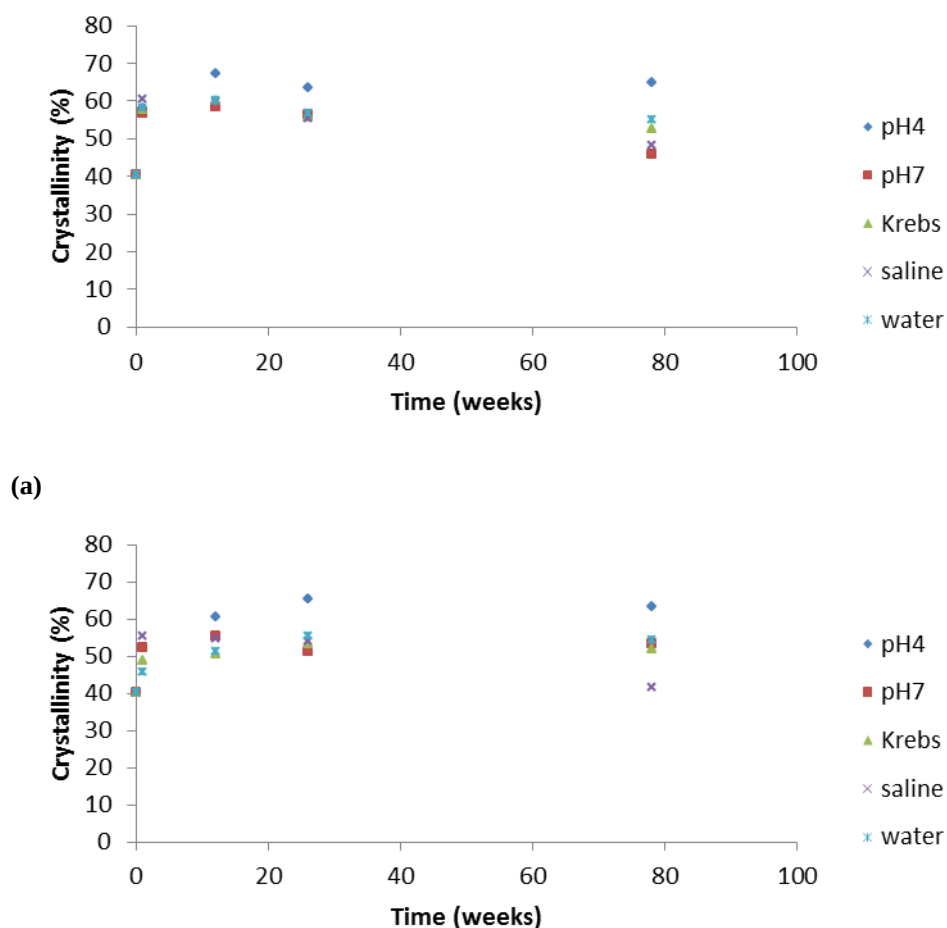


Figure 4-31Graph of crystallinity for (a) PCL/0Fe and (b) PCL/5Fe after 78 weeks storage at 37°C, in pH4, pH7, Krebs, saline and water

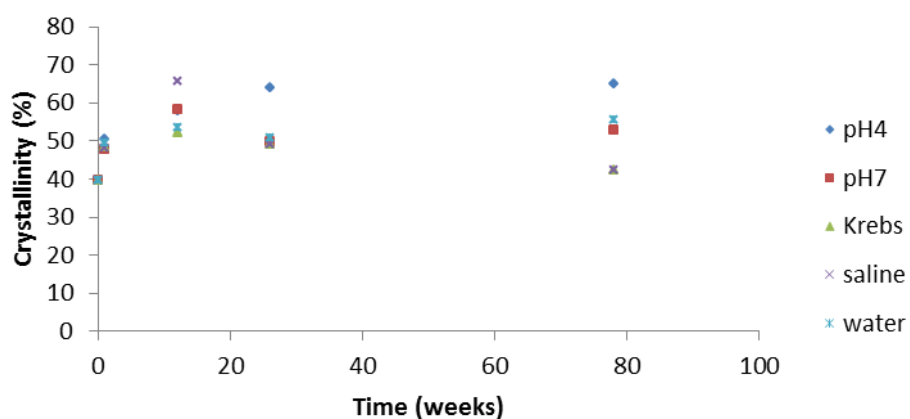


Figure 4-31(c) Graph of crystallinity for PCL/ 10Fe after 78 weeks storage at 37°C, in pH4, pH7, Krebs, saline and water

4.10 FTIR Analysis

4.10.1 PBG

The 0Fe, 5Fe and 10Fe PBGs FTIR spectra were compared, Figure 4-32.

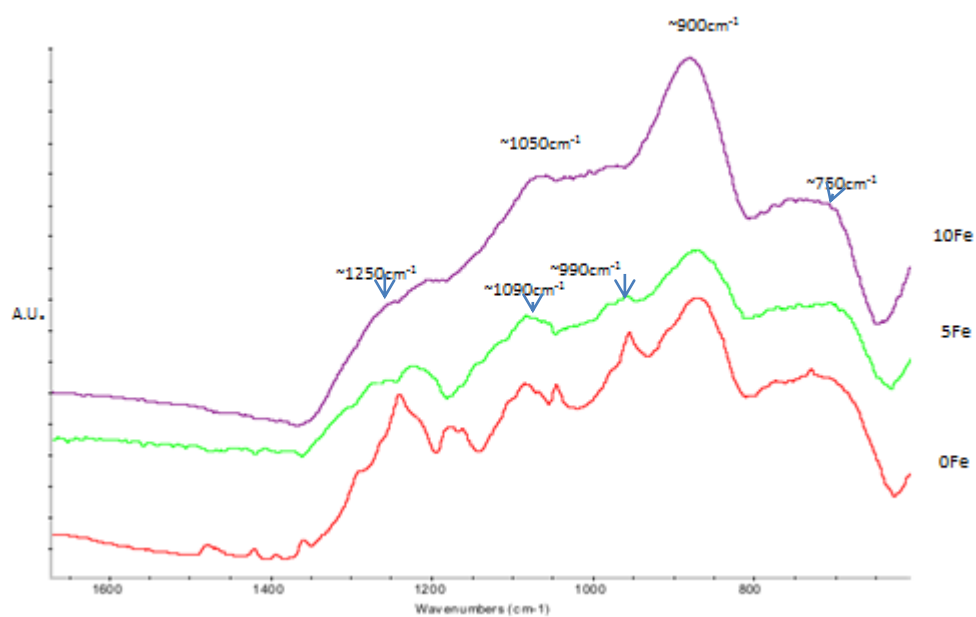


Figure 4-32 FTIR spectra for 0Fe, 5Fe and 10Fe PBG

Table 4-12 shows a comprehensive list of typical PBG absorption peaks associated with structural assignments. The main peaks for 0Fe, 5Fe and 10Fe PBGs from Figure 4-32 are also assigned.

Wavenumber (cm ⁻¹)	0Fe	5Fe	10Fe	Assignment	Reference
~457-477				Bending harmonics of O-P-O linkages	[285]
~517				Harmonics of $\delta(\text{P=O})$ modes	[285]
~596				Stretching of P-O-P mode	[258]
~640	631			$\delta(\text{O-P-O})$ units	[285]
~627, ~633, ~647				Bending harmonics of Fe-O bonds in FeO_4 units	
~688				$\nu_s(\text{P-O-P})$ in long chain of (Q^2 units)	[285]
~715				Stretching vibration of P-O-Ca	
~750-753				$\nu_s(\text{P-O-P})$ in Q^1 units	[260]
~776				$\nu_s(\text{P-O-P})$ in Q^1 units	[260]
~868-900	876	878	887	$\nu_{as}(\text{P-O-P})$ groups	[262]
~990-996				ν_s of NBOs in Q^0 tetrahedra	[261]
~1058	1048			Motion of NBOs (PO_3) in Q^1 tetrahedra	[261]
~1090	1084	1084	1070	Symmetric stretching vibration of O-P-O	
~1107				ν_s of PO_3 groups (Q^1 units)	[262]
~1167	1160 & 1179			$\nu_s(\text{P-O-P})$ of NBOs in Q^2 tetrahedra	[262]
~1250	1243	1226		Asymmetric stretching vibration of O-P-O	
~1262				$\nu_s(\text{P=O})$ in Q^2 tetrahedra	[285]
~1294	1296			$\nu_{as}(\text{P=O})$ modes	[241]
~1317-1334				Harmonics of $\nu_{as}(\text{P=O})$ modes	[286]
~1290, ~1361	1366			Stretching vibration of P=O	
~1663				H_2O bending vibration	[127]

Table 4-12 Frequencies and assigned bands for 0Fe, 5Fe and 10Fe PBGs [237, 240, 257, 403]

It can be seen that the peaks at $\sim 700\text{cm}^{-1}$ and $\sim 1110\text{cm}^{-1}$ are very broad. This can be explained due to the fact that they consist of two overlapping peaks due to the presence of 50% network modifiers. The 0Fe PBG exhibits peaks at $\sim 1366\text{cm}^{-1}$, $\sim 1296\text{cm}^{-1}$, $\sim 1243\text{cm}^{-1}$, $\sim 1179\text{cm}^{-1}$, $\sim 1160\text{cm}^{-1}$. However, the PBG containing 5%w/w Fe does not exhibit these defined regions. The bands at $\sim 1366\text{cm}^{-1}$, $\sim 1291\text{cm}^{-1}$, $\sim 1243\text{cm}^{-1}$ and $\sim 1179\text{cm}^{-1}$ have disappeared and a broad band between 1182cm^{-1} to 1360cm^{-1} is observed. This is likely to contain asymmetric stretching vibrational O-

P-O bonds, symmetrical vibrations of P=O in Q² tetrahedra and various stretching vibrations of the P=O bonds. For the 10%w/w containing iron PBG, the peaks are less visible, at ~1250cm⁻¹, ~1070cm⁻¹, with a gradual shift to lower wavelength, leaving a broad band from ~1351cm⁻¹ to ~1180cm⁻¹. There is also band broadening for the region 1180cm⁻¹ to 1046cm⁻¹ for both 10Fe and 5Fe PBG. This area encompasses the motion of non-bridging oxygens (NBOs), PO₃⁻, in Q¹ tetrahedra and PO₃⁻ Q¹ units, symmetric vibrational stretching of the O-P-O group and symmetrical vibrational of P-O-P bonds of NBOs in the Q² tetrahedra. The bands at ~1366cm⁻¹ and ~1291cm⁻¹ (in 0Fe) are characteristic of the stretching vibration of P=O and at ~1250cm⁻¹ to the asymmetric stretching vibration of O-P-O. The disappearance and merging of bands, in the regions described above occurs with increased iron addition. This is due to the breaking of the P=O bonds, the reduced force constant between P and O and the formation of more non bridging oxygen ions [240,410,483].

4.10.2 PCL/PBG Composite

The IR spectra for PCL, PCL/0Fe, PCL/5Fe and PCL/ 10Fe, before storage in aqueous media, can be seen in Figure 4-33.

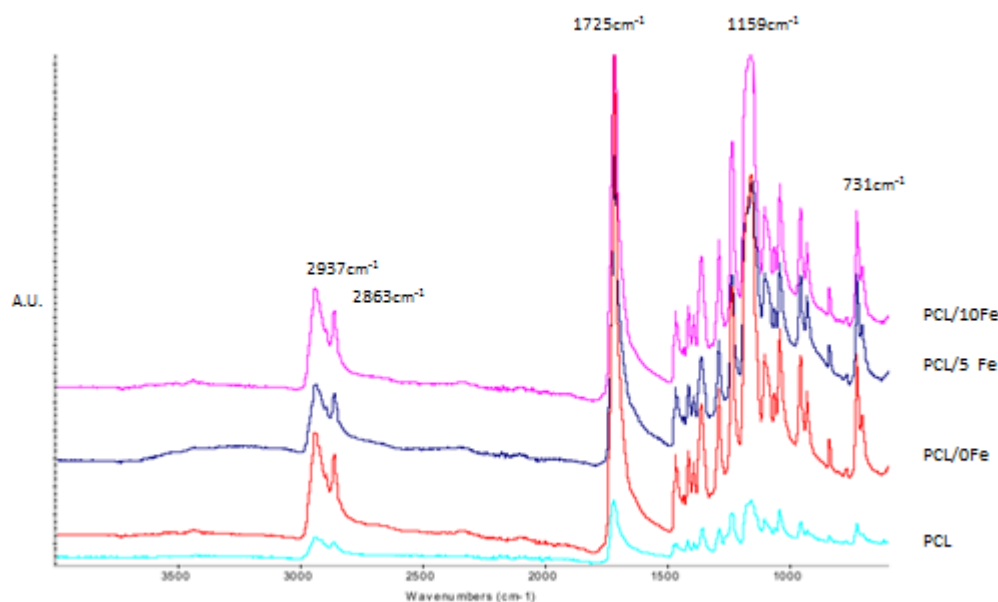


Figure 4-33 IR spectrum of PCL, PCL/0Fe, PCL/5Fe and PCL/10Fe before storage in aqueous media

A more detailed spectrum can be seen for peaks between $\sim 1470\text{cm}^{-1}$ and $\sim 700\text{cm}^{-1}$ in Figure 4-34.

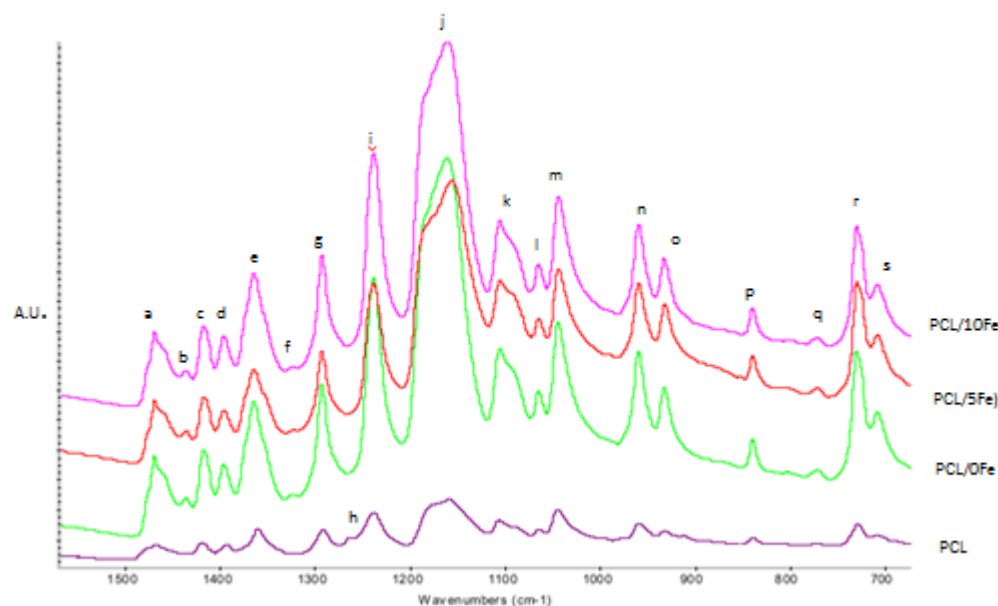


Figure 4-34 IR spectrum of PCL, PCL/0Fe, PCL/5Fe and PCL/10Fe before storage in aqueous media

Table 4-13 details the peaks observed for PCL and PCL/0Fe, PCL/5Fe and PCL10Fe (before storage in aqueous media) with key associated wavenumber and identification. Figure 4-35 details the peaks observed after storage in water.

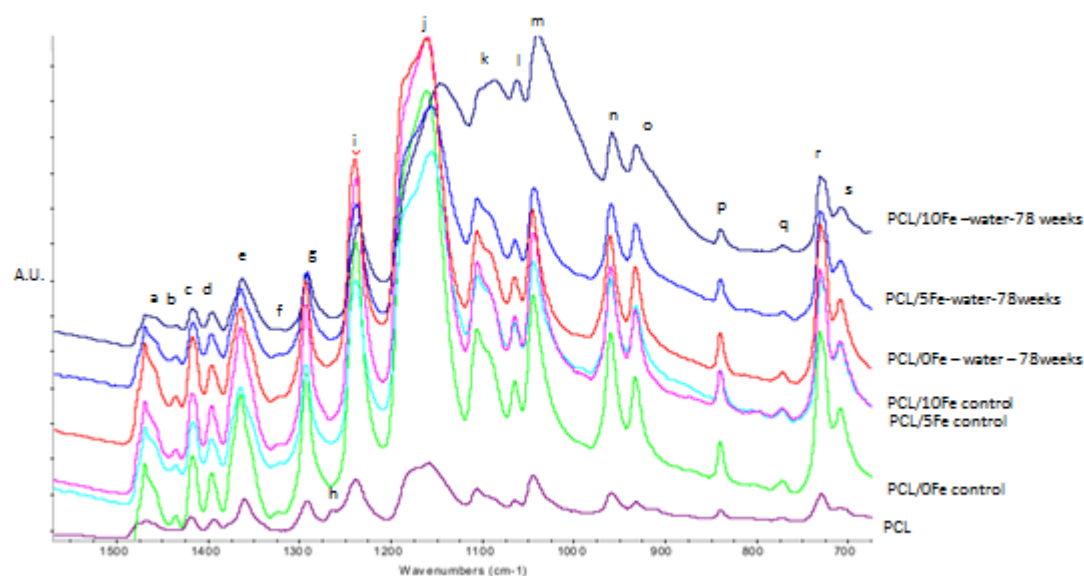


Figure 4-35 IR spectrum of PCL, PCL/0Fe, PCL/5Fe and PCL/10Fe, after storage in water

Table 4-13 details the structural vibrations assigned to the wavelengths observed for PCL, PCL/0Fe, PCL/ 5Fe and PCL/10Fe before storage in aqueous media (refer to Figure 4-34).

	Wavelength				Structural Vibration
	PCL	PCL/0Fe	PCL/5Fe	PCL/10Fe	
	2940	2940	2937	2940	CH ₂ symmetric vibration
	2914	2895	2895	2895	CH ₂ stretching region
	2866	2863	2863	2866	CH ₂ asymmetric vibration
	1722	1719			-C=O crystalline domain, ester vibration
a	1469	1470	1470	1470	(δ CH ₂)
b	-	1436	1436	1437	Symmetric stretching vibration of P=O
c	1420	1418	1417	1418	CH ₂ band vibration
d	1394	1396	1397	1398	CH ₂ band vibration
e	1360	1365	1366	1365	CH ₂ band vibration
f*	-	1325	1325	1325	Harmonics of vas(P=O) modes
g	1292	1294	1294	1294	(wCH ₂)
h	1266	-	-	-	CH ₂ band vibration
i	1240	1239	1239	1239	COO ester vibration
j	1158	1156	1163	1161	-C-O stretching
k	1108	1107	1107	1107	O-C vibration
l	1064	1065	1066	1065	γ -C-O stretch
m	1046	1046	1046	1045	O-C vibration
n	960	961	961	961	O-C vibration
o	931	933	934	934	CH ₂
p	840	840	840	840	CH ₂
q	773	773	772	773	CH ₂
r	730	732	732	733	CH ₂ rocking
s	706	709	709	709	CH ₂

Table 4-13 Frequencies and assigned bands for PCL/0Fe, PCL/5Fe and PCL/10Fe before storage in aqueous media

The results indicate that before composite storage in aqueous media, the PCL component of the composite is dominant, Figure 4-34. After 78 weeks storage in water, the resulting IR spectra for the majority of samples appeared to be similar to

the spectra before storage, Figure 4-35. Associated frequencies and assigned bands are provided in Table 4-14.

Detailed in Figure 4-35	Wavenumber (cm ⁻¹)		
	PCL/0Fe	PCL/5Fe	PCL/10Fe
a	1470	1470	1470
b	1436	1437	1435
c	1417	1418	1419
d	1397	1398	1398
e	1365	1365	1364
f	1325	1325	1325
g	1293	1293	1293
h	-	-	-
i	1237	1239	1239
j	1162	1156	1156
k	1107	1107	1104
l	1065	1064	1063
m	1045	1044	1041
n	960	959	959
o	932	932	932
p	841	840	840
q	772	772	772
r	731	730	729
s	709	709	709

Table 4-14 Frequencies and assigned bands for PCL/0Fe, PCL/5Fe and PCL/10Fe after storage in water

The FTIR technique used for examining the composites directly was not optimal for examining the PCL/PBG structure. This is partly due to the quantity of PCL within the composite (90%w/w) and high molecular weight (Mn42,500). The diamond tip on the Golden gate apparatus may not always have touched the glass fibre due to the smaller amount within the composite and distribution. An alternative or improved technique is to use KBr discs. This was not conducted because the samples were difficult to grind into a powder and it was not known if any PCL/PBG interactions would be compromised. If the experiments were to be repeated KBr discs would be produced. Although the majority of samples showed a PCL dominance in the spectra,

due to the number of samples analysed there were regions observed where PBG and PCL were visible, Figures 4-36 to 4-40.

Figure 4-36 shows a spectrum of PCL, 0Fe and PCL/0Fe stored for 1 month in pH4 buffer solution.

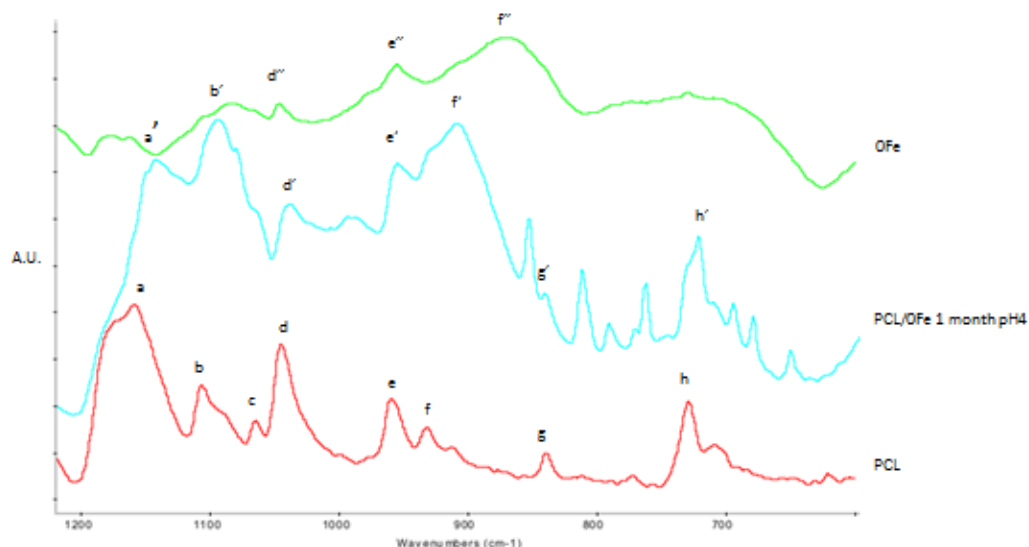


Figure 4-36 IR spectrum of PCL and 0Fe PBG before immersion in pH4 buffer and PCL/PBG, after storage in pH4 buffer for 1 month at 37°C

The IR spectrum of the PCL/0Fe composite (stored in pH4 buffer for 1 month), Figure 4-36, showed presence of PCL and PBG. Due to band broadening, there appears to be overlap in some regions of the PCL and PBG components of the composite matrix.

Comparison of the PCL control sample, 0Fe PBG control and PCL/0Fe was conducted. There is a shift for peak (a) (PCL) to a lower wavelength (from 1158cm^{-1} to 1142cm^{-1}). This peak is associated with -O-C stretching for the PCL component of the matrix. There is also a shift for peak (b) (associated with -O-C- vibration) to lower wavelength, from 1108cm^{-1} to 1094cm^{-1} . The shift is due to the presence of PBG which has a broad band over this region and associated peak at 1106cm^{-1} due to stretching of P-O. The γ -C-O stretch (c) is less defined and appears as a shoulder in the PCL/0Fe sample. Peaks (b) and (c) (PCL) start to merge into one broader peak for the PCL/0Fe composite. This is due to the contribution of the PBG element of the composite matrix. The intensity of d' and e' (PCL/0Fe), associated with -O-C vibration, have decreased and shifted to a lower wavelength due to the association

with asymmetric P-O-P groups linked to small metaphosphate rings. Peak f', associated with CH₂, shifts from 931cm⁻¹ to 908cm⁻¹ for the PCL/0Fe composite due to the symmetric bending mode of P-O-P groups linked with a linear metaphosphate chain (observed at ~870cm⁻¹). The original peaks observed for the PCL control sample (e to g) broaden in the PCL/0Fe sample due to the presence of PBG. The series of peaks (between g-h) are associated with the symmetric bending mode of P-O-P.

	Wavelength (cm ⁻¹)			Comments
	PCL	PCL/0Fe 1month pH4	0Fe	
a	1158	1142	-	-C-O stretching
b	1108	1094	1106	O-C vibration and ν_s of PO ₃ groups (Q ¹) units
c	1064	1065	-	γ -C-O stretch
d	1046	1039	1046	O-C vibration and asymmetric P-O-P groups linked to small metaphosphate rings
e	960	955	955	O-C vibration and asymmetric P-O-P groups linked to large metaphosphate rings associated with Q ² unit
f	931	908	870	CH ₂ and symmetric bending mode of P-O-P
g	840	840	-	CH ₂
h	730	721	733	CH ₂ rocking and stretching vibration of P-O-P associated with Q ¹ unit

Table 4-15 IR absorption bands from ~1200cm⁻¹ to ~600cm⁻¹, assigned to Figure 4-36 for PCL, PCL/0Fe stored for 1 month at 37°C in pH4 buffer and 0Fe PBG Fibres

One month and 6 months data for PCL/0Fe composites stored in pH7 buffer solution also show presence of PCL and PBG, Figure 4-37. Associated wavenumbers and assigned structural bands for Figure 4-37 are provided in Table 4-16.

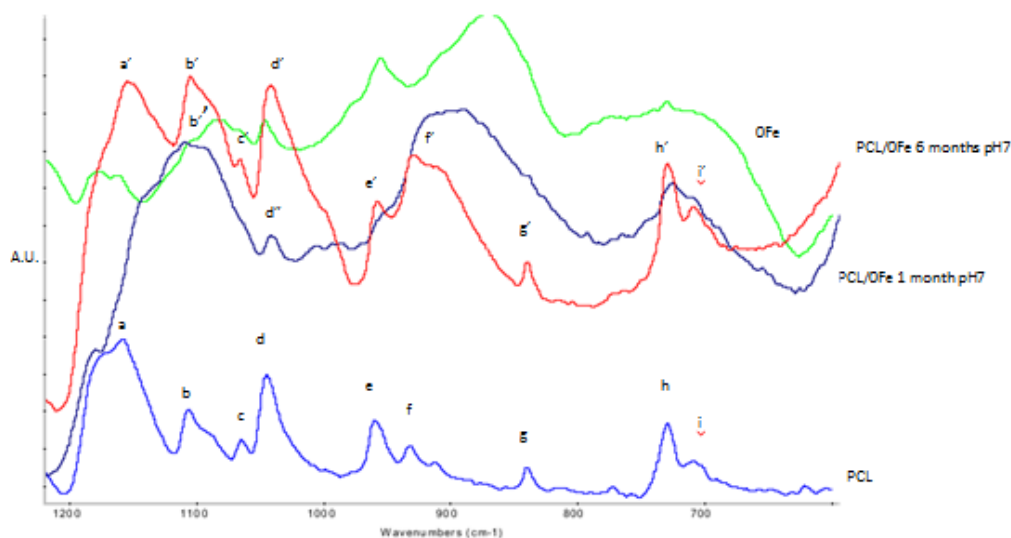


Figure 4-37 IR absorption bands from $\sim 1200\text{cm}^{-1}$ to $\sim 600\text{cm}^{-1}$ for PCL control sample, PCL/0Fe stored for 1 month and 6 months at 37°C in pH7 buffer and 0Fe PBG control sample

Of particular interest are the peaks normally present for PCL (between $\sim 1158\text{cm}^{-1}$ and 1109cm^{-1}) which converge into 1 peak. This is more indicative of the PBG structure which shows a band from 1142cm^{-1} to 1055cm^{-1} associated with the stretching vibration of P-O, non-bridging oxygens and stretching vibration of PO_2 groups. The peak at 1040cm^{-1} is still present which indicates the presence of the O-C vibration within PCL. One broad band is observed for the PCL/0Fe sample as opposed to a peak seen at 960cm^{-1} , 932cm^{-1} and 840cm^{-1} for PCL. The broad band from the PBG influence (870cm^{-1} to 906cm^{-1}) is likely to cause this behaviour due to P-O-P asymmetric stretching vibrations. The spectra for PCL/0Fe, stored for 6 months in pH7 buffer, shows some peaks associated with PBG but the peaks primarily resemble those of PCL. This is likely to be due to the leaching of PBG from the polymer matrix. There are slight shifts for peaks (a), (b) and (d) to a lower wavelength (from 1158cm^{-1} , 1108cm^{-1} and 1046cm^{-1} to 1154cm^{-1} , 1105cm^{-1} , 1042cm^{-1} respectively), due to the presence of $\text{vs}(\text{P-O-P})$ of NBOs in Q^2 tetrahedra, vs of PO_3 groups (Q^1 units) and motion of NBOs (PO_3) in Q^1 tetrahedra, Table 4-16. The region associated with CH_2 has broadened and the intensity increased due to the influence of asymmetric stretching vibration of P-O-P. The assigned peaks are similar to the PCL/0Fe samples stored in pH4 buffer solution only with absence of the series of peaks (between g-h) which were associated with the symmetric bending mode of P-O-P.

	Wavelength				Comments
	PCL	PCL/0Fe 1 month pH7	PCL/0Fe 6 months pH7	0Fe	
a	1158		1154		-C-O stretching
b	1108	1109	1105	1106	O-C vibration and ν_s of PO ₃ groups (Q ¹ units)
c	1064		1066		γ -C-O stretch
d	1046	1040	1042	1046	O-C vibration and asymmetric P-O-P groups linked to small metaphosphate rings (Q ²)
e	960	956	958	955	O-C vibration and asymmetric P-O-P groups linked to large metaphosphate rings (Q ²)
f	931	-	931	-	-
	-	-	-	870	CH ₂ and asymmetric bending mode of P-O-P
g	840	-	839	-	CH ₂
h	730*	726	730	733	CH ₂ rocking and stretching vibration of P-O-P
i	706	-	709	-	CH ₂

Table 4-16 IR absorption bands from ~1200cm⁻¹ to ~600cm⁻¹, assigned to Figure 4-37 for PCL, PCL/0Fe stored for 1 month and 6 months at 37°C in pH7 buffer and 0Fe PBG fibres

One month and 12 months data for PCL/5Fe composites in pH7 buffer solution showed PCL and PBG presence, Figure 4-38. The PCL/5Fe composite (stored for 1 month in pH7 buffer at 37°C) shows peaks similar to 5Fe PBG and after 12 months storage the composite starts to resemble PCL, with less distinguishable PBG peaks, due to leaching of PBG. Table 4-17 provides information on the wavenumbers and associated structural assignments, it illustrates peak shifts, band broadening and disappearance of peaks are due to the presence of PBG.

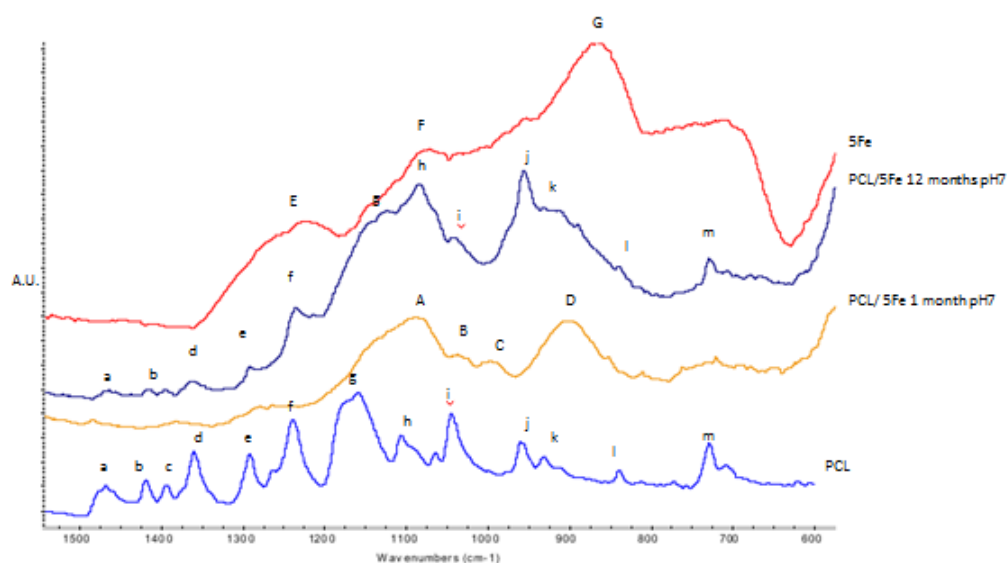


Figure 4-38 IR Absorption Bands from $\sim 1500\text{cm}^{-1}$ to $\sim 600\text{cm}^{-1}$ for PCL control sample, PCL/5Fe stored for 1 Month and 12 Months at 37°C in pH7 Buffer and 0Fe PBG Control Sample

	Wavelength			Comments	
	PCL	PCL/5Fe 1 month pH7	PCL/5Fe 12 months pH7	5Fe	
a	1469	1485*	1470	-	(δCH_2)
b	1420	-	1417	-	CH_2 band vibration
c	1394	1383*	1396	-	CH_2 band vibration
d	1360	-	1363	-	CH_2 band vibration
e	1292	1280*	1292	-	($w\text{CH}_2$)
f	1240	-	1235	1229(E)	COO ester vibration, asymmetric stretching of P-O-P groups linked to small metaphosphate rings
g	1158	-	1125	-	-C-O stretching
h	1108	1109 (A)	1083	-	O-C vibration
i	1046	1040 (B)	1042	1075(F)	O-C vibration, stretching vibration of P-O
j	960	956 (C)	957	-	O-C vibration, shoulder
k	931	-	930	-	CH_2
l	840	899(D)	940	868(G)	CH_2 rocking, asymmetric stretching vibration of P-O-P
m	730	-	726	-	CH_2

*Peaks are barely visible

Table 4-17 IR absorption bands from $\sim 1500\text{cm}^{-1}$ to $\sim 600\text{cm}^{-1}$, assigned to Figure 4-38 for PCL, PCL/5Fe stored for 1 month and 12 months at 37°C in pH4 buffer and 5Fe PBG fibres

Six months and 18 months data is presented for PCL/10Fe composites stored in pH7 buffer solution, Figure 4-39. The PCL/10Fe composite (stored for 6 month in pH7 buffer at 37°C) shows peaks similar to 5Fe PBG with some shifts to the lower wavelength due to the presence of PCL. The PCL/10Fe composite (stored for 18months in pH7 buffer) shows evidence of PCL and PBG. However, peaks presence and intensities associated with PCL are more obvious due to the leachables of PBG. Table 4-18 provides information on the wavenumbers and associated structural assignments, it illustrates peak shifts, band broadening and disappearance of peaks are due to the presence of PBG.

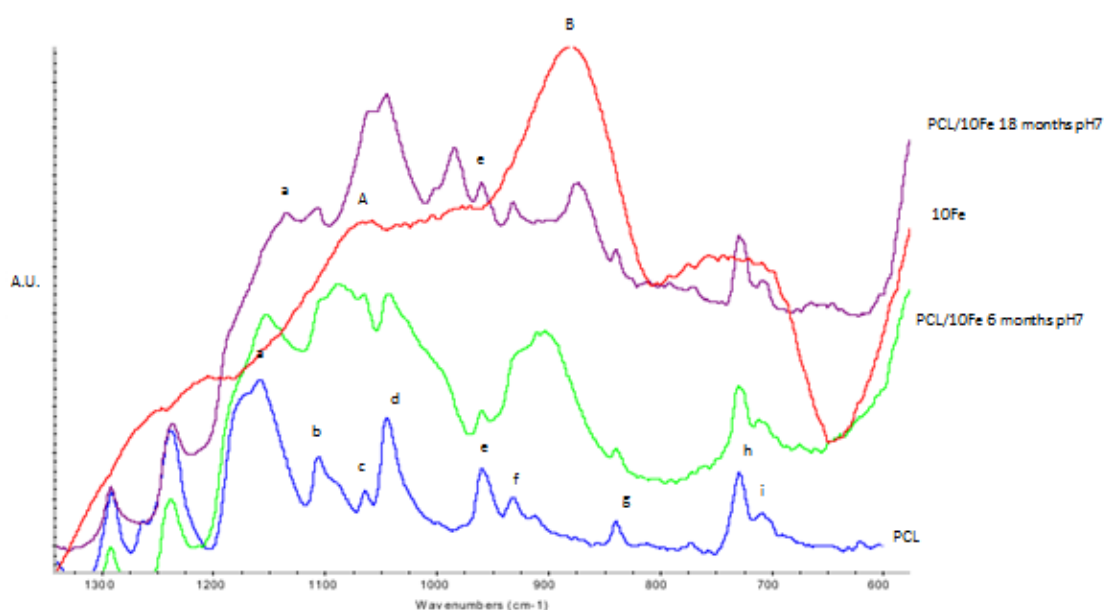


Figure 4-39 IR absorption bands from $\sim 1300\text{cm}^{-1}$ to $\sim 600\text{cm}^{-1}$ for PCL control sample, PCL/10Fe stored for 6 months and 18 months at 37°C in pH7 buffer and 10Fe PBG control sample

	Wavelength				Comments
	PCL	PCL/10Fe 6 month pH7	PCL/10Fe 18 months pH7	10Fe	
	1176	-	-	-	Doublet disappeared
a	1158	1154	1134	-	-C-O stretching
b	1108	1106	1108	-	O-C vibration
c	1089	1087	1083	-	γ -C-O stretch
	1064	1066	1062	1068	O-C vibration and Motion of NBOs (P0 ₃) in Q ¹ tetraheda
d	1046	1043	1046	-	O-C vibration
e	960	960	985	-	O-C vibration, shoulder
	-	-	960	-	ν_{as} (P-O-P) groups
f	931	929	931	-	-P-O-P symmetric bending mode
	-	-	875	880	Asymmetric stretching vibration of P-O-P groups
g	840	839	839		CH ₂
h	730*	730	729		CH ₂ rocking
i	706	710	708		CH ₂

Table 4-18 IR absorption bands from $\sim 1300\text{cm}^{-1}$ to $\sim 600\text{cm}^{-1}$, assigned to Figure 4-39 for PCL, PCL/10Fe stored for 6 months and 18 months at 37°C in pH4 buffer and 10Fe PBG fibres

The peak at $\sim 1725\text{cm}^{-1}$, Figure 4-40, is associated with vibration of a carbonyl bond ($\nu\text{C=O}$).

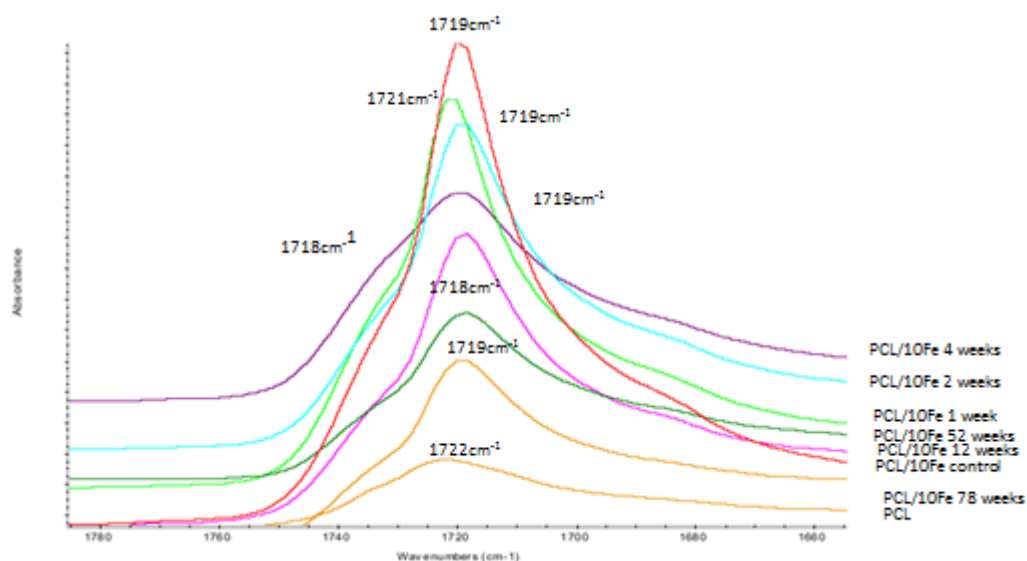


Figure 4-40 IR spectrum of PCL/10Fe after storage in aqueous media at 37°C for 18 months

1720cm⁻¹ has been reported to be a crystalline domain and associated with ester vibration [344]. It has also been reported that at 1718cm⁻¹ a well ordered crystalline band (νC=O) has been observed and at 1724cm⁻¹ an imperfect crystalline state is present. Hence, as the wavelength increases a more amorphous structure is observed. Figure 4-40 shows an IR spectrum of the wavelength region ~1720cm⁻¹ and Table 4-19 provides information on the shifts in wavelength for the PCL/PBGs after storage in aqueous media. PCL showed an absorbance for (νC=O) at 1722cm⁻¹. The addition of PBG within the PCL matrix shows a decrease in wavenumber for all composites (1719cm⁻¹, 1718cm⁻¹ and 1720cm⁻¹, PCL/0Fe, PCL/5Fe and PCL/10Fe respectively).

Time (weeks)	Wavelength (cm ⁻¹)				
	pH4	pH7	Krebs	Saline	Water
1	1718	1718	1718	1718	1719
2	1718	1717	1718	1719	1719
4	1720	1718	1718	1718	1718
12	1720	1718	1719	1719	1718
26	1720	1719	1723	1719	1719
52	1718	1718	1718	1720	1720
78	1723	1719	1718	1718	1718

PCL/0Fe control: 1719cm⁻¹ for (νC=O)

Table 4-19(a) PCL/0Fe wavelength for (νC=O) after storage for up to 78 Weeks, at 37°C, in aqueous media. All figures are within either -2cm⁻¹ or +1cm⁻¹ of the control. The two numbers highlighted in blue are within +4cm⁻¹ of the control.

Time (weeks)	Wavelength (cm ⁻¹)				
	pH4	pH7	Krebs	Saline	Water
1	1719	1721	1721	1719	1718
2	1720	1720	1719	1718	1719
4	1718	1719	1720	1718	1718
12	1718	1718	1718	1719	1719
26	1720	1724	1719	1719	1718
52	1720	1722	1718	1718	1718
78	1720	1719	1718	1720	1718

PCL/5Fe control: 1720cm⁻¹ for (νC=O)

Table 4-19(b) PCL/5Fe wavelength for (νC=O) after storage for up to 78 weeks, at 37°C, in aqueous media. All figures are within either $\pm 2\text{cm}^{-1}$. The number highlighted in blue is within $+4\text{cm}^{-1}$ of the control.

Time (weeks)	Wavelength (cm ⁻¹)				
	pH4	pH7	Krebs	Saline	Water
1	1721	1719	1719	1719	1721
2	1719	1718	1719	1718	1719
4	1718	1720	1719	1719	1719
12	1719	1719	1719	1719	1718
26	1718	1720	1720	1719	1720
52	1718	1720	1720	1716	1718
78	1721	1719	1718	1721	1719

PCL/10Fe control: 1720cm⁻¹ for (νC=O)

Table 4-19(c) PCL/10Fe wavelength for (νC=O) after storage for up to 78 weeks, at 37°C, in aqueous media. All figures are within either -2cm^{-1} or $+1\text{cm}^{-1}$ of the control. The number highlighted in blue is within -4cm^{-1} of the control.

Minimal shifts in wavenumber are observed after PCL/PBG composite storage in aqueous media. The composites are composed of crystalline and amorphous regions. The resolution used for analysis was 4cm^{-1} and the majority of the data sets (from 1 week to 78 weeks storage in aqueous media) are within 1718cm^{-1} to 1722cm^{-1} . However, 3 results (highlighted in blue) are higher than this range (1723cm^{-1} and 1724cm^{-1}) and one below (1716cm^{-1}) indicating a slight tendency towards a more crystalline and an amorphous state respectively. After 1 month storage there is a

general tendency for peak broadening in the carbonyl vibrational region which is due to strong hydrogen bonding.

CHAPTER 5 – DISCUSSION

5.1 Introduction

The aim of this project was to develop a biodegradable composite for bone implantation applications due to the problems associated with metals, certain polymers [23, 25-27] and a limited supply of human bone (which also has its own associated risks) [12-17]. Particular focus was aimed at extractables and leachables from the composites, after storage in different aqueous media. Any species which could extract from a material or leach within the body needs to be studied to determine whether the rate of release of that species will pose a toxicological risk.

PCL/PBG composites were chosen for the study. PCL has a longer degradation rate, than other polyesters such as PLA and PGA, so is more attractive matrix for these studies. PCL is also FDA approved and the PBG is composed of ions (phosphate, calcium and sodium) already found in bone. Various aspects of the materials chosen can be tailored in order to alter degradation rates. For this study the addition of iron within the PBG structure improves durability, and an objective was to investigate whether this also affects the species which are extracted or leached from the composites.

5.2 Leachables from PBG

Leachables from the PBG were initially studied in order to determine whether any one of the three PBG materials was more durable. The durability was described as the resistance of the PBG to attack by a specified reagent. The study was conducted over 216 hours for degradation comparison with current literature. As far as the author is aware, the particular compositions studied in this thesis:

- i. $50\text{P}_2\text{O}_5\text{-}35\text{CaO-}5\text{Na}_2\text{O-}10\text{Fe}_2\text{O}_3$
- ii. $50\text{P}_2\text{O}_5\text{-}40\text{CaO-}5\text{Na}_2\text{O-}5\text{Fe}_2\text{O}_3$

iii. 50P₂O₅-45CaO-5Na₂O

have not been documented in the literature. PBG with calcium and sodium [74, 121, 401, 406-409, 419, 420, 448] have been studied and also calcium, sodium and iron, but with different compositions to those used for this MPhil study [414, 68]. In the literature, the presence of iron increased the PBG durability by two orders of magnitude. The results for the PBG leachables experiment (Figure 4-1), showed an increased rate of release of over one order of magnitude for the 10Fe PBG material compared to 0Fe and 5Fe PBG. The order of leaching was phosphate >calcium> sodium > iron. This was expected due to the quantities of the species within the PBG structure. The concentration of the leached species, tended to plateau after 75 hours which was similar to the results from Abou Neel [414].

It was noted that the PBG leachables results (section 4.1) were an order of magnitude greater than the literature. Two methods for ion detection are detailed in the literature [40, 236, 290, 414]. These are ion chromatography and ICP. The studies, documented in this thesis, used the ICP method due to availability, ease of use and ability to detect at low levels. The two techniques have similar detection limits. IC can detect: 0.3ppm Na⁺, 1.1ppm Ca²⁺ and 0.012ppm Fe³⁺ (Dionex ICS -3000, conductivity detection [454]), ICP and can detect 0.5ppm Na⁺, 0.05ppm Ca²⁺, 0.1ppm Fe³⁺ and Phosphate 4ppm [452]. Both methods are commonly used in industry. Several factors can contribute to different amounts of ions released. These include % w/w of the components used to manufacture the PBG, manufacturing techniques, history of the glass, has it undergone heat treatment or different pulling speeds and the dimensions. The results in section 4.1 were also an order of magnitude greater than the results reported in section 4.3 (extractables from PCL/PBG). Within this section, the theoretical %w/w of PBG lost due to ion release is calculated. The extractables were conducted on composites which have approximately 10 times less %w/w PBG present per gram of material (due to 90:10 ratio PCL/PBG). Hence, the amount of ions leached from the PBG alone is expected to be ten times more than the composite. This is confirmed by the extractables and leachables data and also seen to be in line with the theoretical calculations of the amount of glass present.

The 10Fe PBG showed a weight loss which was a third less for the 10Fe material compared to 0Fe and 5Fe PBG. This confirms the higher durability and slower

dissolution rate of the 10Fe material. Appendix B details weight losses and degradation rate found in the literature for various PBG compositions. Table 5-1, however, shows the degradation rate of the PBGs studied in this thesis compared to similar PBG compositions (in common units, %mm⁻²h⁻¹).

Composition	Fibre Diameter	Degradation Rate (%mm ⁻² h ⁻¹)	Reference
50P45Ca5Na	61µm	4.14	Table 4-1
50P40Ca5Na5Fe		3.52	
50P35Ca5Na10Fe		1.23	
50P30Ca20Na	32µm	11.04	[414]
50P30Ca19Na1Fe		1.94	
50P30Ca18Na2Fe		1.41	
50P30Ca17Na3Fe		0.49	
50P30Ca16Na4Fe		0.23	
50P30Ca15Na5Fe		0.17	
50P30Ca20Na	13µm	32.29	
50P30Ca19Na1Fe		3.84	
50P30Ca18Na2Fe		2.73	
50P30Ca17Na3Fe		0.75	
50P30Ca16Na4Fe		0.44	
50P30Ca15Na5Fe		0.21	
50P46Ca6Na	30µm	98.21	[68]
50P46Ca3Na1Fe		72.92	
50P46Ca2Na2Fe		7.44	
50P46Ca1Na3Fe		4.46	

Table 5-1 Degradation rate for various phosphate glasses stored in H₂O at 37°C

Table 5-1 also confirms that the addition of iron reduces PBG weight loss however, the weight loss results are not linear due to the complexity of the interactions between the different amount of species within the PBG structure. As the iron content increases the PBG durability increases due to the formation of more hydration resistance P-O-Fe bonds. 10%w/w material is more durable than 0Fe and 5Fe. The

Fe^{3+} can be present in both tetrahedral and octahedral co-ordination thus acting as a network former within the P-O-P backbone [413,423]. Vitreous phosphate glasses can be classified as polymerised 3 dimensional networks that are composed entirely of chains/ rings of PO_4 tetrahedra, which can be attached to a maximum of three neighbouring tetrahedra. The addition of modifying oxides will lead to the depolymerisation of the network resulting in a cleavage of the P-O-P bonds and creating non-bridging oxygen in the glass. However, the addition of Ca^{2+} ions provide ionic cross-linking between the non-bridging oxygen of two different chains. Increasing the bond strength of this ionic link can also account for the enhancement of the glass durability. Appendix B shows PBG structures containing titanium and gallium increase the PBG durability by creating polyhedra between adjacent phosphate chains in the glass network. This is due to smaller ionic radii (0.61 Å) than Na^+ and Ca^{2+} (1.02 Å and 1.0 Å) a smaller combined charge for Na^+ and Ca^{2+} [434, 449]. Strontium however, tends to lower durability due to a larger ionic radius compared to other ions (1.18 Å) hence, perturbing the atomic arrangement of the glass network and weakening the structure.

The results, in Table 5-1, also show that the fibre diameter has a considerable impact on the durability. Bitar *et al.*, [68] reported 50% weight loss from 50P46Ca6Na PBG (30µm diameter) whereas 50P45Ca5Na (61µm diameter) showed only a 10% decrease in weight.

Three PCL/ PBG composites were fabricated from the PBG fibres, Section 3.1, using a compression moulding technique. The PCL was removed by combustion based on a standard test method (ASTM 2584 – 94, Method for Ignition Loss of Cured Reinforced Resins). The test confirmed that the PCL/ PBG ratio was approximately 90:10.

5.3 Extractables and Leachables

Extractables must be analysed from the composite material and leachables over the entire shelf life of the product [43-45, 266-190]. Extractables testing (via ultrasonication for 48 hours) was conducted, as an accelerated study, in water, pH4

and pH9 buffer solutions. The results were analysed by ICP-OES. 48 hours was chosen as a suitable time for the amount of ions extracted to reach a plateau. There was some variation in the amount of ions extracted depending on the extraction medium. However, due to only one sample being used per extractable medium there are limitations in the interpretation of the data and statistical analysis is not possible. It was not clear whether acidic, basic or neutral conditions favoured the extraction of a particular ion. From the graphical data, Figures 4-5 to 4-8, it can be seen that PCL/10Fe produced a lower amount of extracted ions, during the 48 hour extraction time, compared to PCL/0Fe and PCL/5Fe. Calcium was an order of magnitude less, sodium was a third less, iron and phosphate approximately half. The reasons for the higher glass durability have already been discussed in this chapter. The results have been converted to estimated, or theoretical, amounts of ions expected, relative to the weight loss, in order to compare the composites directly. Table 4-3 shows the percentages of ions extracted from the PCL matrix. The highest amount is for PCL/0Fe, then PCL/5Fe and least observed for PCL/10Fe. The absence of PBG fibres within the polymer matrix is also confirmed by SEM images of the composites, discussed later in this chapter. The degradation rate, Figures 4-5 to 4-, is within ~24 hours for sodium, calcium and iron but quicker for phosphate, approximately 18 hours. The order of extracting was $P > Na > Ca > Fe$. Phosphate could be first due to breakage of the phosphate backbone within the PBG. Calcium is divalent and forms chelate cross links which decreases the dissolution rate compared to sodium and Fe-O-P bonds are more resistant to hydration than P-O-P bonds [69, 414, 429, 431, 432]. The results reported in Table 4-3 concur with the literature.

Current literature documents storage of PCL/PBG composites in deionised water and phosphate buffer for up to 31 days [40, 72-74, 236]. Leachables testing was conducted on the PBG component, of the three composite materials, after storage in pH4 and pH7 buffers, krebs, water and saline for 78 weeks at 37°C, then analysed by ICP-OES, section 4.4. The results can be found in Figure 4-9 to Figure 4-12. The total amount of leachables observed increased over time. Similarly to the extractables, there was some variation in the amount of ions extracted depending on the extraction medium. However, due to only one sample being used per extractable medium (n=1)

there are limitations in the interpretation of the data and statistical analysis is not possible. It was not clear whether a particular aqueous medium had a propensity for a specific leached ion. For a more meaningful statistical analysis a sample size of 10 would be preferential. The leachables final concentrations from the composites are an order of magnitude less than the leachables results from the glass. The amount of PBG in the composites is an order of magnitude less than the glass alone (90:10, PCL/PBG). This indicates that as the PBG is dissolving from the polymer matrix, channels are being created within the polymer matrix allowing access of more water to dissolve the PBG fibres. The order of leaching is the same as for the PBG leachables (P>Na>Ca>Fe) and this has been explained due to the amount of the oxides originally present in the PBG structure and breakage of the phosphate backbone within the PBG, divalent calcium ions forming chelate cross links which decreases the dissolution rate compared to sodium and Fe-O-P bonds being more resistant to hydration than P-O-P bonds [69, 414, 429, 431, 432]. The PCL/10Fe composite is leaching approximately half the ions of the PCL/0Fe samples and the PCL/5Fe lies between the two.

The amount of leached ions from the PCL/PBG composites was compared to the literature, Table 5-2. The quickest rate of ion release was up to 12 weeks for PCL/0Fe, PCL5Fe and PCL/ 10Fe, section 4.4.

Composition	Reference	Na (ppm)	Ca (ppm)	Fe (ppm)	P (ppm)
PCL/45P24Ca31Na PCL/45P28Ca27Na PCL/45P32Ca23Na PCL/45P36Ca19Na	[74]	150-400	90-150	-	130-330
PCL/50P40Ca9Na1Fe PCL/50P40Ca7Na3Fe PCL/50P40Ca5Na5Fe	[72]	140-280	36-71	N/R	403-791
PCL/50P40Ca10Fe	[236]	N/R	20	10	20*
PCL/50P45Ca5Na0Fe	-	280-340	620-2310	0-4	2870-4600
PCL/50P40Ca5Na5Fe	-	400-450	340-2100	0-70	2820-4040
PCL/50P35Ca5Na10Fe	-	320-360	310-1100	0-40	1000-3710

N/R: Not reported. * Unable to provide an accurate value due to some phosphate ions not being monitored.

Table 5-2 Summary of range in total leachables results from the literature and thesis

It is not possible to make a direct comparison of the composites reported in Table 5-2 due to several factors which will influence the amount of ion leaching. However, the results from section 4.4 are slightly higher than the literature but within the same order of magnitude for the range of sodium, calcium and iron reported. The amount of phosphate ions leached for PCL/0Fe, PCL/5Fe and PCL/10Fe is higher but within the same order of magnitude as reference [72]. The reasons for the differences can be due to the different glass structures which will have different chemical durability. This will stem from different amounts of phosphate, the longer the chain the slower the degradation rate, the presence of iron which has a more resistant bond to hydration than P-O-P [414] and calcium chelate cross linking, which will also lower degradation rate. Prabhakar et al [74], studied $5PxCa(0.55-x)Na$ [74], Ahmed et al [52] investigated $50P40Ca5Na5Fe$, Alani *et al.*, looked at $50P40Ca9Na1Fe$, $50P40Ca7Na3Fe$ and $50P40Ca5Na5Fe$ [72], and Mohammadi et al [236] studied $50P40Ca10Fe$. The amount of PCL in the composites also differed with PCL/PBG ratios of 80:20 [74,72]. The ratio was not provided for the $50P40CaFe$ PBG containing composite [236]. The composite produced by Alani *et al.*, [52] utilised PLA instead of PCL, the ratio was 75:25 [52]. The molecular weight (M_w) of the PLA ranged from 90,000- 120,000 [52] and PCL was M_w 65,000 [236]. The M_w was not provided by the other authors [72, 74]. It has been well documented that the greater the M_w (e.g. 65,000) the less likely a polyester is to degrade. Biodegradation only takes place during the later stage involving a low molecular weight ($M_n < 5,000$) [434]. The manufacturing of the composites was also not harmonised. Two techniques were used. These were solvent dispersion with the PCL and PBG to form thin films, then compression conducted with several films to form a composite [72, 74] and pressed film for the polymer alone which was then compression moulded with PBG matts at 38 bar compression (100°C for the PCL composite and 210°C for the PLA composite), [52, 236]. The surface area of the polymer composites are also not equivalent and it has been reported that the higher the surface area the faster that degradation will occur. The dimensions were 24 x 3 x 0.5mm [72, 74] and 32 x 15x 1.6mm [52]. The composites used in section 4.4 were similar to 10 x 10 x 1.6mm [236] as their dimensions were approximately 10 x 10 x 1.5mm. However, the PBG for the 10 x 10x 10.6mm [236] were annealed to reduce the process induced stress in

order to improve durability. The results of leached calcium and iron have reduced so this appears to have been beneficial.

The total leached amounts of each species over the 78 week period, Table 5-2, were within toxicological safety limits. Calcium oxide was observed at a maximum of 1310 ppm or 1.310 mg/L over the duration of 78 weeks. The normal blood concentration range for calcium (ionized form) as cited by the National Institute of Health is 60 - 140 mg/L (2.05 - 2.5 mmol/L). Based on these values and assuming complete absorption, the maximal amount of additional calcium contributed by the PCL/PBG composite would be <2% of the normal human blood concentration. No adverse effect is expected from the addition of this limited amount of calcium. Sodium was observed at a maximum of 450 ppm or 0.45 mg/L over the duration of 78 weeks. The normal blood concentration range for sodium as cited by the National Institute of Health is 2760 - 3560 mg/L (135 - 144 mmol/L). Based on these values and assuming complete absorption, the maximal amount of additional sodium contributed by the PCL/PBG composite would be <0.02% of the normal human blood concentration. No adverse effect is expected from the addition of this minimal amount of sodium. Iron was observed at a maximum of 70 ppm or 0.07 mg/L over the duration of 78 weeks. The normal blood concentration range for iron as cited by the National Institute of Health is 0.400 - 1.55 mg/L (40 - 155 ug/dL). Based on these values and assuming complete absorption, the maximal amount of additional iron contributed to the blood by the PCL/PBG composite would be <18% of the normal human blood concentration. No adverse effect is expected from the addition of this amount of iron. Phosphorus was observed at a maximum of 4600 ppm or 4.6 mg/L over the duration of 78 weeks. The normal blood concentration range for phosphorus as cited by the National Institute of Health is 25 - 48 mg/L (2.5 - 4.8 mg/dL). Based on these values and assuming complete absorption, the maximal amount of additional phosphorous contributed by the PCL/PBG composite would be approximately 18% of the normal human blood concentration. No adverse effect is expected from the addition of this limited amount of phosphorous.

The leachables data for the PCL/PBG materials correlates with the weight loss data, section 4.6 which shows that the degradation order of the materials are PCL/0Fe> PCL/5Fe> PCL/10Fe >PCL. The average final weight loss, taken across the 5

aqueous media, corresponds to weight losses in the range of 11%w/w, 8%w/w, 5%w/w and 2.96%w/w respectively. PCL undergoes bulk degradation, via an initial hydrolysis mechanism [219, 313] this is due to the hydrophobic nature and high crystallinity of the polymer, the process is also autocatalysed [80]. The rate of degradation is determined by the initial molecular weight of the polymer [320]. Gel permeation chromatography (GPC) is a key technique for investigating the change in molecular weight of the PCL after storage in the aqueous media. As this was not conducted during analysis it is not possible to conclude how much composite degradation is attributed to the PCL this would need to be addressed in further work.

The weight loss of the composites results, in section 4.6, was compared to the literature (Appendix B, Table B4 and Table B5). The studies ranged from 2 hours in de-ionised water with a weight loss of 27% w/w [73] to the longest studies conducted over 60days with a 0.83% weight decrease. As with the leaching of ions data, it is difficult to compare the weight losses across composites directly for reasons already discussed. The set of data in Table B4-B5 contains composites with different PBG structures, the ratio of PCL/ PBG ranges from 60:40 [41], 82:18 to 94:6 [73], 80:20 [74] and 90:10 [456]. The PCL/PBG which only showed 0.83% weight loss in 60 days contained 90%w/w PCL with a phosphate, sodium, magnesium, calcium and iron based glass [456]. The low weight loss indicates that this particular PBG is highly durable, more so than the PCL/PBG composites reported in section 4.6. In some cases there has been evidence of a weight increase within the literature [74, 236] which was also observed during weight loss analysis, section 4.6. This was explained by 2 mechanisms. The first involved water uptake occurring within the matrix and at the filler matrix interface with eventual dissolution of the PBG resulting in weight loss. Since the solubility of the glasses can be relatively low, weight gain can be dominant at early time points (dependant on the PBG structure). Once the polymer matrix is saturated, weight becomes predominant due to glass dissolution. This weight gain will be associated with slight dimensional changes (which were not measured here) and which would need to be considered during the development of a biomaterial implant.

All aqueous solutions containing the PCL/PBG composites showed a decrease in pH over the 78 week storage period to a final pH of between 3.4 and 4.5, section 4.7. The final pH for pH7 buffer solution was slightly higher at 5.52. The literature explains that phosphoric acid (H_3PO_4) is produced from the PBG and 6-hydroxycaproic (insoluble in water) from the PCL. The pH shift to more acidic, section 4.7, occurred more quickly (~ 1 week) for the saline and water solutions. This could be due to no presence of buffer, which would counteract the release of acidic species. A similar profile was observed for all samples (except for the aqueous solutions containing PCL which showed a higher pH). This is likely to be due to the absence of PBG in the structure resulting in a more acidic environment. Within the literature, a variety of pH values have been reported for several PBG structures, Table 2-19. These range from 8.6 [448] to 3.5 [408] dependant on the PBG structure. The results from section 4.7 are within this range which would indicate that the PBG does have a larger impact on pH change of surrounding solution than the PCL component of the composite. There have been reports of the pH increasing and decreasing within the literature. This has been observed in Figure 4-20. An explanation could be that the pH starts to return to neutral as the PBG dissolution slows down or is more or less complete due to the presence of less phosphate ions being released [52]. Modification of the PBG structure can also control the release of the phosphate species [236]. Acid/ base reactions can aid glass dissolution by disrupting the ionic interactions between chains due to protonation of phosphate chains [401]. These reactions are said to control the surface pH of the glass. Water is then able to penetrate the glass faster, resulting in rapid chain hydration and uniform dissolution. Hydrolysis reactions result in the cleavage of P-O-P bonds and have the potential to totally disrupt the polymeric phosphate network to produce orthophosphate (PO_4^{3-}).

So far, there has been considerable focus on the PBG component of the PCL/PBG composites. Although GPC was not conducted in order to monitor the changes in Mw due to PCL/PBG degradation, it is important to understand the degradation of PCL. The diffusion rate of water into PCL can be described by Ficks second law and polymeric groups can be easily hydrolysed, leading to chain scission. Ester hydrolysis can be either acid or base catalysed [80]. Polyester hydrolysis has been

traditionally modelled using a first order kinetic mechanism. Each polymer molecule with its own carboxylic and alcohol end groups, is broken in two, randomly in the middle at a given ester group. So the number of carboxylic end groups will increase with degradation time, while the molecules are being split by hydrolysis. The hydrolysis is dependent on molecular scission, temperature, surface area, molecular weight, crystallinity and the pH of aqueous media. The PCL/PBG composites, have been stored at 37°C (elevated temperature) and are surrounded by an acidic aqueous medium. As cleavage occurs, shorter chains will be formed with more carboxylic end groups. This contributes to acidity which in turn accelerates the rate of hydrolysis resulting in autocatalysis and degradation of the PCL component [289].

As far as the author is aware, there has not been a comprehensive study of leachables for the PCL component (using GC/MS) within PCL/PBG composites documented in the literature. However, there have been numerous studies on thermal bulk degradation and breakdown products of PCL, in particular looking into the proposed mechanism and fragmentation of the PCL structure [299, 322, 333-335]. GC/MS was utilised in order to produce a list of fragments and intensities from the thermal breakdown of PCL, Table 2-13. PCL is known to hydrolyse producing 6-hydroxycapronic acid but little else is documented [221]. Leachables testing was conducted on the PCL component of the three composite materials. The composites were stored in pH4 and pH7 buffers, Krebs, water and saline for 78 weeks at 37°C, then analysed by GC/MS, section 4.5. The results showed that unpolymerised caprolactone was present in all PCL, PCL/0Fe, PCL/5Fe and PCL/10Fe samples (Figure 4-16). The amount of caprolactone present was greatest for PCL followed by PCL/0Fe and PCL/5Fe. The PCL/10Fe results were an order of magnitude lower than the other two composites and PCL. Two oligomers, $\text{CH}_3\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{COO}^+$ (m/z 115) and $\text{CH}_3(\text{CH}_2)_4\text{COO}(\text{CH}_2)_5\text{COO}^+$ (m/z 229) were also detected, Figure 4-17 and Figure 4-18. The leachables from the oligomer with m/z 229 were detected as half that of the oligomer with m/z 115. The overall amounts of these oligomers leached were similar for all materials analysed. However, there are limitations in the interpretation of the results. PCL is more soluble in dichloromethane (DCM) so due to diethyl ether being used as the extraction medium, all leachables may not have been

recovered during the extraction process (from aqueous solution into an organic solution for analysis). There is no evidence of zinc, or any other catalysts used for the polymerisation of PCL, observed in the GC/MS traces. This may also be due to a poor choice of extraction medium compared to DCM. As mentioned in section 4.5.3, there were also no standards for the oligomers available so the leachables amounts were determined based on PCL which may have a different response factor (Rf).

The results from section 4.5.3 were compared to Table 2-13, proposed fragmentation patterns and intensities after heating PCL. The comparison has been tabulated in Table 5-3. There were eleven organic species detected during PCL leachables analysis, section 4.5.3, which has not been documented in the literature. Six of these ions had the same empirical formula as those seen during thermal degradation. It can be seen from Table 5-3 that two structures have been proposed by Luderwald [333] for ions with m/z values 41, 55 and 69. When section 4.5 was written only one structure was considered as the most probable for a leachable. Thermal analysis can involve oxidation hence, the potential reason for slightly different breakdown products. The proposed ions for m/z 97 and 115 are similar but for 229 Luderwald has suggested a structure containing four oxygens for thermal decomposition as opposed to a suggested three oxygen structure for a leachables product.

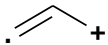
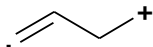
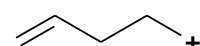
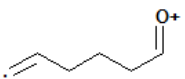
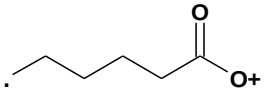
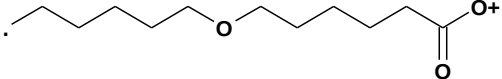
Ion (m/z)	Proposed Structure (from Section 4.5)	Literature [333]
41		C ₃ H ₅
55		C ₄ H ₇ , CH ₂ =CH-C≡O
69		C ₅ H ₉ , CH ₂ =CH-CH ₂ -C≡O
97		H ₂ C=CH-(CH ₂) ₃ -C≡O
115		HO-(CH ₂) ₅ -C≡O
229		HO-(CH ₂) ₅ -CO-O-(CH ₂) ₅ -C≡O

Table 5-3 Comparison of ions detected in the literature (for thermal analysis) and proposed structures for leachables found for PCL/0Fe, PCL/5Fe and PCL/10Fe composites

The additional five leachables species observed during leachables analysis can be seen in Table 5-4.

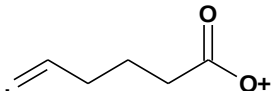
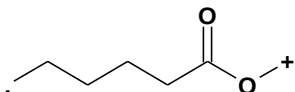
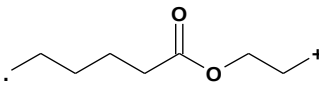
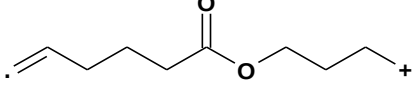
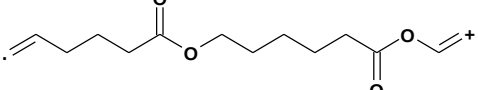
Proposed Structure (from Section 4.5)	Ion (m/z)
	113
	128
	156
	169
	253

Table 5-4 Proposed structure of leachables from PCL/PBG composites when stored in aqueous media, at 37°C, for up to 78 weeks

5.4 Material Characterisation

The density, of the PCL and PCL/PBG composites was examined using a helium system, pycnometer, in order to avoid solvent use and the morphology of PCL/PBG was examined by SEM.

The original morphology of the PCL/PBG composites was fairly smooth, section 4.8.3, which was also reported in the literature [74, 73]. However, after 1 week there was evidence of glass dissolution from all samples. This was also observed by Kim *et al.*, [73]. The literature reports a gradual loss of glass fibres from PCL and PLA composites up to 6 weeks in H₂O [40, 52, 417]. At this time point, section 4.8.3, there are still fibres present in the PCL/ PBG structures. For the PCL/0Fe material there is evidence of PBG degradation occurring from one week (which is confirmed by leachables data, section 4.4). A pore of 1.7µm diameter is observed, Figure 4-24(b), and the surface of the PCL is also less smooth indicating some surface degradation of the polymer. At 4 weeks there is a cavity of approximately 1.7µm to 183µm. Larger channels can be seen at 26 weeks where PBG was present. At 52 and 78 weeks pores within the PCL matrix are easily visible, diameter 30µm. Looking back at the equivalent leachables results (Section 4.4) it can be seen that the sodium release is starting to plateau but the calcium, iron and phosphorous are still rising which concurs that there is still phosphate glass within the structure at 52 weeks. At 78 weeks it is expected that there is still a small amount of PBG present within the PCL matrix because the leachables have not fully reached a plateau, Figure 4-9(a) to Figure 4-12(a), the pH of the storage mediums are still acidic, at 78 weeks, indicating presence of phosphate groups (hence, HP₃O₄ generation). Also, the density of PCL/0Fe (Figure 4-23(a)) is still higher than PCL (1.1246g/cm³). A similar pattern is observed for PCL/5Fe composite samples, although after 1 week pore sizes were observed from 50 to 100µm, at 2 weeks a cavity was observed measuring ~30µm in diameter and 0.1mm in length. At 52 weeks, Figure 4-27, shows clear evidence of some PBG fibres still present within the PCL matrix alongside pores of ~20µm. At 78 weeks it is expected (for the same reasons as the PCL/0Fe material) that some PBG is still within the composite structure. SEM images of PCL/10Fe appear to show minimal topography. At 26 weeks a rupture is apparent (due to the SEM vacuum) which

contains PBG fibres and at 78 weeks a cavity is observed (<10 μ m diameter, >220 μ m in length) still containing PBG.

As far as the author is aware there is no literature available on the density of PCL/PBG composites stored in aqueous media for 78 weeks. Any measurements conducted are often performed using an Archimedes principle with solvent displacement [414, 419, 420, 423] as opposed to a helium method used for studies conducted in section 4.8.2. The density of bulk glass is known to be influenced by the cross link density and packing of atoms in the glass. Therefore, it gives an indication of the degree of the change in the glass structure with change in the glass composition associated with addition of modifying metal oxides. As iron was added to the PBG the density increases due to formation of Fe-O-P bonds which has a dual nature of tetrahedral and octahedral coordination. Results from table 4-6 confirm this behaviour. The density of 0Fe is 2.6423gcm⁻³, 5Fe is 2.7335gcm⁻³ and 10Fe is 2.8562gcm⁻³. The addition of these PBGs to PCL increases the density from 1.1246gcm⁻³ to 1.2309gcm⁻³, 1.2606gcm⁻³ and 1.2354 gcm⁻³ respectively. After storage in aqueous media the density of all composites decreased, Figure 4-23, indication the leaching of PBG. It is also expected that the PCL has started to degrade due to SEM images of surface erosion and autocatalysis from pH acidic storage solutions. At the end of 78 weeks storage, in aqueous media, the density of all composites was still above that of PCL. A statistical analysis cannot be conducted due to one sample per storage medium and per time point (n=1). There are therefore limitations in the data interpretation. However, if an average density is taken across all storage media at 78 weeks the resulting densities are within the range PCL/0Fe 1.1697 gcm⁻³, PCL/5Fe 1.1988 gcm⁻³ and PCL/10Fe 1.2288gcm⁻³. These results indicate that there is still PBG within the PCL matrix after 78 weeks in the order of PCL/0Fe< PCL/5Fe < PCL/10Fe.

In order for a material to be suitable for bone implantation it is required to degrade at an appropriate rate for bone growth and proliferation [244, 259] and it should also maintain mechanical integrity. Due to the leaching of PBG ions from the PCL matrix pores have been created, section 4.8. A porous network can facilitate migration of cells, permit a nutrient supply, remove cellular waste products effectively and permit

vascular invasion to maintain electrolytic balance within the body [85,259]. The reported degradation time of 12 hours is lower than most literature reports, which is often a request of degradation time being over 6 weeks in order to facilitate cell growth and retain mechanical properties. The density measurements from Table 4-6 were used to calculate the porosity and pore volume, Table 4-7. After 78 weeks storage, the porosity values ranged from PCL/0Fe 4.12 to 6.45%, PCL/5Fe 4.00 to 5.87% and PCL/10Fe 0.41 to 2.50%. The pore volumes were: PCL/0Fe 3.50% to 5.61%, PCL/5Fe 3.04% to 4.95% and PCL/10Fe 0.33% to 3.44%. These results, combined with observed pore size, from the SEM analysis indicate that these materials would not be suitable as scaffold type materials for bone implantation (100-350 μ m pore size, 38-98% porosity), [259]. The pore size, is too small (a cell is in the order of 10-40 μ m), the porosity is 2 orders of magnitude lower than required and the pore volume one to two orders of magnitude too low. For fracture fixation devices, which are non-load bearing, this should not pose a problem.

An understanding of the composite thermal stability and thermal degradation behaviour is important for processing and application [348]. There is considerable literature available for PCL which details proposed degradation mechanisms and also resulting fragmentation patterns. [299, 332, 333, 335, 336-339, 346-353, 359, 360-362, 366]. It was concluded that the degradation was related to the molecular weight (Mn), type of heating (isothermal, non-isothermal) and the surrounding environment (O₂, N₂). A two stage degradation mechanism is favoured which involves random chain scission followed by specific end scission. Typical degradation products include ϵ -caprolactone monomer, oligomers, cyclopropane, various carboxylic acids, 5-hexenoic acid, ketenes, CO₂, CO and H₂O. Although the thermal properties of PBGs have been studied the amount of information is not extensive [121, 235, 236, 406, 408, 411, 430]. There has been focus on the glass transition temperature (T_g), melting temperature (T_m) and crystallinity (Cr), which is dependent on the glass structure and crystalline or amorphous states. The T_m has been shown to increase with the presence of Fe [236]. Although there is thermal information available for PCL and PBG, as far as the author is aware, there is no reported information on PCL with PBG containing iron. The T_g, T_m and (Cr) of the PBG are discussed by Mohammadi *et al.*, [236] and Ahmed *et al.*, [40] however this is

not further investigated as a composite with PCL. One paper was found which involved studies of PCL/ Calcium sulphate (CS) whisker composites prepared by a melt blending and co-precipitation methods [482]. The paper concluded that although the flexural strength of the PCL/CS increases however, the co-precipitation method ‘worsens’ the crystallisation perfection of PCL compared to a melt method. This was because the nucleation effect of the whisker within a melt method was stronger than for a co-precipitation method. Other factors which affect the thermal properties include the calcium content within the PBG. The T_g increases with the addition of more calcium because it is a refractory (heat resistant) material [406]. The T_g also increased with additional phosphate (up to 50mol%) this was believed to be due to a change in the glass structure from ortho, to the longer chain, metaphosphate [243]. The addition of iron into the PBG structure tends to significantly decrease the T_g due to a high charge density [411].

The thermal properties of PCL and PCL/PBG composites was performed using DSC and TGA. Two peaks were observed upon DSC analysis of the PCL and composites, Figure 4-30. The 1st peak was typically associated with the melting point of the crystalline phase of PCL and the 2nd peak showed thermal degradation of the product. This was confirmed by TGA analysis which showed a large decrease in weight at approximately 400°C (Figures 4-31(a) –(c)) for PCL/0Fe, PCL/5Fe and PCL/10Fe. All samples showed a higher weight loss after storage in aqueous media, Table 4-8. The control samples for PCL/0Fe, PCL/5Fe and PCL/10Fe showed a weight loss of 87.3%, 79.8% and 90.3% respectively. These results confirm the thermal stability order of PCL/0Fe < PCL/5Fe < PCL/10Fe. After storage in aqueous media, the weights decrease to 99.3 – 98.9% for PCL/0Fe, 96.6- 89.5% for PCL/5Fe and 93.6 – 91.0% for PCL/10Fe. The T_g, T_m and Cr are documented in Table 4-9 to Table 4-11. However, due to a limited data set (n=1 samples) there are limitations in the data interpretation. The addition of PBG to the PCL matrix decreased the Cr and the T_g, however, the melting and degradation temperatures increased. The melting temperature for PCL was 64.9°C. The addition of PCL/0Fe increased this to 69.0°C, for PCL/5Fe 68.7°C and for PCL/10Fe 70.4°C. The T_g for PCL was -64.3°C. Addition of PBG decreases the T_g to -67.4°C for PCL/0Fe, to -69.6°C for PCL/5Fe and -69.9°C for PCL/10Fe. The increase in T_m and decrease in T_g is due to the

addition of the PBG and also increased cross linked density of the glass network increasing the chemical stability [90, 399]. It has also been suggested that a shorter molecular chain of the PCL during manufacture could cause a nucleation effect thus increasing the nucleation rate and increasing T_g [482]. The crystallinity of PCL was 56.49%. The addition of PBG decreased this to 40.5% for PCL/0Fe, 40.3% for PCL/5Fe and 39.8% for PCL/10Fe. PCL is a semi-crystalline material which contains amorphous and crystalline areas. The addition of the PBG (an amorphous material) has disrupted the ordered crystalline areas within the polymer.

There was minimal variation in the T_m results for the PCL/0Fe, PCL/5Fe and PCL/10/Fe over the 78 week storage period, in aqueous media, for all 3 composites and the interpretation of the data was limited due to a sample of n=1. In general the T_m decreased, Table 4-9 to Table 4-11. It is proposed that the decrease is due to hydrolysis and cleavage of P-O-P bonds disrupting the polymeric PBG network and hydration, potential dissolution of entire chains and the potential bulk degradation of the PCL matrix. The T_g values for the PCL/PBG composites (after storage in aqueous media) increased and this was accompanied with a Cr increase, Table 4-9 to Table 4-11 and Figure 4-32]. Similar behaviour has been documented by Liu *et al.*, for PCL with CS whiskers who suggested that crystallisation is due to a nucleating effect of the PBG thus increasing T_g. Liu *et al.*, further went on to say that an increase in size or number of fibres can also cause crystallisation due to a nucleating effect of the PBG [482]. Changes in T_g from the PBG can also be due to changes from ortho to metaphosphate groups [90]. Although the crystallinity increased for all three PCL/PBG composites, after storage, the results obtained over the 78 time period did not show a clear trend, Figure 4-32. The reasons for this could be due to easier alignment of the polymer chains upon storage at 37°C creating the presence of slightly less amorphous and more crystalline areas within the polymer. PCL is a semi crystalline polymer and the amorphous regions tend to degrade more quickly than semi or crystalline areas. As the amorphous areas degrade, more crystalline areas remain. The amount of amorphous PBG within the polymer structure will also not be uniform throughout the composite. As the PBG dissolves from the polymer matrix, there will also be different regions within each sample which have amorphous and crystalline areas.

FTIR analysis has been used to determine fragmentation patterns from thermal analysis of PCL [336-339]. There are several papers available which have assigned wavenumbers and structural bands to PCL during studies involving drug delivery, phase separation between polymer blends, hydrolytic and enzymatic degradation studies and crystallinity determination [309, 329, 336, 340-343, 345, 354]. There have also been numerous papers written on the FTIR spectral analysis of PBG [62, 66, 237, 238, 240, 403, 410, 484]. Interfacial properties of phosphate based glass with PLA composites have also been studied to see the effect of end functions [49]. However, there are minimal papers on PCL/PBG with iron and, as far as the author is aware, no literature examining the structure of PCL/PBG after storage in aqueous media for up to 78 weeks [41, 495, 496].

The FTIR spectra for the PBG with addition of 5Fe and 10Fe is presented in Figure 4-32. The peaks presented in Figure 4-32, and described in Table 4-12, are all generally believed to be due to vibrations within the phosphate tetrahedron. It is well documented phenomena that as the modifier, such as iron, content increases in the phosphate matrix, probable phosphate structural units change from Q^3 to Q^2 to Q^1 and Q^0 [62]. The PBG appears to contain primarily Q^2 and Q^1 units, associated with metaphosphate and pyrophosphate. The addition of any modifying oxides leads to depolymerisation of a vitreous PBG containing only PO_4 tetrahedra (Q^0). The result is cleavage of the P-O-P bond creating a non-bridging oxygen, which is observed in Figure 4-32. The Q unit depends on the nature and concentration of cations present in the glass. In general the spectra for the PBG is not well defined because the structures are amorphous. The presence of 50% network modifiers within the PBG causes overlapping peaks, observed at $\sim 700\text{ cm}^{-1}$ and $\sim 1110\text{ cm}^{-1}$, Figure 4-32. The 0Fe PBG shows fairly defined peaks at $\sim 1366\text{ cm}^{-1}$, $\sim 1296\text{ cm}^{-1}$, $\sim 1243\text{ cm}^{-1}$, $\sim 1179\text{ cm}^{-1}$, $\sim 1160\text{ cm}^{-1}$. The changing amplitude at $\sim 1243\text{ cm}^{-1}$ (PO_2 asymmetric stretch) demonstrates depolymerisation of the phosphate network with a decrease in P_2O_5 . The fraction of NBOs decrease and cross linking density increases due to formation of P-O-Fe replacing Na_2O [90].

The interpretation of the results for the PCL/PBG composites after storage in aqueous media is limited. The FTIR technique used for examining the composites directly was

not optimal for examining the PCL/PBG structure. Depending on where the diamond tip, from the Golden Gate apparatus, was placed it did not always touch a glass fibre. This was due to 90%w/w PCL (with a high molecular weight, M_n 42,500) within the composite and the in-homogenous distribution of the fibres. At the time of analysis it was difficult to grind the composites into a powder and it was not known if any PCL/PBG interactions would be compromised. Although there were some analysis issues, some samples did show presence of both PBG and PCL, indicating a successful manufacture. An alternative and potentially improved method is to use KBr discs.

PCL, PCL/0Fe, PCL/5Fe and PCL/10Fe were analysed by FTIR, before storage and after storage in aqueous media, in order to investigate any structural changes, Figures 4-34 to 4-39 and Tables 4-13 to 4-18. A qualitative assessment on the peak intensities and changes in Q^i units was not possible due to explained limitations, only band identification and assignment was conducted. The presence of PBG and PCL was observed in all three composites, Figures 4-36 to 4-39. However, observations were made when compared to control samples (PBG and PCL alone). There were various peak shifts to lower wavelengths ~~were observed~~, alongside band broadening and peak disappearance. For PCL/0Fe after 1 months storage in pH4 buffer solution, the peak associated with -O-C- (stretching in the PCL component) shifted from 1158cm^{-1} to 1142cm^{-1} and 1108cm^{-1} shifted to 1094cm^{-1} (-O-C- vibration in PCL). This was believed to be due to the presence of PBG which has broad band in this region (1106cm^{-1}) due to P-O stretching. The peak at 1108cm^{-1} (-O-C- vibration) and 1064cm^{-1} (γ -C-O- stretch) merge in the composite due to PBG vibrational stretching $\text{PO}_3 Q^1$ units. There is also a shift to lower wavelength for peaks at 1046cm^{-1} and 960cm^{-1} (O-C- vibration) due to P-O-P groups linked to small metaphosphate rings. The CH_2 from PCL, shifted from 931cm^{-1} to 908cm^{-1} due to the bending mode of P-O-P groups linked with a linear metaphosphate chain (observed at $\sim 870\text{cm}^{-1}$). Similarly results from PCL/0Fe in pH7 solution showed peak convergence, shifts, disappearance and presence of PBG after 1 months storage. After 6 months storage in aqueous media, the PCL/0Fe composite IR spectra more closely resembled PCL than PCL/PBG. However, peak shifts from 1158cm^{-1} , 1108cm^{-1} and 1046cm^{-1} to 1154cm^{-1} , 1105cm^{-1} and 1042cm^{-1} were believed to be due to presence of vibrational stretching

(P-O-P) of NBOs in Q² tetrahedra, vibrational stretching of PO₃ groups (Q¹) and the motion of NBOs (PO₃) in Q¹ tetrahedra from some remaining PBG component. For PCL/5Fe and PCL/10Fe similar behaviour is observed as for PCL/0Fe however, the peaks more closely resembled the PCL component for PCL/5Fe after 12 months. At the end of the 78 week storage period, in aqueous media, the PCL/10Fe composite still showed presence of a PCL and PBG structure. The results confirm that the changes in T_g in the PBG can be due to changes from ortho to metaphosphate structures. IR showed slight wavelength shifts for the C=O (1720cm⁻¹) bond which can aid identification of crystalline or (higher wavelength) amorphous states. This confirmed that presence of amorphous and crystalline states within the composites, as confirmed by DSC analysis. There was also evidence of peak broadening after storage in aqueous media due to strong hydrogen bonding. The peak shifts, band broadening and disappearance of peaks infers that there is potential interfacial interaction between the PCL and PBG. However, this would require further investigation.

CHAPTER 6 - CONCLUSION

The following conclusion can be made from the work performed for this thesis:

There are limitations throughout the interpretation of this work due to an $n=1$ sample number and improvements are required for some analyses. The following glass structures were studied: 50P45Ca5Na, 50P35Ca5Na5Fe and 50P35Ca5Na10Fe and it was demonstrated that the addition of 10%w/w iron into the PBG structure increased the durability of the PBG by an order of magnitude. This was due to the iron acting as a network former within the P-O-P backbone. The order of leaching was phosphate > sodium > calcium > iron. This was explained by the amount of the oxides originally present in the PBG structure, breakage of the phosphate backbone within the PBG, divalent calcium ions forming chelate cross links (which decreases the dissolution rate compared to sodium) and Fe-O-P bonds being more resistant to hydration than P-O-P bonds. The weight loss for 50P35Ca5Na10Fe was a third less than the other two PBGs and the weight loss for the equivalent composite was half of the non-iron containing composite. The leaching rate of the 10%w/w iron containing composite was approximately half that of the non-iron containing composite. Unpolymerised caprolactone was observed, alongside 2 oligomers, for the organic leachables from the PCL component of the composites. The density of the PCL/PBG composite was greater than that for PCL alone. DSC showed a decrease in crystallinity and T_g when PBG was added to PCL but an increase in melting point due to cross linked density of the PBG network within the composite. After storage, of the composite, in aqueous media the crystallinity and T_g increased which could partially be attributed to realignment of the polymer chains within the PCL under storage in aqueous media at 37°C.

The amount of iron in the glass structure extended the time for dissolution. Elemental analysis showed that the presence of iron within the composite reduced the leachables

rate. Hence, the accumulation of the ions in a local area is lowered due to fewer ions being released over the same time period for iron containing PBG. The leachables were within acceptable toxicological limits and the amount of calcium present (within the PBG) is beneficial for the bone healing process. Hence, this material has potential for use in a bone fixation device.

CHAPTER 7 – RECOMMENDATIONS FOR FUTURE WORK

Considering the work carried out, over the past few years, and current knowledge of totally resorbable phosphate based glass reinforced polymer composites (for bone implantation applications) the following recommendations are made for future work:

The leachables studies were limited to the actual components of the PCL/PBG composites. Processing aids and catalysts should also be investigated. The studies showed limited organic leachables from the composite. Hence, further investigation into quantitative analysis of leached species, using dichloromethane to extract the organic phase from the aqueous leachables, is recommended. In order for a material to be considered for implantation it needs to be sterile. A popular sterilisation technique is by gamma irradiation. Leachables testing would potentially show any degradation products from the materials after sterilisation and alleviate potential safety issues.

The weight loss, of the PBG/PCL composites, showed that the composites containing iron within the PBG structure, were less than the non iron containing composite. With 10%w/w iron, the PCL/PBG weight loss was approximately half that of the non iron containing material. Quantitative leachable ions, from the PBG component of the matrix, were recorded. However, quantitative organic leachables from the PCL were not recorded. Therefore, it was not possible to establish the overall weight losses due to PBG and PCL components. Further studies would involve quantitative analysis of PCL leachables and the use of Gel Permeation Chromatography (GPC) to establish the change in Mn for PCL over time.

Although the composites have been found to have a lower degradation rate when the PBG has a higher iron content, the mechanical properties were not investigated.

Mechanical behaviour is crucial for a bone implant and it must retain its integrity during cell growth and proliferation. Mechanical behaviour of PLA/PBG and PCL/PBG is documented in the literature. Such properties, from this particular composite, have not been reported. Hence, it is suggested that mechanical properties be investigated before and after sterilisation.

FTIR studies have been conducted but the information from these studies is limited due to the experimental design. It would be beneficial to make KBr discs or find an alternative way to create a more uniform sample for analysis. Evaluation of up to 5 different regions, within the sample, would ensure a more representative quantification of abundance and structural changes, over time, for PCL and potential conversion of the phosphate structural units from Q^3 to Q^0 . The use of X-ray photoelectric spectroscopy (XPS) could complement the FT-IR data. XPS can be used to show specific elements at the composite interface and surface. The composite could be examined on the surface and through a cross-section to examine the % change in PBG and PCL after storage in aqueous media. A single filament pull out test could be utilised to monitor the strength of interaction between the fibre surface and PCL matrix after storage in aqueous media over the shelf life of the proposed composite material.

Another area for further research are cytotoxicity studies since the materials studied have not been documented in the literature.

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APPENDIX A – ACTIVATION ENERGY METHODS

$$\ln (g(\alpha)/T_2) = \ln (AR/\beta E_a) - E_a/RT \quad (\text{Coats Redfern Equation})$$

$$\ln (1/(1-c)^n dc/dt) = \ln A - E_a/RT \quad (\text{Chang Equation})$$

$$\ln \beta = \log [0.0048 A E_a / g(\alpha) R] - 1.0516 E_a / RT \quad (\text{Flynn-Wall-Ozawa Equation})$$

$$\ln (dc/dt) = \ln (A) + \ln (1-c) - E_a/RT \quad (\text{Freidmann Equation})$$

$$\ln \beta/T^2 = \ln [A E_a / g(\alpha) R] - E_a/RT \quad (\text{KAS Equation})$$

$$\ln (\beta/T_f^2) = \ln (AR/E_a) - E_a/RT_f \quad (\text{Kissinger Equation})$$

Key

A	Activation energy
β	Heating rate
C	C= 1- weight at ant time – initial weight
dc/dt	Isothermal rate of conversion
E _a	Energy of activation
E α	Energy of activation
R	Gas constant
T	Absolute temperature
T _f	Temperature of DSC melting peak
KAS	Kissinger-Akahira-Sunrose

APPENDIX B – Literature Search: Weight Loss and Solubility

Description	Conditions	Weight Loss (%)					Reference
		7 days	30 days	60 days	180 days		
40P16Ca16Na4Fe24Mg	6 months in deionised water at 37°C	1.45	3.6	5.2	16.3-		[463]
		5hrs	24hrs	50hrs	80hrs	96hrs	
50P30Ca20Na	96 hours in deionised water at 37°C	63	-	-	-	-	[414]
50P30Ca19Na1Fe	Pulling speed 400 m min ⁻¹	1.0	47	57	70	73	
50P30Ca18Na2Fe		5.0	35	52	72	73	
50P30Ca17Na3Fe		3.0	11	19	20	22	
50P30Ca16Na4Fe		3.0	5.0	10	11	12	
50P30Ca15Na5Fe		2.0	4.0	5.0	5.0	5.0	
50P30Ca20Na	Pulling speed 1600 m min ⁻¹	98 (2hrs)					
50P30Ca19Na1Fe		18	98				
50P30Ca18Na2Fe		1.0	65	95 (35hrs)			
50P30Ca17Na3Fe		3.0	18	30	45	60	
50P30Ca16Na4Fe		2.0	10	18	21	27	
50P30Ca15Na5Fe		1.0	5.0	5.0	5.0	7.0	

Table B-1 Literature Weight Loss Results for PBG (Presented as weight loss %)

		Weight Loss (%)				
10 days in deionised water at 37°C		1 day	4 days	7 days	10 days	[68]
50P46Ca4Na		8	50	65	66	
50P46Ca3Na1Fe		5	30	45	49	
50P46Ca2Na2Fe		3	4	5	5	
50P46Ca1Na3Fe		1	1	2	3	

Table B-1 Literature Weight Loss Results for PBG (Presented as weight loss %)

Description	Conditions	Solubility (%mm ⁻² h ⁻¹)	Reference
50P30Ca17Na3Ti	6 months in deionised water at 37°C	1.1	[449]
50P29Ca17Na3Ti1Sr		11.0	
50P27Ca17Na3Ti3Sr		10.5	
50P25Ca17Na3Ti5Sr		9.2	
50P30Ca20Na	405 hrs (16.9 days) in deionised water at 37°C	0.0004	[5]
50P30Ca19Na1Ti		0.0001	
50P30Ca17Na3Ti		0.00002	
50P30Ca15Na5Ti		0.00001	

Table B-2 Literature Solubility Results for PBG (Presented as weight loss %mm⁻²h⁻¹)

Description	Conditions	Solubility (mgmm ⁻² h ⁻¹)	Reference
45P16Ca39Na	5 days in deionised water at 37°C	0.0417	[448]
45P14Ca38Na3Ga		0.0176	
45P15Ca37Na3Ga		0.0135	
45P16Ca36Na3Ga		0.0073	
45P30Ca25Na	250 hrs (10.4 days) in deionised water at 37°C	0.0008	[419]
45P35Ca20Na		0.0007	
45P40Ca15Na		0.00035	
50P50Na	Deionised water at 37°C.*	1.00	[420]
50P10Ca40Na		0.2	
50P20Ca30Na		0.01	
50P30Ca20Na		0.001	
50P40Ca10Na		0.0005	
50P10Ca		0.0003	
50P30Ca20Na	5 days in deionised water at 37°C	0.0015	[399]
50P30Ca17Na3Ag		0.0014	
50P30Ca15Na5Ag		0.0009	
50P30Ca20Na	5 days in nutrient broth (Oxoid) at 37°C	0.0016	[399]
50P30Ca17Na3Ag		0.0013	
50P30Ca15Na5Ag		0.011	

Table B-3 Literature Solubility Results for PBG (Presented as weight loss mgmm⁻²h⁻¹)

Description	Conditions	Solubility (mgmm ⁻² h ⁻¹)		Reference
50P30Ca20Na	625 hrs (26 days) in deionised water at 37°C	0.00189	[407]	
50P35Ca15Na		0.00058		
50P40Ca10Na		0.00056		
55P30Ca15Na		0.00039		
55P35Ca10Na		0.00036		
55P40a5Na		0.00029		
45P30Ca25Na	Deionised water at 37°C (no time specified)	12.68E-06	[406]	
45P35Ca20Na		2.038E-06		
45P40Ca15Na		1.557E-06		
50P30Ca20Na		2..833E-05		
50P35Ca15Na		1.260E-05		
50P40Ca10Na		5.883E-04		
55P30Ca15Na		3.455E-06		
55P35Ca10Na		2.940E-06		
55P40Ca5Na		3.125E-06		
50P10Ca40Na	5 hrs in deionised water at 20°C	3.0E-04	[401]	
50P20Ca30Na		1.8E-05		
50P10Ca40Li		3.6E-05		
50P20Ca30Li		1.8E-06		

Table B-3 Literature Solubility Results for Phosphate Glass (Presented as weight loss mgmm⁻²h⁻¹)

		Weight Loss (%)				Reference
Description	Conditions	7 days	30 days	56 days	60 days	
100% PCL	56 days in deionised water at 37°C	-	-	0.55- 0.67	-	[41]
100%PCL	5 weeks in deionised water at 37°C				**	[52]
100% PCL	28 days in NaOH solution	10.0	~11.0	-	-	[321]
100% PCL	28 days in simulated body fluid	10.0	10.0			
100% PCL	28 days in saline phosphate buffer (SPB) solution	~0.2	~0.75			
100% PCL	28 days in esterase/ SPB solution	7.0	7.0			
		100hrs	250hrs	400hrs	600hrs	
100% PCL	625 (26 days) hours in deionised water at 37°C	0.00	0.00	~0.10	~0.50	[74]
	7 days in deionised water at 37°C	2hrs	25hrs	75hrs	168hrs	
100% PCL		0	0.2	2.0	3.0	[121]

Table B-4 Literature Weight Loss Results for PCL (Presented as weight loss %)

		Weight Loss (%)				
Description	Conditions	7 days	30 days	56 days	60 days	Reference
(90%PCL/ 10% phosphate glass)++						
PCL/ 16Na24Mg16Ca4Fe40P	6 months in deionised water at 37°C	0.32	0.56	-	0.83	[463]
60%PCL/ 40% P or Si glass*						
PCL/ 40 Si	56 days in deionised water at 37°C	-	-	62.07-68.11	-	[41]
PCL/ 30 Si/ 10 P		-	-	59.63 – 59.87	-	
PCL/ 20 Si/ 20 P		-	-	52.35 – 54.59	-	
PCL/ 10 Si/ 30 P		-	-	31.85 – 37.49	-	
PCL/ 40 P		-	-	17.27 – 19.41	-	
22%PCL/ 18% phosphate glass						
50P50Ca	5 weeks in deionised water at 37°C	100hrs	250hrs	400hrs	800hrs	[40]
		8.0	15.0	20.0	2.0	
94% PCL/ 6% phosphate glass						
50P50Ca		2.5	5.0	8.0	8.0	
80% PCL/ 20% phosphate glass						
	625 (26 days) hours in deionised water at 37°C	100hrs	250hrs	400hrs	600hrs	[74]
45P24Ca31Na		20.00	~22.00	~22.00	~19.00	
45P28Ca27Na		~8.00	~9.50	~10.00	~10.00	
45P32Ca23Na		~7.00	~8.50	~9.00	~9.00	
45P36Ca19Na		~0.04	~2.50	~3.50	~5.00	

Table B-5 Literature Weight Loss Results for PCL/ Phosphated glass (Presented as weight loss %)

		Weight Loss (%)				
PCL/ phosphate glass	7 days in deionised water at 37°C	2hrs	25hrs	75hrs	168hrs	[73]
45P20Ca35Na		27	33	37	40	
45P30Ca25Na		2	27	30	35	
45P40Ca15Na		0	10	20	25	
45P50Ca5Na		0	4	8	15	

Table B-5 Literature Weight Loss Results for PCL/ PBG (Presented as weight loss %)

*The structure of the phosphate glass was 40Ca10Fe50P (mol%) and the structure of the silicon glass was 40Ca10Si50P (mol%).

** There was no weight loss for PCL after 5 weeks storage deionised water at 37°C.

+The volume fraction of the PCL to phosphate glass is given in Table 4-x.

++ Mass fraction

APPENDIX C - GC/MS METHOD

6890 Gas Chromatography Method

Oven

Initial temperature: 35°C

Initial time: 2minutes

Maximum temperature: 350°C

Equilibration time: 0.00 minutes

Ramps:	Rate	Final Temperature	Final Time
1	25.00	250	1.00
2	30.00	325	1.00
3	50.00	335	4.00
4	0.0 (Off)		

Post temperature: 0°C

Post time: 0.0 minutes

Run time: 19.30 minutes

Cryo (CO₂)

Cryo: Off

Cryo fault: On

Cryo timeout: 10.00 minutes (On)

Quick cryo cool: Off

Ambient temperature: 25°C

Front Inlet (Split/ Splitless)

Mode: Split

Initial temperature: 280°C (On)

Pressure: 6.76 psi (On)

Split ratio: 5:1

Split flow: 5.0mL/ min

Total flow: 8.4 mL/ min

Gas saver: Off

Gas type: Helium

Back Inlet (Volatiles)

Mode: Split

Initial temperature: 50°C (Off)

Pressure: 0.00 psi (Off)

Total flow: 45.0mL/min

Gas saver: Off

Gas type: Helium

Column 1

Capillary Column

Model Number: SUPELCO EQUITY-5

Equity-5 30m x 0.25mm x 0.25µm

Max. temperature: 320°C

Nominal length: 30.0m

Nominal diameter: 250.00µm

Nominal film thickness: 0.25µm

Mode: constant flow

Initial flow: 1.0mL/min

Column 2

Not installed

Nominal initial pressure: 6.77psi
Average velocity: 36 cm/sec
Inlet: Front Inlet
Outlet:MSD
Outlet pressure: vacuum

Front Detector
Temperature: 150°C (On)
Flow: 40.0 mL/min (Off)
Flow: 450.0 mL/min (Off)
Mode: Constant makeup flow
Flow: 45.0 mL/min (Off)
Makeup Gas Type: Nitrogen
Flame: Off
Electrometer: Off
Lit offset: 2.0

Back Detector
N/A

Signal 1
Data rate: 20Hz
Type: test plot
Save Data: Off
Zero: 0.0
Range: 0
Fast peaks: Off
Attenuation: 0

Signal 2
Data rate: 20Hz
Type: test plot
Save data: Off
Zero: 0.0 (off)
Range: 0
Fast Peaks: Off
Attenuation: 0

Column Comp 1
(No Detectors Installed)

Column Comp 2
(No detectors Installed)

Thermal Aux 2
Use: MSD Transfer Line Heater
Description:
Initial temperature: 300°C (On)
Initial time: 0.00 min
Rate Final temp Final time
1 0.0 (Off)

AUX Pressure
Description:
Gas Type: Helium
Initial pressure: 0.00psi (Off)

AUX Pressure 4
Description:
Gas Type: Helium
Initial pressure: 0.00psi (Off)

AUX Pressure 5
Description:
Gas type: helium
Initial pressure: 0.00psi
Post Run
Post time:0.00min

Front Injector	
Sample washes:	2
Sample pumps:	4
Injector volume:	1.0 microlitres
Syringe size:	5.0 microlitres
Nanolitre adaptor:	Off
Post injection solvent A washes:	2
Post injection solvent B washes:	2
Viscosity delay:	0 seconds
Plunger speed:	Fast
Pre injection dwell:	0.00 minutes
Post injection dwell:	0.00 minutes

Back Injector
No parameters specified

Solvent delay: 4.00 minutes

APPENDIX D – Calculation of ppm for Constituents of Phosphate Glass

Calculation of total phosphate (Cotton et al 1987):

P: - molecular mass = 30.97g

O:- molecular mass = 16.00g

Hence, molecular mass of P_2O_5 = 141.94g

Of this 141.94g phosphorous pentoxide the following % is phosphorous:

$$61.94/141.94 \times 100 = 43.64\%$$

The weight loss from the composite = (A)

The initial weight of the composite is (B)

According to the formulation: $50P_2O_5-45CaO-5Na_2O$ only 50% is P_2O_5 .

Hence, 50% of (A) will be in the form P_2O_5 .

This equates to $(A) \times 50\% = (C)$

Hence, theoretical amount of phosphorous present:

43.64% of (C) = (D) phosphate present per weight (B) of starting material

Hence: $(D/B) = \text{ppm or } \mu\text{g/g}$ phosphate theoretically present.

Calculation of total sodium:

Na: - molecular mass = 22.99g

O:- molecular mass = 16.00g

Hence, molecular mass of Na_2O = 61.98g

Of this 61.98g sodium oxide the following % is sodium:

$$45.98/61.98 \times 100 = 74.19\%$$

The weight loss from the composite = (A)

The initial weight of the composite is (B)

According to the formulation: $50P_2O_5-45CaO-5Na_2O$ only 5% is Na_2O .

Hence, from the weight loss 5% of (A) will be in the form Na_2O .

This equates to $(A) \times 5\% = (C)$

Hence, theoretical amount of sodium present:

74.18% of (C) = (D) sodium present per weight (B) of starting material

Hence: $(D/B) = \text{ppm or } \mu\text{g/g}$ sodium theoretically present.

Calculation of total calcium:

Ca: - molecular mass = 40.08g

O:- molecular mass = 16.00g

Hence, molecular mass of CaO = 56.08g

Of this 56.08g calcium oxide the following % is calcium:

$$40.08/56.08 \times 100 = 71.47\%$$

The weight loss from composite = (A)

The initial weight of composite = (B)

According to the formulation: 50P₂O₅-45CaO-5Na₂O only 45% is CaO.

Hence, from the weight loss 45% of (A) will be in the form of CaO.

This equates to (A) x 45% = (C)

Hence, theoretical amount of calcium present:

71.47% of (C) = (D) calcium present per weight of starting material

This equates to (D)/(B) = ppm or µg/g calcium theoretically present.

Calculation of total Iron:

There was no iron in the low durability material so these calculations are based on the medium durability phosphate glass.

Fe: - molecular mass = 55.85g

O:- molecular mass = 16.00g

Hence, molecular mass of Fe₂O₃ = 159.7g

Of this 159.7g iron oxide oxide the following % is iron:

$$111.7/159.7 \times 100 = 69.94\%$$

The weight loss from composite = (A)

The initial weight of composite = (B)

According to the formulation: 50P₂O₅-40CaO-5Na₂O-5Fe₂O₃ only 5% is 5Fe₂O₃.

Hence, from the weight loss 5% of (A) will be in the form 5Fe₂O₃.

This equates to (A) x 5% = (C)

Hence, theoretical amount of calcium present:

69.94% of (C) = (D) iron present per weight of starting material (B)

This equates to (D)/(B) = ppm or µg/g iron theoretically present.