MELT PROCESSABLE BIOMATERIALS FOR DEGRADABLE SURGICAL FIXATION DEVICES.

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Doctor of Philosophy

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Submitted for the Degree of Doctor of Philosophy

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SUMMARY

There are currently few biomaterials which combine controlled degradation rates with ease of melt processability. There are however, many applications ranging from surgical fixation devices to drug delivery systems which require such combined properties. The work in this thesis is an attempt to increase the availability of such materials.

Polyhydroxybutyrate-polyhydroxyvalerate copolymers are a new class of potentially biodegradable materials, although little quantitative data relating to their *in vitro* and *in vivo* degradation behaviour exists. The hydrolytic degradation of these copolymers has been examined *in vitro* under conditions ranging from "physiological" to extremes of pH and elevated temperature. Progress of the degradation process was monitored by weight loss and water uptake measurement, x-ray diffractometry, optical and electron microscopy, together with changes in molecular weight by gel permeation chromatography. The extent to which the degradation mechanism could be modified by forming blends with polysaccharides and polycaprolactone was also investigated.

Influence of the valerate content, molecular weight, crystallinity, together with the physical form of the sample, the pH and the temperature of the aqueous medium on the hydrolytic degradation was investigated. Its progress was characterised by an initial increase in the wet weight, with concurrent decrease in the dry weight as the amorphous regions of the polymer are eroded, thereby producing an increase in matrix porosity. With the polysaccharide blends, this initial rate is dramatically affected, and erosion of the polysaccharide from the matrix markedly increases the internal porosity which leads to the eventual collapse of the matrix, a process which occurs, but less rapidly, in the degradation of the unblended polyhydroxybutyrate-polyhydroxyvalerate copolymers. Surface energy measurement and goniophotometry proved potentially useful in monitoring the early stages of the degradation, where surface rather than bulk processes predominate and are characterised by little weight loss.

KEYWORDS: **Polyhydroxybutyrate-polyhydroxyvalerate copolymers**
Polysaccharide blends
Bioerosion
Goniophotometry
Surgical fixation devices

This thesis is dedicated to the memory of my grandparents.

ACKNOWLEDGEMENTS

I would like to express my deep-felt gratitude to Dr Brian J Tighe, my supervisor, for the inestimable help, advice, support and encouragement that he provided throughout the course of this work and for having made this research period so fulfilling.

Thanks are also extended to Drs A M Jolly and S.J. Holland, Messers Philip Corkhill and Colin Hamilton, Miss Karen Thomas and other members of Dr Tighe's research group for helping to make my time at Aston so enjoyable. Thanks also to my friends, MKY and Co., for their moral support and encouragement during the course of this work.

I would also like to thank the technical staff of the Chemistry department and both Ann (helping with the typing of this thesis), and Roger Howell (x-ray diffractometry and printing of SEM photographs) for their contributions.

Special thanks to my parents, without whose hard work, great patience, understanding, sacrifices and encouragement, I would never have reached this stage.

Finally, I would like to thank my wife and other members of my family for their help and to my sons, Umar and Mudassar who have made all this hard work worthwhile.

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LIST OF ABBREVIATIONS.

DMF	Dimethyl formamide.
DMSO	Dimethyl sulphoxide.
GA	Glycolic acid.
GAAS	Glycolic acid anhydrosulphite.
GF	Gloss factor.
GPC	Gel permeation chromatography.
I_d	Diffuse reflectance.
IR	Infra red spectroscopy.
I_s	Specular reflectance.
ITM	Initial tensile modulus.
LA	Lactic acid.
MFI	Melt flow index.
Mn	Number average molecular weight.
Mw	Weight average molecular weight.
MWD	Molecular weight distribution.
Mwt	Molecular weight.
NMR	Nuclear magnetic resonance.
PBT	Polybutylene terephthalate.
PCL	Polycaprolactone.
PDS	Poly para dioxanone.
PE	Polyethylene.
PEPBO	Poly(ethylene 1,4 - phenylene - bis - oxyacetate).
PET	Polyethylene terephthalate.
PHB	Polyhydroxybutyrate.

PHV	Polyhydroxyvalerate.
PLA	Polylactic acid.
PP	Polypropylene.
PTFE	Polytetrafluroethylene.
PVC	Polyvinylchloride.
SEM	Scanning electron microscopy.
t_{10}	Time for 10% of original dry weight loss.
t_{50}	Time for 50% of original dry weight loss.
T_g	Glass transition temperature.
THF	Tetrahydrofuran.
Tm	Polymer melting point.
TMC	Trimethylene carbonate.
TS	Tensile strength.
UTS	Ultimate tensile strength.
$W_{1/2}$	Peak width at half height.
YS	Yield strength.
% EY	% elongation at yield.
% EB	% elongation at break.
γ^d	Dispersive component of surface energy.
γ^p	Polar component of surface energy.
γ^t	Total surface energy.
θ	Contact angle.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION.

There is a great need and demand by surgeons for materials which degrade in a controlled manner over a predetermined implantation period, for various surgical applications. Once their aim is fulfilled, biodegradable materials should, ideally, be resorbed by the body in a predictable manner, making subsequent removal unnecessary. Polymer scientists are now attempting to provide such molecularly designed biodegradable polymeric materials. The work in this thesis is aimed at providing a group of materials with such properties.

1.1 SURGICAL FIXATION DEVICES.

Surgical fixation devices can broadly be described as having two functions; wound management following surgery, and structural support/substitution following trauma. Staples, ligators and tape are involved in the former case, whereas screws, pins and plates are used in the latter.

The oldest and perhaps, the most used surgical fixation device is the surgical suture. The earliest use of sutures dates back to 4000 B.C., when the Egyptians probably used linen and catgut for wound closure. Cotton sutures were definitely used in India by 600 B.C.[1], along with plaited horse hair, strips of leather and fibres from tree bark. Galen refers to sutures of silk[1] and catgut[2] being used about 1,800 years ago. Gold wire sutures were used in the Sixteenth Century and silver wire sutures in the Nineteenth Century. In the 1930's, steel wire replaced the weaker and more brittle silver wire[2,3] and cotton was reintroduced in time to provide a satisfactory substitute for silk during the Second World War[4].

The possibility of using mechanical suturing devices, to reduce operating time and for ease of application has always appealed to surgeons for both medical and economic reasons.

At the turn of this century, experimental studies began on the construction of such mechanical devices. In 1894 Abbe used intraluminal glass tubing to join blood vessels[5]. Fischer and Hultl invented the first surgical stapler in 1908. This stapler speeded surgical procedures, even though it weighed 5 kilogrammes[6]. Cushing developed ligating clips, made from silver wire, also in the same year[7]. Although improvements to the basic design of these surgical staplers have been made over the years, all these devices used metallic staples and clips, (silver, gold, steel or tantalum)[8]. This presented problems in that not only did these metallic staples and clips produce extensive inflammatory reaction in surrounding tissue and were very easily dislodged from vessels, but they also caused interference with subsequent post-operative x-rays, and remain as foreign material.

With a rapid growth in the synthesis of new polymers in the 1940's, new materials were available for use by surgeons for the benefit of both patients and surgeons. Although Lister was the first person to produce chromic acid treated catgut sutures, having controlled absorption rate[2], it was not until the 1960's that the first synthetic biodegradable polymer, poly(glycolic acid)[53], was used to make biodegradable sutures.

Sutures have been classified as either absorbable or non-absorbable. An absorbable suture is one which loses its entire tensile strength within 60 days and is degraded in body tissues to soluble products, disappearing from the implant site, usually within 2 to 6

months. A non-absorbable suture on the other hand retains its strength for longer than 60 days, is resistant to biodegradation, (or degrades very slowly), becomes encapsulated in a fibrous sheath, and remains in the tissue as a foreign body[37,38].

The advantages of using biodegradable fixation devices are numerous. Biodegradation of sutures, clips and staples, once their primary function has ceased, is obviously desirable, for both the patient and the surgeon, because it obviates the need for surgical removal, thus speeding patient recovery. Similarly, using biodegradable bone pins and plates, tailored and fabricated correctly could encourage tissue ingrowth, gradually replacing the degraded material with new tissue. For these reasons, there is a substantial incentive to develop biodegradable fixation devices. Indeed, activity in this area has increased in recent years. What does biodegradation mean, in context to the implantation of synthetic material into the body ?

1.2 BIODEGRADATION.

In a recent review by Holland et al [9], the various definitions of biodegradation have been reviewed. As indicated by these authors, there is considerable confusion in the literature as to the meaning of the term 'biodegradation'. Taylor[10] defines 'biodegradation' as breakdown of the polymeric material by living organisms or their secretions, a definition which is similar to those held by Williams[11], Potts[12], Griffin[13] and Kopek et al [17]. Pitt et al [14] and Gilbert et al[15] define biodegradation as simple hydrolytic breakdown of polymeric materials, whereas Gilding[16] on the other hand, reports that the term 'biodegradable polymer' is widely used to convey the meaning of any polymer that degrades in the human body.

Zaikov[18] in a recent article, considers the *in vivo* degradation of a polymer in terms of three degradative components, without giving a definition for biodegradation. The degradative components considered by Zaikov were water, (diffusivity), salt content, together with pH of the physiological environment and enzymatic attack.

In literature covering areas of controlled drug delivery devices, the term bioerosion (bioerodible), is widely used. Heller[19] defines bioerosion as the conversion of an initially water-insoluble material to a water-soluble material which may or may not involve major chemical degradation. Although Langer and Peppas initially made no distinction between bioerodible and biodegradable[21], the term 'erodible' was later discussed[22] in the context of the ability of the polymer matrix degradation to control the rate of drug release. Bioerosion involves physical loss from the polymer matrix, which may be brought about by a variety of physical and chemical processes.

In this work, because the polymer devices will be used in the physiological environment, a much broader definition that could encompass the general definitions of biodegradable and bioerodible is used. The physiological environment is very complex and intricate in design, providing a varied hydrolytic (salt content and pH) and enzymatic environment. It is probable that polymer implants in the body are likely to be degraded by both these agents.

However, it is important to point out here that for polymer degradation occurring *in vivo*, the degradation by-products are of paramount importance. They should be biocompatible i.e. nontoxic, non-immunogenic, non-carcinogenic and non-thrombogenic. Polymer degradation products can be eliminated by the kidneys, but there is a renal cut-off barrier which depends on the molecular weight and nature of the

polymer[126]. The term 'bioresorbable', (bioresorbability), has been recently coined to describe the degradation of polymers that can degrade to low molecular weight metabolites. These metabolites are then capable of entering metabolic pathways, without any risk of toxic reaction. The term bioresorbable has been adopted by some workers for poly(lactic acid), poly(glycolic acid) and copolymers, poly(malic acid) and polymers of other hydroxy acids of the Krebs cycle, (e.g. fumaric, citric, isocitric)[62,127,128].

The definition of biodegradation in this work will refer to hydrolytic and enzymatic degradation processes occurring in a polymer whereby the polymer degrades to nontoxic, low molecular species, which may be normal body metabolites.

1.3 BIODEGRADABLE POLYMERS.

Although a wide range of materials have been used for surgical fixation devices, current trends are towards the development of biodegradable materials for this function. Individual polymers which have been used for this purpose are now discussed, together with mechanisms of biodegradation.

Biodegradable polymers may be divided into natural and synthetic.

1.3.1 NATURAL BIODEGRADABLE POLYMERS.

Natural biodegradable polymers that have been used for surgical fixation devices are either protein or carbohydrate derivatives.

1.3.1.1 PROTEIN DERIVATIVES.

1.3.1.1.1 COLLAGEN.

Catgut sutures have been in use for a considerable time and are generally derived from sheep intestinal mucosa or the intestinal serosa of cattle. The major component of catgut is collagen with mucopolysaccharides and proteoglycans as minor components. Major drawbacks of catgut sutures have become apparent over the years. Due to their natural origin, catgut sutures vary greatly in tensile strength from batch to batch[23]. When left in tissues, its tensile strength varies tremendously and usually decreases quite rapidly, falling to zero between 2 days and 4 weeks. Catgut sutures cause inflammation, because the material contains foreign protein material. Many studies have demonstrated that catgut sutures excite the highest degree of tissue reaction, often severe enough to impede tissue healing[24,25]. The rate of absorption of catgut sutures is very erratic and unpredictable, (from a few days to several months)[31], and depends on the foreign body reaction or inflammatory response the suture material created and on the amount and types of by-products released during its degradation by hydrolytic and proteolytic enzymes[26]. The site of implantation also affects the rate of absorption of the suture, being rapidly absorbed if implanted into the peritoneum or mucus membrane, and somewhat slower in muscles. In addition, the presence of infection is also known to accelerate its absorption rate. Knot security of catgut sutures decreases very promptly, when exposed to body fluids and knotting tends to weaken or fray the sutures.

Lister introduced sterilization techniques for catgut sutures and suggested the chromatizing of these to increase their resistance to degradation. Alexander[27] prepared fray-resistant catgut sutures by chromatizing the catgut with 0.5 - 1% chromium trioxide

and then soaking in an aqueous solution of 1% gelatin prior to twisting. However, significant differences between plain and chromic catgut have not always been noted[28,29]. Bichon et al[30] coated catgut sutures with different polyester-based polyurethanes to reduce the degradation rate of the catgut. They found that a 50μm polyurethane coating based on glutaric acid polyester maintained its original tensile strength for 8 days, after which it decreased, as for plain catgut. The coating also reduced the strong inflammatory reaction observed in the first three days with non-coated catgut, but this occurred again later, although to a lesser extent.

The antigenic properties of catgut, coupled with an increased demand, prompted the search for an alternative biodegradable suture. The removal of the mucopolysaccharide component from catgut was found to lower its antigenicity. Novak[32] extruded collagen fibres from a solution of collagenous material. These fibres were further treated with dextran, to bind the fibres together, forming a monofilament suture, and also to provide protection from proteolytic enzymes.

Further improvements to the manufacture of collagen sutures were developed in the early 1960's. Collagen from bovine tendon or hide is first digested with ficin or protease and then swollen in dilute cyanoacetic acid[16]. The resulting viscous gel is extruded through an acetone bath for coagulation. The coagulated fibril is stretched, twisted and dried or treated with formaldehyde and chromic salts before twisting and drying. Collagenase is thought to play a role in the enzymatic degradation of collagenous materials, but its activity is reduced in the presence of either metal ions, which act as enzyme poisons, or by aldehydes. Use of collagen sutures is limited to ophthalmic applications, because the increased purity of the collagen matrix increases the crystallinity, reduces the water content and plasticizability, leading to increased rigidity and poor handling characteristics.

The collagen suture is manufactured in small diameter sizes and dyed blue for ophthalmic surgical use. Devi et al[33] describe various other methods for producing collagen sutures. Salthouse[26] suggests that collagen is degraded by the same mechanism as catgut.

Over the years surgeons have commented on the disadvantages of using catgut or collagen sutures for wound closure, because of unpredictability in tensile strength and absorbability, coupled with the adverse inflammation produced by these sutures. The use of catgut or collagen sutures in skin closure has been strongly discouraged because of bacterial infection[34]. Although, Halstead[3] in 1913 suggested that catgut sutures were obsolete, an opinion shared by Laufman[35], catgut sutures are still in use today, being particularly predominant in obstetrics and gynaecology[36]. This is possibly due to economic reasons (catgut sutures are particularly cheap in comparison to other synthetic sutures), but more likely because even today, surgeons are not using any scientific criteria in suture selection, but follow 'handed down' advice ('It works for me').

1.3.1.1.2 SILK.

Silk is a natural protein fibre, (fibroin), which is held together by a gum, (sericin). Silk sutures are made by first removing the natural waxes and gums, and then twisting or braiding the clean silk fibres to form the suture strands. It is usually dyed black and coated with wax or silicone rubber to reduce its capilliarity and improve handling[38]. Dermal silk sutures are coated with an insolubilized gelatin coating to prevent tissue ingrowth. Since silk is a foreign protein, it has the disadvantage of causing considerable tissue reaction. As such, silk is mainly used for skin sutures in areas where tissue reactivity is minimised by early removal of the sutures. When silk is used internally, it

often loses a third or more of its original tensile strength within six months[24]. Although silk starts to degrade within a few weeks[29], complete absorption occurs in nearly two years[39]. One reason for this long period could be because silk is a crystalline polymer. Clark[40] suggests that with the development of synthetic sutures, silk sutures should be replaced because of their inferior tensile properties and tissue inflammation, but because old habits die hard, silk sutures are still in common use.

1.3.1.2 CARBOHYDRATE DERIVATIVES.

Although many examples of carbohydrate derivatives are mentioned in the literature as possible materials for surgical sutures, few have been commercialised. The carbohydrate derivatives that have been proposed for use as suture materials are based on amylose, alginic acid, cellulose and dextran.

1.3.1.2.1 ALGINIC ACID.

Sodium and calcium salts of alginic acid are readily formed, sodium alginate being soluble in water and calcium alginate insoluble. Bonniksen[41] produced double alginates by randomly introducing sodium and calcium ions into alginic acid. Fibres of the double alginate were spun and used as surgical sutures. Although Bonniksen suggests that the alginate fibres were biodegradable, there is no mention of the degradation products, or time for total absorption.

Blaine[42] suggests that although gels, films, gauze and foam made from calcium alginate are biodegradable and cause minimal tissue reaction, sutures of calcium alginate have very low wet strength. Skelton et al[43] have also made sutures from calcium

alginate, but these are commercially not available.

1.3.1.2.2 AMYLOSE.

Barger and Mumma[76] extruded sutures of amylose under high temperature and pressure. Although they claimed that the sutures were biodegradable, neither the tensile strength or the degradation time of these was stated.

1.3.1.2.3 DEXTRAN.

Bishop[44] extruded fibres of the ethyl ether of dextran and suggested that these could be useful in surgery. Use of dextran as a binder of collagen fibres has been mentioned[32].

1.3.1.2.4 CELLULOSE.

Cotton sutures are still in use today and are prepared by twisting long staple fibres[38]. They handle well and exhibit good knot security but are the weakest of sutures currently used[4] and cause extensive inflammation. These sutures begin to lose strength within two weeks and are weakened and absorbed slowly within a few years[40]. Postlethwaite[39] suggests that the degradation of cellulose sutures is slower than that of silk sutures. This slow degradation of cotton arises from the high crystallinity, which in turn is due to hydrogen bonding, coupled with the presence of stable β-glycosidic linkages in cellulose (see Appendix A).

Chemical modification of the cellulose molecule, (the chief component of cotton and linen), to change its degradation rate has been attempted by several workers. Yackel and

Kenyon[45] oxidised cellulose using nitrogen dioxide, and found that oxidised cellulose degraded faster than native cellulose. Frantz[46] suggests the use of oxidised cellulose in haemostatic preparations. The degradation rate of oxidised cellulose was found to be related to the time of oxidation[46], i.e. absorption rate depends on the content of carboxyl groups. Cotton oxidised for 7 hours was absorbed in 4 - 6 weeks, whilst that oxidised for 21 hours was absorbed in 26 days. This oxidised cellulose contained 15 to 24% of carbonyl context and had mechanical strength far too low for surgical sutures.

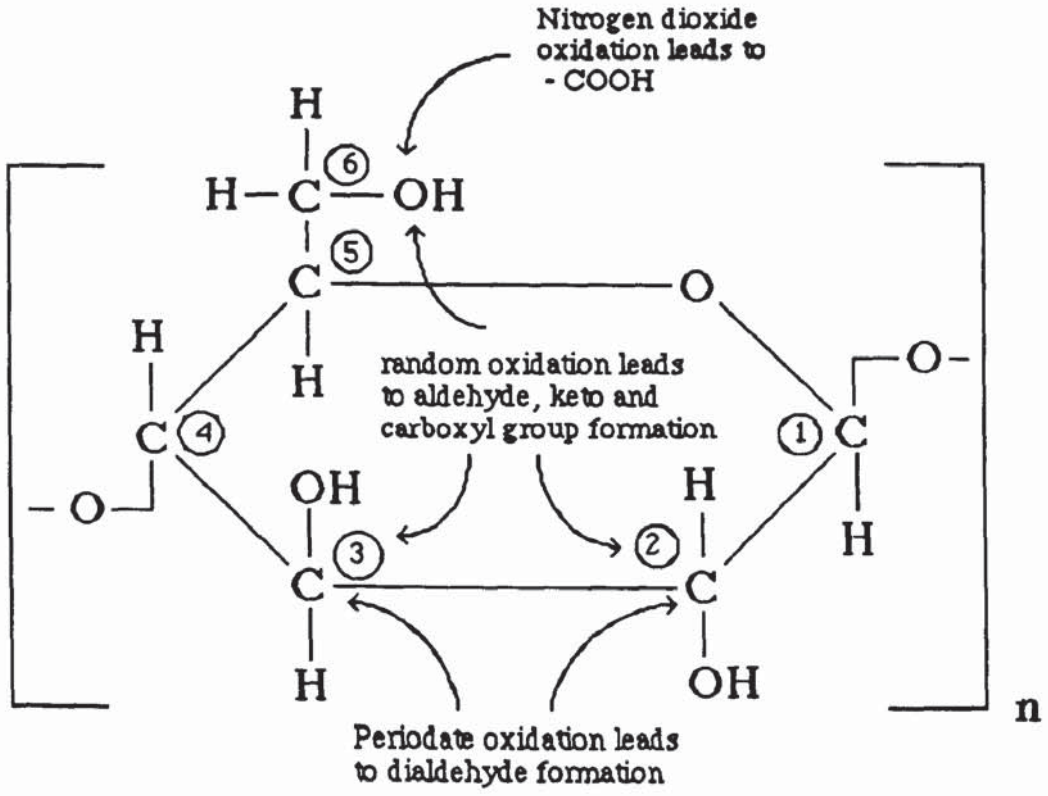


Figure 1.1 Various oxidation positions in the cellulose molecule.

Eberl[47] produced surgical sutures from oxidised cellulose, having a carboxyl content of 4 to 25%, but surgical manipulation with these sutures was difficult. Other work on surgical sutures from oxidised cellulose is also documented[33].

Smith[48,49] made resorbable sutures from cellulose acid ethers. Sodium salts of cellulose acid ethers, such as cellulose glycolic acid ether, were dissolved in acidic aqueous alcohol solutions to free the acid ether. The carboxyl group of the acid ethers were crosslinked with polyvalent metal salts derived from aluminium, chromium, iron or zinc. Strands of these crosslinked acid ethers were extruded, stretched and heated under tension to produce absorbable sutures. These sutures were absorbed in 4 to 90 days, the rate of absorption being affected by the cation. Sutures made with aluminium cations absorbed in 4 days. Various oxidation points on the cellulose molecule are shown in Figure 1.1.

1.3.2 SYNTHETIC BIODEGRADABLE POLYMERS.

Many synthetic biodegradable polymers have been developed over the years, although the major polymers used for fabrication of surgical fixation devices that have found commercial acceptance are based on polyesters and polyamides, (Table 1.1).

Other synthetic polymer derivatives that have been used for biodegradable sutures are mentioned by Kronenthal[50]. These are derivatives of polyvinyl alcohol, polyaminotriazoles and polyamino acids. Although sutures of polyglutamic acid were relatively weak and readily absorbed, the corresponding methyl and benzyl esters were stronger but not sufficiently absorbable. Crosslinking polyvinyl alcohol with formaldehyde is stated to increase the dimensional stability of the fibres. Other attempts at changing the degradation rate of polyvinyl alcohol fibres, by combination with various polymers such as cellulose, starch, gelatin and albumin are also mentioned.

The amide and ester groups present in polyamides and polyesters, respectively, are both

susceptible to hydrolytic degradation. The hydrolytic instability of the individual polyesters and polyamides depends, amongst other factors, on specific molecular structures and hydrophilicities[11]. Aromatic polyesters, such as polyethylene terephthalate (PET), in general, are less sensitive to moisture than aliphatic polyesters,

Table 1.1 Synthetic Biodegradable Polymers.

Polymer name	Commercial name	Structure
Poly(ε caprolactone)	----	$--(-O-CH_2)_5-CO--)_n$
Poly(glycolic acid)	Dexon® (sutures)	$--(-O-CH_2-CO--)_n$
Poly(lactic acid)	----	$--(-O-\underset{\displaystyle Me}{\overset{\displaystyle H}{\overset{\displaystyle |}{\underset{\displaystyle |}{C}}}}--CO--)_n$
Poly(glycolic-co-lactic acid)	Polyglactin 910 or Vicryl® sutures, Lactomer staple	$-(O-CH_2CO-O-\underset{\displaystyle H}{\overset{\displaystyle Me}{\overset{\displaystyle |}{\underset{\displaystyle |}{C}}}}-O-)_n$
Polydioxanone	PDS (sutures) Absolok® (clip)	$-(-O-(CH_2)_2-O-CH_2-CO-)_n$
Poly(hydroxybutyrate)	Biopol®	$--(O-\underset{\displaystyle Me}{\overset{\displaystyle H}{\overset{\displaystyle |}{\underset{\displaystyle |}{C}}}}-CH_2-CO--)_n$
Poly(hydroxyvalerate)	(used as comonomer with the hydroxybutyrate)	$--(O-\underset{\displaystyle Et}{\overset{\displaystyle H}{\overset{\displaystyle |}{\underset{\displaystyle |}{C}}}}-CH_2-CO--)_n$
Poly(caproamide)	Nylon 6	$-(NH-(CH_2)_5CO--)_n$
Poly(hexamethylene adipamide)	Nylon 6/6	$-(NH-(CH_2)_6NHCO-(CH_2)_4-CO)_n$

because of the greater hydrophobicity of the aromatic group, and indeed aliphatic polyesters are known to degrade faster than aromatic polyesters. PET fibres are reported

to lose only 50% of their tensile strength in 10 ± 2 years, and it is estimated in dog and man, total absorption takes 30 ± 7 years[54]. At the other extreme, sutures of glycolic acid are completely absorbed within 4 months[20].

A detailed study of the individual polyesters and polyamides that have found extensive use as biodegradable surgical fixation devices will be presented here.

1.3.2.1 POLYAMIDES.

Polyamides have received attention as biodegradable polymers because of the general assumption that the amide linkage is subject to attack by both nonspecific amidases and hydrolysis.

Both Nylon 6, (poly(caproamide)) and Nylon 6.6, (poly(hexamethyl adipamide)), in the form of monofilament sutures are used for wound closure. They are known to degrade very slowly over several years in physiological environment, although they are classified as non-absorbable sutures. They have good handling properties and cause minimal tissue reaction[34]. Williams[129] found that Nylon 6,6 degrades faster in tissues of an acute inflammatory response. Degradation of Nylon 6 is characterised by surface cracks soon after implantation, by bulk aqueous amide cleavage, and by enzyme-catalysed surface erosion[130]. Gilding[132] and Williams[11] describe the mechanisms of the hydrolytic degradation of Nylon 6.

1.3.2.2 POLY (α-ESTERS).

Polyesters, in particular those derived from α-hydroxy carboxylic acids, such as glycolic

acid, were noted by William Carothers[55] to be potentially low-cost, tough, fibre-forming polymers, with one disadvantage, hydrolytic instability. This unique property of poly(α-hydroxy acids) has been used since 1966 for the fabrication of biodegradable surgical fixation devices[51].

Hydroxy acids, being bifunctional molecules, may be condensed, (by direct self polyesterification), to form low molecular weight poly(α-hydroxy acids), e.g. from lactic and glycolic acids. However, lactic and glycolic acid on mild heating form cyclic dimers, known as lactides and glycolides, respectively. These cyclic dimers, when subjected to a catalytic ring-opening procedure, polymerise to high molecular weight poly(α-hydroxy acids).

1.3.2.2.1 POLY(GLYCOLIC ACID) (PGA).

The first synthetic absorbable sutures were made from poly(glycolic acid), in 1967[53], and since 1970 these sutures have been available commercially under the trade name Dexon®. PGA is the most hydrophilic of all the polyesters and with a high molecular weight, (Mw > 20,000), is a hard, tough, crystalline polymer, melting at about 224-226°C with a glass transition temperature, (Tg), of 36°C. PGA is insoluble in most common polymer solvents. PGA having an Mw range of 20,000 to 145,000 is useful for fibre extrusion[20] and such a high molecular weight polymer is synthesised by the ring opening polymerisation of glycolide at 220°C, using a tin catalyst[20], (Figure 1.2). A recent patent[119] discloses the synthesis of PGA from formaldehyde and carbon monoxide in the presence of methanesulphonic acid, as a catalyst.

Extensive work relating to the degradation of PGA, (mostly in the form of Dexon® sutures), has been reported[61,79,81-84,114-116,121-125]. The normal crystallinity of Dexon® sutures has been reported to be 50%[61], and this high crystallinity not only controls the mechanical properties of the polymer, but also its biodegradation characteristics. A further consequence of the high crystallinity is that Dexon® sutures are

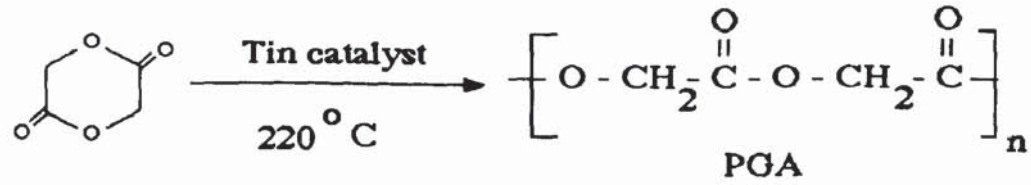


Figure 1.2 Synthesis of PGA by ring opening of the glycolide

rigid and are available as multifilament (braided) sutures. Handling characteristics of Dexon® sutures are reported to be superior to those of catgut sutures.

In vitro degradation of Dexon® sutures has been investigated by several workers, amongst these have been Chu[81-84], and Reed and Gilding[61]. Chu[81-84,114-116] describes the *in vitro* degradation as a two-stage process. The first stage from Day 0 to Day 21, consists of diffusion of water into amorphous regions of the polymer, with random hydrolytic chain scission of the ester groups occurring. As a result of the amorphous regions being degraded, an apparent increase in the crystallinity occurs. This is explained by the chain fragments of the hydrolysed amorphous regions having a lesser degree of entanglement, and by their ability to re-align themselves. The second stage starts from Day 21 to Day 49. Maximum crystallinity occurs around the transition between the first and second stage of degradation. By approximately Day 49, tensile strength, (TS), was lost completely, although 58% by weight of the suture remained.

The rate of hydrolysis is increased in strongly alkaline environments[82,114-116] with respect to normal physiological or acidic conditions. A growing oligomeric fraction of the molecular weight distribution, (MWD), as the degradation proceeds is indicated by Reed and Gilding[61], using gel permeation chromatography (GPC).

Effects of buffer salt concentration on PGA degradation have been reported by Zaikov[18]. The rank order of degradation rates being : 1 mol/l concentration (buffer) > *in vivo* (rabbit) > 0.5 mol/l > 0.1 mol/l >> water.

The effect of enzymes on the degradation of PGA *in vitro* has been reported by Williams[117]. Of a total fourteen enzymes tested *in vitro*, four enzymes, namely ficin, carboxypeptidase A, alpha-chymotrypsin and clostridiopeptidase A, affect the rate of degradation of PGA. The studies of Salthouse and Matlaga[79] revealed the presence of levcine aminopeptidase and acid phosphatase at the site of PGA implants, and in addition Herrmann[118] suggests that tissue esterases play an important part in PGA degradation. Williams[125] suggests that the presence of bacteria reduces the rate of degradation of PGA sutures, both *in vitro* and *in vivo*.

Sharma and Williams[121] demonstrated that lipids, (e.g. butyric, caproic, heptanoic and stearic acids), can increase the initial degradation of PGA sutures *in vitro*. The authors suggest that both lipids and enzymes play a part in the initial degradation of PGA sutures.

Recent work by Williams[122] has found a sharp difference between the *in vitro* and *in vivo* degradation rates, (as measured by tensile strength), of PGA sutures over the first two days. *In vivo* (rat) studies showed an appreciably higher reduction in TS over the first two days, after which loss followed *in vitro* TS-time profile results. When samples

were exchanged after two days the same enhanced reduction in TS occurred *in vivo*, whilst the *in vitro* sample showed the expected reduction in TS.

Sterilisation of PGA sutures by gamma irradiation has been shown to decrease the molecular weight of the PGA[83], and as a consequence, degradation of PGA has been shown to be accelerated[123].

Possible mechanism for PGA degradation is discussed by Chu[115] and by Gilding and Reed[75]. Glycolic acid (GA) produced as a result of the hydrolysis of PGA *in vivo* is metabolised in the tricarboxylic acid cycle and is eventually removed from the body by the lungs as carbon dioxide and water[120].

Surgical fixation devices of PGA, other than sutures, have not been commercialised because of the fast degradation rate of PGA. However, a recent patent[124] attempts to address this, by suggesting PGA/barium sulphate composites with a controlled *in vivo* degradation rate.

1.3.2.2.2 POLY(LACTIC ACID), (PLA).

Lactic acid is present in nature either as an intermediate or as an end product in carbohydrate metabolism. Lactic acid (LA) (Figure 1.3a) with its assymetric carbon atom is optically active, and so exists in both the D(-), L(+) and the racemic DL forms. The L(+) form is metabolised in the body. The terms D and L are derived by analogy from the structures of (-) and (+) glyceraldehyde, respectively, the (+) and (-) specifying the direction of rotation of the sodium D line. However, on dimerisation the direction of the sodium D line is reversed, hence the D(-) - lactic acid gives the D(+) - dilactide and the

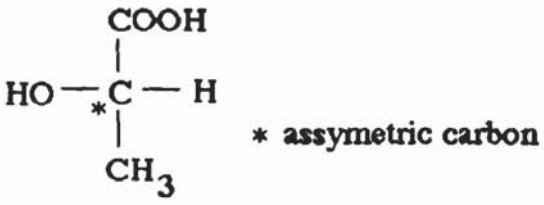
L(+) - lactic acid gives the L(-) - dilactide. Since ring opening of the lactides is commonly used in polylactide synthesis, (Figure 1.3b), an apparent complexity arises because poly(L(+) - lactic acid) and poly(L(-) - dilactide) are structurally equivalent. For this reason, the abbreviations P(D)LA, P(L)LA and P(D,L)LA are sufficient, unless it is specifically intended to show the synthetic origin of the polymer. Thus poly(L(-) - dilactide) and poly(D(+) - lactide) are sometimes used.

Poly(L)LA is a tough inelastic, semi-crystalline polymer having a crystallinity of ≈37%[61] and Tg and Tm of 36°C and 180°C, respectively. Combined with the effect of crystallinity and increasing hydrophobicity due to the extra methyl group compared with PGA, water uptake is restricted to about 2%[16] and degradation of P(L)LA is slower than that of PGA. Reed and Gilding[61] showed that in their work on P(L)LA *in vitro* degradation, 10 - 15% loss in weight occurred in 16 weeks. However, Vert et al[62] in a recent work throw considerable doubt on the results of Reed et al[61] and on those of several previous workers. The degradation of P(L)LA was found to be substantially longer than had been previously supposed, (<10% weight loss in 13 months). Indeed, these findings were substantiated by the work of Jamshidi et al[63], who reported that P(L)LA fibres exhibited no change in either tensile strength or weight for 5 months, at 37°C in pH 7.4 phosphate buffer.

Poly(D,L)LA is amorphous, a property which can be used to good effect in drug delivery applications[9], since it has only one morphological phase, which allows an even distribution of the active component throughout the device. P(D,L)A undergoes a solid-melt transition in the region of 130-135°C. The D(-) monomer is relatively costly and has received little attention for polymer synthesis.

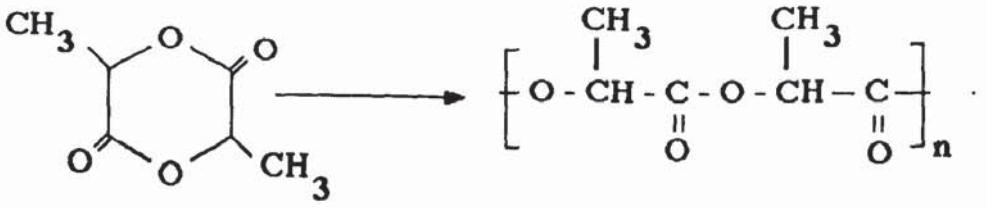
Kulkarni et al [51,52] prepared both P(D,L)LA and P(L)LA, through ring-opening of the lactides, and investigated their potential use as surgical repair material in the form of films, rods and sutures. Pins from these polymers were used for fracture repairs in dogs[52]. The P(L)LA was found to degrade slower than the P(D,L)LA because of its increased crystallinity. Tissue reaction around the implants showed minimal inflammatory response, and the pins virtually disappeared from the fracture site after 8 months.

Getter et al[57] reported on plates and screws of PLA for the repair of canine mandibular



Lactic acid

Figure 1.3a Lactic acid



Lactide **PLA**

Figure 1.3b Synthesis of PLA from ring opening of the lactide

fractures. The implants were found to be partially degraded after six weeks and completely absorbed after 32 weeks. Cutright et al[56] reported that cross sectional area of PLA sutures used in monkeys decreased from 8.9 mm^2 at 2 weeks to 0.3 mm^2 at 12 weeks. The same authors[58] employing sheets of PLA to repair blowout fractures of the

orbital floor in monkeys, found slight degradation of PLA after 38 weeks.

Recent work on the evaluation of poly(L(-)-Lactide) for bone fixation devices has been published by Tunc et al[64,65,66]. Leenslag et al[131] describe the preparation, physical and degradative properties of compression moulded plates and screws of P(L)LA. *In vivo* implantation after 11 weeks (in sheep and dogs), showed massive degradation of the polymer, which was attributed, among other factors, to the microporous structure of the implants.

Interest in the use of carbon-fibres for use as replacement tendons and ligaments has recently increased[67]. Carbon fibres were found to provide both strength and biocompatibility, but underwent premature mechanical degradation. To overcome this problem carbon fibres were coated with P(L)LA[68]. These composites of carbon fibre - PLA provide strength and are biodegradable. Evaluation of bone plates of carbon fibre - P(L)LA composites suggest that the rate of loss of mechanical properties with time would seem adequate for the canine but too rapid for human use[69]. However, as the bone plates degrade and become less rigid, stress atrophy is eliminated and also the need for additional surgery to remove the device.

A graft containing carbon fibres and an organic polymer, prepared from physical mixtures of P(L)LA and a segmented polyurethane has been used to repair large wedge-shaped meniscal lesions in dogs[70]. The porous P(L)LA - polyurethane matrix made the graft easy to handle and also promoted the ingrowth of tissue.

Holland et al[9] in their review mention work of several researchers who have used PLA for drug delivery. Asano et al[71] describe the release of a contraceptive drug from hot-

pressed P(D,L)LA discs. A mixture of P(DL)LA and powdered des-Gly^{10}-[D-Lev^{6}]-LH-RH ethylamide, a synthetic agonist of luteinizing hormone-releasing hormone (LH-RH), was melted at 70°C, the molten mixture charged into a teflon tube, and cooled to 20°C. This hot-pressed disc was implanted subcutaneously in the back of male Wistar rats. The release of LH-RH agonist was found to be slightly faster than the rate of *in vivo* degradation of P(DL)LA itself. Effective efficacy of the LH-RH agonist could be maintained during the first 7 weeks for P(DL)LA having a Mn of 1850 and during the first 11 weeks for P(DL)LA of Mn 2200.

Smith and Hunneyball[72] prepared microspheres, by emulsion techniques, of P(DL)LA containing prednisolone. Microparticles of P(DL)LA containing the same drug were prepared by melt mixing, cooling and grinding the resulting solid to 1 -10 μm. Release of prednisolone was found to be faster from the microspheres than from the microparticles. Melt fusion of the drug into P(DL)LA matrix resulted in the drug to be released slowly over 60 days. This could be due to a compaction effect of the drug in the matrix, which presents a greater barrier to water, impeding the release of the drug by diffusion.

Vert et al[62] list several factors which can affect the mechanical and biological properties of PLA implants. These factors are molecular weight, configurational structure, residual monomer and low molecular weight compounds, processing, annealing, sterilization and crystallinity. Indeed, as P(DL)LA is amorphous, it finds a use in drug delivery applications, but the same property renders P(DL)LA useless for sutures, as it possesses no dimensional stability. P(L)LA, on the other hand, because it is semi-crystalline, finds use as sutures. This also explains the relative difference in the degradation of P(DL)LA and P(L)LA, which are both degraded hydrolytically, by ester hydrolysis, in an aqueous media. Enzymatic degradation of P(L)LA has been investigated by Williams[73], who

suggests that several enzymes degrade PLA. Sutures of P(L)LA were subjected to various enzyme solutions. Hydrolysis of P(L)LA was faster in the presence of pronase, proteinase K and bromelian, than in ficin, esterase, trypsin, lactate dehydrogenase or even aqueous media.

Composites of P(DL)LA and hydroxyapatite for biodegradable bone fillers have been reported by Higashi et al[90]. Rat femurs were exposed and holed with a dental burr. A pellet of P(DL)LA/hydroxyapatite and P(DL)LA oligmer/hydroxyapatite was implanted in both femurs. *In vivo* studies showed that with P(DL)LA oligmer/hydroxyapatite composite, rapid absorption of the PLA oligmer occurred in 8 weeks and the incorporated hydroxyapatite appeared to play an active role in new bone formation. In P(DL)LA/hydroxyapatite composites, the P(DL)LA was absorbed at a slower rate (≈ 1 year), but the surgical defect in the cortical bone was completely repaired in 20 weeks.

Studies on degradation routes of PLA using radio labelled ^{14}C techniques[52,59,60] showed that as in the case of PGA, the polymer is eliminated via the respiratory route rather than in urine or faeces.

Use of the differing rates of hydrolysis of PGA and PLA has been exploited in the synthesis of PGA-PLA copolymers, which have been used for both controlled drug delivery systems and surgical fixation devices.

1.3.2.2.3 COPOLYMERS OF GA WITH LA.

Attempts to determine the degradation rate of (L)LA/GA copolymers quantitatively were

reported by Cutright et al [74] for (L)LA/GA copolymers of various ratios. Cylinders of 1.6 x 1.75 mm were inserted into the femurs of rats, rats sacrificed every 20 days up to 220 days, and the rate of degradation evaluated by histologically measuring height and width of the pellets. P(L)LA implants were 40% degraded at 220 days, whereas the LA/GA copolymer degradation rate was related to the GA content : 100% degradation at 180 days for 25% GA and 100% degradation at 120 days for 75% GA. PGA was only 18% degraded at 220 days, which is inconsistent with the degradation characteristics for PGA sutures.

Gilding and Reed[75] report that most of the copolymer composition range consists of amorphous polymers. The (DL)LA/GA series of 0 to 70% GA copolymer compositions are amorphous, whilst for the (L)LA/GA system 25 to 75% GA compositions are amorphous. They also found that for copolymers of (L)LA/GA system, over the whole compositional range of 0 to 100%, copolymer ratios rich in P(L)LA or PGA are very much more stable to hydrolytic attack than the intermediate compositions[61]. Gilding and Reed[75] predicted, on considering water uptake by the copolymer series, that as the 70% GA copolymer was the most hydrated, it would be the most likely to degrade preferentially.

Since LA/GA copolymers were first reported in the literature[77], extensive studies on sutures of these have been carried out. Commercial sutures of P(L)LA/GA copolymers made from 92% molar % GA and 8% LA, are known as Vicryl® or Polyglactin 910 (Ethicon Inc.). Craig et al[78] studied the *in vivo* properties of Polyglactin 910. In comparison to PGA sutures, although Polyglactin 910 sutures were found to be stronger, weight loss of PGA sutures was reported to be faster than Polyglactin 910 sutures. Salthouse and Matlaga[79] studied the *in vivo* absorption of Polyglactin 910 sutures and

the role of cellular enzymes in the degradation. They concluded that the *in vivo* degradation occurs by the same mechanisms as *in vitro* degradation, i.e. hydrolysis. They also postulated that *in vivo* hydrolytic degradation of Polyglactin 910 sutures would result in release of lactic and glycolic acids, which subsequently would be metabolised to carbon dioxide and water by macrophage oxido-reductase enzymes. Several radioactive studies show that the degradation products are eliminated mainly through respiration as carbon dioxide[59,80].

Recent studies on the effect of pH on the *in vitro* degradation of Polyglactin 910 sutures have been reported[81]. The suture was found to degrade significantly faster in a pH 10 buffer than in either pH 5.2 and pH 7.4. Sutures in pH 7.4 retained their breaking strength over a longer period of time, compared with sutures in pH 5.2 and pH 10. Chu[81] suggests that the hydrolytic degradation of Polyglactin 910 copolymer is catalysed by either hydrogen or hydroxy ions. In a comparative study investigating the *in vitro* degradation of PGA (Dexon®) and Polyglactin 910 (Vicryl®) sutures, following the affect of γ-irradiation on tensile properties and changes in pH, Chu[83] suggests that the degradation of these sutures involves more than one single stage. The first stage starts in the amorphous regions, and once these are degraded, the second stage starts in the crystalline regions[84]. Fredericks and co-workers[85] in their work on the *in vitro* degradation of Vicryl® sutures found that the first stage of hydrolytic degradation involving the amorphous regions lasts for the first 28 days, after which the crystalline domains are degraded. The degradation is accompanied by an increase in crystallinity which has a maximum value between the two stages. There was no preferential attack on either the lactide or glycolide units of the polymer backbone despite the difference in their hydrophilicity.

In comparison to other commercially available sutures, both Vicryl® and Dexon® sutures were found to have lost their tensile strength after 28 days in physiological conditions[82]. Interest in the use of absorbable surgical staples has grown recently, because of the obvious advantages of using such staples for wound closure. The advantage is reduction in overall operative time, avoiding necessity of successive procedure for removal which results in increase of patient comfort.

In 1982, the development of an absorbable staple, Polysorb 55 lactomer copolymer, (80% LA and 20% GA)[88], fitted to a TA 55 stapler (Auto Suture Co.), allowed the stapler to be used in areas in which steel wire was prohibited, such as the vagina or urinary tract[89]. Hirashima et al[86] report the use of lactomer copolymer staples in gastrointestinal surgery. The lactomer copolymer degrades by a hydrolytic mechanism and maintains a tensile strength greater than 50% for over 2 months, which is more than adequate for wound healing in intestine, bladder, vagina or uterine ligaments.

Recent patents suggest the injection moulding of surgical devices from various LA/GA copolymers. Kaplan and Muth[87] found that surgical devices made from high LA content and low GA content had strength and reasonable degradation rates because of low crystallinity. With increasing L(LA) content, the staples retained their strength and weight over a longer period of time. Smith et al[88] report on making surgical fasteners of the staple/receiver type from blends of PLA and PGA. PLA and PGA are dry blended and fed into the injection moulder, preferably in a ratio of 30% to 70%, respectively. The injection moulded staple has a crystallinity of 15 to 45%. The receiver is moulded from poly(p-dioxanone), and it is suggested that staples made from PGA/PLA blends, having a high GA content, are stiffer and degrade faster than both poly(p-dioxanone) clips and lactomer staples.

1.3.2.2.4 POLY(CAPROLACTONE), (PCL).

Poly(ε-caprolactone), (PCL), is a crystalline polymer with a melting point of 63°C and a glass transition temperature of - 60 to - 71°C [91]. Pitt, Schindler and co-workers[92] showed that PCL degrades *in vivo* similarly to P(D,L)LA, with random chain scission by ester hydrolysis; the process being autocatalysed by the generation of carboxylic acid end groups. The critical Mn at which linear molecular weight loss with time is disrupted with an accompanying loss of tensile strength is about 5000. No weight loss was observed until this same point. Work by Woodward et al[93] supports the idea that intracellular degradation of PCL is the principal *in vivo* degradation pathway once the molecular weight (Mn) of the polymer falls to 3000, whilst the work on the microbial degradation, (P. *pullans*), by Fields et al[94] shows the necessity of low polymer weight for enhanced degradation.

Pitt et al[92] and Jarrett et al [95] both found that the residual crystallinity of PCL decreases with time, with the amorphous regions of the polymer being eroded first : hence bulk erosion is predominant.

Extensive work involving the use of PCL in release of various drugs has been reported[9]. Commercial applications of drug release from PCL matrix have been exploited for long term contraceptive control[9], because *in vivo*, from an initial Mn of 50,000, three years are required for total removal from the body of PCL.

Because PCL of high molecular weight is a tough, flexible, crystalline polymer, (extension at break of ≈ 750%[91]), use of pure PCL in surgical fixation devices has been limited. Wise and Gregory[95] mixed fibres of PCL with zinc carboxylate and

reported that this composite had good potential as an orthopedic cement.

Various copolymers of PCL with PLA have been reported to degrade faster than the homopolymer[96,97,98] and their use in drug delivery systems is suggested. Copolymers of PCL and PGA have been recently described by Shalaby and Jamiolkowski[100]. *In vivo* degradation of sutures extruded from these copolymers showed that with high PCL content, breaking strength was retained over a longer period of time[101].

1.3.2.2.5 POLY(DIOXANONES) AND POLY(OXALATES).

Polymers in this series, some of which are lactide/glycolide hybrids, can be prepared from dioxane-diones and oxalates. Figure 1.4 illustrates their preparation and structures[9].

Augurt and co-workers prepared the unsymmetrically substituted 1,4-dioxane-2,5-diones[102,103]. The 3-methyl member of the series is a hybrid of half a lactide and half a glycolide molecule fused together. The 'dimer' is then polymerised to give a polymer with a similar GA,LA,GA,LA,..... sequence. The authors claim it is suitable for use as an absorbable surgical suture or as a bone pin.

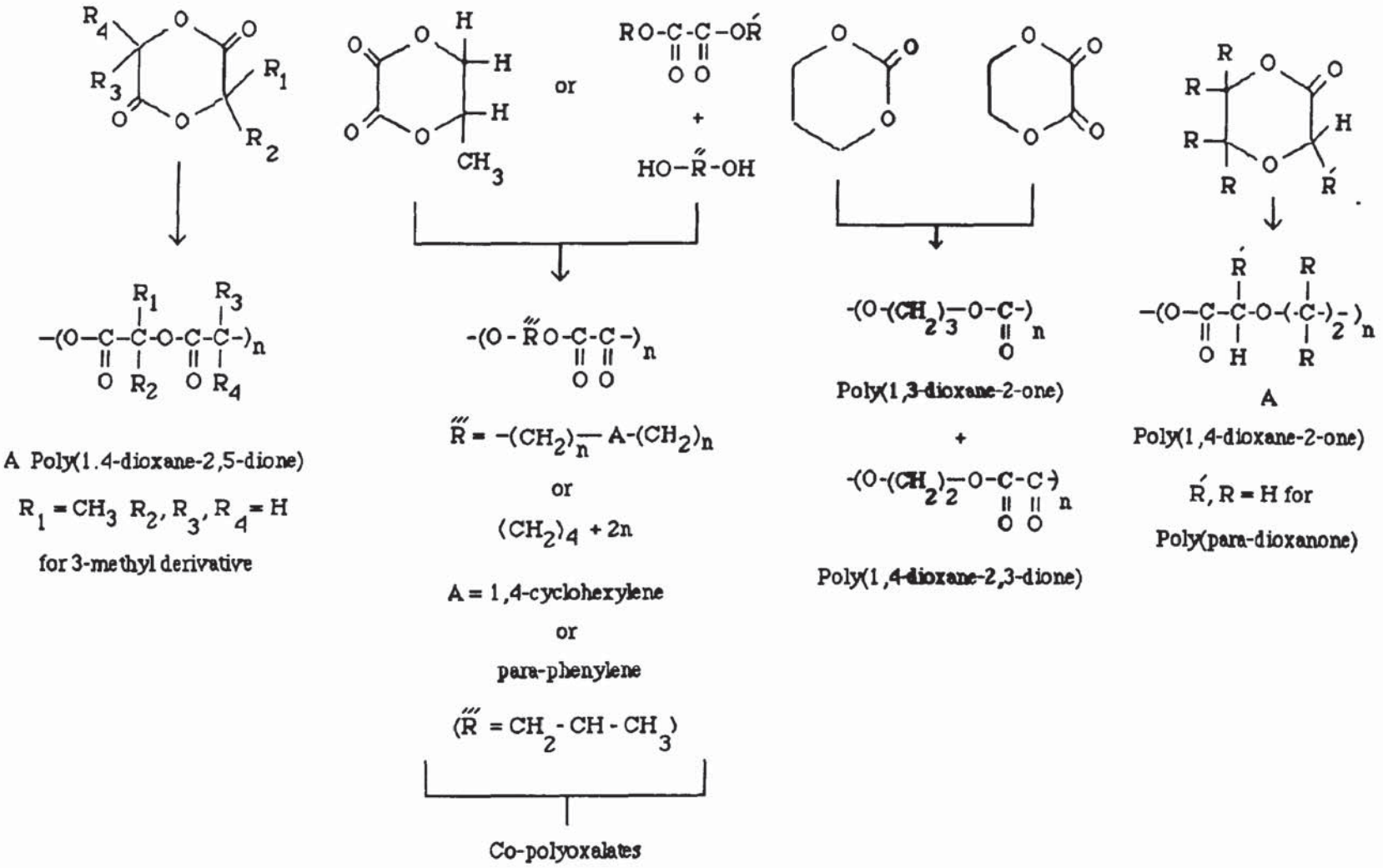
Shalaby and Jamiolkowski fabricated copolyoxalate polymers[104] and Gilding discusses a poly(oxalate) suture made by Ethicon[16]. The suture undergoes ester hydrolysis to yield oxalic acid and propylene glycol. Gilding states that the hydrophobicity lies between that of PGA and PLA, and, as it is less crystalline than PGA, he predicts absorption of a monofilament suture would be quicker than PGA,

(Dexon®) sutures. The copolyoxalate polymers listed by Shalaby and Jamiolkowski are prepared by ester interchange reactions between a mixture of diols and an ester of oxalic acid, preferably diethyl oxalate.

Rosensaft and Webb[105,106] prepared triblock polymer structures by copolymerising what they claim to be L(-)LA (=L(+)LA?) or 1,3-dioxanone-2-one (trimethylene carbonate, TMC) with GA to form TMC/GA/TMC (or LA/GA/LA) structures, with polymer chains rich in TMC for compositions of <15mol% TMC, >85% GA. Higher TMC ratios up to 57.5% mol% can be made. A decrease in *in vivo* (rat) tensile strength as the TMC ratio was increased from 48.5% to 57.4% was observed for these higher TMC/GA copolymers, and it is reported that these TMC/GA copolymers have a greater *in vivo* (rat) strength than PGA when in suture form. Poly(1,4-dioxane-2,3-dione) can be used instead of TMC. Rosensaft and Webb[105,106] say that the major use of these dioxanones is for making absorbable surgical sutures, and Katz et al[107] describe a new commercial suture material, Maxon®. Maxon® is a copolymer of TMC and glycolide (32.5% TMC), and is reported to have better handling properties than the equivalent PGA Dexon® suture. Maxon® sutures retained their breaking strength *in vivo* over a longer period than PGA sutures.

Poly(para-dioxanone) is primarily used as the absorbable suture material PDS[108], because it has superior TS properties to PGA, and unlike PGA and Polyglactin 910, it has the ability to form monofilaments. *In vivo* (rat) degradation work on PDS sutures[108] showed a slow linear cross-sectional area profile loss for five months, followed by a complete loss during the sixth month. Corresponding ^{14}C studies on weight loss showed that it is slow for the first twelve weeks, with major loss occurring between 12 to 18 weeks, and complete degradation after 26 weeks. Since there is

Figure 1.4 Various polydioxanones and polyoxalates[9]



correlation between the *in vitro* and *in vivo* degradation, (breaking strengths and molecular weight), this suggests that the degradation mechanism involves non-enzymatic hydrolysis of the ester bonds, i.e. homogeneous erosion. With a reported crystallinity of 37%[109], the degradation mechanism of PDS sutures could be similar to PGA, with the amorphous regions preferentially eroded. However, ^{14}C studies[108] indicate that *in vivo* degradation products, unlike those of PGA and PLA, are removed via urine (93%). As a result of the ether linkage, PDS sutures, unlike Dexon and Polyglactin 910 sutures, are flexible and as such are available as monofilament sutures in a full range of sizes. Several clinical investigations of PDS sutures in wound closure of a wide range of surgical procedures have been reported[110,111]. A ligating clip made from PDS has been reported by Schaefer et al[112]. They observed that the absorption of PDS in the clip form was extended to approximately 210 days and the main degradation product was 2-hydroxyacetic acid. Minimal tissue reaction was observed.

Compatible blends of PDS with poly(alkylene phenylene-bis-oxyacetate) have been prepared by Koelmel et al[113]. PDS and poly(ethylene 1,4-phenylene-bis-oxyacetate), (PEPBO), were melt blended in a single screw extruder. The PDS/PEPBO blends, containing 5 to 20% PEPBO, were fabricated into sutures. With increasing PEPBO content, *in vivo* (rat) degradation of these sutures decreased.

1.3.2.2.6 POLY (D(-)-3-HYDROXYBUTYRIC ACID) (PHB).

Poly-β-hydroxybutyric acid is an optically active aliphatic polyester produced by different strains of bacteria, such as Azobacter *beijeinckii*[133], Bacillus *megaterium*,

Rhodospirillum *rubrum* and Bacillus *cereus*[134]. The polymer serves as an energy and carbon storage product in much the same way as glycogen in mammalian tissue[150]. Grassie et al[135] describe how low molecular weight PHB may be prepared synthetically. Although Lemoigne[134] was the first to isolate PHB from cells of Bacillus *megaterium* in 1927, only recently has it been possible to obtain this thermoplastic polyester in reasonable amounts. ICI have developed pilot scale facilities to manufacture PHB from Azobacter *beijeinckii* cultures[136,150], using a variety of feedstocks, such as hydrolysed corn syrup[145]. These bacteria have been persuaded to make a range of entirely novel PHB random copolymers with polyhydroxypentanoic acid, (which is more commonly referred to under its trivial name of polyhydroxyvaleric acid (PHV)), from 0 to 30% PHV, which are available under the trade name Biopol®.

The PHB-PHV copolymers exhibit an unusual property known as isodimorphism[148,152], which means that the repeat units of each polymer can be accommodated in the crystal lattice of the other, resulting in copolymers that have high crystallinities (>60%)[148].

Gilding[133] found that PHB from Azobacter *beijeinckii*, with a molecular weight > $2x10^6$, showed a decrease in molecular weight after processing, and *in vitro* hydrolysis resulted in no apparent degradation after six weeks. However, early work by Kronenthal[137] showed an onset of degradation, *in vivo* (in an unspecified species), after eight weeks. Degradation studies of PHB, apart from this, have been scarce in the literature. Holmes[150] gives a broad indication about the extent and nature of enzymatic involvement in the degradation of PHB and PHB-PHV copolymers.

Recent work by Williams et al[138] on the *in vitro* and *in vivo* (rats) degradation of

PHB and PHB-PHV sutures, (of unspecified molecular weights), showed no apparent change in tensile properties over a six month period. The thermal degradative properties of PHB have been extensively reported by Grassie et al[135,139,140].

With the recent increased availability of PHB and PHB-PHV copolymers various studies have been reported. Unlike conventional thermoplastics, there are no catalyst residues in PHB polymers. The major impurities are inorganic nitrogen, phosphorus and sulphur-containing compounds, which are present at concentrations of less than 200ppm[144]. As a result of the high purity, PHB has been studied as a model system of spontaneous polymer nucleation and crystallisation[141,142]. The PHB-PHV copolymers have been similarly studied[144,145,146,148,149]. The piezoelectric properties of PHB and PHB-PHV copolymers have been investigated by Fukada and Ando[143] and nuclear magnetic resonance used by Doi et al[147] to determine the sequence distribution of monomeric units in PHB-PHV copolymers.

PHB and particularly its copolymer series with PHV, have reasonably good melt stability and therefore can be processed using conventional polymer melt processing techniques[136]. They should therefore be suitable for exploitation as melt processable biodegradable polymers for surgical fixation devices.

1.4 SCOPE OF THIS WORK.

The aim of this work at the outset is to produce biodegradable, melt processable precursors, which could be used to fabricate biodegradable surgical fixation devices, having controlled degradation profiles.

Synthesis of new polymers for a particular need can be carried out either by :

a) developing new monomers and different polymerisation techniques; or

b) by physical blending of two or more polymers.

Poly (α-esters), such as PGA, although they are biodegradable, are brittle, difficult to melt process, and added to this, the degradation rates of these are difficult to control. Additionally, because these polyesters have to be melt processed at relatively high temperatures, (due to the high melting points), thermal degradation of these polyesters is likely to cause a reduction in their molecular weights, which will affect the properties of the subsequent moulded article. As such, injection moulding of PGA and PLA is carried out under rather complex conditions (e.g. vacuum and nitrogen atmosphere are normally used), to reduce thermal degradation.

Ballard and Tighe[151] developed a novel technique to synthesise poly(α-esters) via the cyclic anhydrosulphite derivative of the appropriate α-hydroxycarboxylic acid. The anhydrosulphites can be converted to poly(α-esters) through ring-opening polymerisation, which is catalysed by bases, aprotic and protonic nucleophiles.

Esterification of naturally occurring biodegradable carbohydrates, such as amylose and cellulose, has resulted in a very useful group of thermoplastic materials (e.g. cellulose acetate). Although these esters are melt processable, the degradation rates are very slow.

As the basis of one strategy it is hoped that by combining the melt processability of derivatised amylose and cellulose with the rapid hydrolytic instability of poly(α-esters), new melt processable, biodegradable precursors could be made via ring-opening

polymerisation of anhydrosulphites (the catalysis being achieved by the abundant hydroxyl groups present in the polysaccharides). These precursors could be used to fabricate surgical fixation devices. Attempts at this chemical modification are described in Chapter 7 of this thesis.

As part of a second strategy an initial physical blending study (Chapter 3), suggested that this method could be used to change the degradation properties of a polymer. The Biopol® range of polyesters are stated to be biodegradable and have melt processability similar to that of polypropylene. These polyesters were chosen as the matrix polymers for further work. Other advantages of using the PHB-PHV series of polymers in physical blending are:

a) availability of the polymers in large quantities;

b) structural variation in the series and

c) availability of polymer in various molecular weights.

Very little information is available on the factors affecting the degradation properties of these copolymers. Investigation of such factors will be carried out using techniques, as they become available. The initial aim is to follow polymer hydrolysis by weight loss and water uptake by the samples.

Using polysaccharides having a wide range of solubility and degradation properties, it is hoped to make a range of melt mixed blends with the PHB-PHV copolymers. Physical and degradative properties of these blends are to be assessed using various techniques.

Blends of PHB-PHV with poly(caprolactone) will also be prepared, and physical and

degradative properties of these investigated.

It is hoped that this work would go some way towards a deeper understanding of the degradative pathway of PHB-PHV hydrolysis, and also provide an insight into methods of altering the physical and degradative properties of these copolymers with various fillers. Information thus gained, would be useful in developing melt processable, biodegradable material for specific end uses ('molecularly designed' biodegradable polymeric materials for specific uses), with a range of physical and degradative properties.

CHAPTER TWO

EXPERIMENTAL

2.1 INTRODUCTION.

This experimental chapter covers the materials and techniques used in the preparation of polymer blends, together with methods used to study and characterise their properties. Materials and techniques used to characterise products produced by chemical modification are also mentioned here, whilst the actual experiment work is covered in Chapter 7 of this thesis.

2.2 PHYSICAL BLENDING.

2.2.1 MATERIALS.

Polyhydroxybutyrate-co-polyhydroxyvalerate (PHB-PHV), copolymers of 0% to 20% hydroxyvalerate content and weight average molecular weights, (Mw), 36,000 to 800,000 used in this study were obtained from Marlborough Biopolymers, ICI, Teeside, UK, and are known under the trade name of "Biopol". Samples were supplied either in powder or "all-bran" form, in both nucleated (1% w/w Boron nitride or hydroxyapatite), and non-nucleated forms, and are listed in Table 2.1.

Polycaprolactone (PCL) was supplied by Aldrich Chemical Co. Inc. in pellet form, (weight average molecular weight of 54,500 and number average molecular weight (Mn) of 20,500). **Polyglycolic acid** was obtained in suture form, (Dexon"S" ®), from Davis & Geck (Cyanamid of Gt.Britain), Gosport UK, and both **polyglycolic-co-**

lactic acid (90/10 sutures, "Vicryl" ®), and **polydioxanone** (PDS ®, sutures) from Ethicon Ltd, Edinburgh, U.K. Other polymers used are listed in Table 2.2.

Table 2.1 Grades of PHB-PHV copolymers used.

Molecular weight (Mw x10^3)	% PHV Content	Batch number	Physical form
36	20	P/V/1 BXT/9/4-2	Powder
100	12	P/V/2 BXT/9/05/037-1	Powder
170	12	P/V/2 BXT/9/05/037-1	Powder
300	20	BX P/V/1-B	Powder
300	20	P/V/1	Powder(+1% Apatite)
330	20	BX P/V/11 + 1% HAP	All Bran(+1% Apatite)
350	12	P/V/2	Powder(+1% Apatite)
350	12	P/V/2BX P/V/2(T/9/0.5-27)	Powder
350	12	BX PV12T-58+1% HAP	All Bran(+1% Apatite)
400	7	BXP/V/7 (EE)	Powder
750	10	P7/46	Powder
800	0	BXG/V/9 (EE)	Powder

(N.B. The hydroxyvalerate (PHV) content was recalculated down by the supplier in 1986. Before this the 12% was 17% and the 20% was 30% PHV. If comparisons are made with previous published work on PHB-PHV copolymers, then this difference in the reclassification of the PHV content should be remembered).

Polymer	Grade	Supplier
Nylon-12	Vestamide L1600	Huls UK
Polypropylene	GMW 22	I.C.I
Polybutylene terephtahalate		Celenase

Table 2.2 Other melt processable polymers used.

Table 2.3 List of polysaccharides used.

Polysaccharide	Grade	Physical form	Supplier
Alginic acid		Particulate	Sigma
Amylopectin	A7780	Particulate	Sigma
Amylose	A9262	Particulate	Sigma
α-Cellulose		Fibrous	Sigma
Dextran		Fibrous	Sigma
Dextrin	80% soluble	Particulate	Sigma
Sodium Alginate		Particulate	Hopkins & Williams
Soluble Starch		Particulate	Hopkins & Williams

Table 2.4 Other materials.

Material	Grade	Supplier
α-Amylase		Sigma
Citric Acid	SLR	Fisons
Dextran Blue 2000		Sigma
Dipotassium hydrogen phosphate	Anhydrous, GPR	B.D.H.
Norwegian Talc		Anchor Chemical Co.
Potassium dihydrogen phosphate	Anhydrous, GPR	B.D.H.
PTFE		Bloore
Sodium Azide		Sigma
Sodium Carbonate	Analar	Sigma
Sodium hydrogen carbonate	Analar	Sigma
Methylene Iodide (Diiodomethane)		B.D.H.

The polysaccharides used in this study are listed in Table 2.3, and like all the polymers, were used without further purification. Other materials used are listed in Table 2.4.

2.2.2 BLENDING.

Conventional polymer processing machinery was used in preparing polymer blends. Initial feasibility of blending polysaccharides with polymers was investigated using the Hampden RAPRA Torque Rheometer (Chapter 3). Further blending was carried out on the Iddon 2-roll mill or the Bridge Micromill. Blending of the PHB-PHV copolymers with polycaprolactone was carried out only on the micromill.

2.2.2.1 HAMPDEN RAPRA TORQUE RHEOMETER.

This is essentially a small scale internal mixer (mixing screws contra-rotating), having a capacity of 36 grams. A mixing cycle of three minutes, rotor speed of 100 revs/minutes, and batch size of 35 grams was used to blend amylose and dextran with the appropriate polymer, at mixing chamber temperatures shown in Table 2.5. The mixing chamber was closed to the atmosphere by a pneumatically operated ram, to ensure efficient mixing.

Polymer	Mixing Temperature(°C)
Polypropylene (I.C.I., GX543M)	180
Nylon-12	178
PHB-PHV, copolymer (10% PHV, Mw = 750K)	165

Table 2.5 Temperatures used for the blending of polymers in the Torque Rheometer.

The blend was converted into pressed films and discs, following the methods in section 2.2.3.

2.2.2.2 IDDON 2 ROLL MILL.

The Iddon 2-roll mill (Iddon Brothers, Leyland, UK), is a double geared, two speed laboratory mixing mill, consisting of two robust horizontal, polished rolls, 6 inches in diameter and 12 inches wide.

A batch size of 40 g, milling time of 5 minutes and friction ratio of 1.3:1, (ie ratio of back roll speed to front roll speed), were the set conditions under which the polysaccharides were blended with the polymers, at the appropriate temperatures, as listed in Table 2.6. Table 2.7 shows the various loadings of the polymer blends produced.

Table 2.6 Temperatures used for the blending of polymers on the 2-roll mill.

Polymer	Temperature of back roll (°C)
Polypropylene (GMW22)	175
Nylon-12	173
PHB-PHV, 10% PHV, (Mw = 750K)	173
PHB-PHV, 12% PHV, (Mw = 350K)	134
PHB-PHV, 20% PHV, (Mw = 300K)	110

Weight of Polymer (g)	Weight of polysaccharide (g)	% polysaccharide in blend (w/w)
40	0	0
36	4	10
28	12	30
20	20	50

Table 2.7 Loadings used to prepare various polysaccharide blends.

Loadings up to 30% polysaccharides were blended only for the 12% PHV and 20% PHV

PHB-PHV copolymers. Milled blends were then fabricated into films, discs or into injection moulded samples.

2.2.2.3 THE BRIDGE MICROMILL.

The micromill, (David Bridge Co. Ltd., Castleton, Rochdale, UK), as the name suggests, is a scaled-down version of the 2-roll mill, (Plate 2.1), and unlike the former, it is ideal for efficient mixing of small quantity of material. Due to the small size of the rolls (2 inches in diameter and 6 inches wide), in comparison to the Iddon 2-roll mill, the pressure exerted on the polymers by the rolls is smaller. The slight disadvantage of this is that a higher roll temperature is needed to soften the polymers, than was previously used for milling on the Iddon 2-roll mill.

The blending of 12% and 20% PHV PHB-PHV copolymers, (Mw molecular weights 350,000 and 300,000, respectively), with the polysaccharides was carried out using a batch size of 40g, at 140°C and 128°C, respectively for the two copolymers, for 5 minutes. Loadings of 10% and 30% w/w polysaccharide blends were prepared.

Although the melting point of polycaprolactone is 63°C, this was blended with the above two PHB-PHV copolymers at the stated temperatures. Batches, 40g in size were prepared according to Table 2.8. With increasing amounts of polycaprolactone the blends became increasingly viscid, and as a result it was more difficult to take off the material from the rolls.

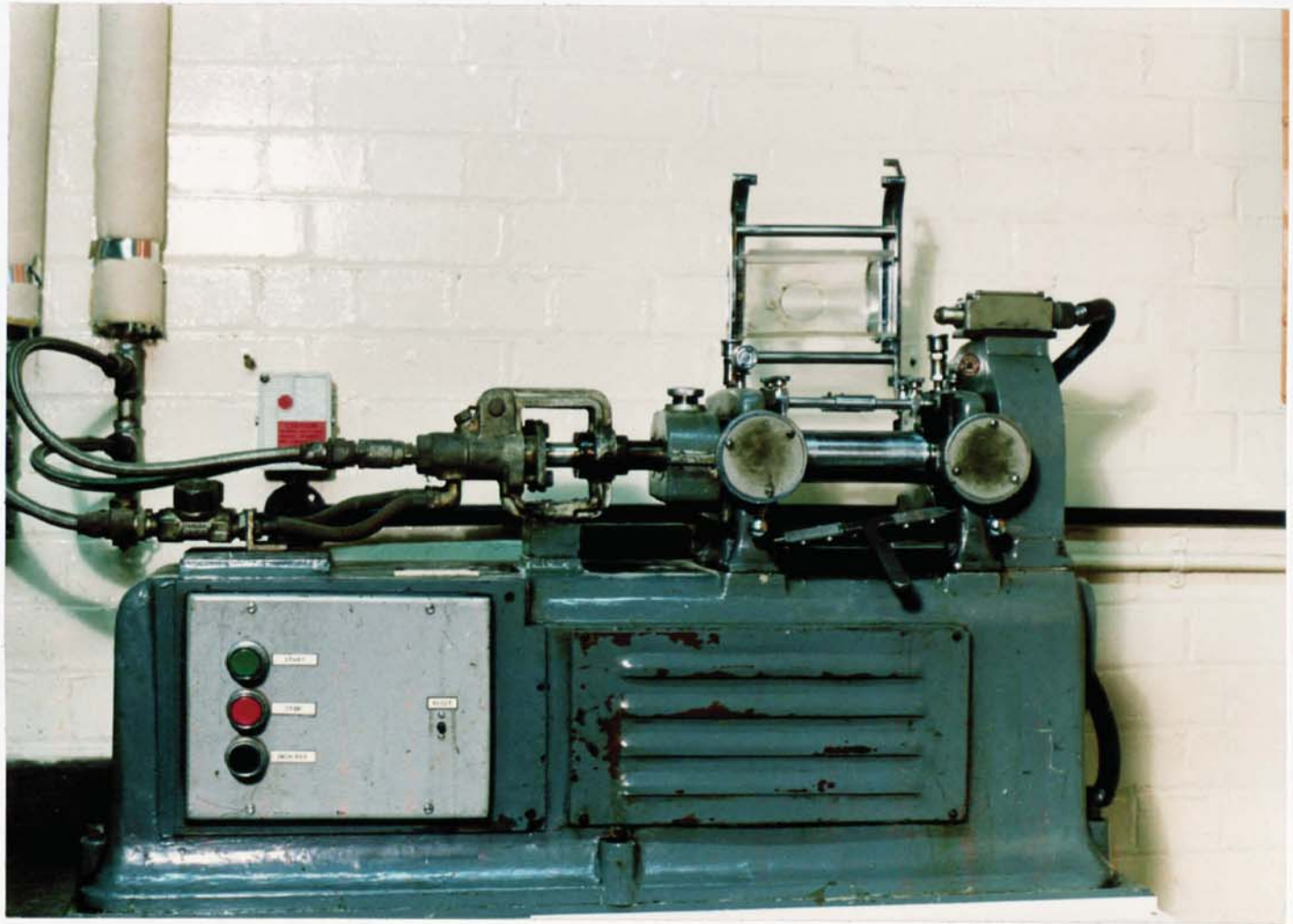


Plate 2.1 The Bridge Micromill

PHB-PHV copolymer (g)	Polycaprolactone (g)	% Polycaprolactone in blend (w/w)
40	0	0
36	4	10
26.8	13.2	33
20	20	50
13.2	26.8	67
4	36	90
0	40	100

Table 2.8 Loadings used to prepare PHB-PHV/Polycaprolactone blends.

2.2.3 CONVERSION OF POLYMER BLENDS.

Conventional compression moulding and injection moulding techniques were used to convert the polymer blends into either films, discs or injection moulded pieces.

2.2.3.1 MELT PRESSED FILMS.

Films of the polymer blends were compression moulded, using a electrically heated, self-contained upstroking hydraulic press (No. 205/SC, Bradley & Turton Ltd, Kidderminister, UK). The sample was placed between two steel plates lined with cellophane (used as mould release agent), and preheated to the appropriate temperature for $2^1/_2$ minutes (at zero pressure) to allow for the material to flow. Pressure was increased to 25 tons in^{-2} and maintained for 2 minutes. Immediately thereafter the platens were cooled to room temperature by running cold water, while maintaining full pressure. The pressed film was removed from the cellophane sheets. The temperature at which the individual polymers were pressed is tabulated in Table 2.9.

Table 2.9 Temperatures used for compression and injection moulding of pure and blended polymers.

Polymer blends	compression moulding (°C)	Injection moulding (°C)
PHB-PHV/Polycaprolactone blends	-	140 or 150
Polybutylene terephtalate/Amylose blends	236	-
Polypropylene (GX543M)/Amylose blends	180	-
Polypropylene (GMW22)/polysaccharides	175	195
Nylon-12/polysaccharides blends	180	-
PHB-PHV 800K Homopolymer (pure)	185	-
PHB-PHV 400K 7% PHV (Pure)	175	-
PHB-PHV 350K 12% PHV (blends)	140	150
PHB-PHV 300K 20% PHV (blends)	130	140
PHB-PHV 170K 12% PHV (pure)	140	-
PHB-PHV 100K 12% PHV (pure)	140	-
PHB-PHV 36K 20% PHV (pure)	130	-
Polycaprolactone (pure)	--	150

2.2.3.2 MELT PRESSED DISCS.

Discs were prepared accordingly to the above method, using a mould, (2 mm thick steel plate with 2 cm diameter holes evenly spaced). Dried material was loaded into the holes, cellophane film placed between the mould and the backing plates, and following the previous method of film preparation, discs were moulded.

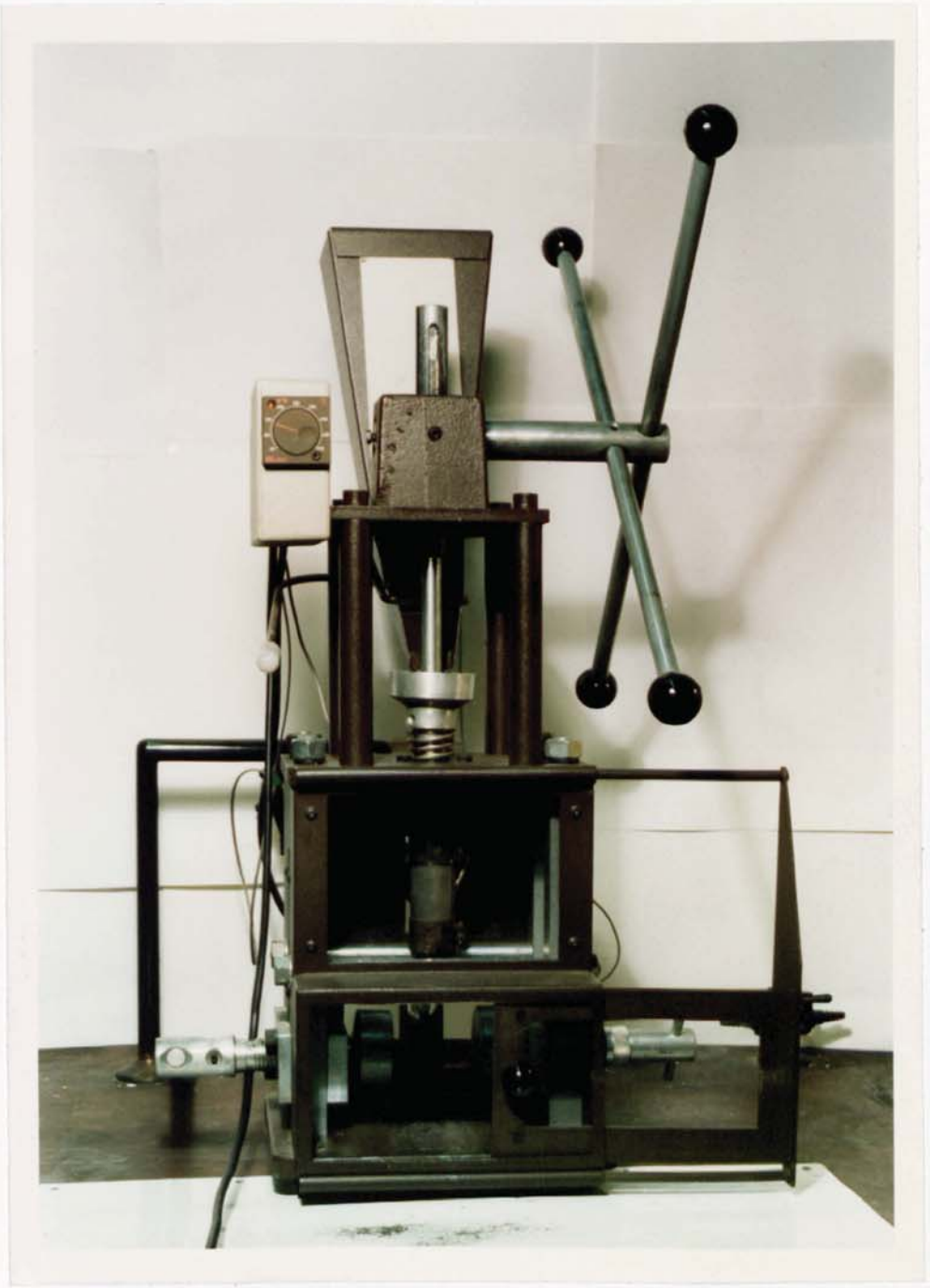


Plate 2.2 The SP 1 bench top injection moulder.

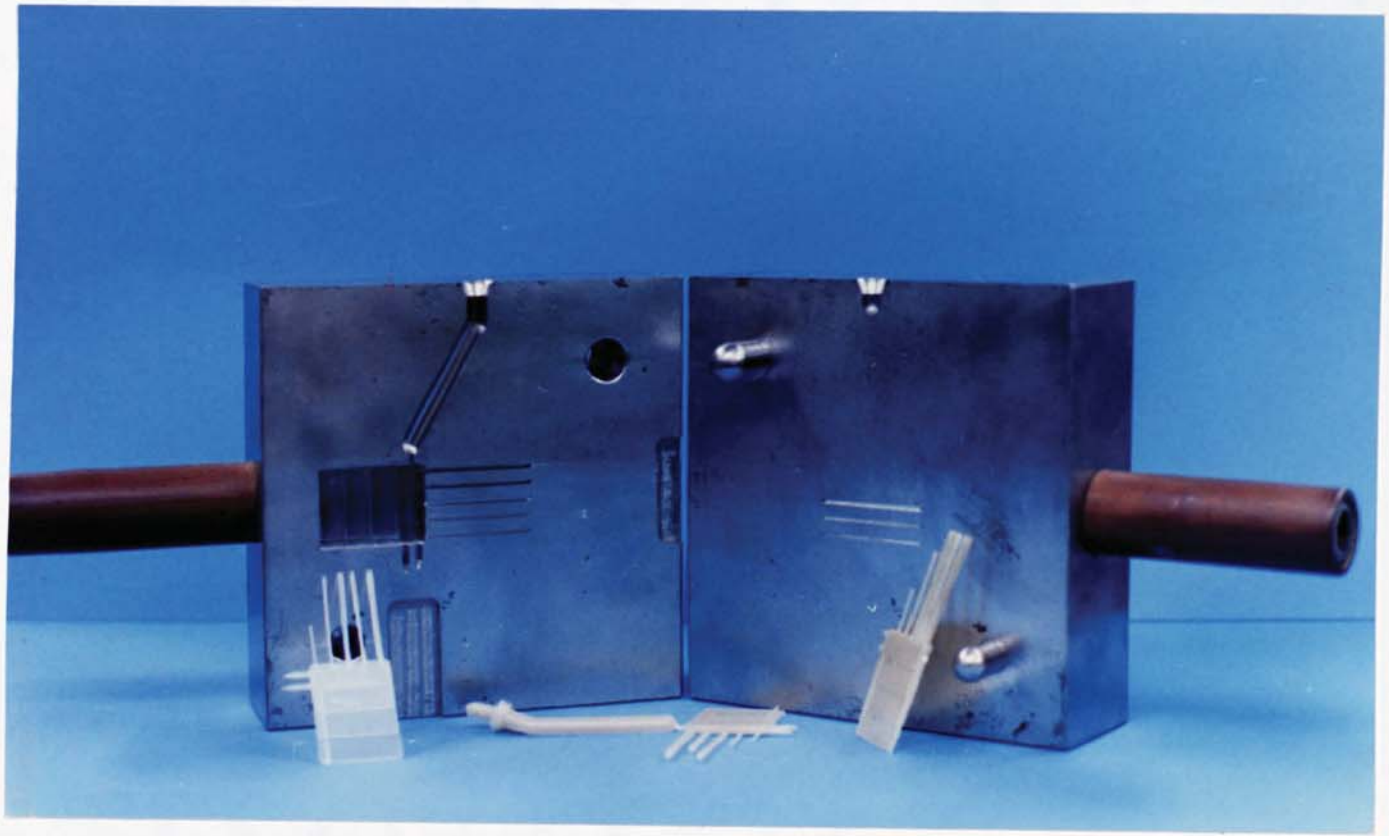


Plate 2.3 The mould used for injection moulding of samples used for the degradation studies.

2.2.3.3 INJECTION MOULDED SAMPLES.

Injection moulding of PHB-PHV/polysaccharide and PHB-PHV/polycaprolactone blends was performed, on a electrically heated bench top injection moulder (SP I, Small Power Machines Co. Ltd, Chippenham, UK), (Plate 2.2). For tensile properties, samples were moulded in a typical dumb-bell shaped mould. For degradation work the stepped mould illustrated in Plate 2.3 was used. The size of the injection moulded plaque made by this mould was 2.5 x 1.5 x 0.15 cm. The thickness of the stepped plaque varied from 1.5 mm at the thickest step to 1.25 to 1.0 and to 0.5 mm at the thinnest step.

The SPI is ram type, manually operated, injection moulder, having a shot size of 20 grammes. Nucleated PHB-PHV 12% (Mw = 350,000) and 20% PHV (Mw = 300,000) copolymers were injection moulded at barrel temperatures of 150°C and 140°C, respectively, using a hot mould (60 - 80°C). The increased valerate content in the 20% PHV copolymer, reduces nucleation rate, which in turn slows the crystallisation. Hence, injection moulding of pure 12% and 20% copolymers is not practical because of the length of time that would be needed before mouldings could be removed from the mould. PHB-PHV copolymers were obtained from ICI ready nucleated with 1% w/w boron nitride or calcium hydroxyapatite, or milled on the two-roll mill with 1% w/w Norwegian Talc. The polysaccharide blends with the PHB-PHV copolymers were injection moulded also at the above temperatures, using a 60°C heated mould.

The PHB-PHV/polycaprolactone blends were injection moulded at 150°C for the 12% PHV blends and 140°C for the 20% PHV blends using a heated mould (≈50°C). Pure polycaprolactone was injection moulded at 150°C, using a warm mould (≈25°C), due to the low melting point of polycaprolactone.

2.3 TENSILE PROPERTY TESTS.

Tensile testing of the injection moulded samples described in Chapters 4, 5 and 6, was carried out using the Instron tensometer, (model TM-M, Instron Ltd, High Wycombe, UK). Five injection moulded dumb-bell test pieces of cross-sectional dimensions 4×10^{-3} m by 1×10^{-3} m were used for each determination at room temperature (20°C). The cross-head speed was 5 mm min^{-1} and gauge length 10 mm. The following properties were evaluated for each sample, (Figure 2.1);

	Units	
Yield Strength (YS) = $\frac{\text{Load at yield (N)}}{\text{Cross sectional area } (m^{-2})}$	MPa	Equation 2.1
Initial Tensile Modulus (TM) = $\frac{\text{Initial stress } (Nm^{-2})}{\text{Initial strain}}$	MPa	Equation 2.2
Ultimate Tensile Strength (B) = $\frac{\text{load at break (N)}}{\text{cross-sectional area } (m^{-2})}$	MPa	Equation 2.3
% Elongation at Yield (EY) = $\frac{\text{extension at yield (m)}}{\text{original length (m)}} \times 100$ %		Equation 2.4
% Elongation at Break (EB) = $\frac{\text{extension at break (m)}}{\text{original length (m)}} \times 100$ %		Equation 2.5

The effect of hydrolytic degradation on the tensile properties of the injection moulded samples was monitored by immersing tensile pieces in aqueous pH 7.4 buffer solution at 37±0.1°C, (see section 2.4). Samples were periodically removed, washed with distilled water, dried with filter paper and tested as before.

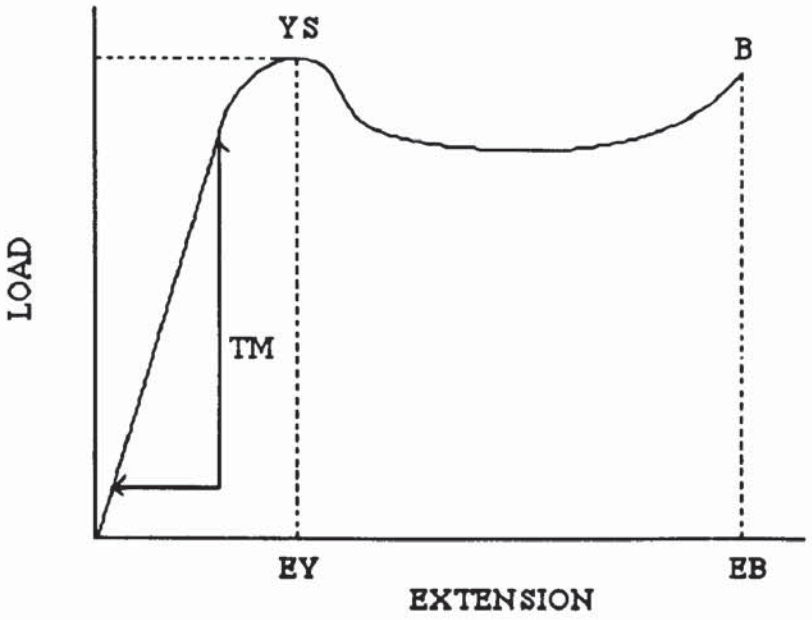


Figure 2.1 **Typical load/extension curve for a polymer showing the various parameters.**

2.4 DEGRADATION STUDIES.

2.4.1 HYDROLYTIC.

Three aqueous solutions were used for the hydrolytic degradation study of the samples, a) Citric acid/K_2HPO_4, b) KH_2PO_4/K_2PO_4 and c) $NaHCO_3/Na_2CO_3$, which provide acidic, common physiological and alkaline pH's. The buffers were made up according to the methods in Dawson[153] and the corresponding pH's of these buffers were 2.3, 7.4 and 10.6, at 37°C, respectively, which changed marginally at 50°C and 70°C. Samples were placed in small bottles containing 50 mls of buffer and maintained at 37±0.1°C, 50±0.1°C or 70±0.1°C, in water baths. The PHB-PHV/polycaprolactone blends were

maintained at 50±0.1°C for accelerated degradation studies. Samples were removed periodically, washed with distilled water and placed between filter paper to remove the surface water. The samples were weighed wet and again after drying overnight in a vacuum oven at 80°C. The PHB-PHV/polycaprolactone blends were dried at 50°C, under vacuum for 2 days. Polymer bulk degradation was monitored using the gravimetric technique outlined in section 2.5.1.

Although all the buffers were made up in distilled water, the pH 7.4 buffer was susceptible to fungal growth. To overcome this problem, pH 7.4 buffer used at 37°C had 0.1% w/w sodium azide added, as a preservative.

2.4.2 ENZYMATIC.

Amylose, soluble starch and to a lesser extent dextran are hydrolysed by α-amylase enzyme, which is present, for example, in blood plasma. Degradation of PHB-PHV/ polysaccharide blends of amylose and soluble starch was carried out by incubating injection moulded samples in 5 mls of 2% w/w α-amylase solution in pH 7.4 buffer (containing 10 mM NaCl), at 37±0.1°C for successive periods of 24 hour intervals. On removal, the samples were washed, surface water wiped, weighed wet and then after drying, returned to fresh enzyme solution.

2.5 DEGRADATION MONITORING TECHNIQUES.

Degradation of the samples was followed conveniently by gravimetric methods, (water uptake and dry weight loss). For the PHB-PHV/polysaccharide and PHB-

PHV/polycaprolactone blends, the additional techniques of goniophotometric analysis, scanning electron microscopy, (SEM), optical microscopy and contact angle measurements were used to monitor surface erosion. Whilst SEM is a destructive technique, the other techniques are non-destructive, so that degradation could be monitored continuously. Bulk degradation was also monitored by changes molecular weight and crystallinity.

2.5.1 GRAVIMETRIC.

Buffer uptake and weight loss of the samples subjected to hydrolytic or enzymatic degradation was followed by measurement of the wet (hydrated) and dry (dehydrated) weights. Samples were periodically removed from the appropriate buffers, thoroughly washed with distilled water, surface water removed by filter paper, and weighed wet and again after drying. The PHB-PHV/polycaprolactone blends were dried in a vacuum oven at 50°C for 48 hours, while the rest were dried at 80°C overnight. Buffer uptake and dry weight loss were calculated using Equations 2.6 and 2.7.

$$\% \text{ Change in wet weight} = \frac{\text{wet weight time T - initial dry weight}}{\text{initial dry weight}} \times 100 \qquad \text{Equation 2.6}$$

$$\% \text{ Dry weight loss} = \frac{\text{dry weight time T - initial dry weight}}{\text{initial dry weight}} \times 100 \qquad \text{Equation 2.7}$$

2.5.2 GONIOPHOTOMETRIC ANALYSIS.

This is a non-destructive, comparative technique for monitoring changes in surface gloss, and is in some ways complementary to microscopic techniques. It enables quantitative as

Figure 2.2 Diagrammatic representation of a Goniophotometer [154].

Plate 2.4 BBC microcomputer and Goniophotometer.

distinct from qualitative, information to be obtained, enabling surface changes to be followed during the degradation process.

The technique involves a collimated beam of monochromatic light impinging at an angle of 45° on to the surface of the sample together with a rotating photocell which records the intensity of scattered light as a function of scattering angles. The apparatus used in this study was an automated goniophotometer, which had been considerably modified[154]; a diagrammatic representation of this is shown in Figure 2.2 and Plate 2.4 shows the setup in practice.

Samples were mounted behind a 2 x 3 mm slit in a metal plate, which was at 45 degrees to the beam of collimated light. The intensity of surface reflected (scattered) light was measured by a photocell at angles between 0 and 138 degrees to the sample surface. The resulting intensity and angle of reflectance were relayed to a Sharp MZ80 microcomputer, and spectra of incident light intensity (I) against the reflectance angle (a) were plotted by the computer, together with the calculated relevant parameters that can be derived from goniophotometric curves. Modifications to the computer link were made during the course of this work, with a BBC microcomputer (model Master 512), replacing the Sharp. A more powerful program, coupled with the increased flexibility of the BBC microcomputer, enabled better presentation of goniophotometric curves.

2.5.3. CONTACT ANGLE MEASUREMENTS.

Surface energy parameters were obtained by contact angle measurements involving two

standard liquids, which have differences in their polar and dispersive surface energy components, on polymer surfaces. A drop of water was formed on the clean surface of the sample, mounted on a glass slide, using a hypodermic needle. The contact angle formed on either side of the drop on the surface, measured directly using a goniometer eyepiece fitted to a cathometer, and at least six readings were taken for each sample. This procedure was repeated using the other liquid, and from the measured (averaged), contact angle values for both of the liquids on the polymer surface, the polar (γ^p) and dispersive (γ^d) components of the surface free energies calculated using the Owens and Wendt method[155]. The calculations were assisted by using a computer program[156].

Water and methylene iodide were the pair of liquids used to measure contact angles of all the polymer blends. The values for the polar and dispersive components of the liquids used to calculate the surface tension components of the polymer surface are shown in Table 2.10[156];

	Polar (γ^p) (mNm^{-1})	Dispersive (γ^d) (mNm^{-1})	Total (γ^t) (mNm^{-1})
water	51.0	21.8	72.8
Methylene Iodide (Diiodomethane)	1.3	49.5	50.8

Table 2.10 The polar and dispersive components of surface free energy for water and methylene iodide.

2.5.4 . SCANNING ELECTRON MICROSCOPY (SEM).

The distribution of the polysaccharides in the PHB-PHV/polysaccharide blends and polycaprolactone in the PHB-PHV/polycaprolactone blends was investigated using the Stereoscan 5150 scanning electron microscope. Samples were coated with a layer of gold, using a spluttering technique to produce an electrically conductive surface.

SEM's of degraded samples were obtained to study the effect differing degradation conditions had on the morphology of the polymer matrix. Samples once gold coated for SEM could not be used for further degradative work, because SEM is a destructive process. SEM's were normally carried out on polymer samples that exceeded the useful degradation monitoring stage.

2.5.5 LIGHT MICROSCOPY.

Light microscopy, which could be regarded as a low magnification SEM, and visual (ie photographic) record of the different stages of degradation of the various polymer blends was also carried out. The Leitz DiaLux 20, microscope was used to record the topography of the blends (degraded and undegraded).

2.5.7 GEL PERMEATION CHROMATOGRAPHY (GPC).

Molecular weight and molecular weight distribution of the samples was performed by RAPRA Technology Limited, Shawbury, using gel permeation chromatography.

Chloroform was used as the solvent.

2.5.8 X-RAY DIFFRACTOMETRY.

Crystallinity of polymer samples was measured by x-ray diffractometry. X-ray diffraction measurements were made using a Philips x-ray diffractometer, operating at 40 kV and 20 mA. Nickel-filtered Cu K α radiation (λ = 0.1542 nm) was used.

The crystallinity was calculated from the diffracted intensity data in the range $2\theta = 10 - 50^{\circ}$, by using the method of Fredericks et al[85]. A baseline was drawn through the two lowest points of the curve (at 50° and 10°). The areas associated with the amorphous and crystalline regions were estimated from the trace. The percentage crystallinity was calculated using;

$$\% \text{ crystallinity} = \frac{Ac}{(Ac + Aa)} \times 100 \qquad \text{..... Equation 2.8}$$

where Ac is the area of crystalline reflections and Aa the area of amorphous scattering.

2.6 HYDROLYTIC DEGRADATION OF SUTURES.

Degradation of commercial sutures, Dexon "S"®, (multifilament, grade 5 metric), Vicryl®, (multifilament, grade 5 metric) and PDS®, (grade 2 metric, clear monofilament), was monitored by gravimetric methods. Lengths of sutures (50 cm), were placed in 50 mls of buffers (see sec 2.4.1), at 37±0.1°C. The sutures were removed periodically, washed thoroughly with distilled water, dried for several hours in a vacuum oven at 60°C, before being weighed.

2.7 CHEMICAL MODIFICATION.

2.7.1 MATERIALS.

Chemical	Supplier
Amylose	Aldrich
α-Cellulose	Sigma
Copper(II) Chloride GPR	B.D.H.
Glycolic Acid (solid)	Koch-Light Ltd.
Gylcolic Acid (67% w/v solution)	B.D.H.
Thionyl Chloride	B.D.H
Diethyl Ether (anhydrous) AR Grade	Fisons
1,4-Dioxane (dry)	Fisons
2,6-Dimethoxypyridine	Aldrich
Tetrahydrofuran (T.H.F.)	B.D.H.
Dimethyl Sulphoxide (D.M.S.O.) (dry)	B.D.H.
Dichloromethane	B.D.H.
Chloroform	B.D.H.
Dimethylformamide (D.M.F)	B.D.H.

2.7.2. METHOD.

The synthesis of glycolic acid anhydrosulphite (via the copper (II) glycolate route), and its subsequent use in the attempts at the chemical modification of amylose and cellulose are described in detail in Chapter 7.

2.7.3 INSTRUMENTAL TECHNIQUES.

Infra red Spectra - Perkin - Elmer infra-red grating spectrophotometers (models 297 and 599), were used to obtain spectra of samples either as KBr discs or as thin films between NaCl plates (for liquids). Air was used as a reference.

Nuclear Magnetic Resonance Spectra - Proton spectra were recorded using a Perkin-Elmer R12B spectrometer operating at 60 MH_Z. Tetramethylsilane (TMS) was used as the internal reference.

Differential Thermal Analysis (DTA) - DTA of the samples was performed using the Stanton-Redcroft (model 617A) DTA. A heating rate of 10°C per minute was used.

CHAPTER THREE

INITIAL FEASIBILITY STUDY OF POLYMER BLENDING

3.1 INTRODUCTION.

Polymer Scientists are continually involved with the improvement and development of materials to meet growing demands and needs of modern society, either by developing new monomers and different methods of polymerisation, or by combining existing polymers in such a way that the resulting material has certain properties superior to those of the individual components. The latter approach has led to a class of materials known as polymer blends, polyblends or polymer alloys, which are physical mixtures of two or more structurally different homopolymers, or copolymers whose individual components are linked together by various intermolecular forces, such as Van der Waal's forces, dipole - dipole interactions, hydrogen bonding, or by chain entanglement.

Polymer blends have become a large and important factor in thermoplastics market, competing head on with traditional materials. The reason for their success lies in the fact that unlike in the synthesis of new polymers, for which vast resources are needed in developing new monomers and polymerisation techniques, minimum development costs are needed to develop new polymer blends, since existing traditional polymer processing techniques can be used. Commercially useful materials can be developed by simply melt mixing already existing polymers.

Commercial polymer blends are available which have been designed to meet particular needs, such as impact resistance, flame retardant or even to reduce the cost of expensive engineering thermoplastics i.e. either for economic or property advantage purpose. The incentives for making a blend are economic pressures for higher performance at a reasonable price plus the advantage of quickly offering a product as the market develops.

Blending of two or more polymers towards the formulation of new materials, closely tailored to meet the requirements of specific end uses is a new and potentially interesting idea for the biomedical field.

3.2 PREPARATION OF POLYMER BLENDS.

Several methods have been used to prepare polymer blends, relying on the intimate mixing of the components in either a melt, solution, or latex form [157-159]. Mixing is aided by diluents, suspending mediums or high temperature.

In solution mixing, a diluent is added to lower the temperature and shear-force, for satisfactory mixing. This method is attractive in that it provides intimate molecular contact between the components, and is useful when the component polymers to be blended are prepared by solution polymerisation.

Latex blending is an important technique for the preparation of commercial polymer blends. In latex mixing, polymers are present as suspended microspheres, the coagulation of neighbouring spheres is prevented by the suspending medium. After extensive mixing of the latexes, the product, containing an intimate mixture of polymers, is isolated. Acrylonitrile-butadiene-styrene (ABS), resins are prepared in this manner.

By far the most common technique used for the preparation of commercial polymer blends, is that of melt mixing. The advantages of this technique are that it not only enables polymer blends to be prepared quickly, using existing plastics and rubber

technology machinery, but above all this technique avoids the contamination and removal problems of diluents. In melt mixing, the ultimate degree of mixing achieved will be dependent on the magnitude of the shear rate developed in the mixing equipment, length of mixing, temperature and rheological properties of the component polymers [160]. Proper temperature control is necessary to achieve efficient mixing and to avoid potential thermal degradation of the polymers. For amorphous polymers the processing temperature must be well above the glass transition temperature (T_g), of each component, and above the melting point (T_m), of mixtures containing semicrystalline polymers. At present, the majority of blending operations are performed with intensive mixers (e.g. Banburys [161]), open roll mills, or various single and multi-screw extruders. Because the heat and shear that is provided by these, for the dispersion of the components, could cause degradation of the polymers, leading to cross-linking, block and graft formation, melt blending is used for systems in which thermal degradation does not ordinarily occur.

3.3 CRITERIA FOR CHOOSING POLYSACCHARIDES.

In choosing naturally occurring biodegradable polymers for blending with synthetic polymers, the most important constraint is the thermal stability of the natural polymer. Normal processing temperatures encountered in the conversion of raw polymers into finished article range from 150 - 300°C, and as such, incorporated natural polymers should be able to withstand these temperatures for short periods without undergoing thermal degradation.

Protein-based materials are thermally unstable to be considered, on the other hand many carbohydrates are thermally stable up to 270°C. These carbohydrates are a class of

biologically important polysaccharides such as cellulose, starch and dextrans. Another advantage of using polysaccharides (apart from their cheapness), as the natural polymers for blending is that, unlike proteins, they are inert and do not cause adverse tissue response when implanted in the body. Furthermore, some of the polysaccharides are degraded by enzymes in the body.

Cellulose and wood flour have been used over the years as inert fillers in thermosetting resins, not because of there biodegradability, but because of their low cost. More recently, attention has focused on the intentional use of carbohydrates, such as starch, as degradable "filler" in common thermoplastics. The materials produced have been of particular interest and of commercial importance in agricultural uses, such as mulch to improve crop yields, provide weed control, control soil moisture and reduce nutrient leaching. Since Polyethylene (PE) and Polyvinylchloride (PVC) films used for mulch cannot be reused and do not degrade between growing seasons, they must be removed from the field and disposed of at an considerable expense.

Griffin[162,163], using commercial compounding and processing developed techniques to combine starch with PE, to produce blown films. These films had the combined properties of PE (flexibility and strength) and of starch (biodegradability). Subjection to α-amylase treatment resulted in increased porosity in the films and consequential increase in the rate of biodegradation of these films. Otey[164-167] prepared degradable plastic mulch films from starch-PVA, starch-poly(ethylene-co-acrylic acid) compositions and starch graft copolymers with PVC, and observed similarly the increased degradation of the films made from the starch blends.

The idea of using the carbohydrates as degradable "fillers" with thermoplastics for

biomedical applications has not been fully exploited, whereas chemical modifications, or incorporation of blocks of carbohydrates to produce degradable materials has been examined by Gilbert and Stannett[98, 168,169].

The incorporation of polysaccharides, by blending, with thermoplastics which are hydrolysable to some extent, such as polyesters, could lead to a whole new range of materials. The biodegradation rate would be controlled by the polysaccharide used, and to a lesser extent the matrix polymer. Materials having desired characteristics with respect to physical properties and degradation rate could be produced by using different polysaccharides.

Although there are several different techniques available for preparing polymer blends, the choice of method for the blending of polysaccharides with thermoplastic polymers is somewhat limited. Precipitation in co-solvents is not possible, because the polysaccharides are only soluble in aqueous or strong polar solvents, due to intensive hydrogen bonding, whereas the intended polymers to be used are only soluble in non-polar organic solvents. Mechanical melt mixing should provide good dispersion of the solid polysaccharide particles, in the polymer matrix.

An initial feasibility study was undertaken to investigate the possibility of blending polysaccharides with different polymers. This study not only looked at the availability of the machinery for blending and processing of the polysaccharide blends, but also at the physical properties and susceptibility to degradation of the materials produced. Blends with known hydrolysable and non-hydrolysable polymers, (polyesters, polyamides and polyolefins), were prepared.

3.4 POLYBUTYLENE TEREPHTHALATE/AMYLOSE BLENDS.

Polyethylene terephthalate (PET), is easily processable and has good mouldability. It is susceptible to hydrolysis, and is known to degrade in the body after 10 years[54]. Similarly, polybutylene terephthalate (PBT), is easily processable, susceptible to hydrolysis[170], and is mouldable. Bearing in mind the high melting point of PBT, (227°C), blending with polysaccharides such as amylose would be of interest to see what affect high processing temperature has on the polysaccharide. Polysaccharides decompose below their melting point, and start to discolour at about 150°C and above 250°C are completely decomposed. The affect of high temperature and also processing conditions on amylose were investigated by mixing a master batch of PBT and amylose in the Banbury mixer, (David Bridge & Co. Ltd., Castleton, Rochdale, UK), and various dilutions made using the Buss Ko-Kneader, (PR 46, Buss AG, Basle, Switzerland), a continuous mixer-extruder.

A master batch of PBT/amylose blend was prepared by blending 1300g PBT with 415g amylose in the Banbury mixer, at 210°C, for 5 minutes. This master batch of 24% w/w amylose loaded PBT was used to prepare various loadings of PBT/amylose blends, ranging from 0% to 36% w/w amylose, using the Buss Ko-Kneader, fitted with granulator, at barrel temperature of 220°C. Dextran blue, a water soluble dye was incorporated into the blends, (0.5% w/w), on the Buss Ko-Kneader, so that degradation of the blends (i.e release of dye), could be monitored spectrophotometrically. The thermal degradation of the dye was found to be minimal.

Fabrication of the PBT/amylose blends was difficult. Ideally, injection moulded samples

of these blends would have been very useful for the investigation of the physical and degradative properties, but with the type of injection moulders and moulds initially available, it was difficult to produce enough samples for a comprehensive study. However, films of these blends were pressed, using conventional melt pressing techniques, (section 2.2.3.1), and these were then used to provide information regarding physical and degradative properties. In this particular case, because of the high melting point of PBT, materials normally used as non-stick release agents with steel plates, could not be used. At 230°C, the temperature at which PBT was pressed, both cellophane and Melinex sheets were inadequate because they fused with the polymer melt. Polytetrafluroethylene, (PTFE), can withstand temperatures up to 300°C, but because it is a soft polymer, under pressure, this stretched and was inadequate as a non-stick release agent. PBT films made using PTFE sheets were uneven. Use of silicone or PTFE release sprays on highly polished steel plates, failed to produce films that were large enough to cut tensile pieces from.

Although quantitative physical measurements of these films were not possible, qualitative observations suggest that with increasing amylose content, the films become more brittle. Initial hydrolytic degradation carried out in aqueous pH 7.4 phosphate buffer, showed slight increase in wet weight with increasing amylose content, over a period of 20 days, at 37°C. This result is encouraging in that the initial water uptake could be regarded as the start of the hydrolytic degradation process. Continual absorption of water by the amylose particles would lead to swelling, resulting in decrease in the physical properties and eventual eruption of the matrix. This was observed after 20 days in pH 7.4 buffer at 37°C, when the films became soft and fragmented.

In the preparation of PBT/amylose blends, using a two-step method, (i.e. Banbury mixer

and Buss Ko-Kneader), PBT and amylose are subjected to high temperatures and high shearing pressures for long periods of time, which may adversely effect their physical properties. Although the mechanical hot blending technique was found to be very successful in the preparation of the PBT/amylose blends, a simple one stage hot blending technique was needed, not only to reduce thermal degradation of polysaccharides and the polymers, but in addition to allow the blending of small amounts of material to be carried out. The RAPRA torque rheometer and the 2-roll mill are used to hot blend polymers, and both of these can be used to prepare small batch sizes. Blending techniques were established using these mixers.

The success in the production of PBT/amylose blends suggested that this technique could be used with other polymers, resulting in a whole new spectrum of polymer blends, the physical and degradative properties of which could be altered by altering the type of polysaccharide, (i.e. changing physical nature of the polysaccharide or its' solubility), or by changing the matrix polymer, (i.e. from a slowly hydrolysable to a completely hydrolysable polymer). Also, in order to eliminate or reduce processing difficulties encountered in using polymers with a high melting point, the ideal polymer should melt below 200°C. The polymers chosen for further study were polypropylene, (which has good processability, but is non-hydrolysable), poly(lauryl lactam), (better known as Nylon 12, and it has good processability , but is slowly hydrolysed), and polyhydroxybutyrate, (a novel polyester). Polyhydroxybutyrate and its' copolymers with polyhydroxyvalerate, known commercially as Biopol® [136,145,150], are a potentially interesting series of copolymers, in that they are not only thermoplastic, but they are also biodegradable. They can be processed and fabricated using conventional polymer processing techniques. Due to the high price and availability of these polyesters, for the evaluation of the blending techniques, and investigation of the affect of different

polysaccharides on the physical properties of the blends produced, the relatively cheaper polypropylene and Nylon 12 were used.

3.5 POLYPROPYLENE/POLYSACCHARIDE BLENDS.

Polypropylene, (PP), (GMW 22, injection moulding grade), was blended with amylose, amylopectin and cellulose on a 2-roll mill, at 175°C. The 2-roll mill is more effective in blending and allows a larger batch size to be made, compared with the Torque Rheometer. Each batch was 400g and made up according to Table 2.7. The Melt Flow Index, (MFI), of PP/amylose blends was measured using the British Standard 2782 procedure[171]. Although the MFI is an important indicator for the thermal degradation of a polymer, the MFI was used to evaluate the flowability of the PP/amylose blends. With increasing amylose loadings, the MFI of the blends decreased, (Graph 3.1), suggesting that high loaded blends would be difficult to injection mould. Polymer flow properties are important in injection moulding, where the polymer melt is injected under pressure into a mould. Further work, including a detailed investigation of the blending techniques and physical properties of polypropylene/polysaccharide blends, was restricted to 30% w/w loadings of the polysaccharide. Tensile pieces of the PP/polysaccharide blends were injection moulded using the Mercia Manimold, at 195°C.

With high loaded blends, the barrel temperature was increased, due to the reduced flow of the polymer melt. Tensile properties were measured using the E-type tensometer, (Tensometer Ltd, Croydon, UK), at a cross-head speed of 25 mm/min, and the results are displayed in Table 3.1.

Cellulose is a fibrous polysaccharide and imparts strength to the PP/cellulose blends.

Graph 3.1 MFI of PP/Amylose blends

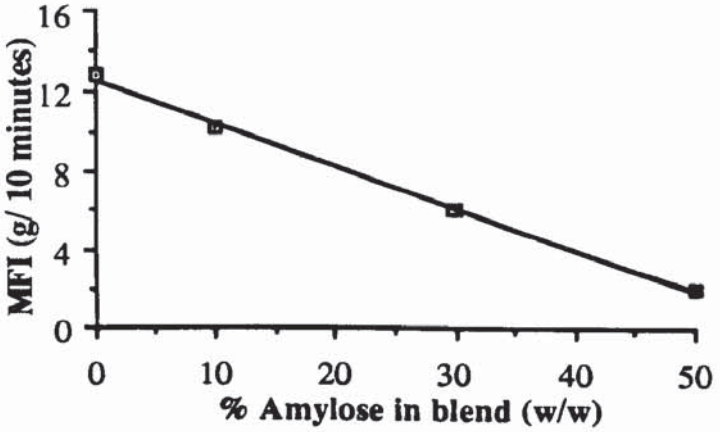
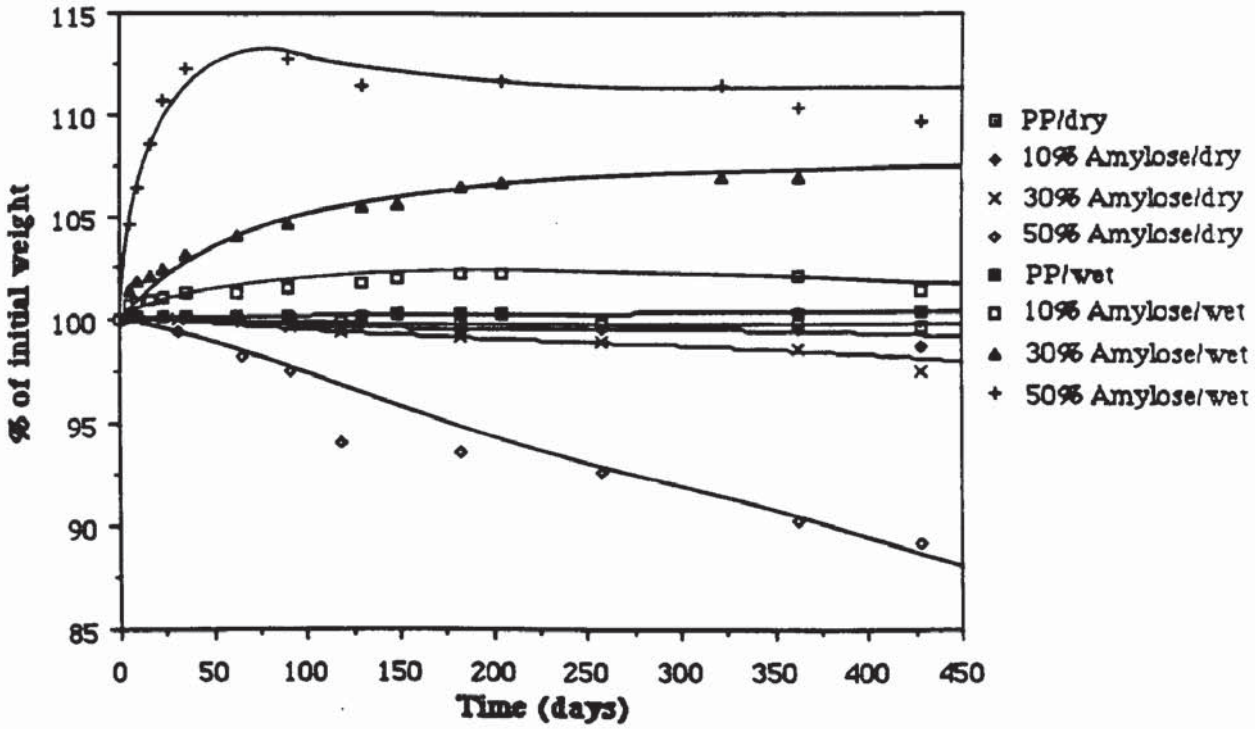


Table 3.1 Physical Properties of Polypropylene/polysaccharide blends.

Blend	Yield Strength (MPa)		Ultimate Tensile Strength (MPa)		% Elongation at Break	
	Day 0	Day 83	Day 0	Day 83	Day 0	Day 83
Polypropylene	32	32	17.2	19.3	351	109
10 % Amylopectin	28.6	28	19.6	19.3	230	108
30 % Amylopectin	22.5	20.7	17.8	17.2	104	123
10 % Amylose	25.8	25.8	19.1	18.9	75	74
30 % Amylose	18.6	20.4	16.7	18.5	34	41
10 % Cellulose	30	28.5	24.3	24.5	46	48
30 % Cellulose	31.9	28.2	31.9	27.3	20	20

Addition of 10% of cellulose to PP, causes a slight decrease in the yield strength, but with 10% amylose or amylopectin there is pronounced effect. With higher polysaccharide

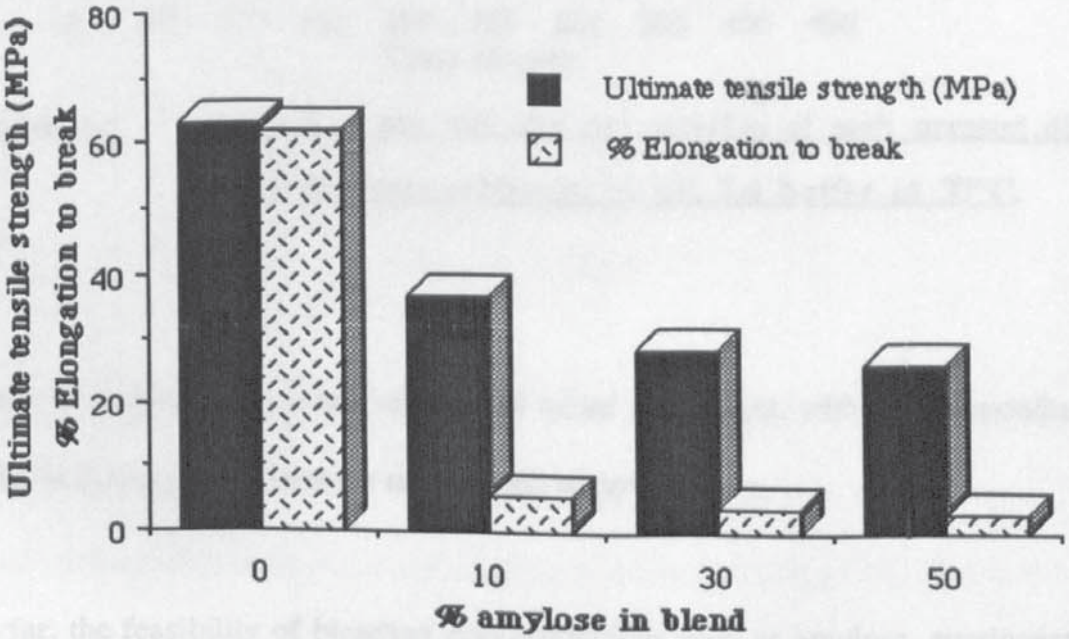


Graph 3.2 Changes in the wet and dry weights of melt pressed discs of polypropylene (PP)/amylose blends in pH 7.4 buffer at 37°C.

loadings, blends with cellulose become increasingly tougher, whilst those with amylose and amylopectin become increasingly brittle. This again is due to the fibrous nature of cellulose, compared with the particulate amylose and amylopectin. After being immersed in aqueous pH 7.4 phosphate buffer, the physical properties of these blends have slightly decreased, (Table 3.1). Hydrolytic degradation of PP/amylose blends was monitored by buffer uptake and dry weight loss, in pH 7.4 phosphate buffer at 37°C, using moulded discs, (approximate weight 1.4g, 3 mm thick and 2 cm wide). The results are shown in Graph 3.2. The blending of Nylon 12 with amylose and dextran was similarly investigated.

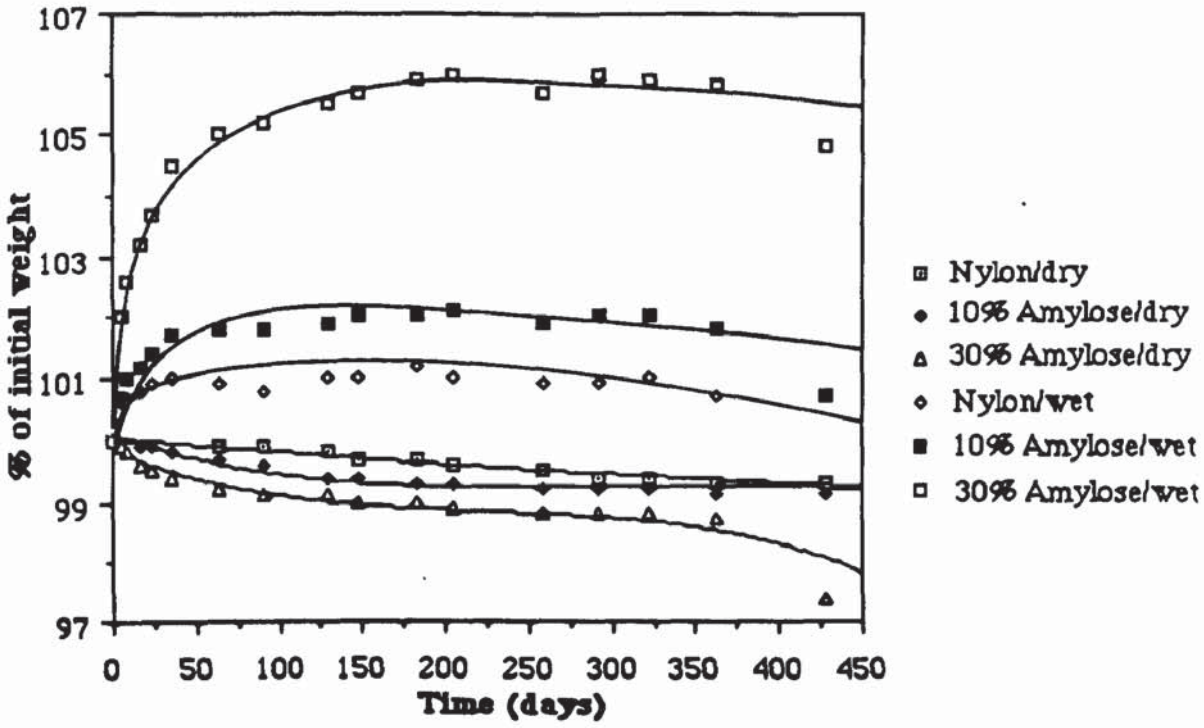
3.6 NYLON 12/POLYSACCHARIDE BLENDS.

Nylon 12/polysaccharide blends were prepared using the torque rheometer and the 2-roll mill, and fabricated into films and discs, using methods described in section 2.2. The tensile properties of the films, using dumb-bell shaped test pieces, (cut from pressed films), were assessed using the Instron Tensometer at a cross-head speed of 5 cm/min and gauge length of 3 cm. With increasing amylose content, the physical properties of the blends decreased. The results are displayed in Graph 3.3.



Graph 3.3 Tensile properties of Nylon 12/Amylose blends

Hydrolytic degradation of the Nylon 12/polysaccharide blends, using melt pressed discs (approximate weight 1.5g, 3 mm thick and 2 cm in diameter), immersed in pH 7.4 phosphate buffer at 37°C, was monitored by buffer uptake and dry weight loss studies. Although the wet weight of the discs increased with time, dry weight loss of the discs was minimal. After 200 days, the wet weight of 30% amylose loaded blend has increased to 6.5%, whereas loss in the dry weight is only 1%, (Graph 3.4). In comparison to this,



Graph 3.4 **Changes in the wet and dry weights of melt pressed discs of Nylon/amylose blends, in pH 7.4 buffer at 37°C.**

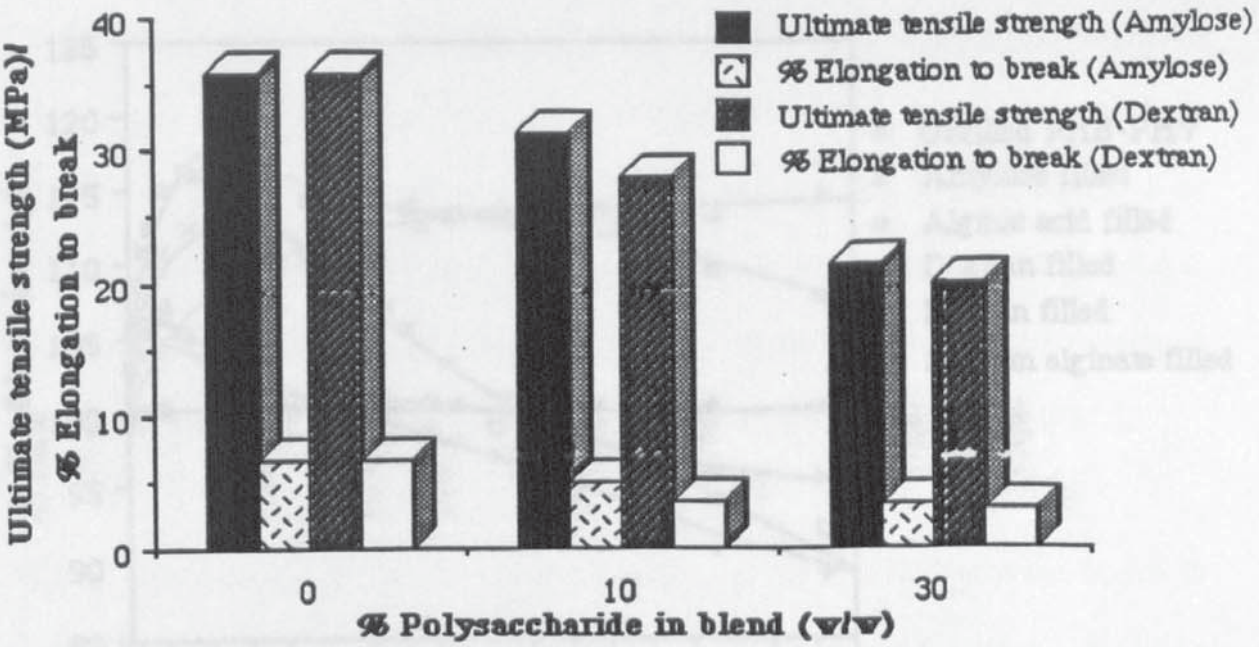
unblended Nylon 12 had lost only 0.3% of its' dry weight, with a corresponding 1% increase in wet weight over the same period of time.

So far, the feasibility of blending polysaccharides such as amylose, amylopectin and cellulose with common thermoplastics such as polypropylene, PBT and Nylon 12, using readily available conventional processing machinery has adequately been demonstrated. Incorporation of polysaccharides with these non-hydrolysable or slowly hydrolysable polymers should increase the degradation of the composite blend. These polymer blends could be used for applications where strength, combined with a slow degradation rate is needed. As the degradation products of these polymer blends may be toxic, use of these is limited to applications were this will not be of importance. However, for biomedical applications, the degradation products of a polymer are of paramount importance. Ideally

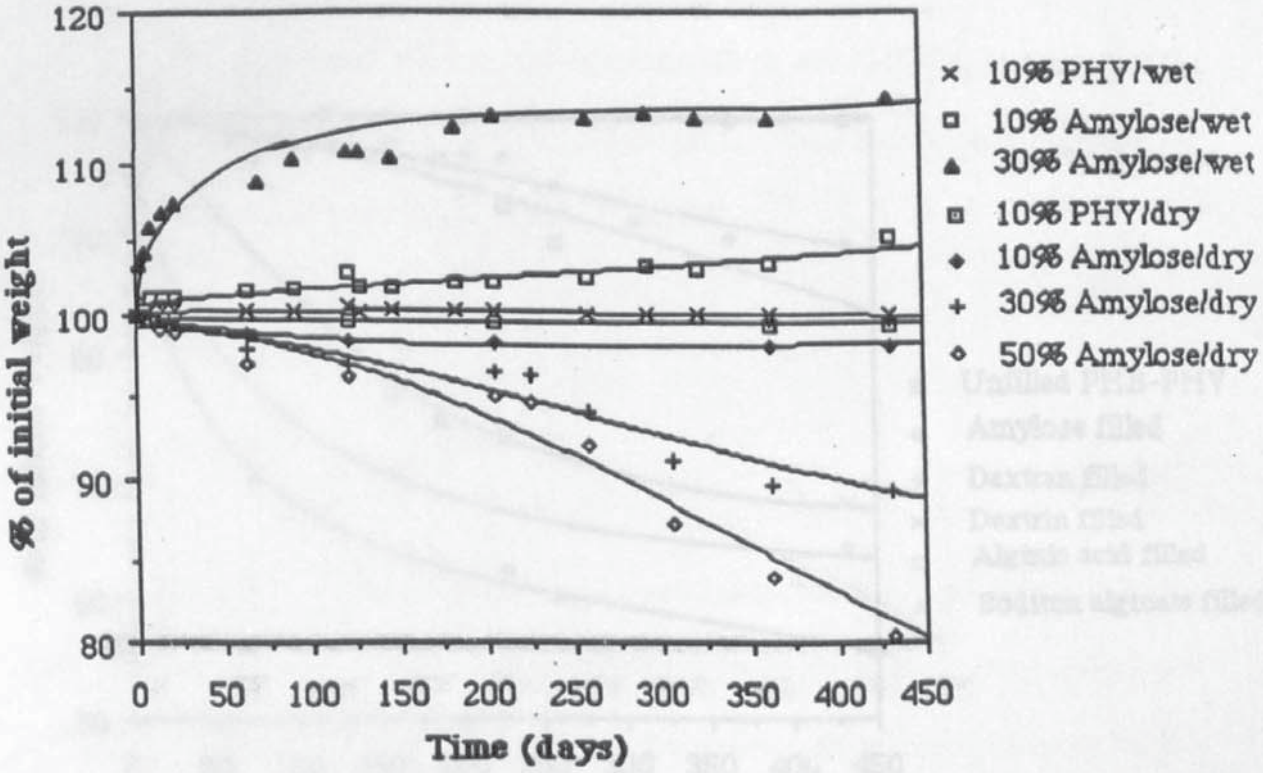
these should be non-toxic and easily assimilated by the body. In this aspect, poly(hydroxybutyrate), (PHB), and its' copolymers with poly(hydroxyvalerate), (PHV), are particularly outstanding in that they are reputed to biodegrade to degradation products which are normal mammalian metabolites[150]. By taking advantage of the thermoplastic properties and biodegradability of PHB and its' copolymers, interesting polymer blends with polysaccharides could be made, having different degradation rates, which could depend upon the copolymer content and/or the polysaccharide used. Techniques already developed for the production of polysaccharide blends with Polypropylene and Nylon 12 could be used for the preparation of these PHB-PHV copolymer blends with polysaccharides.

3.7 PHB-PHV/POLYSACCHARIDE BLENDS.

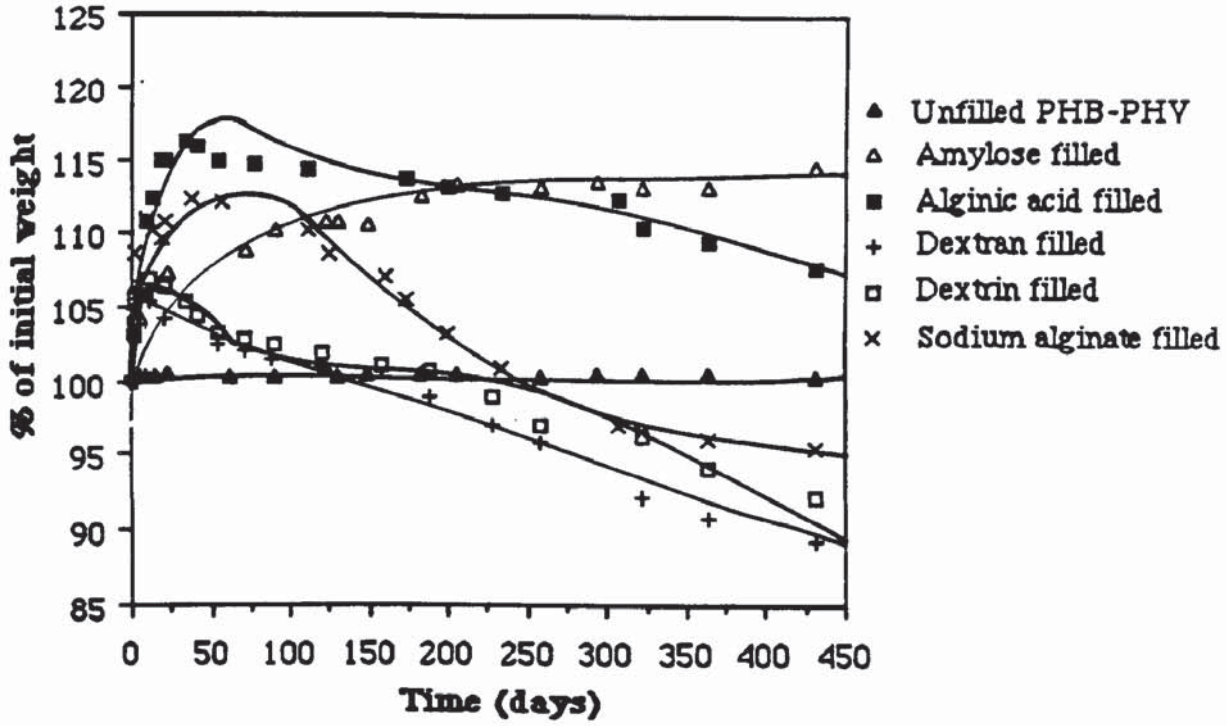
A PHB-PHV copolymer sample of 10% PHV and weight average molecular weight, (Mw), 750,000 Daltons was initially milled on the 2-roll mill, in order to assess the processability of this polyester and feasibility of blending polysaccharides with it. Little information was available regarding the processing of these polyesters, so a copolymer of high molecular weight and valerate content was used, hoping that the thermal and physical shear that the polyester would undergo during processing would not adversely affect its molecular weight and other physical properties. Indeed,the 10% PHV copolymer mixed for 30 minutes at 162°C in the torque rheometer, showed very slight decrease in molecular weight, (molecular weights determined by RAPRA Technology Limited, Shawbury). Hence it is conceivable that the molecular weight of a sample blended on the 2-roll mill for 5 minutes with the polysaccharides will not be drastically affected.



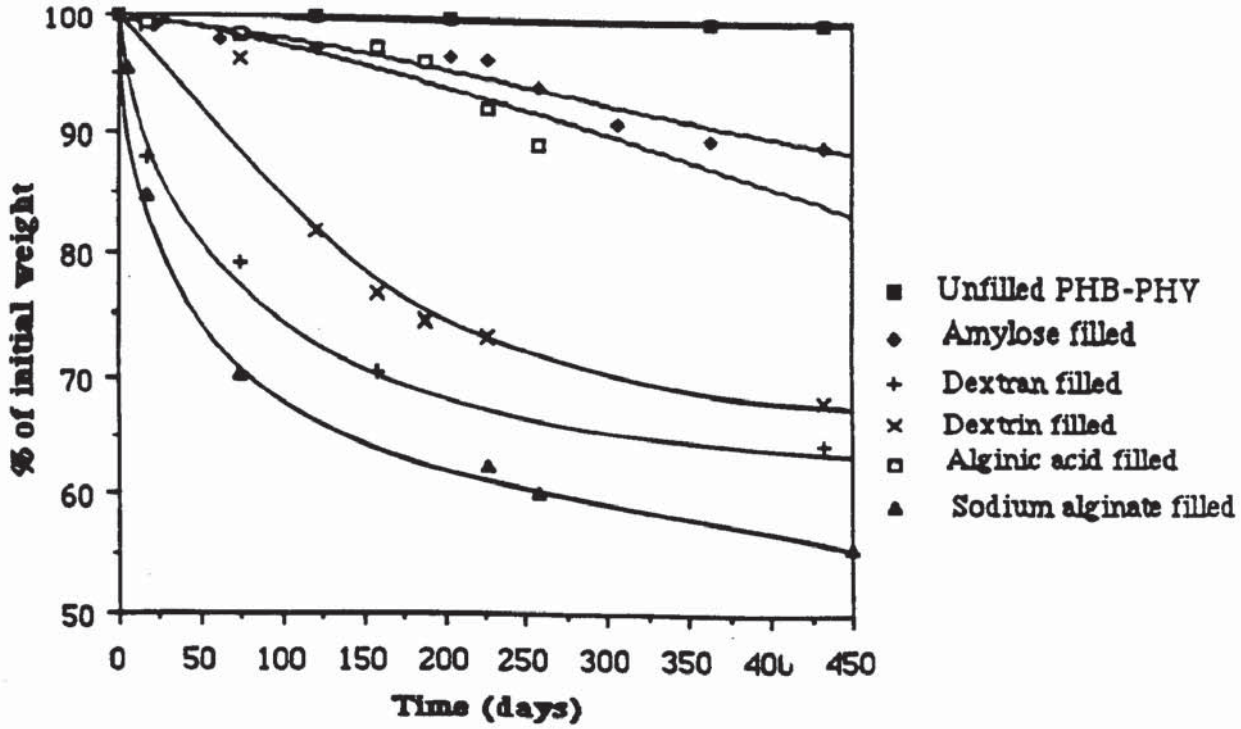
Graph 3.5 **Tensile properties of PHB-PHV (10% PHV) blends with amylose and dextran.**



Graph 3.6 **Changes in the wet and dry weights of melt pressed discs of PHB-PHV (10% PHV) blends with amylose in a pH 7.4 buffer at 37°C.**



Graph 3.7 The changes in the wet weight of melt pressed discs of 10% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C



Graph 3.8 The changes in the dry weight of melt pressed discs of 10% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C

Blends of amylose and dextran with the 10% PHV (Mw = 750 x10^3) copolymer were prepared, as described in section 2.2.2.2, (Table 2.7), and discs and films of these fabricated. Tensile properties of the PHB-PHV/amylose and dextran blends were performed on pressed films, using the Instron tensometer, at a cross-head speed of 5 cm/min and gauge length of 3 cm. Incorporation of amylose and dextran causes a significant drop in the ultimate tensile stress and % elongation at break (Graph 3.5).

The hydrolytic degradation of the melt pressed discs of the 10% PHV/amylose blends in pH 7.4 buffer at 37°C was monitored by gravimetric methods and the results are shown in Graph 3.6. Graphs 3.7 and 3.8 illustrate the changes in the wet and dry weights of the 10% PHV copolymer filled with either 30% amylose, dextran, dextrin, sodium alginate or alginic acid (melt pressed discs, approximate weight 1.8 g, 3 mm thick and 2 cm in diameter), in pH 7.4 buffer at 37°C. The PHB-PHV copolymer absorbs very little water itself, but with the incorporation of a polysaccharide in the PHB-PHV matrix, buffer uptake is dramatically increased. The effect of the polysaccharide on water uptake and dry weight loss is apparent from Graphs 3.6, 3.7 and 3.8.

3.8 DISCUSSION.

Of the several different blending techniques investigated, the 2-roll mill seems to be the most practical method for mixing polysaccharides with thermoplastic material. The 2-roll mill consists of two robust horizontal cylindrical polished rolls, either oil or steam heated, (Plate 2.1), counter-rotating and separated by a narrow gap called the "nip". The feed is dropped between the two rolls and is dragged down from the rolling reservoir or "bank" into the nip and adheres as a band onto one of the rolls. There is therefore a high laminar

shear flow through the nip: this develops a substantial pressure profile which causes the bank upstream of the nip to roll, thus promoting radial mixing. There is no mechanism for axial mixing, so the band is cut at an angle to the roll axis and is simply folded over, or feed through the rolls vertically.

Mixing is a function of a) the roll speeds, b) the amount of turnover (folding) between passes, c) number of passes and d) the gap size between the rolls. The value of (d) is decreased during processing. The value of (b) and (c) are functions of the amount of material in a batch and time of milling. For this reason, batch sizes were chosen to provide efficient mixing, and indeed it was for this reason that a smaller 2-roll mill, (the micro-mill), was used in further work. Also, the heated 2-roll mills were found to be easy to use and provided adequate wetting of the components, which is necessary for effective mixing of components.

Melt viscosity of polymer melts tends to decrease above the melting point (Tm) of the polymer. Blending of polyesters with polysaccharides above the Tm of the polyester will reduce the efficiency of mixing (i.e. dispersion), because the melt viscosity of polyesters decreases dramatically above their melting point. Efficient mixing is possible if blending is carried out just below the Tm of the polyester, at an temperature where the polymer just begins to soften, under the combined effects of heat and pressure exerted by the rolls.

This initial feasibility study also showed that with increasing polysaccharide loadings, the melt flow of the polymer blend decreased. For injection moulding purposes, it is desirable to have good melt flow of the material, in particular so that all parts of the mould are completely filled. For further work, maximum loadings of 30% w/w of polysaccharides with PHB-PHV copolymers were used.

Fabrication of the polymer blends into medical devices was envisaged to be carried using injection moulding techniques. This enables production of intricate designed moulded parts on a large scale from a single mould. Close examination of the properties of the PHB-PHV/polysaccharide blends could be easily carried out by injection moulding tensile test pieces in an appropriate mould, and careful consideration was needed as to the type of injection moulder that would be ideal for this purpose. Certainly, to reduce thermal degradation of the components, residence time of the material in the injection moulder should be minimum. Using a screw-type injection moulder this would not be possible and generally, these type of moulders have a large shot size. Ram type injection moulders, of the type shown in Plate 2.2, have a small barrel lengths, i.e., a small shot size, so the time spent by the material in the barrel is much less than in the screw-type injection moulders. The SP 1 injection moulder was found to be very convenient for this work because it has a small barrel (approximate capacity of 18 g), and is a electrically heated, bench top, ram-type injection moulder.

Although a large number of polysaccharides are available commercially, amylose, dextran, dextrin, sodium alginate and soluble starch were selected for further detailed work. Cellulose is a fibrous polysaccharide that imparts toughness to the polymer blend, and whereas it is very slowly hydrolytically degraded in physiological environments, enzymic degradation of cellulose is not possible in the body. As the ideal polymer blend would be used to make surgical fixation devices having reasonable strength, coupled with a reasonable degradation rate, the use of cellulose was ruled out for further work. However, for use in veterinary surgery, in animals where enzymes are present to degrade cellulose, cellulose blends could be quite promising. Also, because the degradation of polypropylene and Nylon 12, (filled and unfilled), is slower than the corresponding PHB-PHV blends, (probably because of the difference in the inherent hydrophilicity of the matrix polymers), polypropylene and Nylon 12 blends with polysaccharides could

also be used in applications where the rate of the composite blend is unimportant.

Alginic acid, although a hydrophilic polymer, has an pH of 2.3. Using this in blends for surgical devices will be detrimental to the formation of new tissue.The polysaccharides selected for further work have differing rates of solubility/hydrolysis or enzymatic degradation in mammalian tissue.

A wide range of polysaccharide blends with polypropylene or Nylon 12 would probably have interesting and useful properties, but by making PHB-PHV/polysaccharide blends, the scope of making a much wider range of useful blends becomes possible. The PHB-PHV copolymers are available in a range of molecular weights and PHV content. However, because very little work had been published on the hydrolytic degradation of PHB and PHB-PHV copolymers, it was decided that a detailed study investigating the factors involved in the hydrolytic degradation of these polyesters was necessary. This particular study is described in the next chapter, together with subsequent work on the PHV-PHV/polysaccharide blends, using PHB-PHV copolymers to form a group of potentially useful materials. These would have a suitable range of degradation rates and mechanical properties that are needed for the potential use in the fabrication of surgical fixation devices.

3.9 CONCLUSIONS.

Polysaccharides can be blended with thermoplastic polymers such as PBT, PP, Nylon 12 and the PHB-PHV copolymers using conventional polymer processing machinery. The 2-roll mill was found to provide the best mixing, combined with ease of use. Cellulose

was found to be a reinforcing polysaccharide compared with amylopectin and amylose. Although incorporation of a polysaccharide into the polymer matrix of PP, Nylon 12 or PHB-PHV copolymer reduced the physical properties, the degradation rate of these blends, (i.e. water uptake and dry weight loss), was increased by the polysaccharides, in comparison with the virgin polymers. Further detailed work was carried out to establish the factors affecting the degradation of PHB-PHV copolymers and subsequent blending of polysaccharides with the ideal PHB-PHV copolymers to form blends with interesting properties.

CHAPTER 4

PHB-PHV COPOLYMERS AND THEIR BLENDS WITH POLYSACCHARIDES:- STUDY OF DEGRADATIVE AND PHYSICAL PROPERTIES.

4.1 INTRODUCTION.

Studies on the PHB-PHV copolymer series, particularly those concerning hydrolytic degradation, are relatively limited. This chapter deals with the hydrolytic degradation of these copolymers, studying the effects of valerate content and molecular weight, amongst other factors that affect their hydrolytic instability. In addition, modifications to the physical and degradative properties of the PHB-PHV copolymers were attempted by forming blends with polysaccharides. The properties of these blends were investigated using various techniques. It was felt desirable to include both aspects of this work in a single chapter, although this inevitably means that this section of the thesis is relatively long. The transition between unmodified and modified systems occurs at the beginning of section 4.3.

4.2 HYDROLYTIC DEGRADATION OF PHB-PHV COPOLYMERS.

4.2.1 MELT PRESSED DISCS.

Initial hydrolytic degradation studies of these copolymers was followed by monitoring the polymer matrix dry weight loss of melt pressed discs, (2 cm in diameter and 2 mm thick), in a pH 7.4 buffer at 70°C, (to facilitate accelerated degradation). Test samples were removed periodically and the wet and dry weight determined as described in Section 2.5.

4.2.1.1 RESULTS AND DISCUSSION.

Degradation of the PHB-PHV copolymer discs in a pH 7.4 aqueous buffer at 70°C was monitored by gravimetric methods. The dry weight loss profiles of several PHB-PHV copolymers illustrated in Graph 4.1, are characterised by an initial slow weight loss, followed by a secondary accelerated degradation phase. It is apparent from the weight loss profiles displayed in Graph 4.1, that degradation of the PHB-PHV copolymers is dependent on several factors. Firstly, comparing the weight loss profiles of the 12 and 20% PHV copolymers (Mw = 350 and 300 $x10^3$, respectively), it is quite evident that the hydroxyvalerate content of the PHB-PHV copolymers makes a certain contribution to the hydrolytic degradation rate of these copolymers. By increasing the hydroxyvalerate content by 8%, (the molecular weight being very similar), the resulting effect on the weight loss is quite dramatic. The 12% PHV copolymer of Mw = 350 $x10^3$, has lost 25% of its original dry weight in 137 days, whereas the 20% PHV copolymer (Mw = 300 $x10^3$), has lost 50% of its initial dry weight over the same period. This dramatic effect of the hydroxyvalerate content on the hydrolytic degradation rate of these two PHB-PHV copolymer samples is illustrated photographically in Plates 4.1 and 4.2, which show melt pressed discs of the 12% PHV copolymer (Mw = 350 $x10^3$), and the 20% PHV (Mw = 300 $x10^3$) copolymer, respectively, after degradation in pH 7.4 buffer at 70°C for 137 days. Undegraded discs of the copolymers are included in these plates for comparison.

The influence of molecular weight on the hydrolytic degradation rate of these copolymers is evident by comparing the molecular weight loss profiles of the two 12% PHV copolymers. By increasing the molecular weight two-fold, the time for 10% of initial weight loss, changes from 15 days for the copolymer of Mw = 170 $x10^3$, to 108 days

Graph 4.1 **Weight loss of PHB-PHV copolymers; melt pressed discs degraded in pH 7.4 buffer at 70°C.**

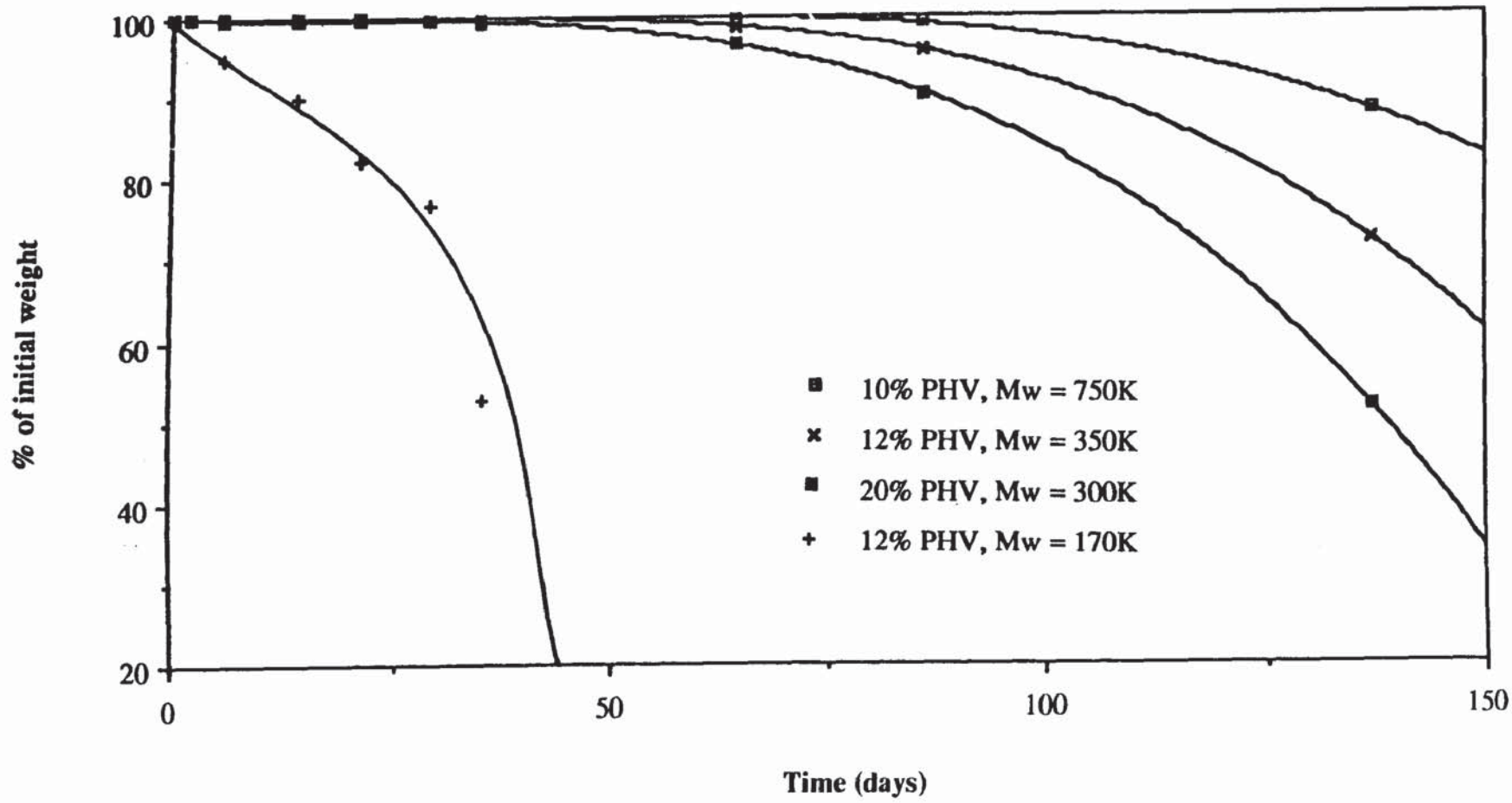


Plate 4.1 12% PHV, (350K) Melt pressed discs; undegraded (left); degraded (right); (pH 7.4 and 70°C, 137 days, weight loss = 25%); (x 1.5).

Plate 4.2 Melt pressed (20% PHV, 300K) discs; undegraded (left); degraded (right), (pH 7.4 and 70°C, 137 days, weight loss = 50%); (x 1.5).

Table 4.1 The weight loss parameters, (t_{10} and t_{50} values) for various PHB-PHV copolymers, (melt pressed discs), in a pH 7.4 buffer at 70°C.

Copolymer	t_{10} (days)	t_{50} (days)
0% PHV, 800 x10^3	78	106
7% PHV, 400 x10^3	72	97
10% PHV, 750 x10^3	134	12% in 137 days
12% PHV, 350 x10^3	108	25% in 137 days
20% PHV, 300 x10^3	88	137
12% PHV, 170 x10^3	15	36

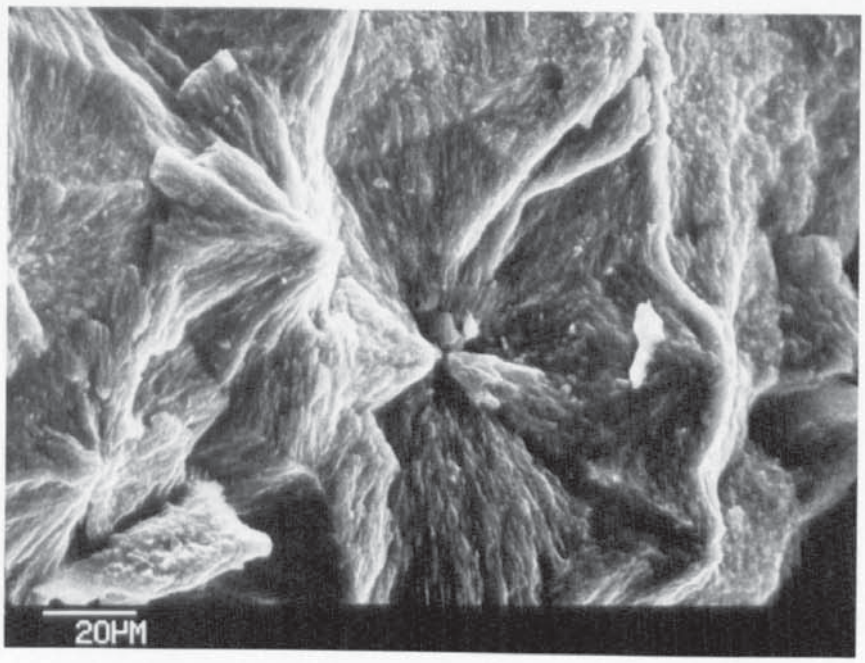
for the 350 x10^3 copolymer. The times for 10 and 50% (t_{10} and t_{50} values, respectively) of initial weight loss of melt pressed discs of the various PHB-PHV copolymers degraded in pH 7.4 buffer at 70°C are shown in Table 4.1.

Holland[172], also found that the degradation rate of these copolymers was dependent on both the molecular weight and valerate content. Holland, using both melt pressed discs and solvent cast films of these copolymers, also suggested the role of crystallinity and/or compaction of polymer matrix in the hydrolytic degradation of these copolymers. He found that the degradation rate of solvent cast films was much faster than the melt pressed discs.

Certainly, it seems that crystallinity plays an important role in the degradation rate of these copolymers. Pure copolymers were used for melt pressing of sample discs, and because the PHB-PHV copolymers are susceptible to processing variations (e.g. rate of cooling from the melt), varying amounts of crystallinity are introduced to samples cooled at different rates from the melt. Discs of the 7% PHV copolymer and the PHB homopolymer were removed from the moulds before crystallisation was complete. As a

result, these two particular samples would be less crystalline in comparison to the other samples, and the hydrolytic degradation rate of these would be faster. This probably explains the faster degradation of lower valerate and higher molecular weight samples

Plate 4.3 SEM of a degraded 10% PHV, 750k PHB-PHV copolymer melt pressed disc, (pH 7.4 buffer at 70°C; dry weight loss after 137 days = 12 %) (x1000)



observed in Table 4.1. Polymer crystallinity has also been associated with the valerate content[144], the crystallinity decreasing slightly with increasing valerate content.

The scanning electron micrograph, (SEM), of degraded 10% PHV (750 x10^3) copolymer disc, (Plate 4.3), shows large spherulites, which are associated with the crystalline regions. The amorphous regions have been completely eroded. Degradation of polyesters, such as glycolic acid, (in the form of Dexon® sutures), have been studied by

several workers[53,61,81-84]. Chu[81-84] found that as Dexon® sutures degrade hydrolytically, crystallinity increases initially and then falls. This two-stage degradation is associated, in the initial phase, by preferential degradation of the amorphous regions, followed by the degradation of the more resistant crystalline regions. Similar degradation profile is probable with these PHB-PHV copolymers.

The changes brought about by processing and after degradation at 70°C in a pH 7.4 buffer to the molecular weights of these PHB-PHV copolymers, measured by gel permeation chromatography (GPC), are shown in Table 4.2. The increase in the Mw/Mn ratio (where Mn is the number average molecular weight), for these copolymers with degradation suggests that hydrolytic degradation of these copolymers occurs by a chain scission process. However, Mn values for these copolymers should be treated with caution because of the exact positioning of the baseline in the GPC calculation procedure. The information in Table 4.2, however suggests that processing has a slight affect on the molecular weight of the PHB-PHV copolymers, and that the combined high valerate content and low molecular weight influence the hydrolytic degradation rate of these copolymers quite markedly. It is also interesting to note that the molecular weight (Mw) of the so-called 10% PHV copolymer as-received powder, is apparently not 750 $x10^3$, but more like 500 $x10^3$. In a recent communication, the supplier has also indicated that this particular sample does not contain 10% of hydroxyvalerate, but possibly 14%. This combined information on the hydroxyvalerate content and molecular weight explains why the difference in the weight loss profiles of the 12% PHV (350 $x10^3$) and the 14% PHV (Mw = 500 $x10^3$) copolymer is much less marked than one would expect if it was instead a 10% PHV (Mw = 750 $x10^3$) copolymer.

Although the hydrolytic degradation of these copolymers has been shown to be

influenced by both hydroxyvalerate content and molecular weight, another important property is controlled by molecular weight. This property is mechanical strength, which for surgical fixation devices is of paramount importance. The effect of molecular weight on physical strength for the PHB-PHV copolymers was evident from the melt pressed discs. Discs of the high molecular weight 10% PHV (Mw = 750 x10^3) copolymer were very strong, whereas those made from the 12% PHV (Mw = 170 x10^3) copolymer were very fragile. After confirmation of the dependence of physical strength on molecular weight, together with a cut-off in the mechanical properties at an Mw of 200 x10^3 for these copolymers [173], further work was limited to using PHB-PHV copolymers of high valerate content and high molecular weight (Mw = 300 - 400 x10^3). This would provide PHB-PHV copolymers with a right combination of reasonable hydrolytic degradation rates and sufficient mechanical properties. The copolymers used for this purpose were the 12% PHV (Mw of 350K) and 20% PHV (Mw of 300K) copolymers.

The importance of crystallinity on the hydrolytic degradation of these copolymers has been mentioned, and polymer fabrication methods are known to affect crystallinity of a sample. It would be interesting to measure the extent of crystallinity induced to samples fabricated by different methods, (i.e. solvent casting, melt pressing, and injection moulding). Furthermore, because these copolymers crystallise very slowly[173], for injection moulding purposes, it would be advantageous to use nucleants to increase the nucleation rate, (i.e. reduce the crystallisation time), in order to reduce the injection moulding time.

The hydrolytic degradation of nucleated injection moulded pieces of 12 and 20% PHV copolymers of Mw 350 and 300K respectively, is described in the next section.

Table 4.2 Changes in the molecular weights of some PHB-PHV copolymers as a result of melt pressing and degradation in a pH 7.4 buffer at 70°C.

ICI Mw values	As-received powder			After melt pressing			After degradation, (137 days)		
	Mw	Mn	Mw/Mn	Mw	Mn	Mw/Mn	Mw	Mn	Mw/Mn
750K (10% PHV)	493K	199K	2.5	460K	165K	2.8	318K	0.94K	3.4
350K (12% PHV)	396K	134K	2.9	365K	142K	2.6	3.58K	1.2K	3.0
300K (20% PHV)	280K	68K	4.1	202K	83K	4.1	2.49K	1.6K	1.6

Table 4.3 Weight loss parameters (t_{10} and t_{50}) for various PHB-PHV copolymers in a pH 7.4 buffer at 70°C.

PHB-PHV copolymer	Solvent cast films (174)		Melt pressed discs		Injection moulded plaque	
	t_{10} (days)	t_{50} (days)	t_{10} (days)	t_{50} (days)	t_{10} (days)	t_{50} (days)
750K (10% PHV)	81	100	134	12% in 137 days	-	-
350K (12% PHV)	78	121	108	25% in 137 days	78	42% in 120 days
300K (20% PHV)	77	96	88	137	80	47% in 120 days
170K (12% PHV)	33	37% in 83 days	15	36	-	-

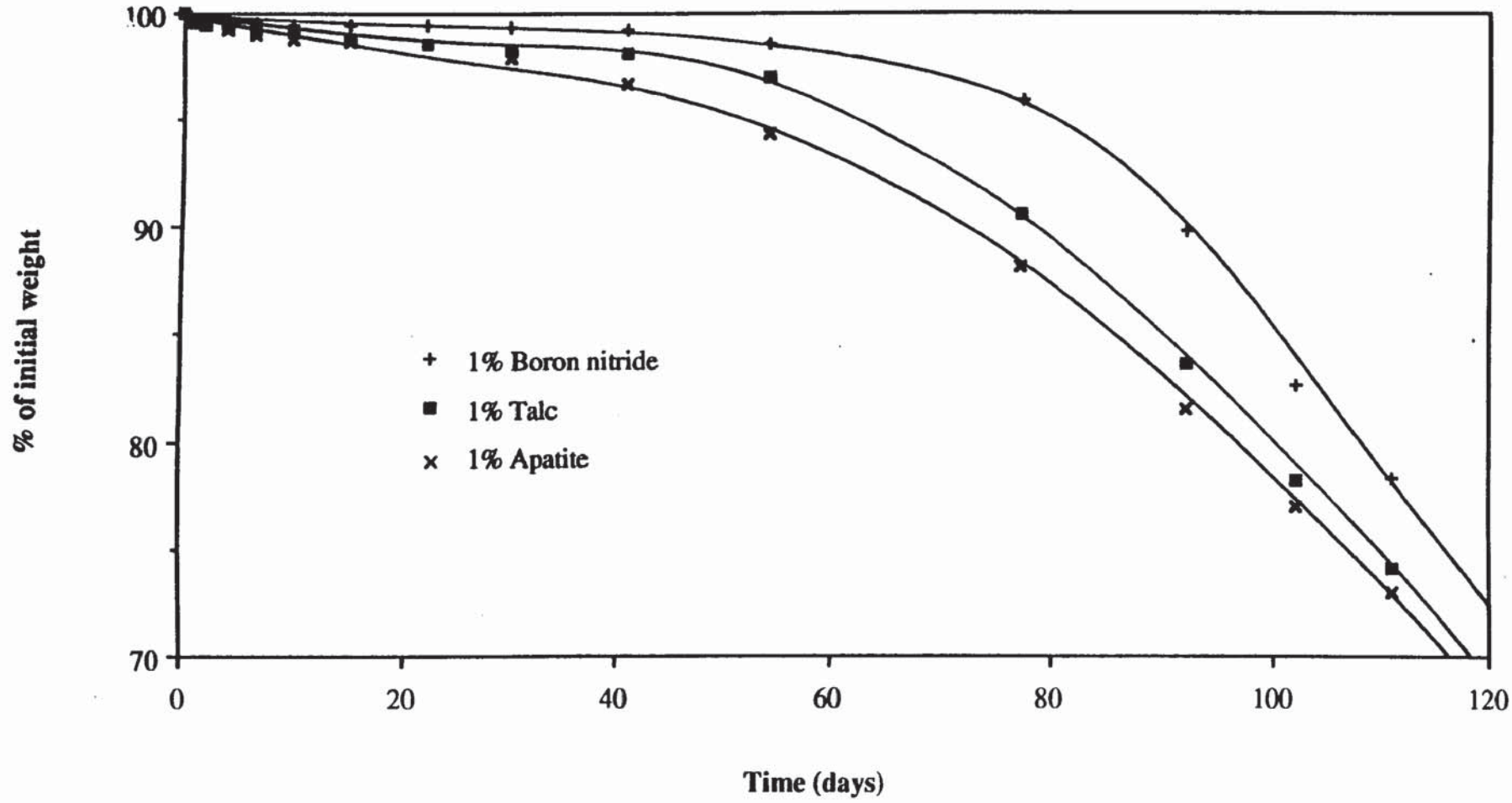
4.2.2 INJECTION MOULDED PLAQUES.

Injection moulded plaques of the 12 and 20% PHV copolymer samples were prepared using nucleated material as supplied by the supplier (nucleated with 1% w/w calcium hydroxyapatite or boron nitride) or the material was nucleated with 1% w/w Norwegian talc by blending with the pure copolymers in powder form on the two roll mill at the appropriate temperatures. The method described in Section 2.2.3.3 was used to injection mould stepped plaques 2.5 cm in length and 1.5 cm in width. The plaques were stepped in four different thickness, from 1.5 to 1.25 to 1.0 and to 0.5 mm at the thinnest step.

4.2.2.1 RESULTS AND DISCUSSION.

The effect of the three nucleating agents on the dry weight loss profiles for 12% PHV (350K) copolymer degraded in pH 7.4 aqueous buffer at 70°C over a period of 120 days is illustrated in Graph 4.2. The initial hydrolytic degradation of injection moulded specimens is again characterised by a slow initial weight loss period, followed by a secondary enhanced phase. Samples nucleated with boron nitride were slightly more resistance to hydrolytic degradation than the samples nucleated with hydroxyapatite or talc. The 20% PHV copolymer (nucleated with either hydroxyapatite or talc), showed a slightly lower resistance to hydrolytic degradation than the 12% valerate copolymer. The general trend for the hydrolytic stability of injection moulded plaques nucleated with the three different nucleants is boron nitride > talc > hydroxyapatite, i.e. the samples nucleated with hydroxyapatite were the least stable to hydrolytic degradation. This is possibly due to the extent of crystallinity induced into the sample by the different nucleating agents. Table 4.4 suggests that samples nucleated with talc are more crystalline

Graph 4.2 **Effect of nucleating agent on the hydrolytic degradation of 12% PHV copolymer;Injection moulded plaques, degraded in pH 7.4 buffer at 70°C.**



and therefore more resistant to hydrolytic degradation, compared to those nucleated with hydroxyapatite. These samples would be expected to show lesser resistance to hydrolytic degradation and this is evident from the weight loss profiles shown for these samples in Graph 4.2.

The effects of different fabrication methods on the hydrolytic degradation of the PHB-PHV copolymers is shown in Table 4.3, which compares the time for 10 and 50% dry weight loss (t_{10} and t_{50} values), of films, discs and injection moulded samples of 12 and 20% PHV, (Mw molecular weights of 350K and 300K, respectively) copolymers after degradation in pH 7.4 buffer at 70°C. The injection moulded samples are more stable to hydrolytic degradation, with solvent cast films being the least stable. The extent of crystallinity induced to the sample as a result of the various fabrication methods was determined by X-ray diffraction. Figures 4.1 and 4.2 illustrate the resulting X-ray diffraction spectra for the 12 and 20% PHV copolymers, respectively. The percentage

Table 4.4 Crystallinities of PHB-PHV copolymer samples fabricated by different methods.

Method of fabrication	% Crystallinity	
	12% PHV (350K) copolymer	20% PHV (300K) copolymer
As- received powder	62±5	60±5
As-received 'All-Bran'	45±5	48±5
Solvent-cast film (174)	48±5	50±5
Melt pressed disc	63±5	61±5
Injection moulded plaque (Talc)	72±5	68±5
Injection moulded plaque (Apatite)	69±5	65±5

Figure 4.1 X-ray diffraction traces for 12% PHV (350K) copolymer fabricated by different methods.

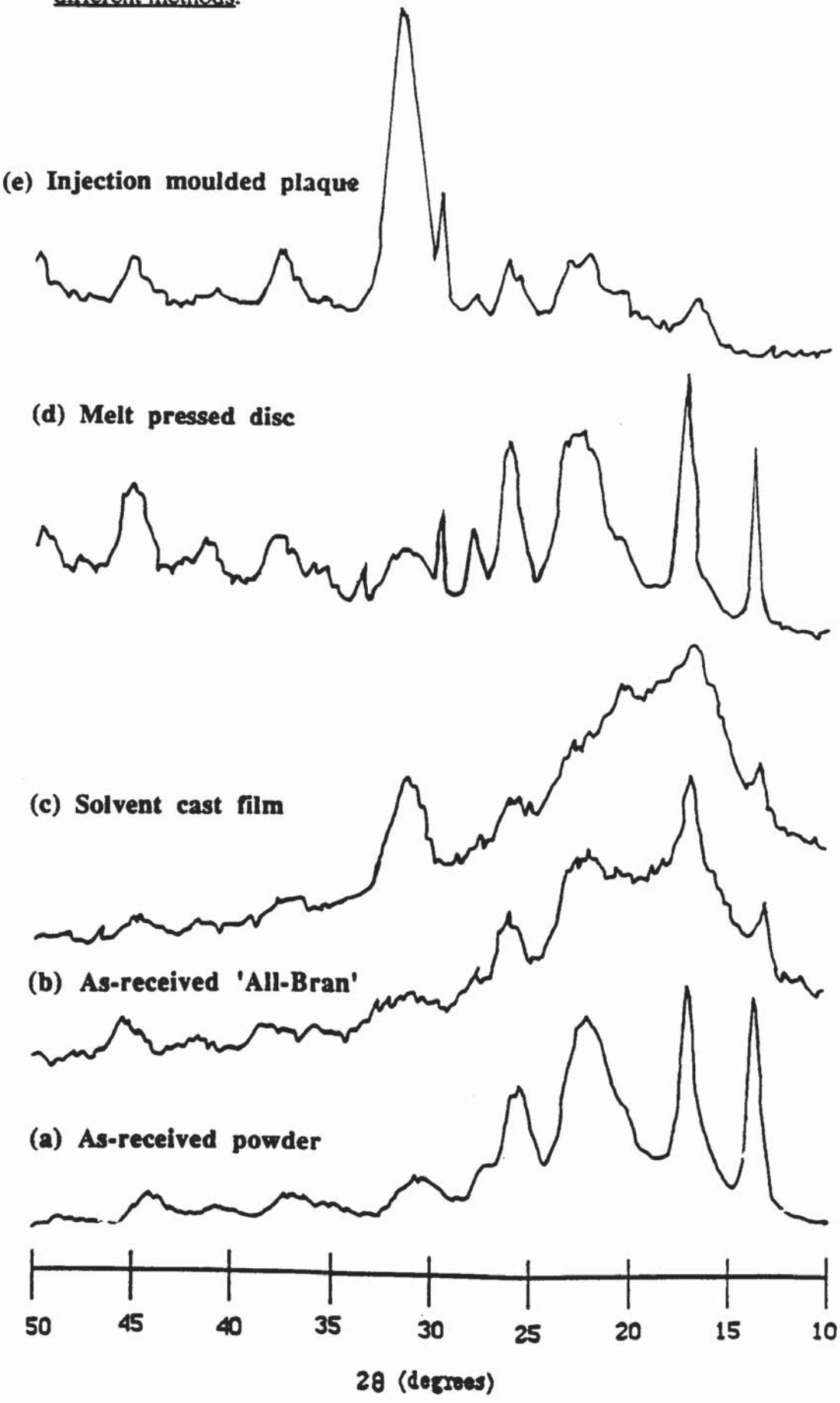
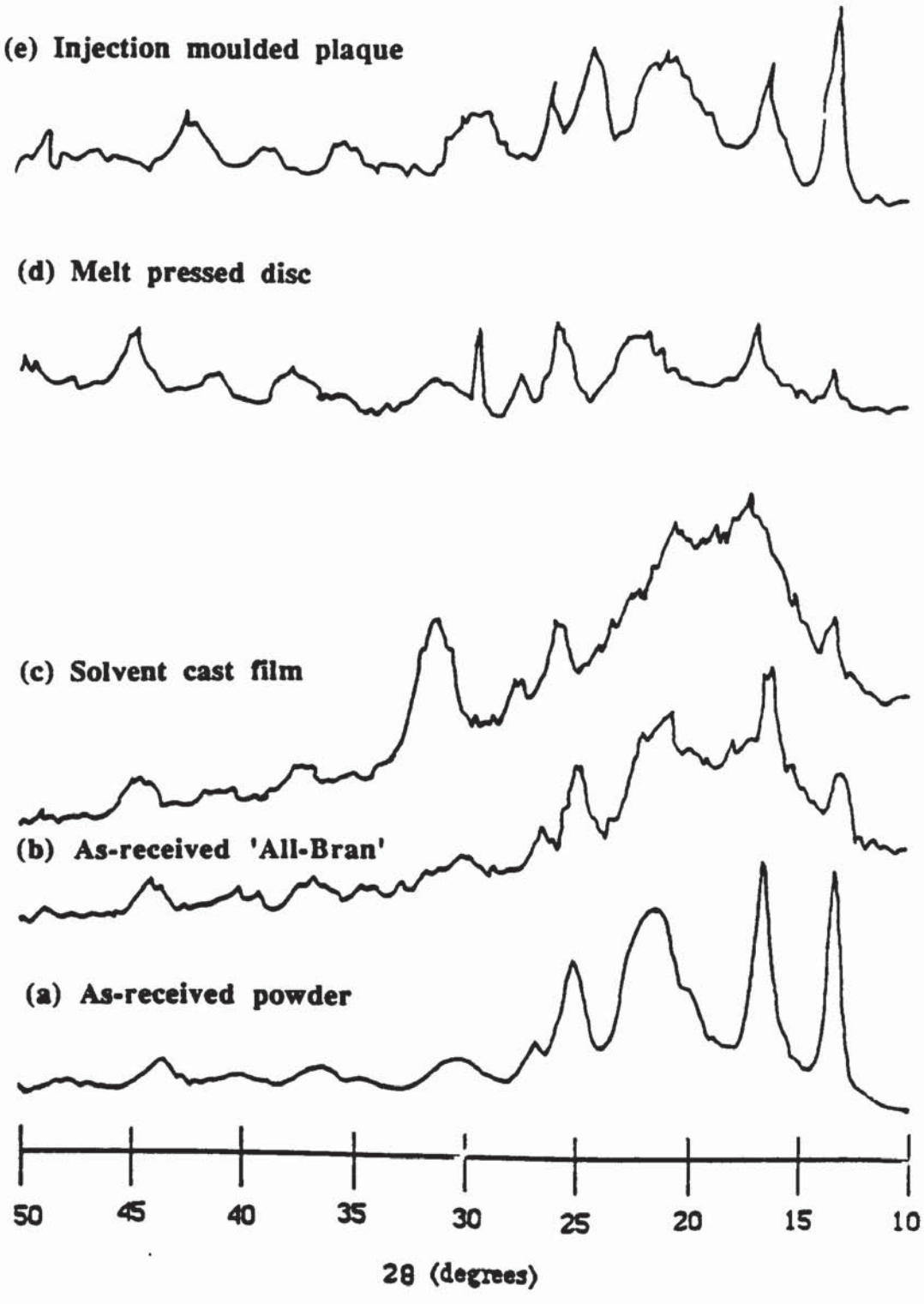


Figure 4.2 X-ray diffraction traces for 20% PHV (300K) copolymer fabricated by different methods.



crystallinities are shown in Table 4.4. The vast difference in the hydrolytic stability of injection moulded and solvent cast films is explained by the differences in the crystallinities of the two samples; injection moulded samples being more crystalline than solvent cast films. The PHB-PHV copolymer samples vary in their 'as-received' crystallinity depending upon the final stages that have been used in the purification and preparation of the material. The fibrous powder form of the raw material is precipitated from methanol in the final stages of preparation, whereas the 'all-bran' form is extruded from a slurry of the polymer in chloroform[173]. These differences in the preparation account for the powder form of the raw material being more crystalline than the all-bran form. Figures 4.1a (powder) and 4.1b (all-bran) illustrate the differences in the crystallinities of the two forms of the raw materials of the 12% PHV copolymer. The corresponding x-ray diffraction traces for the raw materials of the 20% PHV copolymer are illustrated in Figures 4.2a (powder) and 4.2b (all-bran).

Figure 4.1c shows the x-ray diffraction trace for a solvent cast film of the 12% PHV copolymer. The changes brought about by melt processing to the crystallinity of the 12% PHV copolymer are illustrated by Figure 4.1d (melt pressed disc) and Figure 4.1e (injection moulded plaque). The x-ray diffraction traces for the 20% PHV copolymer corresponding to the solvent cast film, melt pressed disc and injection moulded plaque are illustrated in Figures 4.2c, 4.2d and 4.2e, respectively.

The overall crystallinity changes of the various forms of the raw and processed material can be generalised in the following order; as-received material < solvent cast film < melt pressed < injection moulded samples.

After extensive periods of hydrolytic degradation, the crystallinity of the samples

Figure 4.3 Changes in the X-ray diffraction traces for 12% PHV (350K), injection moulded plaques (Talc), samples as result of hydrolytic degradation, (pH 7.4 buffer, 70°C): 120 days; weight loss = 42%.

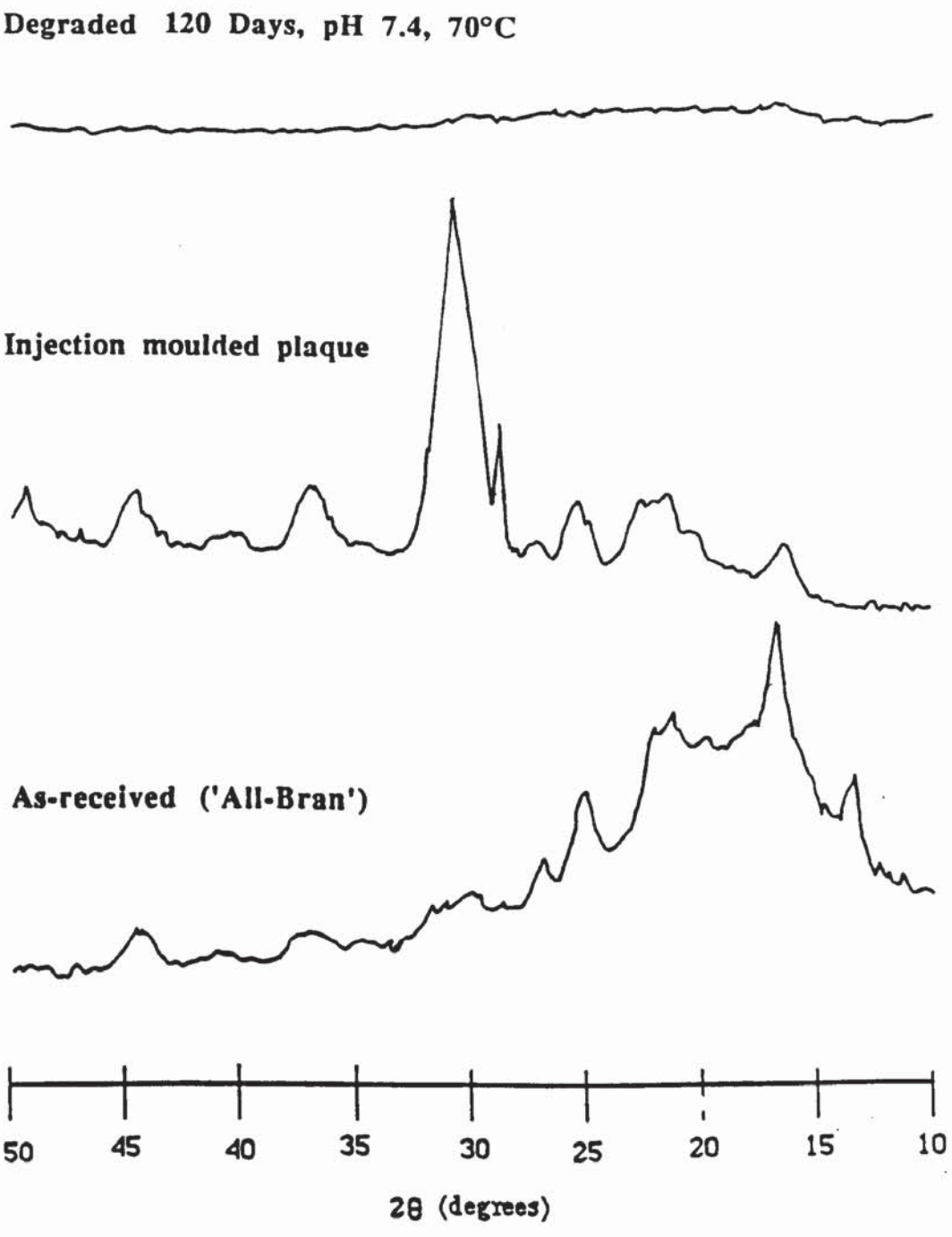


Figure 4.4 Changes in the X-ray diffraction traces for 20% PHV copolymer, injection moulded plaques (Talc), samples as result of hydrolytic degradation,(pH 7.4 buffer, 70°C); 120 days; weight loss = 47%.

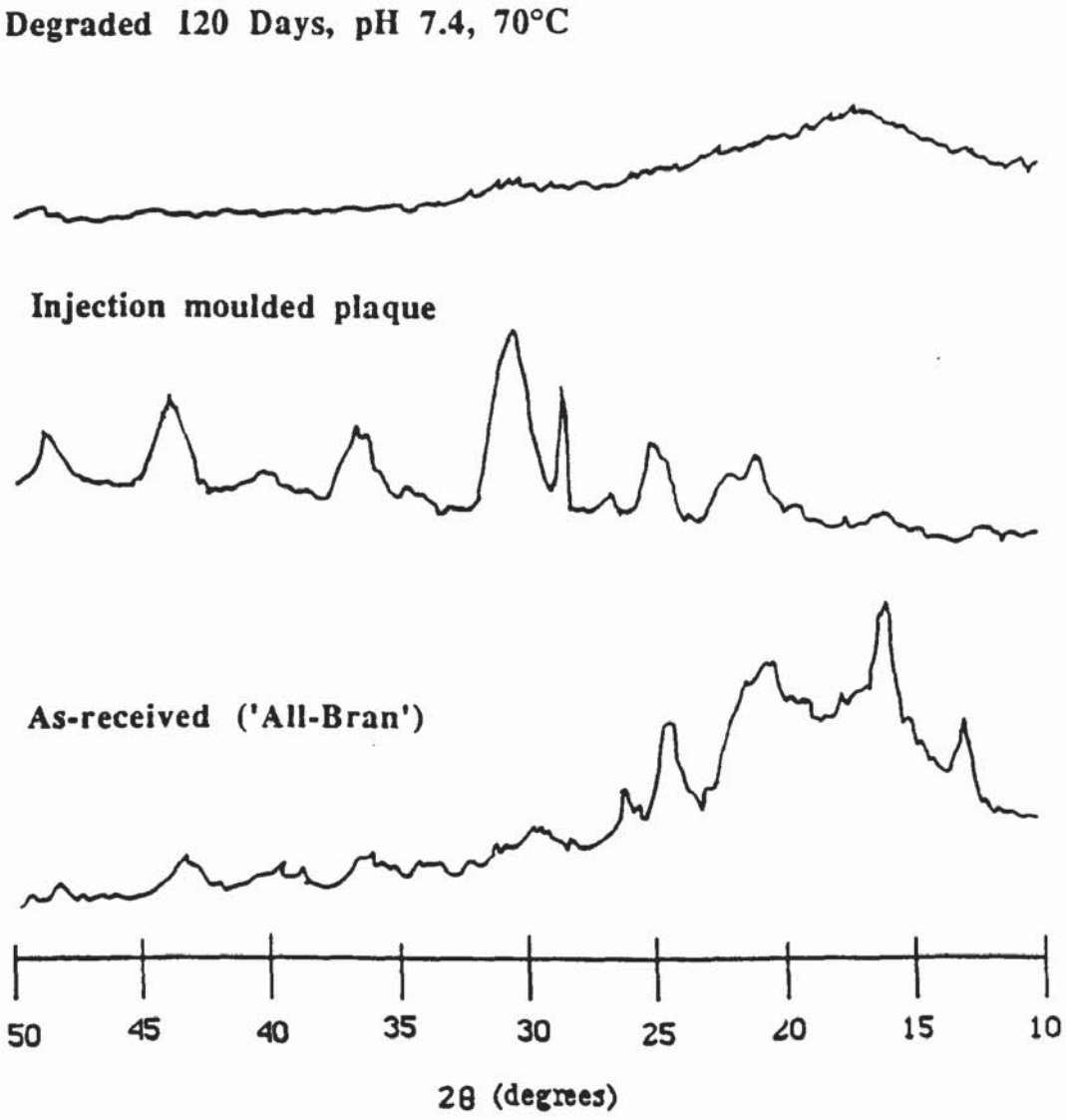


Table 4.5 Effect of processing and degradation on the molecular weights of 12 and 20% PHV copolymers; Injection moulded plaques (Talc nucleated). Degradation conditions: pH 7.4 buffer at 70°C, 120 days.

	12% PHV ('350K')			20% PHV ('300K')		
Parameter	As-received powder	Injection moulded	Degraded pH 7.4, 70°C	As-received powder	Injection moulded	Degraded pH 7.4, 70°C
Mw	280K	192K	4.27K	390K	195K	3.4K
Mn	68K	87K	2.4K	134K	87K	2.1K
Mw/Mn	4.1	2.2	1.8	2.9	2.2	1.6

decreases. Figures 4.3 and 4.4 illustrate the changes with crystallinity as a function of degradation time, for the 12 and 20% PHV copolymers, (talc nucleated, injection moulded plaques), respectively. The low crystallinity of the degraded samples, in comparison to undegraded samples, suggests that after the amorphous regions are initially degraded, degradation of the more resistant crystalline regions occurs. It is interesting to see the contribution the different nucleating agents make to the hydrolytic degradation rate of these copolymers. This is probably due to the physical nature (i.e. particle size) of the nucleating agent. Boron nitride was found to be the best nucleating agent, possibly due to its smaller particle size (≈ 0.5 - 1.0 μm)[173]. The particle size of calcium hydroxyapatite was ≈ 20μm[173], whereas Norwegian talc was found to consist of acicular particles having an average length of ≈ 15μm. The particle size of the nucleating agent could affect the resulting size of the spherulites in the injection moulded specimens and it is a well known fact that the size of spherulites controls the physical properties of the sample.

Tensile tests on injection moulded samples confirmed that samples containing boron nitride were slightly superior to those containing either hydroxyapatite or Norwegian talc. However, because of its toxicity, use of boron nitride in the body is not permitted for obvious reasons. Further work on the injection moulded samples of the PHB-PHV copolymers was limited to using either hydroxyapatite (which is a major inorganic constituent of bone), or talc as the nucleating agents.

The combined effects of milling and injection moulding on the molecular weight of these copolymers was studied using gel permeation chromatography, (GPC). It is important to remember that molecular weight determinations by gel permeation chromatography should not be treated as absolute determinations, because interpretation

of the data from GPC is liable to be affected by some operator decisions[175]. This is apart from the basic problem of using suitable solvents for the polymer in the first place. This probably explains why the molecular weights quoted for the as-supplied material by the supplier are different to the molecular weights determined by RAPRA Technology Ltd for the same material.

The effects of processing on the molecular weight of the 12 and 20% PHV copolymers is illustrated in Table 4.5. The Mw values for the 12 and 20% PHV copolymers after the blending and injection moulding processes have decreased from $3.9x10^5$ and $2.8x10^5$ to $1.92x10^5$ and $1.95x10^5$, respectively. The combined effects of shear and temperature are responsible for this. After degradation in a pH 7.4 buffer at 70°C for a period of 120 days, it is evident from Table 4.5 that the molecular weights of the injection moulded (talc nucleated) samples of these copolymers have decreased quite dramatically. The Mw values for the 12 and 20% PHV copolymers have fallen to $3.4x10^3$ and $4.3x10^3$ from an initial value of $1.9x10^5$, respectively. This again suggests hydrolytic degradation is occurring by chain scission processes.

Comparing Tables 4.2 and 4.5, the effects of processing on molecular weight are also apparent. The injection moulding process causes a decrease in the Mw and Mn values, a decrease which is somewhat more marked than that which is produced by melt pressing. This difference is possibly due to the varying degrees of shear and pressure, as well as the difference in the temperatures which the copolymers experience during the two processes. Melt pressing is a much milder processing technique, which requires a lower temperature and pressure of operation than injection moulding. In injection moulding the polymer is subjected to higher temperatures so that it will flow into the mould and also a higher pressure is needed for complete filling of the mould. As a result of the combined

effects of higher temperature and pressure, molecular weights will be decreased more markedly with injection moulding than with melt pressing. In conclusion, the hydrolytic degradation of the PHB-PHV copolymers has been shown to be affected by molecular weight, valerate content, and mode of sample preparation.

In an attempt to increase the rather slow hydrolytic degradation rate of these copolymers, polysaccharides were blended with these copolymers using melt blending techniques established in Chapter 3. The physical and degradative properties of these blends were investigated. The 12 (Mw = 350K) and 20% (Mw = 300K) PHV copolymers were used for this study because these particular samples provided a combination of suitable basic physical and degradative properties, and above all they had ideal processing temperatures.

4.3 PHB-PHV/POLYSACCHARIDE BLENDS.

4.3.1 INTRODUCTION.

Various polysaccharides were blended with 12 and 20% PHV copolymers, using the methods described in Chapter 2. Physical and degradative properties of these were investigated using injection moulded samples.

4.3.2 PHYSICAL PROPERTIES.

In order to eliminate possible problems in the injection moulding of the PHB-

PHV/polysaccharide blends and also to enable comparison of the physical properties of the blends with the unfilled copolymers, hydroxyapatite nucleated 12 and 20% PHV copolymers were used to prepare these blends. Tensile properties were investigated on small injection moulded dumb-bell shaped test pieces, using the Instron tensometer at room temperature, as described in Section 2.3.

4.3.2.1 RESULTS AND DISCUSSION.

The various physical parameters obtained from tensile test measurements are shown in Table 4.6. With addition of 10% w/w of polysaccharide to the 12 and 20% valerate copolymers, both the yield strength and % elongation to break fall, while initial modulus increases, in comparison to unfilled polymer.

The mode of fracture of the PHB-PHV/polysaccharide blends seems to be basically one of brittle fracture, except for the 20% PHV/10% dextrin blend. This particular blend fractured by a ductile mode of fracture, and as a result the % elongation to break of this blend is higher than the unfilled 20% PHV (apatite nucleated) sample. The yield strength of both unfilled 20% PHV copolymer and the 20% PHV/10% dextrin blend are similar, while the initial modulus for the dextrin was higher than the unfilled polymer.

Tensile properties of the PHB-PHV/polysaccharide blends were limited to investigations involving three polysaccharides, amylose, dextran and dextrin, with a maximum of 10% w/w loadings. These three fillers were used because of their difference in solubility/physical form. The effect of the physical size of the filler on the physical properties of these PHB-PHV/polysaccharide blends is evident when comparing the yield

strength and % elongation to break for the 12% PHV blends with amylose and dextrin blends. The larger particle size of the amylose (≈ 50μm) compared to the smaller dextrin particles (≈ 10μm), decreases the yield strength and the % elongation to break, probably by reducing the compaction of the matrix.

Table 4.6 Tensile properties of PHB-PHV/polysaccharide blends, (injection moulded).

Blend	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
12% PHV (1% apatite)	28.7 ±1.8	12±2	500±30	27.1±1.8	16±2
20% PHV (1% apatite)	19.1±0.2	13±2	310±10	17.3±0.9	22±2
12% PHV/10% Amylose	24.3±1.1	9±2	550±20	21.4±1.0	14±3
20% PHV/10% Amylose	20.0±0.5	11±2	450±50	17.1±0.4	21±2
12% PHV/10% Dextran	25.7±1.1	10±2	580±30	24.7±0.8	12±2
20% PHV/10% Dextran	19.5±0.3	11±2	380±40	17.1±0.4	24±2
12% PHV/10% Dextrin	26.6±1.3	10±2	580±30	23.8±1	24±2
20% PHV/10% Dextrin	19.3±0.7	12±3	450±20	14.6±0.9	77±5

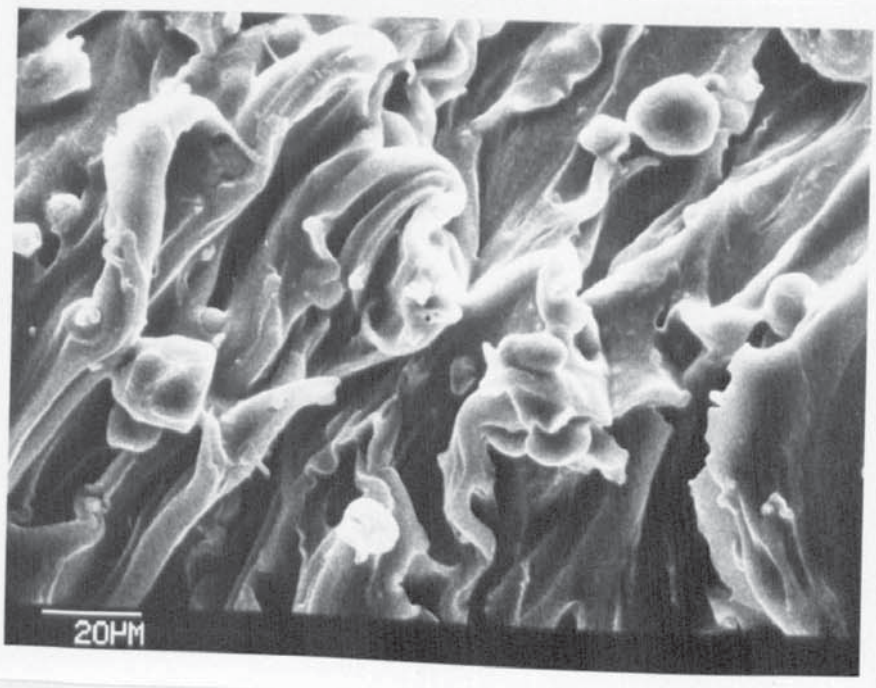
where YS = yield strength (MPa) % EY = % Elongation at yield
ITM = Initial tensile modulus (MPa) UTS = Ultimate tensile strength (MPa)
% EB = % Elongation at break

The 20% PHV/10% dextrin blend shows interesting properties, which could be because a more 'compatible' blend results. The evidence that this particular blend fractures by a ductile mode of fracture is apparent from both visual inspection of the fractured

fragments, and from the SEM of this fractured region, (Plate 4.4). The region of whitening around the fracture area is characteristic of amorphous or semi-crystalline polymers breaking by a ductile fracture, and this whitening is probably due to stress crystallisation occurring. The SEM of this fractured blend shows strands of stretched polymer in the matrix. The small dextrin particles seem to be well distributed and firmly attached to the polymer matrix. The physical properties, i.e. % elongation also reflect this ductile mode of fracture.

Although dextran is fibrous, no apparent advantage is achieved in the physical properties by using this filler. This is probably because the fibres of dextran are not inherently strong enough to influence the physical properties to any great extent.

Plate 4.4 SEM of 20% PHV/ dextrin (10%) blend (injection moulded); fractured surface; x 1000.



It is worth mentioning here the effect of processing on the mechanical properties of the PHB-PHV copolymers and their blends with polysaccharides. The tensile property values for the unfilled 12 and 20% PHV copolymers shown in Table 4.6, are much lower than those quoted by Holmes et al[176-177] because the injection moulded samples used by Holmes et al were annealed before testing. This pretreatment, (annealing at 60°C for 24 hours), ensured that optimum levels of crystallinity were induced into the samples, and because crystallinity is an important parameter in controlling the mechanical properties, any increase in crystallinity will result in an concurrent increase in the tensile strength. A similar treatment of annealing has been used to increase the tensile strength of melt pressed films of PHB homopolymer[178].

Un-nucleated PHB-PHV copolymers were extremely difficult to injection mould, however, attempted mouldings made from these materials were found to exhibit a certain degree of flexibility and whitening, (due to polymer crystallisation under stress), was seen in the fracture region of these samples. The addition of nucleating agents, apart from reducing the injection moulding cycle and producing consistent mouldings, reduces the size of the spherulites in the sample, which ultimately improves the mechanical properties.

Tensile property comparisons with published data on injection moulded samples of these copolymers needs to be viewed with some caution, because the mould size and geometry, together with the type of injection moulder used will make some contributions to the level of crystallinity induced into the sample, and hence to the final values of the tensile properties. The hand operated SP 1 injection moulder used in this study, is unable to provide the same level of pressure for mould closing and filling that is obtainable from hydraulic operated injection moulders that have been used in other studies[176-177].

Both the forward pressure (during mould filling) and the backward pressure (when the mould is full) are critical during the injection moulding cycle, and control the level of mould filling (i.e. compaction of material). Furthermore, the size and the shape of the mould, combined with the temperatures of both the melt and the mould, govern the extent of crystallinity that is induced into a sample, which has a bearing on the final mechanical properties.

Table 4.7 Combined effect of blending and injection moulding on the molecular weight of 12% PHV copolymer.

Sample	Mw x10^3
12% PHV, as-received powder (Initial sample)	357
12% PHV + 1% talc (after blending and injection moulding)	209
12% PHV/Dextrin (10%) (after blending and injection moulding)	279
12% PHV/Dextrin (30%) (after blending and injection moulding)	223
12% PHV/Sodium alginate (10%) (after blending and injection moulding)	276
12% PHV/Sodium alginate (30%) (after blending and injection moulding)	255

Molecular weight is an another important parameter which affects the mechanical properties of these copolymers. Any processing condition that decreases the molecular weight of these copolymers, will in turn affect the final mechanical properties of the fabricated specimen. Table 4.5 shows the combined effects of blending and injection moulding on the molecular weights of 12 and 20% PHV copolymers nucleated with 1% talc. Table 4.7 shows the combined effects of blending and injection moulding on the molecular weights of the 12% PHV copolymers blended with different polysaccharides. At 10% loadings of dextrin and sodium alginate, a dramatic decrease in the molecular

weight of the 12% PHV (as-received powder form) copolymer occurs. At these low levels of polysaccharide loadings, the contributions from the physical nature of the polysaccharide to the reduction in the molecular weight is not apparent. However, at higher loadings, (30%), there is evidence that the physical nature of the polysaccharide plays a role in the decrease in the molecular weight of the PHB-PHV copolymers during processing. Dextrin is a fine particulate filler and will cause more shear of the PHB-PHV copolymer chains during blending than the fibrous sodium alginate. This is observed from the Mw molecular weights tabulated in Table 4.7.

Although the tensile properties of the PHB-PHV/polysaccharide blends shown in Table 4.6 are based on hydroxyapatite-nucleated copolymers, the tensile properties of blends of the PHB-PHV copolymers with polysaccharides made without any added nucleating agent, were similar to these. The levels of the polysaccharides used ensured that adequate nucleation sites were available for polymer crystallisation, so no problems in nucleation of the PHB-PHV copolymers were encountered during injection moulding.

4.3.3. HYDROLYTIC DEGRADATION.

Hydrolytic degradation of the PHB-PHV/polysaccharide blends with 10 and 30% polysaccharide loadings was carried out using injection moulded stepped plaques already described, at 70°C in pH 2.3 and 7.4 buffers, and at 37°C in pH 2.3 and 10.6 buffers.

Degradation of these PHB-PHV/polysaccharide blends under "physiological" condition, i.e. pH 7.4 and 37°C, is of paramount importance. The changes in the physical

properties, combined with the changes in dry weight and molecular weight of these blends, with time, is of real interest if these particular blends are to be used in the physiological environment. As such, it was felt that the importance of this particular study warranted a chapter by itself, i.e. Chapter 6 of this thesis, so no mention will be made of degradation under *in vitro* "physiological" conditions in this chapter.

4.3.3.1 RESULTS AND DISCUSSION.

Degradation of the PHB-PHV/polysaccharide blends was monitored by gravimetric methods as described in Chapter 2. The weight loss profiles of the 10% polysaccharide loaded PHB-PHV blends at 70°C in both pH 2.3 and 7.4 buffers (Graphs 4.3 to 4.6) are characterised by an initial slow weight loss, followed by an enhanced secondary phase. Increasing the valerate content of the PHB-PHV copolymers produced a small, but significant change in the rate of degradation, but did not change the form of the degradation profile.

The weight loss profiles of the 30% loaded blends in pH 2.3 and 7.4 at 70°C are apparently more complex (Graphs 4.7 to 4.10). They are characterised by a rapid initial weight loss, followed by a secondary steady phase and an enhanced tertiary phase. During the initial phase the polysaccharide begins to dissolve or degrade and is thereby removed from the polymer matrix. In the secondary phase, weight loss slows down, before accelerating again in the third phase. The weakened polymer matrix produced by the erosional processes in the first phase and second phases tends to collapse in the third phase, causing a more dramatic increase in the weight loss. Here again the effect of increasing the valerate content of the PHB-PHV copolymers was a small increase in the

Table 4.8 The t_{10} and t_{50} weight loss parameters for PHB-PHV/Polysaccharide blends in a pH 2.3 buffer at 70°C ; (Injection moulded plaques)

12% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	100	10% in 100 days
10% Amylose	52	24% in 100 days
30% Amylose	8	39% in 100 days
10% Dextran	84	17% in 100 days
30% Dextran	6	87
10% Dextrin	63	22% in 100 days
30% Dextrin	1.5	37% in 100 days
10% Sodium Alginate	63	19% in 100 days
30% Sodium Alginate	3	36% in 100 days

20% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	88	13% in 100 days
10% Amylose	41	30% in 100 days
30% Amylose	3	89
10% Dextran	34	22% in 100 days
30% Dextran	1	100
10% Dextrin	3	40% in 95 days
30% Dextrin	1	91
10% Sodium Alginate	46	32% in 100 days
30% Sodium Alginate	1	89

Table 4.9 The t_{10} and t_{50} weight loss parameters for PHB-PHV/Polysaccharide blends in a pH 7.4 buffer at 70°C ; (Injection moulded plaques)

12% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	78	42% in 120 days
10% Amylose	77	109
30% Amylose	16	56
10% Dextran	81	111
30% Dextran	4	94
10% Dextrin	73	113
30% Dextrin	3	89
10% Sodium Alginate	13	97
30% Sodium Alginate	1	22

20% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	80	47% in 120 days
10% Amylose	58	103
30% Amylose	3	20
10% Dextran	65	112
30% Dextran	2	92
10% Dextrin	40	114
30% Dextrin	2	83
10% Sodium Alginate	11	88
30% Sodium Alginate	1	15

Table 4.10 The t_{10} and t_{50} weight loss parameters for PHB-PHV/Polysaccharide blends in a pH 2.3 buffer at 37°C; (Injection moulded plaques)

12% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	2% in 400 days	-
10% Amylose	3% in 400 days	-
30% Amylose	330	-
10% Dextran	1% in 400 days	-
30% Dextran	25	24% in 400 days
10% Dextrin	4% in 400 days	-
30% Dextrin	32	21% in 400 days
10% Sodium Alginate	7% in 400 days	-
30% Sodium Alginate	10	40% in 400 days

20% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	1% in 400 days	-
10% Amylose	4% in 400 days	-
30% Amylose	235	15% in 400 days
10% Dextran	6% in 400 days	-
30% Dextran	12	26% in 400 days
10% Dextrin	160	18% in 400 days
30% Dextrin	9	23% in 400 days
10% Sodium Alginate	345	12% in 400 days
30% Sodium Alginate	5	30% in 125 days

Table 4.11 The t_{10} and t_{50} weight loss parameters for PHB-PHV/Polysaccharide blends in a pH 10.6 buffer at 37°C; (Injection moulded plaques)

12% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	300	-
10% Amylose	135	300
30% Amylose	35	145
10% Dextran	162	44% in 300 days
30% Dextran	5	230
10% Dextrin	145	275
30% Dextrin	15	275
10% Sodium Alginate	25	125
30% Sodium Alginate	2	60

20% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	4% in 400 days	-
10% Amylose	120	245
30% Amylose	19	155
10% Dextran	110	290
30% Dextran	1	122
10% Dextrin	138	265
30% Dextrin	19	157
10% Sodium Alginate	10	94
30% Sodium Alginate	1	12

Plate 4.5 SEM of degraded 12% PHV/30% sodium alginate blend (injection moulded plaque); pH 7.4, 70°C; 15 days; weight loss = 45% (x 500).

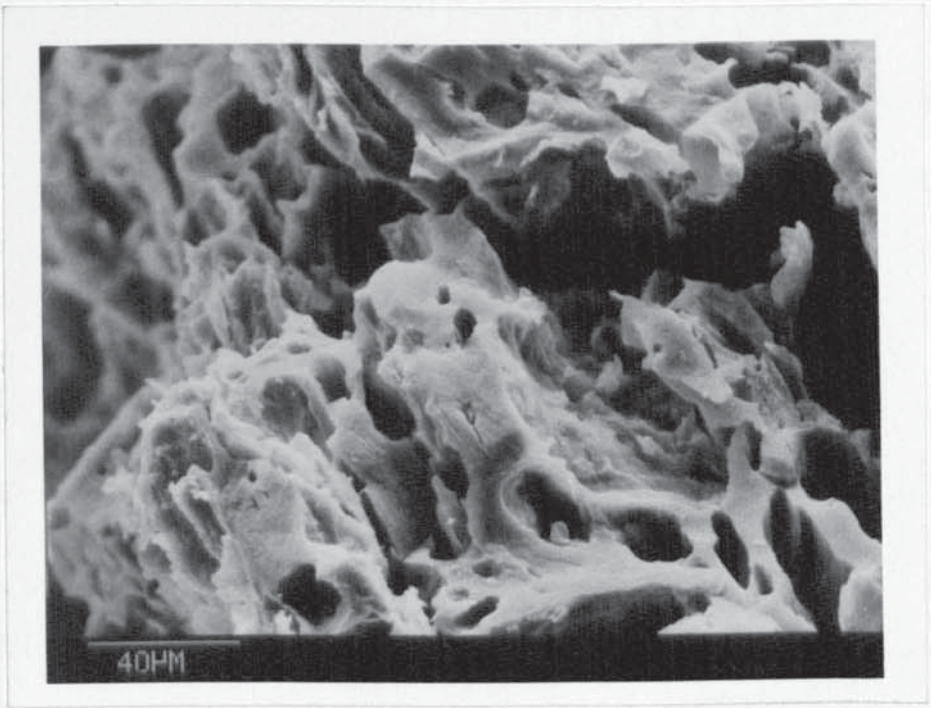
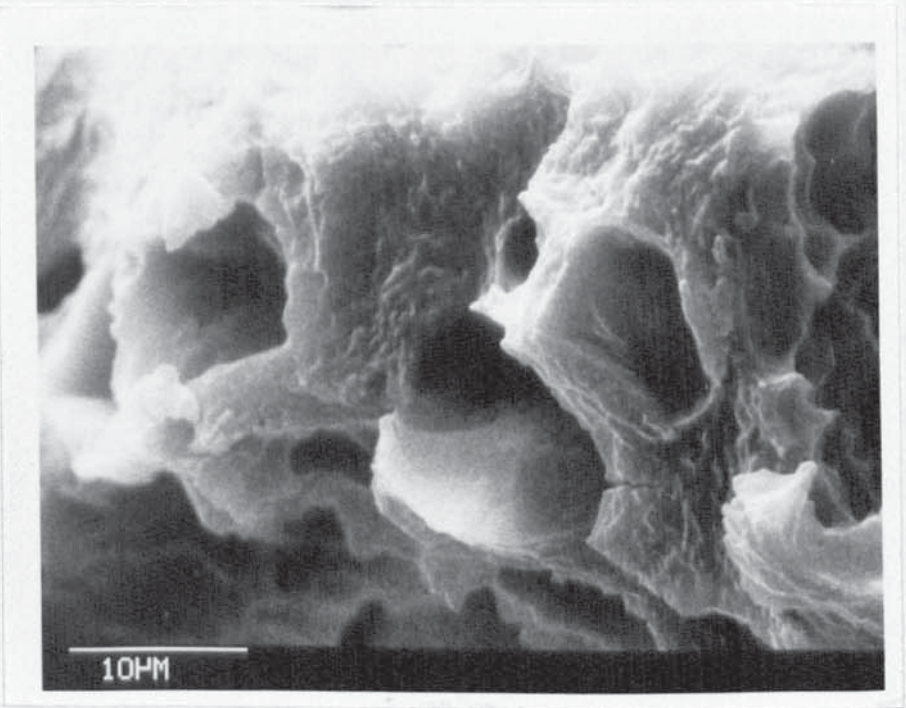


Plate 4.6 SEM of degraded 20% PHV/30% dextrin blend (injection moulded plaque); pH 7.4, 70°C; 41 days;weight loss = 40% (x 2000).



overall degradation rate, but no change in the degradation profile. Comparisons with the 10 and 30% polysaccharide loaded blends indicates that the difference between them lies in an enhanced initial degradation rate in the case of the 30% polysaccharide loadings. Thus phases 2 and 3 in the degradation of the 30% loaded blends correspond broadly with phases 1 and 2 of the 10% loaded blends. Thus the first phase is obviously present in the 10% loaded polysaccharide blends, but is less apparent.

The pH dependence on the degradation of the PHB-PHV/polysaccharide blends is also apparent from the weight loss profiles in pH 2.3 and 7.4 buffer. The weight loss in pH 7.4 buffer is appreciably faster than in pH 2.3 buffer. The overall trend regarding the elution of the polysaccharides is that in pH 2.3 buffer, dextrin is the fastest and dextran the slowest, with both amyiose and sodium alginate being intermediate. In pH 7.4 buffer however, the trend is sodium alginate > amylose > dextrin > dextran. A clearer indication obtained by comparing the time period required for 10 (t_{10}) and 50% (t_{50}) weight loss. This information is presented in Tables 4.8 and 4.9. The weight loss profiles in pH 2.3 buffer at 37°C are illustrated in Graphs 4.11 to 4.14 and the t_{10} and t_{50} values in Table 4.10, respectively.

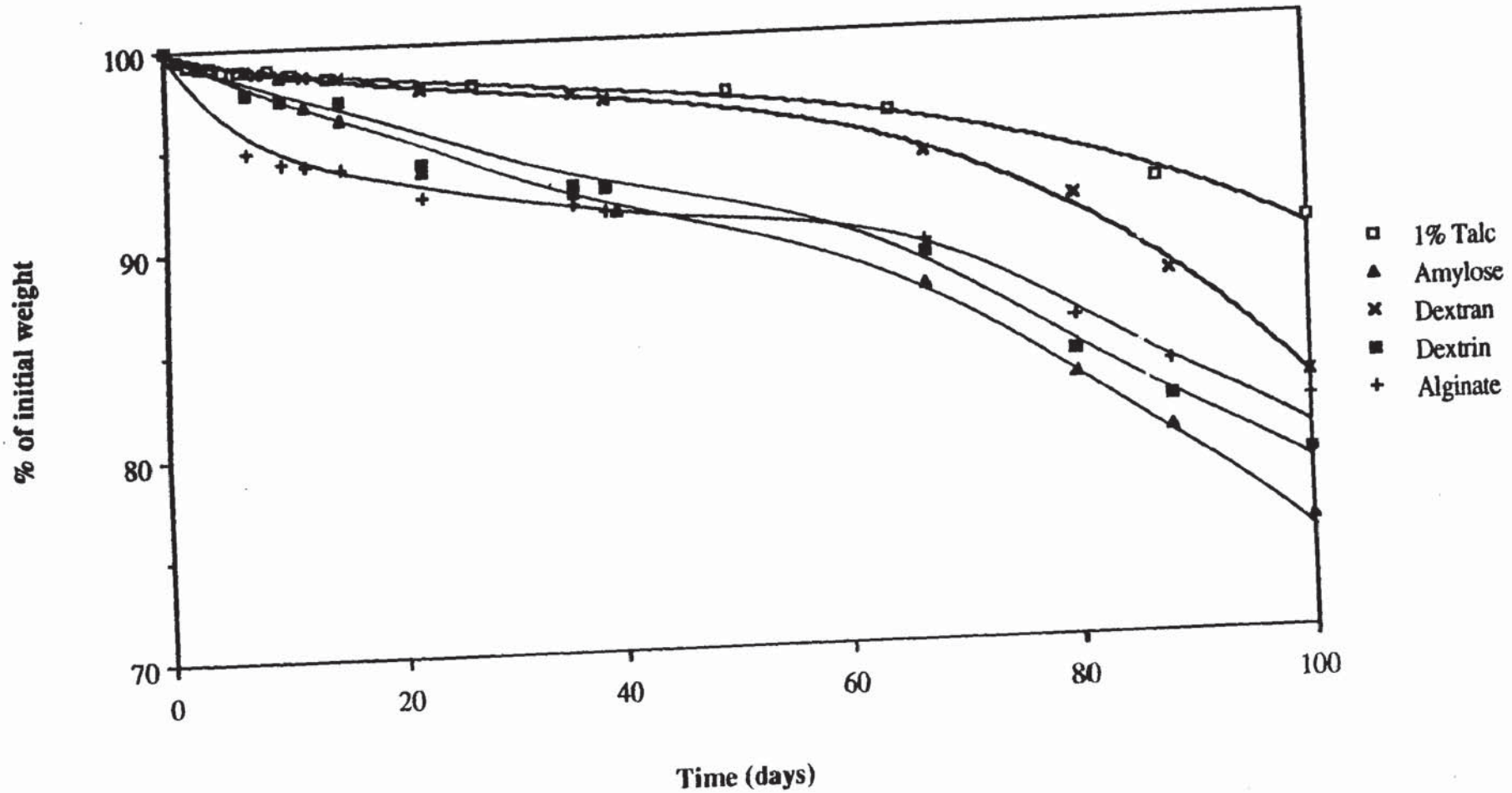
Table 4.9 illustrates that the degradation rate is increased by some 20 to 30-fold at 70°C, in comparison to degradation at 37°C. It is apparent from these results that increasing the acidity of the solution does not provide a method of accelerating the degradation. In contrast, increasing the pH to 10.6 at 37°C, provides a dramatic increase in the rate in comparison to degradation at pH 2.3 and 37°C. The weight loss profiles at pH 10.6 and 37°C are presented in Graphs 4.15 to 4.18, and the t_{10} and t_{50} values in Table 4.11. Although degradation rates at pH 10.6 and 37°C are broadly similar to those observed at pH 2.3 and 70°C, marked differences are observed in the case of individual

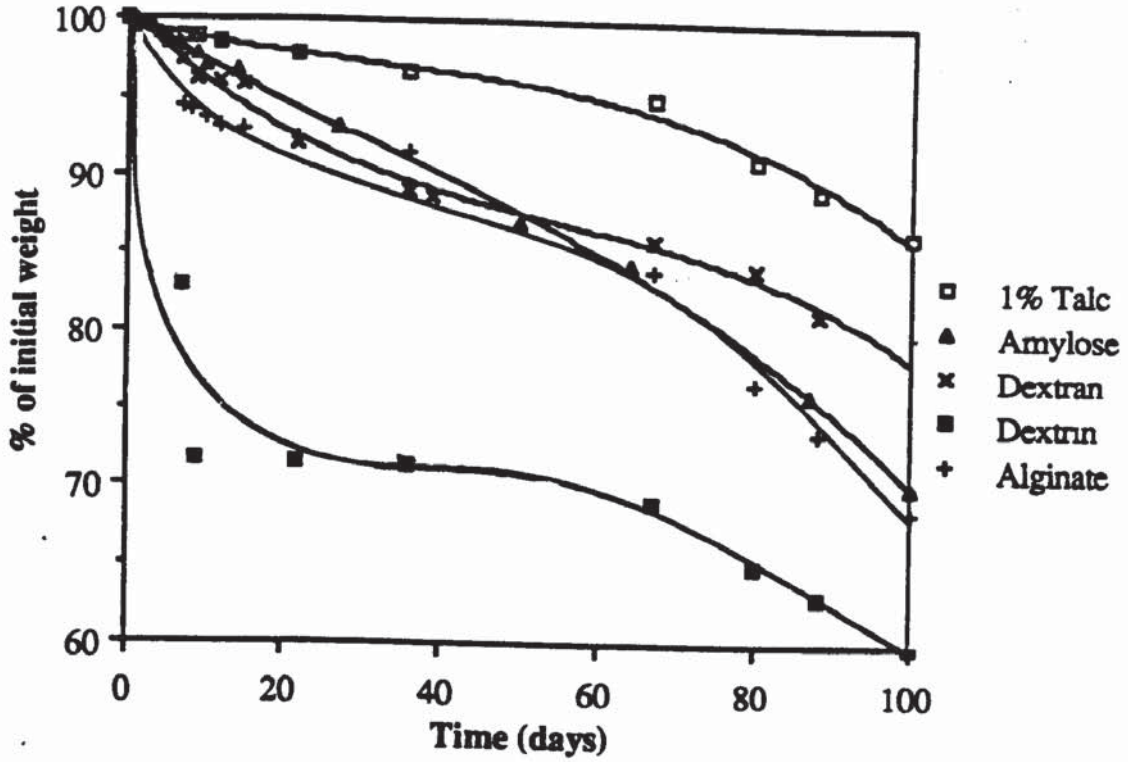
polysaccharides whose relative solubility in acid and alkaline solutions differ considerably.

It is apparent that with the incorporation of the polysaccharide into the PHB-PHV matrix the degradation rate of the PHB-PHV/polysaccharide blends increases dramatically, in comparison to unfilled copolymers. Similarly, the rates of dissolution and degradation of the polysaccharide themselves are pH dependent, which is a reflection of the differences in the known hydrolytic stabilities, and is controlled by the structural difference in the polysaccharide (see Appendix A). Dextran consists of varying amounts of α 1-2, α 1-3, α 1-4 and α 1-6 glycosidic bonds, and as a result is quite soluble. In comparison, although dextrin is soluble because of its branched structure, when this particular polysaccharide is blended into the PHB-PHV polymer matrix, other factors come into play, which govern the rate of dissolution from the matrix. These factors are a combination of physical and structural properties. The fibrous nature of dextran coupled with the mainly α 1-6 glycosidic bonds (which are more stable than α 1- 4 glycosidic bonds), results in dextran elution from the polymer matrix being much slower than the fine dextrin particles. In the case of amylose the contribution made by the actual physical size of the particles contributes to the weight loss more than does the hydrolytic stability of the α 1-4 glycosidic bonds. Amylose is insoluble, (or very slowly soluble) in pH <10, so in pH 2.3 and 7.4 buffers, the mode of weight loss from the blends could involve physical breaking up of the blend matrix, as amylose swells by absorbing the buffers.

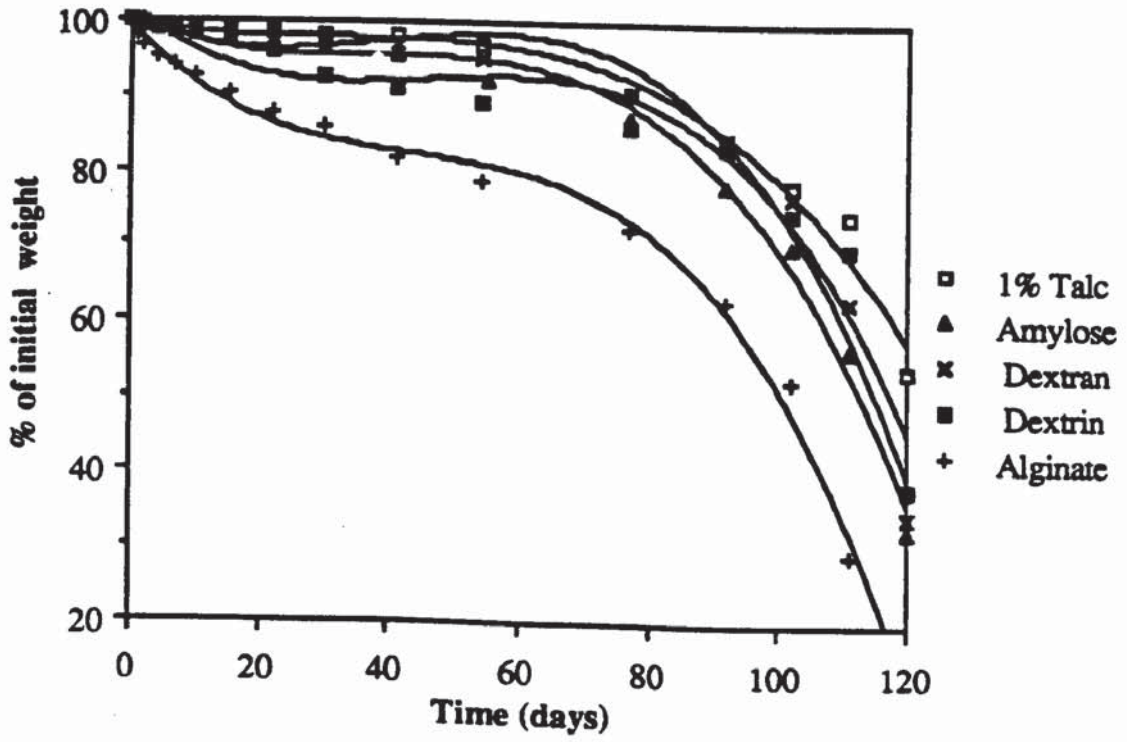
In the removal of sodium alginate from the blends, the combined effects of physical form, solubility and physical structure have to be taken into account. Although sodium alginate consists of both α 1-4 and β 1-4 glycosidic bonds, because of the the lack of

Graph 4.3 **Weight loss of 12% PHV/Polysaccharide (10%) blends; Injection moulded plaques degraded in pH 2.3 buffer at 70°C.**

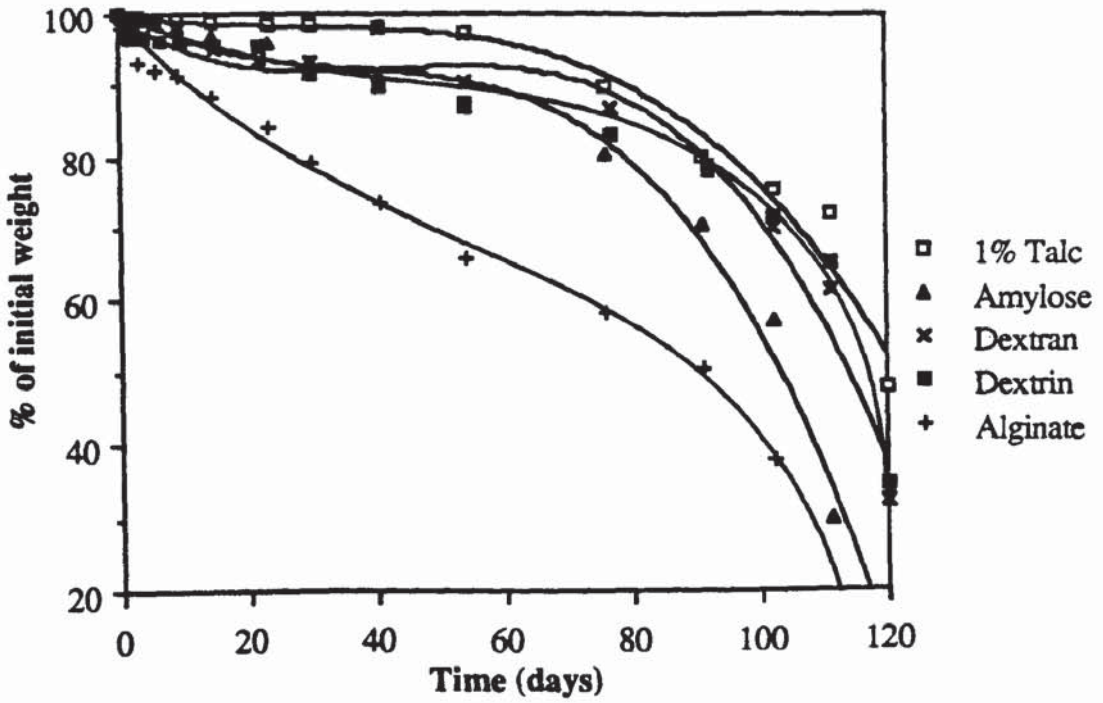




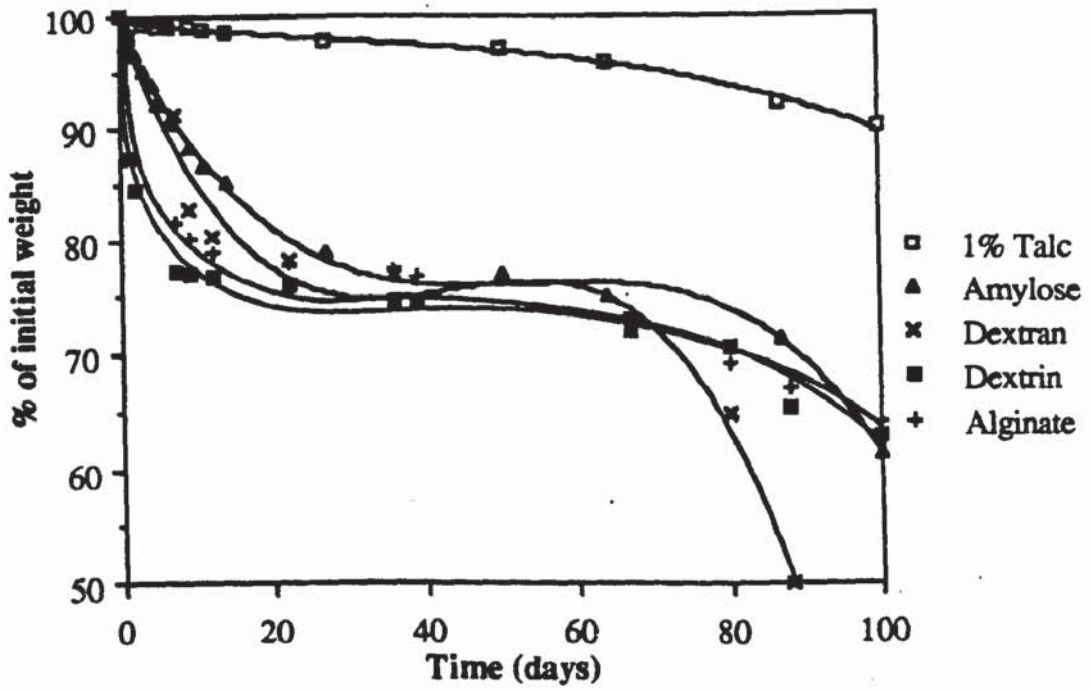
Graph 4.4 Weight loss of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 70°C.



Graph 4.5 Weight loss of 12% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 7.4 buffer at 70°C.

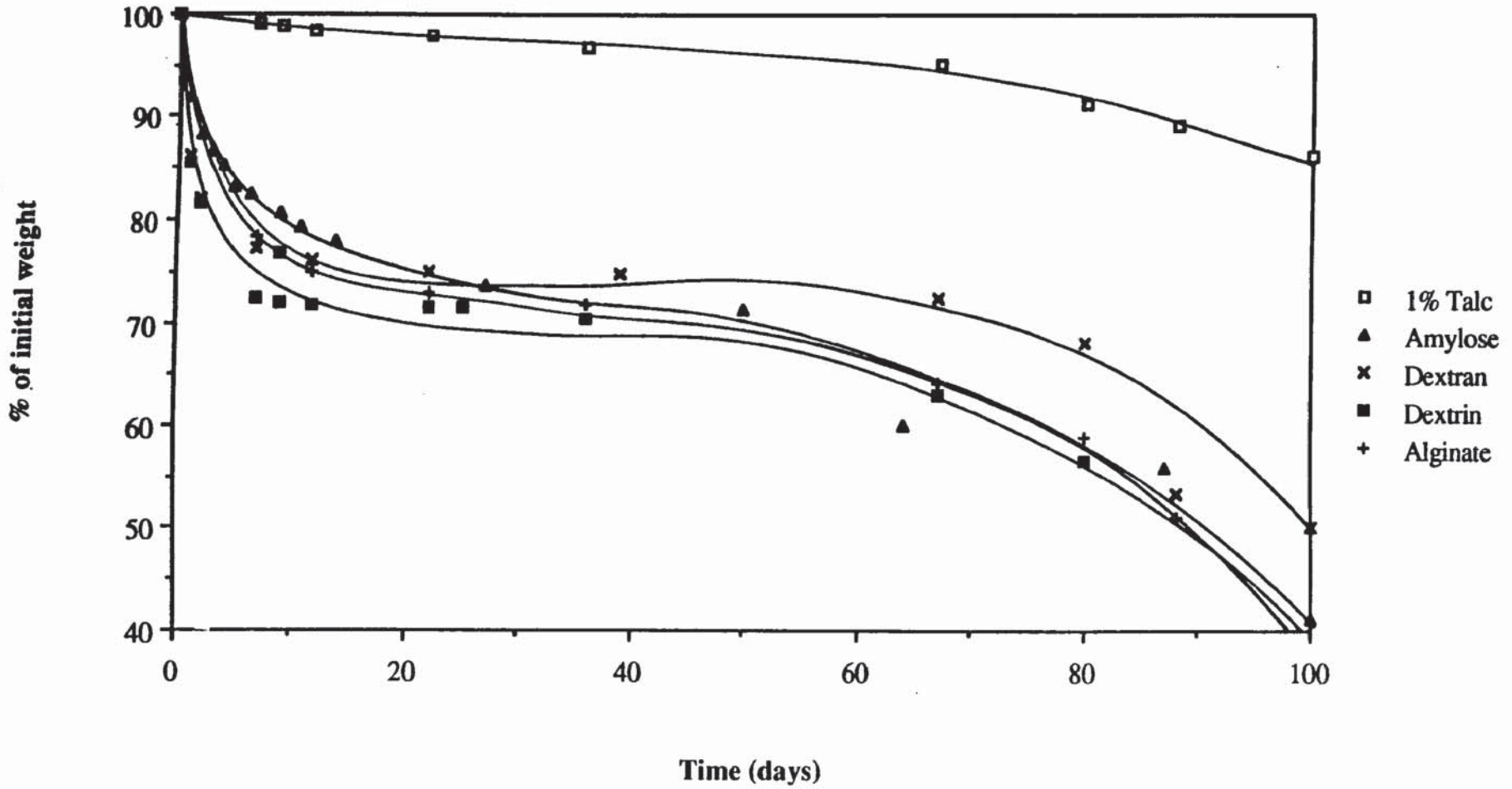


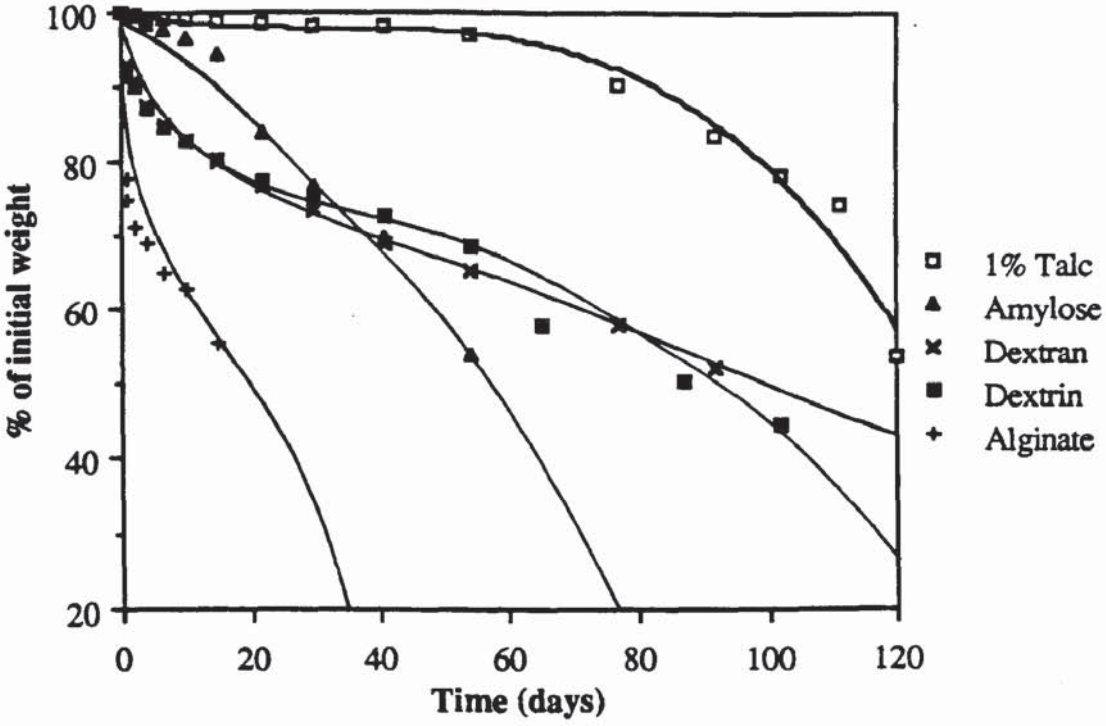
Graph 4.6 Weight loss of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 7.4 buffer at 70°C.



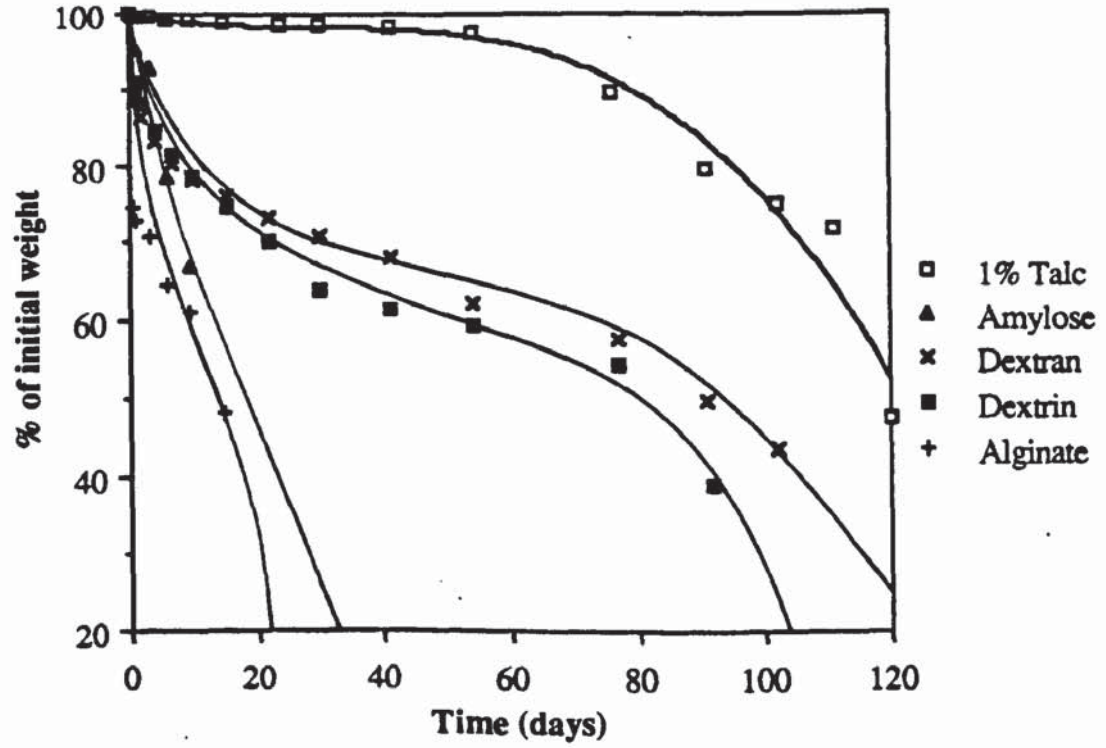
Graph 4.7 Weight loss of 12% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 70°C.

Graph 4.8 **Weight loss of 20% PHV/Polysaccharide (30%) blends; Injection moulded plaques degraded in pH 2.3 buffer at 70°C.**

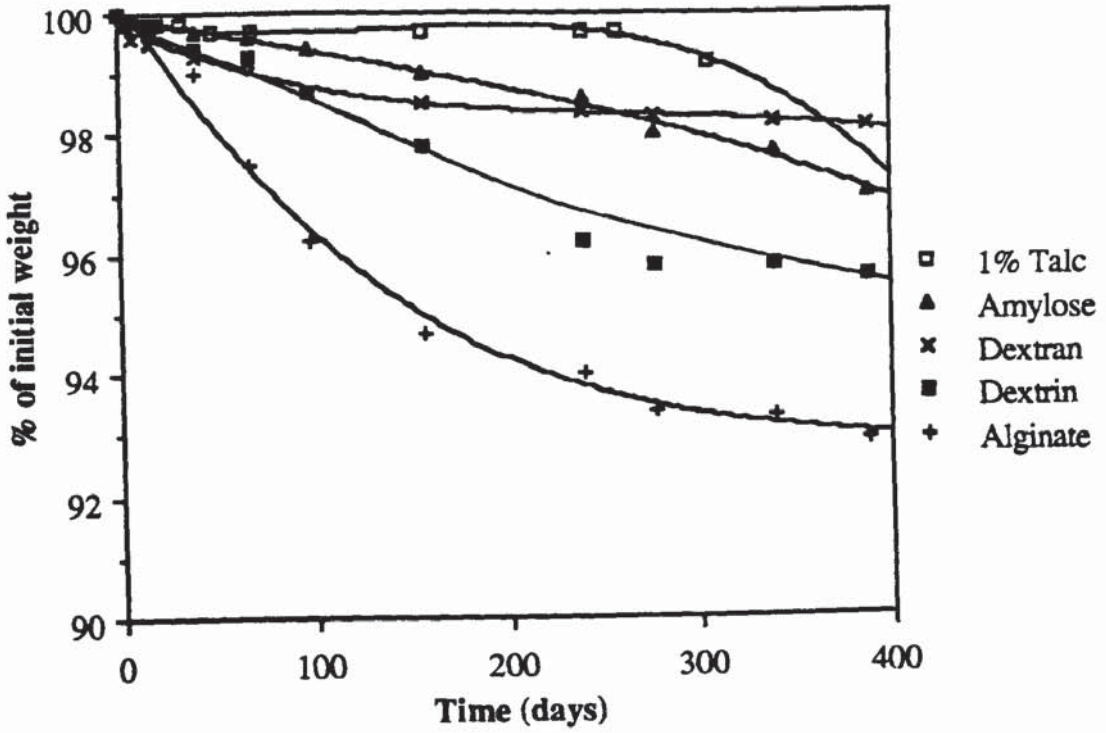




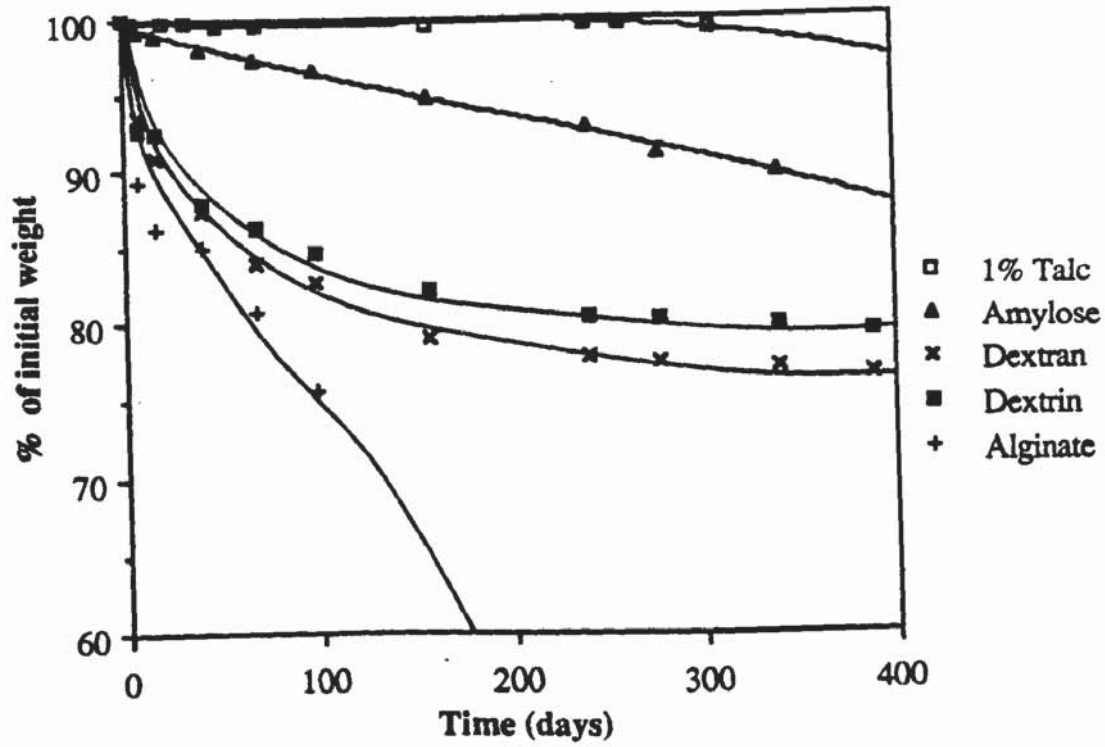
Graph 4.9 Weight loss of 12% PHV/Polysaccharide (30%) blends; Injection moulded plaques degraded in pH 7.4 buffer at 70°C.



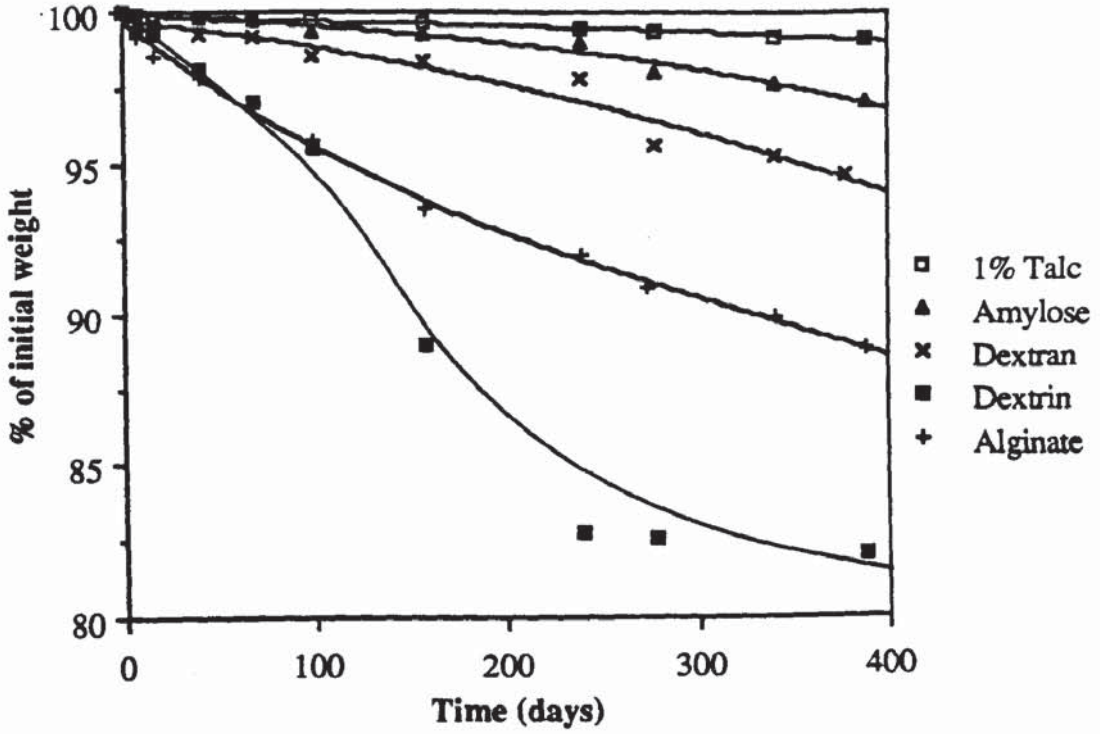
Graph 4.10 Weight loss of 20% PHV/Polysaccharide (30%) blends; Injection moulded plaques degraded in pH 7.4 buffer at 70°C.



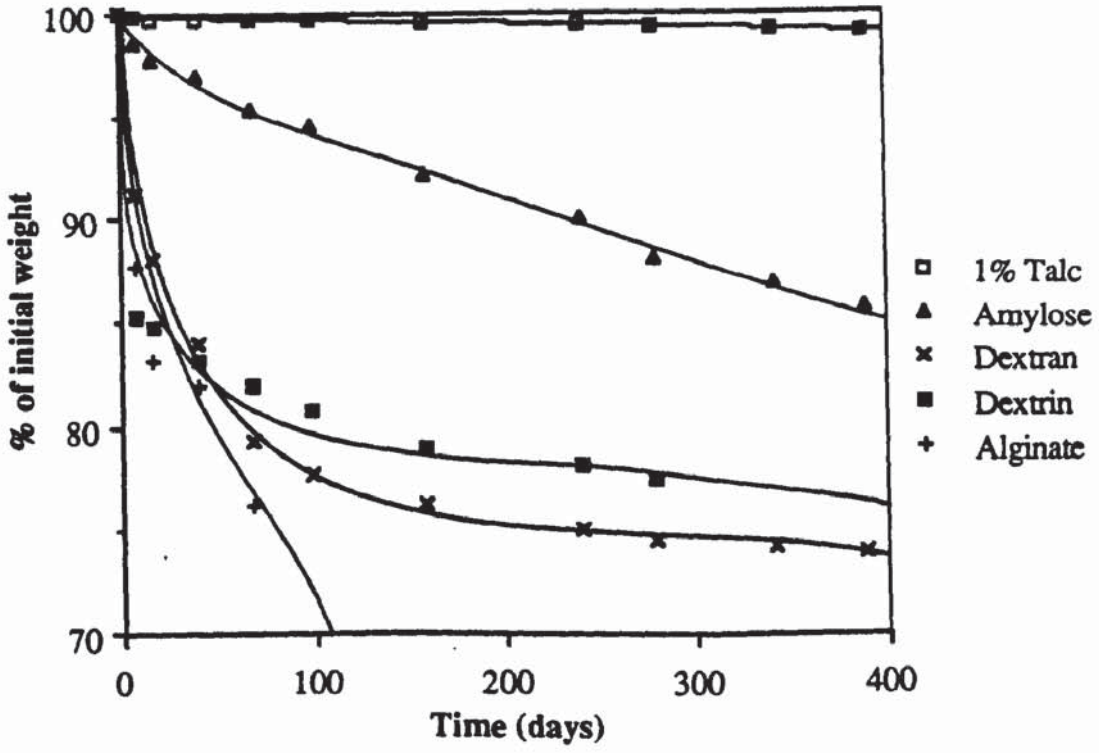
Graph 4.11 Weight loss of 12% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 37°C.



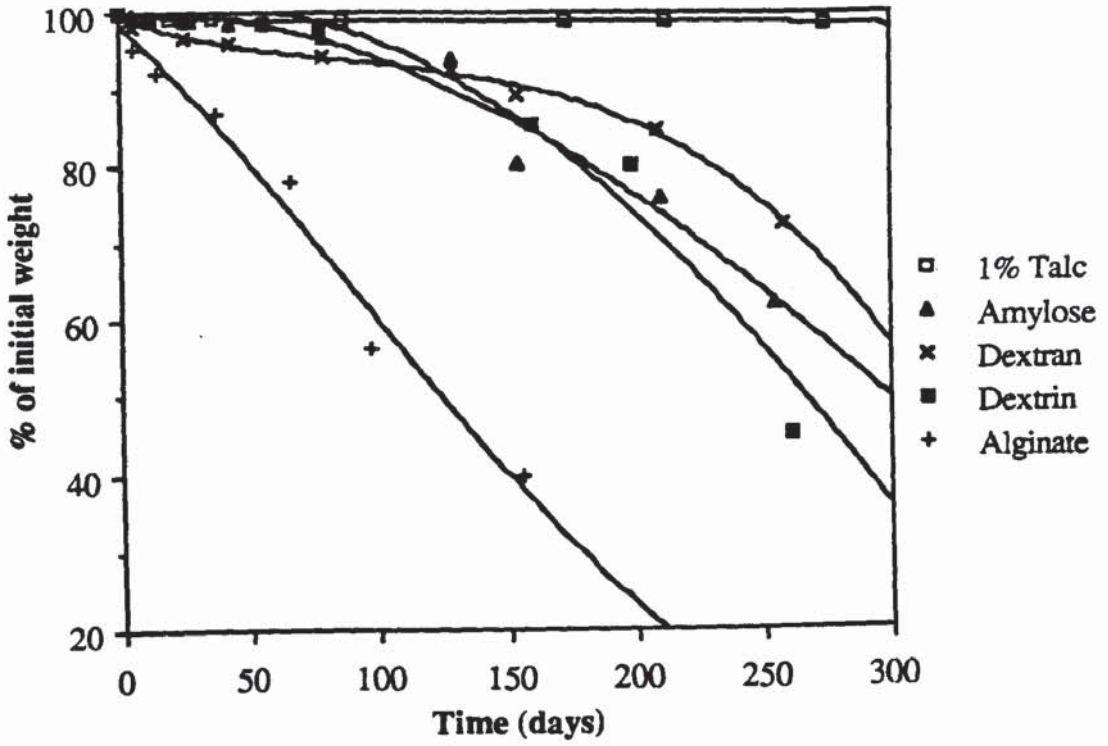
Graph 4.12 Weight loss of 12% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 37°C.



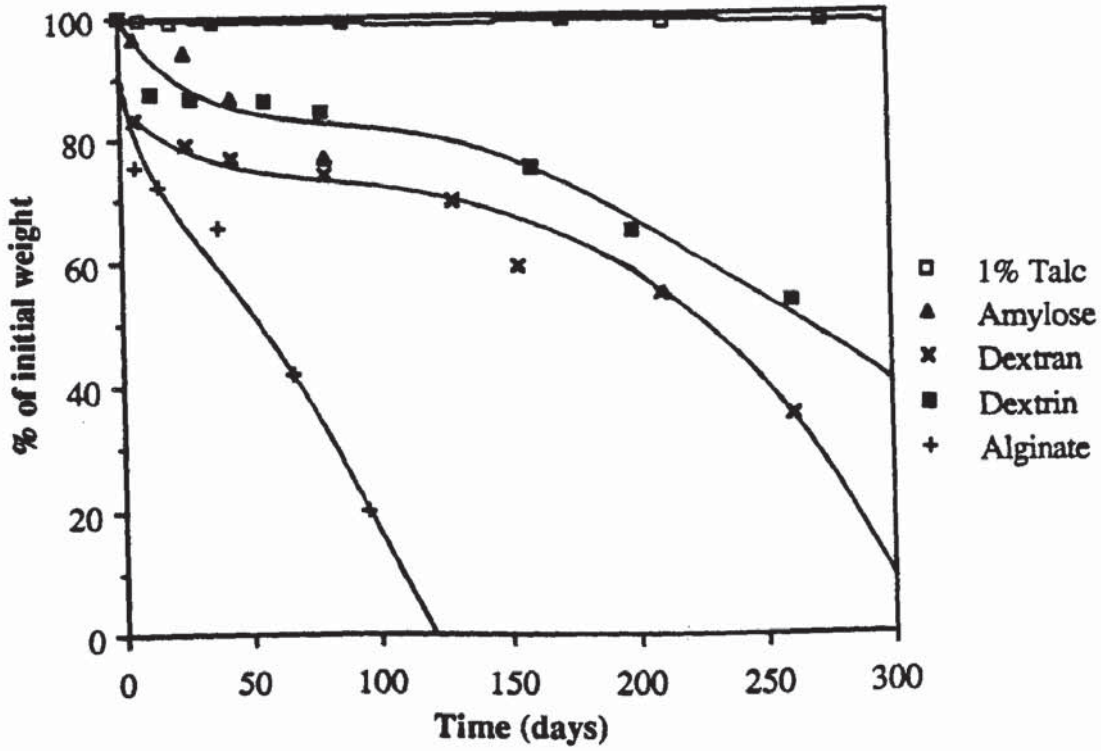
Graph 4.13 Weight loss of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 37°C.



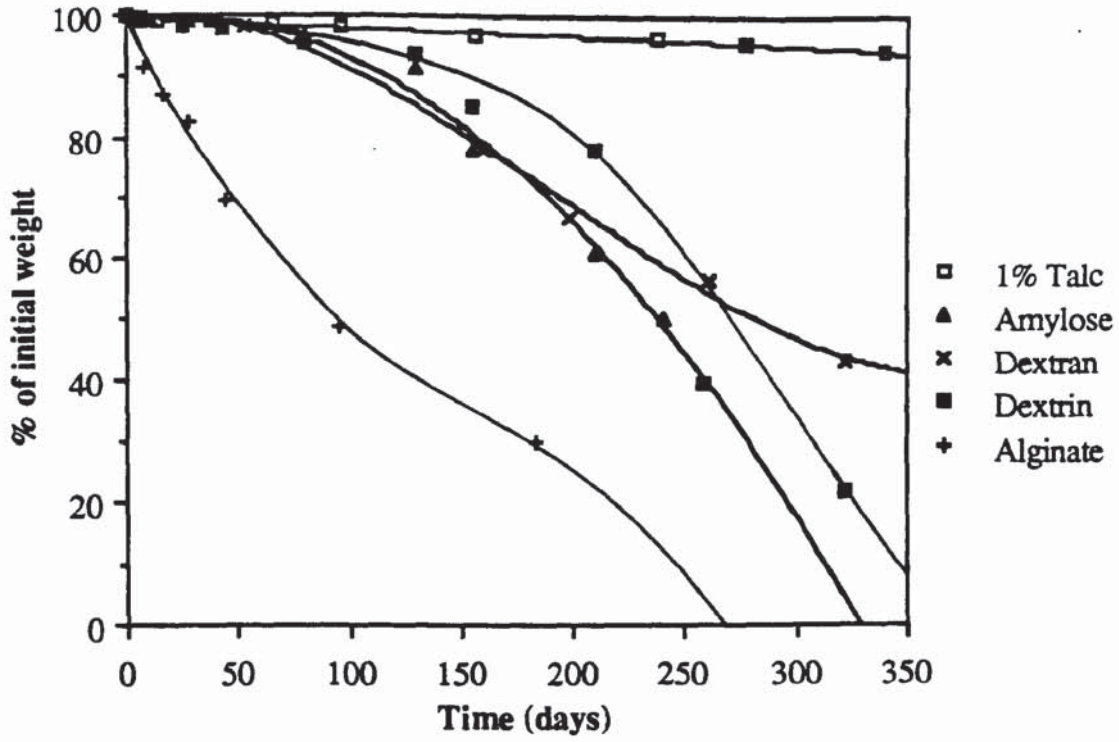
Graph 4.14 Weight loss of 20% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 2.3 buffer at 37°C.



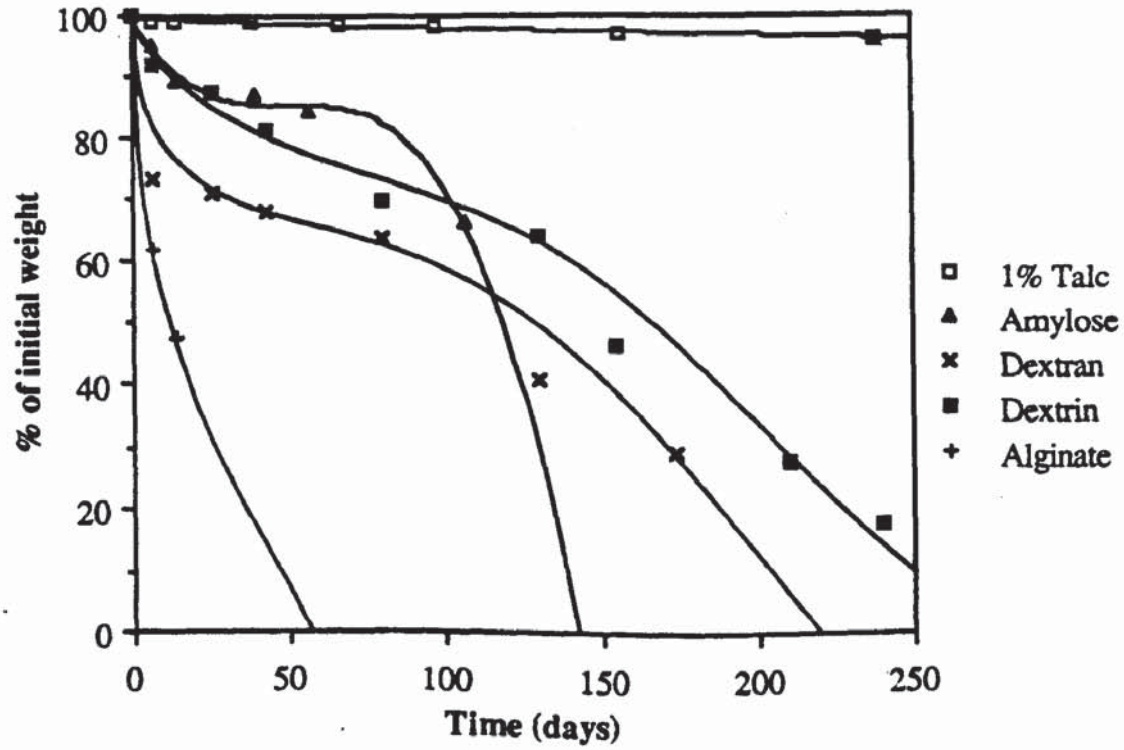
Graph 4.15 Weight loss of 12% PHV/Polysaccharide (10%) blends; Injection moulded plaques degraded in pH10.6 buffer at 37°C.



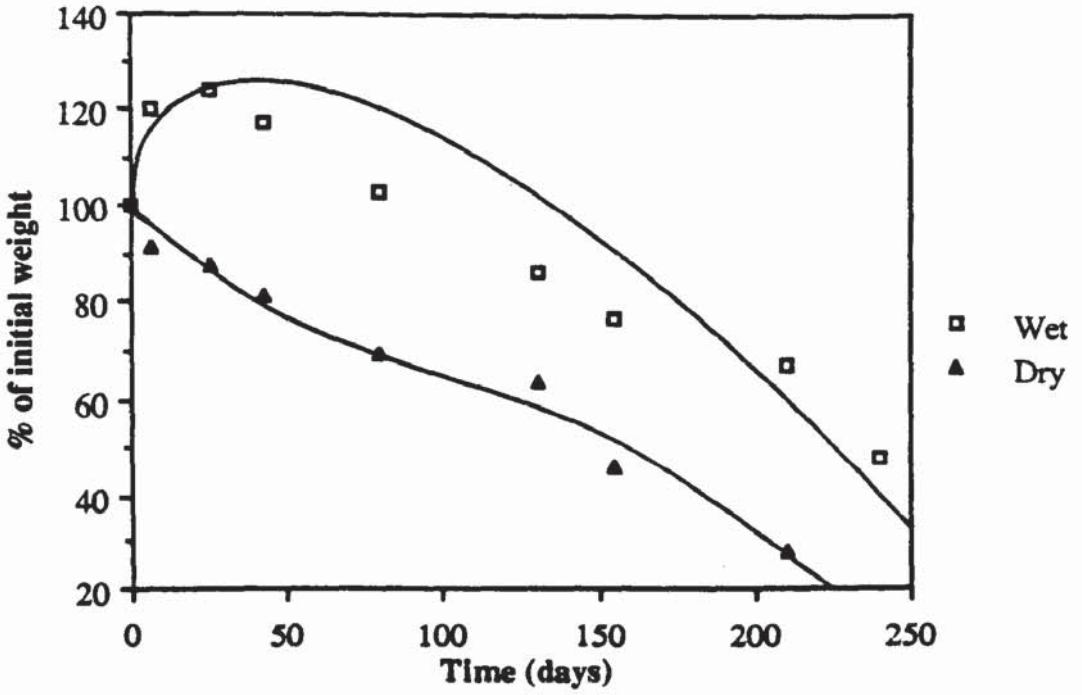
Graph 4.16 Weight loss of 12% PHV/Polysaccharide (30%) blends; Injection moulded plaques degraded in pH10.6 buffer at 37°C.



Graph 4.17 Weight loss of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH10.6 buffer at 37°C.



Graph 4.18 Weight loss of 20% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH10.6 buffer at 37°C.



Graph 4.19 Comparison of progressive 'wet' and 'dry' weight loss of 20% PHV/Dextrin (30%) blend: Injection moulded plaque in pH 10.6 buffer at 37°C.

hydrogen bonding sodium alginate is soluble. If one considers hydrolytic degradation of sodium alginate alone, then increased stability of the β 1-4 glycosidic bonds in comparison to α 1-4 bonds, should make the degradation of sodium alginate blends the slowest of all the polysaccharide blends studied. However, the increased solubility of sodium alginate, combined with its fibrous structure and large particle size, results in its increased dissolution from the blend matrix, leaving a weak hollow matrix, which collapses on itself.

The role played by the polysaccharide in the degradation of the PHB-PHV/polysaccharide blends is clearly important and may in some cases involve the dissolution, in addition to

the degradation of the polysaccharide from the matrix. The contribution of dissolution is evident from SEM studies of degraded 20% PHV/30% dextrin and 12% PHV/30% sodium alginate blends, (Plates 4.5 and 4.6), which clearly show a very porous structure remaining after the polysaccharide has completely dissolved out of the matrix. This considerable increase in the internal porosity was also indicated by measuring the difference between the 'wet' and dry weights of these blends. A typical example is shown in Graph 4.19. Initially, the wet weight increases as the polysaccharide absorbs the buffer, and as the dry weight starts to decrease (due to the polysaccharide dissolving out), the difference between the wet and dry weights should increase because of the increasing porosity. However, the difference shown in Graph 4.19 for the 20% PHV/dextrin (30%) blend, degraded in pH 10.6 at 37°C, is not so dramatic. This is because in the latter stages of degradation, because of the increased porosity, the accuracy of wet weights becomes limited due to the inherent problem of removing excess surface water, without drawing water from the porous matrix. Although weight loss studies are convenient in following degradation of these blends, the accuracy of this technique is limited to the start of the portion preceding the start of the enhanced breakdown of the matrix, which occurs after 30% weight loss. Further weight loss serves primarily as an indication of the rate at which the porous matrix collapses on itself.

The initial stages of degradation, particularly at 37°C are difficult to monitor by just using water uptake and weight loss techniques. Other valuable techniques which magnify the effects of matrix surface hydrolysis are available to follow the initial degradation. Two such convenient techniques that are used in these laboratories to monitor surface changes are goniophotometric analysis and surface energy measurements. The use of these techniques to monitor surface changes in the PHB-PHV/polysaccharide blends is described in the next section.

4.4. TECHNIQUES FOR MONITORING SURFACE DEGRADATION.

4.4.1 INTRODUCTION.

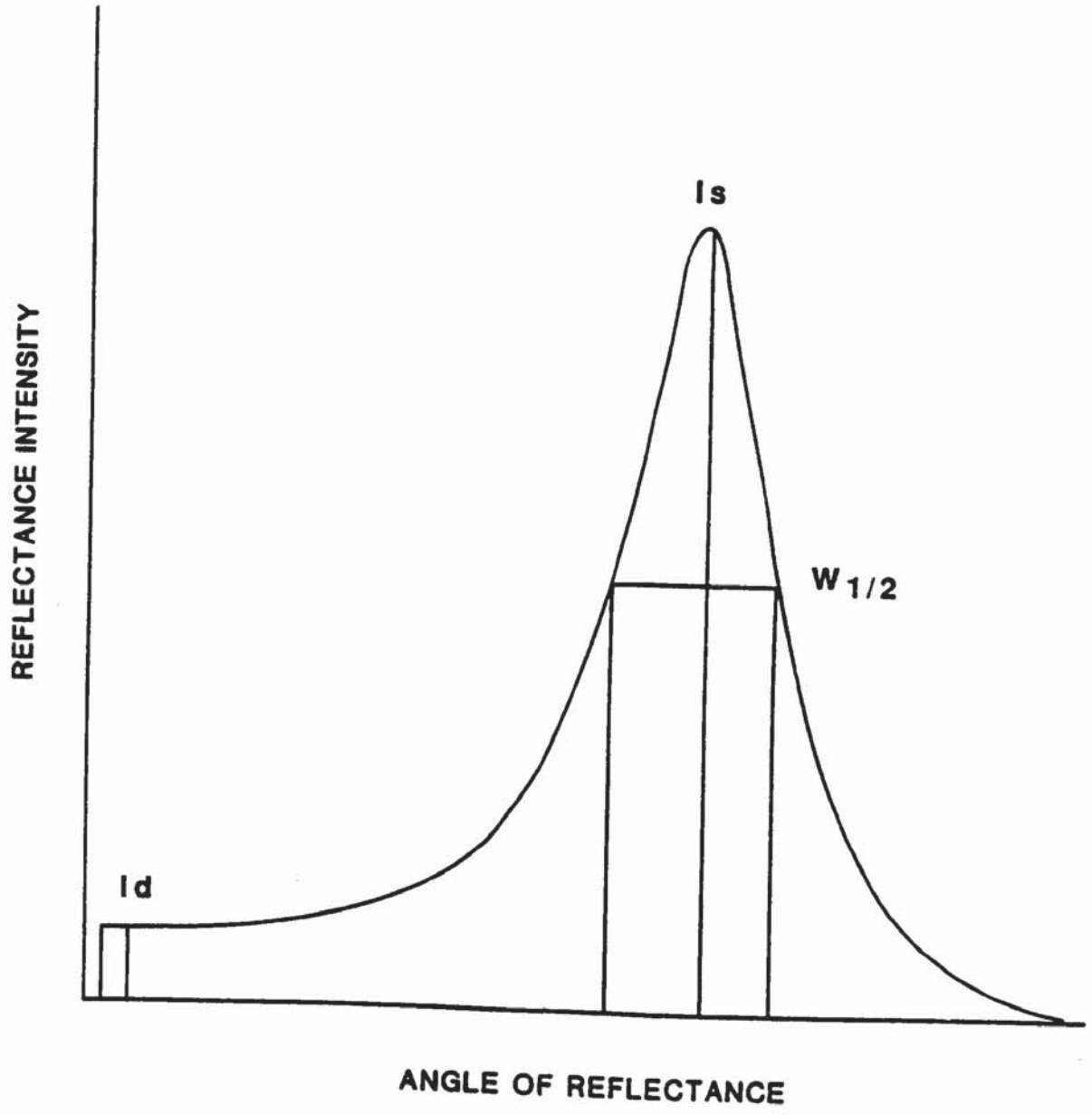
Although SEM provides a very valuable detailed morphological investigation of degraded surface, it has the inherent disadvantage of sample destruction. Both goniophotometry and surface energy measurements (via contact angle measurements), are non-destructive methods however, which enable surface degradation to be monitored over a period of time.

4.4.2 GONIOPHOTOMETRY.

Goniophotometry is a technique of measurement of the intensity of light reflected from a surface, as a function of viewing angle (154). When light is incident upon a surface, it is subsequently reflected by two different processes; either by surface reflection at the surface/air boundary, or by reflection of light which has been scattered by penetration of the sample surface. Surfaces can be said to exist between two extremes; a perfect 'mirror' or a completely matt surface.

If a surface behaves like a perfect 'mirror', all the incident light is reflected, at an angle equal to the angle of incidence, instead of being scattered. This type of reflection is known as 'specular reflectance'. A perfectly matt surface is often defined as a surface which reflects incident light equally in all directions, and this type of reflectance is known as 'diffuse reflectance'. The scattering envelope produced is governed by the rugosity or roughness of the surface and hence this technique is useful in following changes in rugosity resulting from erosional processes which affect the surface.

Figure 4.5 A typical goniophotometric curve for a undegraded PHB-PHV/polysaccharide blend (injection moulded plaque) indicating the various derived parameters.



The application of this technique to study of polysaccharide blends of the PHB-PHV copolymers is illustrated in Figure 4.5, which shows a typical goniophotometric curve for a undegraded blend. Three important parameters may be derived from this curve. These are (i) the intensity of light scattered at the specular angle (I_s), (ii) the diffuse reflectance (I_d) and (iii) the peak width at half-height ($W_{1/2}$), which is at a level midway between the intensities I_s and I_d. The specular angle is the angle of maximum reflectance for those surfaces that are not dominated by imperfections having dimensions similar, or greater than, the wavelength of the incident light beam, whereas diffuse reflectance (I_d) is the reflection normal to the sample surface.

These three quantities, (I_s, I_d and $W_{1/2}$), are combined together to give a very useful single parameter, the so-called 'gloss factor', (GF), (Equation 4.1)[178], which is the most useful single parameter that can be derived from goniophotometric curves.

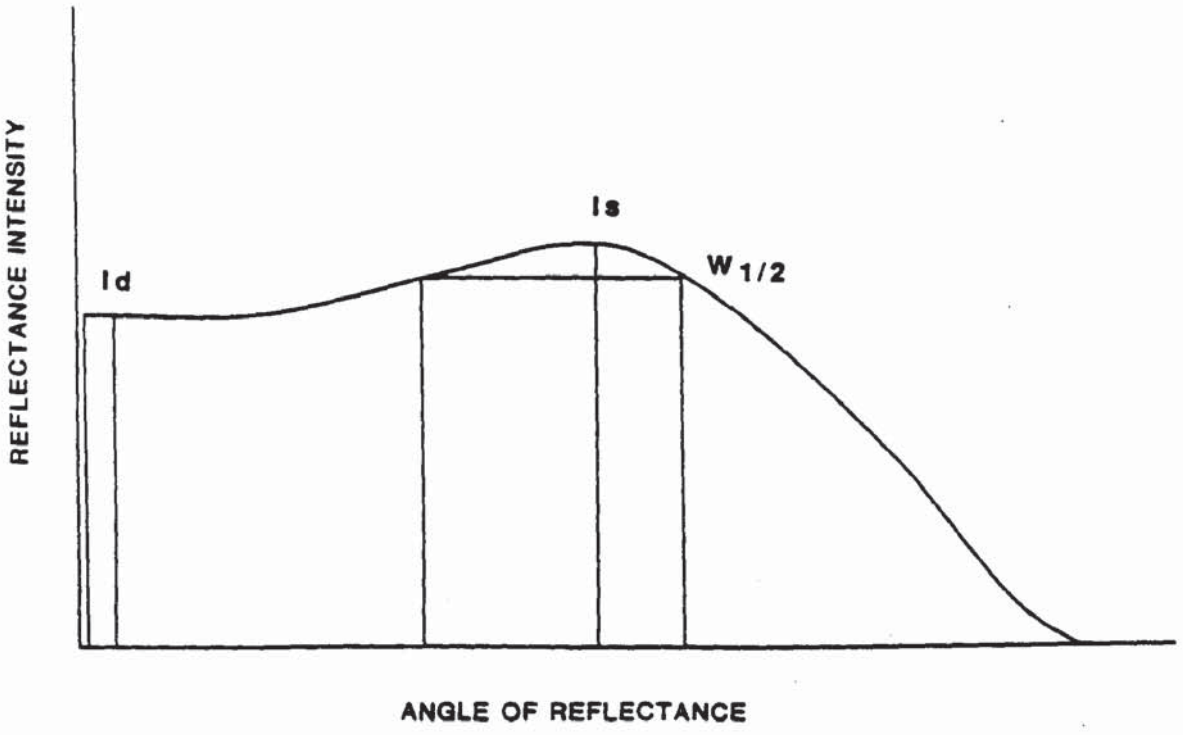
$$\text{Gloss factor (GF)} = \frac{I_s - I_d}{W_{1/2}} \qquad \text{Equation 4.1}$$

The gloss factor of a sample quantitatively reflects the changing nature of a surface, and provides a very useful way of monitoring the reflectance properties of a surface over a period of time. Changes in the size of surface features of less than the wavelength of light can be detected by goniophotometry.

As a surface degrades/deteriorates, surface rugosity increases. Development of surface imperfection of dimensions smaller than the wavelength of light beam used ($\approx 0.5\mu m$), leads to the removal of energy from the beam, which results in a drop in the measured specular peak intensity, (I_s), with little change in peak asymmetry, i.e. $W_{1/2}$. As the size

of the surface imperfections increases to around the wavelength of light, spectrum peak broadening occurs and this is quantitatively seen as an increase in the peak width at half-height, ($W_{1/2}$), together with an increase in I_d and a decrease in I_s. With further degradation, peak dissymmetry occurs and the I_d value approaches the I_s value as the surface nears a 'matt' appearance; (I_s - I_d, sometimes called the mean peak height gives a simple indication of surface gloss). This type of behaviour is observed in the PHB-PHV/polysaccharide blends that have been subjected to extensive hydrolytic degradation. Figure 4.6 shows the goniophotometric curve for a substantially degraded blend.

Figure 4.6 Representative goniophotometric curve of a substantially degraded PHB-PHV/polysaccharide sample (injection moulded plaque).



4.4.2.1 RESULTS AND DISCUSSION.

The usefulness of this technique is perhaps best illustrated in relation to samples that have undergone relatively small degradation. The goniophotometric curves for two injection moulded samples, (12% PHV (talc nucleated) copolymer and 12% PHV/10% sodium alginate blend), after degradation in pH 2.3 buffer at 70°C for 5 days, are shown in Figures 4.7 and 4.8, respectively, and the goniophotometric parameters for these are tabulated in Tables 4.12 and 4.13.

Although the weight loss of the 12% PHV (talc nucleated) injection moulded sample is very small (< 1%) after 5 days immersion in pH 2.3 buffer at 70°C, evidence of surface erosion (surface roughening) is indicated from the goniophotometric curve (Figure 4.7). The I_s value has decreased slightly, with a concurrent increase in the I_d value and decrease in the gloss factor. Increased peak dissymmetry is also apparent. Incorporation of a polysaccharide into the PHB-PHV matrix increasing surface erosion is also evident from Figure 4.8, which relates to a 12% PHV/10% sodium alginate blend (weight loss ≈ 6%). The I_s value and the gloss factor have both decreased, while the I_d value has increased. Onset of peak dissymmetry is also evident. It should be stressed that these changes to the surface can be measured at very low weight loss.

During the initial stages of degradation in which the surface erosion is the major degradation process, sample weight loss monitoring techniques are not very accurate. It appears that goniophotometry is a more sensitive and valuable technique in monitoring this early surface degradation. Certainly, the errors arising from the electronics of the equipment are low, although reproducible sample mounting has to be ensured to maintain the high level of measurement precision.

Figure 4.7 Goniophotometric curves for (a) degraded and (b) undegraded, 12% PHV, injection moulded (talc nucleated) plaques; pH 2.3, 70°C, 5 days.

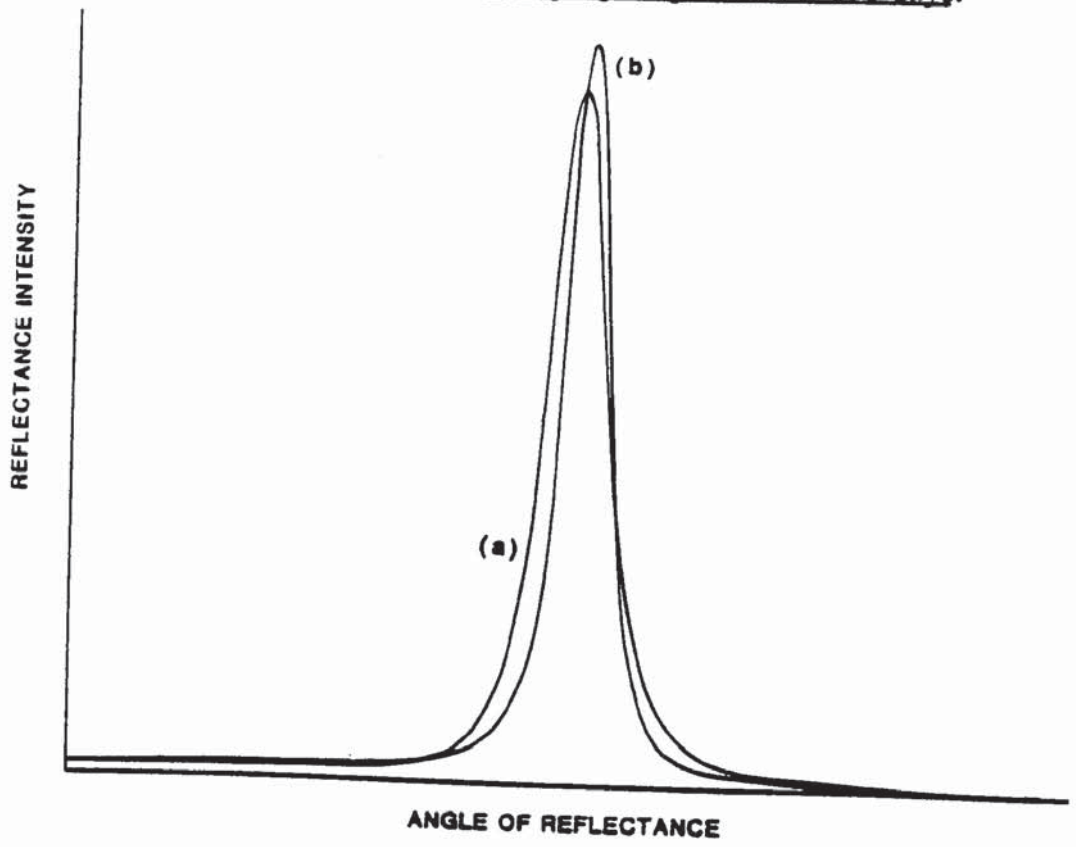


Figure 4.8 Goniophotometric curves for (a) degraded and (b) undegraded 12% PHV/10% sodium alginate blend, injection moulded plaques; pH 2.3, 70°C, 5 days.

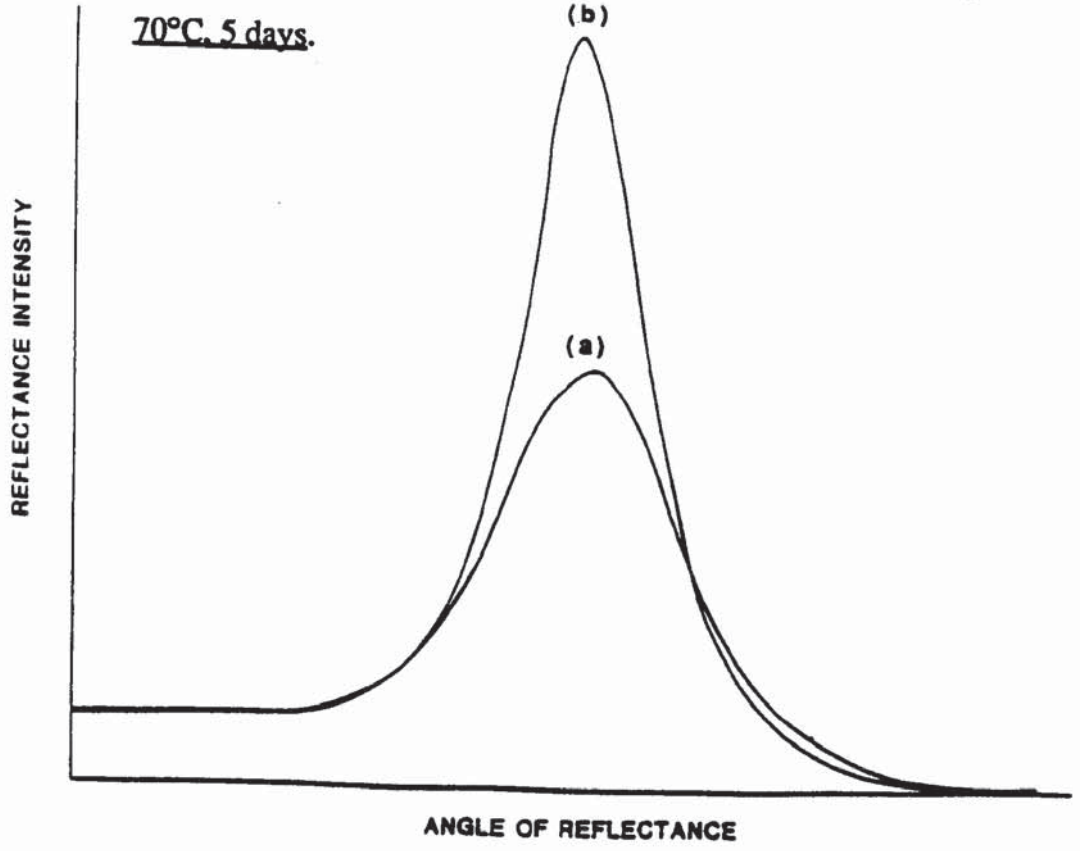


Table 4.12 Goniophotometric parameters for 12% PHV (talc nucleated) undegraded and degraded samples; (pH 2.3, 70°C), (injection moulded plaques).

Parameter	Undegraded.	Degraded (5 days).
I_s	54.8	51.5
I_d	1.0	1.4
$I_s - I_d$	53.8	50.1
$W_{1/2}$	2.3	3.0
Gloss factor	23.2	16.7

Table 4.13 Goniophotometric parameters for 12% PHV/10% sodium alginate blend; undegraded and degraded samples; (pH 2.3, 70°C), (injection moulded plaques).

Parameter	Undegraded.	Degraded (5 days).
I_s	24.6	12.8
I_d	2.2	1.6
$I_s - I_d$	24.4	11.2
$W_{1/2}$	6.4	9.2
Gloss factor	3.5	1.2

In addition to this method for surface characterisation, another technique was used for characterising surface degradation. This was the measurement of surface free energy of the samples by means of the sessile drop contact angle measurement as matrix hydrolysis proceeded.

4.4.3 SURFACE ENERGY MEASUREMENTS.

4.4.3.1 INTRODUCTION

A drop of liquid in contact with the surface of a solid, under normal atmospheric conditions can be considered as having three forces acting on it,(180) one at each of the interfaces that occur, these being liquid/gas, solid/gas and solid/liquid, (Figure 4.9).

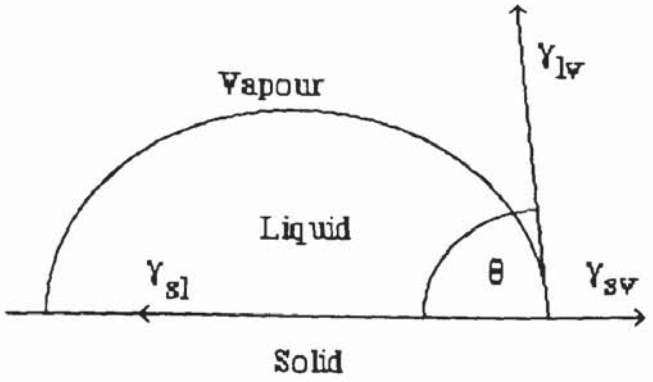


Figure 4.9 Contact angle of a sessile drop on a solid surface.

γ_{lv} is the surface tension at the interface of the liquid and vapor phase, γ_{sl} that at the interface of the solid and liquid, γ_{sv} that at the interface of the solid and vapour and θ the contact angle. This equilibrium is expressed mathematically by the Youngs Equation(181).

$$\gamma_{lv} \cos\theta = \gamma_{sv} - \gamma_{sl} \qquad \text{..... Equation 4.2}$$

Dupre' (182) demonstrated that the work of adhesion (W_A) could be defined in terms of Youngs' Equation:

$$W_A = \gamma_{sv} + \gamma_{lv} - \gamma_{sl} \qquad \text{..... Equation 4.3}$$

Two experimental systems for measuring contact angles exist, liquid/air/solid systems

and liquid/liquid/solid systems. Estimation of solid surface free energy (γ_s) from contact angle measurements presents problems, because this can only be achieved for a solid in a high vacuum. Although surface tension or surface free energy of a polymeric solid is impossible to evaluate absolutely, the problem was solved by Zisman and co-workers(183). Contact angles of a series of liquids with known γ_{lv} on a polymeric surface were measured. A plot of cos θ against γ_{lv} was a straight line relationship and the value of γ_{lv} at the intercept where cos θ =1 was termed as the critical surface tension of wetting, (γ_c), for that polymer. The critical surface tension equals the surface tension of the liquid which just exhibits a zero contact angle on the solid. The difference between solid surface free energy (γ_s) and the solid-vapour interfacial energy (γ_{sv}) is known as the equilibrium spreading pressure (Π_e).

$$\Pi_e = \gamma_s - \gamma_{sv} \qquad \text{..... Equation 4.4}$$

$$\text{and} \quad \gamma_s = \gamma_{sv} + \Pi_e \qquad \text{..... Equation 4.5}$$

$$\gamma_s = \gamma_{lv} \cos\theta + \gamma_{ls} + \Pi_e \qquad \text{..... Equation 4.6}$$

The critical surface tension of wetting (γ_c) is equal to the surface tension of the liquid (γ_{lv}), when cos θ = 1. The critical surface tension of wetting (γ_c) can now be described by Equation 4.7.

$$\gamma_c = \gamma_s - \gamma_{sl} - \Pi_e \qquad \text{..... Equation 4.7}$$

γ_s is the critical surface tension, of the solid in vacuum, γ_{sl} the interfacial tension and Π_e the equilibrium spreading pressure. The critical surface tension, (γ_c), is smaller than the

surface free energy, (γ_s), by the amount ($\gamma_{sl} + \Pi_e$). The interfacial tension and the equilibrium spreading pressure vary with the nature of the testing liquid(183), so γ_c will always be smaller than γ_s. The surface free energy term (γ_s) can be separated into polar (γ_s^p) and dispersive (γ_s^d) components;

$$\gamma_s^t = \gamma_s^p + \gamma_s^d \qquad \text{..... Equation 4.8}$$

where γ_s^t = the total surface free energy

γ_s^p = the polar component of the surface free energy

γ_s^d = the dispersive component of the surface free energy

Owens and Wendt (155), taking into account that the work of adhesion (W_A), between a liquid and a polymer will often have both polar and dispersive energy components, evolved an equation;

$$W_A = \gamma_{lv} + \gamma_s + \gamma_{sl} = 2 (\gamma_{lv}^d \gamma_s^d)^{1/2} + 2 (\gamma_{lv}^p \gamma_s^p)^{1/2} \qquad \text{..... Equation 4.9}$$

$$\text{i.e. } \gamma_{sl} = \gamma_s + \gamma_{lv} - 2 (\gamma_s^d \gamma_l^d)^{1/2} - 2 (\gamma_l^p \gamma_s^p)^{1/2} \qquad \text{..... Equation 4.10}$$

Combining Equations 4.2, 4.3 and 4.10, the well-known Owens and Wendts Expression results(155);

$$1 + \cos\theta = \frac{2}{\gamma_{lv}} \left[(\gamma_s^d \gamma_l^d)^{1/2} + (\gamma_s^p \gamma_l^p)^{1/2} \right] \qquad \text{..... Equation 4.11}$$

where θ is the contact angle, γ_{lv} is the liquid/surface tension, and the subscripts s and l denote the solid and liquid phases. The superscripts d and p refer to the dispersive and polar components.

This method allows the estimation of the surface free energy of polymers, by direct measurement of contact angles formed by two wetting liquids which have been fully characterised for polar and dispersive components. Water and methylene iodide are a pair of liquids that are commonly used for this purpose, because they have large differences in their values for polar and dispersive components.

4.4.3.2 RESULTS AND DISCUSSION.

The changes to the surface free energy components of injection moulded plaques of unfilled copolymer (20% PHV, talc nucleated), and a filled copolymer (12% PHV/30% dextran), in a pH 2.3 buffer at 70°C are illustrated in Table 4.14.

After 7 days immersion and with less than 1% weight loss, the unfilled copolymer is already showing signs of surface degradation. The polar component of surface energy has increased, whilst the dispersive component of surface energy has decreased very slightly. The weight loss for the dextran blend on the other hand, after the same period, is $\approx$ 6%, whilst the corresponding surface energy parameters, have changed dramatically. Most significantly, the polar component has increased by over 50% in this relatively short degradation period.

Incorporation of the polysaccharide increases the surface rugosity of the matrix over a

period of time as the polysaccharide dissolves out. This increases the surface area of the matrix polymer which is in contact with the aqueous buffer. As a result of this increased porosity, the polymer will degrade faster, resulting in an increase in the formation hydroxyl and carboxyl groups. The dramatic increase in the polar component of the surface energy for the filled sample in comparison to the unfilled sample, thus indicates the increase in the degradation rate as a result of incorporating a polysaccharide into the matrix.

Table 4.14 Surface energy parameters for unfilled 20% PHV and 12% PHV/dextran (30%) blend (injection moulded plaques): undegraded and degraded (pH 2.3, 70°C, 7 days).

	unfilled 20% PHV		12% PHV/dextran blend	
Parameter	undegraded	degraded	undegraded	degraded
CA water (degrees)	68	66	61	51
CA MeI (degrees)	26	26	26	26
polar component (γ^p) (mN/m)	7.9	9.0	11.9	18.3
dispersive component (γ^d)(mN/m)	40.9	40.5	39.6	38.0
total (γ^t) (mN/m)	48.8	49.5	51.5	56.3

where CA= average contact angle.

So far, the relative merits of a variety of techniques in studying the degradation of PHB-PHV copolymers, and various blends derived from these, have been described. The hydrolytic degradation of all the PHB-PHV/polysaccharide blends in pH 10.6 buffer at 70°C was investigated by combining all the various techniques, (gravimetric,

photographic, goniophotometry, contact angles and SEM), so that a detailed understanding of the degradation mechanism of these blends degrade could be obtained. This work is described in the next section.

4.5 STUDY OF HYDROLYTIC DEGRADATION BY COMBINED TECHNIQUES.

4.5.1. INTRODUCTION.

The various individual techniques that could be used to follow polymer degradation have already been described. In an attempt to bring all these techniques together, to provide a better picture of the degradation processes, hydrolytic degradation of the PHB-PHV/polysaccharide injection moulded blends was carried out using accelerated degradation conditions, i.e. pH 10.6 buffer and 70°C. Hydrolytic degradation was monitored by using the techniques described in Chapter 2 for a period of 46 days.

4.5.2 RESULTS AND DISCUSSION.

The use of goniophotometry to follow the degradation of 12 and 20% PHV copolymers (injection moulded plaques; nucleated with 1% talc), is illustrated in Figures 4.10a and 4.10b. The time chosen to illustrate progress of degradation was over a period of 800 hours (33 days), by which time both samples were beginning to show signs of physical degradation. The goniophotometric results illustrate the more pronounced degradation of 20% PHV copolymers over this time period in comparison

to 12% PHV copolymer sample.

The comparative change in surface energy of these two samples is shown in the form of a histogram in Graph 4.20. The progress of degradation is reflected in the increase in the polar component of surface energy, which again occurs more rapidly in the case of the 20% PHV copolymer in comparison to the 12% PHV analogue. Some slight progressive decrease in the dispersive component of surface energy parallels the more dramatic increase in the polar component. The sample is becoming increasingly roughous and porous as time progresses. This does not detract from the ease or relevance of goniophotometric monitoring, which as Figure 4.10 shows maybe continued for some 30 days. On the other hand, surface energy measurements involving the use of sessile drop techniques at the polymer surface become progressively more difficult at a much earlier stage. In the case of the samples shown in Graph 4.20, the values are questionable at 10 days and of little value thereafter.

The goniophotometry and surface energy techniques maybe similarly combined in the study of polysaccharide blends. Figures 4.11a and 4.11b show the use of goniophotometry to observe the effect of incorporation of 10% dextrin into the 12 and 20% PHV copolymers, respectively. The parallel surface energy values for these systems are shown in Graph 4.21. It is apparent from both these figures that degradation is occurring much more rapidly in these blends than in the unfilled copolymers. The very dramatic change in the polar component of surface energy between 1 and 3 days is paralled by an observable increase in the diffuse reflectance (I_d) and peak width at half height in the goniophotometric curves. The fact that the usefulness of goniophotometry persists further in the degradation process is illustrated by the progressive transition between 3 and 6 days in Figure 4.11. It was not possible

to obtain further useful surface energy measurements beyond 3 days for these samples.

The goniophotometric curves for 12 and 20% PHV copolymers loaded with 10% dextran are illustrated in Figures 4.12 (a) and 4.12 (b), respectively. The parallel surface energy values for these systems are shown in Graph 4.22. These again illustrate that the 20% copolymer degrades faster than the 12% copolymer. In Figures 4.12 (c) and 4.12 (d), the goniophotometric curves for the 30% dextrin filled blends of the 12 and 20% PHV copolymers, respectively, are illustrated.

The effect of higher filler loadings and more soluble fillers are illustrated in Figure 4.13, which shows goniophotometric curves corresponding to the degradation of the 12 and 20% copolymers filled with 10% sodium alginate and the 12% PHV copolymer filled with 30% dextran. Although it was possible to use goniophotometry for periods well beyond 3 days for these systems, dramatic transition appears to occur in surface energy between 1 and 3 days. This is reflected in the information shown in Graph 4.23, in which measurements carried out after 1 day show a small change, whereas at the 3 day period, surface energy data had either increased dramatically or was un-obtainable because of the porosity of the sample.

Although the use of nucleating agents such as talc or hydroxyapatite, is essential in order to optimise the injection moulding process with unfilled copolymers, the polysaccharides themselves in the levels used here appear to act as quite efficient promoters of the nucleation process. Thus, little difficulty was observed in the moulding behaviour of the polysaccharide-filled PHB-PHV copolymers with and without added nucleating agent. Graph 4.24 shows combined surface energy and

goniophotometric data for 10% dextrin filled PHB-PHV copolymers, (both 12 and 20% PHV), containing 1% talc. The parameters shown are the polar and dispersive components of surface energy and the peak height (I_s - I_d). Although goniophotometry was usefully employed for many days after this point, the surface energy measurements had ceased to become meaningful. The pattern, profile and duration of the degradation process was indistinguishable from that observed for a range of non-nucleated blends.

Despite the fact that both goniophotometry and surface energy measurements make a useful contribution to the study of the overall degradation mechanism for all of the blends of the type studied here, it is clear that the most dramatic changes monitored by these techniques are occurring in the early stages of the degradation process. Since the accelerated (i.e. high temperature and high pH) degradation conditions, in combination with the effect of the polysaccharide fillers produces fairly dramatic changes in other properties within a period of a few days, the particular sensitivity of these surface techniques is less advantageous than is the case with (say) un-blended materials under near physiological conditions. Nonetheless, important information is obtained by goniophotometry and surface energy studies under the conditions described here, and this information provides a valuable basis for predicting the path of degradation by the use of these techniques under "physiological" conditions.

Molecular weight and crystallinity play important roles in controlling the rate of degradation of synthetic polyesters. Attention has been paid to the way in which crystallinity changes during hydrolytic degradation of materials of this type. The effect of processing on both molecular weight and crystallinity of the PHB-PHV copolymers has already been mentioned in earlier sections of this Chapter. Because this Chapter is

concerned with accelerated degradation it is difficult to take samples at precise times within the relatively short span of the degradation process which meaningfully correlate with other degradation parameters. Such studies are best carried out as part of more prolonged degradation experiments. Some aspects of the trend in observed crystallinity associated with the experimental described here are presented in Figure 4.14. The PHB-PHV copolymer samples vary in their 'as-received' crystallinity depending upon the final stages that have been used in purification and preparation of the material. Figure 4.14a shows the x-ray diffraction trace of one of the more crystalline (powder) forms of the raw material. Figure 4.14b shows the small changes that are brought about by injection moulding. During the initial stages of degradation process, the level of crystallinity increases somewhat, as amorphous material is preferentially removed, and ultimately begins to fall as degradation becomes advanced. Figure 4.14c shows the x-ray diffraction trace for 20% PHV copolymer degraded in pH 7.4 buffer at 70°C for 120 days. This sample is almost amorphous. Similar effects are produced when degradation is carried out under more strongly alkaline conditions. Figures 4.14d and 4.14e show diffraction traces for the 12 and 20% PHV copolymers, respectively, after 46 days degradation in pH 10.6 buffer at 70°C. Here again crystallinity has substantially disappeared.

The molecular weight data corresponding to copolymers 'as-received', to injection moulded samples and to the same materials after degradation under conditions corresponding to Figures 4.14c (pH 7.4, 70°C), are shown in Table 4.5. As already stated, it is clear that processing plays an important part in controlling the molecular weight of these polymers. Similarly, the terminal stages of degradation are associated with a dramatic decrease in both molecular weight and crystallinity. This point is illustrated in Table 4.15, which shows the change in molecular weight for both 12 and

20% PHV copolymers after 46 days degradation at 70°C and pH 10.6. The crystallinity of the degraded samples has already been illustrated in Figures 4.14d and 4.14e. The intermediate changes in both molecular weight and crystallinity have been studied in detail in connection with the degradation of the PHB-PHV copolymers under simple *in vitro* 'physiological' conditions in which the time scale of the degradation process extends over some 18 months (Chapter 6 of this thesis). Table 4.15 also shows the effect of incorporation of 10% dextrin on the molecular weight change that occurs in the 12% PHV copolymer at pH 10.6 and 70°C after 46 days. This result should be compared with the information in Table 4.16, which shows that the incorporation of this level of dextrin produces a relatively modest reduction in the t_{50} time from 42 days to 33 days. This provides reasonable supporting evidence for the assertion that the incorporation of the polysaccharide fillers accelerates the existing degradation pathway, rather than producing a dramatic modification in degradation mechanism.

4.6 SUMMARY AND CONCLUSION:

The rate of hydrolytic degradation of the PHB-PHV/polysaccharide blends is affected by both pH and temperature and the rate of degradation of these blends increases considerably in pH 10.6 buffer at 70°C, (by 20 to 30-fold), in comparison to other degradation conditions, such as pH 2.3 and 7.4 at 70°C. Thus the degradation of the PHB-PHV/polysaccharide blends is much faster under alkaline conditions than under acidic conditions, as was the case with the unfilled copolymers.

The initial stages of hydrolytic degradation of the polysaccharide blends studied here

are characterised by a short-term increase in the wet weight and a corresponding decrease in the dry weight. As hydrolysis progresses, both the wet and dry weights begin to decrease. The rate of dissolution of the polysaccharide from these blends is to some extent dependent on pH, particle size and temperature. The changes in the wet and dry weights of the injection moulded plaques of the 12 and 20% PHB-PHV/polysaccharide blends, (10 and 30% loadings), are illustrated in Graphs 4.25 to 4.32. In general, the 20% PHV copolymer was found to degrade faster than the 12% PHV copolymer; polysaccharide blends with these copolymers showed a similar trend.

As the wet weight of these blends increases, the polysaccharide component starts to dissolve and leach out of the blend matrix, resulting in an increase in the surface porosity. This was evident from light microscopy photographs for two typical samples, 12% PHV/10% dextrin (Plate 4.7) and 20% PHV/10% dextran (Plate 4.8), showing the onset of porosity after 6 days immersion in pH 10.6 buffer at 70°C. Visual appearance of the samples suggests surface roughening. This surface roughening, as a consequence of increasing porosity, is also evident from the goniophotometric curves. As the surface rugosity increases, because of increasing dissolution of the polysaccharide from the blend matrix, changes in the goniophotometric scattering envelope occur. The advancement of the surface erosion process leads to an increase in the size of physical irregularities which are not detected visually or by low power light microscopy. When the size of the physical irregularities exceeds the wavelength of the incident light beam ($\approx$ 500nm), the asymmetry of the goniophotometric curves becomes more marked because $W_{1/2}$ increases, together with the intensity of diffuse reflectance, (I_d), as a result of the greater disparity of the scattered beam. This reflected in the progressive changes shown in Figures 4.10 to 4.13.

The initial stages of degradation are characterised by little change in the bulk or mass of the sample. Gradual diffusion of buffer solution (evident from the increase in wet weight), into the bulk occurs, which is accompanied by either dissolution of the polysaccharide and/or progressive chain scission within the polymer matrix. The effect on the surface, apart from increasing rugosity, is an increase in the surface energy, which is a result of an increase in the hydroxyl and carboxyl group concentration as a consequence of ester hydrolysis at the polymer-water interface. This is consistent with observations made on the surface energies of the various samples presented here. The limit of contact angle measurement occurs when the sample surface becomes so porous that it becomes impossible to measure the contact angle formed by a receding drop of water. In general, detailed values of γ^p above 20 mNm^{-1} are imprecise, but useful for indicating the dramatic changes occurring at the surface.

During the initial stages of degradation, when progressive changes at the surface are occurring, as evident from both goniophotometric parameters and surface energy measurements, concurrent bulk erosional processes are at work. These result from the diffusion of products of chain scission processes from the amorphous regions of the matrix. Low molecular weight fragments are able to diffuse out initially, but as degradation proceeds, the matrix becomes progressively more porous, allowing an increasing loss of higher molecular weight products, a process which for the polysaccharide blends would be more enhanced. Combined molecular weight and crystallinity studies give some indication of the nature of the progressive bulk degradation. The detailed changes in both these properties in comparison to other aspects of the change in degradation profile is most easily monitored under *in vitro* physiological conditions, where such changes are much slower.

All the techniques used have shown that the 12% PHV copolymer is more stable than the 20% PHV copolymer, and blends with the polysaccharides show a similar trend. The incorporation of polysaccharide increases the degradation of the polymer matrix, by increasing both the surface and bulk erosion processes. For these blends, as a consequence of the increased porosity with degradation time, larger molecular weight fragments, resulting from chain scission of the polymer matrix, are able to diffuse out more easily, and hence a much more rapid decrease in the molecular weights with degradation time occurs for the blended copolymers. The high initial molecular weight (Mw) of $2.7x10^5$ for a 12% PHV/10% dextrin blend decreases to $4.3x10^3$ in 46 days at 70°C in pH 10.6 buffer, whereas for the unfilled 12% PHV (talc nucleated) sample, after the same time period, the molecular weight is $1.5x10^4$. Tables 4.2, 4.5 and 4.15 illustrate the molecular weight data obtained by gel permeation chromatography (GPC) on unprocessed, processed and degraded polymers. Mn values and thus values of Mw/Mn are very sensitive to the exact positioning of the baseline in the GPC calculation procedure. This, together with the progressive removal of low molecular weight fragments from the eroding matrix means that greatest reliance should be placed on values of Mw and Mn-based data should be treated with caution.

Scanning electron microscopy provides dramatic illustrations of the differences between the various physical forms of different polysaccharide fillers and their contribution to acceleration of the erosional degradation of the blends. The porous 'sponge' or honeycomb like matrix left behind after the polysaccharide dissolves out of the blend is clearly seen by comparing the SEM of 20% PHV copolymer filled with 30% dextran after 9 days degradation in a pH 10.6 buffer at 70°C (Plate 4.9b), with the SEM of the undegraded sample (Plate 4.9a). This is also evident from the SEM of 12% PHV copolymer loaded with 30% dextrin after 12 days degradation under the

same conditions (Plate 4.10b), with the SEM of the undegraded sample (Plate 4.10a). The honeycomb structure will, as a consequence of further hydrolytic degradation, collapse on itself, causing physical break-up of the polymer matrix. The changes in the degradation pathway are best illustrated by a sequence of photographs. The fragmentation process is evident from photographs of injection moulded plaques taken after various periods of hydrolytic degradation in a pH 10.6 buffer at 70°C.

This pictorial evidence allows some visual comparison to be made of the relative degradation of filled and unfilled polymers, of the relative degradation of the 12 and 20% copolymers and there blends and of the effect of filler loading. Thus, the comparison of Plate 4.11 (12% PHV copolymer) with Plate 4.12 (20% PHV copolymer) illustrate the more rapid degradation of injection moulded plaques fabricated from 20%, relative to 12% PHV copolymer. Plates 4.13 and 4.14 show the fate of plaques fabricated from the same copolymers containing 10% dextrin. Again the degradation of the 20% copolymer based-blend is somewhat more rapid than that of the material based on the 12% copolymer. The greatly enhanced degradation produced by 30% polysaccharide loadings is evident from the photographs for the higher loaded blend. The comparative goniophotometric and surface energy data are shown in Figures 4.10 to 4.13 and Graphs 4.20 to 4.24, respectively. Some broad appreciation of the extent of increase in degradation produced by polysaccharide loading may also be obtained from these plates. A simple comparison of the extent of erosion between 10 and 30% polysaccharide loadings (Plates 4.13 to 4.25), illustrates this point. Comparison of the extent of sample erosion between 6 and 10 days for the blends shows a dramatic enhancement produced by higher levels of polysaccharide loading. It is interesting to note that there is a broad similarity in the physical degradation of the plaques produced from the various filled and unfilled materials.

This is consistent with the view that the blending techniques used here are simply accelerating the underlying physical stages of the degradation mechanism.

Figure 4.10 Goniophotometric curves for a) 12% PHV and b) 20% PHV copolymers (talc nucleated plaques), degraded in pH 10.6 buffer at 70°C.

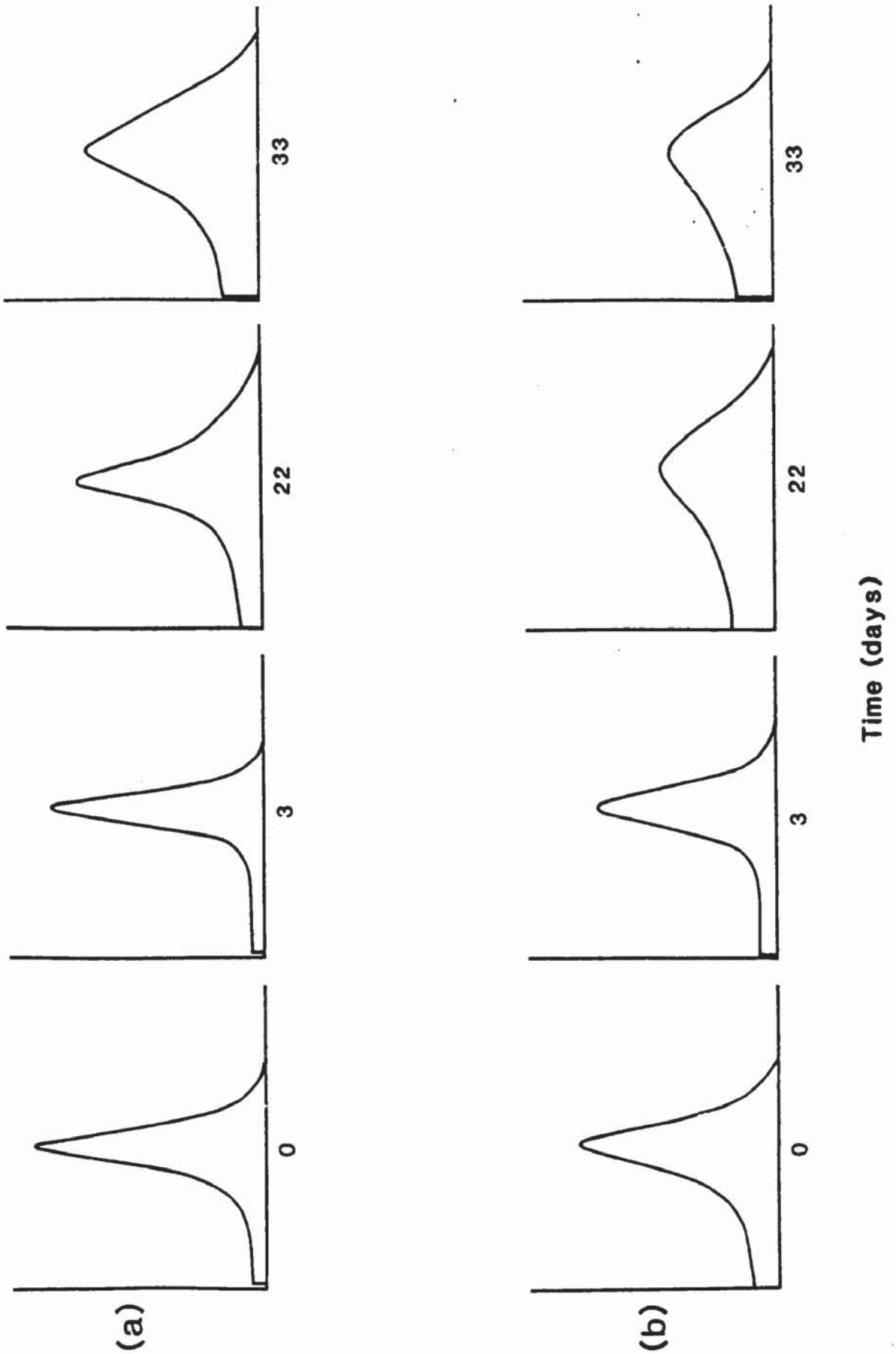


Figure 4.11 Goniophotometric curves for 10% dextrin blends with a) 12% PHV and b) 20% PHV copolymer (plaques), degraded in pH 10.6 buffer at 70°C.

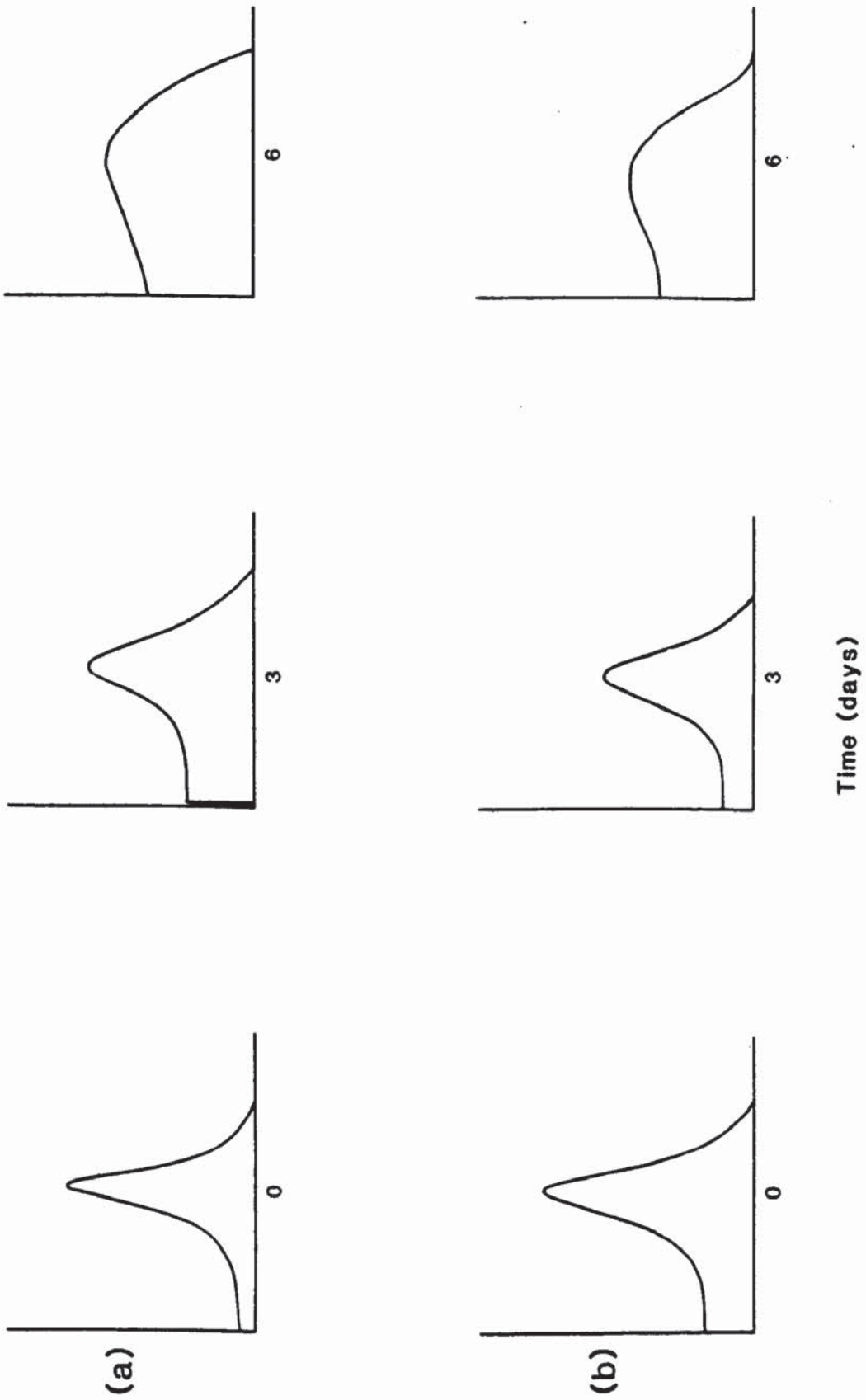


Figure 4.12 Goniophotometric curves for 12% PHV and 20% PHV copolymer blends with 10% dextran (a and b) and 30% dextrin (c and d) (plaques), degraded in pH 10.6 buffer at 70°C.

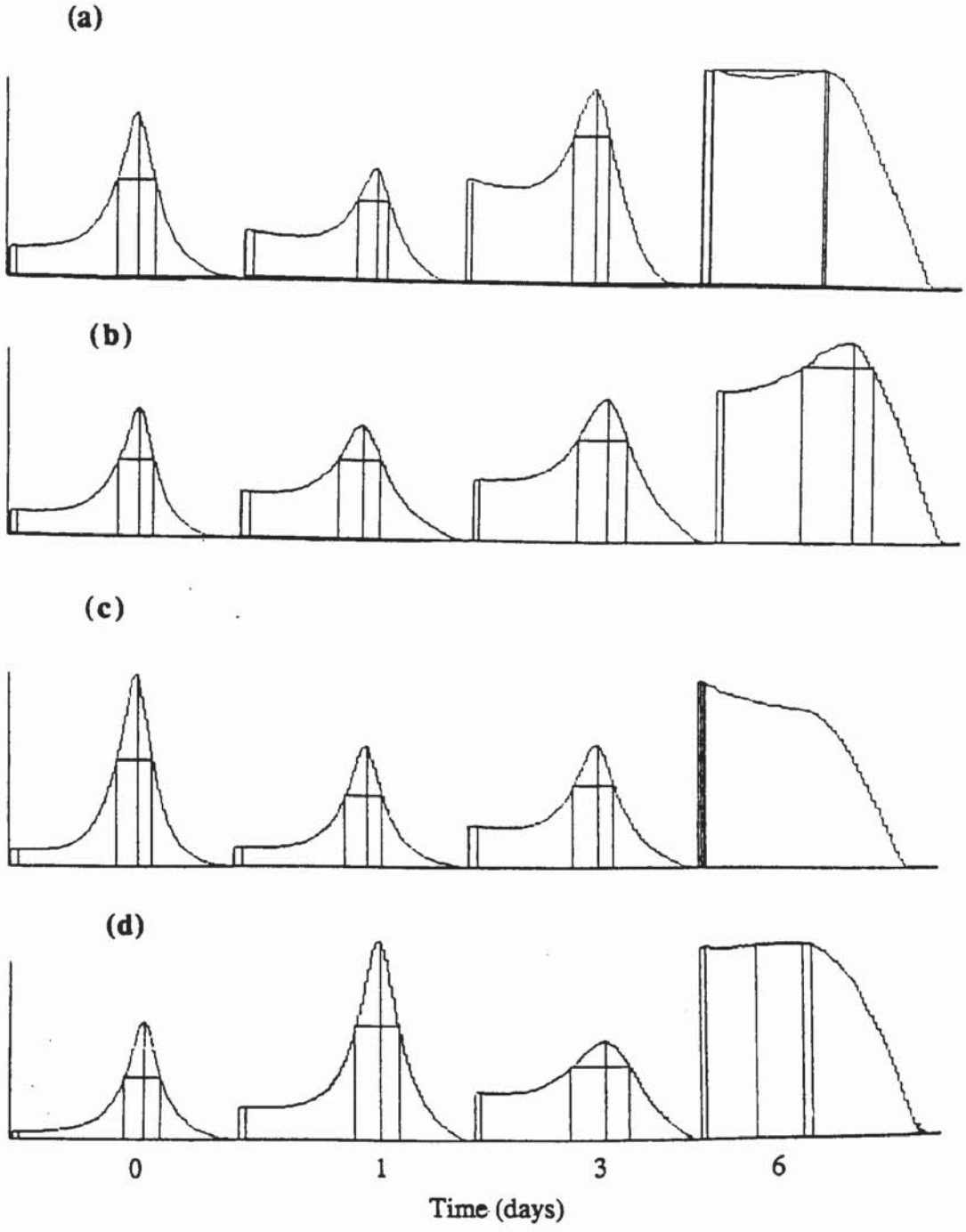


Figure 4.13 Goniophotometric curves for 12% PHV and 20% PHV copolymer blends with 10% sodium alginate (a and b) and 30% dextran (c) (plaques), degraded in pH 10.6 buffer at 70°C.

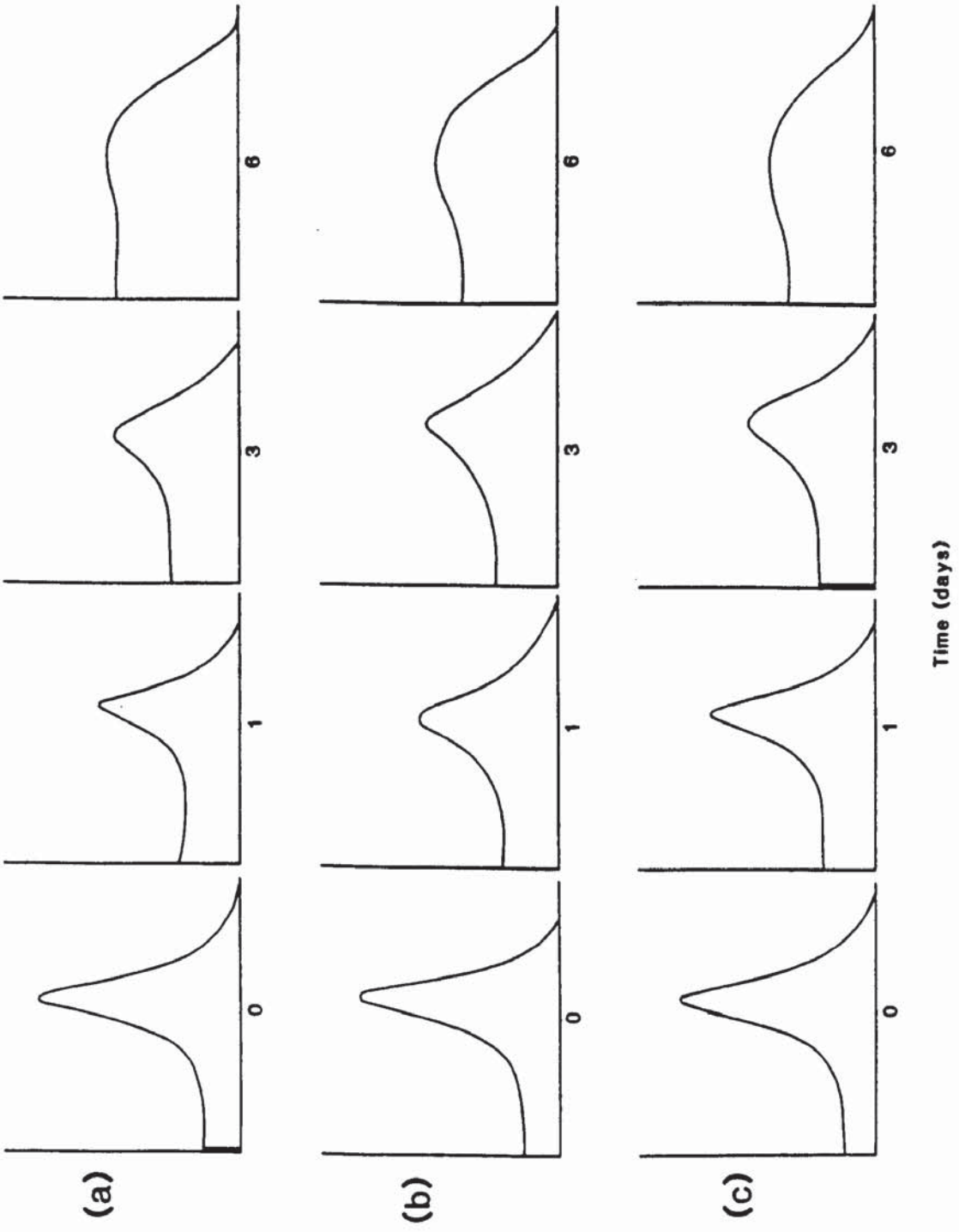
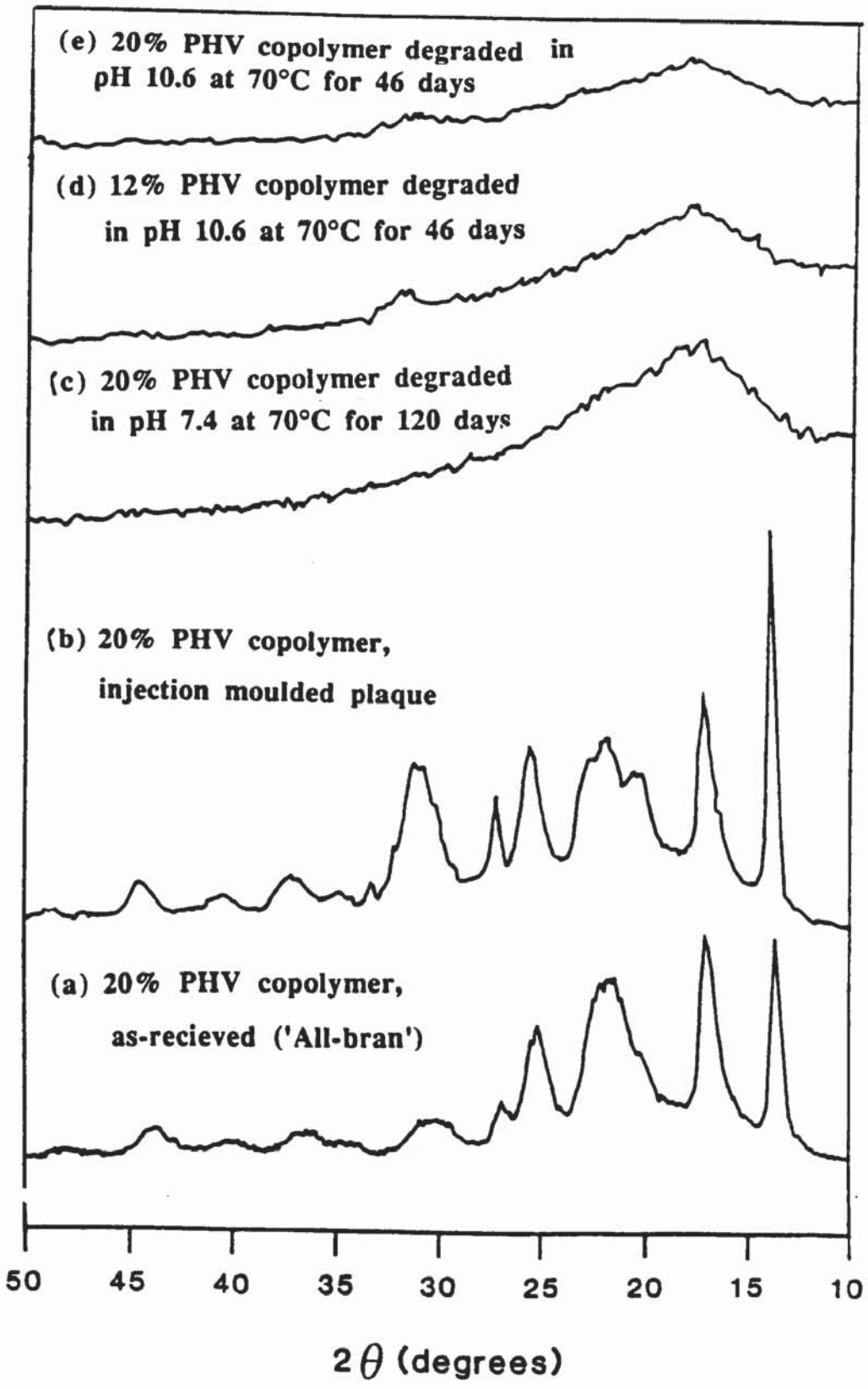
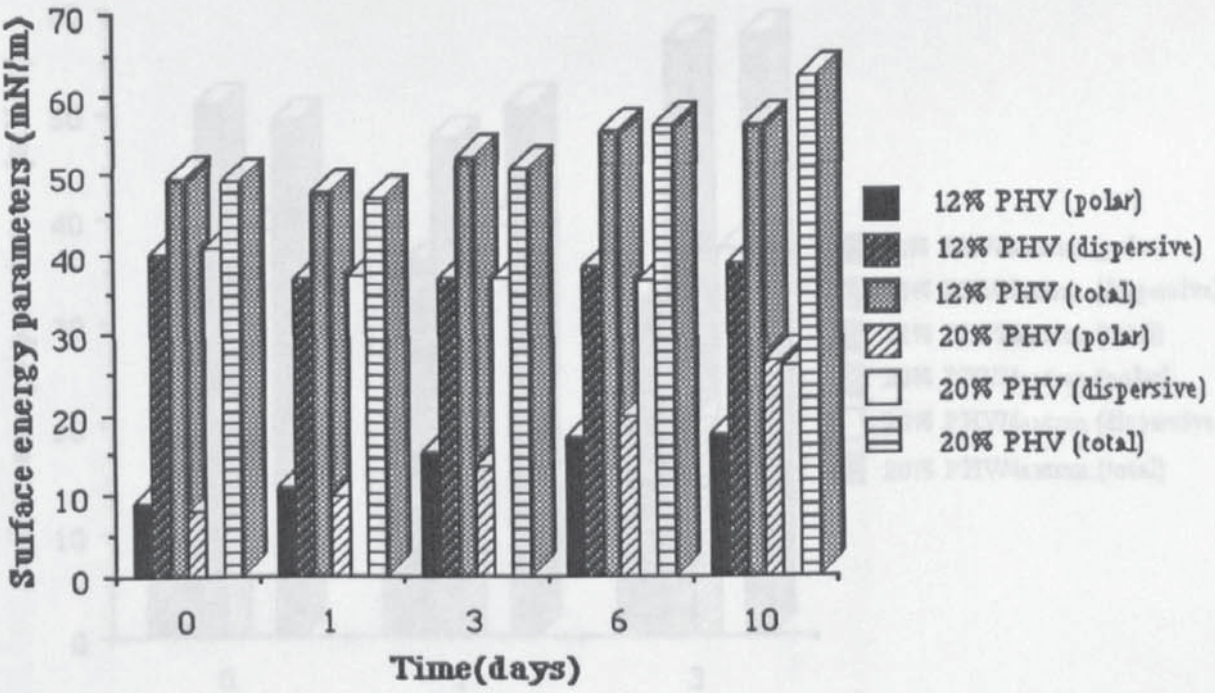
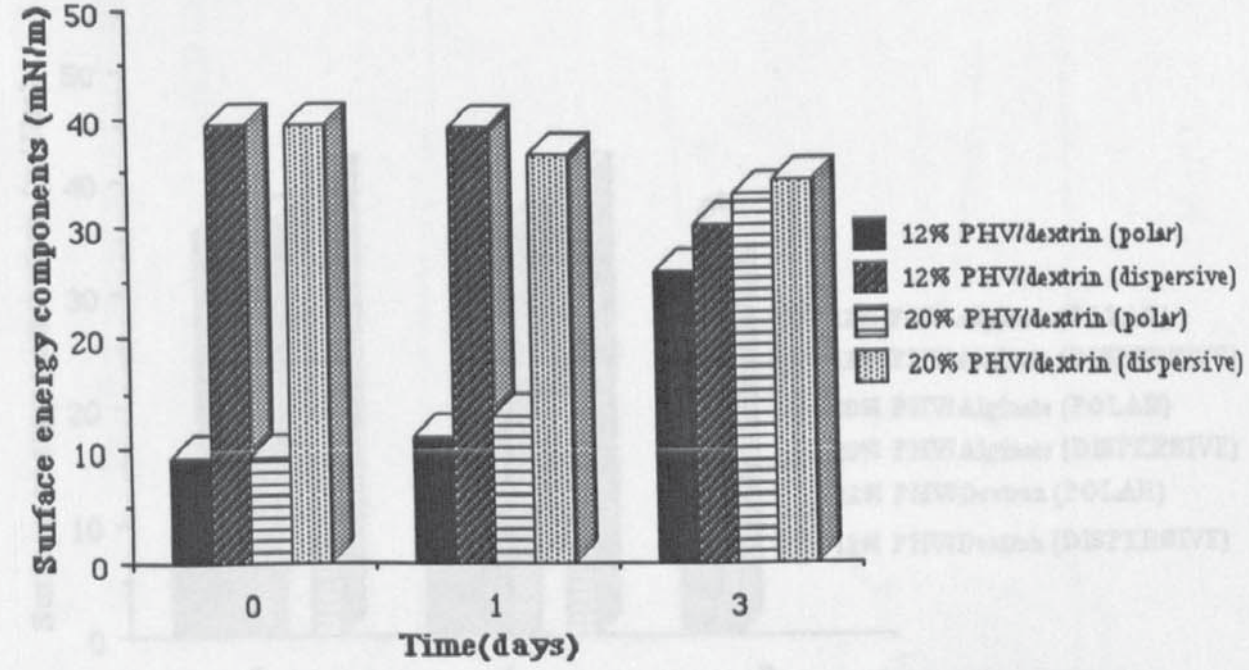


Figure 4.14 X-ray diffraction traces of various as-received, processed and degraded 12 and 20% PHV copolymers.

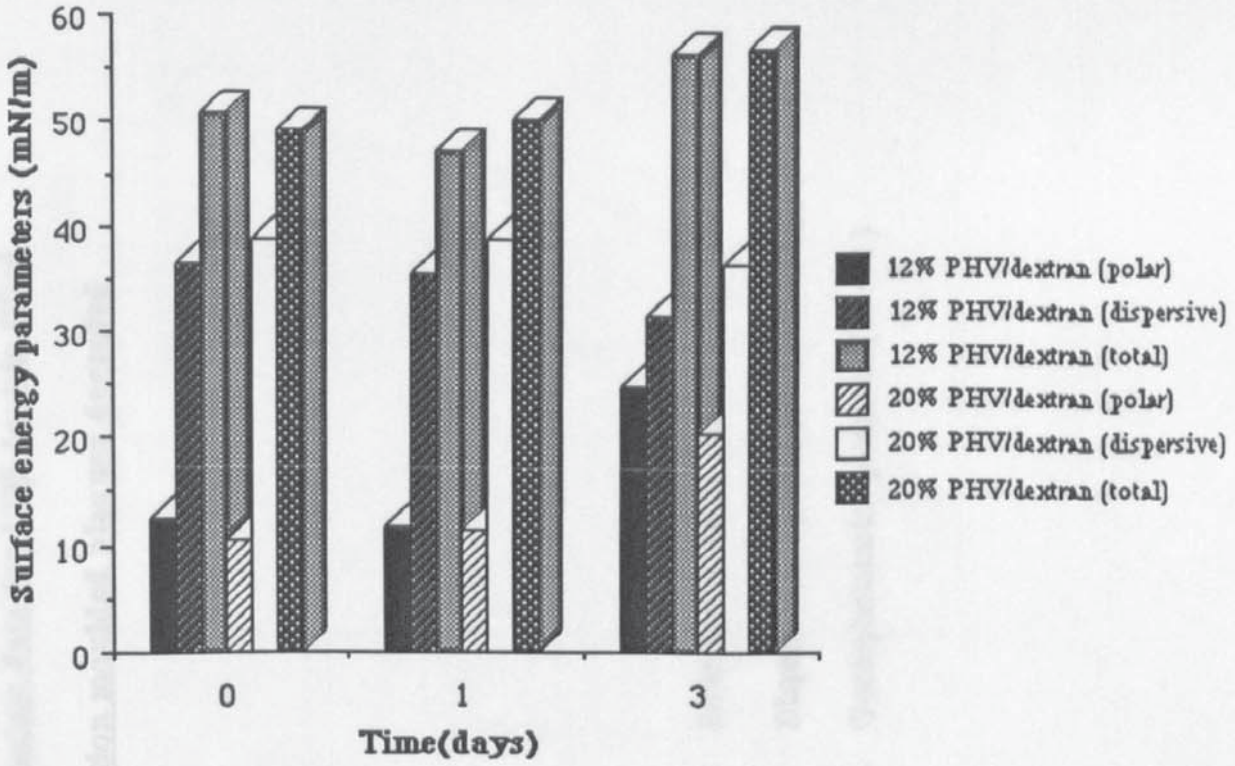




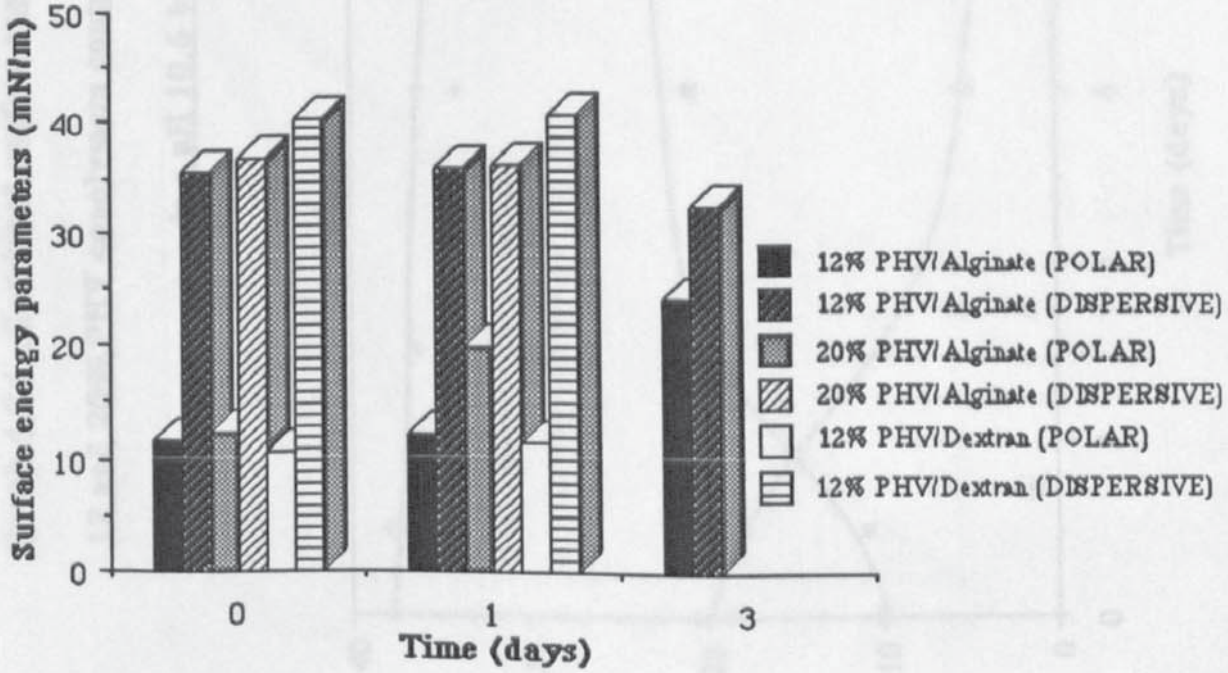
Graph 4.20 Changes in the surface energy components of 12 and 20% PHV copolymers (Talc nucleated): Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



Graph 4.21 Changes in the surface energy components of 12 and 20% PHV copolymer blends of dextrin (10%): Injection moulded plaques degraded in pH 10.6 buffer at 70°C.

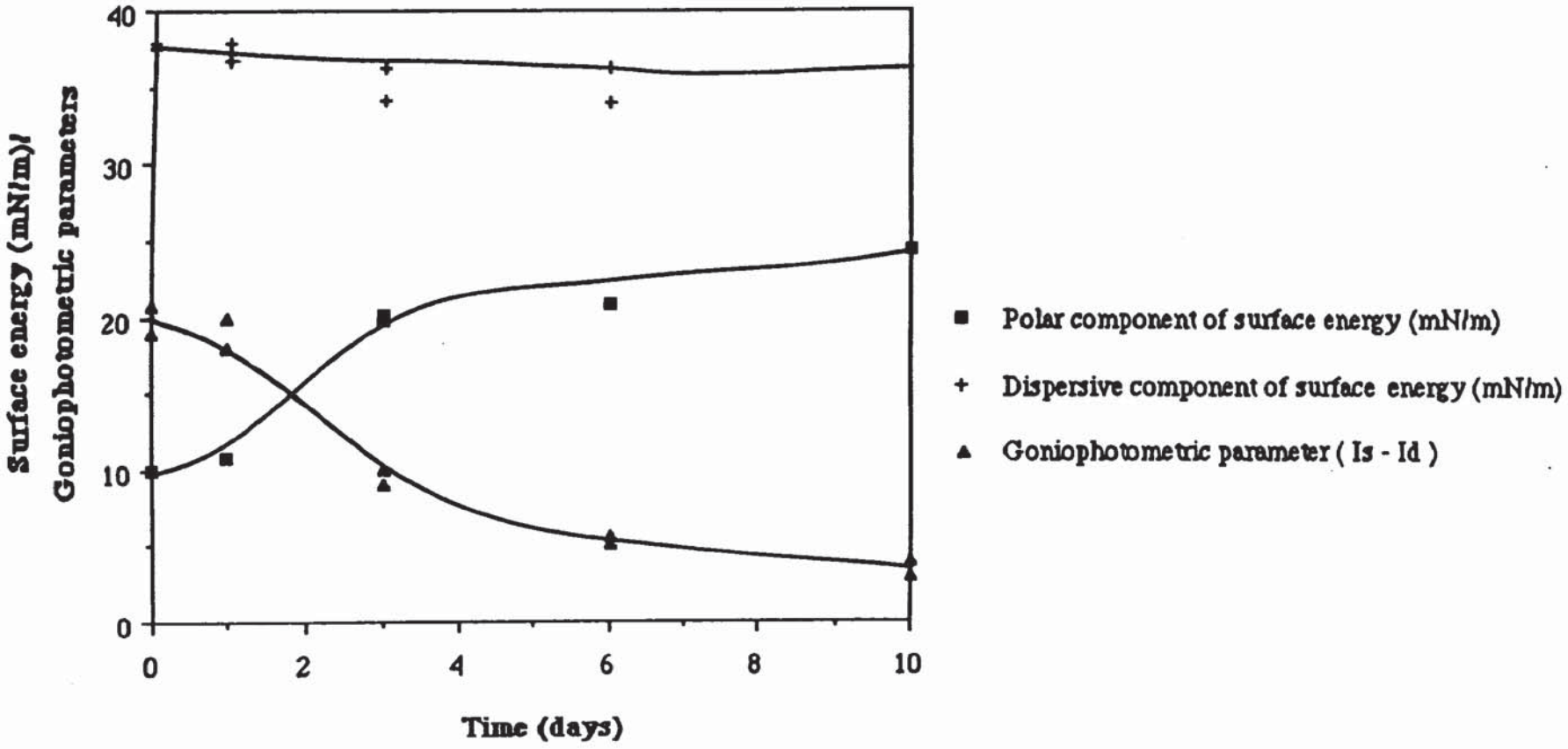


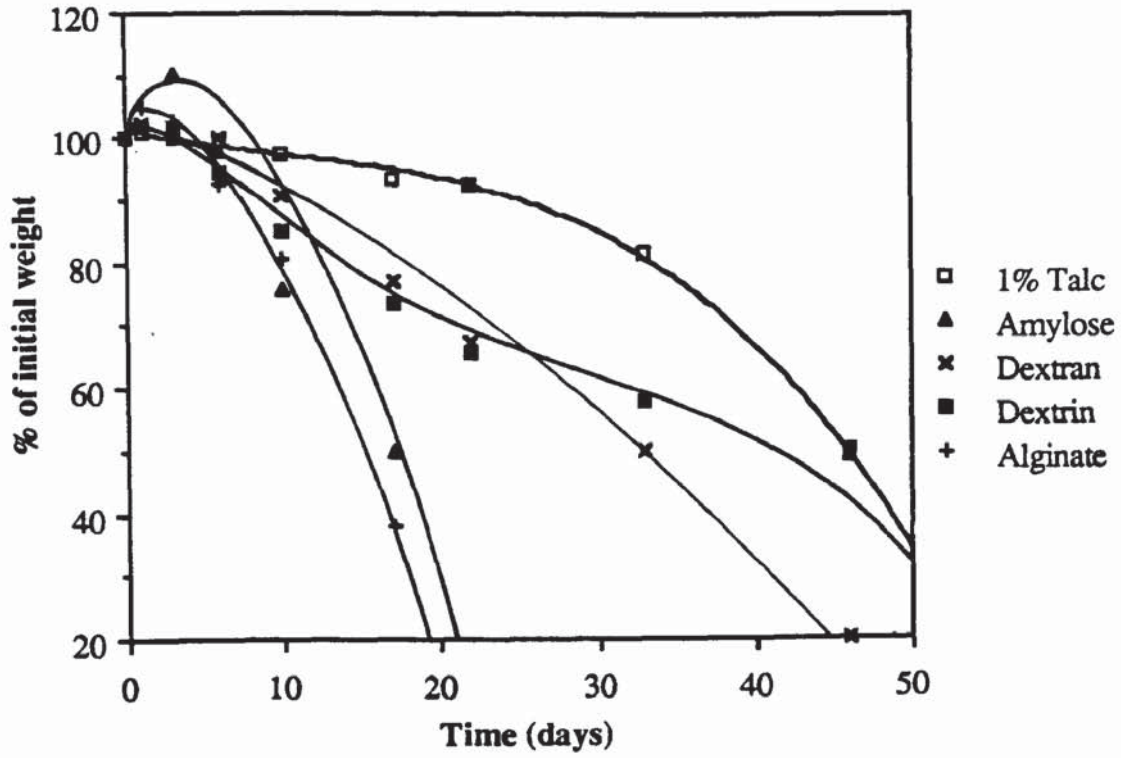
Graph 4.22 Changes in the surface energy components of 12 and 20% PHV copolymer blends of dextran (10%); Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



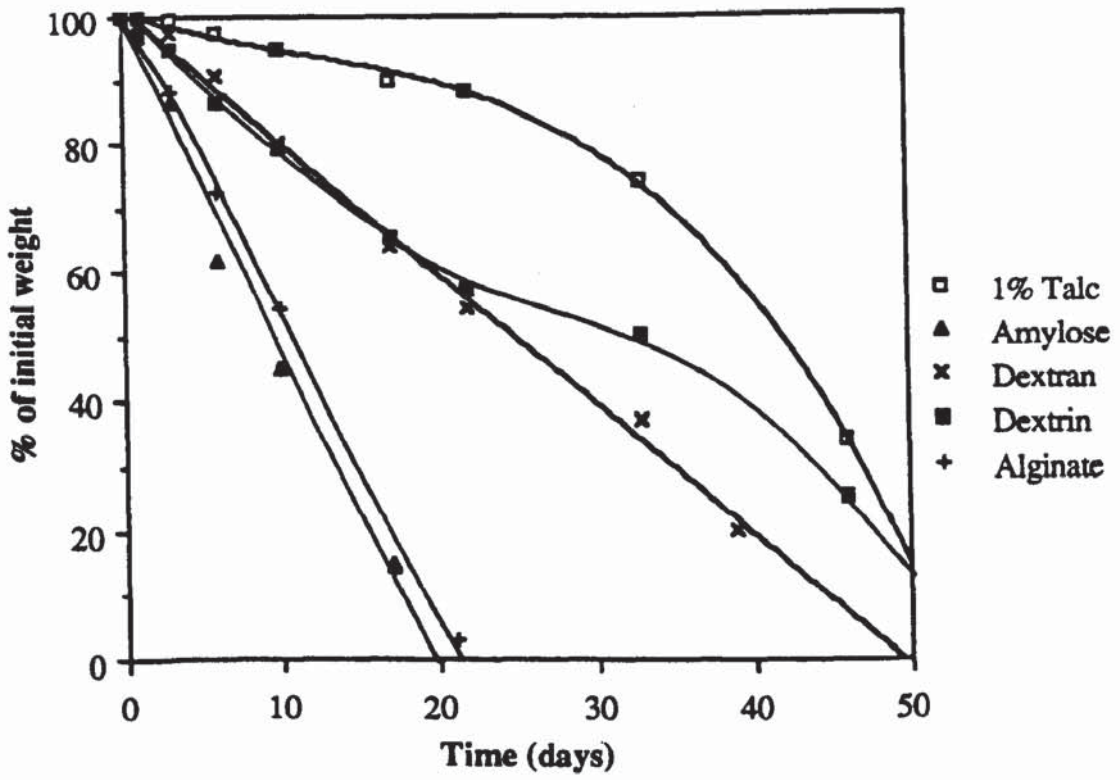
Graph 4.23 Changes in the surface energy components of 12 and 20% PHV copolymer blends of sodium alginate (10%) and dextran (30%); Injection moulded plaques degraded in pH 10.6 buffer at 70°C.

Graph 4.24 Combined surface energy and goniophotometric data for 10% dextrin filled 12 and 20% PHV copolymers containing 1% talc: Injection moulded plaques degraded in a pH 10.6 buffer at 70°C.

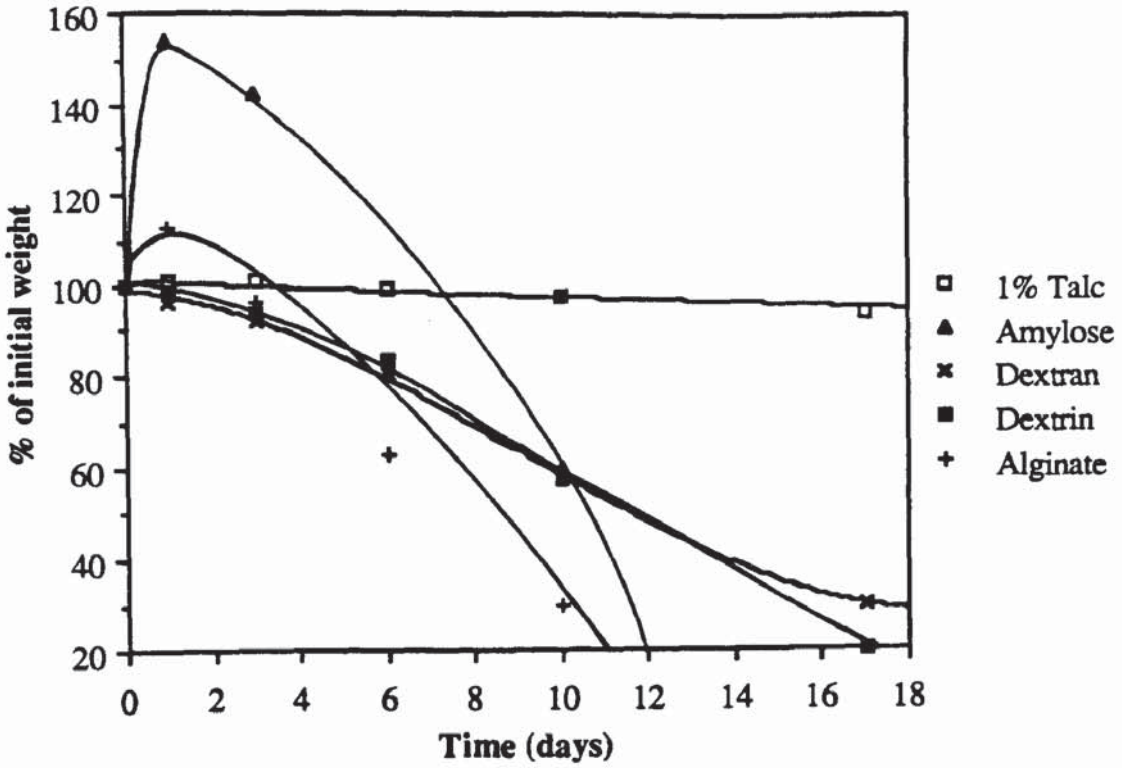




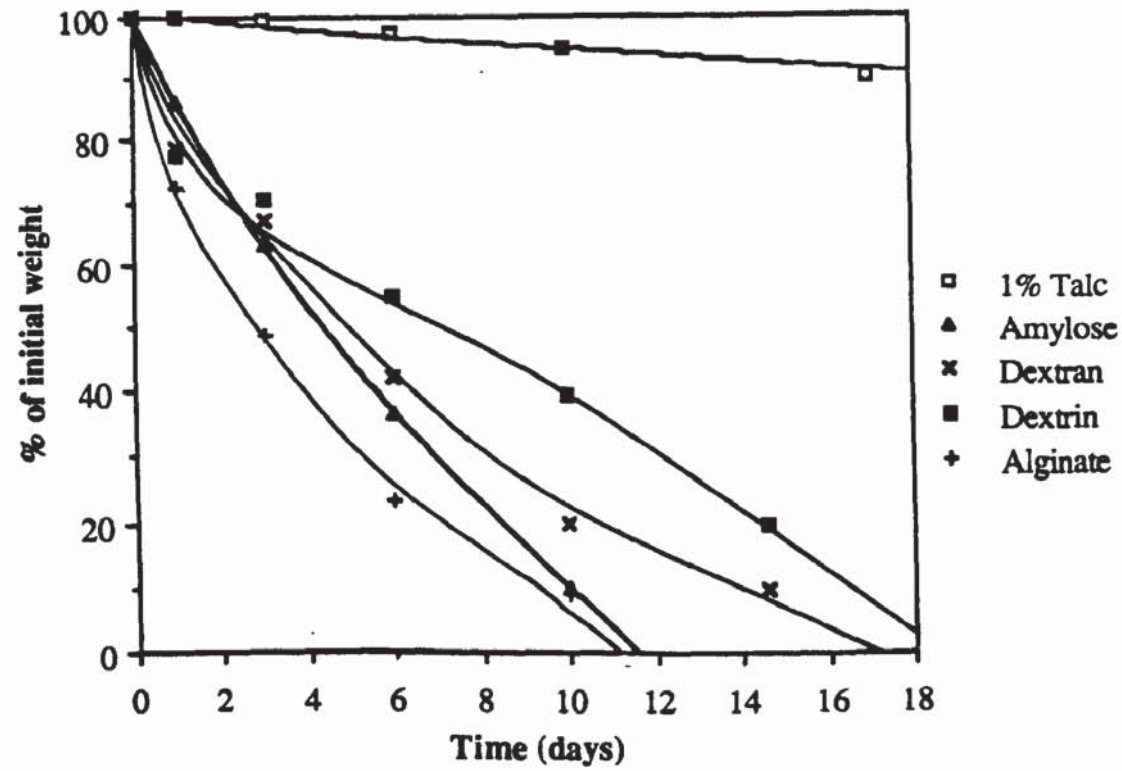
Graph 4.25 Change in wet weight of 12% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



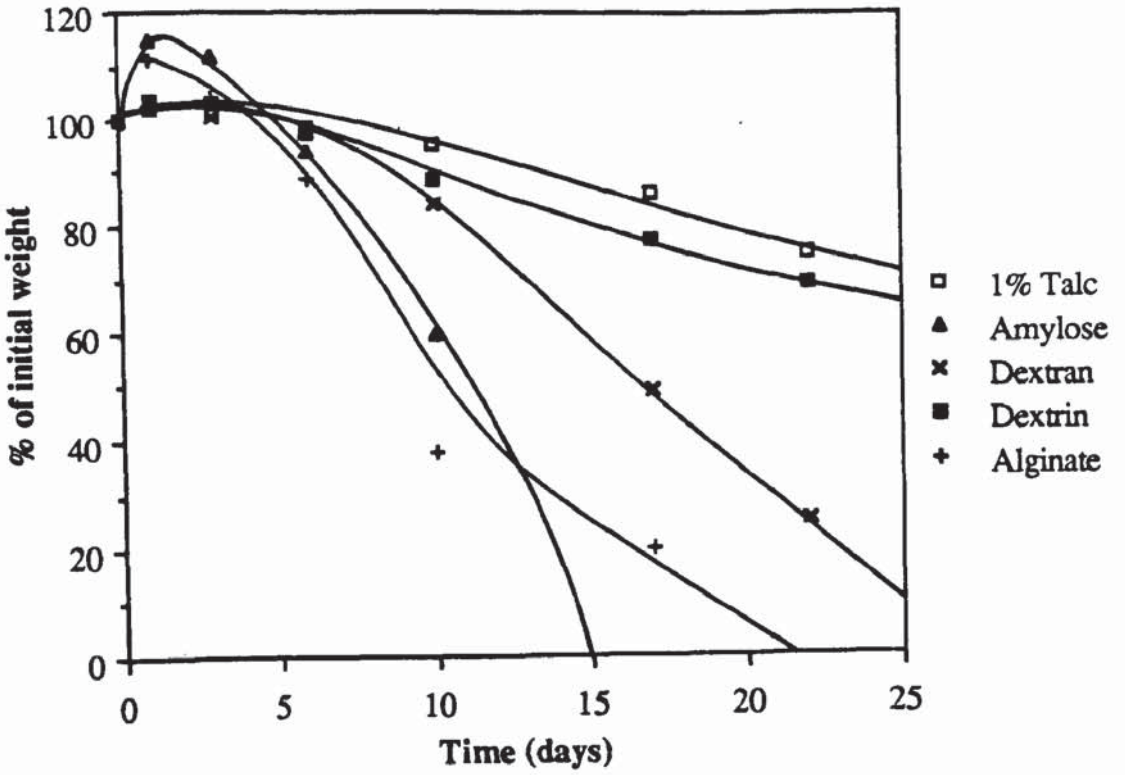
Graph 4.26 Change in dry weight of 12% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



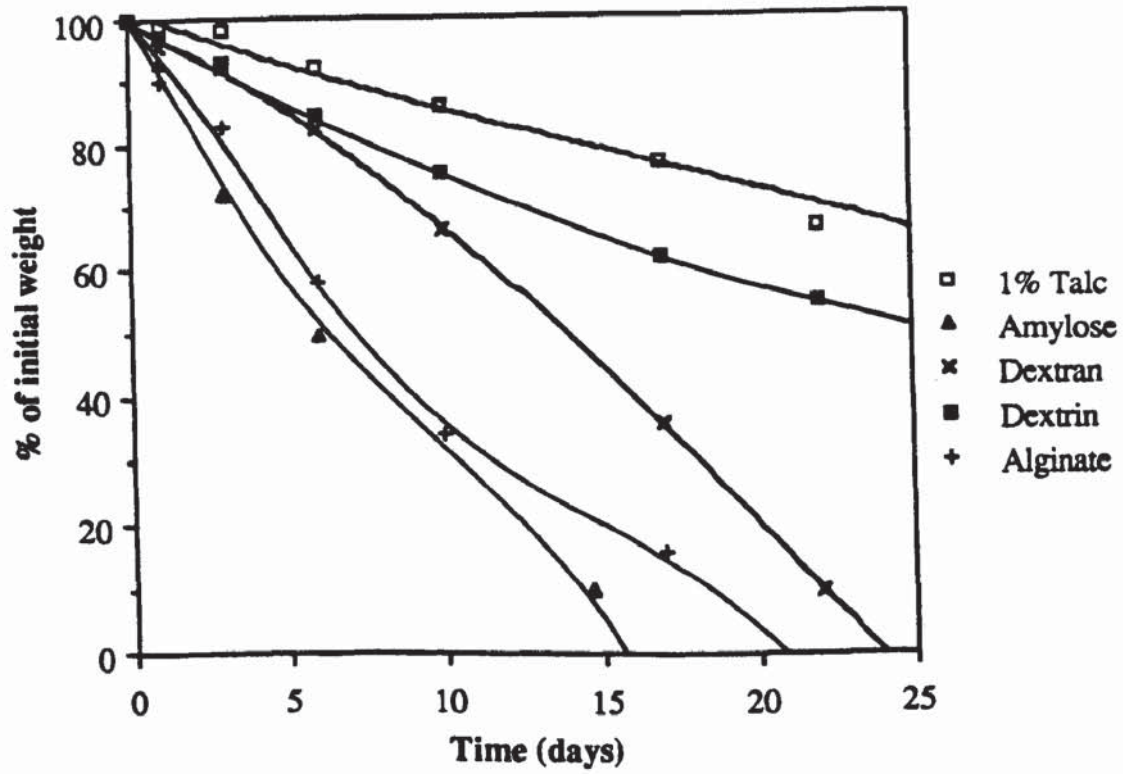
Graph 4.27 Change in wet weight of 12% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



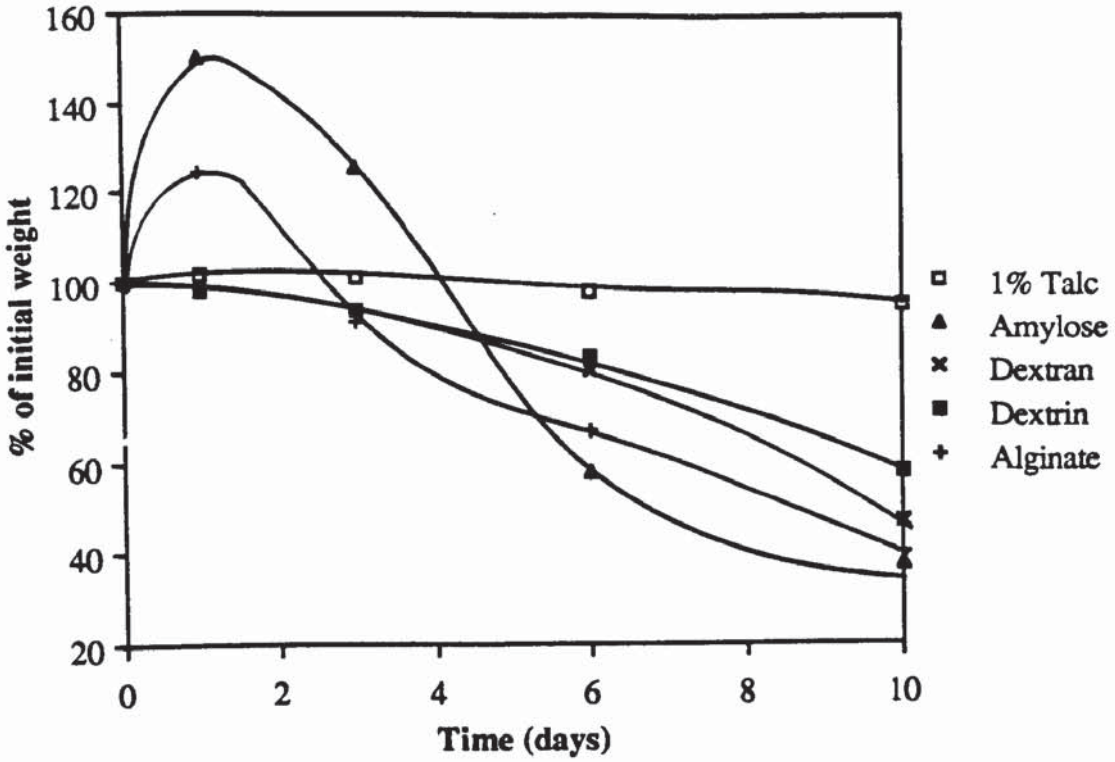
Graph 4.28 Change in dry weight of 12% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



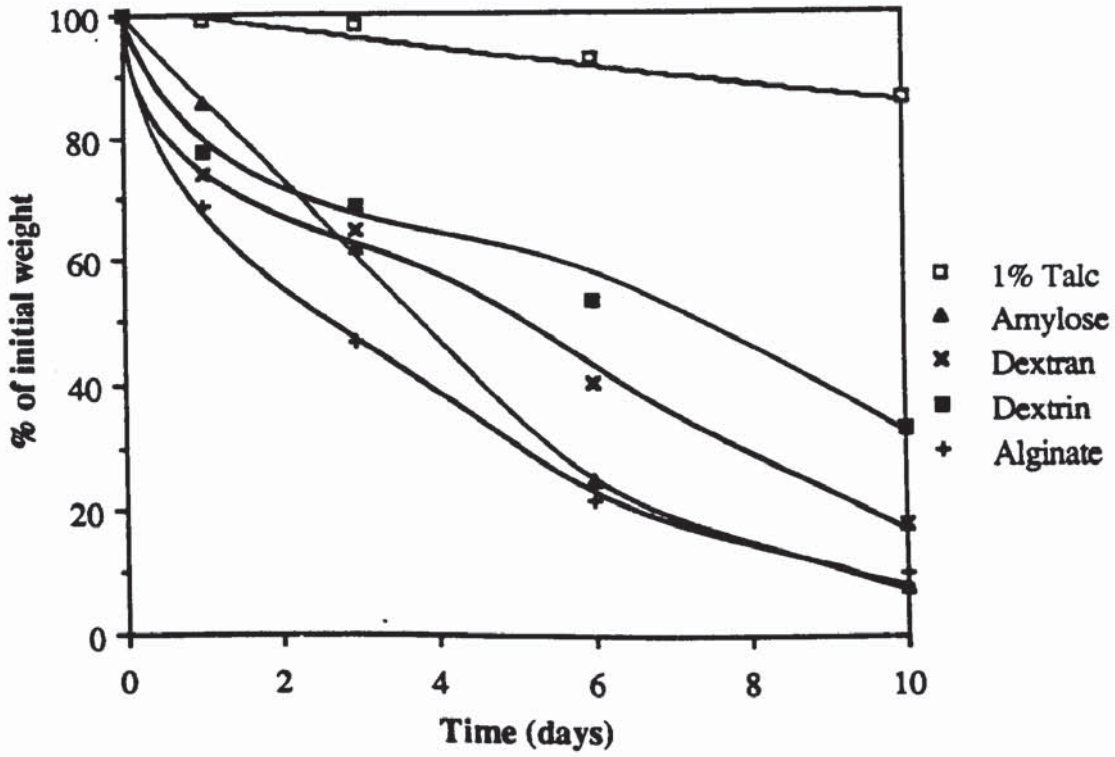
Graph 4.29 Change in wet weight of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



Graph 4.30 Change in dry weight of 20% PHV/Polysaccharide (10%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



Graph 4.31 Change in wet weight of 20% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.



Graph 4.32 Change in dry weight of 20% PHV/Polysaccharide (30%) blends: Injection moulded plaques degraded in pH 10.6 buffer at 70°C.

Table 4.15 Molecular weights of degraded injection moulded plaques (filled and unfilled).

Degradation conditions: pH 10.6 buffer at 70°C, 46 days.

	12% PHV ('350K')		20% PHV ('300K')		12% PHV/dexrtin (10%) blend.	
Parameter	Injection moulded	Degraded pH 10.6; 70°C	Injection moulded	Degraded pH 10.6; 70°C	Injection moulded	Degraded pH 10.6; 70°C
Mw	192K	15K	195K	12.2K	270K	4.3K
Mn	87K	2.7K	87K	2.8K	107K	1.5K
Mw/Mn	2.2	5.5	2.2	4.3	2.5	2.9

Table 4.16 The t_{10} and t_{50} weight loss parameters for PHB-PHV/Polysaccharide blends in a pH 10.6 buffer at 70°C (Injection moulded plaques)

12% PHV

12% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	20	42
10% Amylose	2	10
30% Amylose	0.5	4.5
10% Dextran	5	27
30% Dextran	0.5	5
10% Dextrin	5	33
30% Dextrin	0.5	7
10% Sodium Alginate	3	12
30% Sodium Alginate	0.5	2.5
10% Dextrin + 1% Talc	6	36

20% PHV

20% PHV/Polysaccharide Blends	t_{10} (days)	t_{50} (days)
1% Talc	12	35
10% Amylose	1	6
30% Amylose	0.5	3.5
10% Dextran	3.5	14
30% Dextran	0.5	4.5
10% Dextrin	4	29
30% Dextrin	0.5	6
10% Sodium Alginate	2	8
30% Sodium Alginate	0.5	2.5
10% Dextrin + 1% Talc	5	31

Plate 4.7 Light microscopy photographs of degraded 12% PHV/dextrin (10%) blend (injection moulded plaque) showing the onset of porosity after 6 days degradation in a pH 10.6 buffer at 70°C. (x 165).

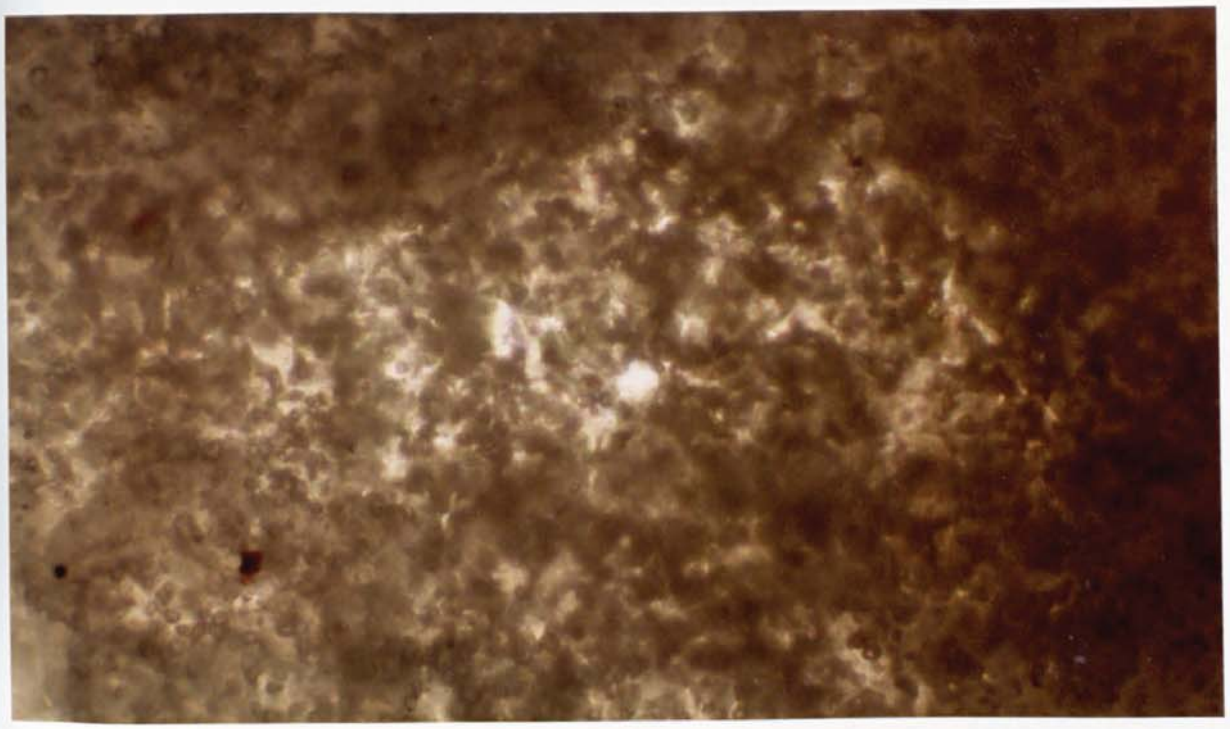


Plate 4.8 Light microscopy photographs of degraded 20% PHV/dextran (10%) blend (injection moulded plaque) showing the onset of porosity after 6 days degradation in a pH 10.6 buffer at 70°C. (x 165).

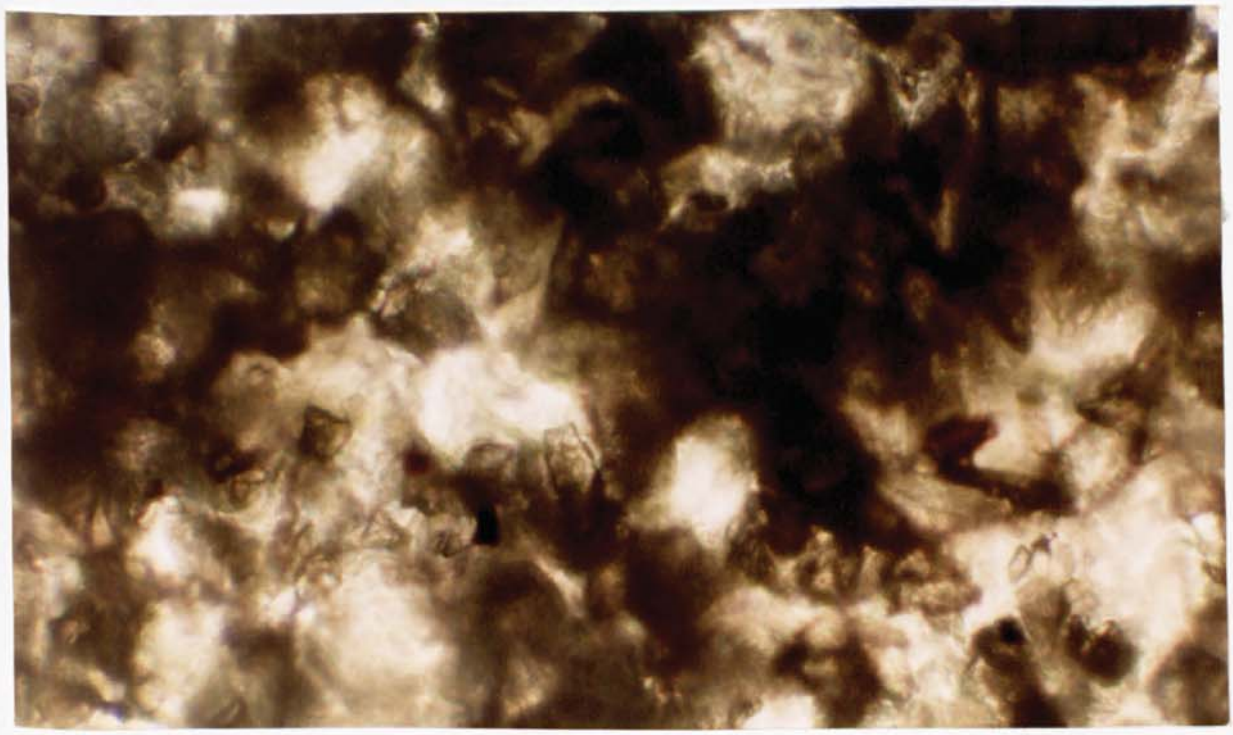


Plate 4.9a SEM of 20% PHV/dextran (30%) blend, (injection moulded plaque); undegraded (fractured surface); x 200.

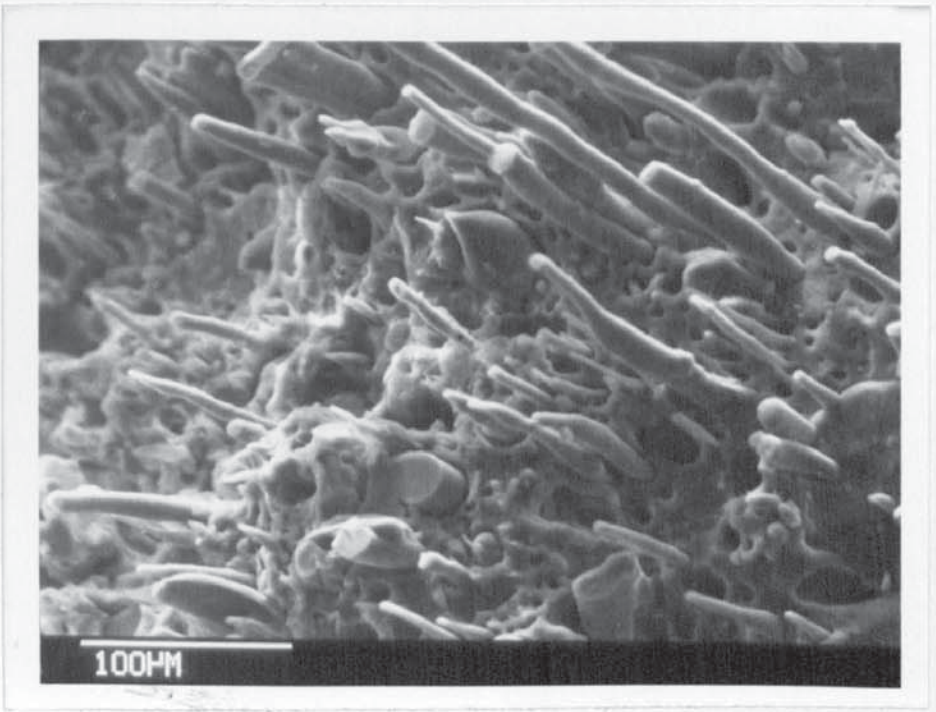


Plate 4.9b SEM of 20% PHV/dextran (30%) blend after 9 days degradation in a pH 10.6 buffer at 70°C (injection moulded; fractured surface); x 1000

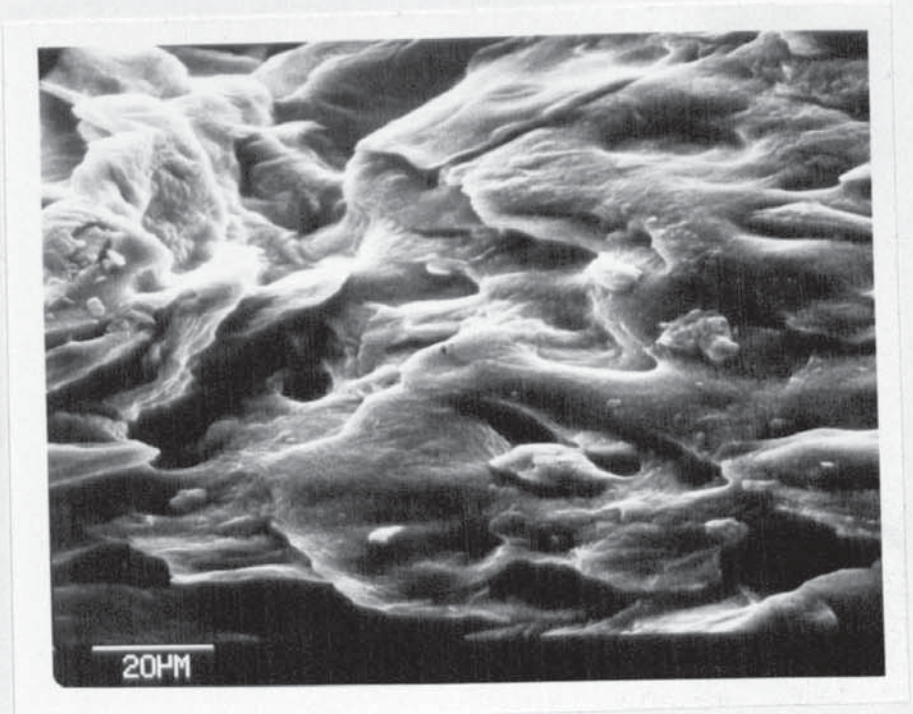


Plate 4.10a SEM of 12% PHV/dextrin (30%) blend, (injection moulded plaque); undegraded (fractured surface); x 500.

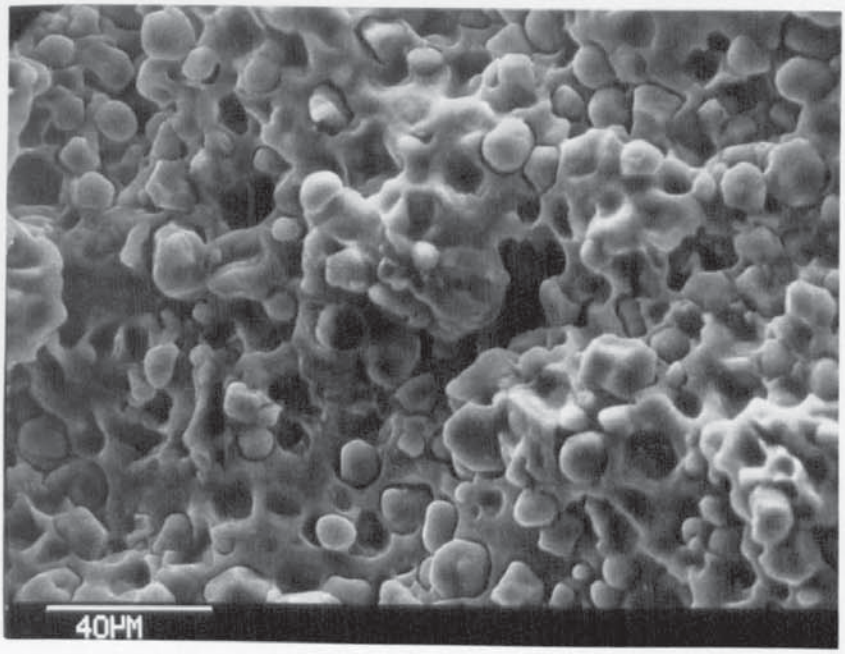


Plate 4.10b SEM of 12% PHV/dextrin (30%) blend after 12 days degradation in a pH 10.6 buffer at 70°C (injection moulded plaque; fractured surface); x 2000.

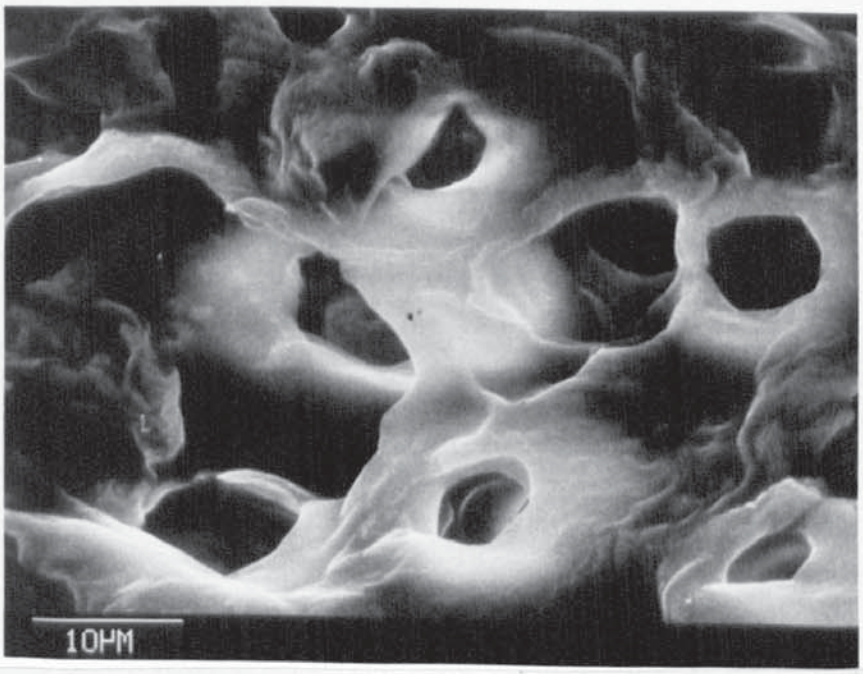


Plate 4.11 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

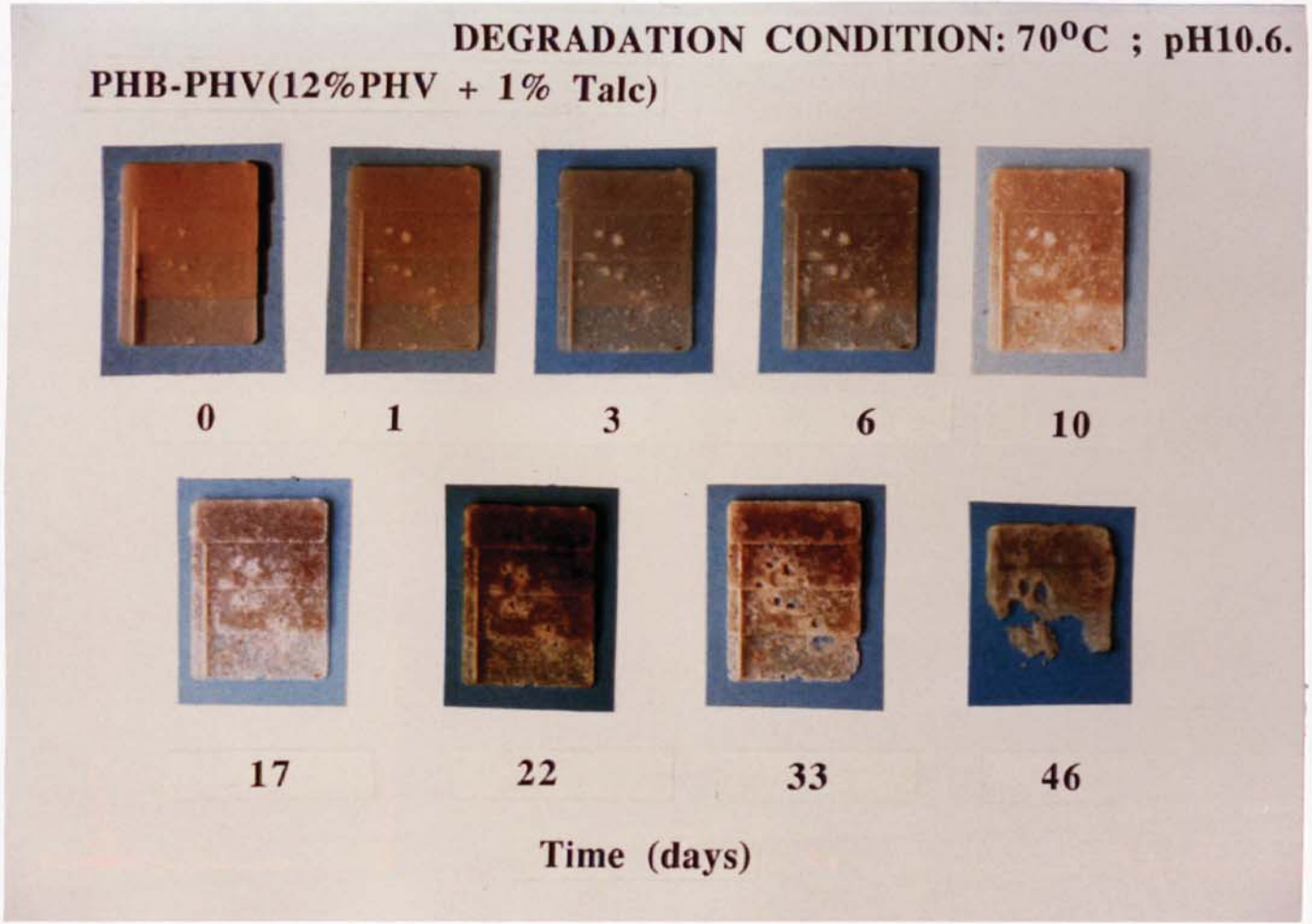


Plate 4.12 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

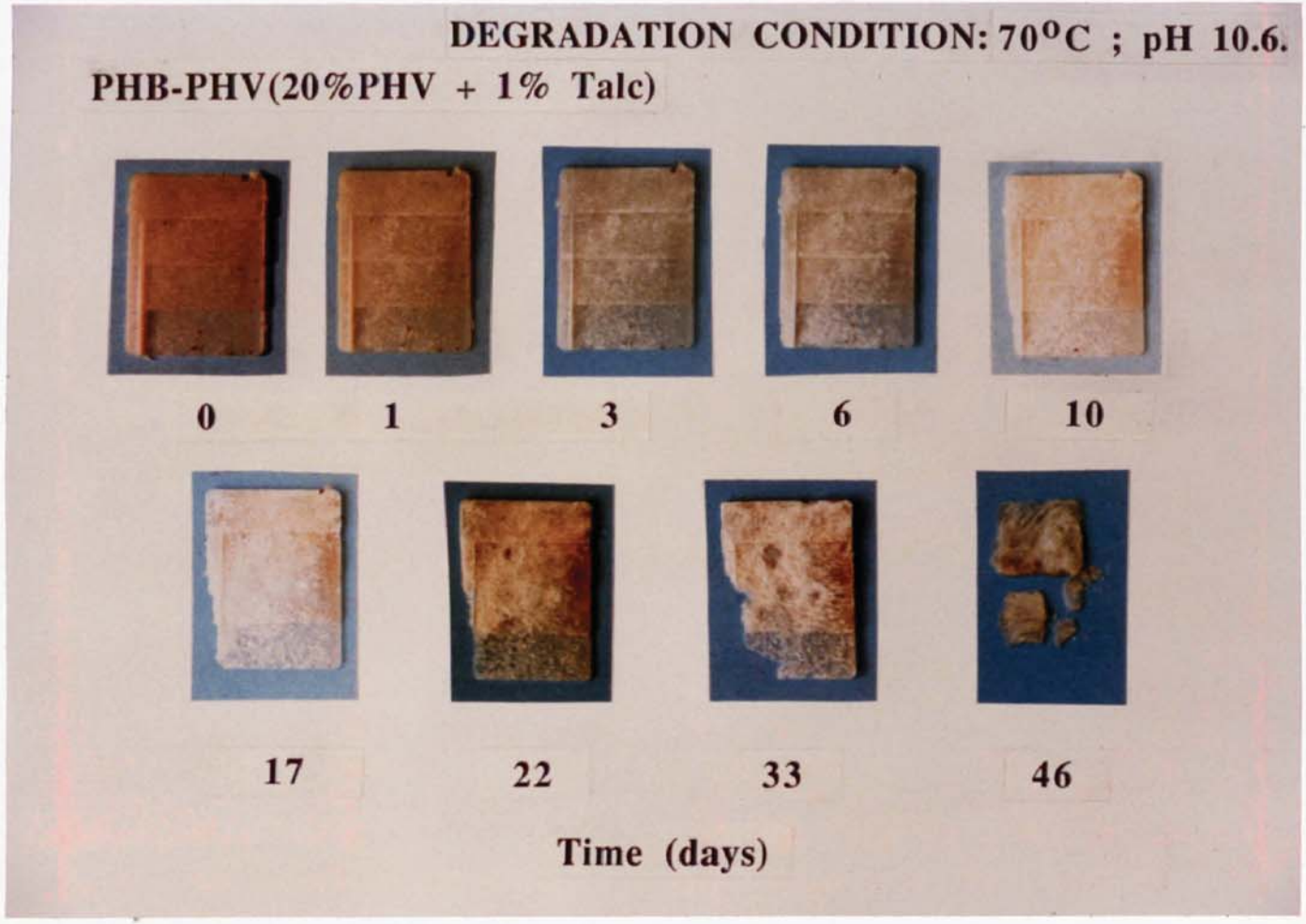


Plate 4.13 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

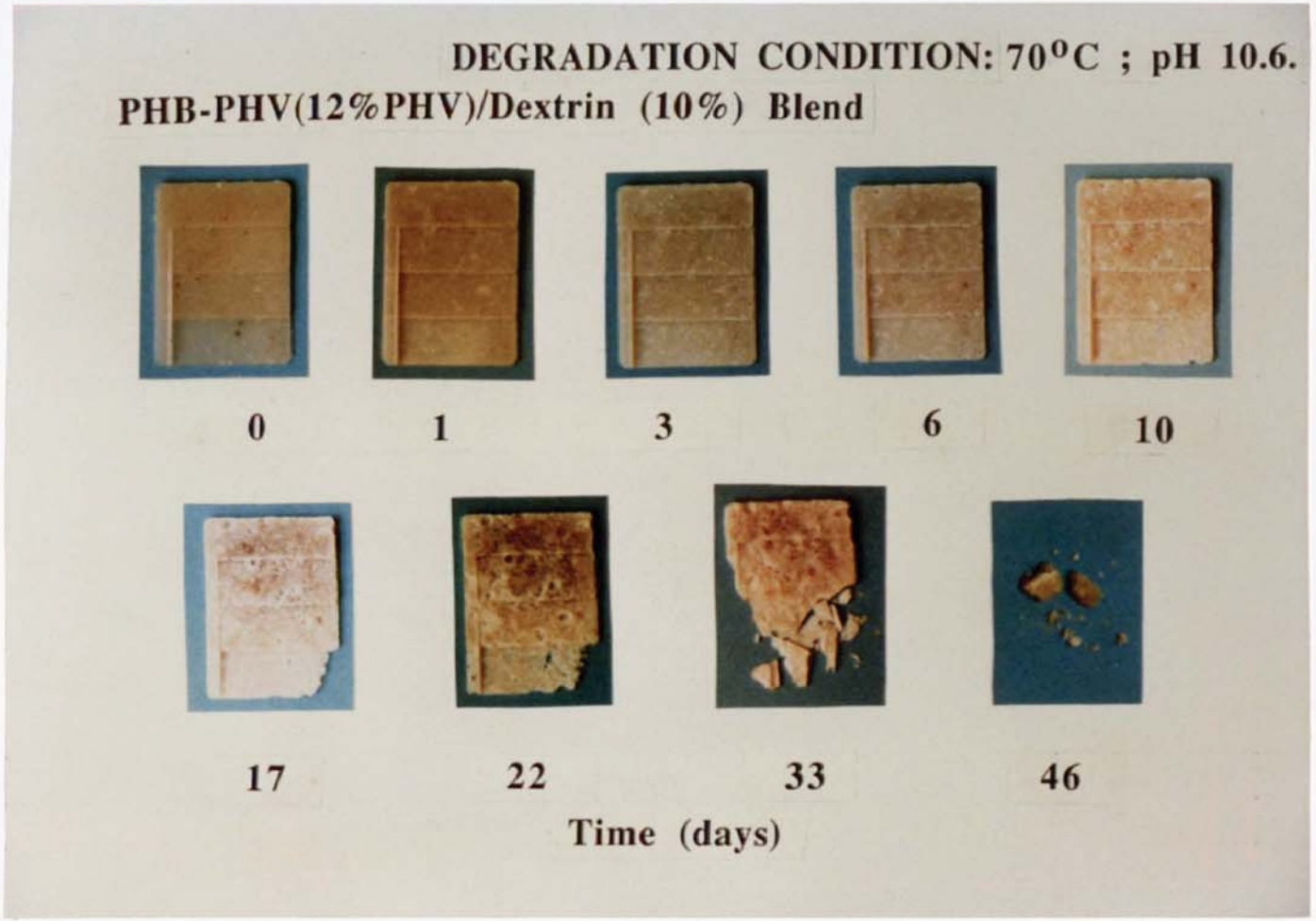


Plate 4.14 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

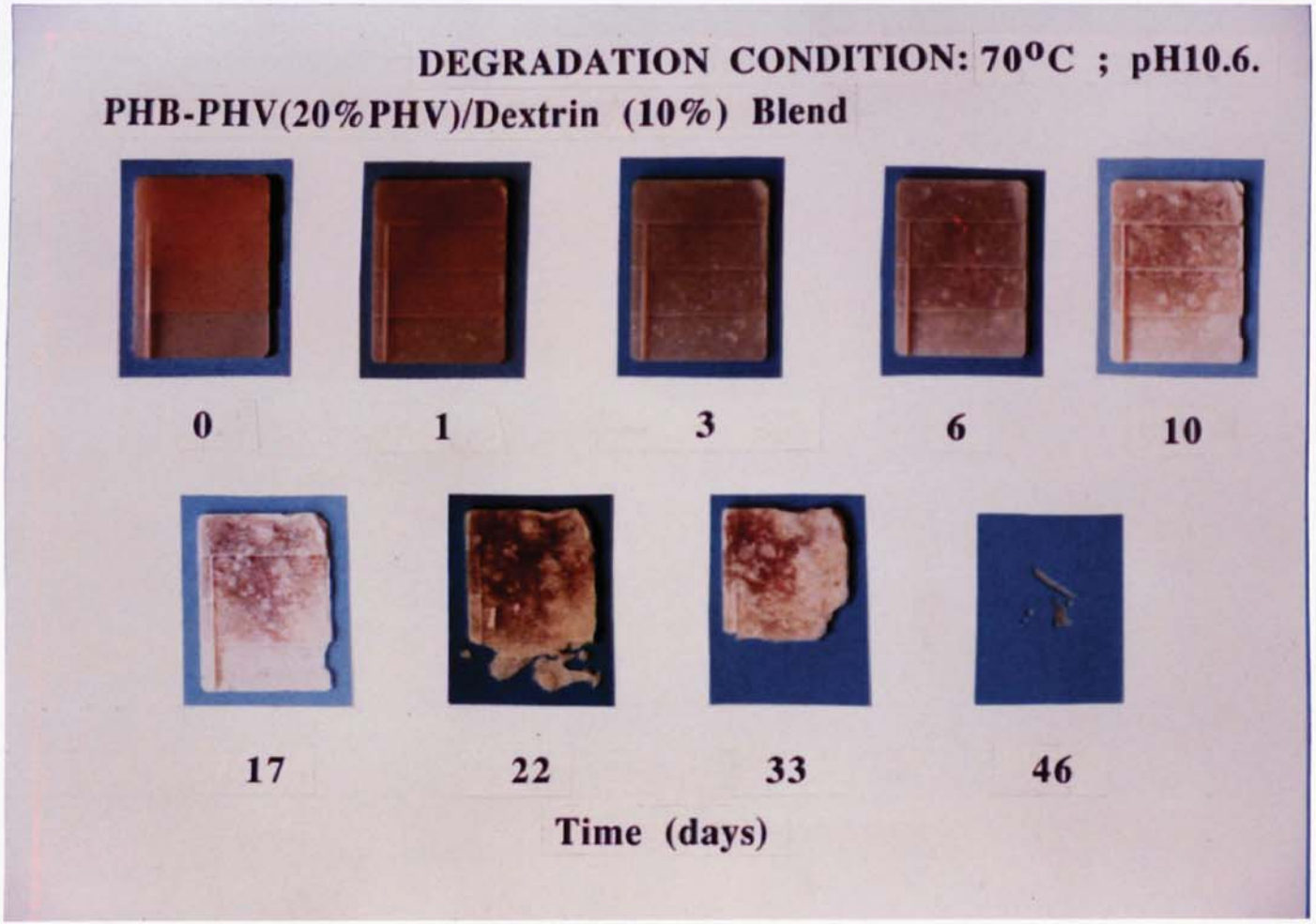


Plate 4.15 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

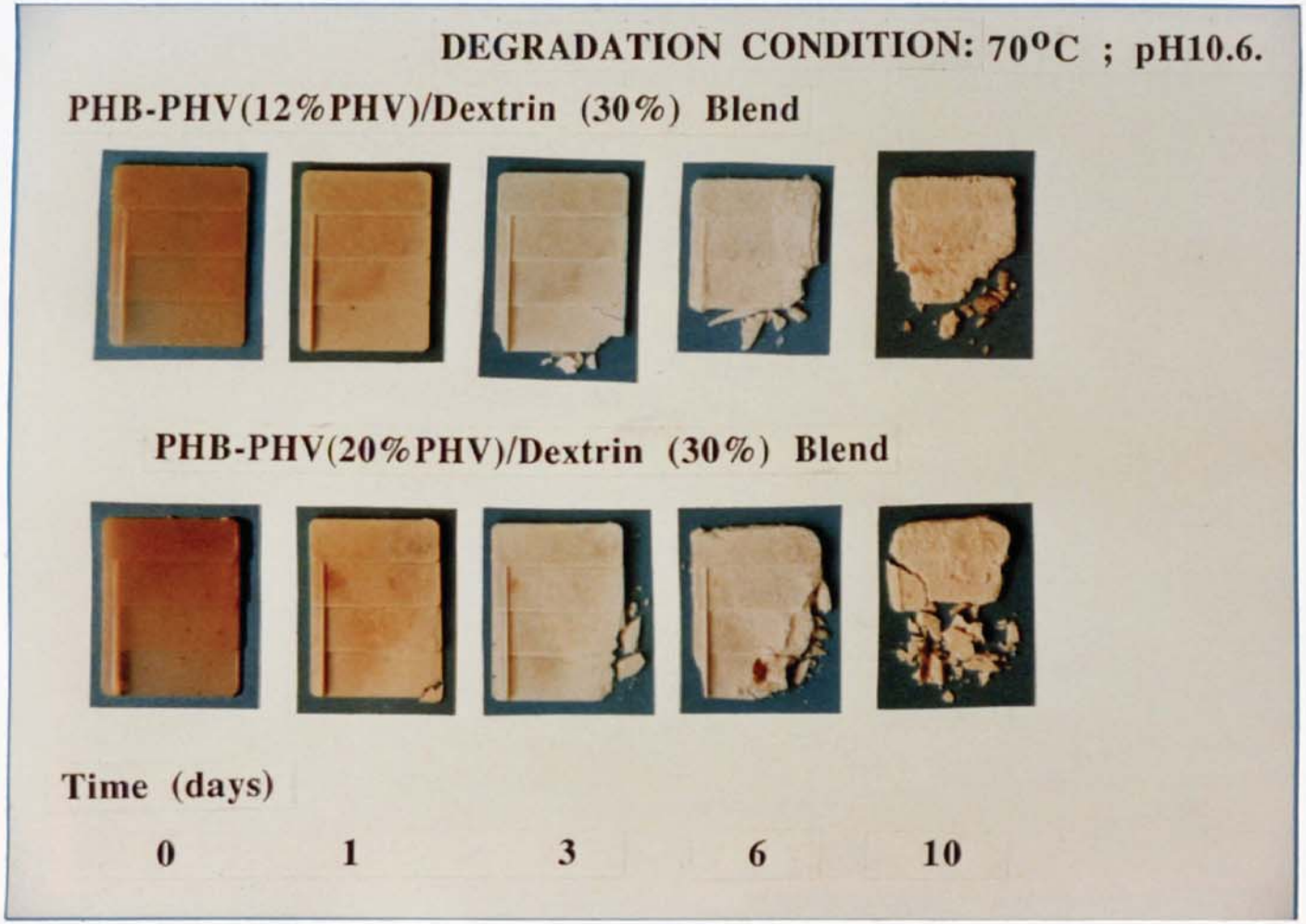


Plate 4.16 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

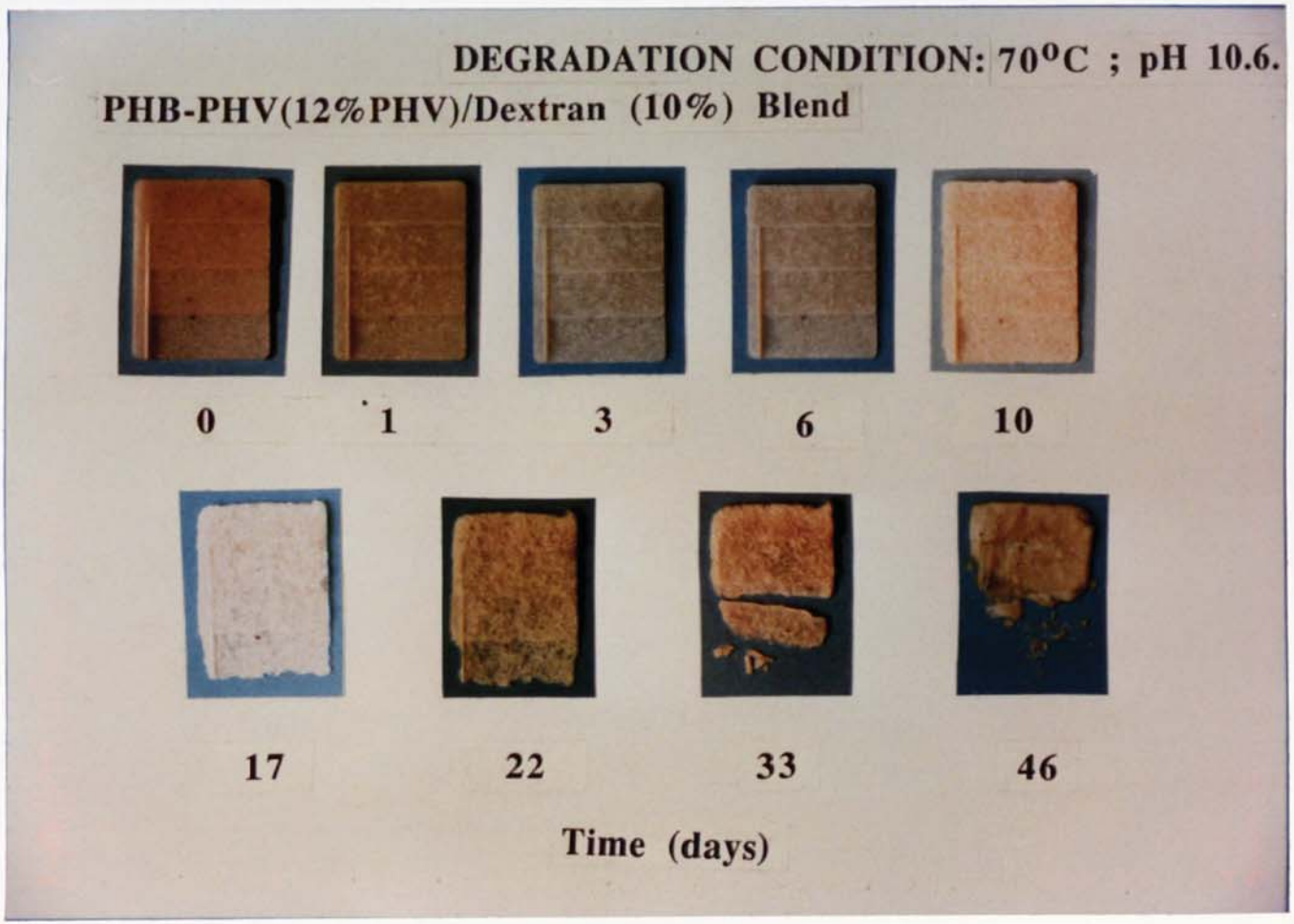


Plate 4.17 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

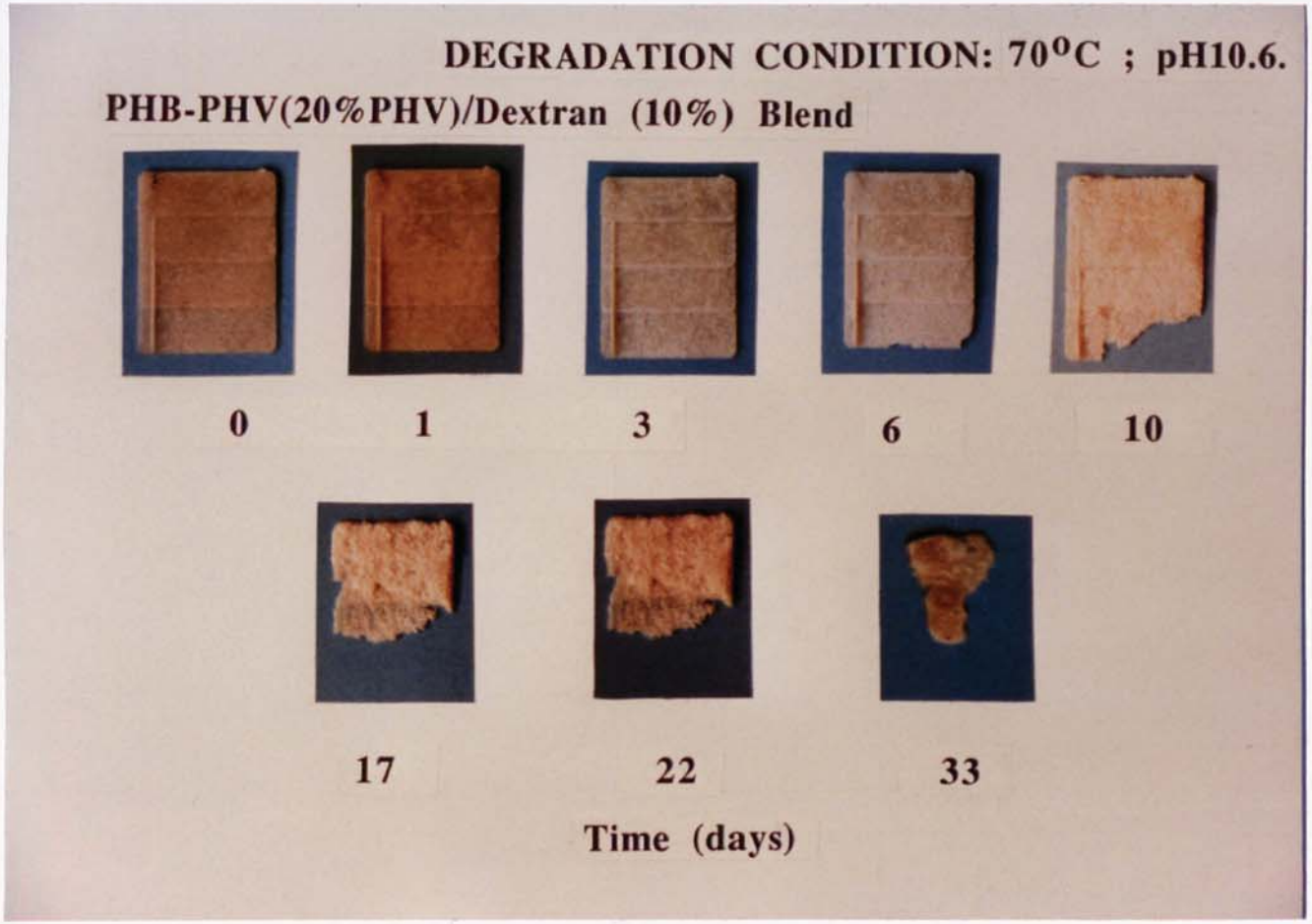


Plate 4.18 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

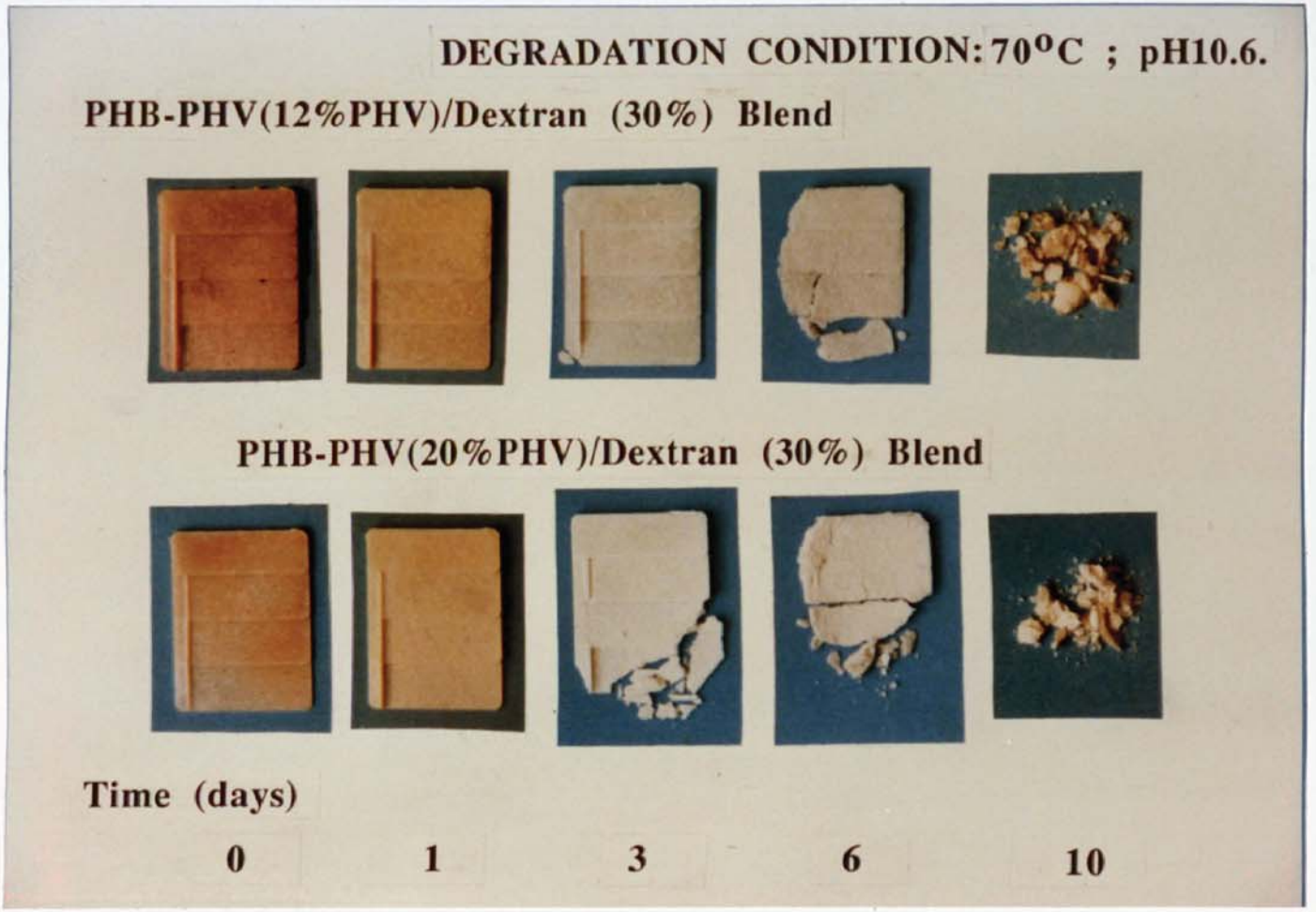


Plate 4.19 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

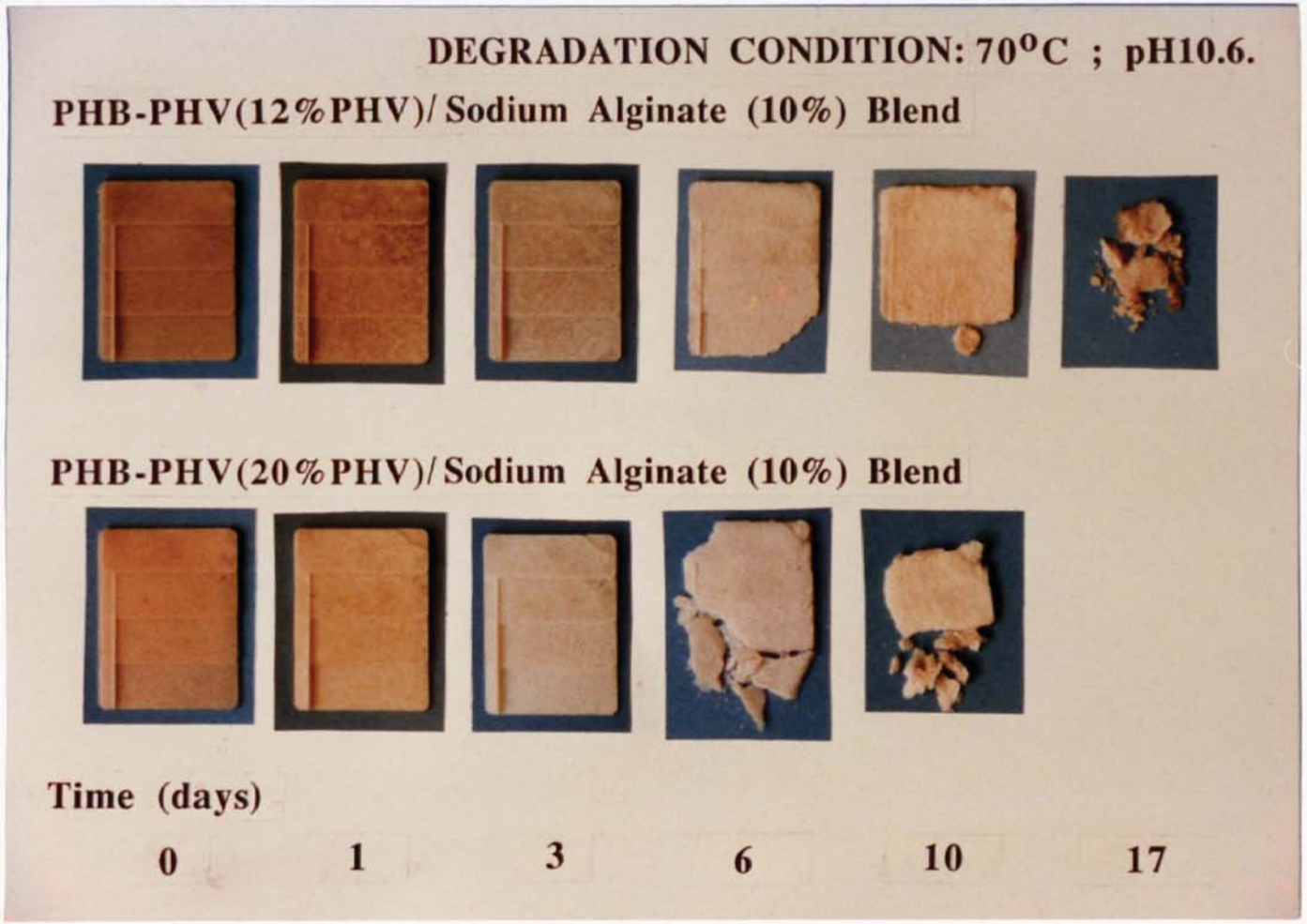


Plate 4.20 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

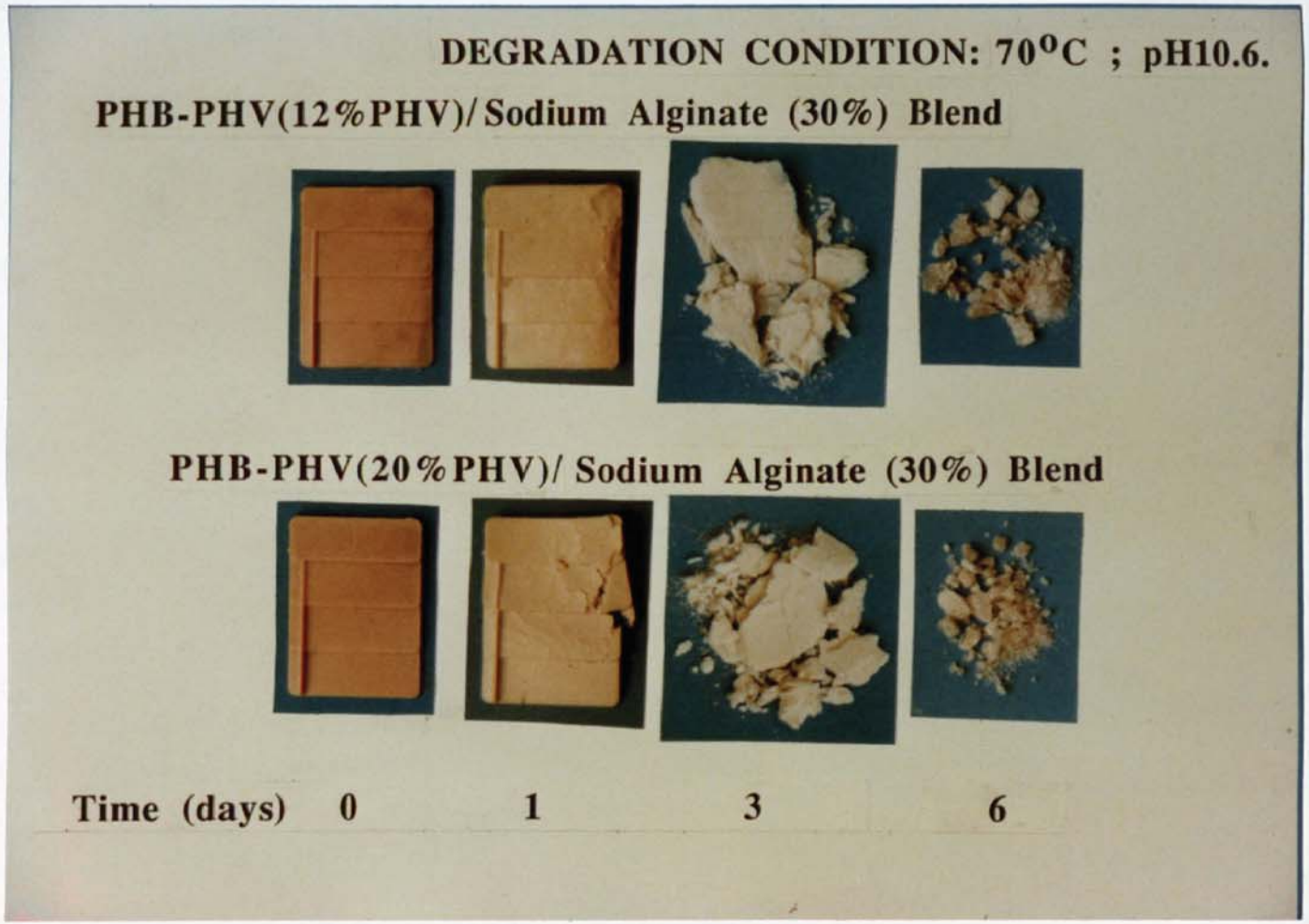


Plate 4.21 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

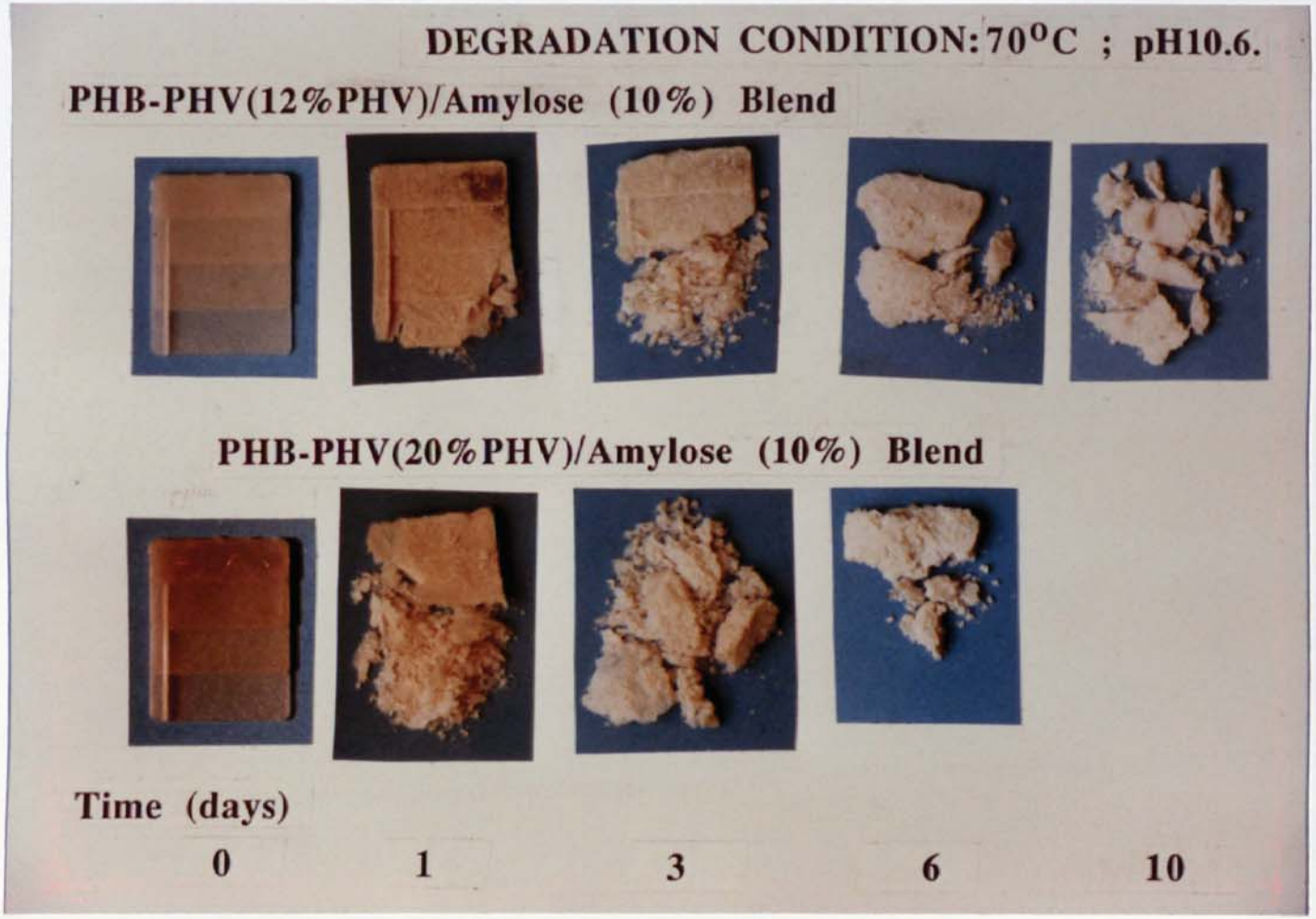


Plate 4.22 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

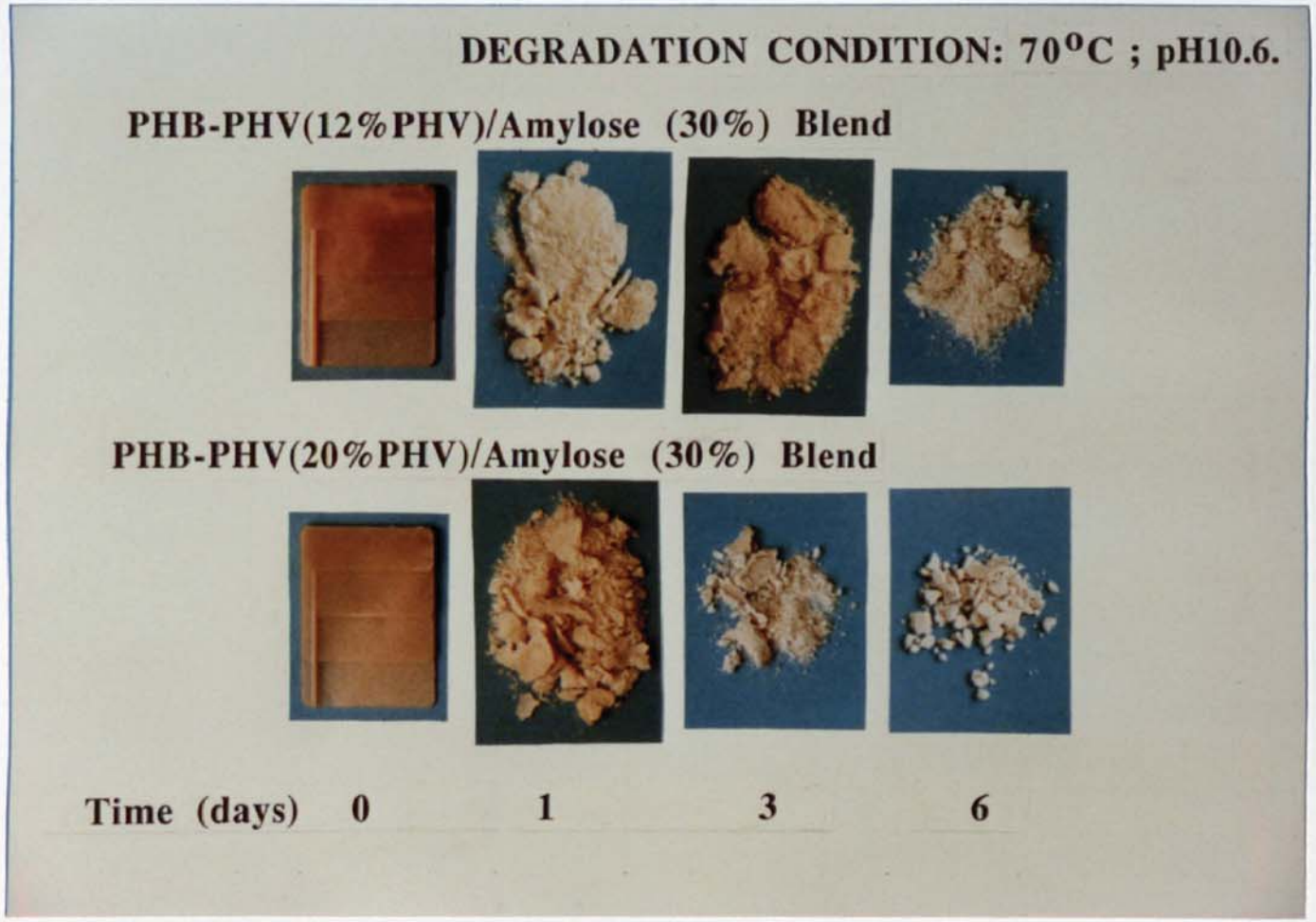


Plate 4.23 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

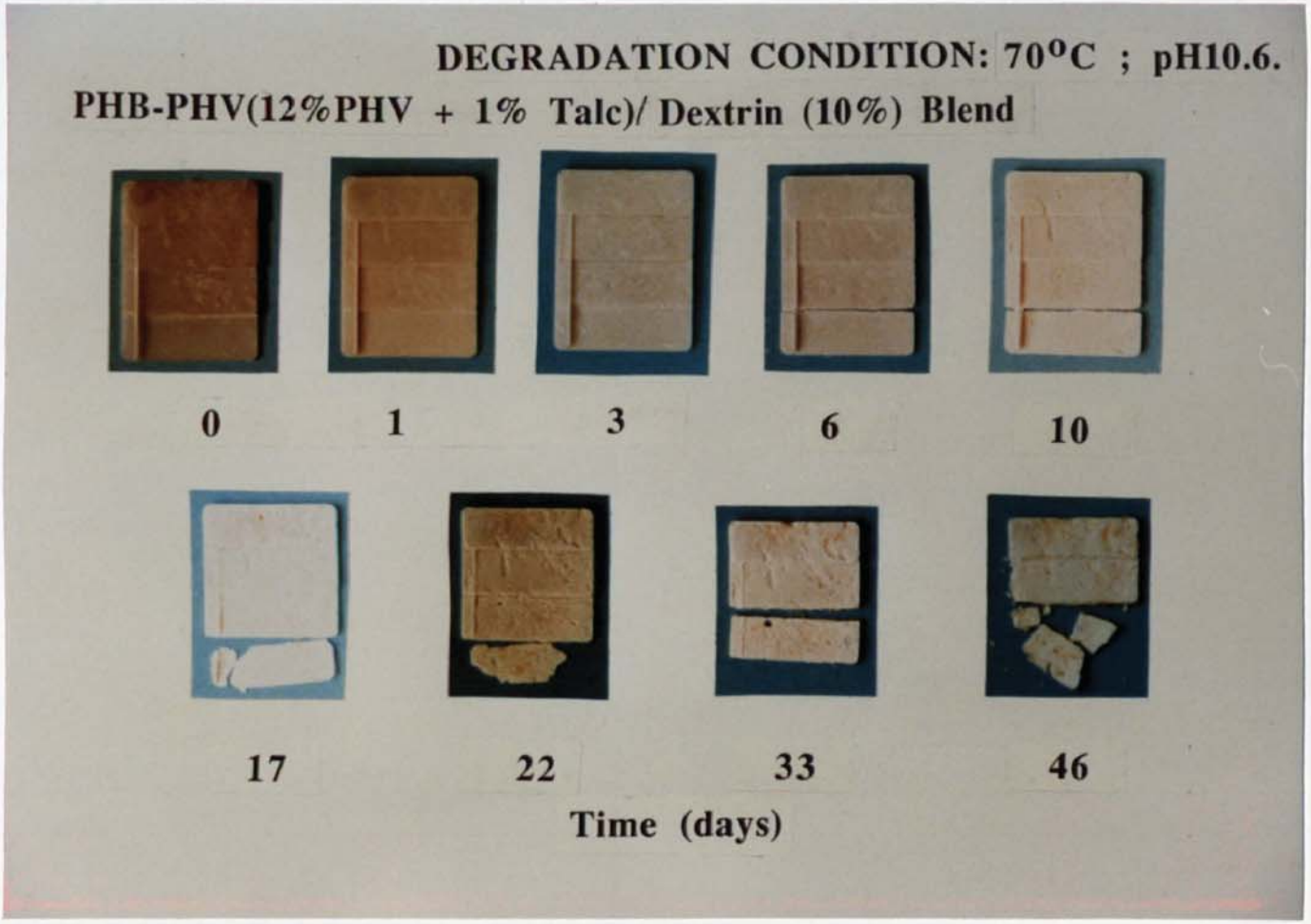


Plate 4.24 Injection moulded plaques: Photographic illustration of hydrolytic degradation.

CHAPTER 5

PHB-PHV/POLYCAPROLACTONE BLENDS:

PHYSICAL AND DEGRADATIVE

PROPERTIES OF A NOVEL RANGE OF MATERIALS.

5.1 INTRODUCTION.

The basic mode of operation for surgical fixation devices, such as clips and staples, requires the device to have a certain degree of strength and the ability to undergo a degree of flexing without fracture. These property requirements are difficult to correlate with tensile parameters obtained from simple stress/strain curves, but some comparison can be made on the basis of initial tensile moduli, which reflect the stiffness of different candidate materials. Although physical properties of polyhydroxybutyrate (PHB) can be varied by hydroxyvalerate (PHV) units, the extent to which these maybe modified is limited. Similarly, the use of the blending techniques described in Chapter 4 to incorporate polysaccharide materials which influence the degradation profiles is also to some extent limited by the nature of the polysaccharide materials. However, it would be interesting to produce a range of materials with a wide spectrum of physical properties by using the blending techniques. These materials could be used for specific applications where a particular combination of physical property and degradation rate is needed.

In this chapter, the use of blending techniques to modify the properties of PHB-PHV copolymers by incorporation by another synthetic biodegradable polymer (polycaprolactone) is described. The aim of this chapter is to investigate the physical properties of materials so produced and at the same time to study the influence of polycaprolactone on the degradation behaviour of these. It should be emphasized that the main thrust of this chapter deals with the development of new novel biomaterials, rather than on investigation of degradation mechanism pathway.

Although a common method of increasing the processability and lowering of the initial tensile modulus (i.e. decrease in stiffness), of a polymer is to use plasticisers during

processing operations, for biomedical applications careful selection of plasticisers is needed, so that potentially toxic substances are not used. Whereas polysaccharides were used primarily to change the degradation properties of the PHB-PHV copolymer series, other polymers could be used as polymeric plasticisers to change the physical properties. One such potentially interesting polymer is the polyester polycaprolactone, (PCL). PCL has proved to be a unique polymer over recent years, enjoying wide exploitation of its unique ability to blend with many polymers over wide composition ranges [91, 184].

PCL is a crystalline, tough and flexible polymer[91] and because of its low glass transition temperature (T_g = - 60 to - 70°C), and ability to increase molecular mobility of the polymer chains, PCL resin has been used as a polymeric plasticiser[185]. PCL is difficult to melt process by itself, because of its' low melting temperature (Tm = 63°C). However, coupled by its low melting temperature, ease of blending and added advantage of being biodegradable[92-94], blends of PCL with the PHB-PHV copolymers with interesting properties could be produced. These materials could be of great interest for various medical applications. Although various copolymers of ε-caprolactone with DL-lactide and glycolide have been described[96-101], physical blends of PCL and the PHB-PHV copolymer series have not been reported.

An initial feasibility study at Aston, on PHB-PHV/PCL blends made by solvent casting techniques[186], suggested that these blends had very interesting properties. The scope and potential of these PHB-PHV/PCL blends made by melt blending techniques was investigated. The physical and degradative properties of injection moulded samples of these blends were followed by using the methods described in Chapter 2 and the results are discussed in this chapter.

5.2 RESULTS AND DISCUSSION.

5.2.1 PHYSICAL PROPERTIES.

Tensile tests on mini-dumb bell injection moulded test pieces of the PHB-PHV/PCL blends were limited to loadings of 10% and 90% w/w PCL in the blend, using 12% PHV (Mw = 350 x10^3) and 20% PHV (Mw = 300 x10^3) PHB-PHV copolymers, (nucleated with 1% w/w hydroxyapatite). Various physical parameters derived from the tensile tests of these blends (at room temperature), are tabulated in Table 5.1.

The effect on mechanical properties produced by the introduction of a small amount of PCL into the matrix of PHB-PHV copolymers of differing valerate content is very similar. For both the 12 and 20% PHV copolymers, the addition of 10% PCL reduces both the yield strength and initial tensile modulus, with a concurrent small increase in the % elongation at break, in comparison to unblended PHB-PHV copolymer.

With increasing PCL content in the PHB-PHV/PCL blends, one would expect the properties of the blend to be more influenced by the PCL component than the PHB-PHV component in the blend. Hence one would expect the properties of the blends containing 90% PCL to be similar to PCL. This is reflected in comparatively similar values for the yield strength and initial modulus of PCL and blends high in PCL. However, the ultimate tensile strength of PCL is higher than the corresponding values for the blends containing 90% PCL, which can be explained in terms of polymer crystallinity. PCL is injection moulded at ≈ 100°C above its Tm, and the sudden cooling under pressure ensures that PCL is very crystalline, as evident from x-ray diffractograms. The incorporation of 10% PHB-PHV copolymer into the PCL matrix decreases the crystallinity marginally, because

of the vast differences in the Tg and Tm of the two components. The PHB-PHV copolymers crystallise out of the melt first, at temperatures that are above the Tm of PCL.

Table 5.1 Physical properties of PHB-PHV/PCL blends (Injection moulded).

Blend	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
12% PHV	28.7±1.8	12±2	500±30	27.1±1.8	15±2
20% PHV	19.1±0.2	13±2	310±10	17.3±0.9	22±2
PCL	16.3±1.2	18±6	150±20	20.1±1.2	400±60
12% PHV/PCL 90/10	19.9±0.1	13±2	300±30	19.9±0.1	13±2
12% PHV/PCL 10/90	15±0.8	43±5	150±10	15±0.8	43±5
20% PHV/PCL 90/10	18±1.2	18±4	300±40	19.2±0.7	400±50
20% PHV/PCL 10/90	15.3±0.3	31±2	150±10	15.3±0.3	31±2

Where YS = yield strength (MPa), % EY = % elongation at yield, ITM = initial tensile modulus (MPa), UTM = ultimate tensile modulus (MPa) and % EB = % elongation at break.

SEM's of the 90% PCL loaded blends show discrete phases, so it is probable that the blends high in PCL are incompatible, in both the technological and thermodynamic terms. Figures 5.1 and 5.2 illustrate the X-ray diffractograms of PCL and various blends with the PHB-PHV copolymers. PCL, along with the 12% PHV/PCL 10/90 and 20% PHV/PCL 10/90 blends is very crystalline, (90%, 88% and 86%, respectively), whilst the 12% PHV/PCL 90/10 and the 20% PHV/PCL 90/10 blends are less crystalline, (70% and 68% crystallinities, respectively), (Table 5.2). The effect of the increasing

hydroxyvalerate content, on the % crystallinity is also apparent from Table 5.2. This is in align with the results presented in Chapter 4, which show that increase in the valerate content reduces crystallinity of the PHB-PHV copolymers.

The role played by PCL in the low PCL content blends, (i.e. the 12% PHV/PCL 90/10 and 20% PHV/PCL 90/10 blends), certainly suggests one of a polymeric plasticiser. Both the initial tensile modulus and the yield strength is decreased, with a corresponding slight increase in the % elongation at yield and at break for the blends, in comparison to the unblended copolymers. The x-ray diffractograms for these blends, (Figures 5.1 and 5.2), indicate that these blends are less crystalline than the unblended copolymers.

This fact is again substantiated by SEM studies, which shows no phase separation between the two components. It is probable that some degree of compatibility has been introduced into these blends containing 10 % PCL. The evidence that certain compositions of the PHB-PHV/PCL blends, (most probably the 12% PHV/PCL 90/10 and the 20% PHV/PCL 90/10 blends), are indeed thermodynamically compatible to a certain degree, is also substantiated by optical photographs of solvent-cast films of these blends(186). Films of up to 50% PCL in the PHB-PHV matrix were clear and with increasing PCL content, the opacity of the films increased. Although films made from amorphous compatible polymers are generally clear, transparency by itself is not sufficient evidence for polymer compatibility. Attempts were made at determining the Tg of the blends, (in order to ascertain additional proof for compatibility), using DSC, without reasonable success. However, the combined evidence of x-ray diffractometry, optical and electron microscopy suggests that PHB-PHV/PCL blends with low PCL content are compatible.

Figure 5.1 X-ray diffraction patterns of 12% PHV/PCL blends (Injection moulded)

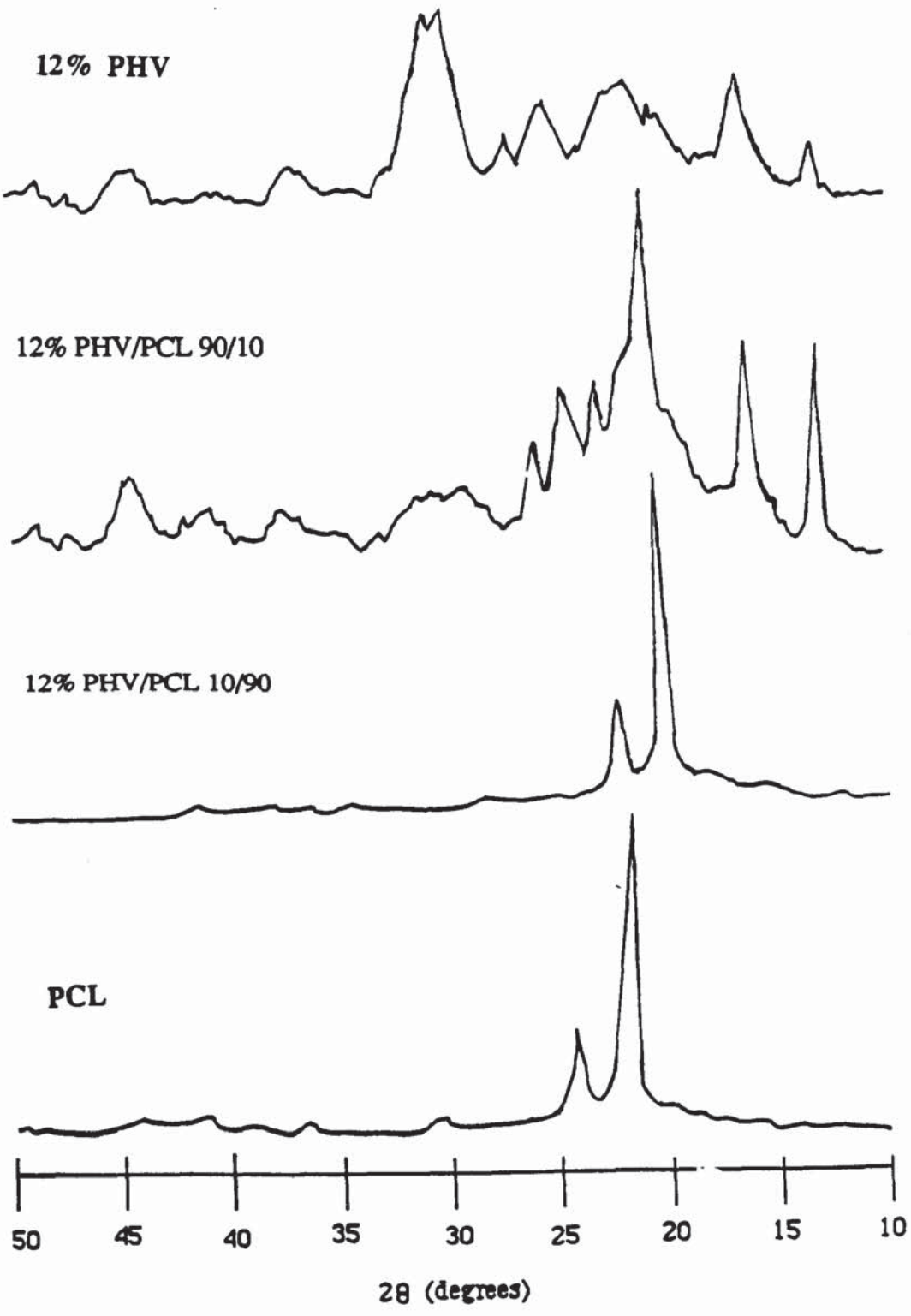


Figure 5.2 X-ray diffraction patterns of 20% PHV/PCL blends (Injection moulded)

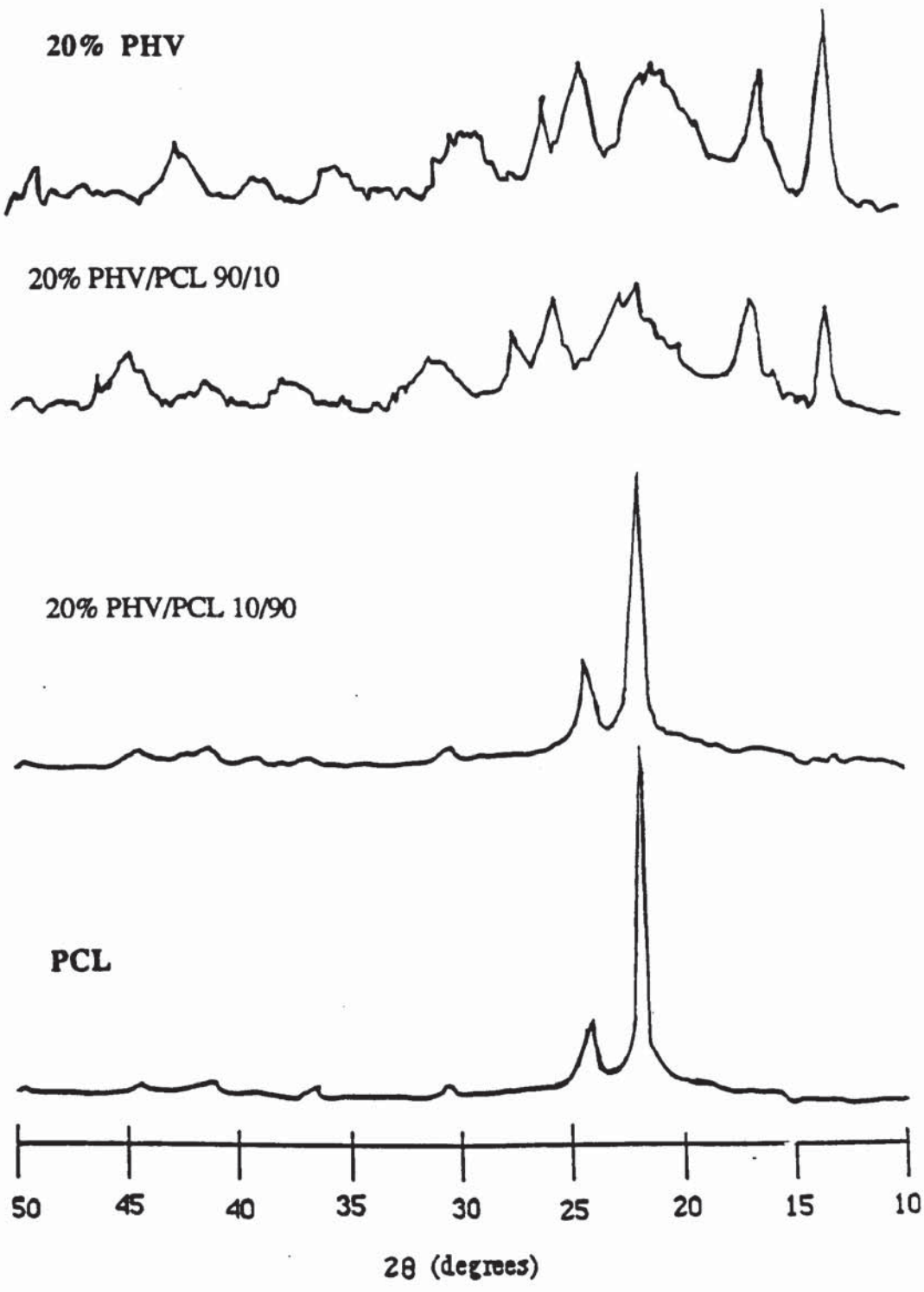


Table 5.2 % Crystallinities of PHB-PHV/ PCL blends (injection moulded).

Blend	% Crystallinity.
12% PHV (Apatite)	69 ± 5
12% PHV/PCL 90/10	70 ± 5
12% PHV/PCL 10/90	88 ± 5
PCL	90 ± 5
20% PHV/PCL 10/90	86 ± 5
20% PHV/PCL 90/10	68 ± 5
20% PHV (Apatite)	65 ± 5

Although it was possible to injection mould PHB-PHV/PCL blends, without using nucleating agents, considerable time was needed to allow the mouldings to crystallise before the mouldings could be removed from the mould. Also it was difficult to produce consistently perfect mouldings. The results in the previous chapter, concerning the hydrolytic degradation of PHB-PHV melt pressed discs, indicate the importance of the effect produced by the rate of cooling (or the degree of crystallinity) on the degradation rates of un-nucleated PHB-PHV copolymers. In an attempt to alleviate these problems and also to produce consistent injection mouldings, PHB-PHV copolymers containing 1% w/w hydroxyapatite were used to prepare the PHB-PHV/PCL blends. However, PHB-PHV/PCL blends without the nucleating agent were found to have a slightly lower initial tensile modulus and yield strength, than blends containing the nucleating agent. Unless otherwise stated, all the work presented in this chapter is based on hydroxyapatite nucleated injection moulded samples.

5.2.2 HYDROLYTIC DEGRADATION PROPERTIES.

This chapter deals with the hydrolytic degradation of the PHB-PHV/PCL blends, at 50°C in aqueous buffers of pH 2.3, 7.4 and 10.6, and at 37°C in pH 2.3 and 10.6. Hydrolytic degradation and physical changes under *in vitro* 'physiological' conditions are presented in Chapter 6.

Degradation of the injection moulded samples of the PHB-PHV/PCL blends in various buffers was monitored by gravimetric methods as described in section 2.5. The time for 10% (t_{10}), dry weight loss for these blends at 50°C in pH 2.3, 7.4 and 10.6 buffer is shown in Table 5.3.

The weight loss profiles at 50°C for these blends is characterised by a initial slow rate, followed by a secondary enhanced phase. Due to the low Tm of PCL, 50°C was chosen as the upper limit for accelerated degradation of these blends. The effect of temperature on degradation of the PHB-PHV copolymers is also apparent when comparisons are made with the t_{10} values presented for the 12 and 20% PHV copolymers in Table 5.3 and with the results presented in the previous chapter. PCL degrades faster than either 12 or 20% PHV copolymer, probably because of its lower molecular weight, (Mw = 68,000, determined by RAPRA Technology on Aldrich PCL of Mw = 54,000).

The degradation of the PHB-PHV/PCL blends at 50°C can be generalised to be fastest under alkaline conditions and slowest under acidic conditions. Graphs 5.1 and 5.2 illustrate the weight loss profiles for 12% PHV/PCL and 20% PHV/PCL blends at 50°C in pH 10.6 buffer. The effect of pH on the degradation for one blend is shown in Graph 5.3. Degradation under alkaline conditions is about 2-3 times faster than under acidic

conditions.

A closer examination of the weight loss profiles of the PHB-PHV/PCL blends suggests the presence of a complex compositional dependence of the hydrolytic degradation of these blends. The hydrolytic stability of the 12% PHV/PCL blends in pH 2.3 and 7.4 buffer at 50°C is in the following order; 12% PHV copolymer > 12% PHV/PCL 90/10 > 12% PHV/PCL 10/90 > 12% PHV/PCL 33/67 > 12% PHV/PCL 50/50 > PCL. The 12% PHV/PCL 50/50 blend is the least stable, whilst the 12% PHV/PCL 90/10 blend is the most stable to hydrolytic degradation. A similar trend is shown for the 20% PHV/PCL blends in pH 2.3 and 7.4 buffer at 50°C. However, in pH 10.6 buffer at 50°C, the 20% PHV/PCL blends degrade faster than either PCL or the 20% PHV copolymer, with the 20% PHV/PCL 50/50 blend being the fastest and the 20% PHV/PCL 90/10 blend the slowest.

A possible explanation as to the faster degradation rate of the 50/50 composition blends, in comparison to the other compositions, is that at this particular loading the PHB-PHV and the PCL components are incompatible with each other. This incompatibility, combined with possibly a low level of crystallinity, increases the degradation rate of this particular blend. Indeed, the optical photographs of the solvent cast films[186] of this particular blend shows possible phase separation. This again indicates the complexity of the factors affecting the hydrolytic degradation mechanism involved in these series of blends.

Although the x-ray diffractograms of the 20% PHV/PCL 90/10 blend indicate that it is of a lower crystallinity than the 20% PHV/PCL 10/90 blend, the 20% PHV/PCL 90/10 blend is more stable than the 20% PHV/PCL 10/90 blend. This is possibly because the

Table 5.3 Weight loss parameter (t_{10}) for 12 and 20% PHV/PCL Blends in pH 2.3, 7.4 and 10.6 buffers at 50°C.

12% PHV/PCL Blends

Blend	pH 2.3	pH 7.4	pH 10.6
12% PHV	6% in 250 days	241	201
12% PHV/PCL 90/10	6% in 250 days	237	156
12% PHV/PCL 50/50	222	185	98
12% PHV/PCL 33/67	232	212	137
12% PHV/PCL 10/90	240	219	163
PCL	207	178	154

20% PHV/PCL Blends

Blend	pH 2.3	pH 7.4	pH 10.6
20% PHV	231	216	147
20% PHV/PCL 90/10	229	212	137
20% PHV/PCL 50/50	210	184	90
-----	---	---	---
20% PHV/PCL 10/90	250	193	106
PCL	207	178	154

role played by the differences in the crystallinities between the two blends in the hydrolytic degradation rate, is overcome by some other factor. This is the large difference between the molecular weight of PCL and the PHB-PHV copolymers. The molecular weight of the PHB-PHV copolymers is ≈ 5 times more than that of the PCL and the contribution of the high molecular weight of the PHB-PHV copolymers in comparison to the low molecular weight of PCL, overrides the relatively smaller difference in the crystallinities of the two compositions of high and low PCL content. The 12% PHV/PCL blends behave similarly. It is quite obvious that a complex set of factors are involved in the hydrolytic degradation of these PHB-PHV/PCL blends, and because more time is needed to study the factors involved, no attempt will be made at predicting a degradation mechanism for these blends.

The t_{50} times for these blends are not presented because only one blend, (20% PHV/PCL 50/50 in pH 10.6 and 50°C), underwent 50% weight loss in the time period of this study. This again illustrates the rather more stable nature of the PHB-PHV/PCL blends in comparison to the PHB-PHV/polysaccharide blends.

The weight loss profiles for the 20% PHV/PCL blends in pH 10.6 at 37°C , (Graph 5.4), are illustrated to indicate the rather slow degradation of these blends at 37°C. In pH 10.6 buffer at 37°C, weight loss is less than 5% after 250 days for the most unstable blend, whereas in pH 2.3 at the same temperature, the weight loss is less than 2%. The 12% PHV/PCL blends showed a similar trend under these conditions.

Although the study of the hydrolytic degradation of the solvent cast films of the PHB-

PHV/PCL blends at 50°C, in pH 7.4 buffer was only for a short time[186], the rate of degradation of these solvent cast films was found to be faster than the injection moulded samples over a similar time scale. This again is due to the differences in the crystallinity, (and polymer matrix compaction), induced by the two different methods of fabrication, which was apparent from the density measurements made on these samples. The density of the samples was measured by using a density gradient column prepared from carbon tetrachloride and xylene (mixed isomers), in CCl_4 : xylene ratios ranging from 17:8 (v/v) to 4:21 (v/v), giving a density range of 0.99 to 1.29 g/cm^3. The solvent cast samples were found to be less dense than the injection moulded samples.

Surface energy measurements (by contact angle measurements), on the injection moulded samples of the PHB-PHV/PCL blends indicate an decrease in the polar component of the surface energy, with increasing PCL content. This suggests that the surfaces of the PHB-PHV/PCL blends with high PCL content will be the most hydrophobic. This increased hydrophobicity will reduce the wettability of the surface, which in turn should decrease the surface degradation rate of the high PCL content blends. However, it is apparent from the weight loss data for these blends in the various buffers, that the contribution of the surface energy to the degradation rate is over ridden by the overwhelming combined effects of the lower molecular weight of the PCL and the contributions of crystallinity. Another possible important factor is that although there is a difference in the polar component in the series of PHB-PHV/PCL blends, the actual difference in the interfacial tension between water and the two polymers (i.e. PHB-PHV and PCL), is so small to have any real effect on the overall wetting of the surfaces. The contact angles, together with the calculated surface parameters for the 20% PHV/PCL blends are tabulated in Table 5.4, and illustrated graphically in Graph 5.5.

Table 5.4 Surface energy parameters for 20% PHV/PCL Blends (Injection moulded).

Parameter / Blend:	20% PHV copolymer	20% PHV/PCL 90/10 blend	20% PHV/PCL 50/50 blend	20% PHV/PCL 10/90 blend	PCL
CA water (degrees)	61	64	65	70	71
CA MeI (degrees)	35	31	27	26	25
(γ P) (mN/m)	13.4	10.9	9.7	6.9	6.4
(γ^{d}) (mN/m)	35.7	38.0	40.0	41.3	41.9
(γ^{t}) (mN/m)	49.1	48.9	49.7	48.2	48.3

where CA = average contact angle,

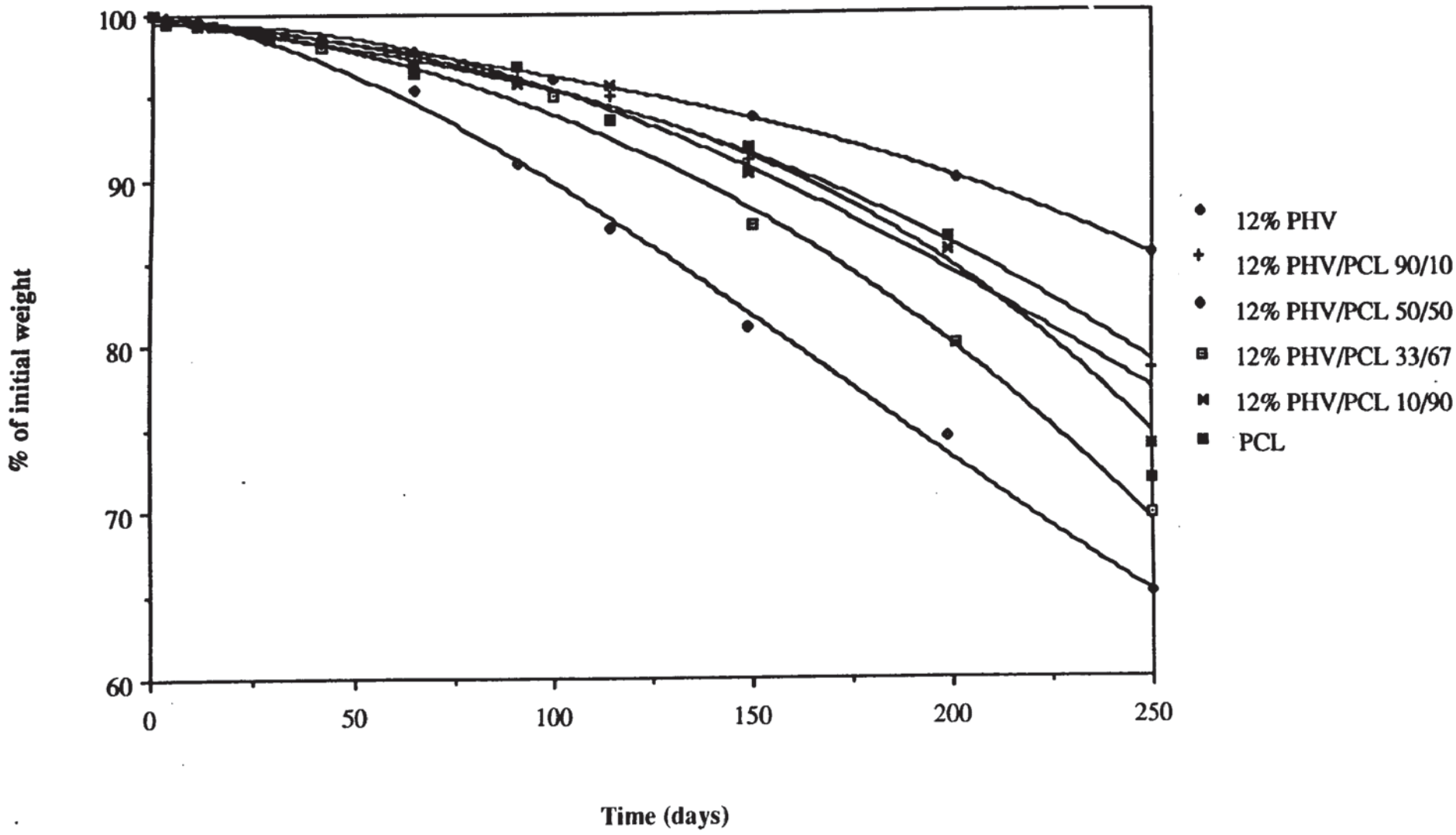
(γ P) = polar component of surface energy,

(γ^{d}) = dispersive component of surface energy,

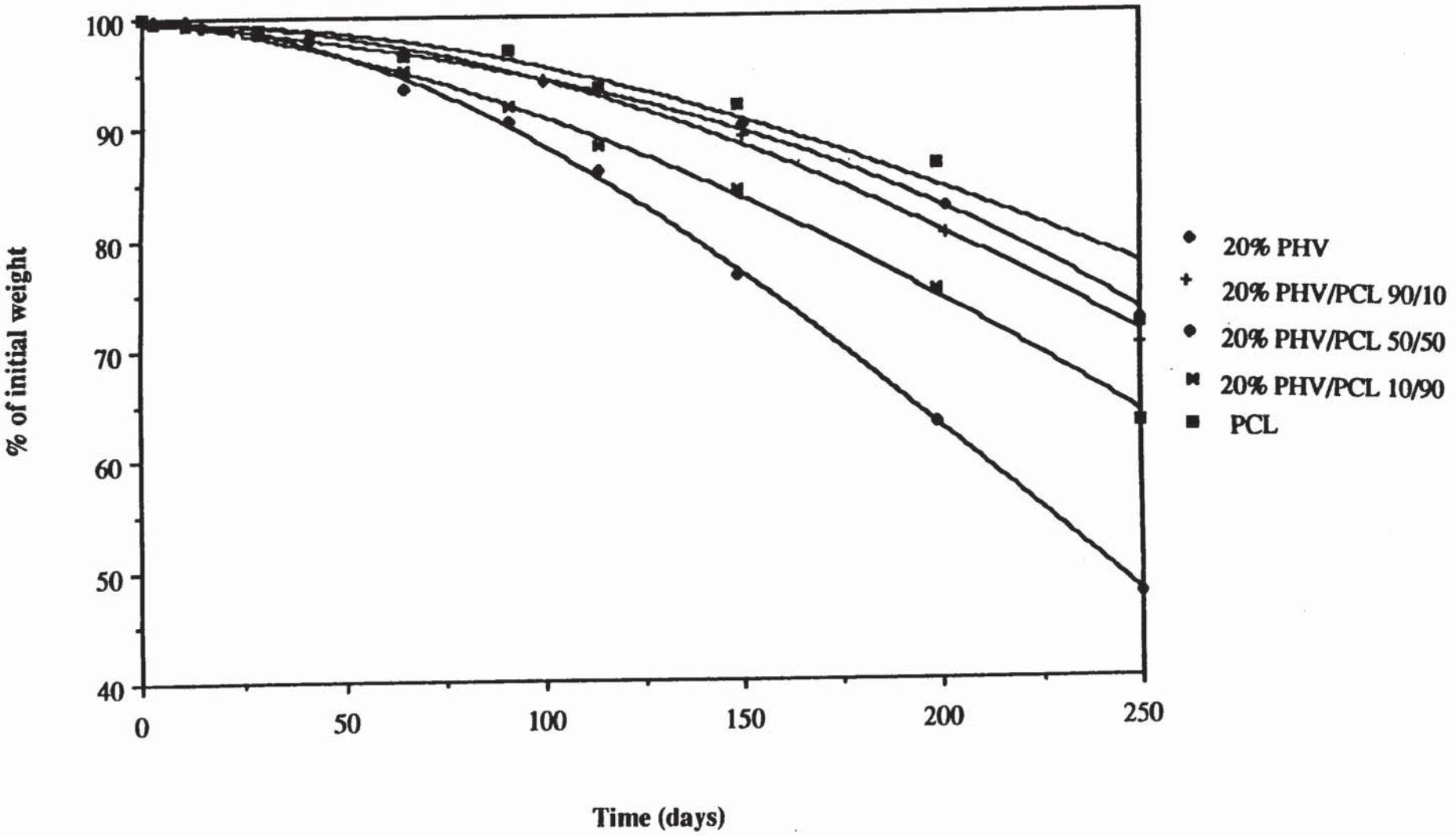
and (γ^{t}) = total surface energy.

In summary, this chapter has illustrated the production of a wide range of novel materials by melt blending two synthetic biodegradable polyesters. The materials produced have very interesting physical and degradative properties. Further work is needed to establish the complex mechanism involved in the hydrolytic degradation of these materials. However, these materials are a basis for increasing the potential and scope of materials available for various biomedical applications. This chapter again illustrates the powerful, but relatively simple and inexpensive technique that melt blending is for the production of novel materials for biomedical applications using existing polymers.

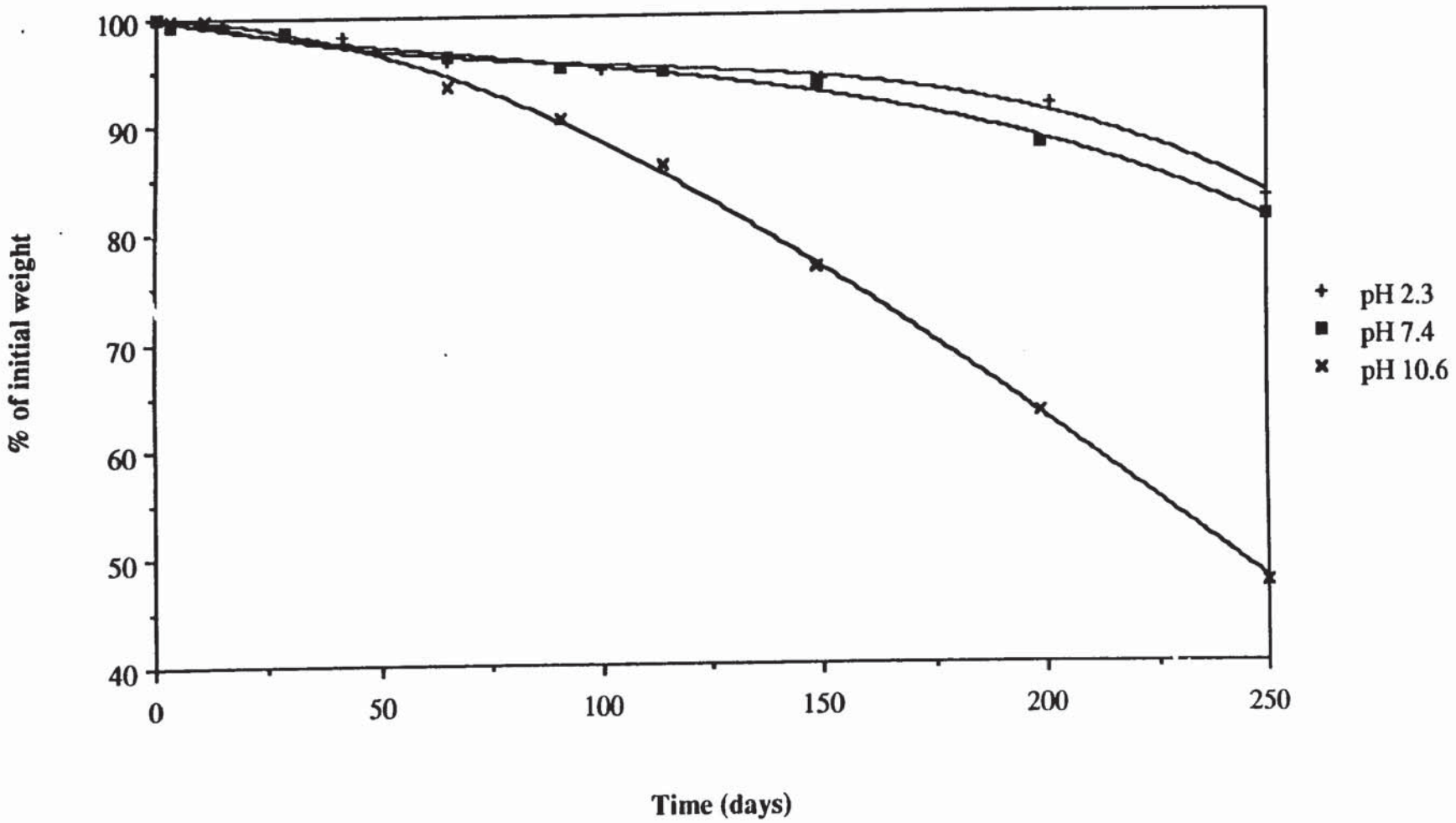
Graph 5.1 **Weight loss of 12% PHV/Polycaprolactone (PCL) blends; Injection moulded plaques degraded in pH 10.6 buffer at 50°C.**



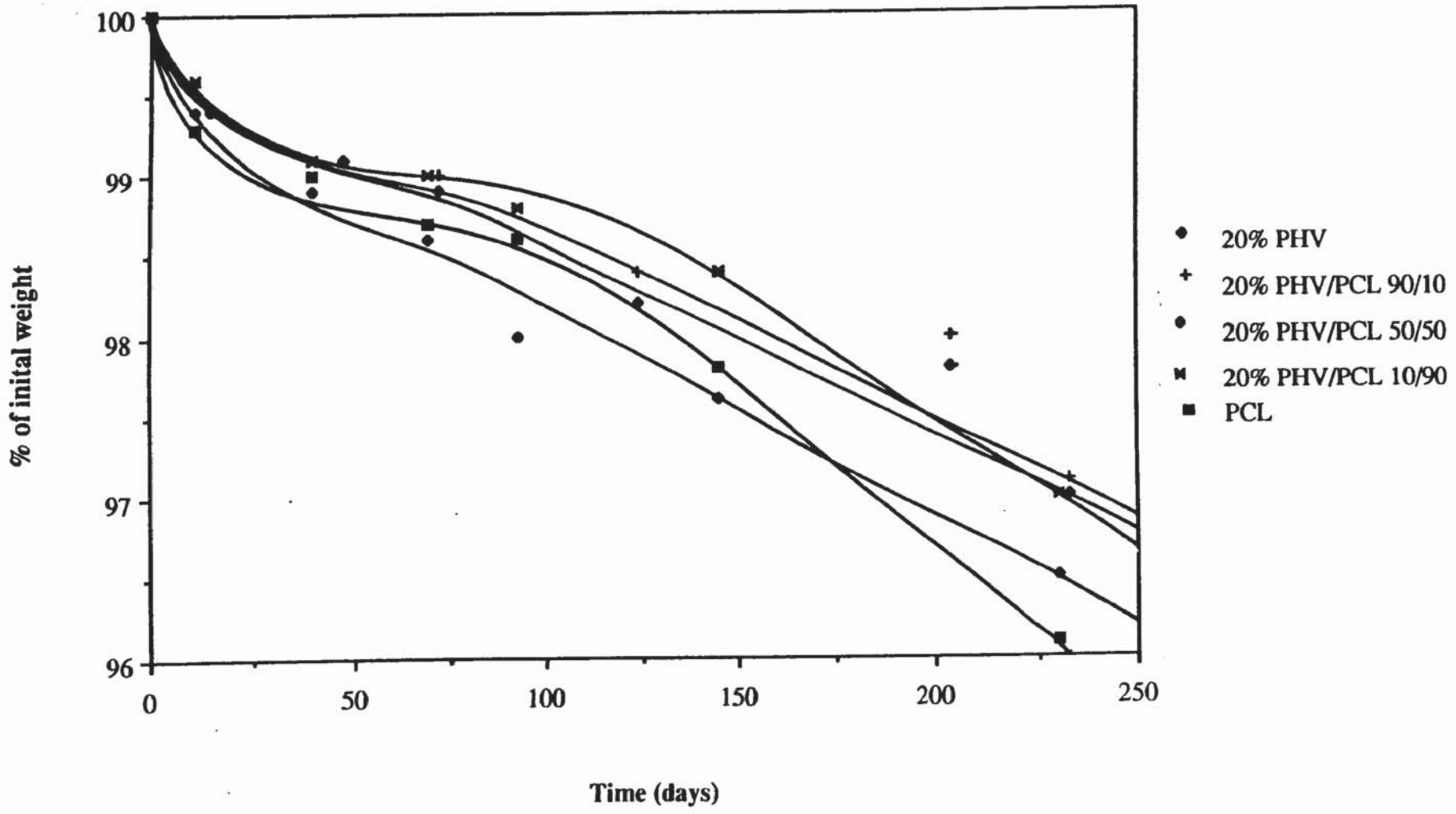
Graph 5.2 Weight loss of 20% PHV/Polycaprolactone (PCL) blends; Injection moulded plaques degraded in pH 10.6 buffer at 50°C.



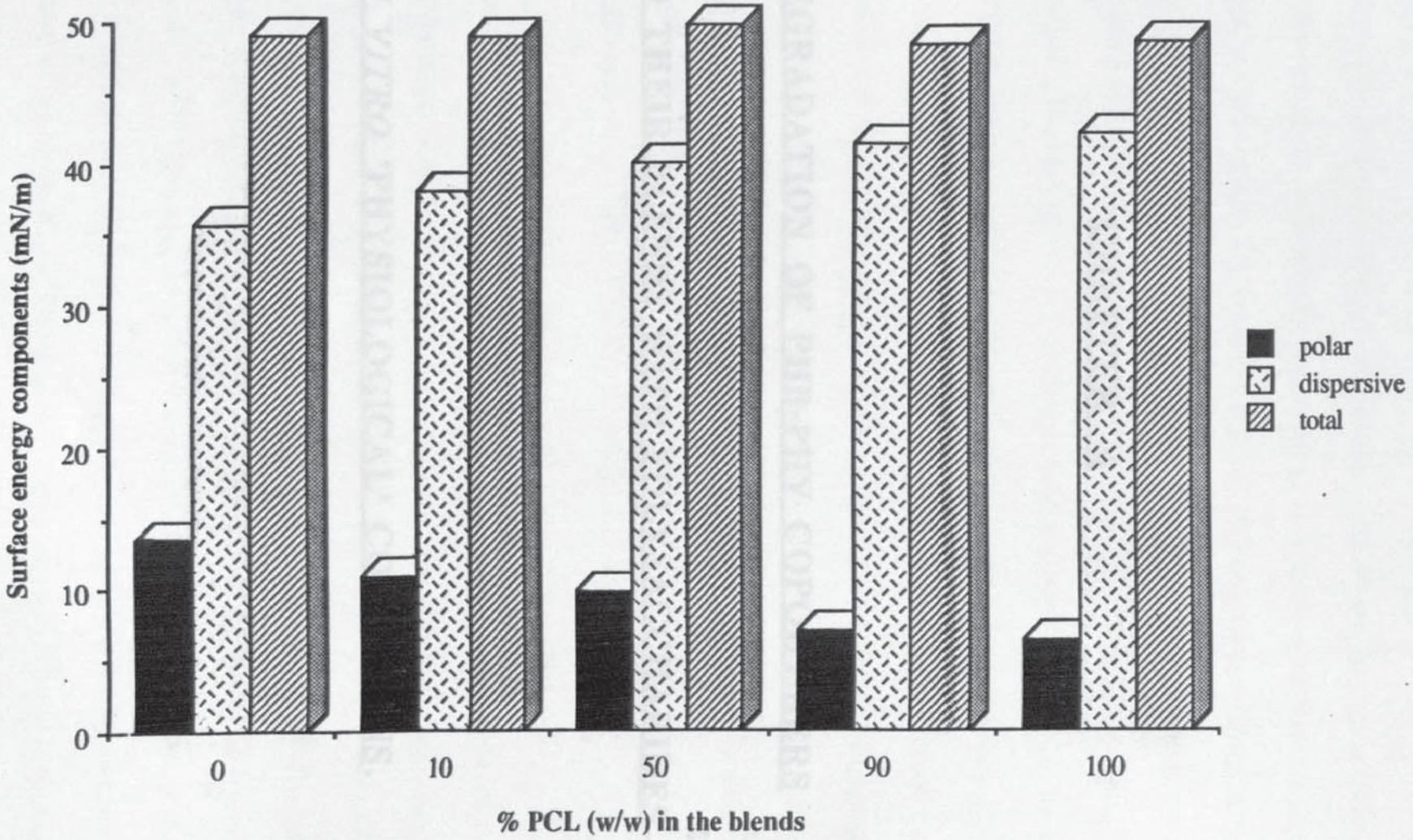
Graph 5.3 **Effect of pH on the hydrolytic degradation of 20% PHV/PCL 50/50 blend; weight loss of injection moulded plaques degraded in pH 10.6 buffer at 50°C.**



Graph 5.4 Weight loss of 20% PHV/PCL blends; Injection moulded plaques degraded in pH 10.6 buffer at 37°C.



Graph 5.5 Changes in the surface energy parameters as a function of PCL content for 20% PHV/PCL blends.



CHAPTER 6

DEGRADATION OF PHB-PHV COPOLYMERS AND THEIR BLENDS WITH POLYSACCHARIDES AND POLYCAPROLACTONE UNDER *IN VITRO* 'PHYSIOLOGICAL' CONDITIONS.

6.1 INTRODUCTION.

Although the physiological environment is very intricate and complex, *in vitro* modelling of this complicated system is frequently simplified to an aqueous system containing salt(s) of physiological strength, kept at physiological (37°C) temperature. The *in vitro* system is by no means an accurate duplication of the physiological environment, however, it does have several advantages over the *in vivo* system. These include a) easy accessibility for following polymer degradation over a period of time, b) more reproducibility of the system, (pH, salt content and temperature), c) selective control of individual conditions to be followed, and d) wider number of samples to be used for investigation. Use of the *in vitro* systems to follow degradation under physiological conditions is a good initial and fundamental step, but there is a further need to correlate the *in vitro* work with the *in vivo* work. For polymers that degrade by relatively simple hydrolytic chemical mechanisms, degradation under *in vitro* conditions has been observed to give reasonably direct correlations with degradation *in vivo* (63,129).

Recent published information on the degradation of the PHB/PHV series has failed to observe any significant extent of hydrolytic degradation under *in vivo* conditions, under the time scale of the studies. Both Williams et al(138, 187) and Doyle et al(188) deduced little or no degradation *in vivo* from studying the tensile properties, which depend predominately upon the integrity of the crystalline regions of the matrix structure. Williams et al(138, 187) have also suggested, that the hydrolytic degradation rate of the PHB-PHV copolymers decreases with increasing hydroxyvalerate content, again based on observations of changes in the tensile parameters. Other reports(189) appear to treat the PHB/PHV copolymer series as being inert but compatible. None of the work published to date, has attempted to identify the extent to which alteration of physical form

has an effect on susceptibility to degradation, and yet there is no uniformity in the forms of sample used. It is with these points in mind that a fuller analysis of surface and bulk properties of the PHB-PHV copolymers and the way in which these are modified under stimulated 'physiological' degradation conditions, (pH 7.4 and 37°C), will be given in this chapter. The hydrolytic degradation of the PHB-PHV copolymers, the PHB-PHV/polysaccharide and PHB-PHV/PCL blends under these conditions, will be discussed in this chapter, using the various techniques described in Chapter 4 to monitor changes in the surface and bulk properties. The effect of the physical form (and processing) on the subsequent hydrolytic degradation rate will also be shown.

Contributions by enzymes and specific ions on the hydrolytic degradation rate of the PHB-PHV/polysaccharide blends was also investigated using blends of amylose (enzymatic) and sodium alginate (calcium ions). The information thus obtained would be a useful indicator as to the performance of the PHB-PHV/polysaccharide blends under *in vivo* conditions.

6.2 RESULTS AND DISCUSSION.

6.2.1 PHB-PHV COPOLYMERS AND THEIR BLENDS WITH POLYSACCHARIDES.

6.2.1.1 HYDROLYTIC DEGRADATION.

One of the problems of making comparisons of degradation, whether under simulated physiological or accelerated hydrolysis conditions, is that the form of the sample affects

its subsequent behaviour. It is well known that this includes molecular weight and percentage crystallinity, a fact that has already been discussed in detail in Chapter 4. Even when some parity is achieved in these parameters, the surface to bulk ratio of each specimen has a marked effect upon the rate of degradation. This is particularly important when bulk rather than surface properties are being studied and becomes apparent when comparing the hydrolytic degradation rates of melt pressed discs and injection moulded plaques of PHB-PHV/polysaccharide blends.

The comparative changes to the wet and dry weights of melt pressed discs of 12 and 20% PHV/polysaccharide blends (10% filled), in a pH 7.4 buffer at 37°C, are shown in Graphs 6.1 to 6.4. The most important observation that can be made from these graphs is that the hydrolytic degradation period is considerably longer (approximately 18 months) than has been reported for any hydroxybutyrate based material. Since a marked deviation from the initial linearity of the degradation profile occurs at around 300 days, it is not surprising that some earlier studies[138, 187-189], based on shorter degradation periods, have concluded that polyhydroxybutyrate and its copolymers with hydroxyvalerate are relatively inert to hydrolytic degradation.

Earlier observations made in Chapter 4, relating to the effect of the introduction of polysaccharides on the degradation rate of PHB-PHV copolymers are also apparent from these graphs. The trends viewed under accelerated conditions are reflected in the results obtained from near 'physiological' *in vitro* conditions. Incorporation of 10% polysaccharide into the PHB-PHV matrix results in a dramatic increase in the degradation rate, in comparison to the unfilled polymer. The relative effects that different polysaccharides have on the degradation rate have been shown in accelerated degradation studies to be somewhat dependent on the temperature and pH selected, since these affect

the relative solubility and susceptibility to hydrolytic degradation of the various polysaccharides in differing ways. At 37°C/pH 7.4 it can be seen that amylose has one of the least disruptive effects on the polymer matrix, whereas a 10% sodium alginate loading significantly increases matrix deterioration; although being a water soluble filler a certain solubilisation would be expected to occur during the early stages of hydrolysis and in this case the most soluble polysaccharide demonstrates a significantly enhanced rate of degradation. Comparison of Graph 6.2 with Graph 6.4 again confirms that the 20% copolymer is more susceptible to hydrolysis in the presence, as in the absence, of added polysaccharide. In these two figures the degradation profile of an unfilled polymer matrix is included to facilitate comparisons. Graphs 6.1 and 6.3 illustrate the changes in the wet weight for these 10% filled blends.

Accelerated degradation conditions indicated that the hydrolysis rate was influenced by the level of polysaccharide loading introduced into a polymer blend. This is again reflected in Graphs 6.6 and 6.8 where the considerable effect of a 30% loading of the four different polysaccharides is shown. These higher loading show a not unexpected increase in matrix breakdown. This can be ascribed to the greater porosity and decrease in cohesion of the polymer matrix as polysaccharide levels increase. The relative effects of the four polysaccharide at the 10% loading are also observed at these higher 30% loadings, with the same order of polysaccharide fillers with respect to the ease of hydrolysis and consistency in the percentage contribution of the additives to the overall degradation process. The dramatic changes to the wet weight by the blends containing 30% of polysaccharide is to be expected, in comparison to the 10% filled blends. Graphs 6.5 and 6.7 illustrate the changes to the wet weight of these 30% filled blends. The initial rapid increase in the wet weights for the amylose and sodium alginate filled blends is followed by a dramatic decrease in the case of the alginate blend, whereas for the amylose

filled blend, wet weight continues to increase. This difference is in direct relation to the solubility of the two polysaccharides under these conditions. Sodium alginate is very soluble and amylose is insoluble under these conditions. The profiles for dextran and dextrin filled blends are intermediate between the two extremes displayed by amylose and sodium alginate filled blends, a trend which is in-line with the solubilities of these two polysaccharides under these conditions. Monitoring changes in the wet weight during latter stages are subject to a lesser degree of accuracy than the initial stages, due to the increase in the porosity of the samples as a direct consequence of the polysaccharide dissolution from the matrix.

The changes in the dry and wet weights of injection moulded plaques of the PHB-PHV/polysaccharide blends, (10 and 30% filled) under these conditions are shown in Graphs 6.9 to 6.16. Initial observations between the melt pressed and injection moulded samples suggests that the pattern and profiles of water (buffer) uptake and weight loss of these samples are similar. However, closer examination indicates that there are differences in the hydrolytic degradation rates of the samples prepared by different methods. The weight loss of the injection moulded samples is slightly faster than the corresponding melt pressed samples, whereas the increase in the wet weight of the melt pressed samples is higher. These differences in the gravimetric changes can be explained when consideration of the surface area/bulk ratio of the melt pressed discs and injection moulded plaques is made. During the initial stages of degradation, there is little difference between the weight loss profiles of the melt pressed and injection moulded samples, because the dry weights of the melt pressed and the injection moulded samples are very similar. However, because the surface area/bulk ratio ratio of the injection moulded samples is ≈ 7 times that of the melt pressed discs (due to the large surface area of the stepped injection moulded plaques in comparison to the melt pressed disc), during

the latter stages of degradation the difference between the decrease in the dry weight of the discs and injection moulded plaques becomes more apparent. As the initial stages of the degradation of the PHB-PHV/polysaccharide blends have been shown in the preceding chapter to be due to surface degradation, this large difference in the surface area/bulk ratio ratio between the melt pressed disc and injection moulded plaques accounts for the differences in their degradation rates. The pictorial illustrations of the hydrolytic degradation of the 12 and 20% PHV copolymers and there blends with polysaccharides shown in Chapter 4 also indicate the importance of surface area/bulk ratio in the degradation of these samples. The degradation of the injection moulded plaques initiates from the thinnest (and highest surface area/bulk ratio) to the thickest (and lowest surface area/bulk ratio) section of the stepped plaque. The t_{10} and t_{50} times for PHB-PHV/polysaccharide blends (10 and 30% filled), for both the melt pressed discs and the injection moulded plaques are illustrated in Table 6.1.

The three stages of hydrolytic degradation, which were observed under accelerated conditions (Chapter 4), are also visible under these 'physiological' conditions. The rapid decrease in the dry weight during the initial stages when the polysaccharide dissolves out of the matrix, is more pronounced for the blends with the higher polysaccharide loadings. During these early stages of degradation, the difference between the t_{10} values for the discs and the injection moulded plaques is small. However, during the latter stages of degradation, a considerable difference is apparent.

The extent of matrix compaction induced by the different processing techniques used for the fabrication of these PHB-PHV/polysaccharide samples should have an effect on the degradation rate of the blends. Hence one would expect melt pressed discs to degrade faster than injection moulded plaques, because the matrix of the latter is packed much

more densely due to the higher pressure used in the fabrication of injection moulded plaques. However, the contribution of surface area/bulk ratio to the hydrolytic degradation overrides the effects of polymer matrix compaction and this is graphically illustrated in Graph 6.17, which shows the weight loss of discs and injection moulded plaques of 20% PHV (1% talc nucleated) copolymer and 20% PHV/amylose (10%) blend degraded in pH 7.4 buffer at 37°C.

Although by the incorporation of a polysaccharide into the PHB-PHV matrix, the degradation rate of these copolymers is dramatically increased, the evidence that this is also affected by both molecular weight and valerate content is illustrated in Graph 6.18. The weight loss profiles of various copolymers of differing molecular weight and valerate content, together with the polyhydroxybutyrate homopolymer are shown for melt pressed discs degraded in pH 7.4 buffer at 37°C. Melt pressed discs were used for this comparison because compression moulding (melt pressing) of the various PHB-PHV copolymers and homopolymer, was much easier in comparison to injection moulding. This was to minimize polymer degradation during the fabrication of the samples.

The effect of different nucleating agents on the hydrolytic degradation rate of these copolymers is illustrated in Graph 6.19, which shows the weight loss profiles of injection moulded plaques of the 12% PHV copolymer, nucleated with 1% hydroxyapatite or 1% talc. Although the relative effects of hydroxyapatite and talc as nucleating agents may be thought to exert their influence primarily through a direct involvement in crystallinity levels, this may not be the complete story. Certainly, there is a slight difference in the crystallinity of the samples nucleated with talc or hydroxyapatite, as shown in Table 4.4 (Chapter 4). However, the relatively small acceleration in matrix breakdown produced by the introduction of 1% talc or 1% hydroxyapatite into the 12%

PHV copolymer, for example, and reflected in Graph 6.19 is an indication on a small scale of the much more dramatic effects produced by the polysaccharides (Graphs 6.10, 6.12, 6.14 and 6.16). With higher loadings of talc or hydroxyapatite (e.g. > 5%), when these materials act as fillers rather than simply as nucleating agents, the resulting blends (or composites), would be expected to behave similarly to the most stable of the PHB-PHV/polysaccharide blends.

The consistency of the experimental information derived from gravimetry is extremely good. Despite this, the method has several shortcomings (increasing friability of the samples at extended stages of degradation), and even after one and a half years degradation at 37°C and pH 7.4, only a 5% weight loss has been observed for injection moulded samples of the more stable PHB-PHV matrices. It is quite clear that polymer matrix changes are occurring within this time period that are not seen in the weight loss data and two further sources of information magnify these changes.

Techniques used in Chapter 4 have illustrated that the relative physical and chemical contributions to the degradation mechanism produce relatively larger alterations in the surface compared to the bulk of the matrix. It is therefore, particularly important that changes in the surface properties during the early stages of degradation are monitored. A second consideration is that modification to the bulk properties that occur concurrently (but not necessarily) in parallel with weight loss are capable of providing important information relating to the degradation process. Hence, it is appropriate to attempt to gather information relating to changes in molecular weight, crystallinity and mechanical properties. Thus although mass-erosion (weight loss) indicates the progress of the degradative process, practical applications for these materials do not involve complete weight loss over a short period but relatively slow degradation e.g. fixation devices. It is

however, important to accelerate and magnify early stages of matrix breakdown and this can be achieved by monitoring changes in the polymer surface. As already indicated in Chapter 4, the calculation of the surface energy by sessile drop contact angle measurements is one highly sensitive method of following these changes. An increasing value of the polar component of surface energy indicates a growing concentration of hydrophilic groups at the polymer surface - presumably hydroxyl in the case of matrix hydrolysis. Graph 6.20 shows the changes in the surface energy components with degradation time under physiological conditions for injection moulded 12 and 20% PHV (apatite nucleated) samples. Changes in the polar component of surface energy for 10% filled polysaccharide blends with 12 and 20% PHV copolymers are illustrated in Graphs 6.21 and 6.22, respectively. Again a significant increase in the polar component is seen over 180 days for all the blends, in comparison to the un-filled copolymers. The dramatic increase in the polar component for the amylose-based blends is in marked contrast with the weight loss for these being less than 2% over the same period.

The other excellent technique for measuring the changes occurring on a polymer surface was found to be goniophotometry, which measures the intensity of a reflected light source off the matrix surface at varying angles of incidence. The resultant profile very accurately describes changes in the surface features. Figures 6.1 to 6.4 show the changing profiles of reflectance intensity against viewing angle for injection moulded 12 and 20% PHV copolymers (apatite nucleated) and blends with amylose (10%). The various profiles at different intervals of time are overlaid. The maximum reflectance (I_S - the specular reflectance) decreases as the magnitude of the surface imperfections increase, together with a broadening of the peak shape. These changes are evident in these figures even after relatively short hydrolysis times. The slight shift in the goniophotometric curves after various periods of degradation for each sample are due to difficulties in

overcoming reproducible sample mounting. The gloss factor, derived from the goniophotometric profiles can be used for accurately indicating a slight alteration of the matrix surface after a short time span. Graph 6.23 shows the changes in the gloss factor for the 12% PHV copolymer and its blends with amylose, dextran and dextrin (all 10% filled). Graph 6.24 illustrates the corresponding changes in the gloss factor for the 20% PHV copolymer and its blends with the polysaccharides. It is evident from the profiles in these graphs that dramatic changes in the surface gloss are occurring at low levels of weight loss.

Bulk properties were monitored during the hydrolytic degradation of the specimens by the study of tensile parameters. Tables 6.2 and 6.3 summarise the various tensile properties of injection moulded samples against degradation time under *in vitro* physiological conditions. The relatively small reduction in tensile strength over the time scale of the study demonstrates that although considerable changes can be observed using surface based measurements, the bulk properties of the matrix, as yet, are unaffected.

With exposure to pH 7.4 buffer at 37°C, there is no dramatic change in the tensile strength of the un-filled copolymers and their blends with polysaccharides over a period of 250 days. However, there is a small change in the yield strength and initial tensile modulus for some of the blends during the first 60 days, and thereafter, a more progressive decrease is observed. For the 20% PHV/dextrin (10%) blend, the % elongation at break drops quite markedly over the period of 0 to 250 days.

Although it is important to obtain tensile property data, this information is a poor indicator of the degradation process, and for crystalline polymers of this type the mechanical properties are dependent on crystallinity and molecular weight, whereas the

degradation processes in the initial stages are more concentrated in the amorphous regions and surface layers of the matrix. It is quite clear in this context that the mechanical strength of polyhydroxybutyrate and its copolymers is susceptible to fabrication techniques and changes in crystallinity and morphology, as already shown in Chapter 4. The samples examined here were prepared under conditions not designed to produce optimum degrees of crystallinity but rather of samples in which the amorphous regions were capable of contributing a reasonable weighting to the rate of hydrolytic degradation.

Changes in the molecular weight of the specimens were monitored using Gel Permeation Chromatography (GPC). Inherent limitations of the gel permeation chromatographic technique and additional solubility problems, caused by handling the polysaccharide blends, indicate that these results are not as reliable as those obtained by other techniques. The weight average molecular weights (Mw) were, however, reproducible and provide a good basis for understanding the progression of the degradation process.

The molecular weight of the various processed samples was always examined before hydrolytic degradation was undertaken. The changes in Mw for injection moulded samples of the 12 and 20% PHV copolymers (apatite nucleated samples) are illustrated in the form of a histogram in Graph 6.25. A steady decrease in the molecular weight during the early stages (i.e. first 100 days) of the degradation process under *in vitro* 'physiological' conditions occurs and this trend appears to continue up to approximately 250 days, shortly after which a more dramatic decrease in molecular weight occurs. The most striking feature of the molecular weight profiles is that the point of dramatic decrease corresponds to the point where there is a change in the weight loss profiles. Table 6.4 shows the changes in the Mw for the injection moulded plaques of both talc and apatite nucleated 12 and 20% PHV copolymers. These results indicate that the

copolymers nucleated with talc are more resistant to hydrolytic degradation than those nucleated with apatite. The decrease in the molecular weight with degradation for the 12% PHV blends with dextrin and sodium alginate (both 10% filled, injection moulded plaques), is also shown in Table 6.4. The dramatic changes in the molecular weight are reached more rapidly for the polysaccharide blends at a point that is remarkably coincident with the marked change in tensile properties for these blends. The solubility of the polysaccharide also has a marked influence on the decrease in the molecular weight, as shown in Table 6.4. Comparing the dextrin and sodium alginate blends with the un-filled 12% PHV copolymer, a more dramatic decrease in the molecular weight of the PHB-PHV matrix is observed for the blends with the more soluble (sodium alginate) polysaccharide. This is in align with the weight loss profiles which indicate a faster degradation of the sodium alginate blend in comparison to the dextrin blend. The effect of the more soluble polysaccharide on the molecular weight is that as the polysaccharide dissolves out of the polymer matrix, a hallow porous structure is formed. This enables the removal of low molecular weight species of the PHB-PHV copolymer degradation products. Increasing the loading of the more soluble polysaccharide (sodium alginate) to 30%, results in a faster decrease in the dry weight (i.e. degradation rate), with a concurrent decrease in the molecular weight. The molecular weight (Mw) of the 12% PHV/sodium alginate (30%) blend falls from an initial value of 255 $x10^3$ to 9.1 $x10^3$ after 177 days. The corresponding decrease in dry weight is ≈ 40%. Increasing the loading to 30% of an sparingly soluble polysaccharide (amylose), has the same effect on the molecular weight that 10% loading of a slightly more soluble polysaccharide (dextrin) has; for example, the Mw for the 20% PHV/amylose (30%) blend (injection moulded plaque), decreased from an initial value of 251 $x10^3$ to 26.5 $x10^3$ after 429 days.

The changes in the molecular weight of the melt pressed discs of the 12 and 20% PHV

copolymers, illustrated in Table 6.5, again provides evidence that a dramatic change in the bulk properties of the copolymers occurs after ≈ 250 - 300 days degradation in pH 7.4 buffer at 37°C. This coincides with the change in the weight loss profiles. The contributions of surface area/bulk ratio on the degradation rate is also apparent from the Mw values of the melt pressed discs and injection moulded samples of the 12 and 20% PHV copolymers. The decrease in the Mw values for the injection moulded plaques is slightly more than the melt pressed discs of the two copolymers, over the same time period.

It is exceedingly difficult to derive accurate values for the number average molecular weight (Mn) from GPC data, especially as the degradation process proceeds. Values for Mw/Mn (the polydispersity index) in the initial polymer as supplied depend, to a large extent, upon the suppliers sample preparation technique, but are typically in the region of 2.5 to 3.5, and it is a feature of the degradation process that the residual polymer does not show any noticeable tendency to increase its polydispersity index value. This implies that the degradation process, concentrated in the amorphous regions leads fairly rapidly to extractable soluble species, leaving the residual matrix relatively unaffected.

Changes in matrix crystallinity were determined using x-ray diffractometry and are presented in Tables 6.6 and 6.7. It can be seen that the underlying increase in the crystallinity up to 250 - 300 days is due to extraction of the amorphous regions, which corresponds to ≈ 2% weight loss in the case of the unfilled copolymers. The upward trend in % crystallinity appears to halt and undergo a slow reversion between 350 - 450 days. This correlates to the change in the weight loss profiles previously described. Although there are significant errors (± 5%) in the absolute accuracy of the % crystallinity values by the method employed, this error is consistent within an series of

measurements, therefore the reproducibility of the values is relatively good. For this reason, the values shown for the various samples is believed to reflect an actual change.

Changes in matrix crystallinity with degradation for the 12 and 20% PHV copolymers, (melt pressed discs and both talc and apatite nucleated injection moulded samples), are illustrated in the x-ray diffraction traces shown in Figures 6.5 to 6.12. The crystallinity values (Tables 6.6 and 6.7 for the injection moulded plaques and melt pressed discs, respectively), increase slightly with increase in degradation time. This is further evidence that the degradation of these copolymers starts with the removal of the amorphous regions and is followed by gradual degradation of the more resistant crystalline regions.

Comparison of Figure 6.5 with 6.7, which illustrates the x-ray diffraction patterns for injection moulded plaque and tensile test samples for the 12% PHV copolymer (both apatite nucleated), respectively, shows the effect that both mould size and geometry have on the crystallinity of the samples. However, it is apparent from comparisons of the x-ray diffraction traces of the 20% PHV copolymer (Figures 6.6 and 6.8), that this difference is only evident in samples of the lower PHV copolymer. The probable explanation for this is due to the contributions of the differences in the rate of crystallisation of the copolymers, size and temperature of the moulds, molecular weight and melt viscosity of the copolymers.

The well resolved x-ray diffraction patterns for the injection moulded tensile sample of the 12% PHV copolymer, suggests that this smaller sample crystallises much faster than the larger plaque sample. With the 20% PHV copolymer, which crystallises somewhat more slowly, the contributions made by the size and geometry of the mould are

negligible. The slight changes in the x-ray diffraction patterns for the 12 and 20% PHV copolymers nucleated with either apatite (Figures 6.7 and 6.8, respectively) or talc (Figures 6.9 and 6.10, respectively), due to the nucleating agents, indicate that samples nucleated with talc are more crystalline than those nucleated with apatite. Figures 6.11 and 6.12 illustrate the changes in the x-ray diffraction traces for melt pressed discs of 12 and 20% PHV (talc nucleated) copolymers after degradation in pH 7.4 buffer at 37°C for various periods of time.

The x-ray diffraction pattern for the 12% PHV copolymer filled with 10% dextrin (injection moulded plaque, Figure 6.13), is similar to that for un-filled (apatite nucleated) 12% PHV copolymer. The crystallinity of the polysaccharide filled copolymers increases with degradation time (Figure 6.13 and Table 6.6). This increase in crystallinity is not so dramatic as one would expect, in comparison to the un-filled copolymer, because the rate of dissolution of the dextrin from the matrix under these conditions is slow. As a result, the increase in the internal porosity is not significant enough to allow faster degradation of the crystalline regions. However, in the case of a more soluble filler (e.g. sodium alginate), the faster rate of dissolution of the polysaccharide from the matrix will increase the internal porosity much quicker, allowing degradation of the crystalline regions to be performed much earlier.

Although the tensile properties of the 12 and 20% PHV copolymer and their blends with polysaccharides do not show much change over a period of 250 days under *in vitro* 'physiological' conditions, these samples do provide evidence of surface degradation processes occurring (Plates 6.1 to 6.8). In these pictorial illustrations, which show tensile pieces tested after various periods of degradation in a pH 7.4 buffer at 37°C, a undegraded test piece is shown for comparison.

6.2.1.2 ENZYMATIC DEGRADATION.

Investigation of the enzymatic degradation of the PHB-PHV/polysaccharide blends was limited to studying the effect of α-amylase on the PHB-PHV/amylose blends. The α-amylase used in this study was impure and of low activity. A 2% w/v solution of the enzyme was used to provide an enzyme solution of sufficient activity over a 24 hour period. Sodium chloride ions (10 mM) were used to activate the α-amylase.

The dramatic effect of α-amylase on the degradation of the 20% PHV/amylose (30%) blend, (injection moulded plaque), is illustrated by the weight loss profiles in Graph 6.26. For comparison, the weight loss of the same blend under *in vitro* 'physiological' conditions is also shown. Under these *in vitro* conditions, the weight loss from the blend is very slow and change in the weight loss profile is evident after some 70 days, by which time the sample has lost ≈ 5% of its weight. On the other hand, under enzymatic conditions the degradation rate is much faster, with 10% of the weight being lost after 7 days and 50% after 70 days. As the enzymatic degradation of amylose proceeds, the weak hollow polymer matrix collapses, causing increase in the weight loss. Graph 6.26 also suggests that esterases, (in this case α-amylase), possibly contribute to the degradation of the PHB-PHV copolymers under enzymatic conditions. After 100 days degradation, the un-filled 20% PHV copolymer has lost <1% of its weight under *in vitro* 'physiological' conditions, whereas under *in vitro* enzymatic conditions, 4% weight loss occurs.

Further evidence that α-amylase causes a dramatic increase in the degradation rate of the PHV/amylose blends is obtained from molecular weight determinations. The Mw value for the 20% PHV/amylose (30%) blend under enzymatic conditions falls from an initial

value of 251 $x10^3$ to 19.9 $x10^3$ after 71 days degradation. Under *in vitro* 'physiological' conditions, the molecular weight falls by the same magnitude after 429 days. For the un-filled 20% PHV copolymer, the Mw value decreases from 436 $x10^3$ to 297 $x10^3$ after 71 days under enzymatic degradation, whereas under *in vitro* 'physiological' conditions, it falls to a value of 279 $x10^3$ after 112 days.

Enzymatic degradation of the PHB-PHV copolymers has also been shown by Holland(172) and Korsatko(190). It is probable that under *in vivo* conditions, the degradation of the PHB-PHV/polysaccharide blends would occur by a combination of both hydrolytic and enzymatic mechanisms. Enzymatic degradation of amylose and dextran *in vivo* will occur by α-amylase and dextranase enzymes, respectively. In summary, degradation of the un-filled PHB-PHV copolymers *in vivo* will be predominately by hydrolytic mechanism, with possibly smaller contributions by low level esterases. However, for the PHB-PHV/polysaccharide blends, degradation by enzymatic mechanism will predominate for polysaccharides which have specific enzymes present *in vivo*, with lesser contribution from hydrolytic mechanism. For polysaccharides that do not have any specific enzymes, degradation by hydrolytic mechanism will predominate.

6.2.1.3 EFFECT OF SPECIFIC IONS.

The replacement of sodium ions by calcium ions in sodium alginate to form a insoluble calcium alginate gel, is a well known fact. The presence of free calcium ions in tissue fluids may dramatically influence the *in vivo* degradation of the PHB-PHV/sodium alginate blends. The effect of free calcium ions on the degradation of the alginate blends was investigated using a 0.1 M pH 7.4 tris buffer containing 25 mM calcium chloride.

The tris buffer was chosen for this particular experiment because with the normal pH 7.4 phosphate buffer used throughout this work, precipitation of calcium phosphate occurred as soon as calcium chloride (used as the source of calcium ions) was added to the phosphate buffer. As a result, all the free calcium ions were removed from the solution and exchange of the sodium ions by the calcium ions in sodium alginate was not possible. The concentration of the calcium ions used (25 mM), is ≈ 5 times the normal level of calcium ions present in tissue fluids.

Graph 6.27 illustrates the dramatic stabilization effect of calcium ions on the weight loss of injection moulded plaques of 12% PHV/sodium alginate (30%) blend, in comparison to the same blend degraded in pH 7.4 phosphate buffer. In the initial stages of degradation, surface bound sodium alginate is able to dissolve into the buffer, but then the degradation rate is retarded somewhat by the replacement of sodium ions by calcium ions. This is evident from the 50% weight loss in 150 days for this blend in the phosphate buffer in contrast to a 20% weight loss in tris buffer (containing 25 mM calcium chloride), over the same time period. The stabilization affect of the calcium ions on the degradation rate of the PHV/sodium alginate blends is also evident from the molecular weight studies. The Mw value for the blend degraded in phosphate buffer decreases from 255 $x10^3$ to 9.9 $x10^3$ after 177 days degradation, whereas in tris buffer, the Mw falls to 22.4 $x10^3$ after 217 days. The insoluble calcium alginate gel reduces the removal of low molecular fragments from the PHB-PHV polymer matrix. The *in vivo* degradation rate of the PHB-PHV/sodium alginate blends, therefore, could be affected by the presence of calcium ions in tissue fluids.

6.2.1.4 COMPARISON WITH COMMERCIAL BIODEGRADABLE MATERIAL.

Three commercially available biodegradable materials, polyglycolic acid, polyglycolic-co-lactic acid copolymer and poly(p-dioxanone), in the form of sutures, (Dexon, Vicryl and PDS, respectively), were used for comparative hydrolytic degradation studies with the PHB-PHV copolymers and their blends with polysaccharides. Although extensive work on the hydrolytic degradation of these commercially available materials has been reported, there is difficulty in comparing published data under *in vitro* 'physiological' conditions for these materials and with the PHB-PHV copolymers and their blends with polysaccharides. This arises not only because of the obvious differences in the physical form of the sutures and the injection moulded plaques, but also because of the experimental variations regarding the make-up of the buffers, with regards to pH and ionic strength. In order to at least standardize these two latter conditions, degradation of Dexon, Vicryl and PDS was monitored by gravimetry, in the 0.1 M pH 7.4 phosphate buffer used throughout this study.

Graph 6.28 illustrates the weight loss profiles of Dexon, Vicryl and PDS under *in vitro* 'physiological' conditions. Although these materials are all crystalline polyesters and apparently degrade by a hydrolytic process, it is difficult to make absolute comparisons between them. Relatively similar degradation rates for these three polymers are observed which is in some way a reflection of their relatively similar molecular weights (4 - 6 $x10^4$). Some specific comparison could be made between Vicryl and Dexon in that the former is less crystalline and more hydrophobic. Since these two materials are of a similar physical form (multifilament), it seems that the effect of crystallinity marginally outweighs the effect of hydrophobicity. Hence the faster degradation rate of Vicryl in comparison to Dexon. Extending the argument to include PDS, the combination of a

relatively high molecular weight and reduced concentration of ester groups in the backbone produces a somewhat smaller degradation rate. In comparing the degradation rate of the PHB-PHV copolymers and their blends with polysaccharides with this small group of commercially available suture materials, inherent difficulties arise. It is certainly impossible to make direct comparisons in a situation where crystallinity, molecular weight and physical form are similar for these group of materials.

The most appropriate comparison that can be made with commercial sutures is that under conditions where molecular weight and composition of the PHB-PHV copolymers are chosen to produce acceptable mechanical properties. For this reason, the weight loss profiles of injection moulded plaques of the 20% PHV (apatite nucleated) and the 20% PHV/sodium alginate (30%) blend are shown for comparison. The 20% PHV un-filled copolymer is less prone to degradation than the most stable (i.e. PDS) of the commercially available suture materials studied. In direct comparison, it is obvious that the 20% PHV/sodium alginate blend degrades faster than the PDS during the initial stages. During this early stage of degradation, dramatic weight loss is due to the polysaccharide dissolving out of the matrix in the case of the alginate blend, leaving a hallow porous structure which during the latter stages of degradation collapses on itself. It is during these latter stages of degradation that the hydrolytic degradation rate of PDS is much faster than the porous PHB-PHV matrix. The information presented in Graph 6.28, illustrates the tremendous potential and scope of altering the degradation rate of these slowly degrading PHB-PHV copolymers, by blending with polysaccharides, the physical form, solubility and enzymatic degradation of which can be used to control the degradation rate of the blends under *in vivo* conditions.

6.2.2 PHB-PHV/PCL BLENDS:

6.2.2.1 HYDROLYTIC DEGRADATION.

The surface and bulk properties of the PHB-PHV/PCL blends in a pH 7.4 buffer at 37°C were investigated using combined techniques of tensile strength, weight loss, goniophotometry, surface energy and molecular weight. This should enable evaluation of the physical and degradative properties under 'physiological' conditions of these blends and also indicate to the further usefulness of these blends.

The weight loss profiles for the 12 and 20% PHV PHB-PHV/PCL blends shown in Graphs 6.30 and 6.32 illustrate the rather stable nature of these blends, in comparison to the PHB-PHV/polysaccharide blends under similar conditions. The rather small change in the dry weight for these blends makes it difficult to accurately predict compositional dependence of the hydrolytic degradation rate under these conditions. The weight loss profiles shown could be regarded as the initial phases of hydrolytic degradation, during which the surface, rather than the bulk degradation processes, are predominant. This initial phase is comparable to the initial phases seen during the hydrolytic degradation of the PHB-PHV/polysaccharide blends under the same conditions.

During these early stages of hydrolytic degradation, the contributions made by high levels of PCL on the PHB-PHV/PCL blends is apparent. Blends high in PCL content are slightly less stable than those containing lower levels of PCL. The least stable blends again seem to be those containing 50% of each component.

The changes to the wet weight for these blends are shown in Graphs 6.29 and 6.31.

These graphs illustrate that these blends, in comparison to the PHB-PHV/polysaccharide blends, take up much less buffer. This is expected because the PCL in comparison to the polysaccharides can be considered to be a more hydrophobic component, and hence less likely to absorb water (buffer).

Although the molecular weight of PCL is four times less than that of the PHB-PHV copolymers, the difference in the dry weight loss between PCL and the PHB-PHV copolymers is very marginal at these early stages of degradation. Pitt et al[92, 93, 96, 98], in their studies on the *in-vitro* and *in-vivo* degradation of PCL, found that during the initial stages of degradation, weight loss was slight, but once the Mn value had decreased to 5000, significant weight loss occurred. The molecular weights (Mw) over the initial stages of degradation for these blends have decreased slightly. The Mw value for PCL decreases from 68.8 $x10^3$ to 50.3 $x10^3$ after 112 days degradation, whilst for the 12% PHV/PCL 90/10 blend, the Mw value decreases from 218 $x10^3$ to 183 $x10^3$ over the same period. Similarly, there is a decrease in the Mw value for the 20% PHV/PCL 90/10 blend from 294 $x10^3$ to 249 $x10^3$ after 112 days. This also indicates that PCL blends with the higher valerate PHB-PHV copolymers degrade faster.

However, from the small decrease in the dry weight, it is apparent that molecular weight and valerate content are not the only two factors affecting the hydrolytic degradation of these blends. It is probable that other factors also greatly influence the hydrolytic degradation of these blends. The role played by crystallinity possibly overwhelms the contributions from molecular weight. All the blends, in particular those containing high levels of PCL, were found to be very crystalline from x-ray diffraction studies. Figures 5.1 and 5.2, shown in the previous chapter, illustrate the x-ray diffraction traces for these blends. The high crystallinity of the PCL and blends high in PCL overweighs the

differences in the molecular weights of the PHB-PHV copolymers and PCL. As hydrolytic degradation of polyesters has been shown to start in amorphous regions before the more resistant crystalline regions are degraded, the slow degradation of PCL and blends high in PCL is expected.

Although during the initial stages of degradation there is relatively little weight loss, dramatic changes in the tensile properties of these blends were observed (Table 6.8). For PCL and the 12 and 20% PHV/PCL blends, there is a steady increase in both the yield strength and initial tensile modulus after 7 days immersion in pH 7.4 buffer at 37°C, which is possibly due to an increase in the crystallinity, brought about by the annealing process. The mode of fracture of the blends changes from a ductile mode to one of brittle fracture over this initial period, possibly due to an increase in crystallinity. X-ray diffraction studies on the 12 and 20% PHV/PCL (10%) blends after 7 days immersion in the buffer illustrate the increase in the crystallinity after this very short period of incubation. Figure 6.14 shows the changes in the crystallinity of the 12% PHV/PCL (10%) blend from 0 to 7 days degradation, and Figure 6.15 shows the corresponding changes for the 20% PHV/PCL (10%) blend from 0 to 22 days degradation. The increase in the crystallinity for these blends with degradation time, (Table 6.9), has profound effect on the tensile properties of these blends. The initial tensile modulus increases, with a concurrent drop in the ultimate tensile strength and % elongation at both yield and at break during increased degradation time. Pitt et al[92, 96, 98] suggest that the increase in crystallinity with incubation for PCL may be associated with chain cleavage in the amorphous phase, and crystallisation of the resulting unstrained tie segments, facilitated by the low glass transition temperature (- 60 to - 70°C) of PCL . A similar explanation may be used to account for the changes in the crystallinity of the PHB-PHV/PCL blends.

During the latter stages of degradation (i.e. after 62 days incubation), as the molecular weight decreases, there is a dramatic transition in the tensile properties. For PCL and blends high in PCL content, this occurs after 112 days incubation, whilst for blends high in PHB-PHV content, this occurs after 180 days. The changes to the physical properties of PCL and PHB-PHV/PCL blends (in particular the % elongation to break), are illustrated pictorially in Plates 6.9 to 6.13. These show a series of tensile pieces tested at various intervals of time after immersion in pH 7.4 buffer at 37°C. The dramatic changes in the % elongation to break for PCL and blends high in PCL content are clearly evident from these plates, as are the changes in the 20% PHV/PCL 90/10 blend. A undegraded tensile piece is shown in these plates for comparison.

The combined techniques of surface energy measurement and goniophotometry have been shown to be very valuable in monitoring early stages of degradation of the PHB-PHV/polysaccharide blends, where gravimetric methods are less sensitive at detecting early changes. In the case of these PHB-PHV/PCL blends which degrade much slowly than the PHB-PHV/polysaccharide blends, these surface techniques are extremely important in monitoring early stages of polymer degradation.

Graph 6.33 shows the comparative changes in the goniophotometric and surface energy parameters for PCL over a period of 180 days. With less than 2% weight loss, dramatic changes in the I_s and gloss factor occur. The I_s and gloss factor have decreased, with a concurrent increase in the I_d value over the time period of 0 to 180 days. Similarly, there is a considerable increase in the polar component and concurrent decrease in the dispersive component of surface energy over the same time period.

Graph 6.34 shows the changes in the goniophotometric and surface energy parameters

for the 20% PHV/PCL (10%) blend over a period of 180 days. Even after 22 days immersion, although weight loss is negligible, dramatic changes in the I_s and polar component of surface energy are apparent. Graph 6.35 illustrates the goniophotometric and surface energy parameters of the corresponding high PCL (90%) blend over the same time period. The increase in the surface energy is due to an increase in the hydroxyl and carboxyl groups on the surface, brought about by surface erosional processes. Comparison of Graph 6.34 with Graph 6.35 indicates that the blend high in PCL content degrades faster than the blend containing lower level (10%) of PCL. This information is consistent with the gravimetric results and the molecular weight changes observed for these blends. For the 20% PHV/PCL (10%) blend, the Mw falls from 294 $x10^3$ to 249 $x10^3$, whilst that of the 90% PCL blend decreases from 100 $x10^3$ to 55 $x10^3$ after 112 days under these 'physiological' conditions.

In conclusion, the PHB-PHV/PCL blends exhibit interesting physical and degradative properties under these *in vitro* 'physiological' conditions. Although the hydrolytic degradation rate of these blends is somewhat slower than the corresponding PHB-PHV/polysaccharide blends, these blends have a rather lower initial tensile modulus. Surgical fixation devices (e.g. clips, staples), providing support in the early stages of wound healing for the tissues, should have a certain degree of flexibility to enable proper mechanical function. Combination of PCL and polysaccharide components, at low loadings ($\approx$ 10%) with the high valerate PHB-PHV copolymers (20% PHV), should produce a blend possessing excellent physical properties with reasonable hydrolytic degradation rates. Other PHB-PHV/PCL blends could be used for the fabrication of medical devices, where neither the physical or degradative properties are important, rather the matrix porosity is important. This could be for the fabrication of drug delivery systems.

6.3 CONCLUSION.

Although gravimetric monitoring of the hydrolysis process of the PHB-PHV copolymer is slow and of limited accuracy throughout most of the experiment, an increasing rate of degradation is seen as the hydroxyvalerate content is increased. This falls in line with the findings under accelerated degradation studies shown in Chapter 4. The introduction of polysaccharides in the PHB-PHV matrix considerably increases the lability of the polymer matrices, although there is a considerable difference in degradation rates under *in vitro* 'physiological' conditions between the PHB-PHV series plus blends with polysaccharides and other commercial biodegradable materials. This is clearly illustrated in the times for 10 and 50% weight loss for some of these materials which is summarised in Table 6.10. It can be seen that PDS, the most resilient of the three commercial biodegradable polymers listed, has a t_{50} value which is twice as fast as the most labile PHB-PHV blend, (20% PHV/sodium alginate (30%) blend (injection moulded plaque)).

Surface features are good indicators for the onset of hydrolytic polymer degradation. The surface techniques do however, have limitations with surface energy data of limited use once porosity of the matrix produces an aqueous contact angle of less than 20°. Similarly, the rate of change of goniophotometric parameters is less marked after 150 days of hydrolysis under simulated 'physiological' conditions. Nevertheless, the vastly superior changes in experimental values of surface parameter values (gloss factor from goniophotometry and the polar component of the surface energy from contact angle measurements) compared to simple weight loss, have been shown to be more reliable in detecting changes during the early stages of degradation for the PHB-PHV copolymers and their blends with polysaccharides and PCL. The incorporation of PCL into the PHB-PHV copolymer matrix does not alter the hydrolytic degradation rate of the PHB-PHV

copolymers to the same degree that is achieved by the introduction of polysaccharides into the matrix.

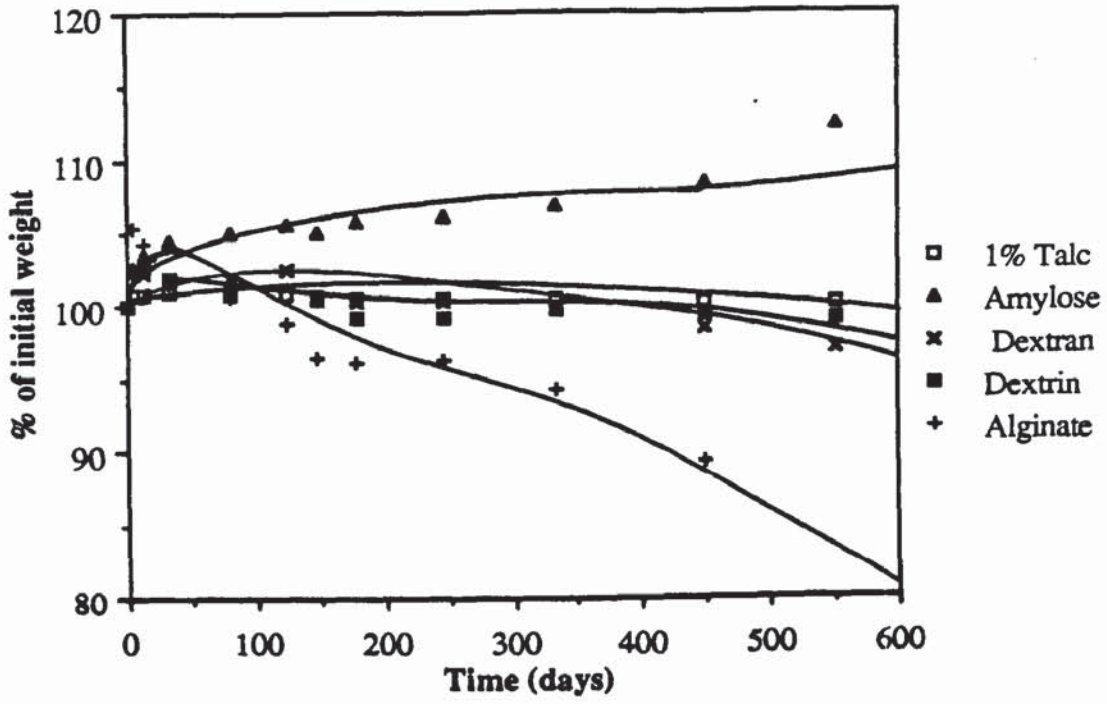
Less rapid changes occur in the bulk properties of PHB-PHV matrices such as crystallinity and tensile strength when compared to the surface measurements alone. However, some significant changes do occur, particularly at enhanced stages of experimental monitoring. Molecular weight does expectedly fall and is an improved method of analysis compared to gravimetry under the experimental conditions in this study, and although tensile strength is relatively unimportant in the determination of the hydrolytic degradation process, it does prove that both the polysaccharide and PCL filled PHB-PHV blends have inherently less mechanical strength than the unfilled copolymers.

The evidence of the collective techniques leads to a suggestion that, up to 100 days, surface properties unlock the door to understanding the effect of hydrolysis on PHB-PHV polymer matrices. At this point, the rate of change of these techniques can be considered as being at a maximum. At approximately 300 days under the simulated *in vitro* 'physiological' conditions, molecular weight and weight loss appear to be at their most efficient in highlighting developments of the hydrolysis in the bulk, and at extended degradation times (> 500 days in this study) tensile strength is of some use in determining differences between the various PHB-PHV polysaccharide blends.

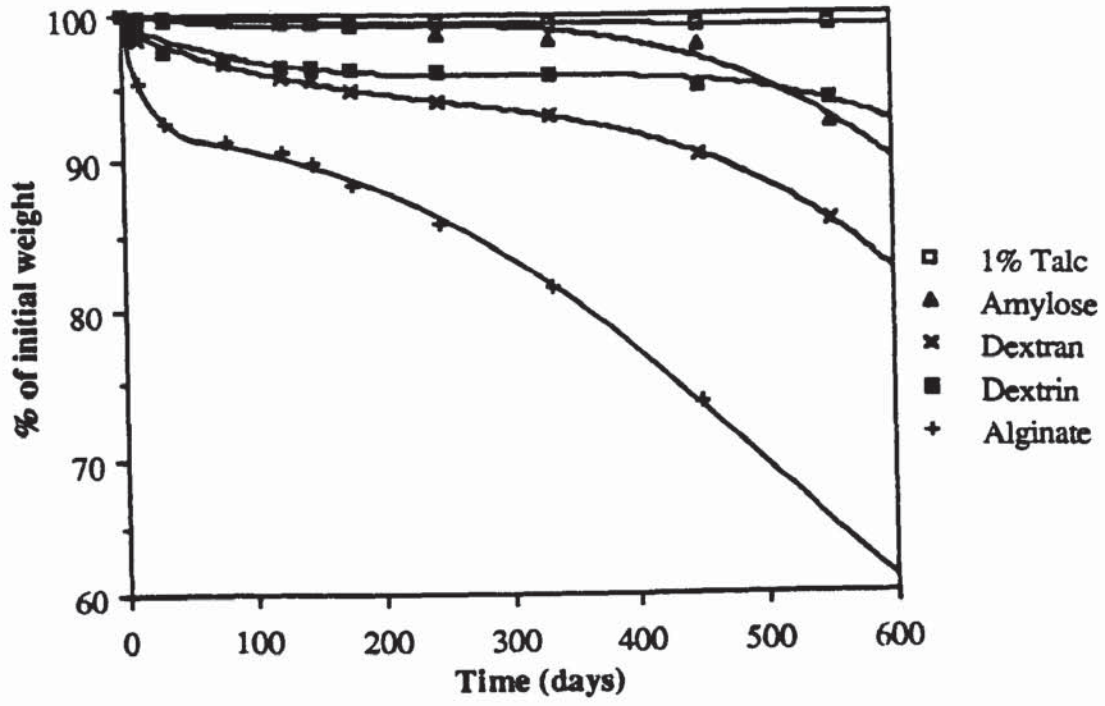
Possible enzymatic degradation of PHB-PHV/amylose blends has also been shown by the action of α-amylase. In comparison to the hydrolytic degradation, where weight loss from the amylose based blends is extremely slow, under enzymatic conditions this is dramatically increased. As a result, decrease in the molecular weight of the PHB-PHV matrix is much faster under enzymatic conditions. Esterase activity on the degradation of

the PHB-PHV copolymers is also probable.

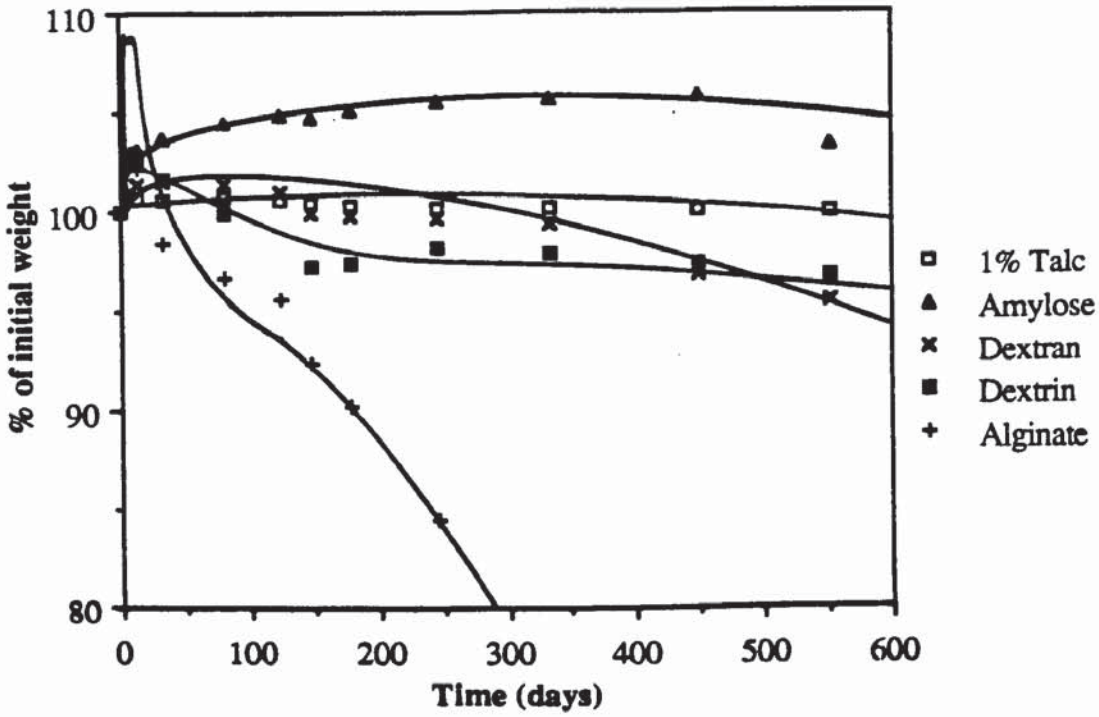
The degradation rate of the sodium alginate based blends was found to be dramatically affected in the presence of calcium ions. This suggests that under *in vivo* conditions, the degradation rate of the sodium alginate blends will possibly be affected by the calcium ions. This again increases the scope of designing materials with controlled rates of degradation for specific applications.



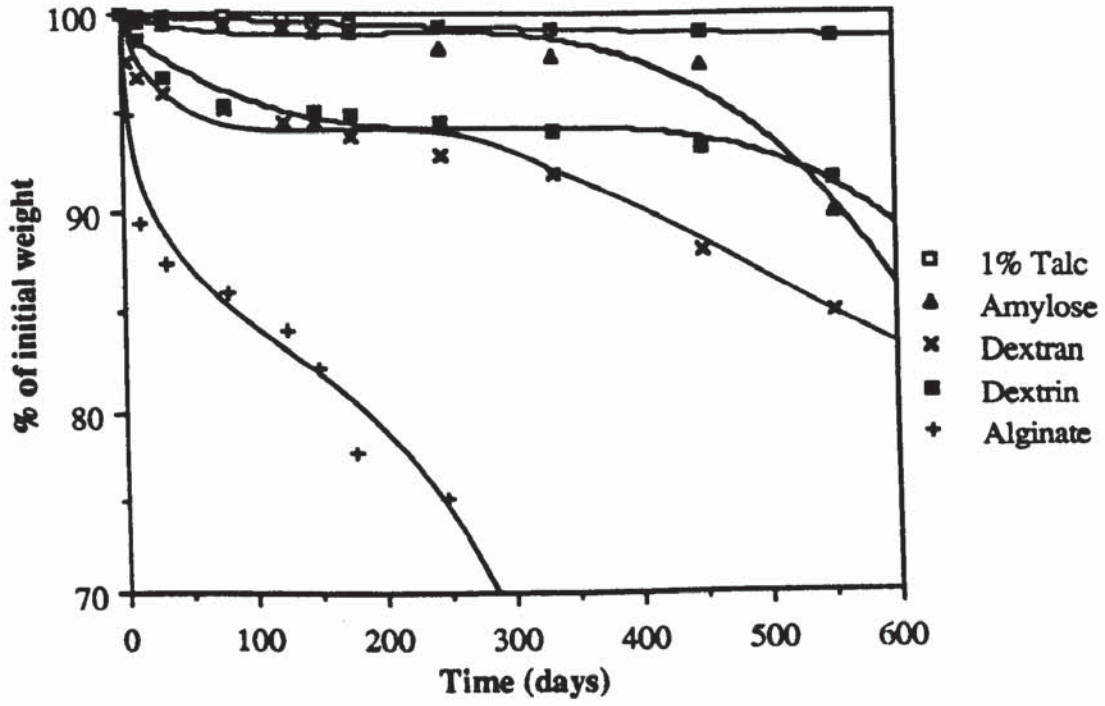
Graph 6.1 Changes in wet weight of melt pressed discs of 12% PHV/ polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



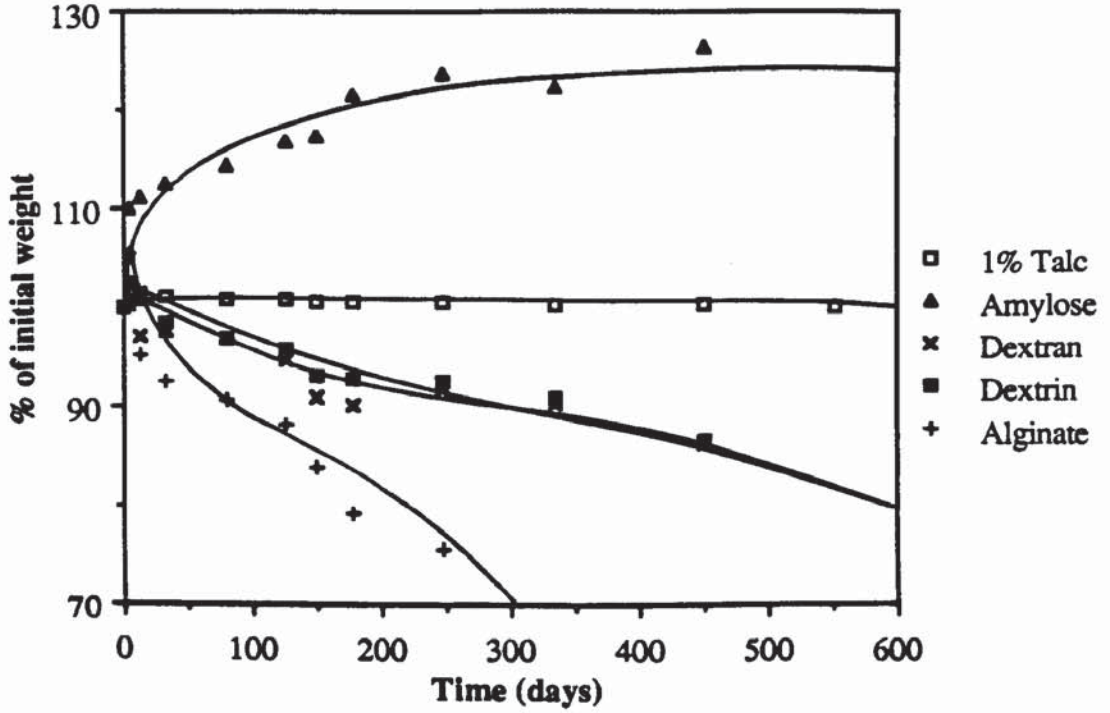
Graph 6.2 Changes in dry weight of melt pressed discs of 12% PHV/ polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



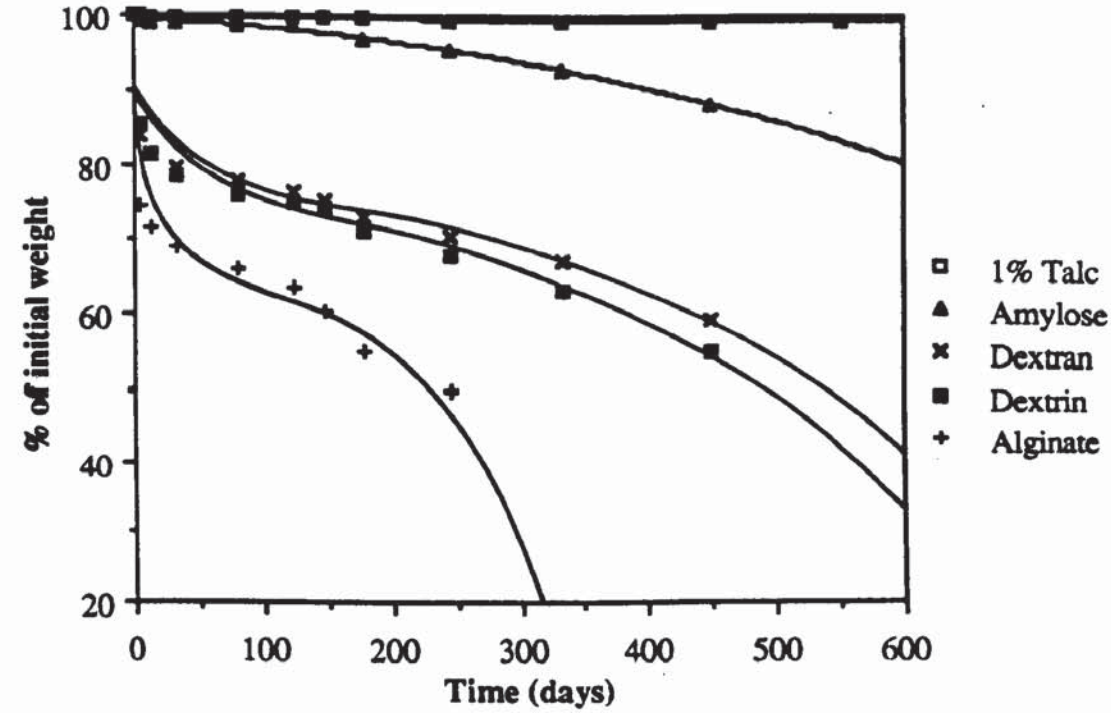
Graph 6.3 Changes in wet weight of melt pressed discs of 20% PHV/ polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



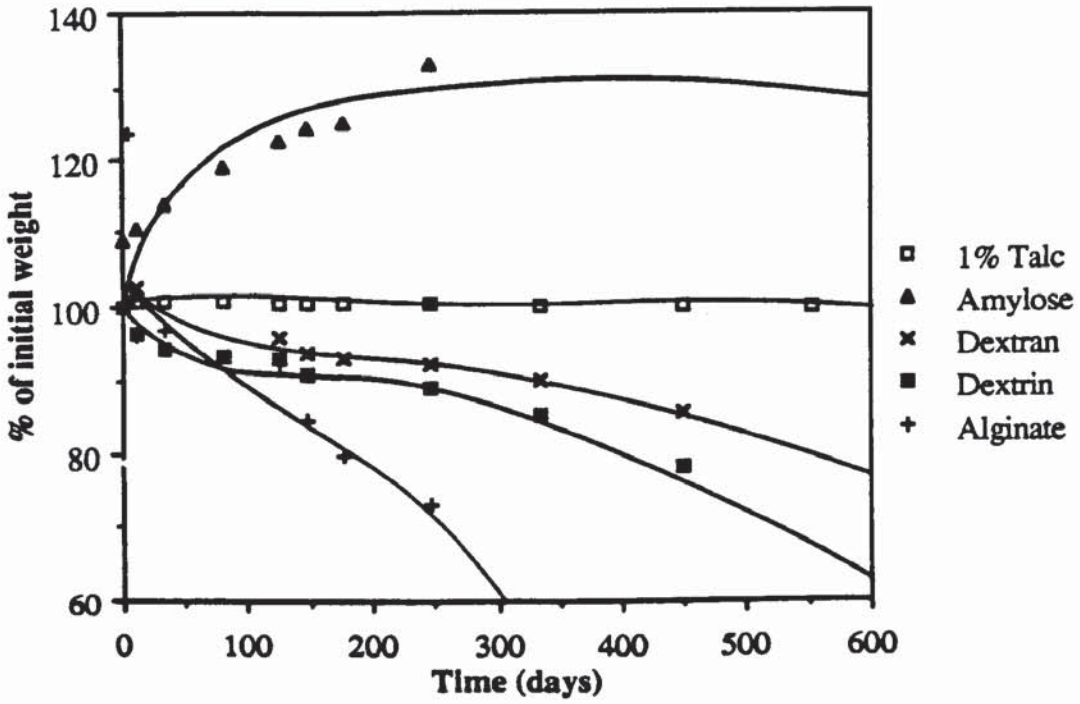
Graph 6.4 Changes in dry weight of melt pressed discs of 20% PHV/ polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



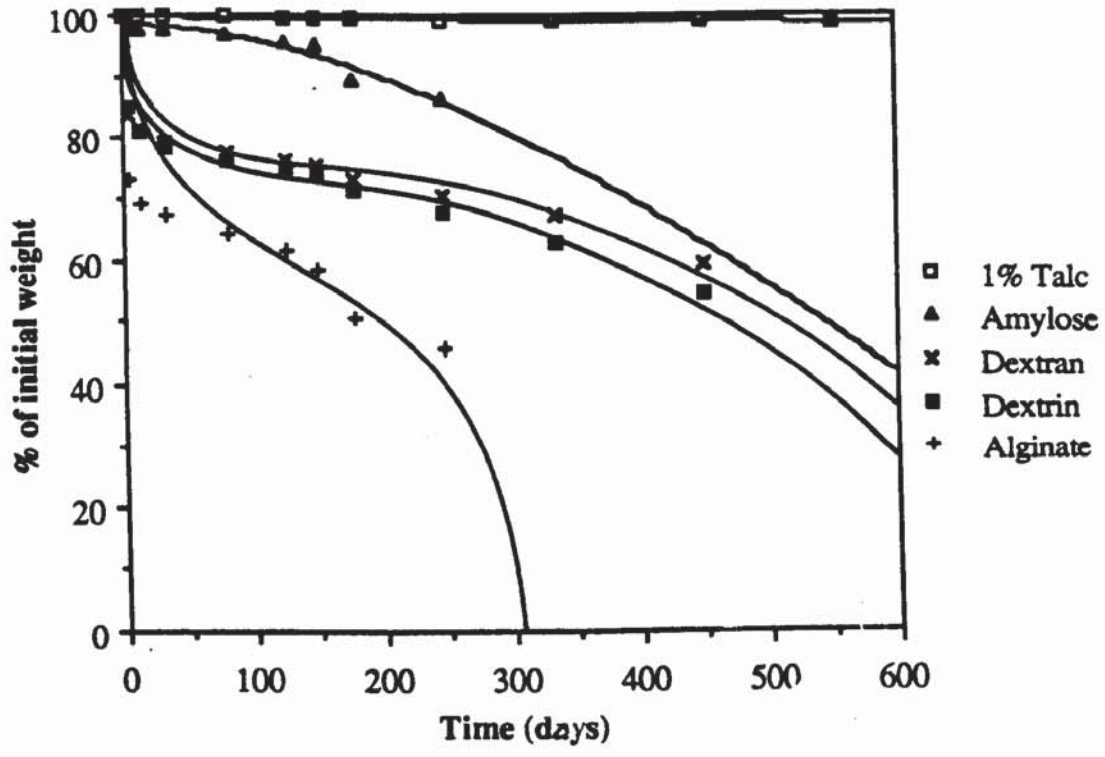
Graph 6.5 Changes in wet weight of melt pressed discs of 12% PHV/ polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



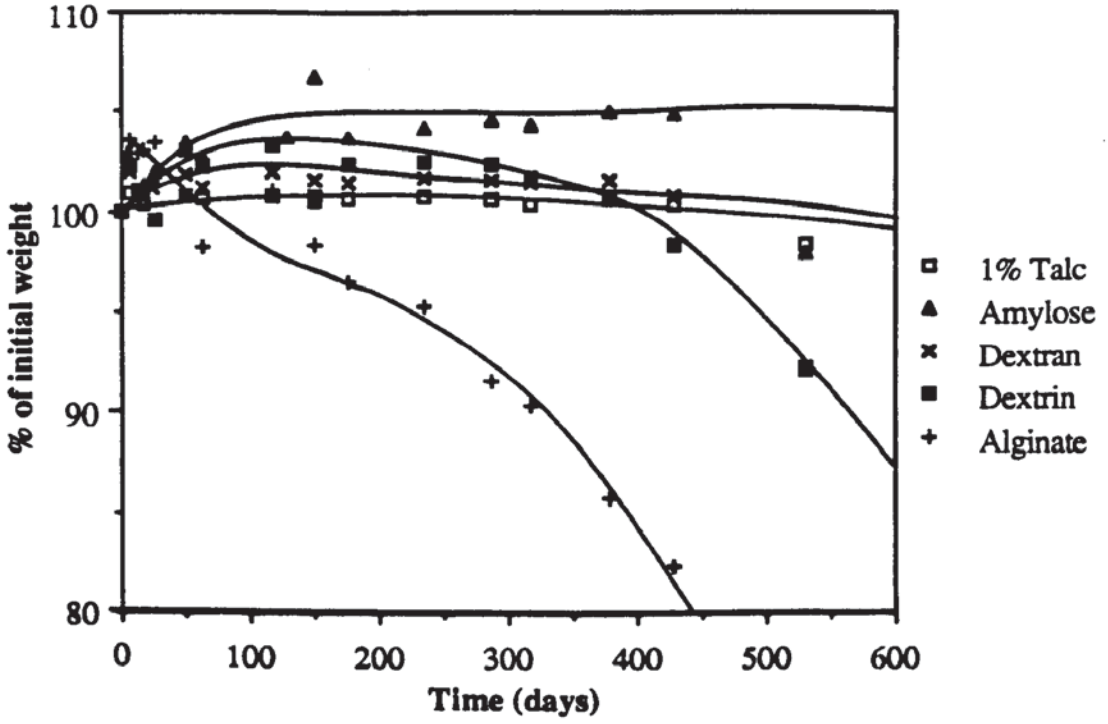
Graph 6.6 Changes in dry weight of melt pressed discs of 12% PHV/ polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



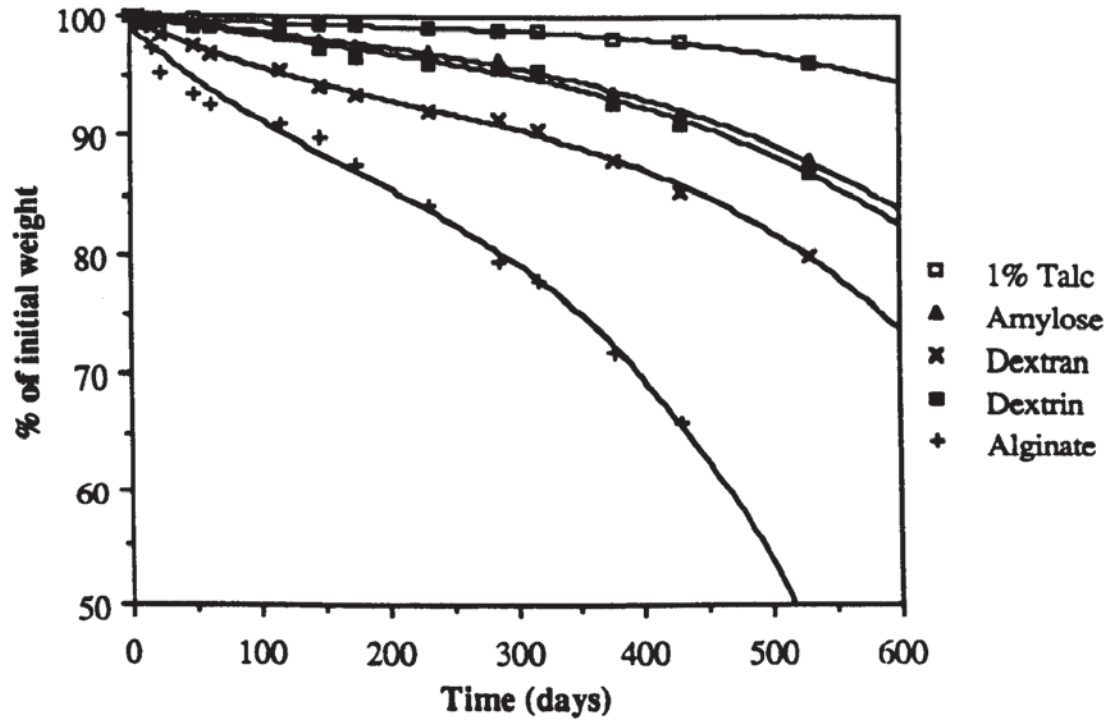
Graph 6.7 Changes in wet weight of melt pressed discs of 20% PHV/ polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



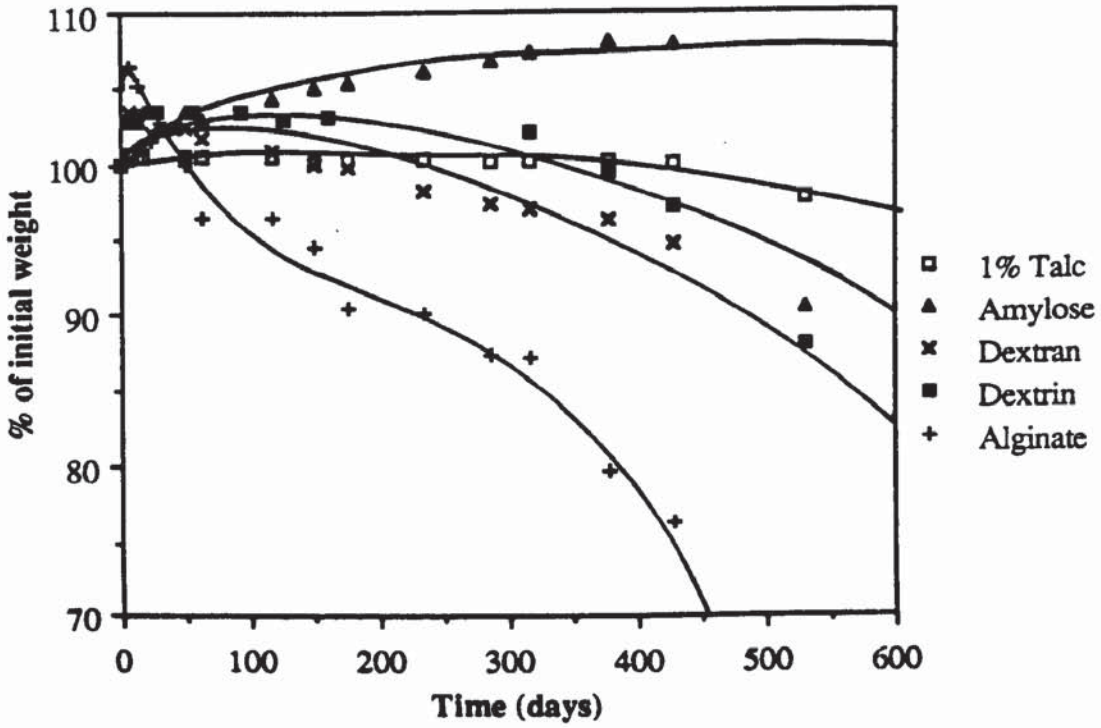
Graph 6.8 Changes in dry weight of melt pressed discs of 20% PHV/ polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



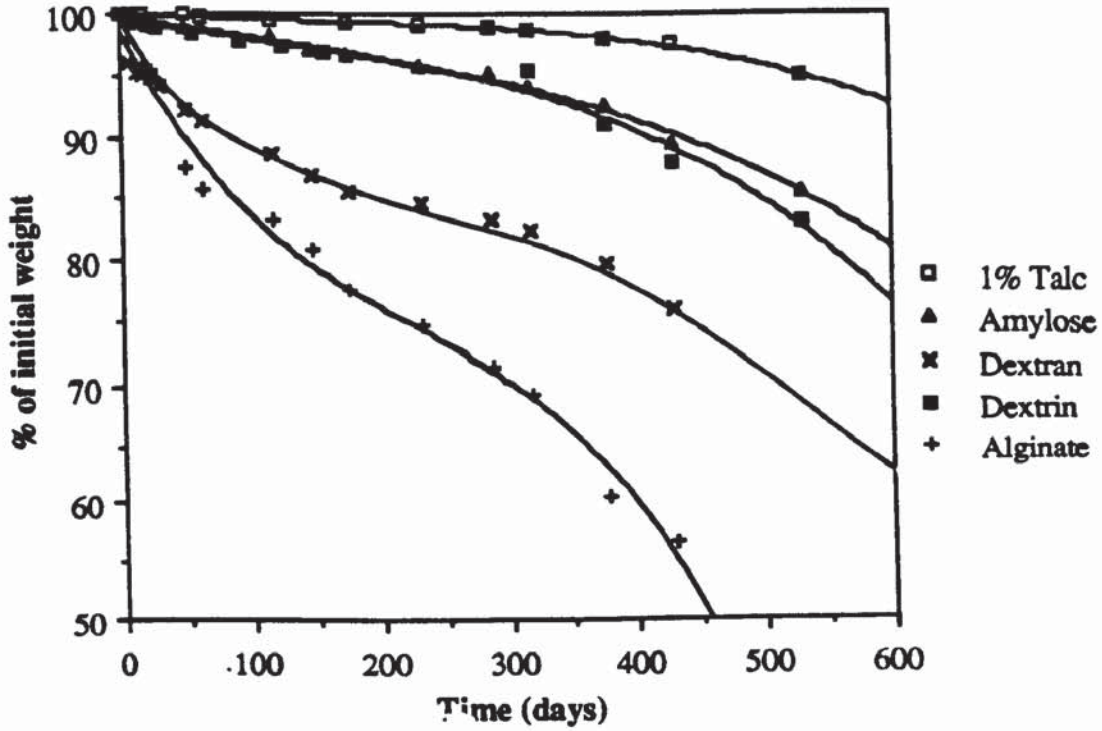
Graph 6.9 Changes in wet weight of injection moulded plaques of 12% PHV/polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



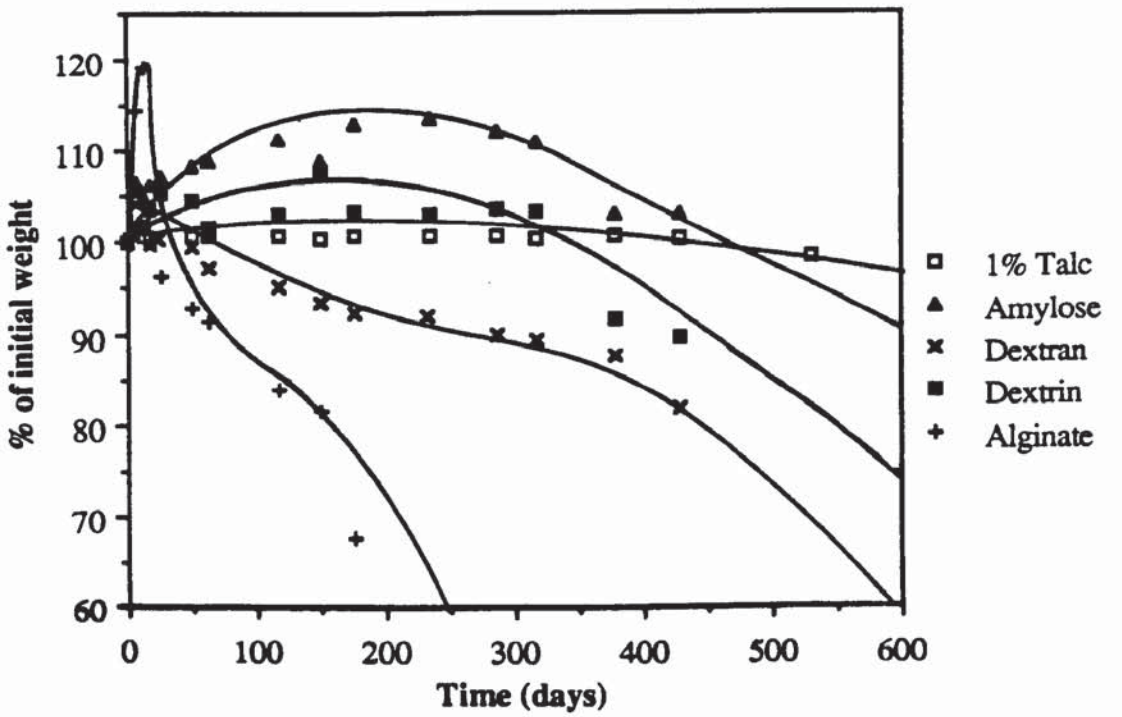
Graph 6.10 Changes in dry weight of injection moulded plaques of 12% PHV/polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



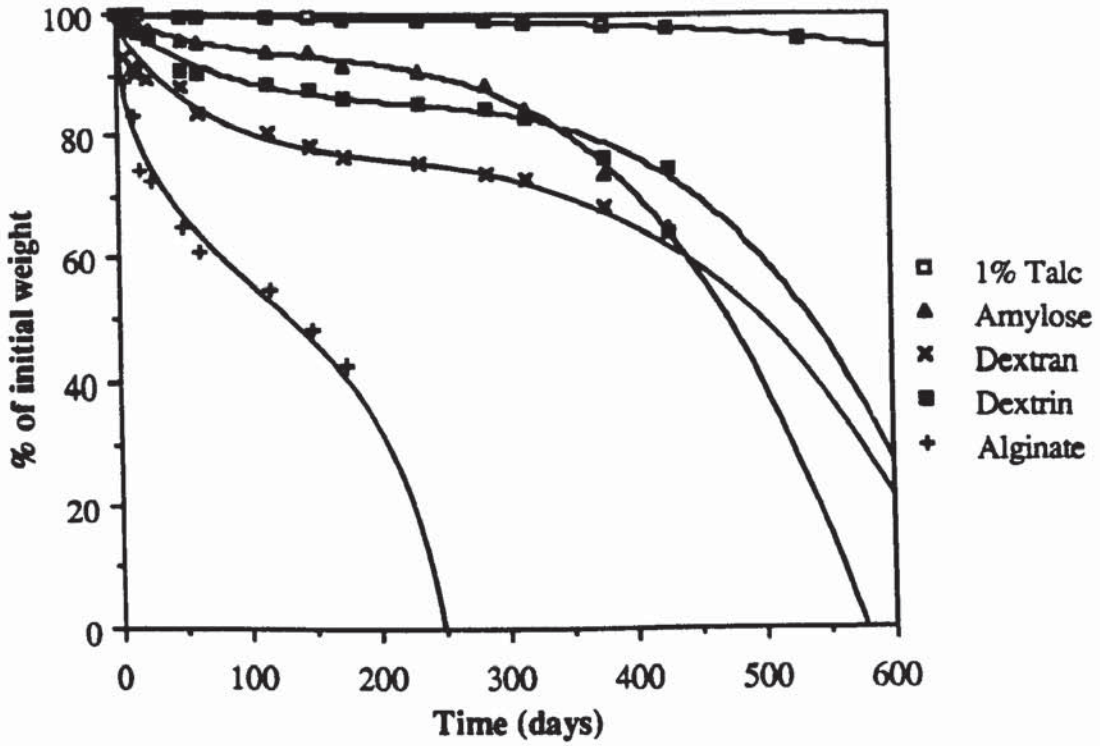
Graph 6.11 Changes in wet weight of injection moulded plaques of 20% PHV/polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



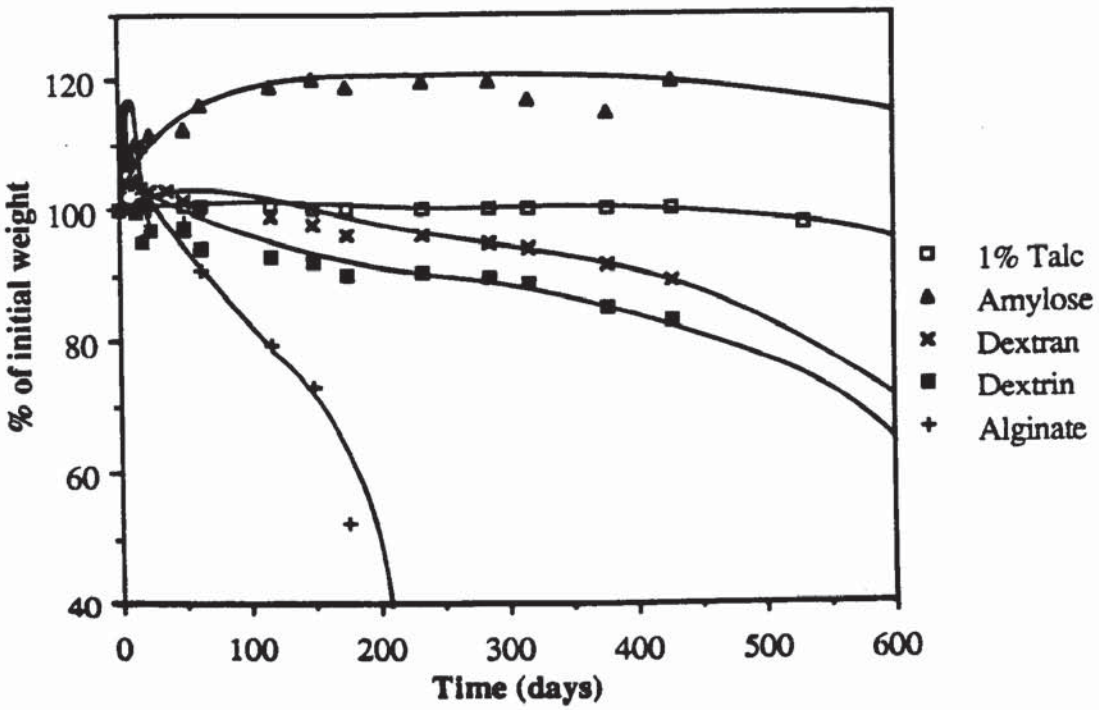
Graph 6.12 Changes in dry weight of injection moulded plaques of 20% PHV/polysaccharide (10%) blends in pH 7.4 buffer at 37°C.



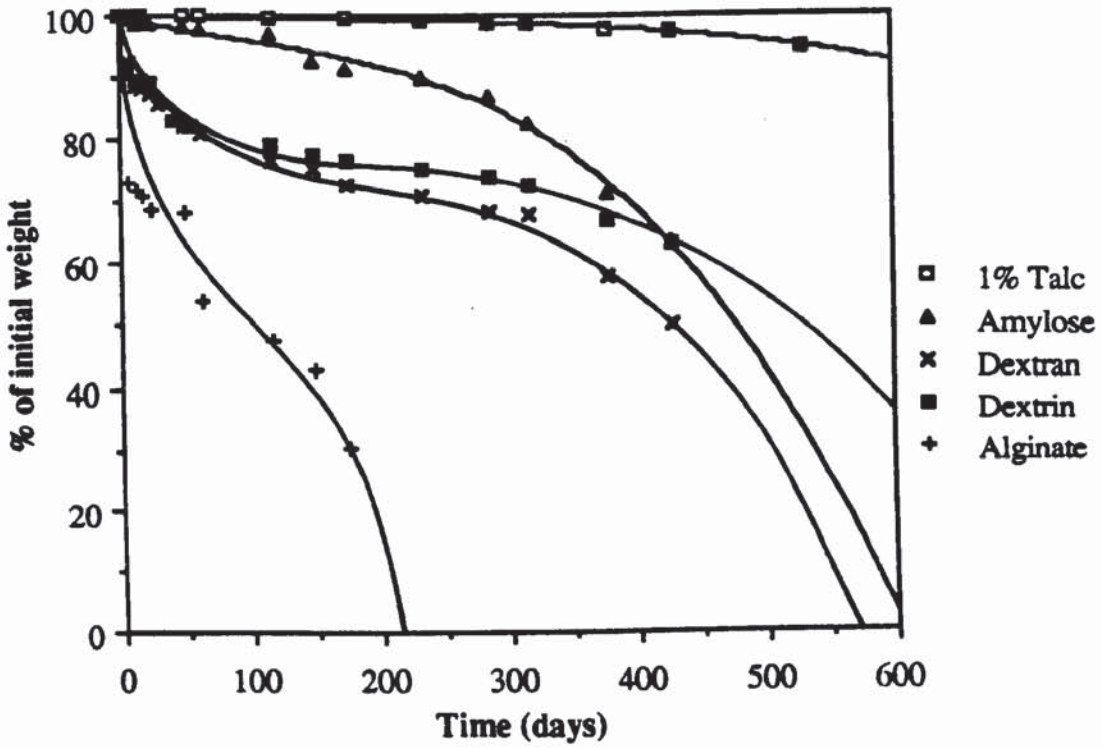
Graph 6.13 Changes in wet weight of injection moulded plaques of 12% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



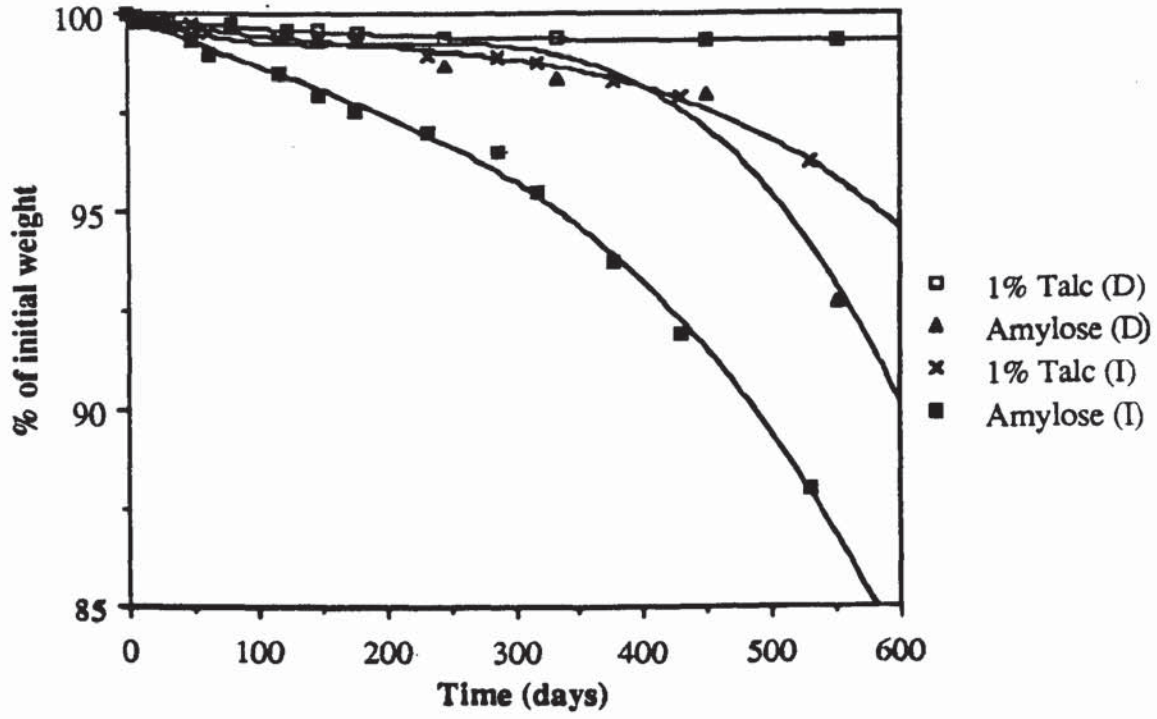
Graph 6.14 Changes in dry weight of injection moulded plaques of 12% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



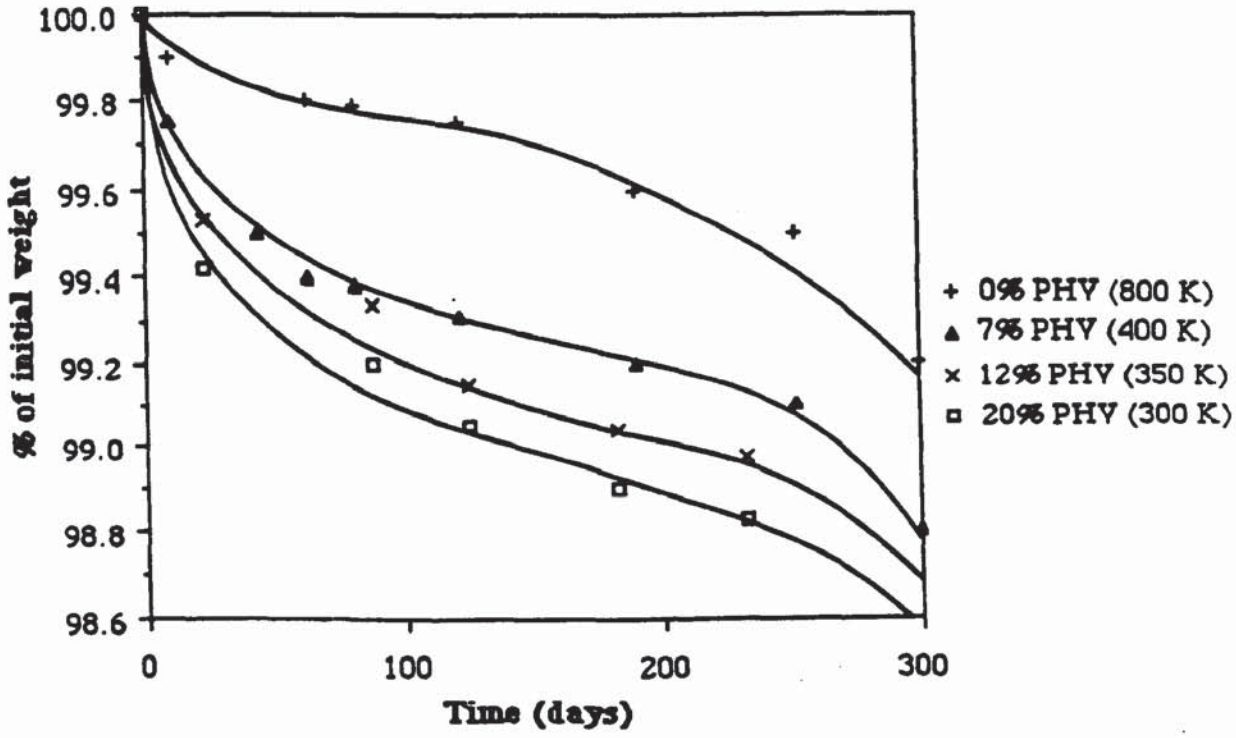
Graph 6.15 Changes in wet weight of injection moulded plaques of 20% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



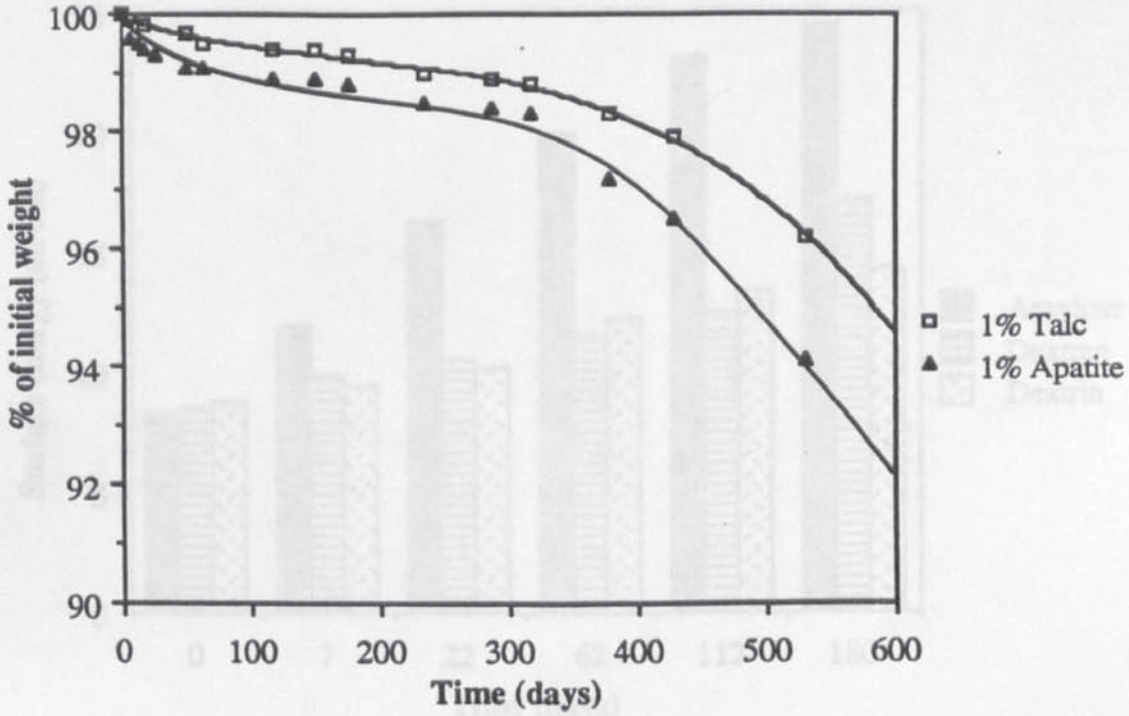
Graph 6.16 Changes in dry weight of injection moulded plaques of 20% PHV/polysaccharide (30%) blends in pH 7.4 buffer at 37°C.



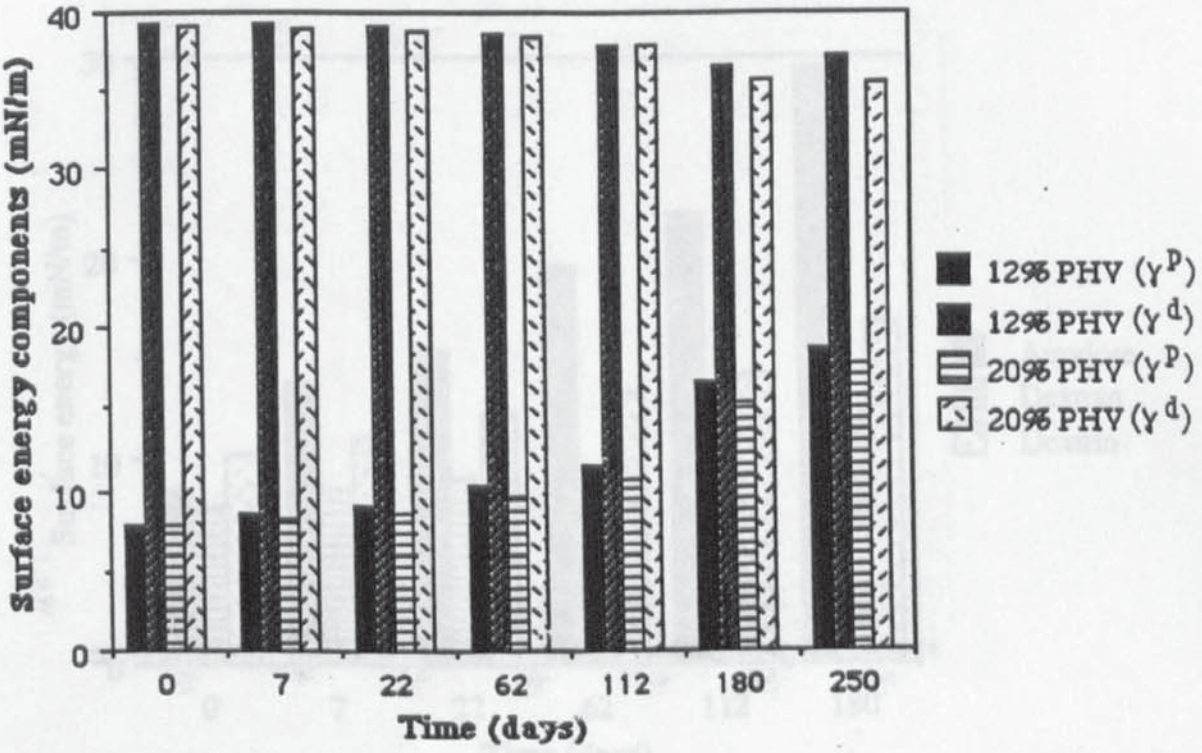
Graph 6.17 Comparison between the dry weight loss from melt pressed discs (D) and injection moulded plaques (I) of 20% PHV (talc) and 20% PHV/amylose (30%) blend in pH 7.4 buffer at 37°C.



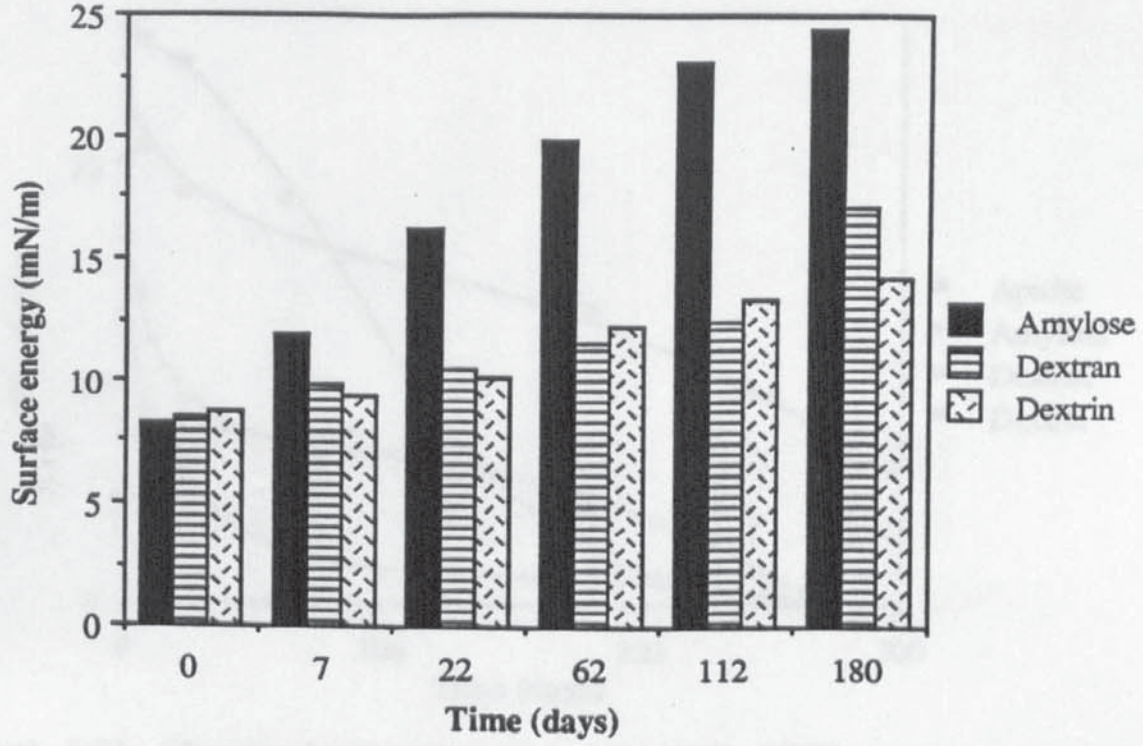
Graph 6.18 Effect of molecular weight and PHV content on weight loss of melt pressed discs of PHB-PHV copolymers (37°C and pH 7.4)



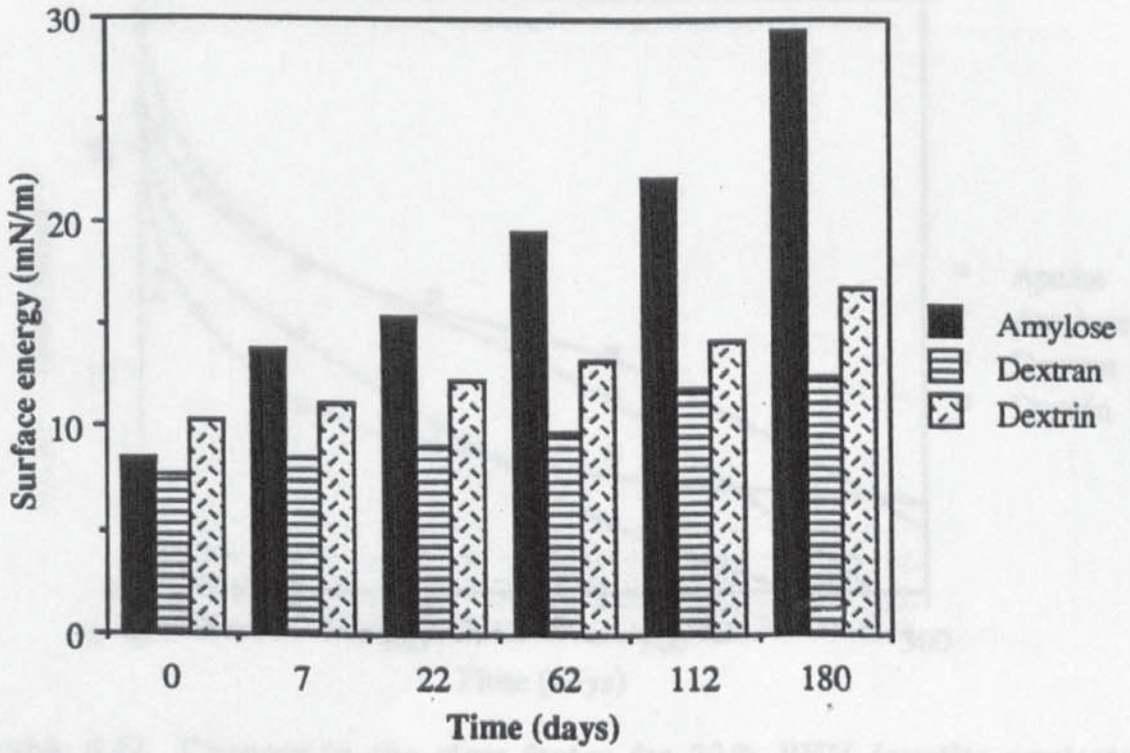
Graph 6.19 Effect of nucleating agent on weight loss from injection moulded plaques of 12% PHV copolymer (37°C and pH 7.4)



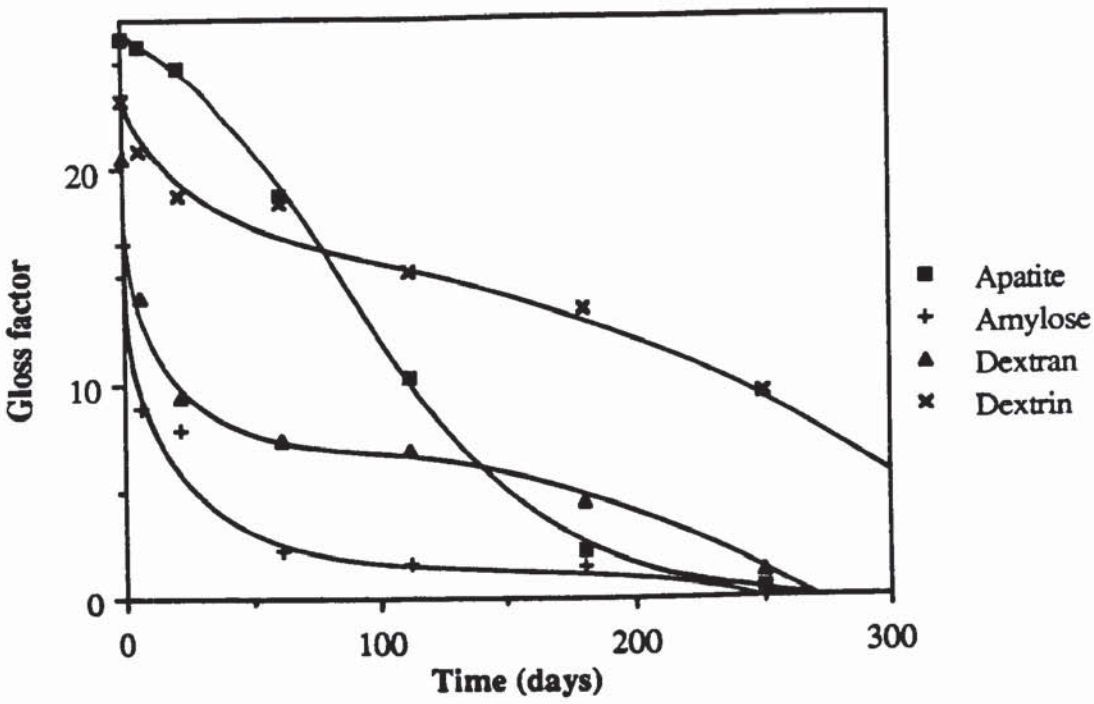
Graph 6.20 Changes in the polar (γ^p) and dispersive (γ^d) components of surface energy for 12 and 20% (apatite nucleated) injection moulded plaques degraded in pH 7.4 buffer at 37°C



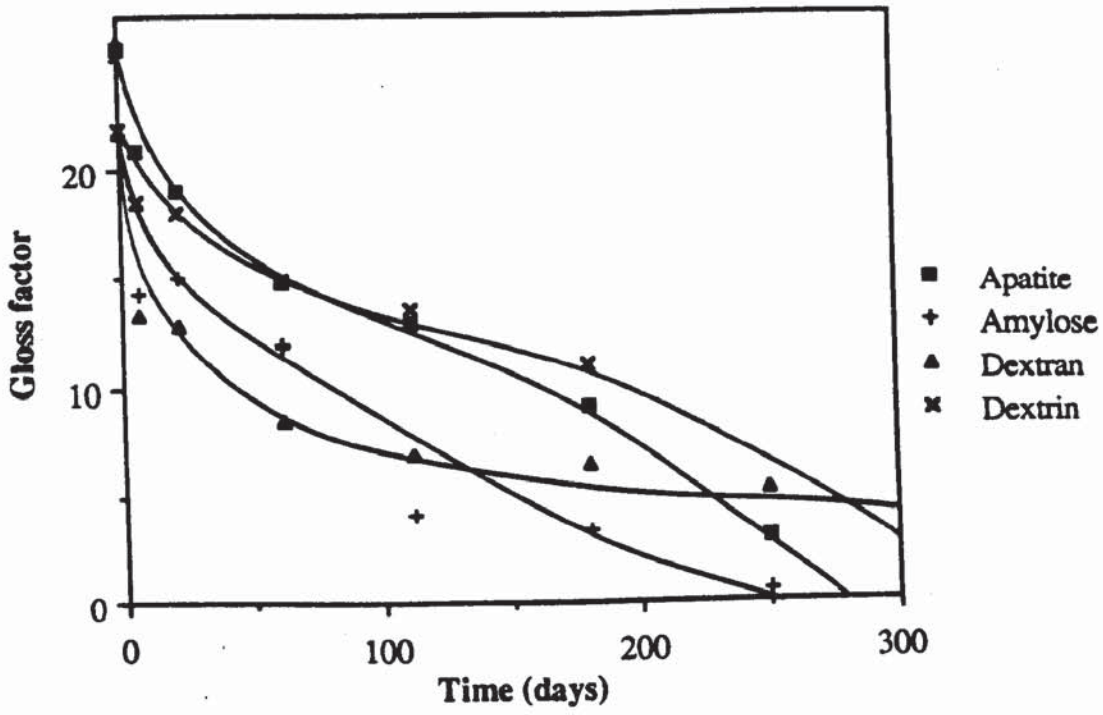
Graph 6.21 **Changes in the polar component of surface energy for 12% PHV/polysaccharide (10%) blends: injection moulded plaques degraded in pH 7.4 buffer at 37°C.**



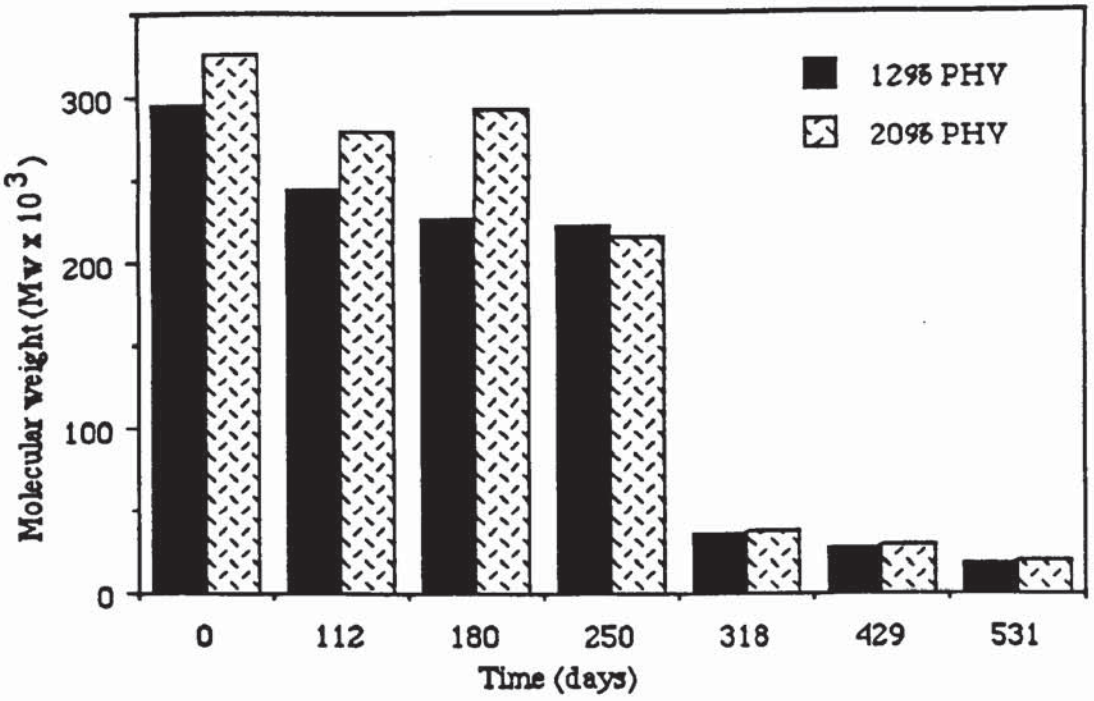
Graph 6.22 **Changes in the polar component of surface energy for 20% PHV/polysaccharide (10%) blends: injection moulded plaques degraded in pH 7.4 buffer at 37°C.**



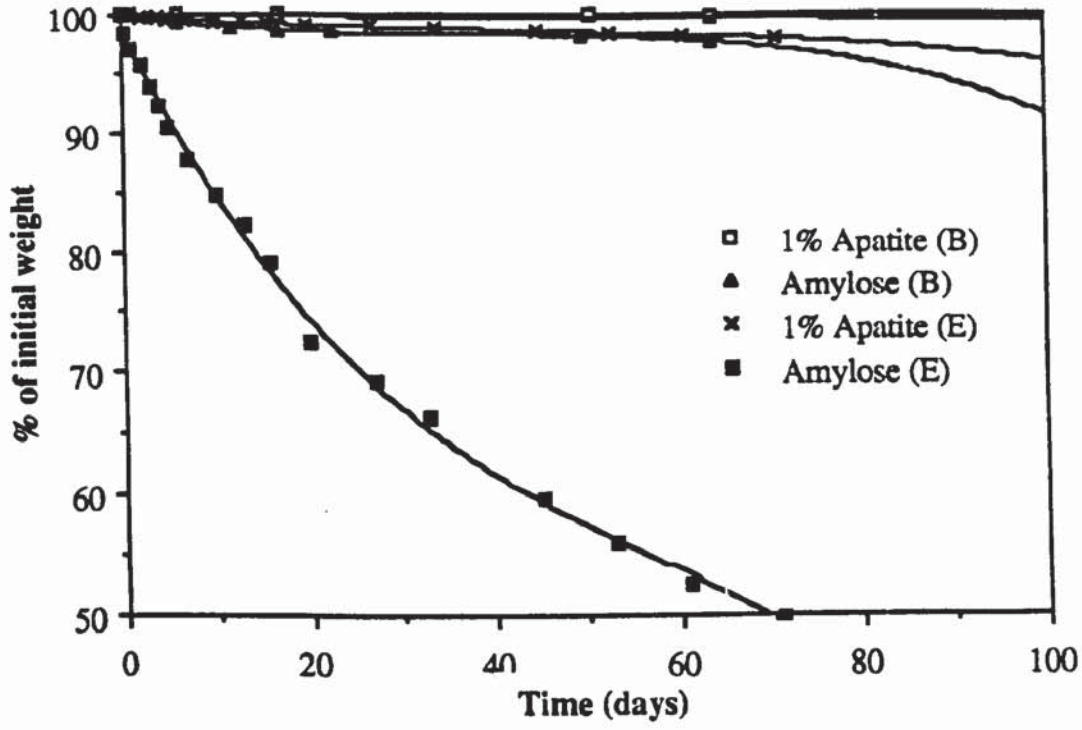
Graph 6.23 Changes in the gloss factor for 12% PHV (apatite nucleated) and blends with polysaccharides (10%); injection moulded plaques degraded in pH 7.4 buffer at 37°C.



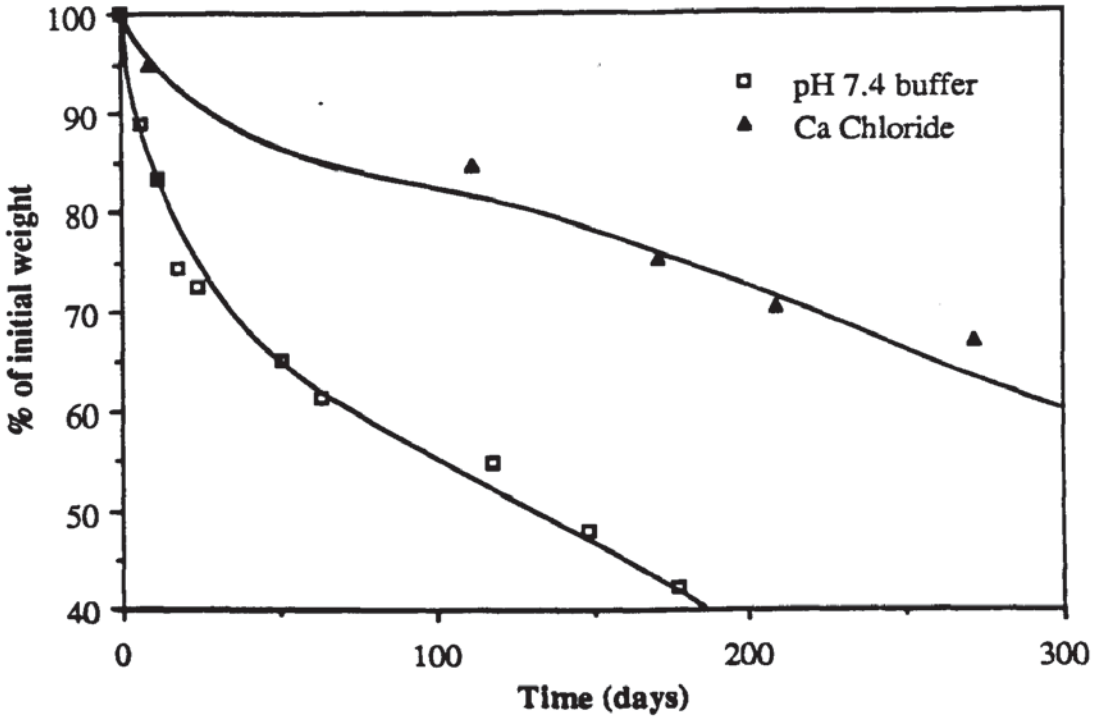
Graph 6.24 Changes in the gloss factor for 20% PHV (apatite nucleated) and blends with polysaccharides (10%); injection moulded plaques degraded in pH 7.4 buffer at 37°C.



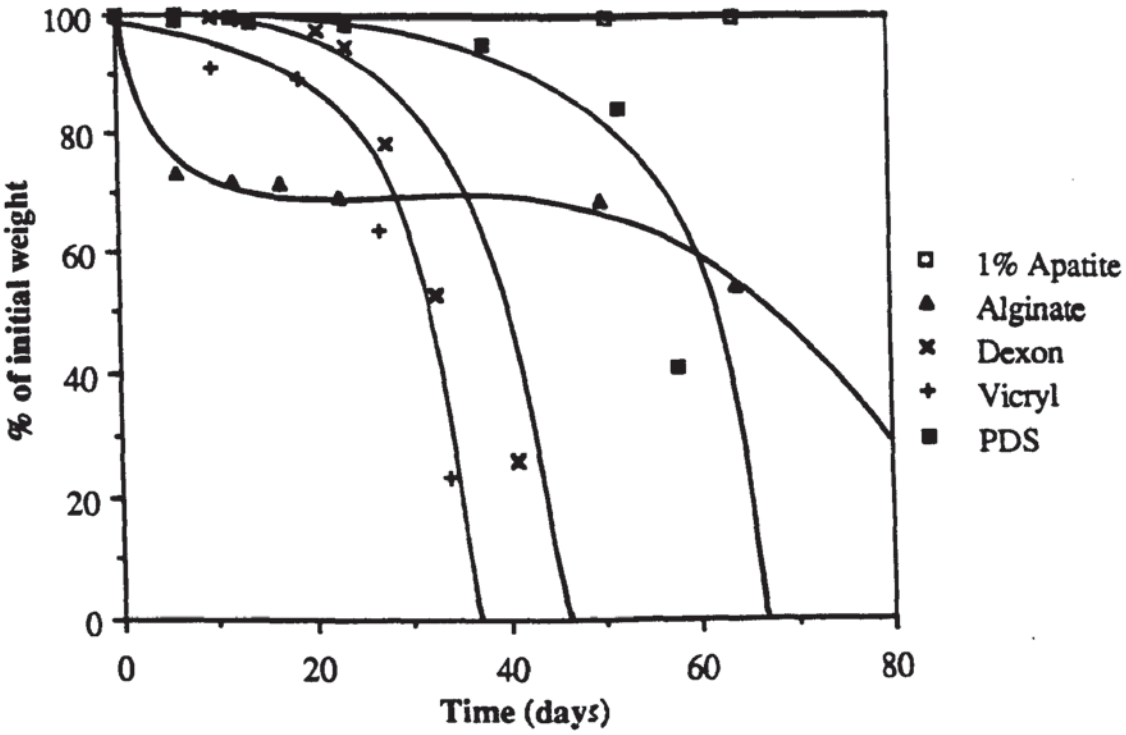
Graph 6.25 Changes in the molecular weight (Mw) of injection moulded plaques of 12 and 20% PHV copolymers (apatite nucleated) degraded in pH 7.4 buffer at 37°C.



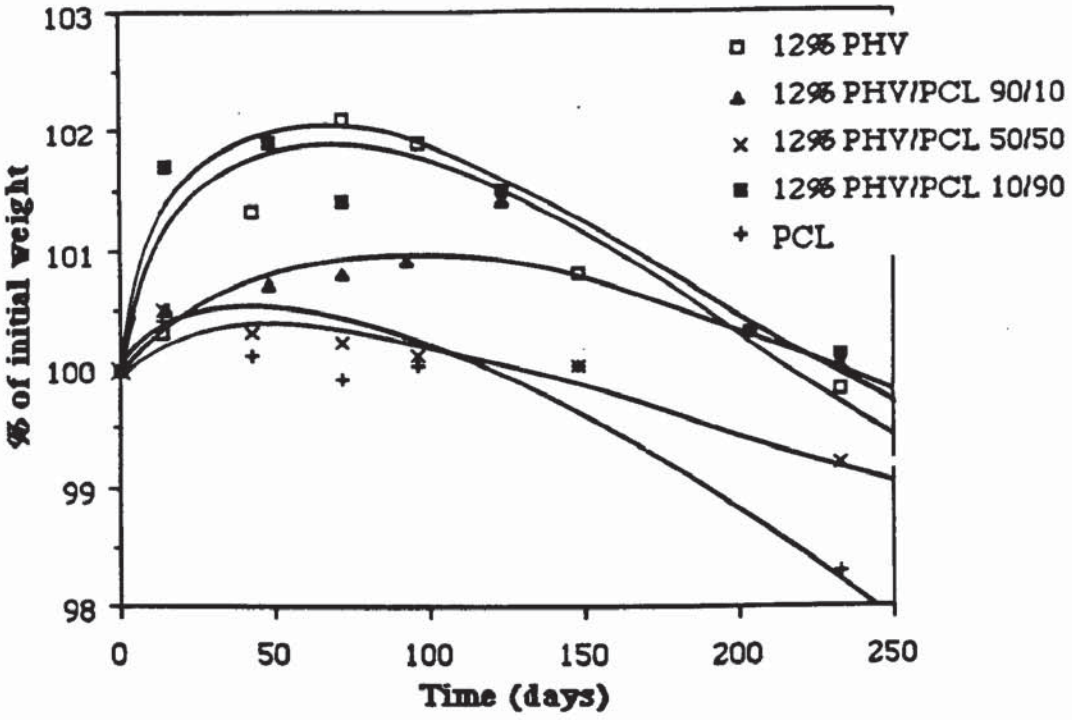
Graph 6.26 Weight loss of injection moulded plaques of unfilled 20% PHV (1% apatite) and amylose (30%) filled blend degraded in buffer (B) and α-amylase (E) at pH 7.4 and 37°C.



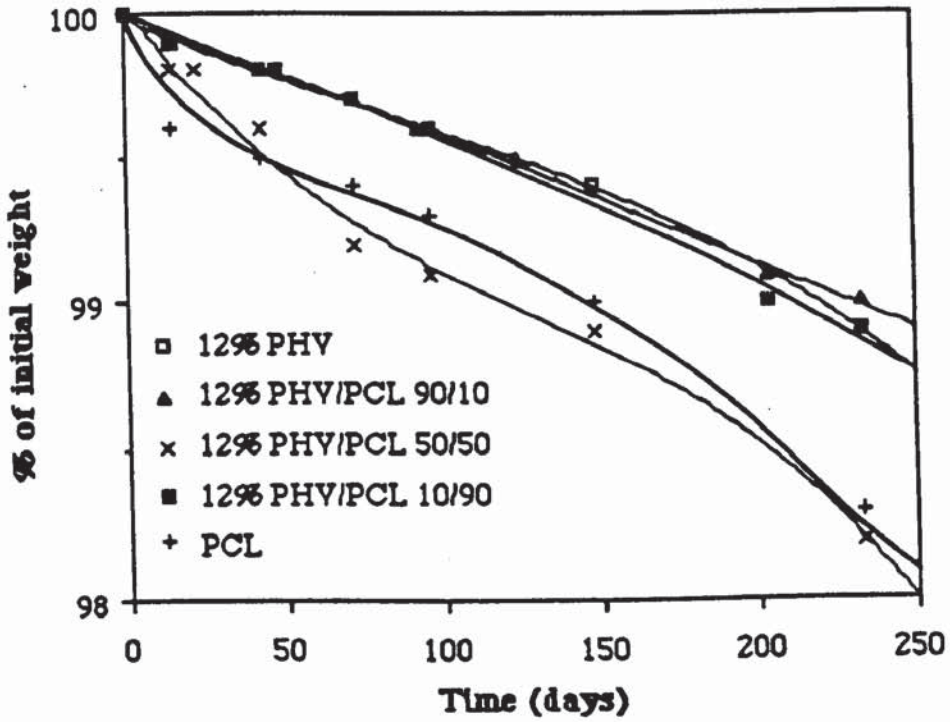
Graph 6.27 Weight loss of injection moulded plaques of sodium alginate (30%) filled blend in pH 7.4 buffers with and without free calcium ions at 37°C.



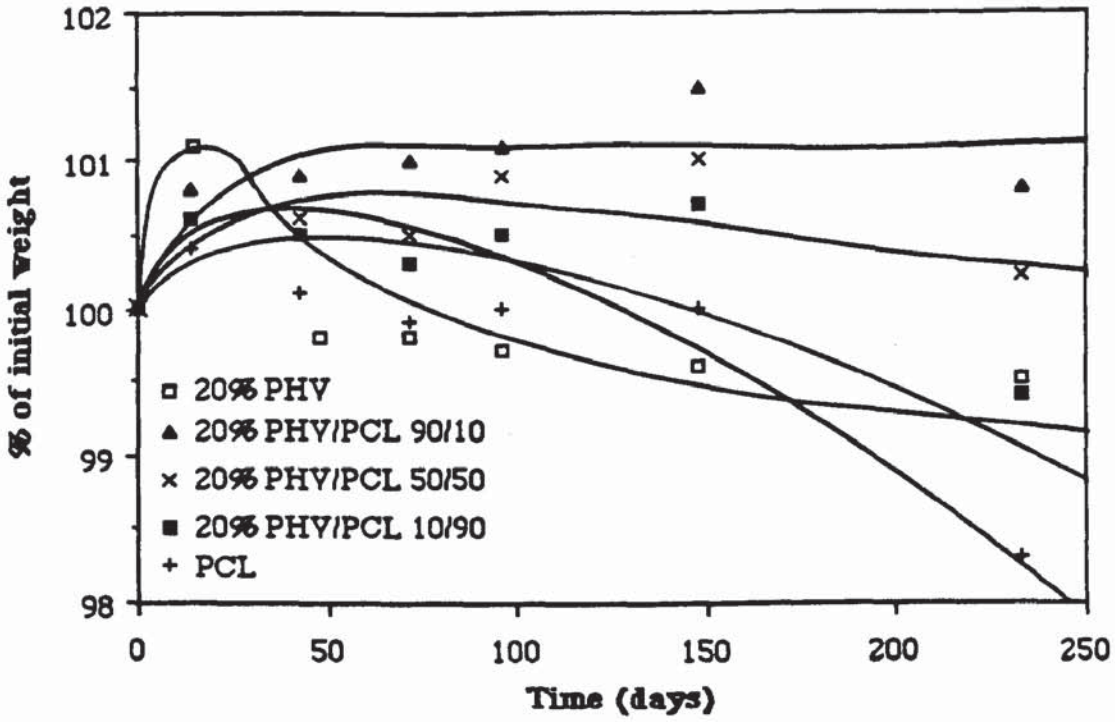
Graph 6.28 Weight loss of sutures and injection moulded plaques of filled (30% sodium alginate) and un-filled (1% apatite) 20% PHV copolymer degraded in pH 7.4 buffer at 37°C.



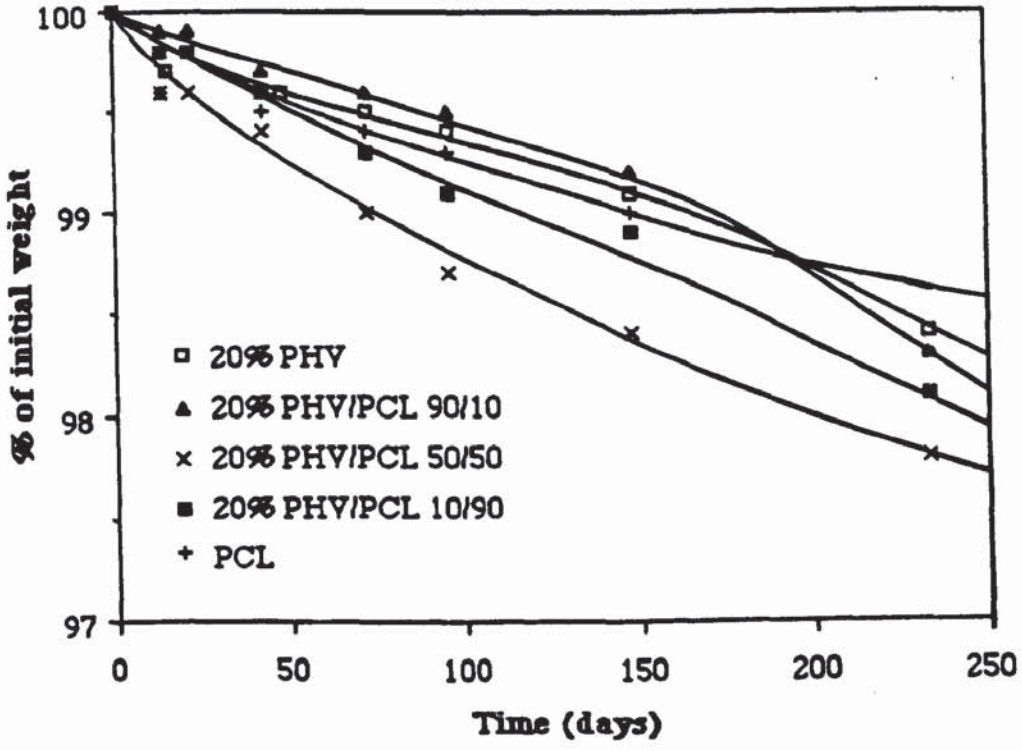
Graph 6.29 Changes in wet weight of injection moulded plaques of 12% PHV/polycaprolactone blends in pH 7.4 buffer at 37°C.



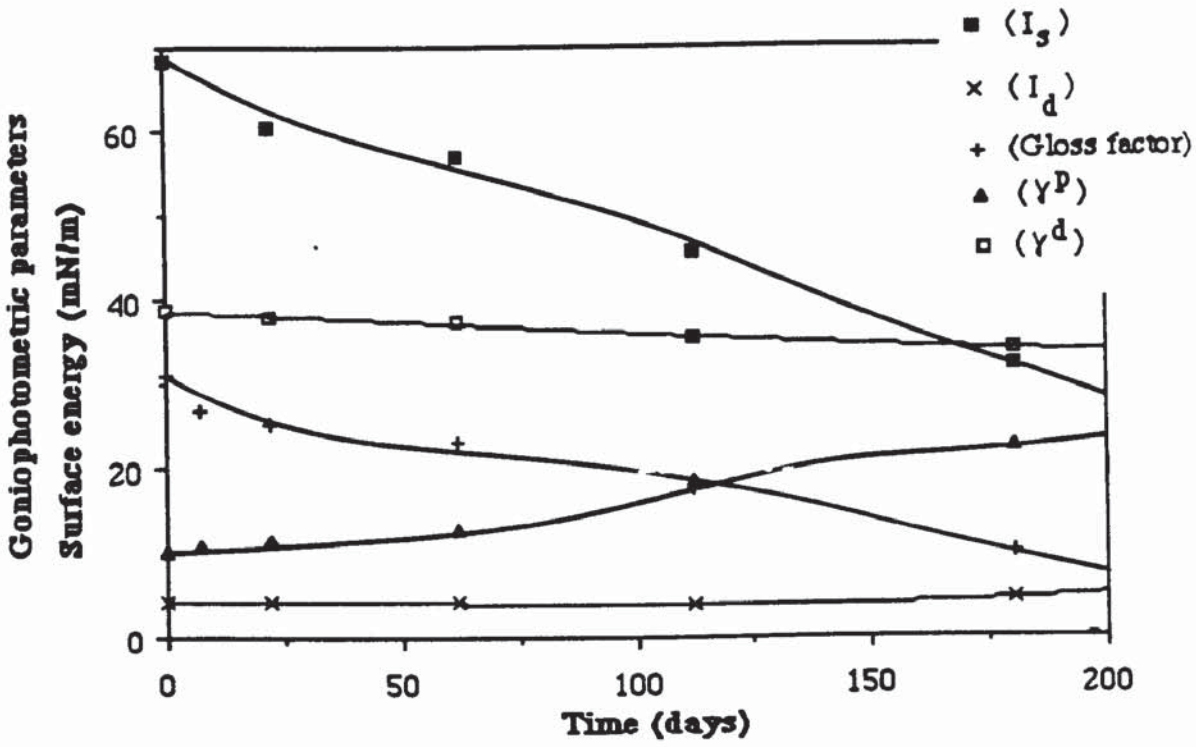
Graph 6.30 Changes in dry weight of injection moulded plaques of 12% PHV/polycaprolactone blends in pH 7.4 buffer at 37°C.



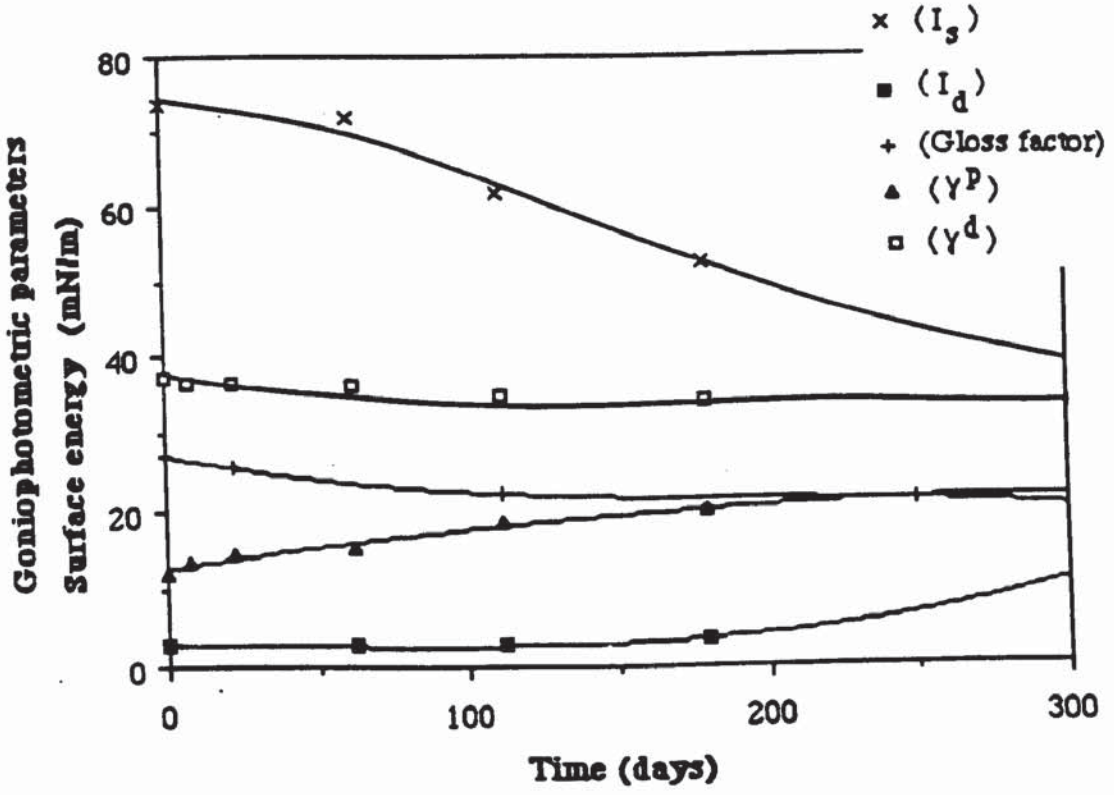
Graph 6.31 Changes in wet weight of injection moulded plaques of 20% PHV/polycaprolactone blends in pH 7.4 buffer at 37°C.



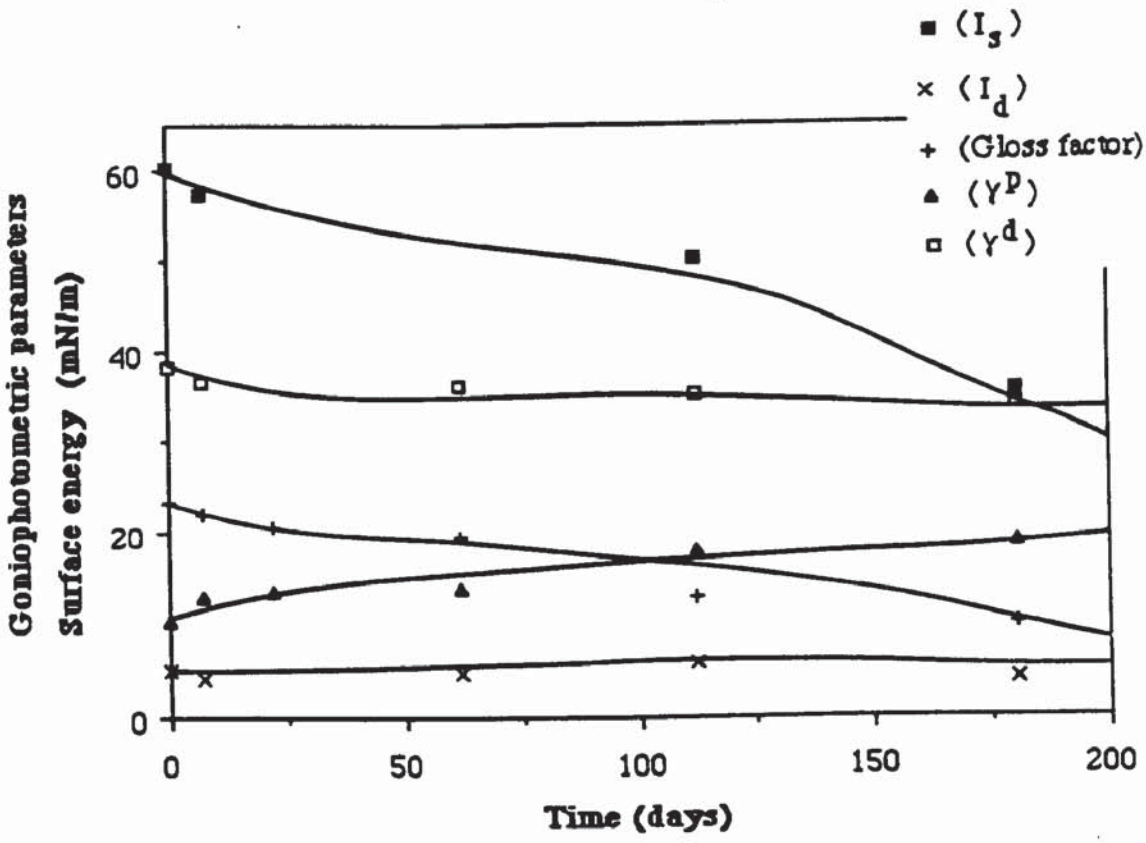
Graph 6.32 Changes in dry weight of injection moulded plaques of 20% PHV/polycaprolactone blends in pH 7.4 buffer at 37°C.



Graph 6.33 Changes in the goniophotometric and surface energy parameters for PCL (injection moulded);pH 7.4 buffer, 37°C.



Graph 6.34 Changes in the goniophotometric and surface energy parameters for 20% PHV/PCL 90/10 blend (injection moulded samples); degraded in pH 7.4 buffer, 37°C.



Graph 6.35 Changes in the goniophotometric and surface energy parameters for 20% PHV/PCL 10/90 blend (injection moulded samples): degraded in pH 7.4 buffer, 37°C.

Table 6.1 The t_{10} and t_{50} weight loss parameters for melt pressed discs and injection moulded plaques of 12 and 20% PHV copolymers and their blends with polysaccharides, degraded in pH 7.4 buffer at 37°C.

Blend	Discs		Plaques	
	t_{10} (days)	t_{50} (days)	t_{10} (days)	t_{50} (days)
12% PHV (1% talc)	1.2% (600 D)	-	5% (600 D)	-
12% PHV (1% apatite)	-	-	8% (600 D)	-
20% PHV (1% talc)	1.5% (600 D)	-	8% (600 D)	-
20% PHV (1% apatite)	-	-	600	-
12% PHV/10% amylose	600	-	484	16% (600 D)
12% PHV/30% amylose	392	20% (600 D)	240	469
12% PHV/10% dextran	465	17% (600 D)	312	17% (600 D)
12% PHV/30% dextran	11	549	25	500
12% PHV/10% dextrin	7% (600 D)	-	462	26% (600 D)
12% PHV/30% dextrin	27	485	84	534
12% PHV/10% alginate	150	593	122	516
12% PHV/30% alginate	4	236	5	131
20% PHV/10% amylose	556	14% (600 D)	431	20% (600 D)
20% PHV/30% amylose	200	542	230	476
20% PHV/10% dextran	409	17% (600 D)	76	45% (600 D)
20% PHV/30% dextran	14	534	9	418
20% PHV/10% dextrin	585	11% (600 D)	410	25% (600 D)
20% PHV/30% dextrin	11	496	14	503
20% PHV/10% alginate	18	425	44	455
20% PHV/30% alginate	2	225	2	100

where D = time in days.

Table 6.2 **Tensile properties of 12% PHV copolymer and blends with polysaccharides after degradation in pH 7.4 buffer at 37°C.**

12% PHV copolymer (apatite nucleated)

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	28.7±1.8	12±2	500±30	27.1±1.8	16±2
7	25.8±2.0	12±2	450±50	25±1.6	13±3
22	25.4±1.1	12±2	500±30	24.6±1.0	15±2
62	27.6±1.0	12±2	490±30	27.6±1.0	12±2
112	27.6±1.2	12±2	500±20	27.5±1.2	12±2
180	24.2±0.8	11±2	530±20	24.2±0.8	11±2
250	26±1.0	13±2	560±20	26±1.0	13±2

12% PHV/10% Amylose blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS) (MPa)	% EB
0	24.3±1.1	9±2	550±20	21.4±1.0	14±3
7	25.2±1.5	10±2	560±40	22.2±0.7	13±2
22	22.5±0.9	11±2	440±30	21.5±0.4	15±4
62	21.6±0.5	10±2	530±50	21.6±0.5	10±2
112	17.1±0.4	9±2	440±40	17.1±0.4	9±2
180	15.6±0.8	8±2	410±40	15.6±0.8	8±2
250	15.2±0.5	7±2	310±40	15.2±0.5	7±2

12% PHV/10% Dextran blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	25.7±1.1	10±2	580±30	24.7±0.8	12±2
7	23±0.9	11±2	520±40	23±0.9	11±2
22	21.3±0.7	11±2	430±10	21.3±0.7	11±2
62	26.5±2.4	10±2	570±10	26.5±2.4	10±2

112	21±0.4	6±2	470±50	18.5±0.6	6±2
180	18.5±0.6	9±2	530±50	17.6±0.4	9±2
250	17.6±0.4	11±2	410±50	16.8±0.5	11±2

12% PHV/10% Dextrin blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	26.6±1.3	10±2	580±30	23.8±1.0	16±4
7	23.8±1.1	9±2	490±10	20.3±1.1	14±2
22	21±0.9	9±2	440±20	19.7±1.0	14±3
62	23±1.5	11±2	520±50	23±1.5	11±2
112	21.1±1.1	10±2	480±30	21.1±1.1	10±2
180	17.5±0.8	10±2	500±10	17.5±0.8	10±2
250	20.7±1.4	12±4	470±20	20.7±1.4	12±4

N.B. YS = yield strength (MPa), % EY = % elongation at yield, ITM = initial tensile modulus (MPa), UTS = ultimate tensile strength (MPa) and % EB = % elongation at break.

Table 6.3 Tensile properties of 20% PHV copolymer and blends with polysaccharides after degradation in pH 7.4 buffer at 37°C.

20% PHV copolymer (apatite nucleated).

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	19.1±0.2	13±2	310±10	17.3±0.9	22±2
7	19.1±0.1	13±2	290±20	18.3±0.8	23±2
22	19.1±0.3	13±2	290±30	18.3±0.6	23±2
62	22.1±0.6	12±2	340±20	21.9±0.2	21±2
112	20.5±0.5	15±2	290±10	18.5±1.1	23±2
180	18.3±0.6	13±2	260±20	18.1±0.1	17±2
250	19.9±0.1	16±2	290±20	18.7±0.1	23±2

20% PHV/10% Amylose blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	20±0.5	11±2	450±50	17.1±0.9	21±2
7	18.9±0.7	12±2	320±10	16.6±0.8	23±2
22	19.1±0.9	12±2	310±10	17.1±0.5	23±2
62	21.6±1.1	12±2	370±30	20.9±0.8	20±2
112	19.6±1.1	12±2	300±10	17.1±0.9	18±2
180	16.1±0.5	15±2	240±20	16.1±0.5	15±2
250	15.7±0.4	14±2	310±10	15.7±0.4	14±2

20% PHV/10% Dextran blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	19.5±0.3	11±2	380±40	17.1±0.4	24±2
7	17.9±0.4	12±2	300±30	16.3±0.8	19±2
22	17.1±0.2	12±2	260±20	15.7±0.4	17±2
62	17.4±0.6	10±2	360±10	17.1±0.1	14±2
112	15.2±0.3	10±2	320±20	13.5±0.9	14±2
180	16.5±0.8	15±2	350±20	16.5±0.8	15±2
250	16.4±0.7	19±2	310±20	16.4±0.7	19±2

20% PHV/10% Dextrin blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	19.3±0.7	12±3	450±20	14.6±0.9	77±5
7	17.5±0.8	15±2	290±10	15±0.8	63±6
22	17.5±0.7	17±2	240±20	14.2±0.8	44±5
62	18.7±1.1	13±2	320±10	17.3±1.1	25±2
112	20.5±1.4	14±2	280±10	17.8±1.0	28±4
180	16.3±0.7	14±2	320±10	15.7±0.5	20±2
250	16.5±0.9	16±2	250±10	16±0.4	22±3

Table 6.4 Changes in molecular weight (Mw) with time for 12 and 20% PHV copolymers and blends with polysaccharides after degradation in pH 7.4 buffer at 37°C (Injection moulded plaques).

Time (days)	12% PHV (1% talc)	20% PHV (1% talc)	12% PHV/dextrin (10%) blend	12% PHV/alginate (10%) blend
0	192	195	279	276
318	-	-	79.1	8.39
429	54.2	65	50.9	6.2
531	31.3	35.2	30.3	-

Table 6.5 Changes in molecular weight with time for 12 and 20% PHV copolymers after degradation in pH 7.4 buffer at 37°C (melt pressed discs).

Time	12% PHV copolymer			20% PHV copolymer		
(days)	Mw	Mn	Mw/Mn	Mw	Mn	Mw/Mn
0	362	142.5	2.5	201	83	2.4
334	134	2.6	51	90.9	2.8	32.4
450	108	35.2	3.0	109	3.1	35
552	34.8	6.3	5.5	57.9	7.3	7.9

Table 6.6 Changes in % crystallinity with time for 12 and 20% PHV copolymers and blends with polysaccharides after degradation in pH 7.4 buffer at 37°C, (Injection moulded plaques).

Time (days)	12% PHV (1% apatite)	20% PHV (1% apatite)	12% PHV (1% talc)	20% PHV (1% talc)	12% PHV/dextrin (10%) blend
0	69±5	65±5	72±5	68±5	68±5
112	71±5	66±5	-	-	-
180	73±5	68±5	-	-	-
250	75±5	72±5	-	-	-
318	77±5	74±5	-	-	-
429	75±5	73±5	78±5	76±5	75±5
531	73±5	71±5	81±5	79±5	78±5

Table 6.7 Changes in % crystallinity with time for 12 and 20% PHV copolymers after degradation in pH 7.4 buffer at 37°C (melt pressed discs).

Time (days)	12% PHV (1% talc)	20% PHV (1% talc)
0	63±5	61±5
319	74±5	73±5
450	76±5	75±5
552	78±5	77±5

Table 6.8 Tensile properties of PHB-PHV/PCL blends after degradation in pH 7.4 buffer at 37°C.

PCL

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	16.3±1.2	18±6	150±20	20.1±1.2	400±60
7	18.5±1.1	32±10	180±10	18.5±1.1	32±10
22	15.4±0.8	12±2	190±10	15.4±0.8	12±2
62	16.5±0.9	10±2	210±10	16.5±0.9	10±2
112	15.4±0.7	11±2	200±20	15.4±0.7	11±2
180	4.3±0.7	5±2	110±30	4.3±0.7	5±2

12% PHV/PCL 90/10 blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	19.9±0.1	13±2	300±30	19.9±0.1	13±2
7	19.5±0.5	11±2	500±10	19.5±0.5	11±2
22	15.4±0.8	13±2	270±50	15.4±0.8	13±2
62	15.5±0.5	7±2	500±10	15.5±0.5	7±2

112	15.2±0.1	6±2	380±50	15.2±0.1	6±2
180	12.8±0.2	6±2	430±10	12.8±0.2	6±2
250	10.4±0.1	6±2	310±50	10.4±0.1	6±2

12% PHV/PCL 10/90 blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	15±0.8	43±5	150±10	15±0.8	43±5
7	14.4±0.3	21±2	170±20	14.4±0.3	21±2
22	13.8±0.2	21±2	140±20	13.8±0.2	21±2
62	10±0.1	5±2	210±10	10±0.1	5±2
112	9.4±0.2	6±2	220±40	9.4±0.2	6±2
180	3.7±0.3	4±2	80±10	3.7±0.3	4±2

20% PHV/PCL 90/10 blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	18±1.2	18±4	300±40	19.2±0.7	400±50
7	21.1±1.8	16±2	250±20	15.9±0.8	36±6
22	17.9±3.1	25±6	220±10	15.9±1.0	66±15
62	21±1.0	11±4	300±10	19.5±0.9	48±8
112	20.1±1.0	13±2	270±10	17.3±1.1	24±4
180	17.5±0.8	11±2	300±30	16.3±0.8	18±3
250	15.6±0.5	17±4	230±10	15.2±0.2	21±5

20% PHV/PCL 10/90 blend.

Time (days)	YS (MPa)	% EY	ITM (MPa)	UTS (MPa)	% EB
0	15.3±0.3	31±2	150±10	15.3±0.3	31±2
7	15.6±0.2	14±2	190±20	15.6±0.2	14±2
22	14.2±0.4	23±2	160±10	14.2±0.4	23±2

62	12.3±0.3	12±2	220±10	12.3±0.3	12±2
112	7.3±0.1	5±2	200±10	7.3±0.1	5±2
180	5.9±0.2	6±2	130±30	5.9±0.2	6±2

Table 6.9 Changes in % crystallinity of 12 and 20% PHV/PCL (90/10) blends after degradation in pH 7.4 buffer at 37°C.

Time (days)	12% PHV/PCL 90/10 blend	20% PHV/PCL 90/10 blend
0	70±5	68±5
7	72±5	71±5
22	-	74±5

Table 6.10 The t_{10} and t_{50} parameters for commercial biodegradable materials and 20% PHV/amylose (30%) blend (plaque) degraded in pH 7.4 buffer at 37°C.

	t_{10} (days)	t_{50} (days)
Dexon	23	36
Vicryl	14	29
PDS	45	61
20% PHV/amylose (30%) blend	7	70

Figure 6.1 Goniophotometric curves for 12% PHV (apatite nucleated; injection moulded), after various periods of degradation in pH 7.4 buffer at 37°C.

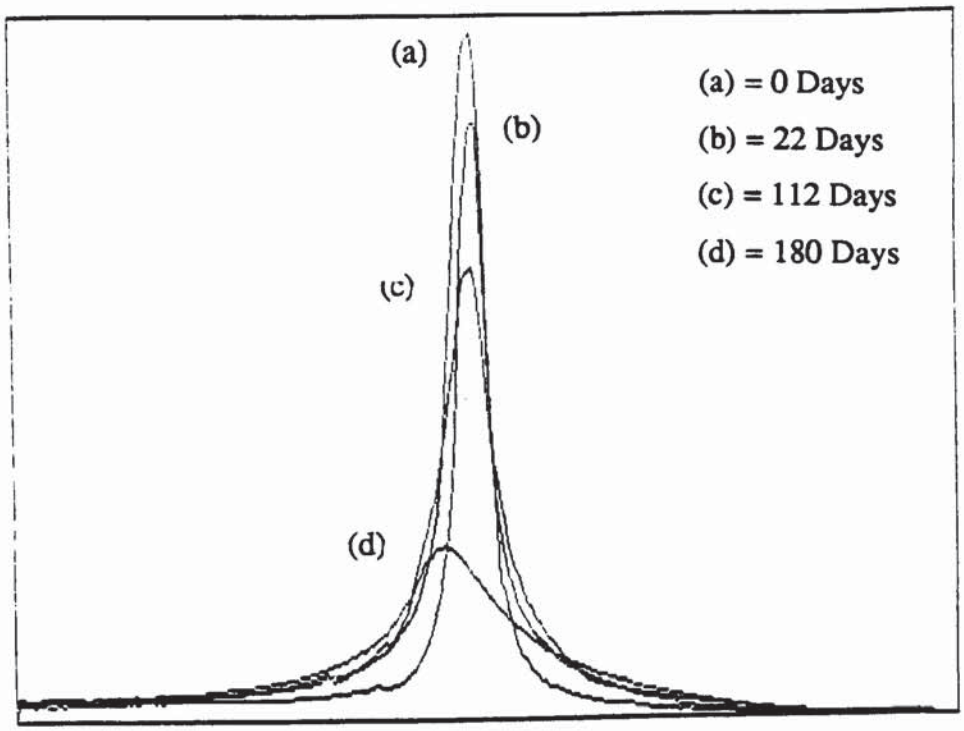


Figure 6.2 Goniophotometric curves for 20% PHV (apatite nucleated; injection moulded), after various periods of degradation in pH 7.4 buffer at 37°C.

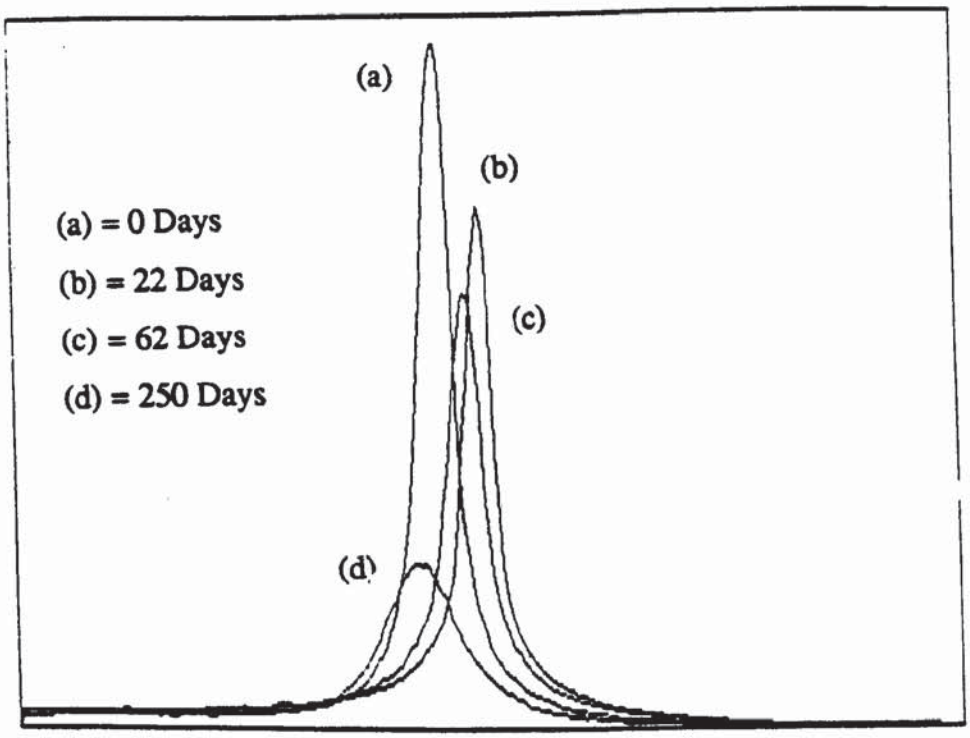


Figure 6.3 Goniophotometric curves for 12% PHV/amylose (10%) blend (injection moulded), after various periods of degradation in pH 7.4 buffer at 37°C.

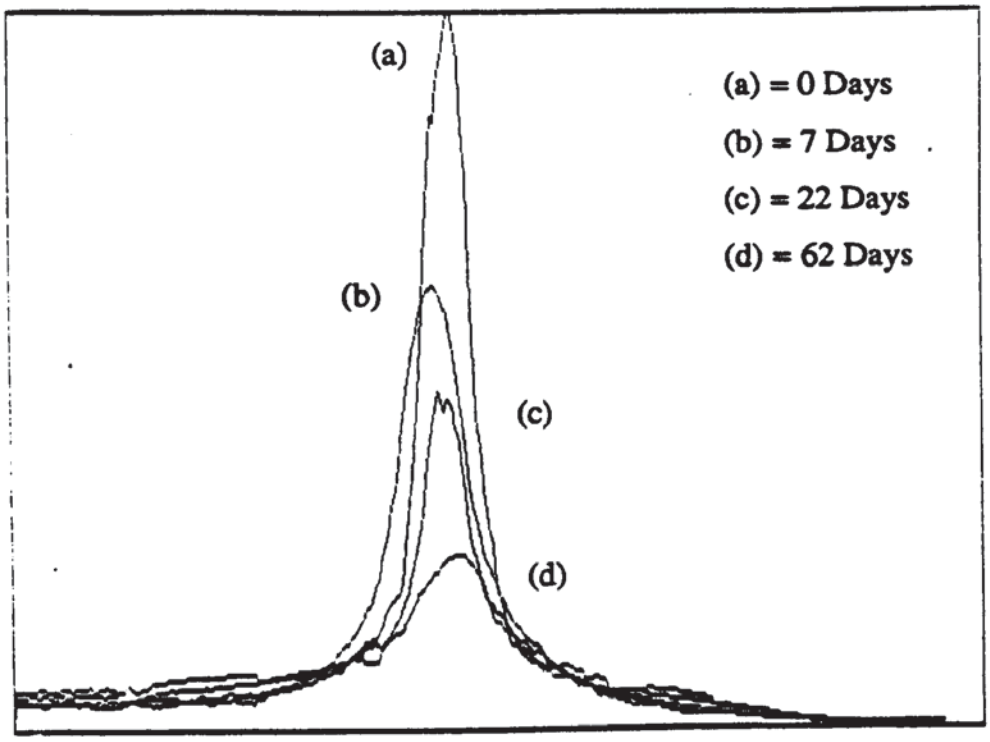


Figure 6.4 Goniophotometric curves for 20% PHV/amylose (10%) blend (injection moulded), after various periods of degradation in pH 7.4 buffer at 37°C.

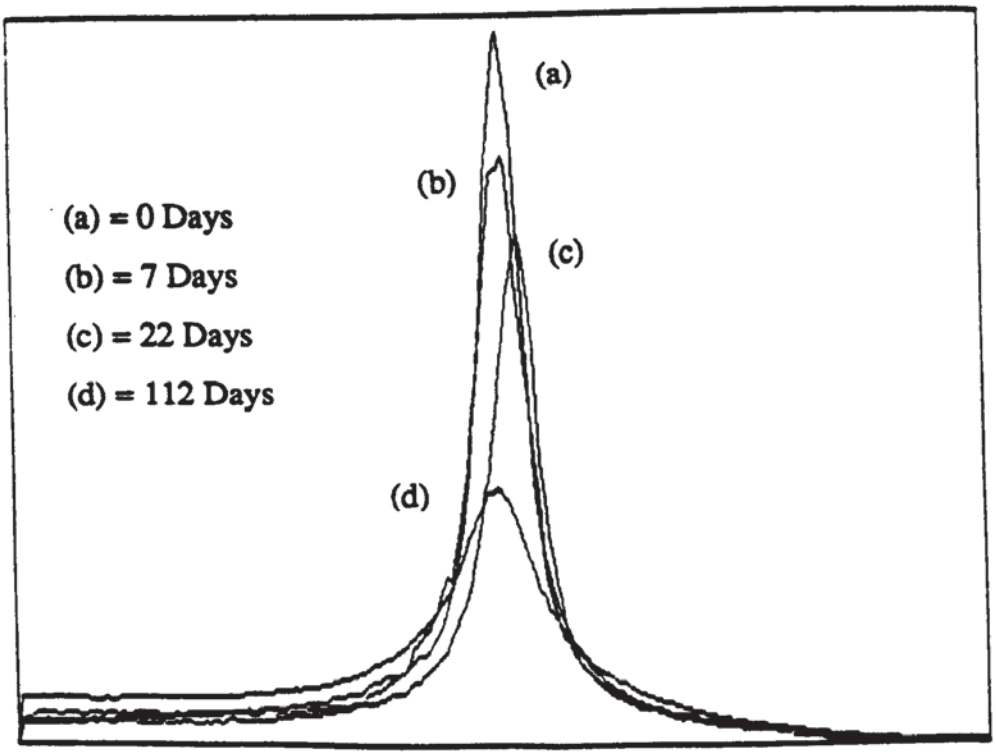


Figure 6.5 X-ray diffraction traces of 12% PHV copolymer (apatite nucleated plaque) after various periods of degradation in pH 7.4 buffer at 37°C.

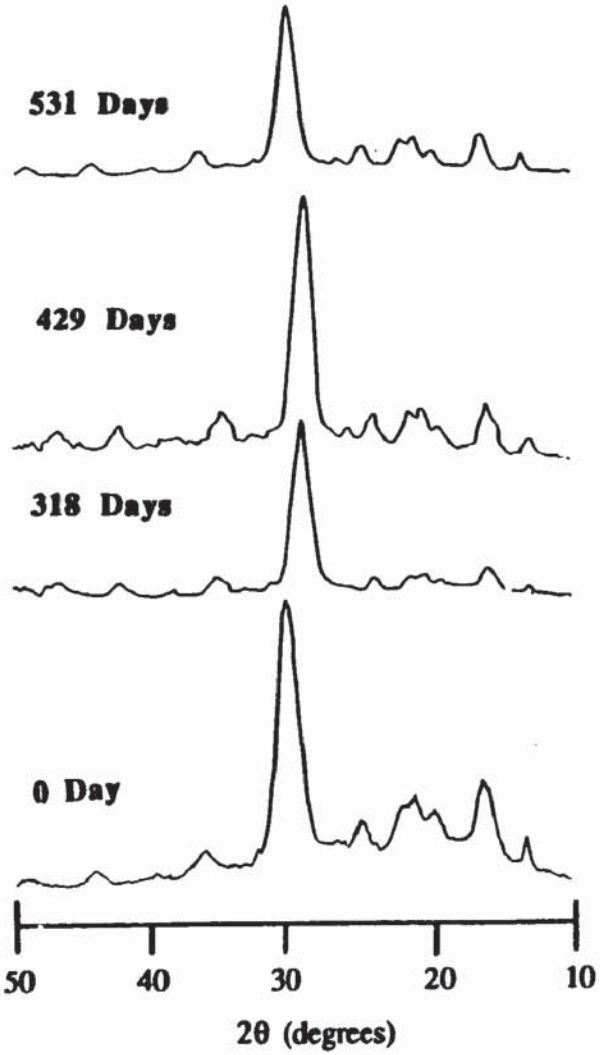


Figure 6.6 X-ray diffraction traces of 20% PHV copolymer (apatite nucleated plaque) after various periods of degradation in pH 7.4 buffer at 37°C.

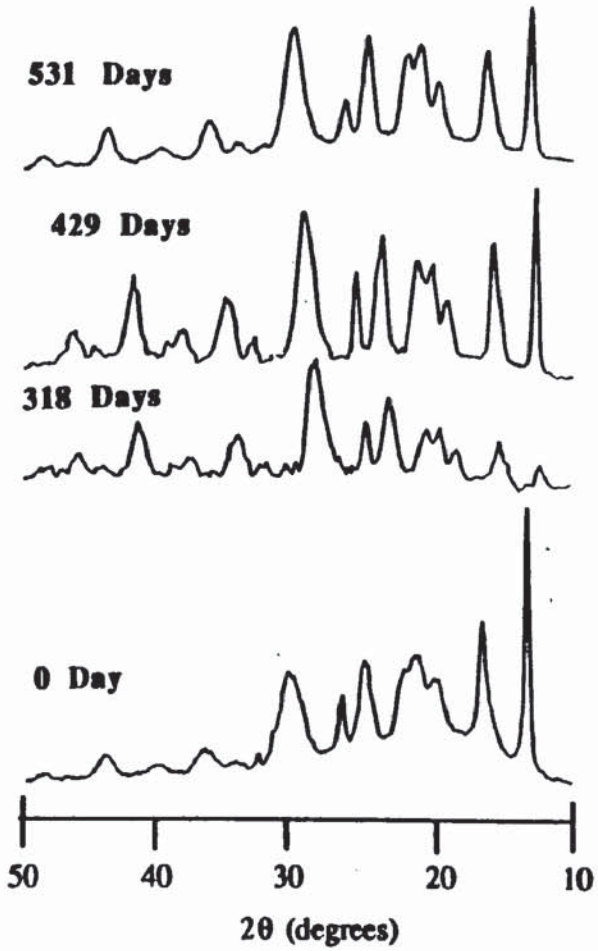


Figure 6.7 X-ray diffraction traces of 12% PHV (apatite nucleated, injection moulded tensile piece), after various periods of degradation in pH 7.4 buffer at 37°C.

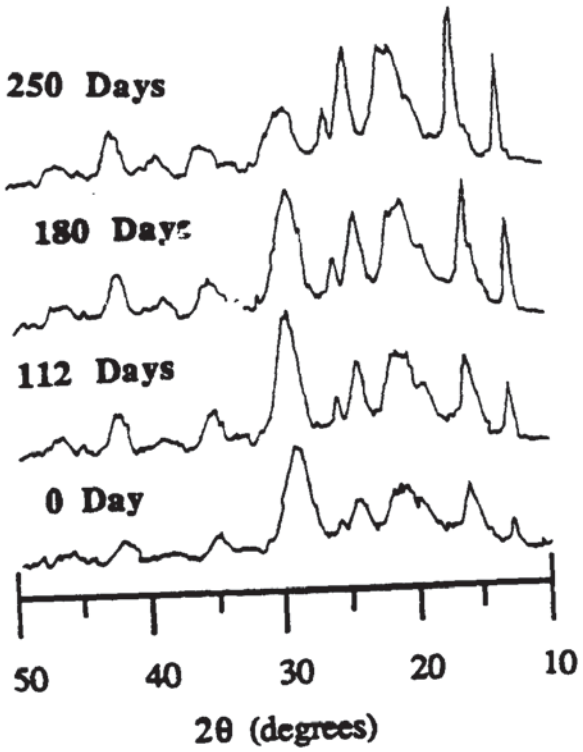


Figure 6.8 X-ray diffraction traces of 20% PHV (apatite nucleated, injection moulded tensile piece), after various periods of degradation in pH 7.4 buffer at 37°C.

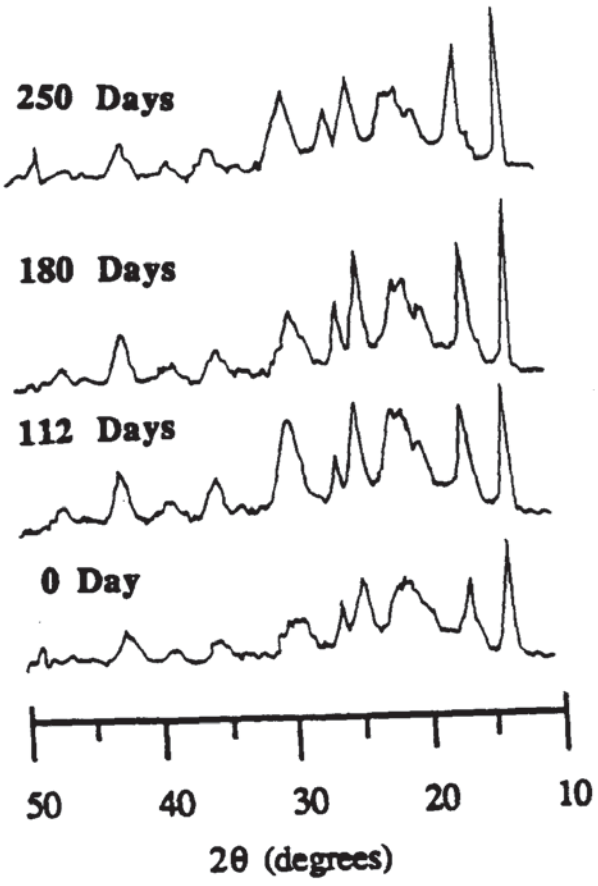


Figure 6.9 X-ray diffraction traces of 12% PHV copolymer (talc nucleated plaque) after various periods of degradation in pH 7.4 buffer at 37°C.

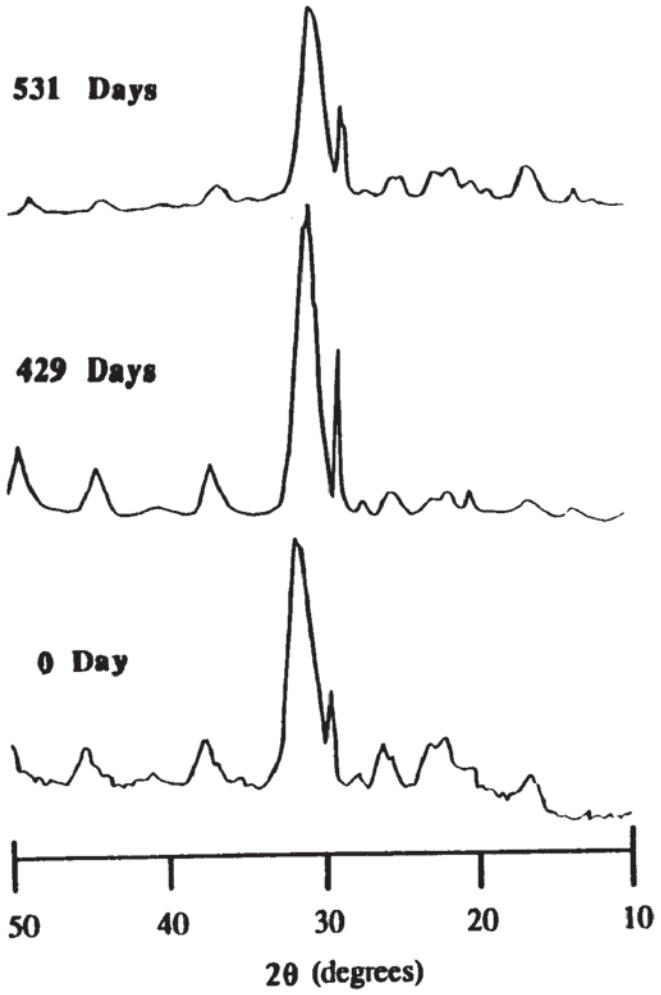


Figure 6.10 X-ray diffraction traces of 20% PHV copolymer (talc nucleated plaque) after various periods of degradation in pH 7.4 buffer at 37°C.

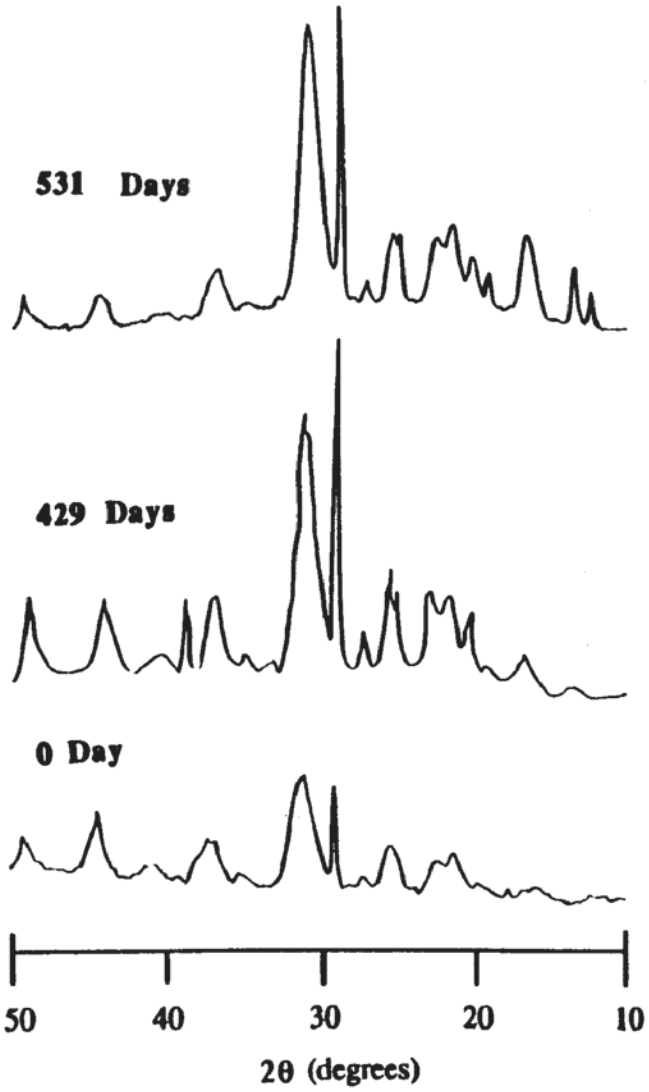


Figure 6.11 X-ray diffraction traces of 12% PHV copolymer (talc nucleated disc) after various periods of degradation in pH 7.4 buffer at 37°C.

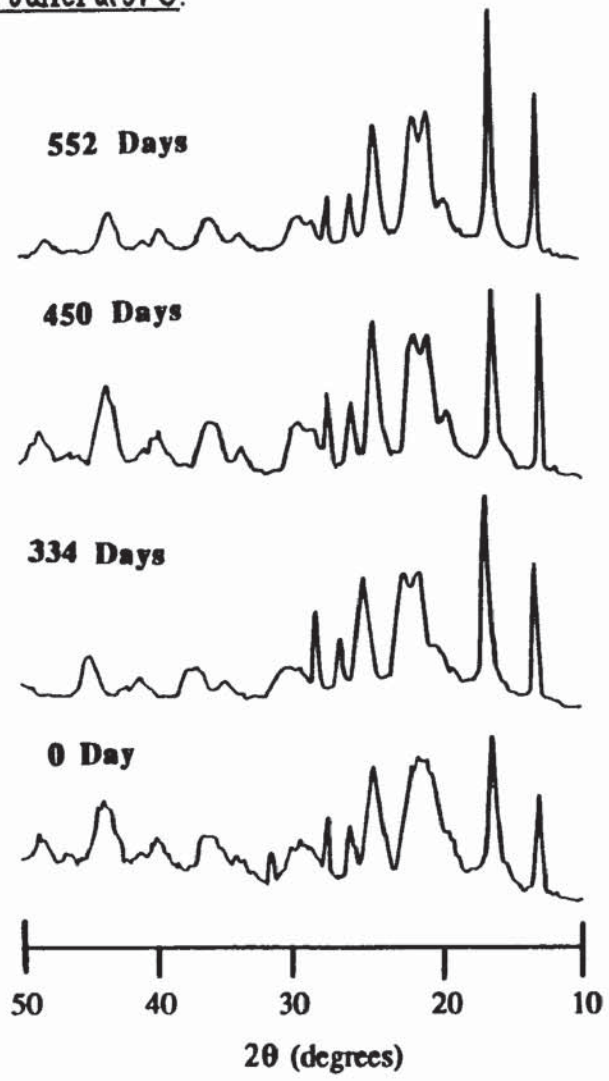


Figure 6.12 X-ray diffraction traces of 20% PHV copolymer (talc nucleated disc) after various periods of degradation in pH 7.4 buffer at 37°C.

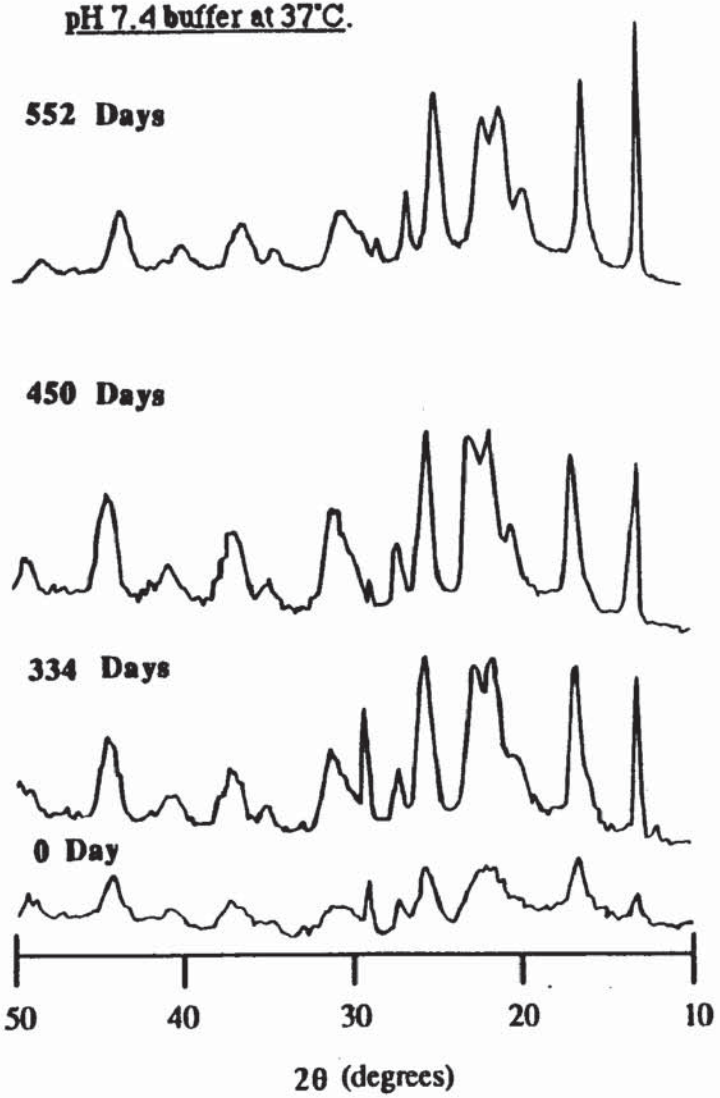


Figure 6.13 X-ray diffraction traces of 12% PHV/dextrin (10%) blend (injection moulded plaque), after various periods of degradation in pH 7.4 buffer at 37°C.

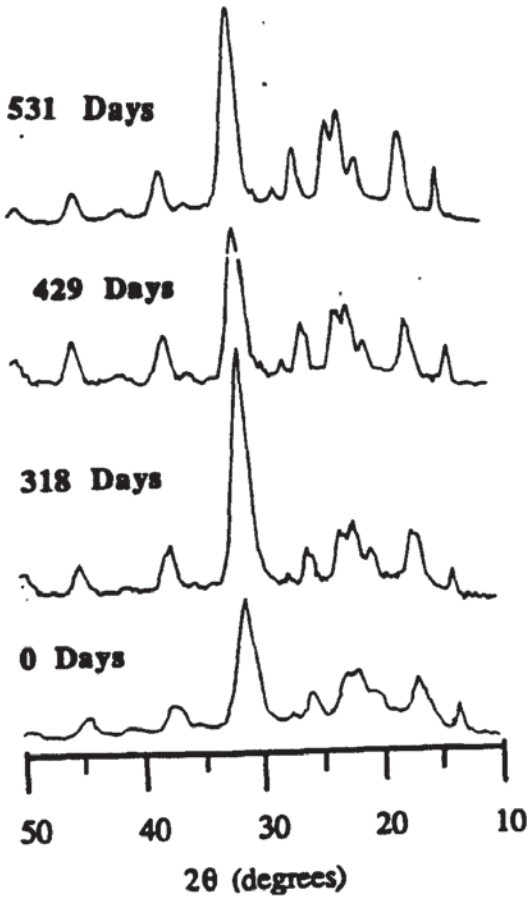


Figure 6.14 X-ray diffraction traces of 12% PHV/PCL 90/10 blend (apatite nucleated, injection moulded tensile piece), after various periods of degradation in pH 7.4 buffer at 37°C.

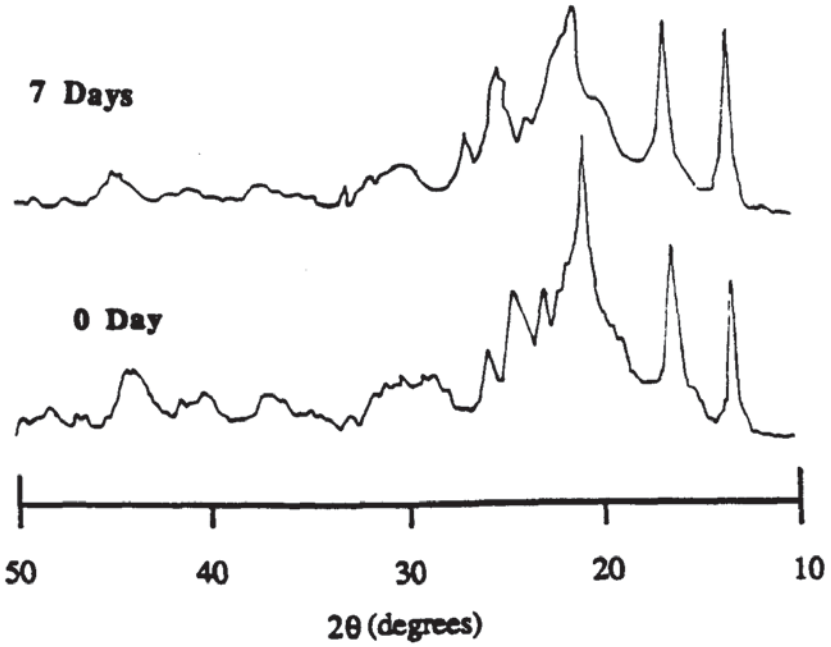


Figure 6.15 X-ray diffraction traces of 20% PHV/PCL 90/10 blend (apatite nucleated, injection moulded tensile piece), after various periods of degradation in pH 7.4 buffer at 37°C.

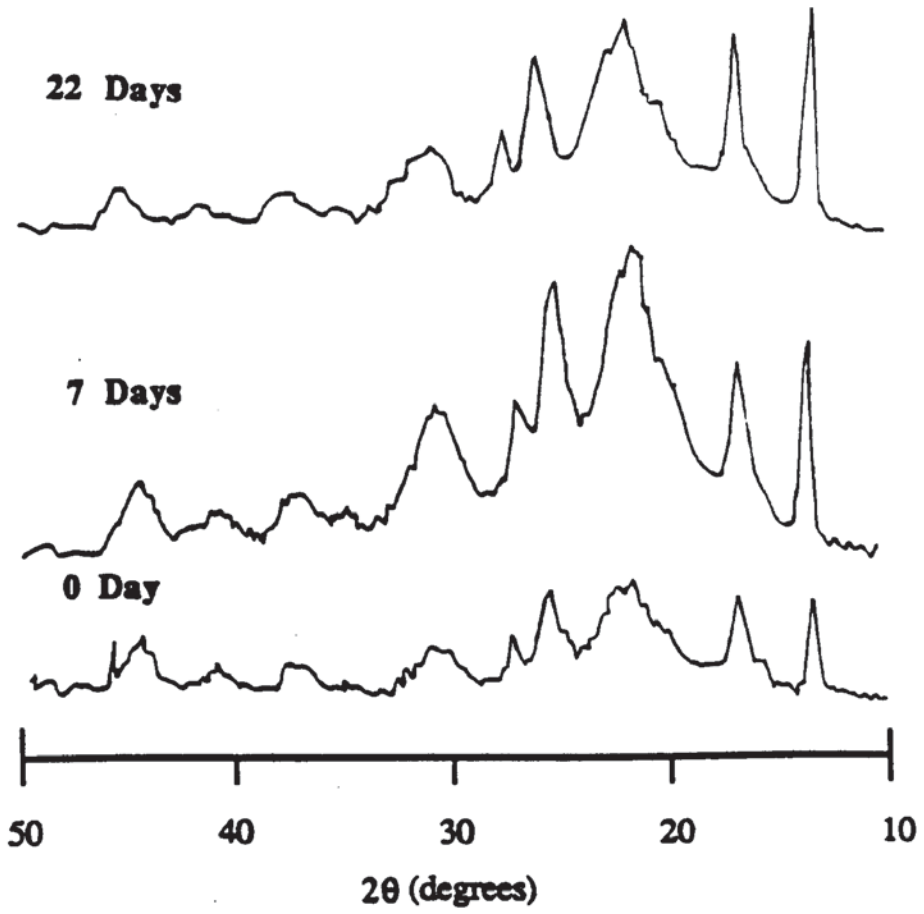
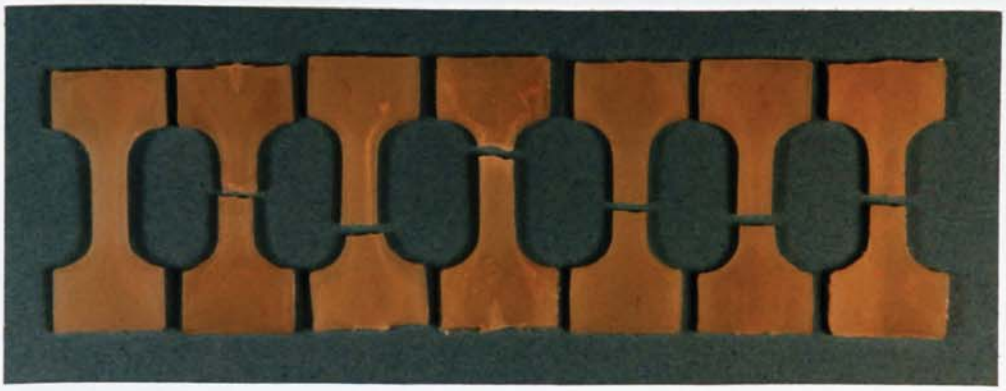
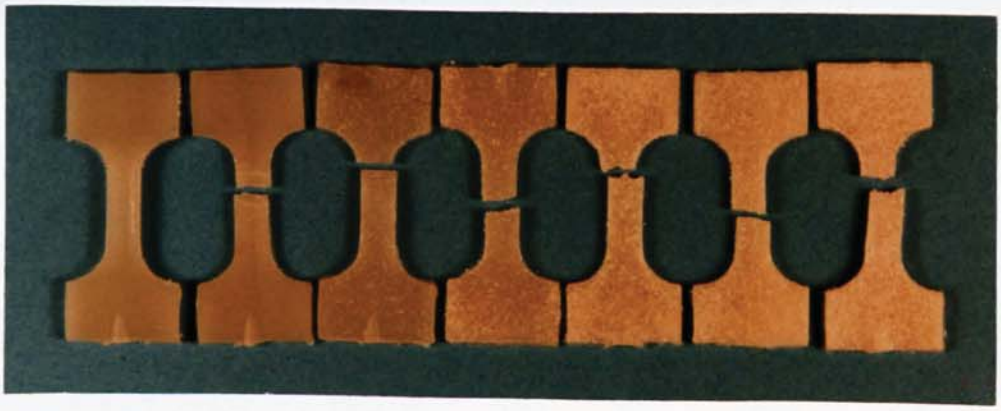


Plate 6.1 Photographic illustration of tensile pieces of 12% PHV copolymer (apatite nucleated), after various periods of degradation in pH 7.4 buffer at 37°C.



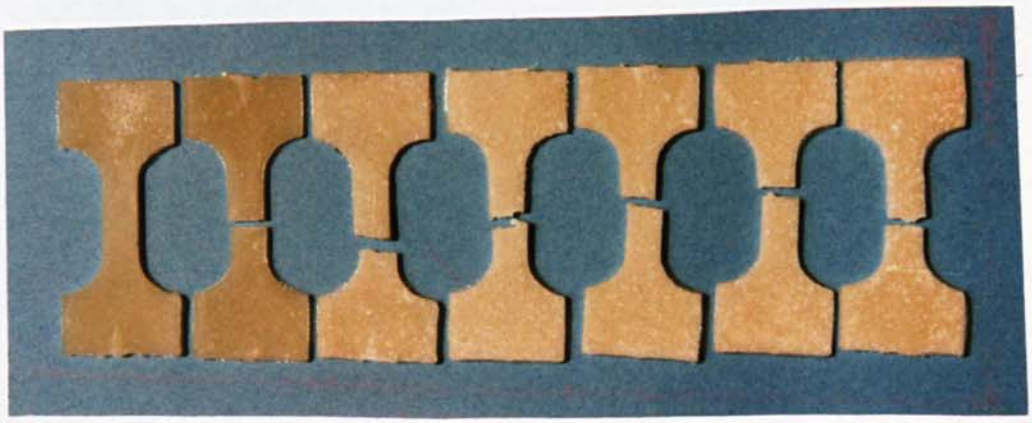
R 0 7 22 62 112 180

Plate 6.2 Photographic illustration of tensile pieces of 12% PHV/amylose (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



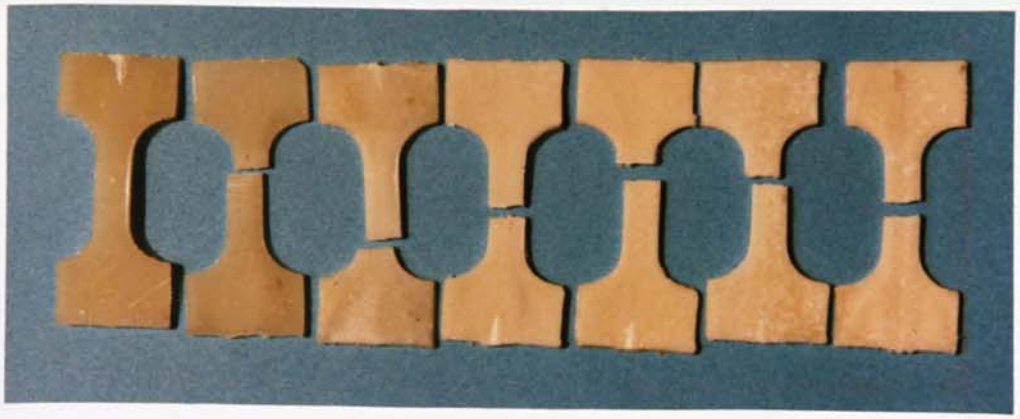
R 0 7 22 62 112 180

Plate 6.3 Photographic illustration of tensile pieces of 12% PHV/dextran (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



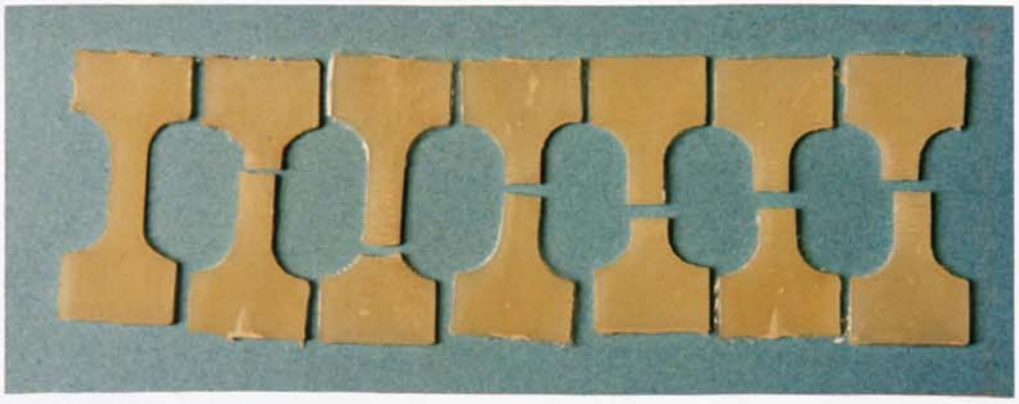
R 0 7 22 62 112 180

Plate 6.4 Photographic illustration of tensile pieces of 12% PHV/dextrin (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



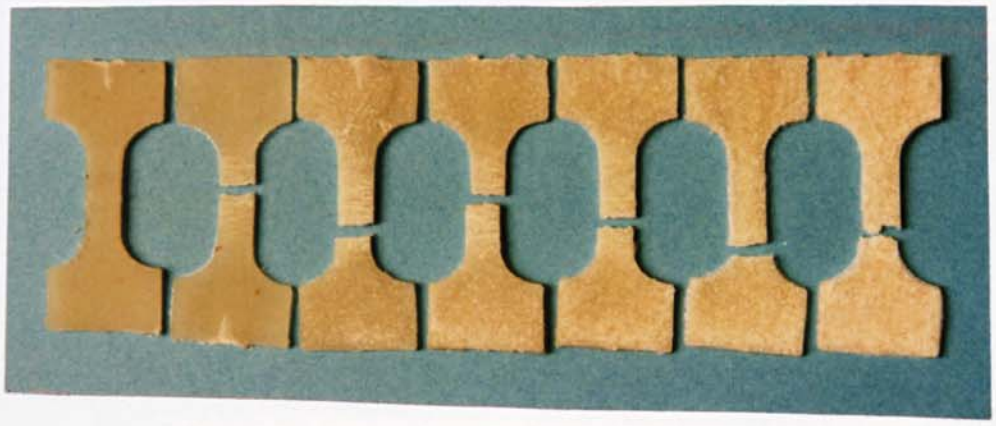
R 0 7 22 62 112 180

Plate 6.5 Photographic illustration of tensile pieces of 20% PHV copolymer (apatite nucleated) after various periods of degradation in pH 7.4 buffer at 37°C.



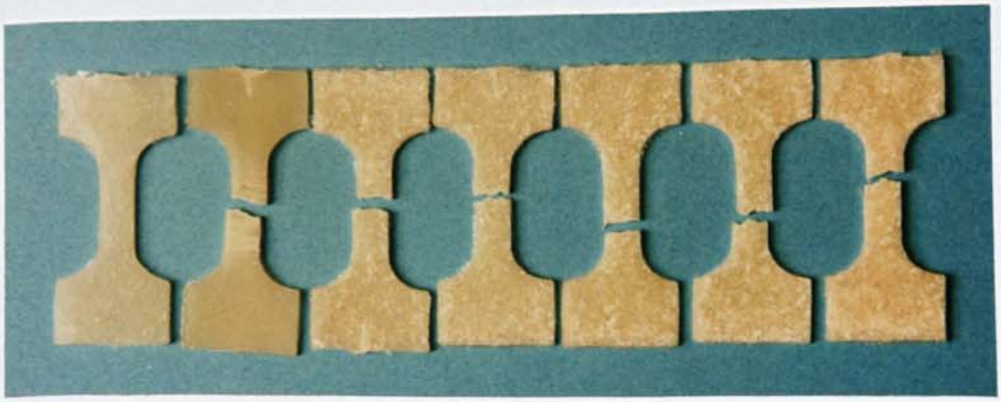
R 0 7 22 62 112 180

Plate 6.6 Photographic illustration of tensile pieces of 20% PHV/amylose (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



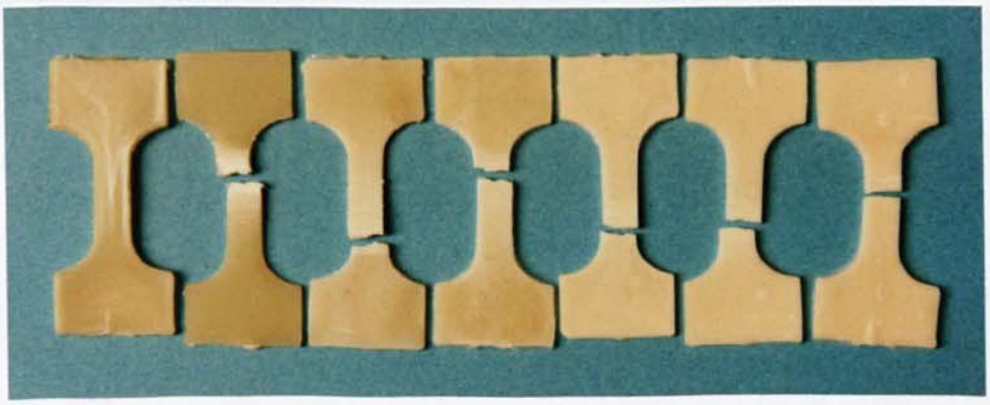
R 0 7 22 62 112 180

Plate 6.7 Photographic illustration of tensile pieces of 20% PHV/dextran (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



R 0 7 22 62 112 180

Plate 6.8 Photographic illustration of tensile pieces of 20% PHV/dextrin (10%) blend after various periods of degradation in pH 7.4 buffer at 37°C.



R 0 7 22 62 112 180

Plate 6.9 Photographic illustration of tensile pieces of 12% PHV/PCL (90/10) blend after various periods of degradation in pH 7.4 buffer at 37°C.

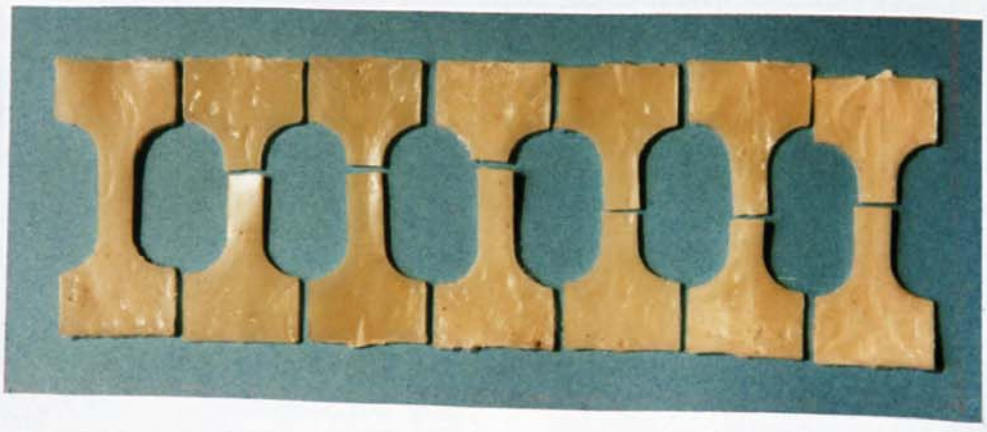


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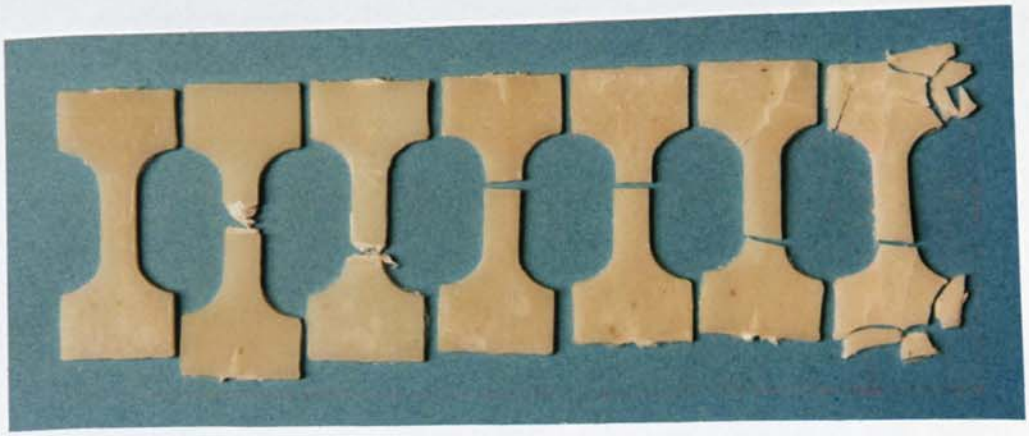
Plate 6.10 Photographic illustration of tensile pieces of 12% PHV/PCL (10/90) blend after various periods of degradation in pH 7.4 buffer at 37°C.

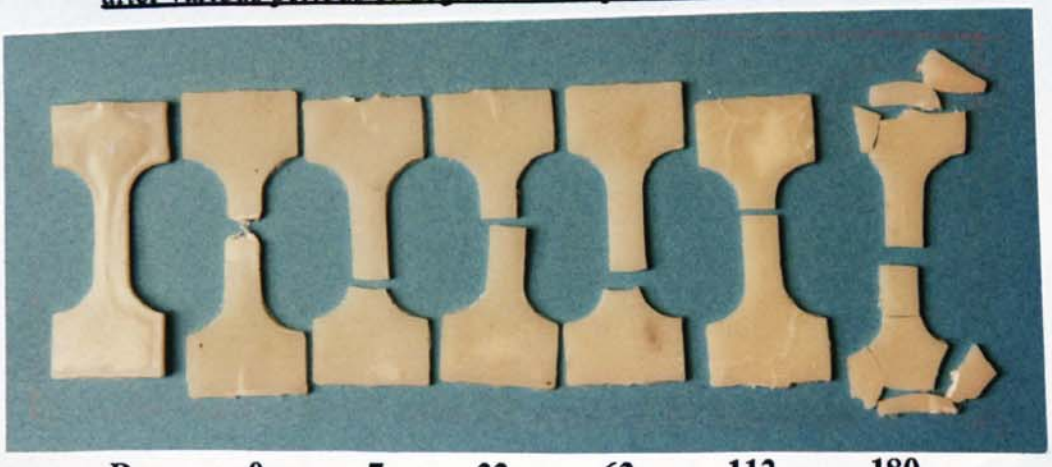


R 0 7 22 62 112 180

Plate 6.11 Photographic illustration of tensile pieces of 20% PHV/PCL (90/10) blend after various periods of degradation in pH 7.4 buffer at 37°C.

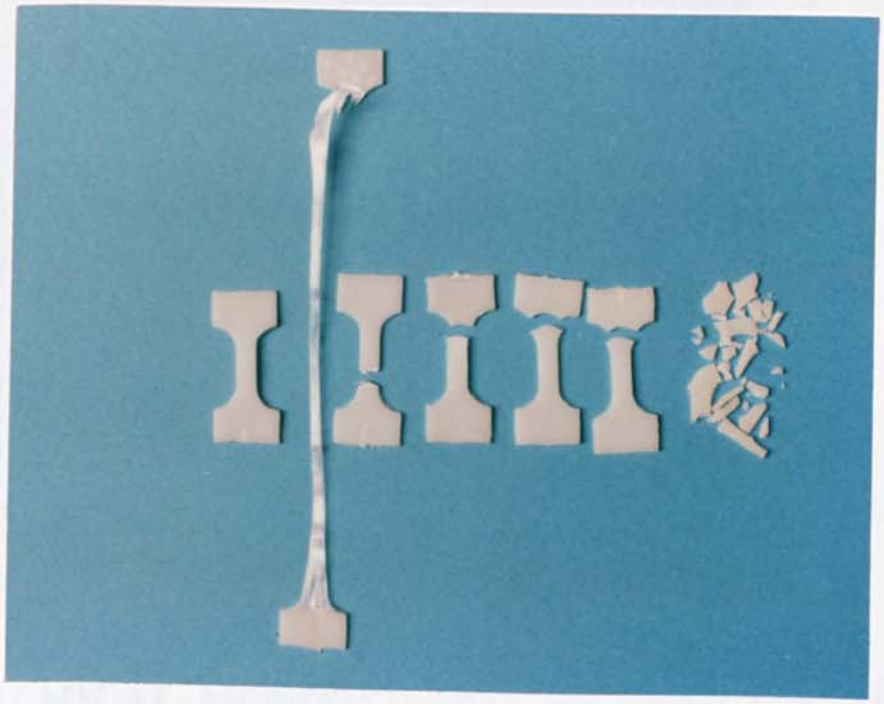
R 0 7 22 62 112 180

Plate 6.12 Photographic illustration of tensile pieces of 20% PHV/PCL (10/90) blend after various periods of degradation in pH 7.4 buffer at 37°C.



R 0 7 22 62 112 180

Plate 6.13 Photographic illustration of tensile pieces of PCL after various periods of degradation in pH 7.4 buffer at 37°C.



R 0 7 22 62 112 180

Note: **In Plates 6.1 to 6.13, an undegraded sample (R) is shown for reference purposes. The numbers below each plate indicate the degradation time (in days) after which that particular sample was tested.**

CHAPTER 7

CHEMICAL MODIFICATION OF POLYSACCHARIDES.

7.1 INTRODUCTION.

Polysaccharides are an important class of carbohydrates. They are naturally occurring biodegradable polymers having two major biological functions, as energy sources and as structural supports in both animal and plant cells. Two of the most important and abundant polysaccharides are starch and cellulose.

Amylose (a component of starch) and cellulose are both linear polymers of D-glucose units (Figure 7.1). In amylose the D-glucose units are linked through α, 1-4 glycosidic linkages, while in cellulose these linkages are β, 1-4 and, as a consequence, because of the β- glycosidic linkages are more stable than the α- linkages, cellulose is more stable to hydrolytic degradation than amylose. The body is capable of degrading amylose enzymatically (by α-amylase), but only slow hydrolytic degradation of cellulose is possible. This difference in the degradation rates of these polymers could be exploited to form a range of materials having various degradation rates. Such materials would be potentially very useful for fabrication into surgical fixation devices, which could degrade at different rates, depending on their particular function.

Both amylose and cellulose could be regarded as polyhydric alcohols, since the anhydroglucose unit contains one primary hydroxyl (C6) and two secondary hydroxyl groups (C2 and C3), (Figure 7.1). The presence of these regularly spaced polar hydroxyl groups in the polymer chains facilitates inter and intra-molecular hydrogen bonding, so both amylose and cellulose decompose on heating before they melt. The consequence of this hydrogen bonding in amylose and cellulose is that both of these biodegradable polymers cannot be fabricated into articles by relatively inexpensive commercial melt processing techniques such as injection moulding, melt spinning and melt extrusion.

Modification of the hydroxyl group(s) in the glucose repeat units of amylose and cellulose, by chemical means, such as esterification [191-193] results in the disruption of hydrogen bonding in the polymer chains. The melting point of the resulting derivatives is below the decomposition temperature of the initial polymer. Derivatives such as amylose and cellulose esters are thus soluble in common organic solvents, possess thermoplastic properties, but the degradation products are not normal body metabolites.

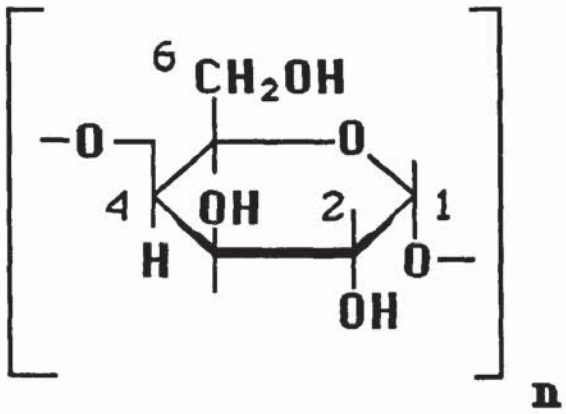


Figure 7.1 D - glucose subunit.

A potentially interesting and new route for the preparation of biodegradable, thermoplastic amylose and cellulose derivatives, for biomedical applications, would be the grafting of biodegradable ester units onto the hydroxyl group(s) in the D-glucose units of the polysaccharide backbone. It would be interesting to prepare, in the first instance, a precursor that is both biodegradable and thermoplastic, which could be used to mould biodegradable surgical fixation devices. The use of naturally occurring α-hydroxy acids (which themselves form biodegradable poly-α-esters) as grafted biodegradable units on the D-glucose unit could lead to a promising group of materials. Lower members of the α–hydroxy carboxylic acid series, such as lactic and glycolic acid, are normal body metabolites. Lactic and glycolic acid, on mild heating form cyclic dimers, known as lactides and glycolides, respectively. When subjected to a catalytic

ring-opening procedure, these dimers polymerise to poly-α-esters, which are hydrolytically unstable (50). In recent years polyglycolic, polylactic and copolymers of these acids have been used in the medical field, because of their hydrolytic instability, as biodegradable polymers(50-59, 62-76). Thus, the grafting of biodegradable poly-α-ester units onto the hydroxyl groups of amylose and cellulose could lead to materials that are both biodegradable and thermoplastic.

A new and potentially useful route of grafting poly-α-ester groups onto the hydroxyl groups in amylose and cellulose is by using the very reactive anhydrosulphite derivatives of the α-hydroxy carboxylic acids. Anhydrosulphites or 1.3.2, dioxanthiolan 4-one-2 oxides are cyclic derivatives (Figure 7.2) which, through ring-opening polymerisation can be converted to poly-α-esters by a number of routes, (Figure 7.3); the ring-opening being catalysed by bases, aprotic or protonic nucleophiles(194). It is probable that in a reaction between the anhydrosulphite and either amylose or cellulose, the presence of a large number of hydroxyl groups in these polymers, will cause ring-opening of the anhydrosulphite, leading to poly-α-ester groups being grafted onto the hydroxyl group

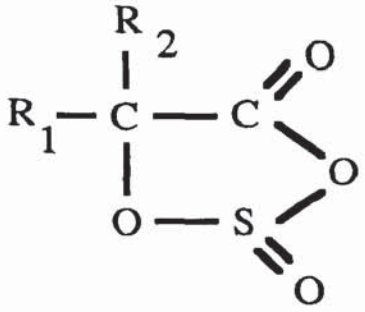
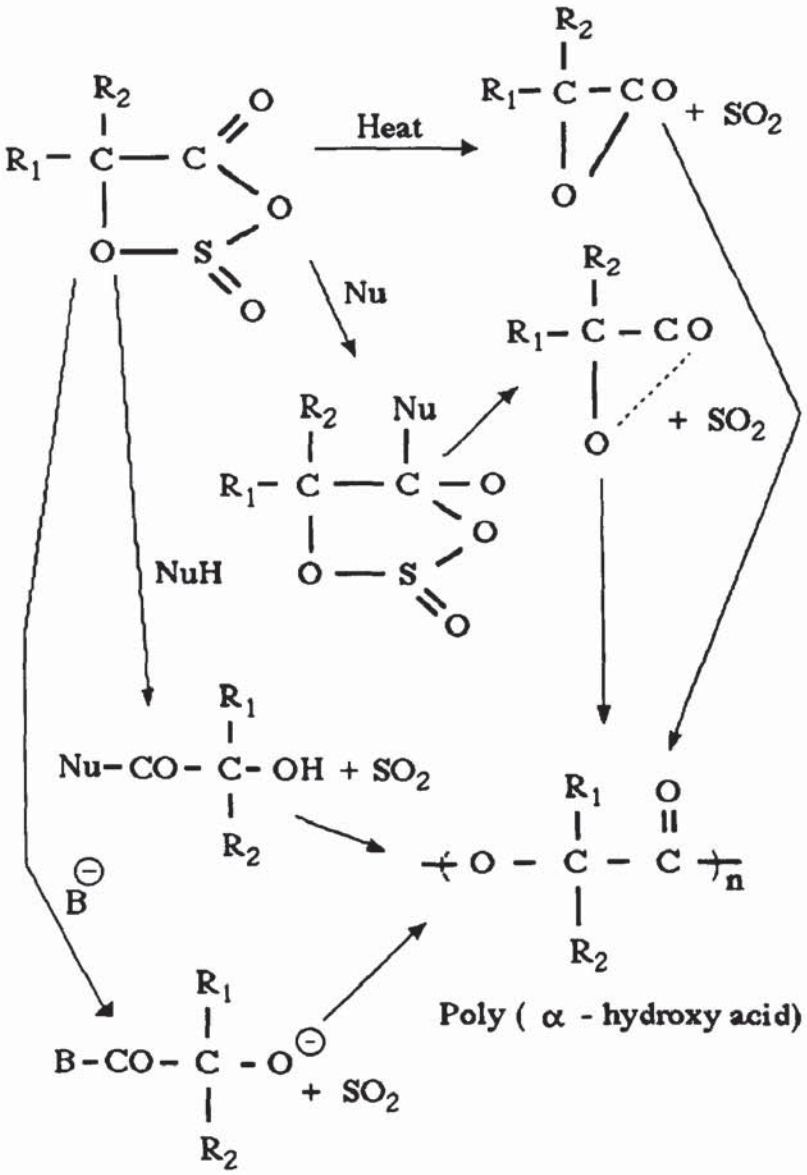


Figure 7.2 Anhydrosulphite of α - hydroxy acid.



Nu = Aprotic nucleophile, e.g. pyridine, triethylamine.

NuH = Protonic nucleophile, e.g. benzylamine, benzyl alcohol.

B^- = Strong base, alkoxide.

Figure 7.3 Various routes for the ring opening of anhydrosulphites.

positions in the glucose unit. An idealised mechanism is shown in Figure 7.4. Ideally, esterification reaction should be carried out in a homogeneous solution of the polysaccharide, using a solvent in which both the anhydrosulphite and the polysaccharide

are soluble. Amylose and cellulose, because of hydrogen bonding, are insoluble in common organic solvents, but soluble in solvents either capable of breaking hydrogen bonding, or acting as proton donors. Such solvents are either aqueous potassium and sodium hydroxide solution, or very polar organic solvents such as dimethyl sulphoxide, pyridine and dimethylformamide.

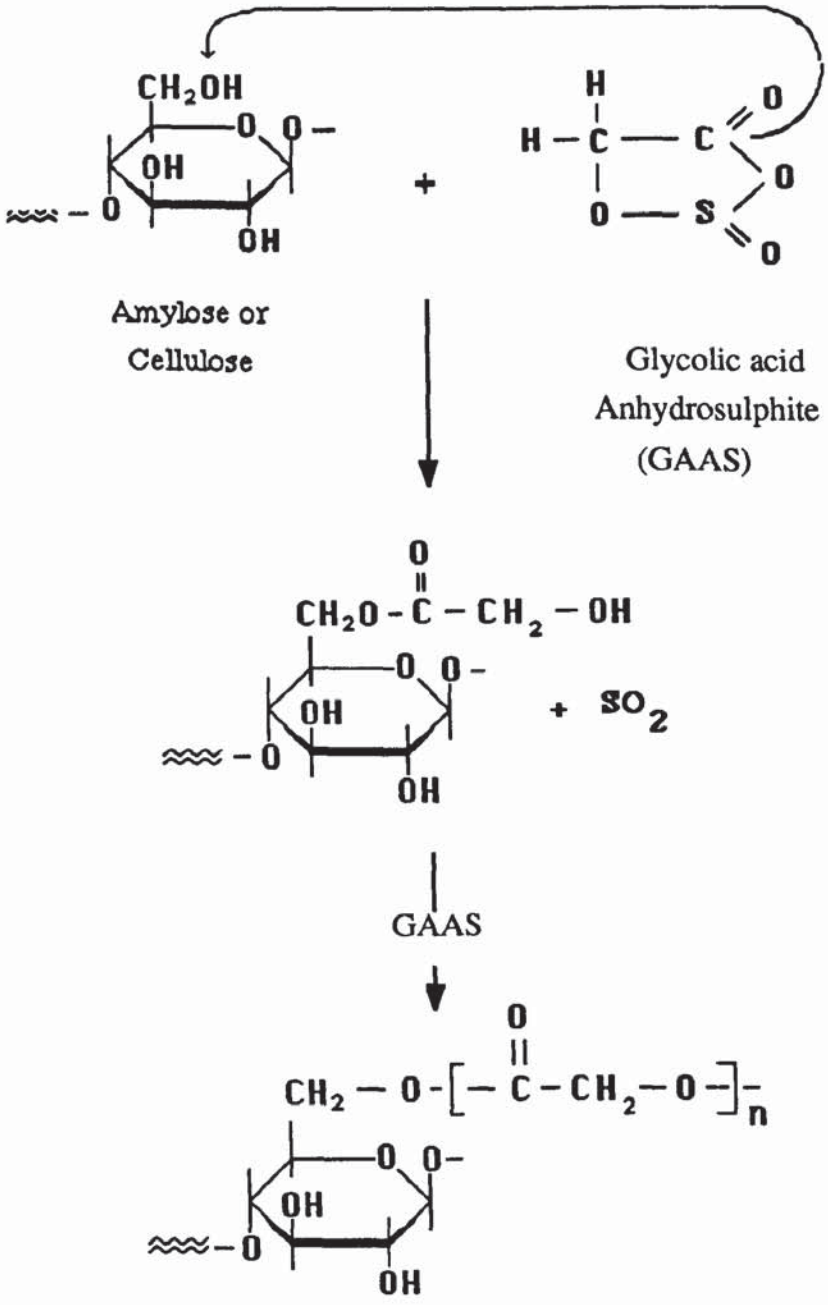


Figure 7.4 Idealised mechanism for the grafting of poly - α - ester groups onto hydroxyl groups in amylose and cellulose.

The ring opening of the anhydrosulphite, as mentioned previously, is catalysed by nucleophilic reagents, so in using the above mentioned solvents, as the reaction medium, problems will be created. These problems are the competing reactions of esterification of the hydroxyl groups in the glucose unit by the anhydrosulphite, and the decomposition of the anhydrosulphite by the solvent. It may be possible to control the latter reaction by carrying out the esterification in a non-solvent for the ester (heterogeneous esterification), or by use of mixtures of solvents. These mixtures of solvents should ideally keep the polysaccharide in solution and also reduce the catalytic effect of the solvents on the anhydrosulphite decomposition.

The following section deals with the synthesis of the anhydrosulphite and subsequent attempts at the esterification of amylose and cellulose by the anhydrosulphite.

7.2 ANHYDROSULPHITES.

7.2.1 INTRODUCTION.

Anhydrosulphites or 1.3.2, dioxanthiolan-4-one-2 oxides may be prepared by two methods. The first method described by Blaise and Montagne[195], is the action of thionyl chloride on an α-hydroxy acid[196-199]. This method, however, leads to several by-products being formed (Figure 7.5). The second route to the anhydrosulphite is by using the novel technique developed by Ballard and Tighe[151], involving the use of metal salts of α-hydroxy acids, especially the copper (II) salts. The copper (II) salts of the α-hydroxy acids can then be reacted with thionyl chloride to yield the anhydrosulphite, with less impurities [194,199-200].

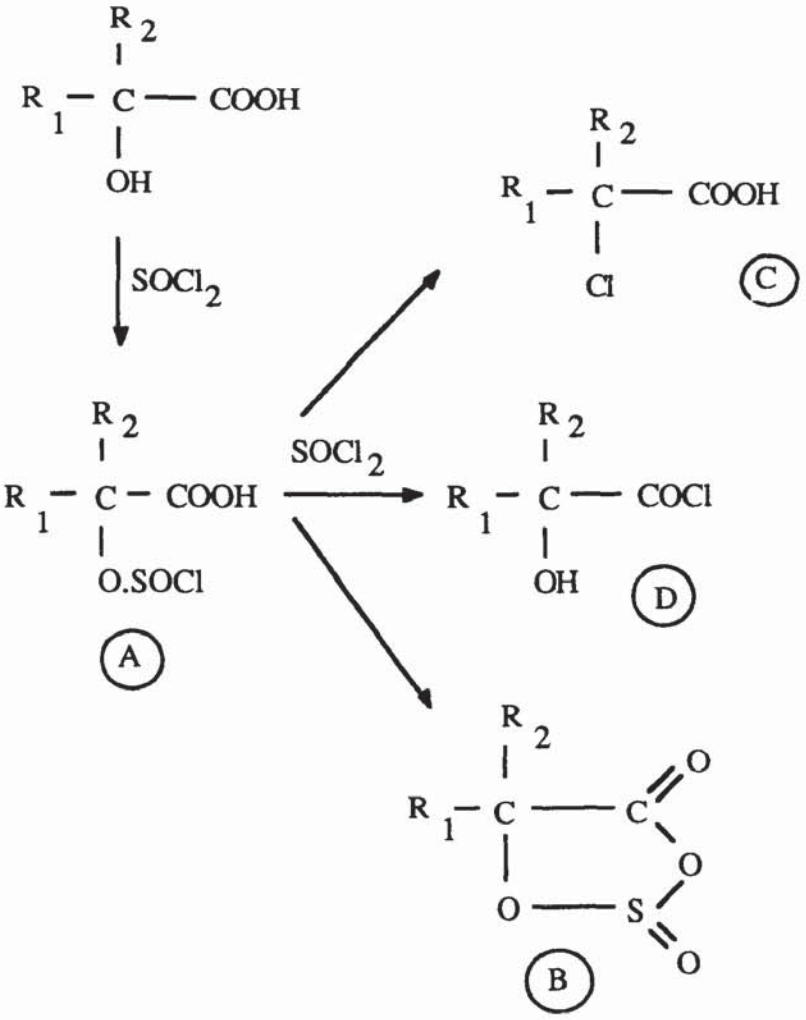


Figure 7.5 Reactions of thionyl chloride with α - hydroxy acids.

7.2.2 SYNTHESIS AND CHARACTERISATION OF CUPRIC GLYCOLATE.

One mole of glycolic acid, dissolved in 150 ml of distilled water was neutralised with ammonium hydroxide and the solution boiled to remove excess ammonia. Copper (II) chloride (0.5 moles) dissolved in 100 ml of distilled water was added slowly to an ice-cooled, neutral solution of the ammonium salt until precipitation of copper (II) glycolate was complete. The salt was washed with distilled water and diethyl ether and finally dried by heating at 100°C under vacuum for 50 hours. The blue colour of the crystals

intensified on drying.

Cuprammonium salts are well known to possess a deep blue colour and could conceivably be present as impurity. However, cuprammonium salts are soluble in cold water and would not be expected to precipitate with copper (II) glycolate as they would be removed by filtration and subsequent washing of the copper (II) glycolate.

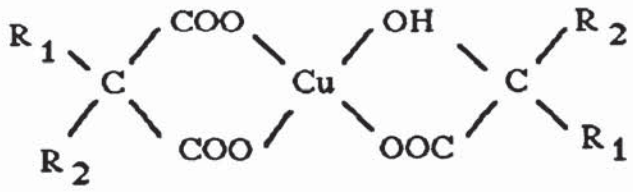


Figure 7.6

Copper (II) salts, (Figure 7.6), can be easily characterised by their infra-red spectra. The free hydroxyl stretching absorption in the region of 3400 wave numbers (Figure 7.7), shown by the free acid is displaced and changed in shape in the copper (II) salt, (Figure 7.8). There are believed to be hydroxyl groups that co-ordinate with the copper (II) metal. The carbonyl stretching frequency at 1740 cm^{-1} in the acid is replaced by the carboxylate anion stretching band which occurs at a lower wave number (1570 cm^{-1}).

The advantages of using copper (II) salts in the synthesis of anhydrosulphite are:-

a) Copper (II) salts are easily prepared and may easily be obtained in an anhydrous state, and,

b) The chloride containing by-products of ring closure are removed as the precipitate of anhydrous copper (II) chloride on filtering.

The reaction involved in the preparation of copper (II) glycolate is shown in Figure 7.9.

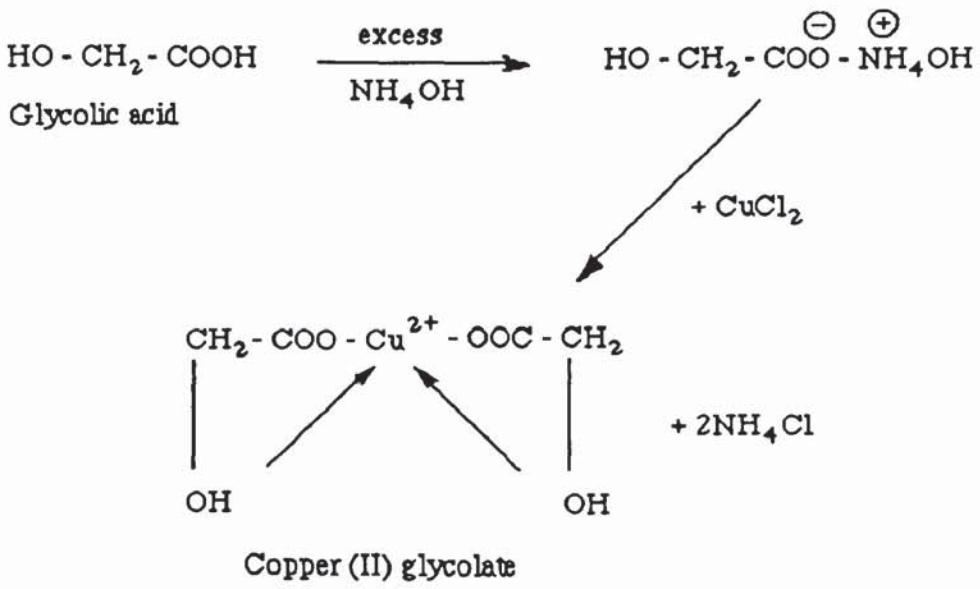


Figure 7.9 Preparation of copper (II) glycolate.

7.2.3 SYNTHESIS AND CHARACTERISATION OF GLYCOLIC ACID ANHYDROSULPHITE.

The anhydrosulphites of α-hydroxy acids can be prepared by either the action of thionyl chloride on the dry α-hydroxy acid, or on the anhydrous copper (II) salt of α-hydroxy acid. This latter approach was used, as described below.

To the dry anhydrous copper (II) glycolate, stirred in 235 ml of anhydrous diethyl ether at 0 - 5°C, 84.6 g of thionyl chloride in 94 ml anhydrous ether was added dropwise over a half-hour period. The resulting precipitate of copper (II) chloride was filtered off and washed with anhydrous ether to remove any anhydrosulphite. The mother liquor was left

for one hour, under reduced pressure (10 - 15 mm Hg), at room temperature and then stirred for 16 hours, under reduced pressure to remove final traces of thionyl chloride and ether.

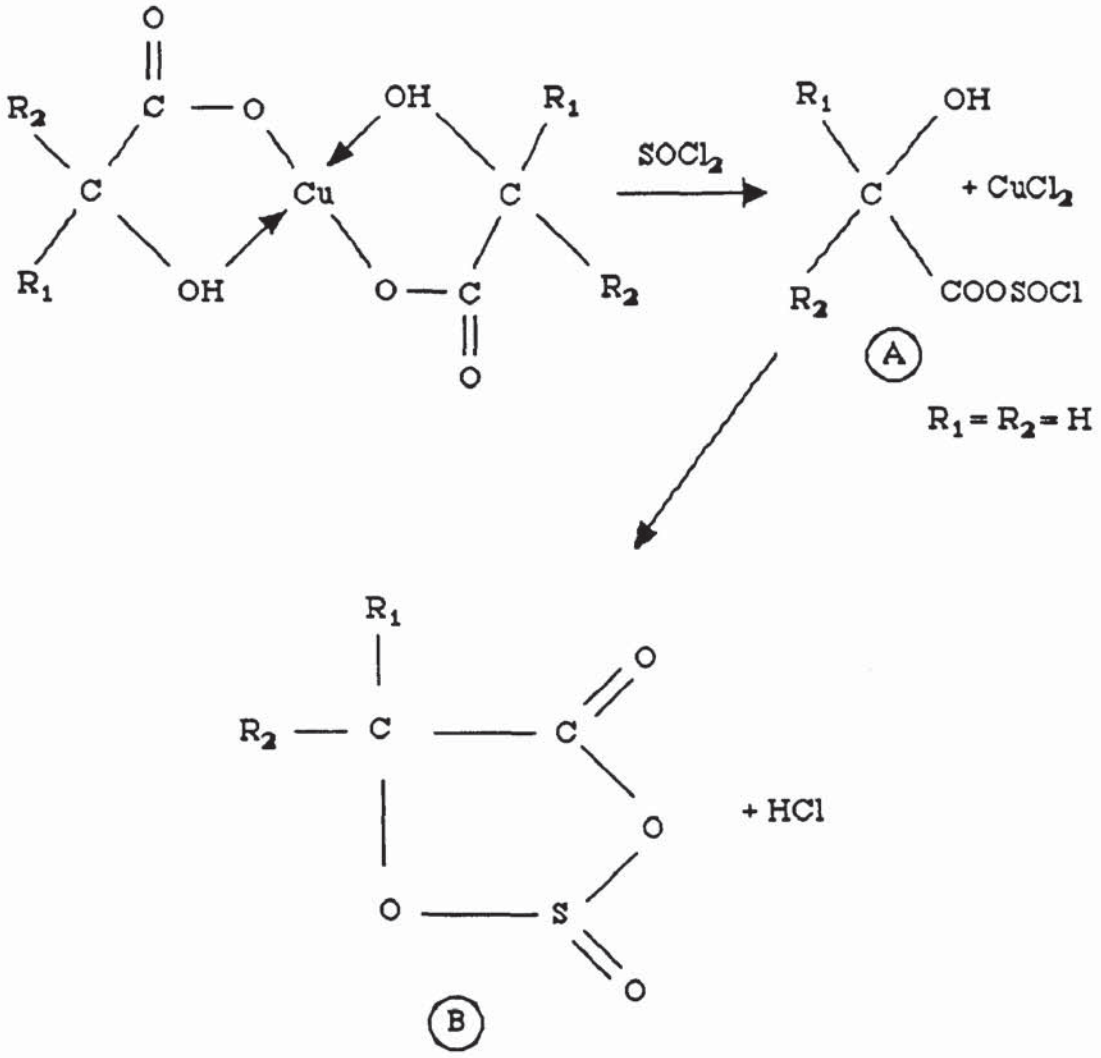


Figure 7.11 Preparation of glycolic acid anhydrosulphite from copper (II) glycolate.

The pale yellow liquid resulting from this operation was characterised by infra-red spectroscopy (Figure 7.10). The mechanism of anhydrosulphite preparation via copper

(II) glycolate is shown in Figure 7.11 [194]. The thionyl chloride attacks the carboxylate anion, rather than the hydroxyl group of the copper (II) salt, with the product intermediate being an acyl chlorosulphinate (A, Figure 7.11). The anhydrosulphite (B), is formed by loss of hydrogen chloride from the acyl chlorosulphinate, and concurrent ring closure.

The designation of the α-chloroacid chloride as a major source of impurity in the anhydrosulphite is substantiated to some extent by the work of Tighe [201] on the reaction between α-hydroxy acids and thionyl chloride. The impurity found in the anhydrosulphite of α-hydroxy isobutyric acid had the formula;

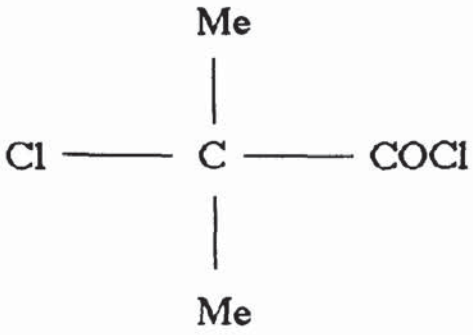


Figure 7.12

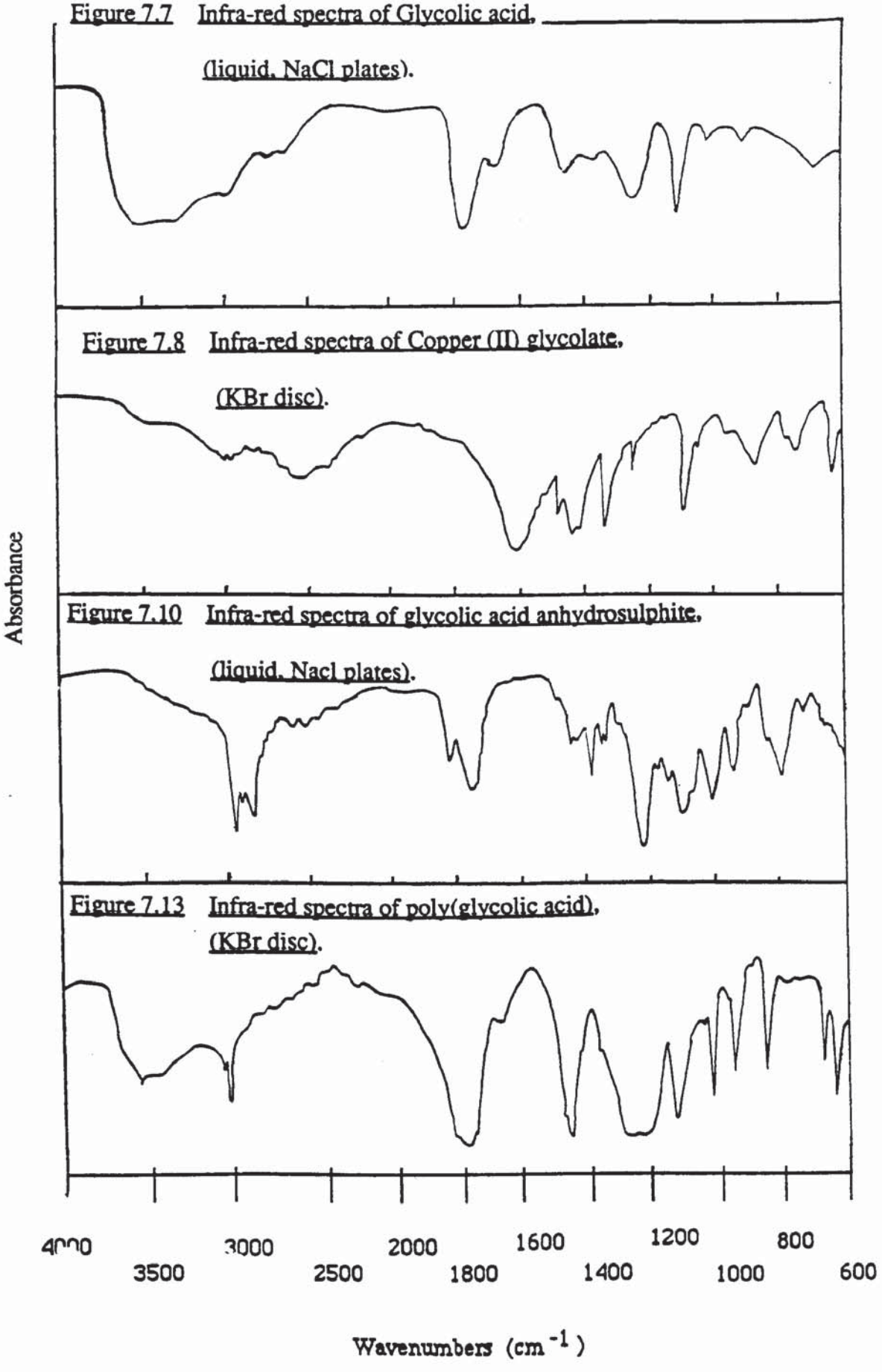
It might therefore be expected, by analogy, that chloroacetyl chloride would be present as an impurity in glycolic acid anhydrosulphite. This was observed in the infra-red spectra of the synthesised glycolic acid anhydrosulphite. Further purification of glycolic acid anhydrosulphite was not undertaken and it was used for preliminary experiments as soon as possible to avoid premature decomposition.

Figure 7.7 Infra-red spectra of Glycolic acid, (liquid, NaCl plates).

Figure 7.8 Infra-red spectra of Copper (II) glycolate, (KBr disc).

Figure 7.10 Infra-red spectra of glycolic acid anhydrosulphite, (liquid, Nacl plates).

Figure 7.13 Infra-red spectra of poly(glycolic acid), (KBr disc).



For all spectra, air used as reference.

These reactions examined were either (a) esterification of hydroxyl groups of amylose and cellulose by the anhydrosulphite, or (b) ester-interchange of the anhydrosulphite with an ester, such as cellulose acetate, to form in the first instance a modified natural polymer with polyester side groups. These reactions are described in the next section.

7.3. ESTERIFICATION OF AMYLOSE AND CELLULOSE.

7.3.1 USING CHLORINATED SOLVENTS.

Initial attempts to achieve esterification of cellulose by glycolic acid anhydrosulphite (GAAS) in methylene chloride were unsuccessful and the end product after 4 hours of refluxing at 42°C, was unmodified cellulose. It was felt that a catalyst may improve the chances of esterification and, hence, pyridine[202] was used.

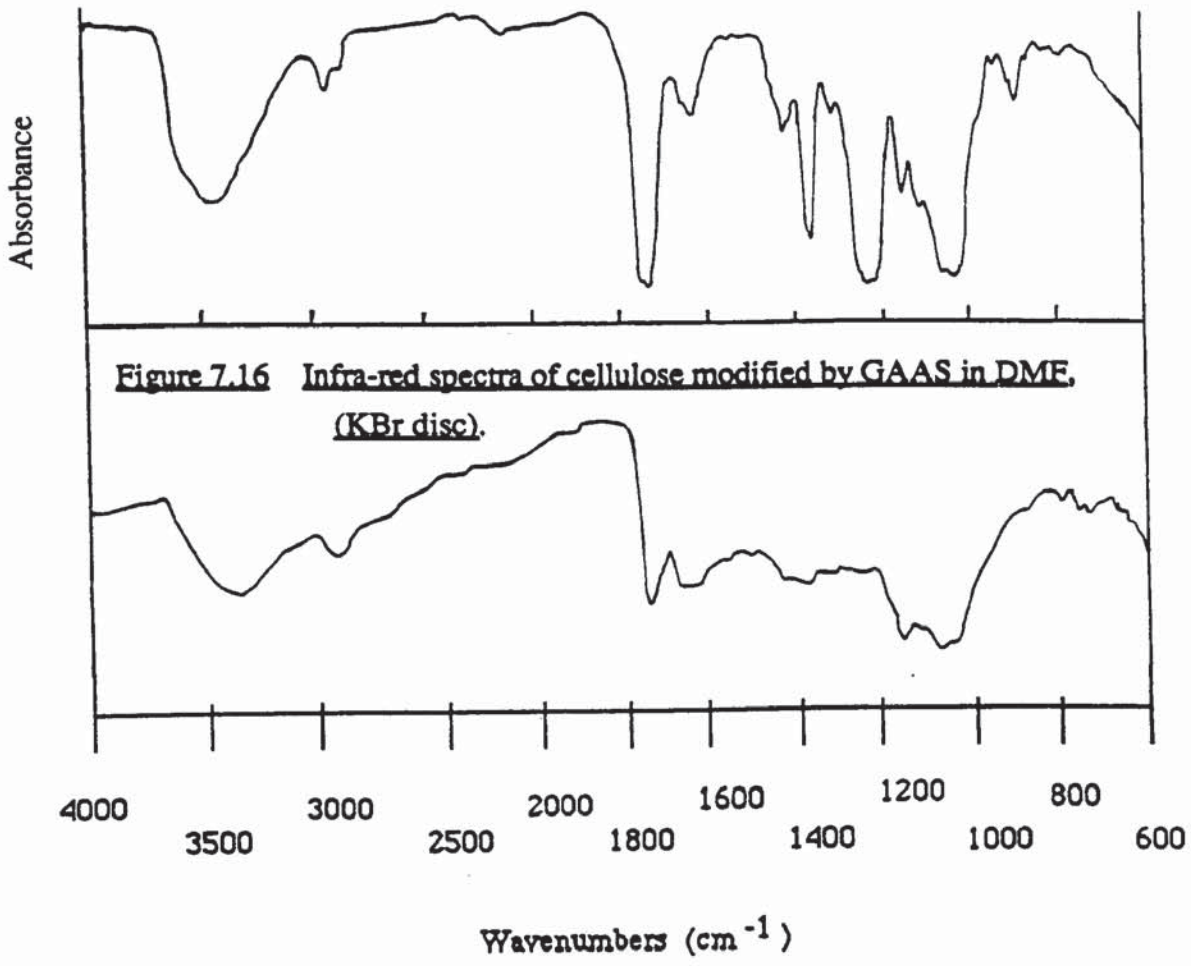
The reaction was carried out by swelling 2 grams of dried cellulose in 50 mls of dry methylene chloride, contained in a 100 ml quick fit flask, stirred at room temperature.

GAAS (3 grams) and 5 mls of dried pyridine were added dropwise to the reaction mixture. This was refluxed for 4 hours at 42°C, after which time the heterogeneous mixture was filtered, the filterate washed with methylene chloride and dried at 100°C. Addition of methanol to the mother liquor caused precipitation of fine black particles, which were filtered, washed and dried. Identification of these two products by infra-red spectroscopy showed that they were polyglycolic acid and cellulose (Figures 7.13 and 7.14, respectively).

Figure 7.14 Infra-red spectra of cellulose, (KBr disc).

Figure 7.15 Infra-red spectra of cellulose acetate, (film cast from chloroform).

Figure 7.16 Infra-red spectra of cellulose modified by GAAS in DMF, (KBr disc).



For all spectra, air used as reference.

Organic bases, such as pyridine, are known to polymerise anhydrosulphites, and because pyridine was found to be a very reactive catalyst, causing rapid decomposition of the anhydrosulphite, the need then was to use bases which had a lower reactivity. Smith[202] studied the reactivity of different bases with α-hydroxy carboxylic acid anhydrocarboxylates and found that the reactivity of substituted pyridines, such as 2,6-dimethoxypyridine was lower than that of pyridine. The reactivity of the anhydrosulphites to pyridine and substituted pyridines is greater than that of anhydrocarboxylates to these bases.

The esterification of cellulose by GAAS was attempted using 2,6-dimethoxy pyridine as the catalyst in different solvents such as chloroform, dioxane and tetrahydrofuran (THF).

The end products of these reactions were unmodified cellulose and polyglycolic acid, in each case. Esterification of amylose under similar conditions resulted in the end products being unmodified amylose and polyglycolic acid.

It is probable that esterification of amylose and cellulose by glycolic acid anhydrosulphite in a more effective solvent or swelling agent for these polymers could be more promising as the anhydrosulphite would be able to penetrate the open structure caused by the breaking up of hydrogen bonds between the polymer chains.

7.3.2. USING SWELLING AGENTS:

Cellulose is swollen by dimethylformamide (DMF) and glacial acetic acid, while amylose is soluble in aqueous solutions of pyridine and is swollen by acetic acid. Esterification of cellulose and amylose by GAAS using these solvents was attempted.

7.3.2.1. PYRIDINE.

GAAS (1.5 g) was added to a suspension of amylose (1.5 g) in 20 mls of pyridine at room temperature. The mixture was refluxed for 2 hours at 110°C, during which time it became very viscous. More pyridine (10 mls) was added and the mixture refluxed for another hour and then filtered. Particulate material was absent from the solution, and addition of methanol to the filterate caused precipitation of fine black particles. Infra-red spectra of the dried product showed it to be amylose.

As mentioned previously, anhydrosulphites are polymerised by organic bases such as pyridine, so it is probable that rapid premature polymerisation of GAAS to the poly-α-ester occurs. Further attempts at esterifying amylose in pyridine were not made, due to the enhanced catalytic effect of pyridine with the GAAS.

7.3.2.2. DIMETHYLFORMAMIDE (DMF).

DMF is a swelling agent for cellulose and hence attempts were made at esterifying cellulose in DMF.

Cellulose (2 g, acetic acid dried) was stirred in 30 mls of DMF. GAAS (11 g) was added over a 15 minute period at room temperature. The white mixture turned to yellow and then brown and viscous, as it was heated at 50°C for 15 minutes. To decrease the viscosity of the solution, further 10 mls of DMF were added and the solution refluxed at 153°C for 112 hours. Addition of methanol to the filterate caused precipitation of fine

Figure 7.17 Infra-red spectra ot cellulose modified by GAAS/glycolic acid. (KBr disc).

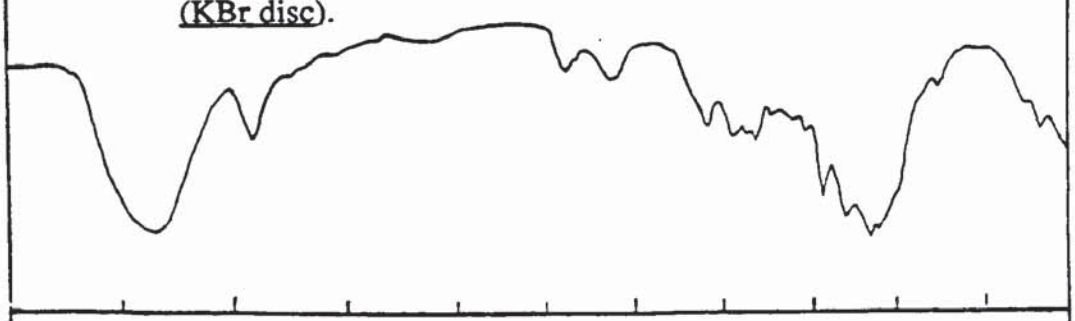


Figure 7.18 Infra-red spectra of cellulose modified by GAAS in dioxane. (KBr disc).

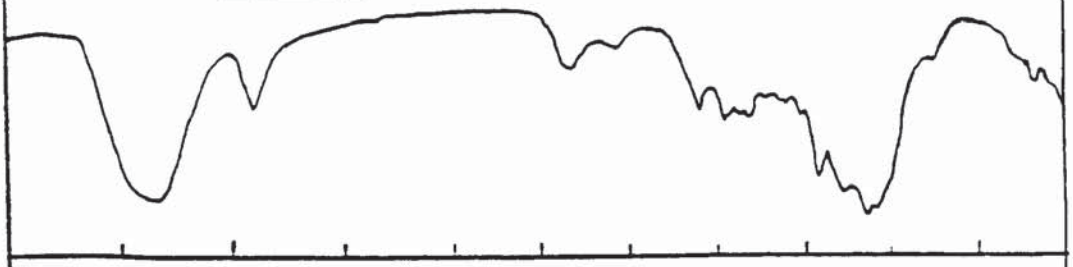


Figure 7.19 Infra-red spectra of glycolic acid. (KBr disc).

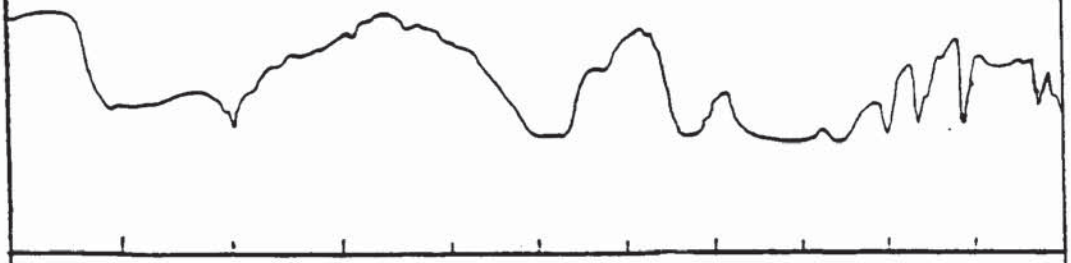
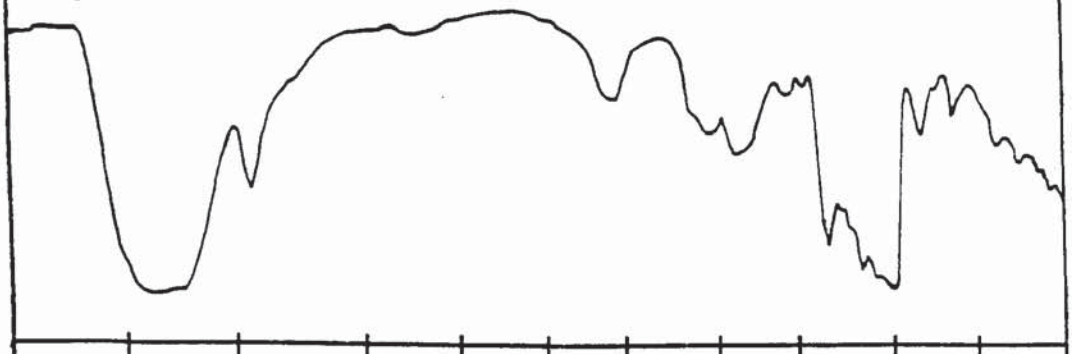


Figure 7.20 Infra-red spectra of amylose. (KBr disc).



For all spectra, air was used as reference.

black particles which were recrystallised from DMF and dried in the oven. The precipitate was found to be insoluble in carbon tetrachloride but soluble in DMSO and examination by infra-red spectroscopy (Figure 7.16) showed the presence of a carbonyl group at 1725 cm^{-1}. Although the NMR spectra (Figure 7.25) of this product (in DMSO) is complex, the (probable) acidic hydroxyl group at $\delta = 8.0$, (chemical shift parameter), reinforces the idea that the original cellulose has been modified. Furthermore, because neither the IR nor NMR spectra of this product correspond with those of cellulose acetate (Figures 7.15 and 7.24), one is led to believe that possibly some substitution of the hydroxyl groups has occurred.

7.3.2.3. ACETIC ACID.

Cellulose is swollen by acetic acid and is used in the esterification of cellulose with acetic anhydride as a solvent for cellulose acetate. Initial attempts were made at the esterification of cellulose by acetic acid alone. After 14 hours refluxing at 115°C, cellulose was not acetylated by acetic acid.

Using a mixture of acetic acid, acetic anhydride and GAAS, with a small amount of 2,6-dimethoxy pyridine, cellulose acetate was formed from cellulose. Esterification of cellulose in acetic acid with increased amounts of GAAS resulted in the formation of cellulose acetate. The amount of acetic acid in the mixture was reduced further and esterification attempted again in the following manner.

Cellulose (2g) was swollen in 20 mls of glacial acetic acid. GAAS (34 g) in 20 mls of dry THF and 0.2 mls pyridine was added to the above mixture, which was refluxed for 4

hours at 65°C and for 2 hours at 112°C. The mixture was filtered, the precipitate washed with water, methanol and acetone. Infra-red and NMR spectra of the product were identical to those of cellulose acetate, so it is most probable that cellulose acetate has been formed.

7.3.3. ESTERIFICATION IN SOLVENTS FOR AMYLOSE.

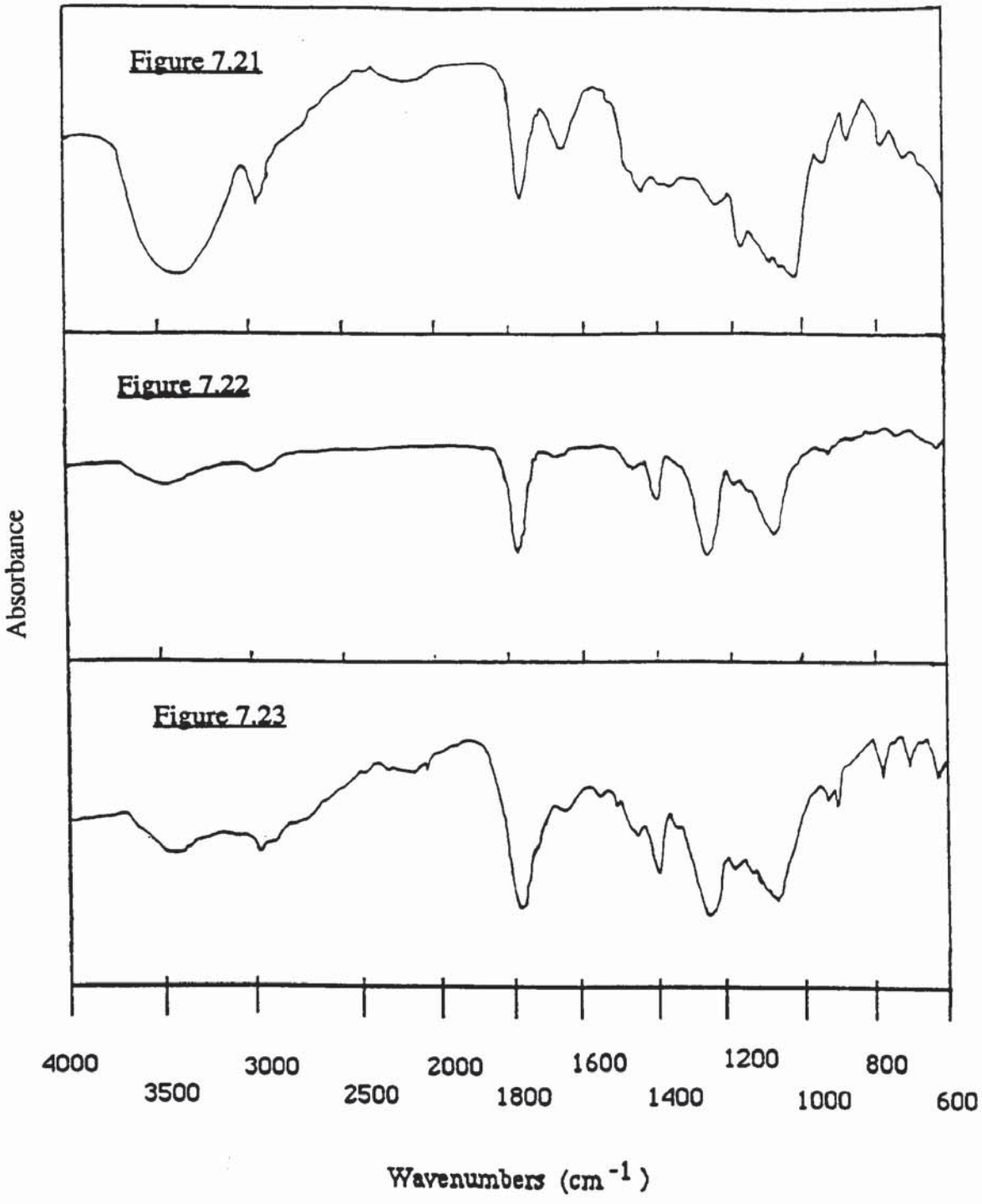
Although amylose is soluble in dimethylsulphoxide, (DMSO), attempts at the esterification of amylose by GAAS in DMSO proved fruitless because of the premature decomposition of GAAS by DMSO.

The addition of GAAS to a solution of amylose in DMSO at room temperature resulted in spontaneous decomposition of GAAS. In order to reduce the rapid exothermic reaction of DMSO with GAAS, the addition of GAAS to the amylose solution in DMSO was carried out at 0°C. This had little effect on the reactivity of DMSO and once again amylose was not esterified after refluxing for 5 hours at 40°C.

In order to further reduce the reactivity of DMSO with GAAS, but at the same time keeping amylose in solution, different co-solvent mixtures of DMSO with other solvents were tried, such as methylene chloride, chloroform, ethyl acetate, diethyl ketone, methyl ethyl ketone, THF and dioxane.

A 5% w/v solution of amylose in DMSO was found to be only compatible up to a ratio of 1:1·5 of DMSO to dioxane, and increasing the dioxane ratio further caused precipitation of amylose from solution. A co-solvent ratio of 1:1 of dioxane to DMSO was used for

Figures 7.21 to 7.23 Infra-red spectra of various modified amyloses. (see text for fuller description). (KBr discs).



For all spectra, air used as reference.

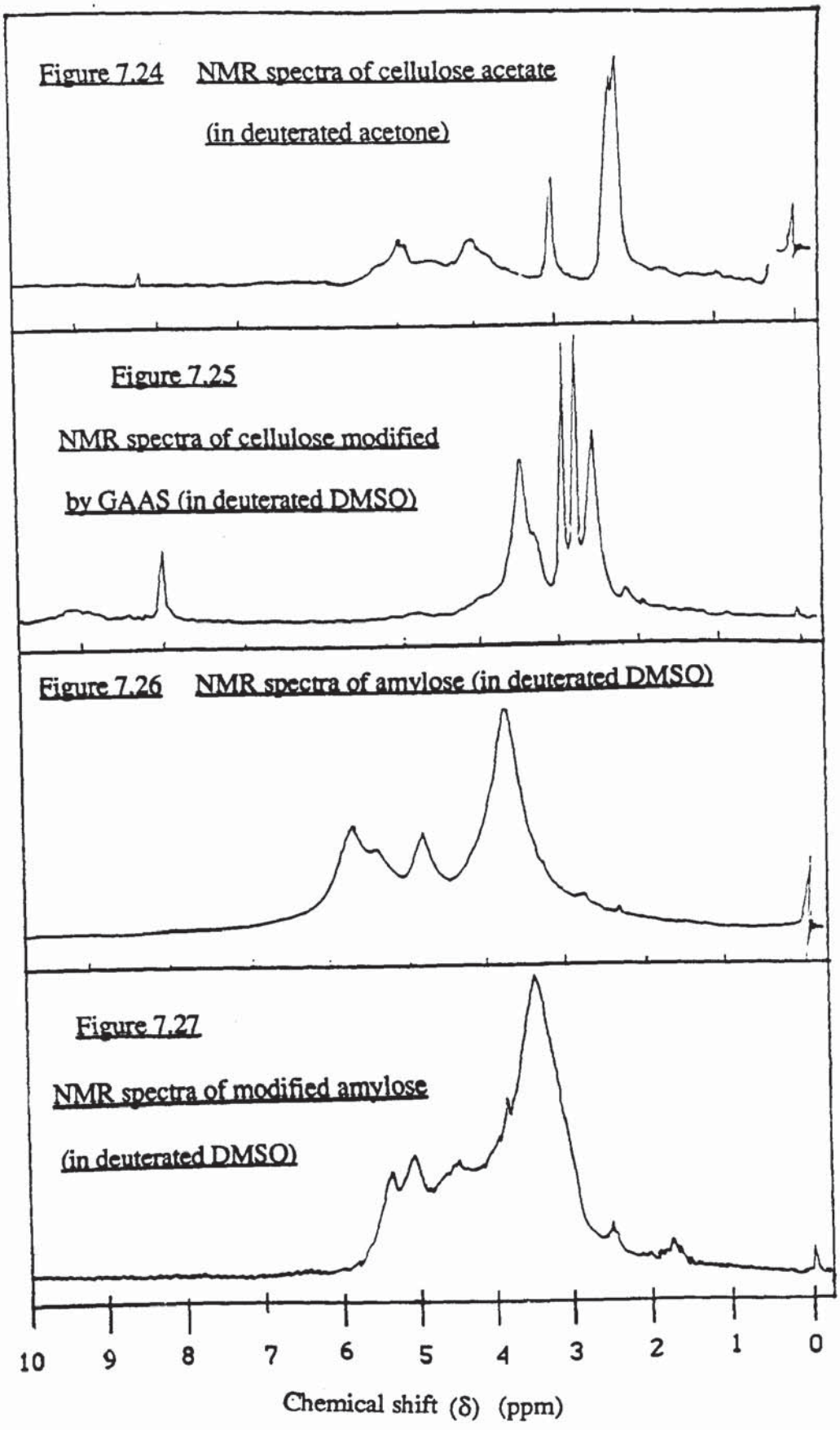


Figure 7.24 NMR spectra of cellulose acetate (in deuterated acetone)

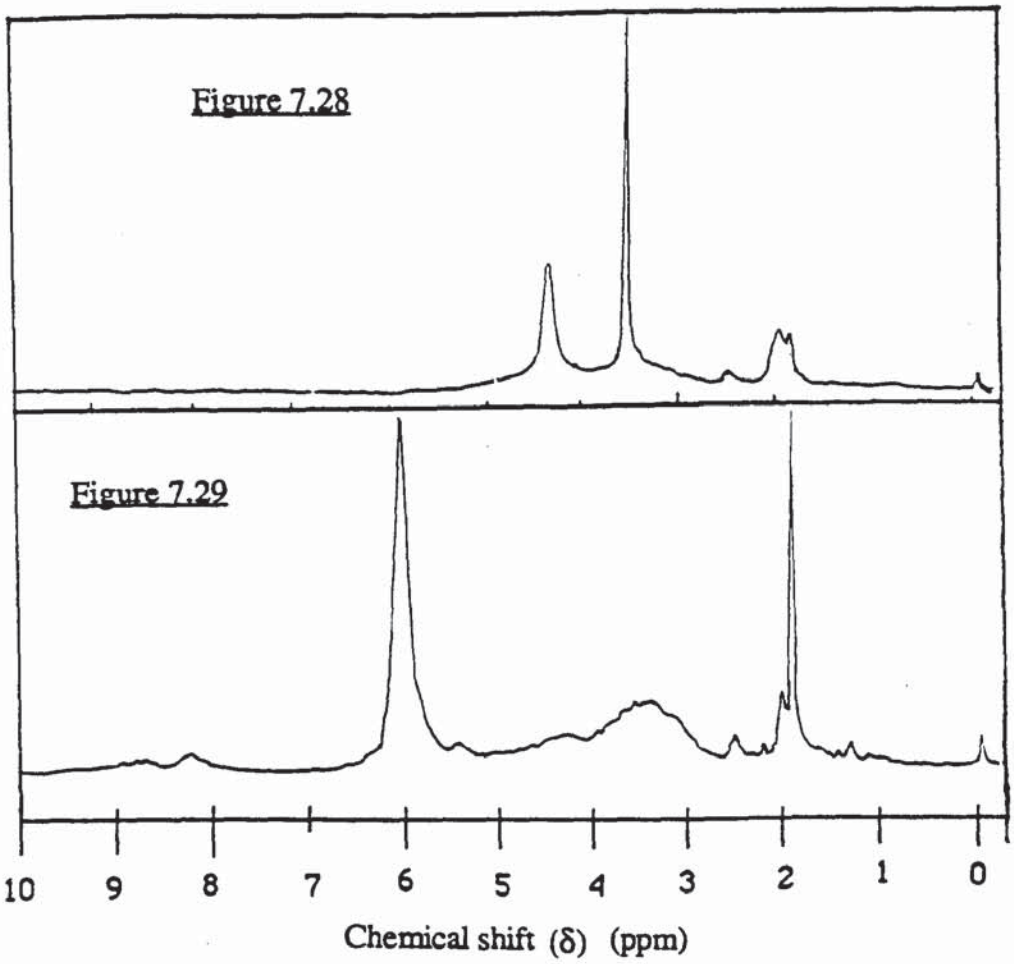
Figure 7.25 NMR spectra of cellulose modified by GAAS (in deuterated DMSO)

Figure 7.26 NMR spectra of amylose (in deuterated DMSO)

Figure 7.27 NMR spectra of modified amylose (in deuterated DMSO)

For all spectra, TMS used as internal reference.

Figures 7.28 and 7.29 NMR spectra of modified amyloses (in deuterated DMSO). See text for fuller explanation.



For all spectra, TMS used as internal reference.

esterification of amylose.

Dry amylose (0.5g) was dissolved in 10 mls of dry DMSO. (GAAS (5g) was dissolved in 10 mls of dry dioxane and this was added slowly to the stirred solution of amylose at room temperature. The solution was refluxed at 100°C for 3 hours and a fine precipitate formed, which did not redissolve. The mixture was cooled, filtered, the precipitate washed with methanol, THF and subsequently dried. Examination by infra-red showed it to be amylose. Small amounts of polyglycolic acid were detected in the mother liquor.

The effect of DMSO on GAAS is much greater than that of amylose on GAAS, so instead of esterification of amylose, decomposition of GAAS to poly(glycolic acid) results. Further experiments in this area were not carried out because of the high reactivity of DMSO for GAAS.

7.3.4. ESTERIFICATION USING GLYCOLIC ACID.

Glycolic acid is soluble in acetone, dioxane and THF. Esterification of cellulose using glycolic acid and an acidic catalyst (analogous to the esterification of cellulose by acetic acid) was carried out by adding 10 g of glycolic acid in 50 mls of dry acetone to 2g dry cellulose. After addition of 2 mls of concentrated sulphuric acid, the mixture was refluxed at 50°C for 21 hours. The heterogeneous mixture was filtered, washed with acetone, and dried. Examination by infra-red spectroscopy showed the presence of a carbonyl group at 1735cm^{-1} (Figure 7.17). Differential thermal analysis thermographs of the sample showed two peaks, one at 137°C and the other at 179°C. It is probable that slight esterification of cellulose has been achieved.

In the acetylation of cellulose, acetic anhydride is used as the acetylating agent, and by comparison, because GAAS could be considered as the anhydride of glycolic acid, esterification of cellulose by using a mixture of GAAS and glycolic acid should induce greater esterification of cellulose than when glycolic acid is used alone. The approach was attempted as below:-

Dried cellulose (2g) was swelled in 40 mls of dioxane containing 15 g of glycolic acid (solid), at room temperature, overnight. 20g of GAAS in 5ml of dioxane was added to the swelled cellulose, and the mixture heated for one hour at 60°C, and then for a further 18 hours at 80°C. Some of the cellulose fibres appeared to have dissolved. The mixture was filtered, the precipitate washed with dioxane, water and THF. The infra-red spectra of this brown precipitate (Figure 7.18), showed the presence of an carboxyl group at 1735 cm^{-1}, and in fact was identical to Figure 7.17. Addition of methanol to the mother liquor, followed by the evaporation of the excess solvent, resulted in a precipitate, which after recrystallisation from dioxane was characterised by infra-red spectroscopy. The infra-red spectra of this (Figure 7.19) was similar to that of polyglycolic acid, so it is probable that the precipitate is low molecular weight polyglycolic acid. The presence of polyglycolic acid as the other product in this reaction again indicates the instability of the anhydrosulphite and in particular, at the ease by which it decomposes into the poly-α-ester.

The esterification of amylose was carried out in a similar manner, using THF as the solvent. The product after 22 hours refluxing at 67°C had a carbonyl group at 1745 cm^{-1} (Figure 7.21) and NMR of this in DMSO, (Figure 7.27), was different from the NMR of amylose in DMSO (Figure 7.26). It is probable that amylose has been esterified to some extent.

7.4. ESTER INTERCHANGE.

Ester interchange (acidolysis or alcoholysis) reactions are well known[203-204] and are used for the preparation of polyesters such as polyethylene terephthalate and polybutylene terephthalate, the interchange being catalysed by heat or both acidic and basic catalysts. Using cellulose acetate ester interchange with GAAS in THF and dioxane was attempted as below.

Dry cellulose acetate (40% acetyl content) was dissolved in 40 mls of hot dioxane. The solution was cooled to 35°C and 11 g of GAAS in 5 mls dioxane followed by 0.1 ml of pyridine added to the stirred solution. Further 5 mls of dioxane were added and the solution refluxed at 100°C for 9 hours, after which it was filtered. Addition of methanol resulted in the formation of a brown precipitate. This was redissolved in hot dioxane and reprecipitated from methanol. The supernatant was left overnight and a precipitate settled out. Both this and the initial precipitate were analysed using IR and NMR. The spectra of these (Figures. 7.22, 7.23, 7.28 and 7.29), compared with those of cellulose acetate, suggest that some initial cellulose acetate has been modified.

It is probable that ester interchange of cellulose acetate by GAAS has occurred, resulting in an ester which may have polyglycolic acid units.

7.5 DISCUSSION.

Amylose and cellulose are often regarded as polyhydric alcohols, possessing two secondary hydroxyl and one primary hydroxyl groups per anhydroglucose unit. The

primary hydroxyl groups are normally regarded as being more reactive than secondary hydroxyl groups, so one would expect the primary hydroxyl group on C-6 of the amylose and cellulose (Figure 7.1), to be esterified more quickly than the secondary hydroxyl groups.

The presence of regularly spaced hydroxyl groups along the backbone chain of the amylose and cellulose molecules provides an ideal environment for hydrogen bonding, which renders the hydroxyl groups inaccessible and together with the regularity of the glucose units, results in the two polymers having high cohesive energy density and being crystalline. In order to achieve the idealised reactions studied here (Figure 7.4), it is desirable to take these polymers into solution. In order to completely dissolve or at least induce some dissolution of amylose and cellulose, polar solvents are needed to break the hydrogen bonds. Such solvents are strong alkaline solutions (e.g. NaOH, KOH), pyridine, DMF, or DMSO, and since common organic solvents such as chloroform, methylene chloride, THF and 1-4 dioxane are not capable of breaking hydrogen bonds, esterification of amylose and cellulose by GAAS in these solvents was not successful. Similarly, esterification in solvents for amylose and cellulose was not possible because of the high reactivity of these solvents (DMSO, DMF, pyridine), for GAAS. As previously mentioned, the ring-opening of anhydrosulphites can be catalysed by bases, aprotic (e.g. pyridine, DMF) and protonic nucleophiles.

Although chlorinated solvents such as chloroform and methylene chloride are used as solvents in the acetylation of amylose and cellulose, they are not solvents for the starting materials, but are solvents for the esters formed. In the acetylation of amylose and cellulose, the acetylating agents (e.g. acetic anhydride/acetic acid), are capable of preferential hydrogen-bond formation, cause swelling of the amylose and cellulose

networks, by breaking the interchain hydrogen bonds. This exposes the hydroxyl groups, which are then vulnerable to the acetylating agents. Although this is one way of increasing the reactivity of amylose and cellulose, unfortunately, the reactivity of the swelling agents used promoted premature decomposition and homopolymerisation of GAAS, without concurrent esterification of the carbohydrate polymers. The fact that cellulose acetate was formed when cellulose was reacted with GAAS in a mixture of acetic anhydride/acetic acid, suggests that acetic anhydride is a more powerful acetylating agent than GAAS, (which could be considered as an anhydride of glycolic acid).

Using glycolic acid/GAAS mixture, (which is analogous to acetic acid/acetic anhydride acetylating mixture, some modification of amylose and cellulose is apparent from the infra-red spectra (Figures. 7.18 and 7.21, compared with Figures. 7.14 and 7.20 respectively). It is probable that glycolic acid acts like acetic acid, breaking hydrogen bonding to swell the amylose and cellulose molecules, and in turn exposing the hydroxyl groups which catalyse the polymerisation of GAAS, forming modified amylose or cellulose containing polyglycolic acid units.

Glycolic acid, besides acting as a swelling agent in these experiments, could possibly react with GAAS to form polyglycolic acid prepolymer, because of the presence of the hydroxyl groups (Figure 7.30). (I) could react with further GAAS to form polyglycolic acid (II), or be involved in the esterification of amylose and cellulose.

It is a well known fact that homogeneous phase bimolecular organic reactions are faster than heterogeneous phase reactions. In the acetylation of cellulose using acetic anhydride/acetic acid, acetic acid only swells the cellulose chains, and surface reactions alone are possible. As the surface cellulose becomes acetylated, the ester formed

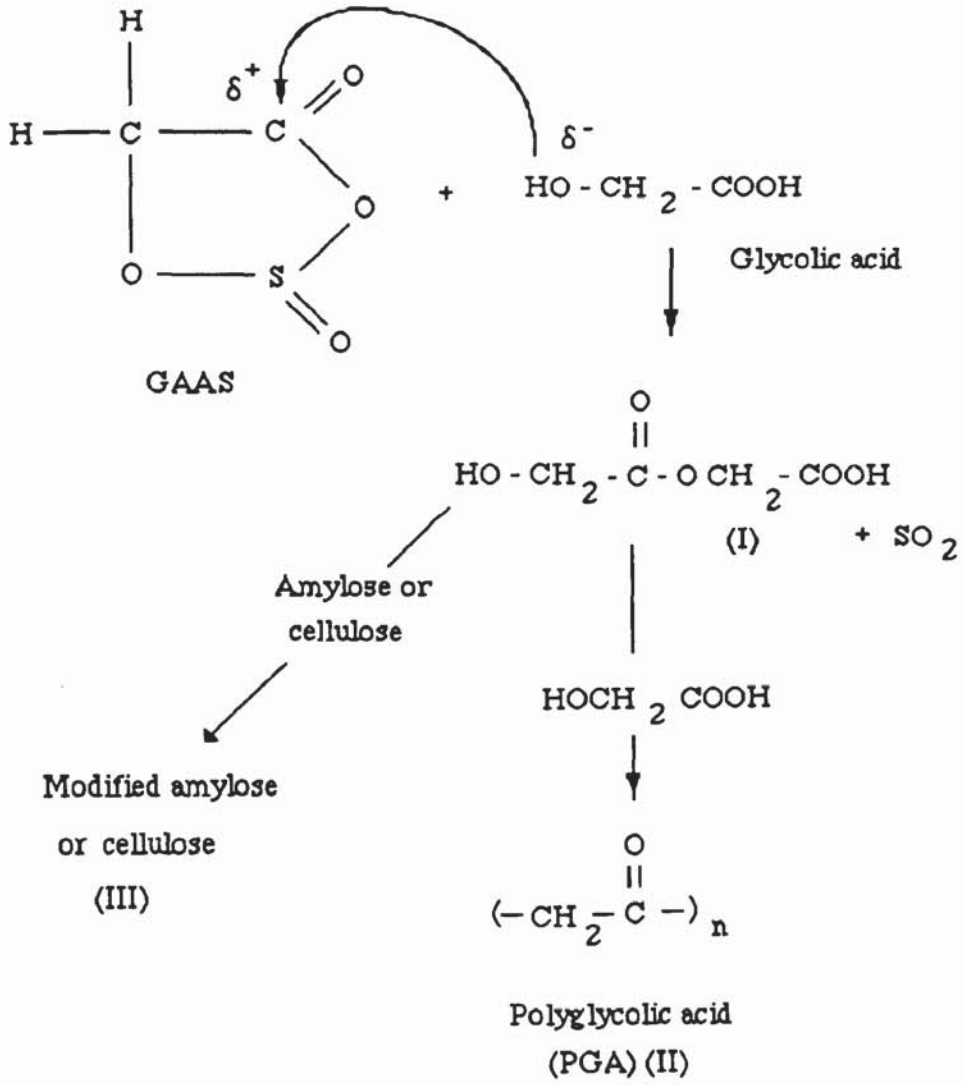


Figure 7.30 Probable reaction mechanism for the reaction of glycolic acid anhydrosulphite with glycolic acid and amylose or cellulose.

dissolves in the acetic acid, and a fresh surface is exposed. This process continues until the whole mass of the cellulose has been acetylated and dissolved in the acetic acid. Esterification of cellulose in a GAAS/glycolic acid mixture probably occurs in a similar manner. Although glycolic acid is a much stronger acid than acetic acid (pKa values of 3.83 and 4.76 respectively [205]), the corresponding anhydrides, i.e. GAAS and acetic anhydride, are likely to be opposite to this. Since GAAS is a weaker anhydride than

acetic anhydride, esterification of cellulose by GAAS/glycolic acid would be slower than

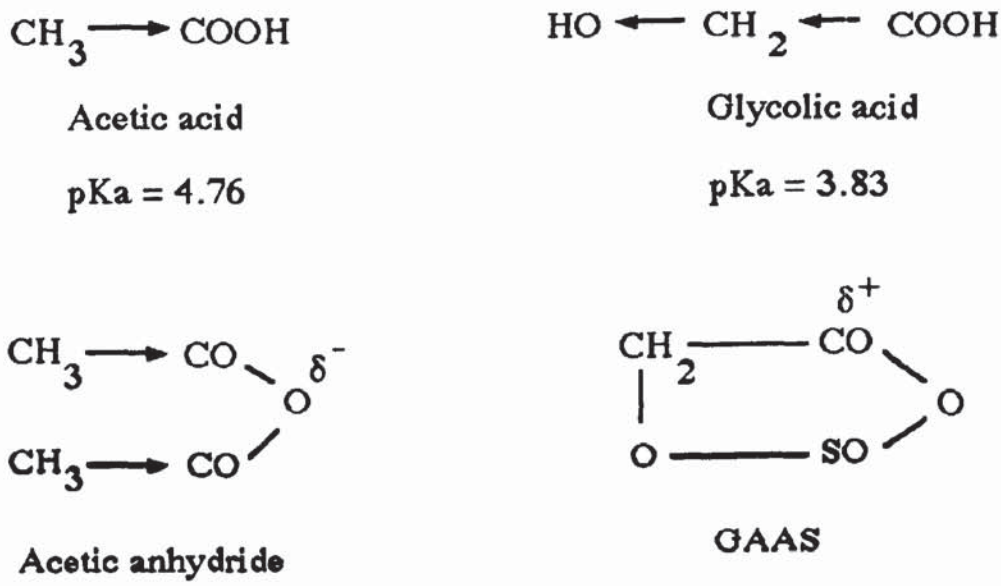


Figure 7.31

the corresponding acetylation of cellulose by acetic anhydride/acetic acid, although the mechanism of esterification would follow the well known acetylation mechanism.

Ester interchange experiments were quite encouraging. It is probable that some ester-interchange has occurred (Figures 7.14 and 7.23), in which some of the acetate groups have been replaced by polyglycolic acid units. Since the degree of substitution governs many properties of cellulose acetate, and because there are three possible acetate groups per glucose unit, complete substitution of all the acetate groups by polyglycolic acid units would be needed to give an ester that would be totally biodegradable. Limited substitution (or interchange) of the acetate groups with polyglycolic acid units does, however, suggest an interesting and promising situation, in which one could synthesis cellulose acetate substituted with different amounts of polyglycolic acid units to give a polymer having varied controllable properties, in particular the biodegradation rate. Cellulose acetate is not biodegradable, and at the other extreme, one would expect cellulose acetate

that is completely substituted by polyglycolic acid units to be totally biodegradable; in between these one could expect a group of polymers with a whole spectrum of degradation rates.

Using cellulose acetate having a lower degree of substitution, together with purer GAAS could possibly achieve the complete substitution of the acetate groups by polyglycolic acid units, resulting in a completely biodegradable ester based on amylose or cellulose, that could be used as a precursor for the melt processable biodegradable surgical fixation devices.

However, because of the time that this synthetic work involving chemical modification of amylose and cellulose would occupy, more immediately promising strategies leading to processable biodegradable polymers were pursued.

7.6. CONCLUSION.

Esterification of amylose and cellulose was achieved by using a mixture of GAAS/glycolic acid. Ester groups, (in the form of polyglycolic acid units), replaced some of the hydroxyl groups in amylose and cellulose and the replacement of these was more likely if the polysaccharides were either in solution or swelled. The role played by glycolic acid was to swell the polysaccharides and promote esterification.

Further attempts should be made in modifying amylose and cellulose, in dioxane or THF, with GAAS/glycolic acid mixture, using purer GAAS.

Ester interchange between cellulose acetate and GAAS is possible. Further attempts should be made at producing cellulose acetate that has all the acetate groups replaced by polyglycolic acid units, using the ester interchain reactions, and possibly starting with a cellulose acetate that has a low acetyl content.

The chemical modification of amylose and cellulose, either by esterification (using GAAS), or by ester interchange reactions, resulting in the grafting of polyglycolic acid units on to the amylose and cellulose molecules, provides a potential precursor material that would be biodegradable, and thermoplastic. However, further time was needed to achieve this, and because the physical blending techniques (described in the earlier parts of this thesis), proved more fruitful, continual detailed work on the chemical modification was ceased. Time permitting, I had hoped to return to this work, but, unfortunately, this was not possible and I hope that the work is taken up by someone else in the near future.

CHAPTER 8

DISCUSSION AND CONCLUSIONS

8.1 CONCLUDING DISCUSSION.

The aim of this work was to make a range of melt processable biodegradable materials, with controlled degradation properties. Two different approaches were used to achieve this; the first being the chemical modification of naturally occurring polysaccharides, such as amylose and cellulose. Attempts were made at grafting poly (α -ester) groups onto the polysaccharides, utilising the novel route of ring-opening polymerisation of anhydrosulphites by the abundant presence of hydroxyl groups in the polysaccharides.

Several problems were encountered with this approach, the first being the dissolution of amylose and cellulose in solvents that did not cause premature decomposition of the anhydrosulphite. The task of finding suitable inert solvents was difficult, because the common solvents or swelling agents for amylose and cellulose caused premature decomposition of the anhydrosulphite. Esterification in an homogeneous mixture would have been ideal, but because of the problems in finding suitable solvents for the polysaccharide, esterification in heterogeneous conditions was attempted. It was hoped that the ester produced during the reaction would dissolve in the non-solvent for the polysaccharides.

Indeed some degree of esterification of amylose and cellulose was achieved by using a mixture of glycolic acid/glycolic acid anhydrosulphite as the reaction medium, (this is analogous to the esterification of amylose and cellulose in a mixture of acetic acid and acetic anhydride). However, the degree of substitution was low because of the competing polymerisation of the anhydrosulphite by the hydroxyl group in glycolic acid rather than

by the hydroxyl groups in the polysaccharides.

Attempts at grafting polyglycolic acid units on to the cellulose by ester interchange reactions between cellulose acetate and glycolic acid anhydrosulphite showed some promise, but here again the level of substitution was low. Further time was needed to achieve a completely chemically modified amylose and cellulose molecule with grafted polyglycolic acid units.

The second approach to designing polymers with controlled degradation rates was the use of physical melt blending techniques to incorporate polysaccharides into the polymer matrix. Initial work considered the incorporation of various polysaccharides into a range of polymers which were hydrolytically stable to a certain degree. This incorporation of the polysaccharide into the polymer matrix was found to exert some influence on the hydrolytic degradation rate. However, the matrix polymers used were not found to be useful for medical applications, because of the extremely slow hydrolytic degradation rates. Even with the polysaccharide blends, the rates were still slow for useful medical purposes. One specific advantage of using naturally occurring polysaccharides is that they present relatively low toxicity in biological systems. This criterion must also be used for the matrix polymers, and is one essential feature of using biodegradable polymers.

Although the physical blending of polysaccharides into the matrix of thermoplastic polymers to change its hydrolytic degradation rate could be used with a wide range of polymers, for medical applications, the degradation products of polymers used *in vivo* is of paramount importance. Ideally, one must use polymers whose degradation products are inert, of low toxicity, or normal body metabolites. In this aspect, PHB and copolymers with PHV, are an interesting group of thermoplastic polymers, in not only

are they biodegradable, but the degradation products (hydroxybutyric and hydroxyvaleric acids), are normal body metabolites. Furthermore, they are available commercially in a wide range of molecular weights and copolymer compositions. The range of melting points from 120°C for the higher valerate copolymers to approximately 180°C for the high molecular weight homopolymer, facilitated the use of conventional plastics processing machinery for these polymers. The 2-roll mill was found to be extremely useful and versatile for blending polysaccharides with the PHB-PHV copolymers.

At the start of this work, although a substantial body of information existed on the synthesis and processability of the PHB-PHV copolymers, published information relating to the hydrolytic degradation mechanism or rate was relatively limited. It is important to gather basic information of the factors affecting the hydrolytic degradation rates of these copolymers before attempting to modify them. Initial work centred on monitoring weight loss from melt pressed discs of copolymers varying in valerate content and molecular weight in a pH 7.4 buffer at 70°C. Degradation was carried out under these conditions in order to assess some of the factors affecting the hydrolytic degradation rate in a relatively short time scale.

The hydrolytic degradation rate from this study was found to be influenced by both molecular weight and valerate content. With increasing valerate content, the hydrolytic degradation rate was found to increase markedly. This was also true when molecular weight was decreased, however, reduction in the molecular weight had a corresponding effect on the mechanical properties. Discs prepared from the 12% PHV copolymer of Mw of 170 x 10^3 were brittle in comparison to the copolymer with an Mw of 350 x10^3. Indeed, there is a molecular weight cut-off at Mw ≈ 200 x 10^3, below which the mechanical properties become inadequate,(173) which has to be avoided if devices

fabricated from these copolymers are used to provide strength or support functions. For this reason, the bulk of this work was centred on using PHB-PHV copolymers of 12 and 20% PHV content with respective molecular weights (Mw) of 350 and 300 $x10^3$.

Crystallinity was also found to play an important part in governing the hydrolytic degradation rate of these copolymers. This was clearly evident when comparisons were made of the same copolymer fabricated by different methods. The degradation rate of the sample fabricated by injection moulding was somewhat slower to that of melt pressed samples. The small difference in the crystallinity and compaction of the matrix induced by the different fabrication technique accounted for the difference in the degradation rates.

A detailed examination of the physical form of the polymer matrix showed that this did indeed affect the hydrolytic degradation rates of these copolymers. The most stable form of the polymer matrix was found to be the injection moulded samples, followed by the melt pressed discs, which in turn were more stable than solvent cast films[174]. The x-ray diffraction traces of these samples fabricated by different techniques (Figures 4.1 and 4.2), together with % crystallinity shown in Table 4.4, illustrate the changes in the crystallinity brought about by processing to these synthetic semi-crystalline polyesters. The order of crystallinity of the samples fabricated by different methods is injection moulded plaque > melt pressed disc > solvent cast film, which is in the same order as the hydrolytic stability of the samples.

To optimise the injection moulding process of the PHB-PHV copolymers, nucleating agents were used. The three nucleating agents used, boron nitride, Norwegian talc and calcium hydroxyapatite (all at 1% w/w loadings) were found to influence both the degradative and physical properties of the moulded article to different extent. The samples

nucleated with boron nitride were found to have the best physical properties and were also the most stable to hydrolytic degradation, whilst those nucleated with apatite degraded faster (Graph 4.2). This faster degradation rate was at the expense of a slight decrease in the physical properties. The nucleation density and spherulite growth rate (and spherulite size), is dependent upon the hydroxyvalerate content, the lower the value of this, the higher the nucleation density and crystal growth rate[173]. Thus, the size of the spherulites (which control the physical and degradative properties) will be the largest in the copolymers high in valerate content. The nucleation density can be increased by the addition of a heterogeneous nucleating agent, the type and concentration of which controls the spherulite size. Boron nitride, because of the smaller particle size, is a much more efficient nucleant than either apatite or talc, hence samples nucleated with this would be expected to be the most crystalline, with those nucleated by the larger apatite particles, being the least crystalline. However, because of the toxicity of boron nitride, talc and apatite were the nucleating agents that were used for most of this work.

The dependence of the nucleation density on the hydroxyvalerate content (and hence, % crystallinity) is most evident when x-ray diffractograms of injection moulded plaques and tensile test pieces of nucleated 12 and 20% PHV copolymers are compared (Figures 6.13 and 6.15, 6.14 and 6.16). The moulds used for the injection moulding of the tensile test pieces and the plaques varied in both size and geometry; the mould used for the plaques being ≈ twice the thickness of the tensile test piece mould. The 20% PHV copolymer, because of its slow crystallisation rate, (even with nucleating agents), will have sufficient time for contributions from surface orientation (including both mould size and geometry), to 'relax' out during the extended time taken for crystallisation, so the x-ray diffraction traces for the plaques and the tensile test samples will be similar. However, in the case of the 12% PHV copolymer, because of its faster rate of crystallisation, the size and shape

of the mould will have dramatic effect on the level of surface orientations present in the sample. Hence, the difference in the x-ray diffraction traces of the plaque and tensile test piece of the 12% PHV copolymer. The large peak at $2\theta = 30^{\circ}$ present in the x-ray diffractograms of injection moulded samples has been assigned to surface orientation effects (173).

The tensile properties of the 12 and 20% PHV copolymers (Mw of 350 and 300 $x10^3$, respectively) were different to the values quoted in the literature(176,177). This difference was possibly due to the combined effects of polymer compaction and crystallinity. Injection moulded tensile test pieces in this study were prepared on a small hand-operated bench-top ram-type injection moulder. The forward and backward pressure applied during the injection moulding process, which controls the compaction and crystallinity of the matrix, is much less than that which is possible from hydraulic-operated injection moulders. Also, the tensile properties quoted in the literature are on annealed samples, which will be more crystalline. This increase in crystallinity will be associated with higher values of yield strength, and lower values of % elongation. Some test pieces were annealed at 50°C overnight, before testing, but although the tensile strength of these specimens was ≈ 20 - 30% higher than the un-annealed samples, the values were still lower than those quoted in the literature. Chu and Browning(206) have also shown that annealing of PGA sutures increases the crystallinity, and hence alters both their physical and hydrolytic degradation properties.

The extent of crystallinity does have marked influence on both the degradation and physical properties of these copolymers. After extensive periods of degradation, crystalline regions are still evident from the SEM's of the degraded samples, as is shown in Plate 8.1, which illustrates small spherulites remaining in the matrix of 20% PHV

copolymer (injection moulded plaque, talc nucleated), after degradation in the pH 10.6 buffer at 70°C for 46 days (the weight loss corresponding to this > 80%). Holmes[177] has shown that the size of the spherulites produced by the PHB-PHV copolymers is as a direct consequence of the processing technique used for the fabrication of the sample. In specimens that are injection moulded, the use of nucleating agents, combined with the processing technique, results in small spherulites being produced. However, in melt pressing, where the sample is allowed to crystallise out slowly, large spherulites are normally present. Plates 4.3 and 8.1 illustrate this difference in the size of the spherulites produced by the two processes.

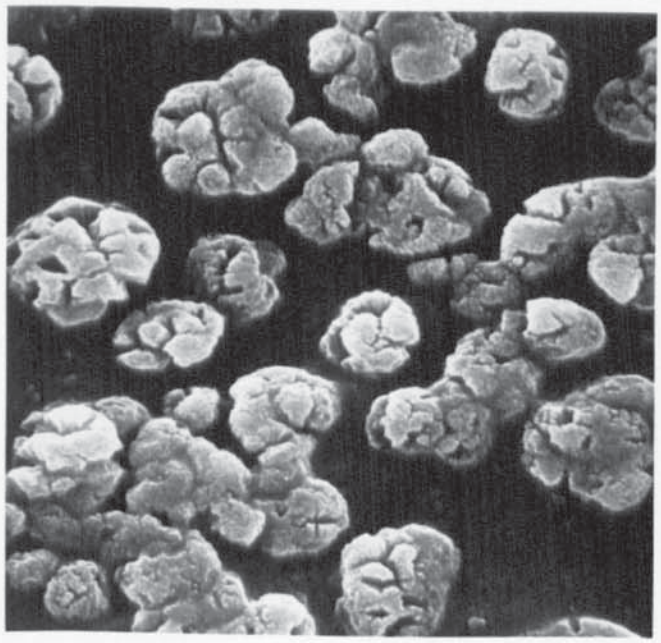


Plate 8.1 SEM of 20% PHV copolymer (injection moulded plaque, talc nucleated), after degradation in the pH 10.6 buffer at 70°C for 46 days (x 4300).

The existence of crystallites after extensive degradation illustrates that the less resistant

amorphous regions are degraded first, followed by the degradation of the more resistant crystalline regions. This two stage degradation, is also present in the hydrolytic degradation of PGA[84] and GA-co-LA copolymers[85].

The mechanism for the degradation of the PHB and copolymers with PHV could be ester hydrolysis by random chain scission of the acyl - (as opposed to the alkyl) oxygen bond in the polymer backbone to yield the free acid and water; the mechanism being either acid or alkaline-catalysed, both of them being bimolecular reactions[207]. The degradation rate of the polyesters, as illustrated in Chapters 4, 5 and 6, was extremely fast at high temperature and pH; while at low pH, the rate was slower.

From the results of this study, a more up-to-date description of the physical degradation mechanism for PHB-PHV hydrolysis in aqueous buffer systems can be presented. The initial stage of degradation is characterised by diffusion of water into the polymer matrix, (evident from initial increase in the wet weight), and preferential hydrolysis of the amorphous regions. Subsequent removal of the soluble oligomeric hydrolysis products by diffusion out of the matrix will then increase the porosity of the matrix with an enhanced hydrolysis process occurring. At this point, changes in the molecular weight of the polymer sample are relatively slow with virtually no measurable differences in mechanical properties. As the process proceeds, the aqueous diffusion within the matrix becomes more efficient as weight loss exceeds two or three percent and more dramatic changes in molecular weight ensue. These do not, however, lead to any marked decrease in crystallinity and the presence of isolated spherulites may, in fact, be seen within the matrix for considerably long periods of time. At extended degradation time, the matrix would be expected to collapse with a final eroding away of the now relatively low molecular weight crystallites that remain.

Although it was possible to change the hydrolytic degradation rate of the PHB-PHV copolymers by varying the valerate content, molecular weight, fabrication technique, pH and temperature, in comparison to the commercial synthetic biodegradable polymers (PGA, GA-co-LA and PDS), these rates were still somewhat slow[174]. To accelerate the hydrolytic degradation rate of these PHB-PHV copolymers, without altering the degradation mechanism, polysaccharides were blended with the PHB-PHV copolymers, using melt blending techniques. It was hoped that this would produce materials having degradation rates approaching those of the more resistant biodegradable polymer, PDS. Results shown in Chapters 4 and 6, illustrate the manipulation of the hydrolytic degradation rate of the PHB-PHV copolymers using various polysaccharides.

Degradation of the PHB-PHV/polysaccharide blends suggested that the hydrolytic degradation rate of the PHB-PHV copolymers was slightly more complex than that proposed for PGA and GA-co-LA copolymers. The first stage of the degradation was characterised by an increase in the wet weight with a concurrent decrease in the dry weight. During this early stage, the polysaccharide is solubilised/swelled and begins to dissolve out or swell from the matrix. This dissolution of the polysaccharide from the matrix results in the formation of a hollow structure, (Plates 4.7 and 4.8), and probable degradation of the amorphous regions. During the second stage, where the weight loss from the blends is static, complete erosion of the amorphous regions is followed by degradation of the more crystalline regions. The weakened polymer matrix produced by the erosional processes, resulting in the first and second stage, tends to collapse in the third phase, and this is seen as a dramatic increase in the weight loss.

Increase in the polysaccharide loading from 10% to 30% w/w, was found to increase the degradation rate dramatically. This difference in the degradation rates was in the enhanced

initial degradation in the case of the 30% polysaccharide loadings, where the first stage of degradation was clearly seen for the higher loaded blends in comparison to the low loaded blends. The degradation rates of the PHB-PHV/polysaccharide blends was also affected by pH and temperature, because the rates of dissolution and degradation of the polysaccharides are themselves pH dependent.

This is a reflection of the differences in their known hydrolytic stabilities which is controlled by structural differences in the polysaccharide and is illustrated photographically in Plates 4.13 to 4.24. The porous honeycomb structure remaining after the polysaccharide has dissolved out of the matrix is clearly seen in the SEM of samples that have undergone extensive degradation (Plates 4.5, 4.6, 4.9b and 4.10b).

The hydrolytic degradation rates of the four polysaccharide fillers could be generalised as sodium alginate > amylose > dextran > dextrin under alkaline condition and sodium alginate > dextran > dextrin >amylose under physiological (pH 7.4) condition. Whilst under acidic condition the corresponding rates are sodium alginate > dextrin > dextran > amylose. This pH dependence of the solubility/degradation of the polysaccharide from the PHB-PHV/polysaccharide blends could be utilised to design medical devices having controlled rates of degradation under specific environments under *in vivo* conditions. PHB-PHV blends with soluble starch (at 10 and 30% w/w loadings) were found to behave similarly to the PHB-PHV blends with amylose under the various conditions of pH and temperature, which is to be expected, because soluble starch contains a certain amount of amylose (Appendix A). These blends were not studied to the same extent as the other polysaccharide blends and so detailed quantitative data of the hydrolytic degradation rates is not shown.

The tensile properties of the 10% polysaccharide filled PHB-PHV blends were shown to change relatively slowly over a period of 0 to 60 days under 'physiological' conditions, after which time a more progressive decrease was observed. With higher filler loadings, the changes in the physical properties will be evident in a shorter period of time. The physical property of the blend could be manipulated by type and amount of polysaccharide used.

Although it is important to study degradation under accelerated conditions (high pH and temperature) to obtain information on hydrolytic degradation rates in a short period of time, one must be able to correlate *in vitro* work with behaviour under *in vivo* conditions. Degradation under pH 10.6 and 37°C could be for predictive purposes, but this cannot replace degradation under pH 7.4 and 37°C for determination of the useful lifetime of a particular matrix. However, the initial stages of degradation (where surface, rather than bulk degradation is predominant), are extremely difficult to monitor by simple gravimetric methods alone. The combination of several surface techniques to monitor and indicate early stages of degradation proved to be invaluable.

Goniophotometry is able to distinguish surface defects at less than the wavelength of light. At the early stages of hydrolytic degradation, increase in surface rigosity (due to surface degradation) is translated in a decrease in the specular reflectance intensity and a corresponding increase in the diffuse reflectance. As degradation proceeds and the size of the surface imperfections reaches the wavelength of light (≈ 500nm), the shape of the goniophotometric curves becomes asymmetrical. Further hydrolysis is shown by the diffuse reflectance approaching the specular reflectance, until a perfectly matt surface is formed. The usefulness of this technique is in following the slow initial degradation rate of the unmodified PHB-PHV copolymers (Figures 4.7, 4.9, 6.1 and 6.2), as well as the

contribution of the various polysaccharides to the degradation rate of the PHB-PHV/polysaccharide blends, (Figures 4.8, 4.10 to 4.12 and 6.3 to 6.4).

Surface energy measurements (determined from contact angle measurements), of the degraded specimens indicate an increase in the polar component of surface energy with increasing periods of degradation, due to an increase in surface carboxyl and hydroxyl groups. In comparison to goniophotometry, the usefulness of this technique was limited to detecting very early stages of surface degradation. There is wide spread evidence and belief that surface energy is an important parameter in relating polymer properties with biocompatibility. The changes in surface energy observed for filled and unfilled PHB-PHV copolymers, under *in vitro* 'physiological' conditions, illustrate the relatively rapid modification of the initial surface energy of these materials. This modification in the surface energy is important if the material is used for bone plates or pins, because it will allow adhesion and growth of cells on the polymer surface[208]. Graphs 8.1 and 8.2 illustrate how the surface and bulk properties of 12 and 20% PHV copolymers (injection moulded, apatite nucleated) samples change with degradation at 37°C and pH 7.4. The transition from surface degradation to bulk degradation is evident from these graphs. Although the surface properties, (gloss factor from goniophotometry and surface energy from contact angle measurements), have changed markedly from 0 to 250 days, little bulk degradation has occurred. However, between 250 - 300 days, changes in the profiles of the dry weight loss, molecular weight and crystallinity start to occur, and bulk degradation begins to predominate. The potential use of both goniophotometry and surface energy (from contact angle measurements) for monitoring the early stages of polymer degradation is clearly evident from these graphs.

Enzymic degradation of PHB-PHV blends with amylose suggested that, *in vivo*, the

contributions to the degradation rate from enzymes (α- amylase) will be more than from simple chemical hydrolysis. Indications are that esterase (α- amylase), also degrades the PHB-PHV copolymers at a rate that is slightly faster than the rate of hydrolytic degradation. It is probable that PHB-PHV blends with dextran *in vivo* will be subjected to degradation by both enzymic and hydrolytic mechanisms. The exchange of sodium ions by calcium ions in sodium alginate suggests that *in vivo*, in the presence of free calcium ions, the *in vitro* degradation rate of the PHB-PHV blends with sodium alginate will be different to the rate under *in vivo* conditions. The possibility exists to make a whole range of PHB-PHV blends with soluble alginate salts (other than the sodium salt), the degradation rate of which is controlled by the solubility of the alginate salt.

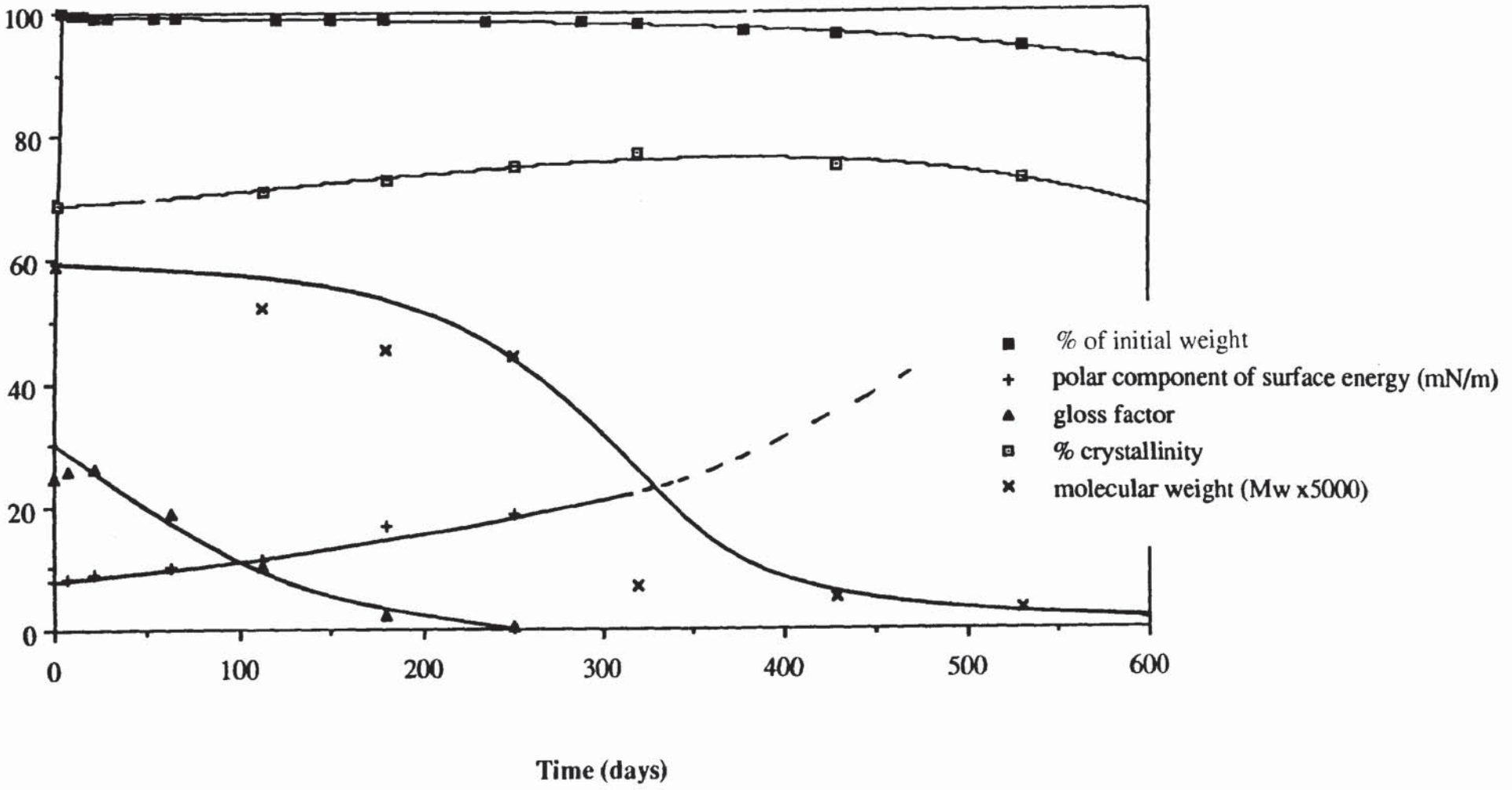
The various factors that affect both the degradation rate and the physical properties of the PHB-PHV copolymers are summarised in Figure 8.1

The physical and degradative properties of the PHB-PHV copolymer blends with PCL were found to have a complex compositional dependence. However, some of the blends produced did have interesting physical and degradative properties which could be utilised for different medical applications. Certainly, these blends are a promising group of materials, the degradative and physical properties of which need to be fully studied.

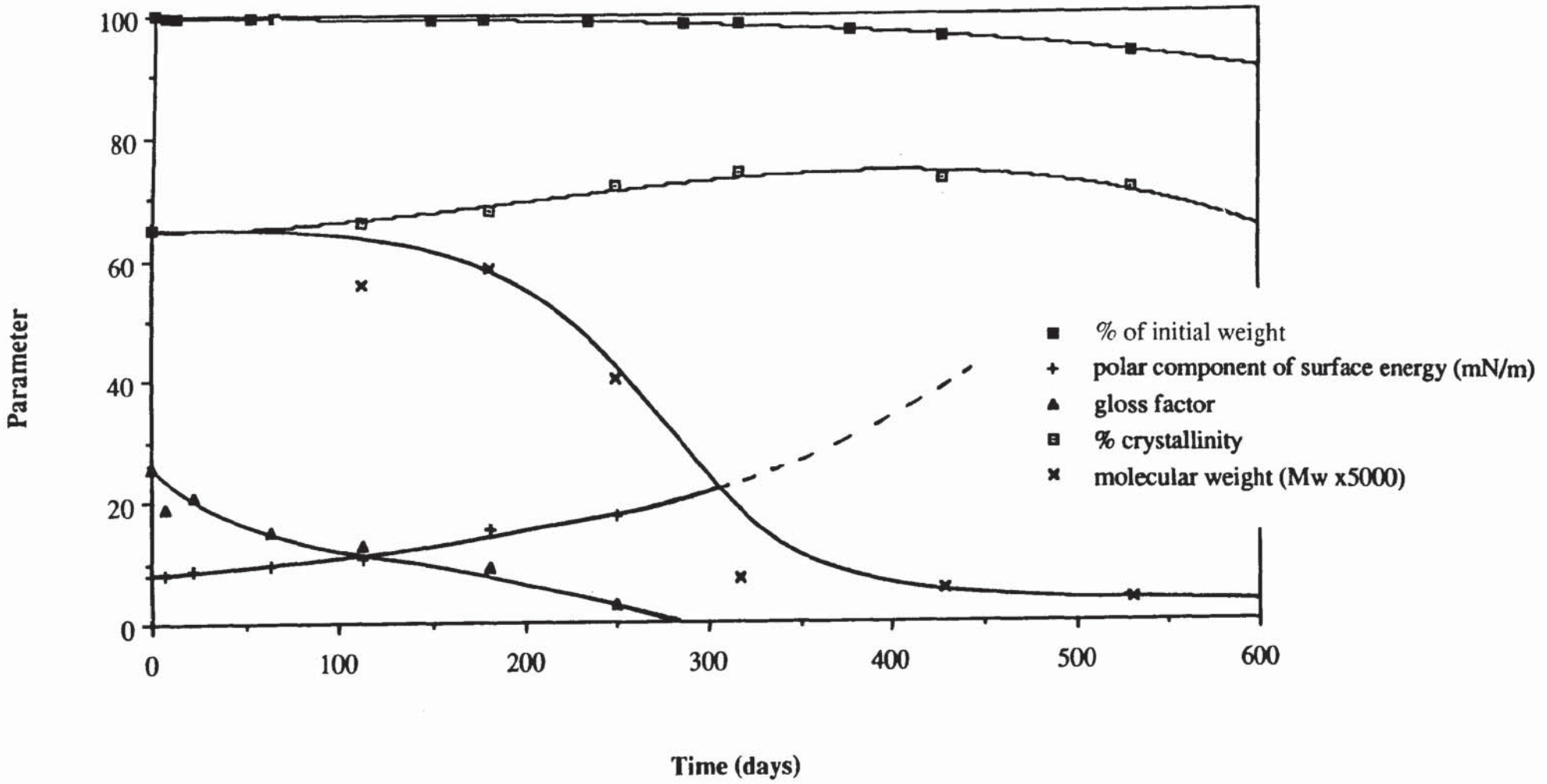
In summary, a wide range of melt processable, biodegradable materials have been produced, the physical and degradative properties of which can be altered by the type and amount of filler used. In the case of the PHB-PHV/polysaccharide blends, the initiation step for the first stage of hydrolytic degradation is built in to the material by the presence of the polysaccharide. The hydrolytic degradation process for these blends needs an aqueous medium for initiation, and hence these materials will be expected to have a long

'shelf-life', as they will be unaffected by the moisture in the air. This is in contrast to commercially available biodegradable suture materials, such as PGA and PDS, which need ambient moisture from the air for initiation of the degradation mechanism. Hence, these materials have a shorter 'shelf-life' and are known to degrade whilst still in the packaging.

Graph 8.1 Changes in surface and bulk properties of 12% PHV (apatite) injection moulded plaques degraded in pH 7.4 buffer at 37°C



Graph 8.2 Changes in surface and bulk properties of 20% PHV (apatite) injection moulded plaques degraded in pH 7.4 buffer at 37°C



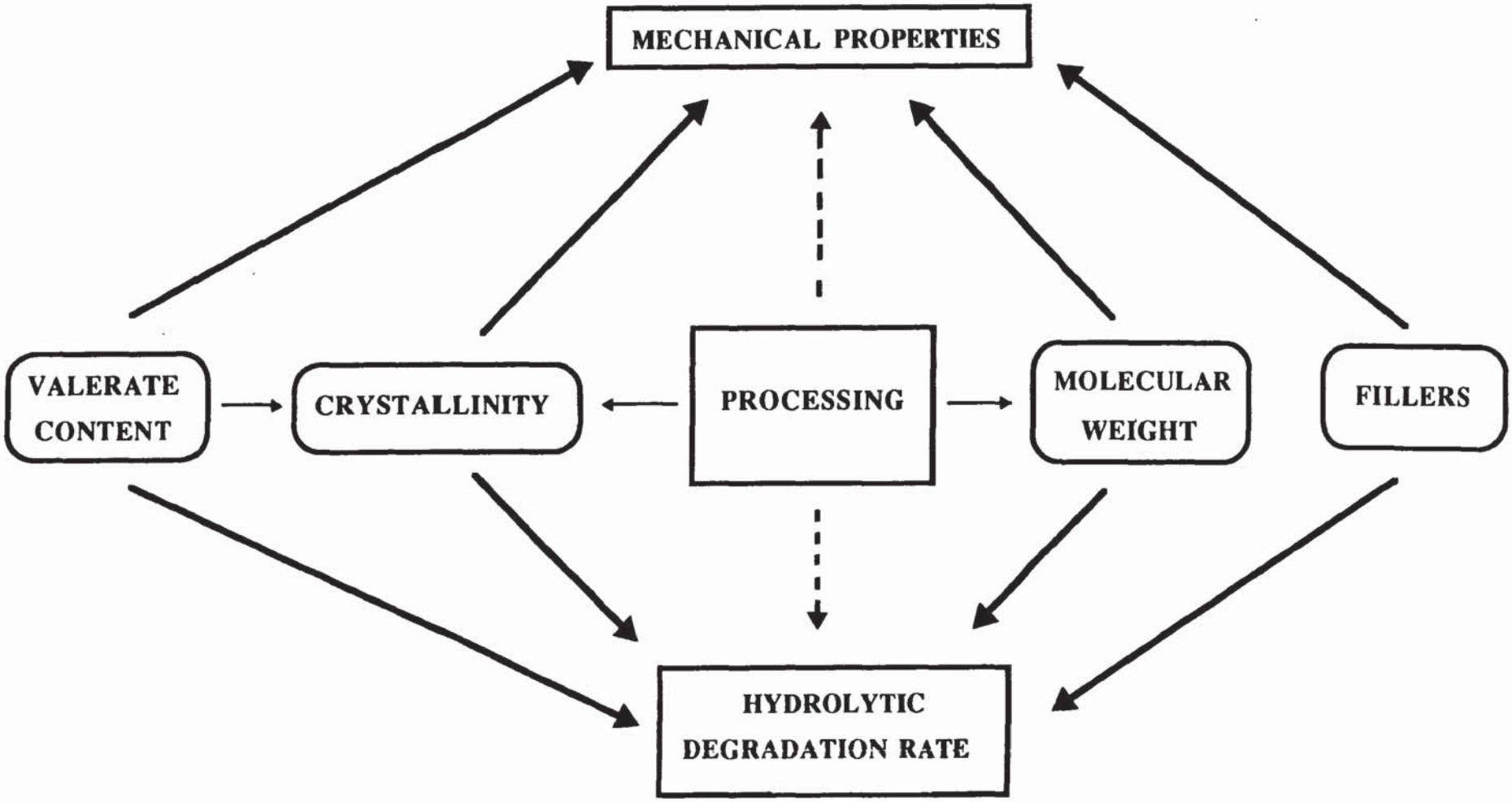


Figure 8.1 Factors affecting mechanical properties and hydrolytic degradation rate of PHB-PHV copolymers.

8.2 CONCLUSIONS.

The hydrolytic degradation rate of the PHB-PHV copolymer series was found to be affected by valerate content, molecular weight, crystallinity, together with the physical form of the sample, the pH and temperature of the aqueous medium. The degradative mechanism is by a random chain hydrolysis process and is characterised by an initial increase in the wet weight, and concurrent decrease in the dry weight, as the amorphous regions of the matrix are eroded, which is in line with the hydrolytic degradation of other polyesters. With the polysaccharide blends, this initial process is increased dramatically, and erosion of the polysaccharide from the matrix increases the internal porosity, which leads to the eventual collapse of the matrix, a process which occurs, but less rapidly, in the degradation of the un-blended PHB-PHV copolymers. The initial stages of the degradation were also found to be markedly influenced by the type and loading of polysaccharide used.

During the early stages of the degradation process, which is characterised by a relatively small weight loss, surface, rather than bulk, processes were found to be predominant. Unlike gravimetry, goniophotometry and surface energy measurements were found to be extremely useful in magnifying these early stages of the degradation process.

Although the hydrolytic degradation rate of the PHB-PHV was found to be relatively slow in comparison to established biodegradable suture materials, incorporation of a soluble polysaccharide into the PHB-PHV matrix was found to give initial degradation rates approaching those of the suture materials. However, differences in molecular weight, crystallinity and specimen form makes direct comparison difficult.

The degradation process was found to be greatly enhanced by α- amylase for the PHB-PHV blends with amylose. Indications were that α- amylase (an esterase) does degrade the PHB-PHV copolymers at a rate that is slightly faster than that of simple chemical hydrolysis.

The complementary techniques of surface studies and monitoring of bulk properties enabled a comprehensive profile of the overall degradation process of the PHB-PHV copolymers and their blends with polysaccharides to be assembled.

The physical and degradative properties of PHB-PHV blends with polycaprolactone (PCL) were found to be influenced by a complex compositional dependence on the two polymers. Blends containing less than 10% w/w of PCL were found to be compatible, the compatibility decreasing with increasing PCL content.

Slight modification of amylose and cellulose by ring-opening polymerisation of the anhydrosulphite, either by esterification reactions or ester-interchange, was possible. The high reactivity of the anhydrosulphite for amylose and cellulose solvents, as well as for glycolic acid, in comparison to the hydroxyl groups in the polysaccharide, rendered incomplete substitution of the hydroxyl groups by the poly -α- ester groups.

8.3 SUGGESTIONS FOR FURTHER WORK.

Although this thesis describes the most up to date information on the *in vitro* hydrolytic degradation of the PHB-PHV copolymers, and methods to control this, further work is needed to substantiate the degradative mechanism of these copolymers *in vivo*. Work described in this thesis suggests that esterases may be involved in the degradation of these copolymers, but a more detailed experimental protocol should be set up to investigate the *in vivo* degradation of these copolymers. The changes in the molecular weight, together with gravimetric analysis, crystallinity and tensile parameters should be used to obtain a clear picture of the degradative mechanism *in vivo*. This work should also indicate the projected life-span of articles made from these copolymers *in vivo*.

Further work is also needed to substantiate that the degradation rates of PHB-PHV/polysaccharide blends (in particular amylose, dextran and soluble starch) *in vivo* are more dependent on enzymatic, rather than on simple hydrolytic mechanisms. It would be interesting to see whether the *in vitro* enzymatic rates for the degradation of PHB-PHV/amylose blends are similar to those under *in vivo* conditions.

The potential of increasing the range of PHB-PHV blends with alginate salts should be investigated. Although the degradation rate of sodium alginate blends may be retarded in the presence of free calcium ions, other soluble alginate salts (e.g. potassium, ammonium, magnesium) are not affected by calcium ions. This means that a whole range of PHB-PHV blends, based on alginate salts with differing degradation rates, is possible.

Further work on the PHB-PHV/PCL blends should establish the complex compositional

dependence of both the physical and degradative properties of this novel range of materials, both *in vitro* and *in vivo*. Recent work at Aston University, utilising the PHB-PHV/PCL blends for controlled drug delivery systems (microcapsules), has shown promising results and it seems certain that compositions that are incompatible can be used to produce microcapsules that have a certain degree of porosity[209].

Initial attempts at producing a three component blend of the PHB-PHV copolymers with PCL and polysaccharides (dextrin) suggested that a whole new range of blends could be produced, utilising the relatively inexpensive and simple method of melt blending techniques; the PCL component acting as an elastomeric plasticiser to provide flexibility, and the polysaccharide to provide a controlled rate of hydrolytic degradation. A PHB-PHV/PCL/dextrin (80 : 10 : 10) blend was found to have both physical and degradative properties intermediate to those of the respective two-phase blends at 37°C and pH 7.4. These blends could be designed to provide the prerequisite requirements of a medical device, by either relatively fast degradation rate and inferior physical properties, superior physical properties but slow rate of degradation, or a balance between these two extremes.

One further application of the PHB-PHV/polysaccharide blends could be in the controlled delivery of drugs. It would be interesting to blend drugs (e.g. antibiotics) on the 2-roll mill with the PHB-PHV copolymers and the polysaccharide and then study the release of these from the fabricated specimens. This potentially interesting idea could be used to incorporate antibiotics into surgical fixation devices, so that the device is able to manage the wound site at the same time as providing the necessary support. Other extensions of this idea could be incorporation of antibiotics (or other drugs) into microcapsules made from the PHB-PHV/PCL blends, and incorporation of the microcapsules into wound

dressings, so that controlled rate of antibiotic release is maintained to keep the wound clean during its healing stage.

Blends of the PHB-PHV copolymers with synthetic polyesters have produced materials with interesting properties. It would be interesting to see if a whole range of compatible polymer blends could be produced by blending PHB-PHV copolymers with other commercial polymers. One such interesting polymer is Elvamide®, a nylon multipolymer resins that have a range of melting points from 105 to 160°C[210]. These resins are extremely flexible and contain carboxyl, amide, and amine groups. Blends of Elvamide with the PHB-PHV copolymers could result in a group of materials of great potential. Another extension of this idea could be the blending of different PHB-PHV copolymers. Here the difference in the molecular weight and the valerate content could be used to produce blends with a wide range of physical and degradative properties.

Further attempts at the chemical modification of amylose and cellulose by the grafting of poly -α- ester groups, using the anhydrosulphites should be attempted in relatively inert solvents. Ester interchange reactions, using low acetyl content amylose and cellulose acetate and glycolic acid anhydrosulphite should be attempted.

Although surgical fixation devices are still in the infancy of the design stage, the materials described in this thesis could form the basis for further developmental work in this area.

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APPENDIX

APPENDIX A

PROPERTIES OF POLYSACCHARIDES.

1 INTRODUCTION.

Polysaccharides are composed of simple sugar units linked by glycosidic bonds, and structurally may be linear, branched or cyclic. Polysaccharides can either occur as homopolymers, composed of a single kind of monosaccharide, or copolymers (random, alternate, or block types, consisting of two different sugars). The commonest type of monomer unit found in the polysaccharides is that of six-carbon aldehydo sugars, in a six-membered pyranose ring (aldohexopyranoses), Figure A 1.

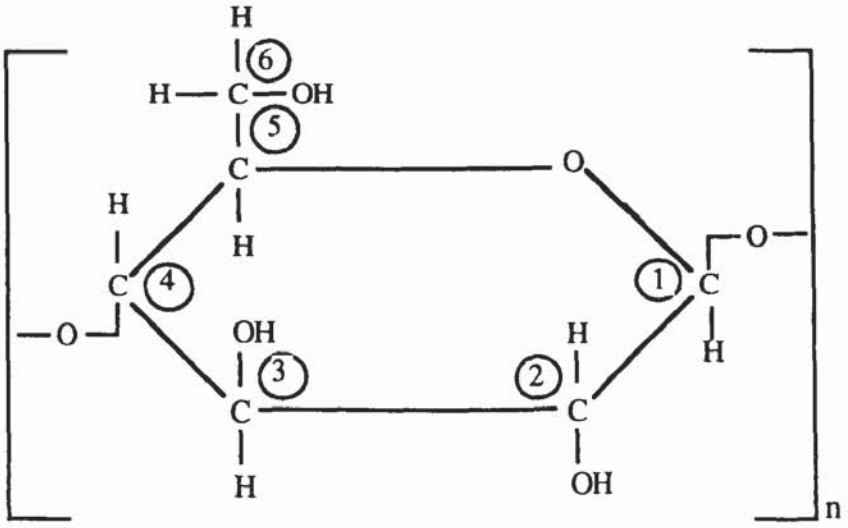

Figure A 1 Six membered pyranose ring.

The pyranose ring exists in two conformations, the chair and the boat form (Figure A 2). The chair form of the pyranose ring is rigid and relatively more stable than the boat form, and is thought to predominate in polysaccharides. The substituent groups in the chair

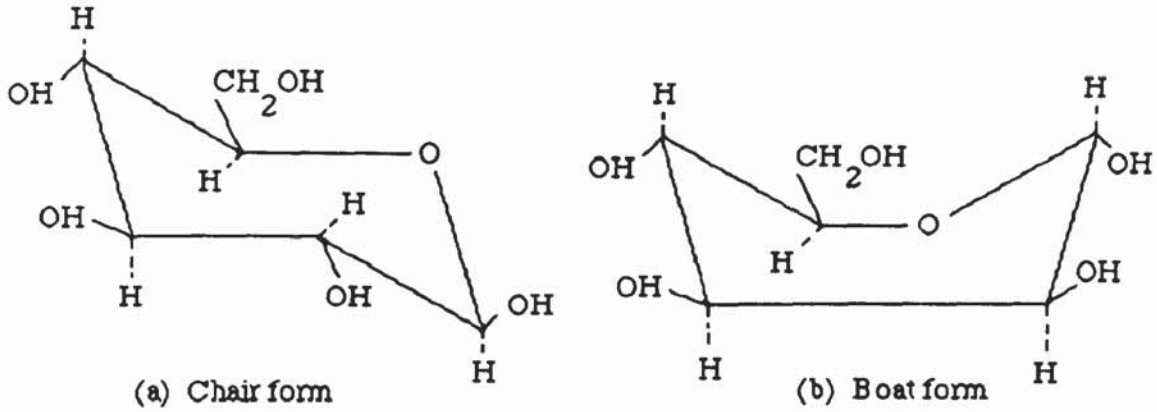

Figure A 2 The two configurations of the pyranose ring.

form are not geometrically and chemically equivalent, and are classed as axial and equatorial. The equatorial hydroxyl groups of pyranoses are more reactive than axial groups, because it is more difficult to insert a bulky grouping into an axial position.

The properties of polysaccharides are not only affected by their molecular structure and the resultant molecular arrangement, but also by the configurations of the individual sugars and the functional groups present [1]. The physical and chemical properties of the polysaccharides are extensively documentated in the literature. This topic will be briefly dealt with here in order to give an insight to the polysaccharides used in this study.

1.1 AMYLOPECTIN.

Amylopectin and amylose are two components of starch. Amylopectin may account for up to 80% of the starch weight in most starch sources, and is isolated from the amylose component by fractionation of the starch granules. Fractionation methods are based on the solubility of the starch components. In some procedures, the more soluble

components are leached from the granule, while in others the less soluble component is precipitated from a starch solution [2]. Complexing agents such as 1-butanol, have been used to precipitate amylose from starch solutions [3]. Starch, (0.5 - 5% starch solids), is dispersed by pressure cooking at 105 - 160°C the starch solution is saturated with pentanol and, on cooling, amylose is separated by centrifugation. Amylopectin is recovered from the liquid phase by concentration and drying [4]. The number-average molecular weight of amylopectin isolated by these procedures ranges from 10^5 to 10^6[5].

A slight confusion exists in the literature as to the solubility of amylopectin in water. It has been suggested that amylopectin is both soluble [6,7,8] and insoluble [9] in water. In fact, amylopectin is soluble in water, forming stable solutions. Amylopectin is a branched polymer of α-D-glucose subunits which are linked by α - (1 – 4) and α - (1 -6) glycosidic linkages. The α - (1– 6) linkage provides a relatively loose jointed structure with a degree of freedom. Additionally, branching in the amylopectin molecule causes irregularity (disorder), and as a result amylopectin is amorphous in nature and soluble in water.

Amylopectin is partially degraded by both α and β amylases, yielding glucose and maltose. Total enzymatic degradation of amylopectin is not possible because neither α or β amylase are able to hydrolyse the α - (1– 6) linkages at the branch points of amylopectin. Hydrolysis with dilute acids yields D-glucose.

1.2 AMYLOSE.

Amylose or poly - (1– 4)-α-glycopyranose, is a linear polymer of D-glucose subunits,

linked by cis α (1 – 4) glycosidic linkages. Each of the D-glucose subunits contains one secondary and two primary hydroxyl groups. The presence of α (1 – 4) glycosidic

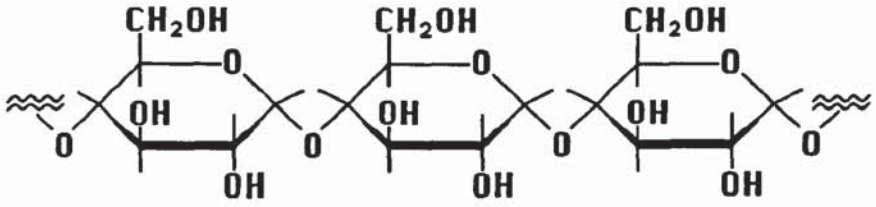

Figure A 3 Amylose.

linkages results in amylose having an open helix structure (Figure A 3), and the combined effect of this with the hydroxyl groups arranged linearly, is that amylose molecules have a tendency to be attracted to each other. This attraction results in hydrogen bonding between the hydroxyl groups on neighboring molecules, which renders the open helix very hydrophobic. When this occurs, affinity for water is reduced, and if amylose is in solution, it will tend to precipitate out at dilute concentrations. In more concentrated dispersions (>1%), the aggregated amylose entraps water and forms a three dimensional polymeric network gel. This process of alignment, hydrogen bond association, and precipitation is essentially a crystallisation process, and is commonly called retrogradation. The rate of retrogradation depends upon a number of factors, such as molecular size, amongst others[10]. It is because of the tendency for retrogradation that amylose is insoluble in water [6,7,8].

Amylose is soluble in dimethyl sulphoxide, urea, dichloroacetic acid, formic acid, aqueous chloral hydrate, ethylene-diamine, formamide and dilute alkali [11,12]. In solution, amylose exists as a coiled or spiral molecule [13]. Amylose reacts with iodine to form a blue complex, unlike amylopectin which forms a red-purple. The iodine

molecule sits in the helical space in the amylose molecule. The colour is related to chain length. A chain length in excess of 40 anhydroglucose units is needed for the blue colour[14].

The number-average molecular weight of amylose varies from 230,000 for amylose from corn starch, to 1,100,000 for amylose from potato starch[5]. Amylose is degraded to maltose by α and β-amylases. α-amylase is found in nearly every tissue in man and requires chloride ions for its activity. Amylose is degraded by dilute acid and alkali. The melting point of amylose is above its decomposition temperature, (because of the hydrogen bonding), so amylose does not melt but decomposes at 273 - 275°C.

1.3 ALGINIC ACID AND ITS' SALTS.

Alginic acid is a polyuronic acid composed of β-D-(1 – 4) mannuronic and α-L-guluronic acid residue (Figure A 4). This structural polysaccharide is found in seaweed as an insoluble complex of potassium, sodium, calcium and magnesium alginate. Alginic acid has a weight-average molecular weight of up to 240,000[15], is insoluble in water, but readily soluble in aqueous solutions of alkali metal hydroxides or carbonates, and gives highly viscous solutions of the alginate salts. The polyvalent alginate salts are insoluble. The alginates are very hydrophilic polysaccharides. A 1% dispersion of alginic acid in water has a pH of 2.9, whilst a similar concentration sodium alginate solution, has a pH of 7.5.

Alginic acid is very resistant to degradation because of the β-D-(1 – 4) linked mannuronic acid residues. The alginates are less stable than alginic acid. Enzymic degradation of

alginic acid is not possible in the body, although alginates, such as sodium alginate, are gradually absorbed by vascular tissues without ill effect and are therefore used as surgical

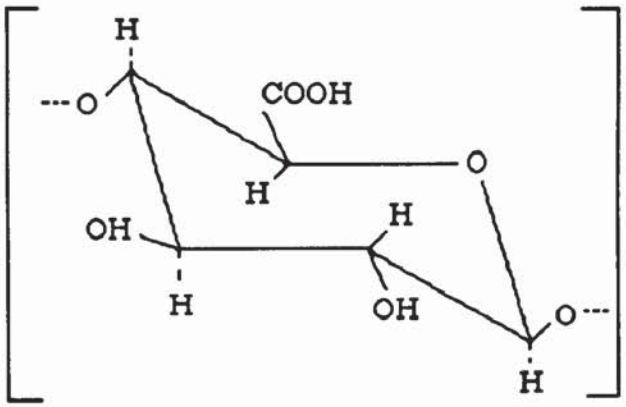

Figure A 4 Alginic acid.

dressings which can be left in the body[16]. Sodium alginate is available in a range of molecular weight (and viscosities), i.e. from 18,000 to 147,000[17]. Alginic acid and the alginates have a browning temperature of 130 - 160°C and a charring temperature of 200 - 240°C.

1.4 CELLULOSE.

Cellulose is the most abundant of naturally occurring organic compounds, for, as the chief constituent of the cell walls of higher plants, it comprises at least one third of the vegetable matter of the world, and is continually created each year through photosynthesis. The cellulose content of such vegetable matter varies from plant to plant. Alpha-cellulose is the term given to pure cellulose of high molecular weight.

Cellulose or poly (1 - 4)-β-glycopyranose, is a linear polymer of D-glucose subunits,

linked by trans β(1 – 4) glycosidic linkages, arranged in the chair confirmation[18], (Figure A 5). Cellulose can be regarded as a syndiotactic polymer, whilst amylose can be regarded as an atactic polymer of polymer of D-glucose units. The β-(1 – 4) linked chains of cellulose, unlike the α (1 – 4) linked chains in amylose, are essentially rigid and straight. As a result, cellulose molecules can readily align themselves side-by-side, and this arrangement is stabilised by intra- and inter-chain hydrogen bonding between the hydroxyl groups on the glucose residues. This intermolecular bonding is so strong that cellulose is insoluble in water, and will not melt at any temperature below that at which it decomposes [19].

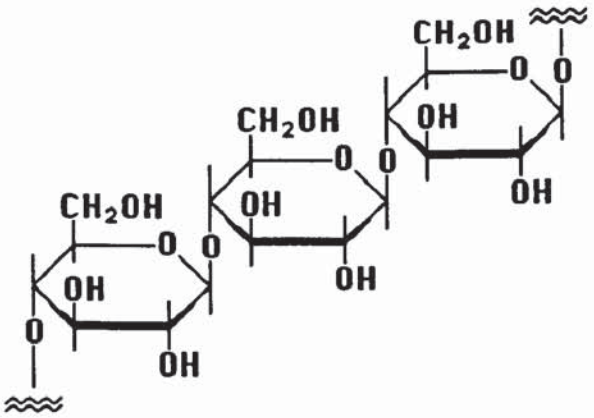

Figure A 5 Cellulose.

The presence of a large number of hydroxyl groups in cellulose makes it hydrophilic and, as a result, when cellulose is immersed in liquids which are capable of preferential hydrogen-bond formation (for example, water, ammonia or acetic acid), the interchain hydrogen bonds are broken, the polymer network becomes swelled and the reactivity of cellulose is increased. Cellulose is soluble in cuprammonium hydroxide, strong acids, quaternary ammonium bases and various other solvent systems[20,21].

The cohesive energy and crystallinity of cellulose is high as a result of the regularity in

the structure and hydrogen bonding. Cellulose is approximately 70% crystalline[22], (amylose is 60% crystalline[23]), and has a weight-average molecular weight of up to 106 [18]. Cellulose is more resistant to hydrolysis than amylose because of the β(1 – 4) glycosidic linkages, and it is because of these linkages that cellulose is not degraded in the body. The enzyme cellulase, which breaks the β(1 – 4) glycosidic bonds is not present in man.

1.5 DEXTRAN.

Dextrans are a class of polysaccharides synthesized from sucrose by micro-organisms of the lactobacillus family[24]. Dextran is a branched polymer, consisting of D-glucose subunits linked by α (1 – 6) glycosidic bonds in the main chain, and by a variable amount of α (1 – 2), α (1 – 3), or α (1 – 4) branch linkages (Figure 6). Dextran is known to have weight-average molecular weights of up to 5 x 10^5[25], and are amorphous[6].

Dextran is soluble in water because of the α (1 – 6) linkages, which confer flexibility to the backbone. High molecular weight dextran is degraded by acids to produce 'clinical dextrans'. Dextran is inert in biological systems and does not affect cell viability. It is because of this, coupled with the hydrophilic and water-solubility properties, that clinical dextrans are used as blood plasma extenders, to maintain or replace blood volume[24]. They achieve this effect by maintaining the colloidal osmotic pressure of plasma. Dextrans of weight-average molecular weights of 40,000 or less are completely excreted in the urine within 48 hours. Above 40,000, dextran remains in the bloodstream for periods of time, depending on its molecular weight, i.e. a 20% of dextran (molecular

weight 40,000), is found in the circulation 6 hours after intravenous injection, while 40%

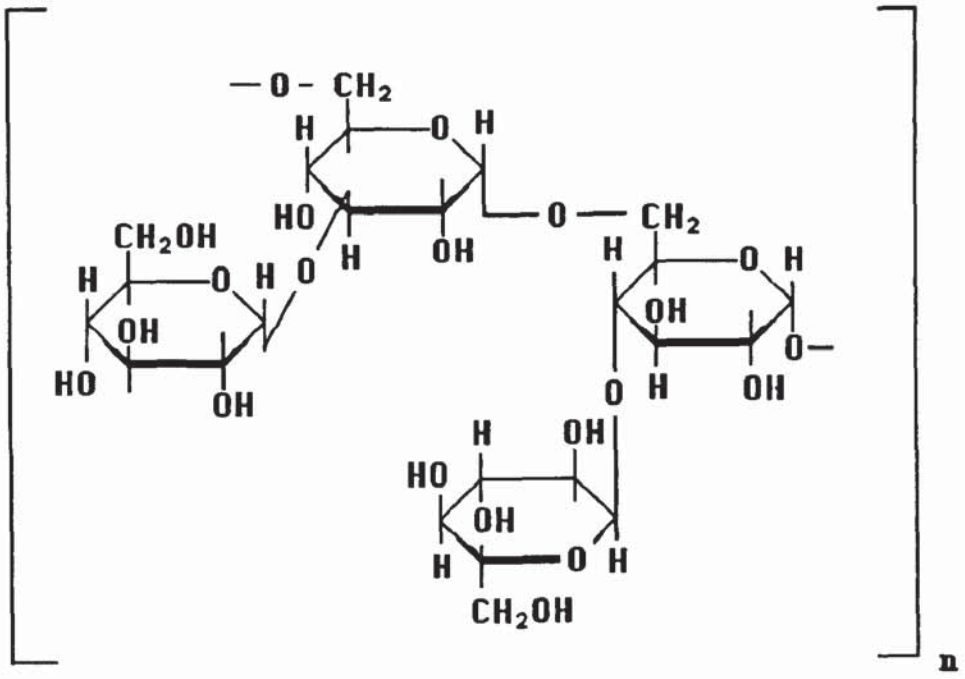

Figure A 6 Dextran.

of dextran (molecular weight 70,000), is present in the blood after 24 hours. Dextran of molecular weight 110,000 can remain in the circulation for two or three days(24). The enzyme dextranase, which is present in every tissue, except blood, breaks down dextran into sugars. The enzymatic degradation of dextran is much slower than the enzymatic degradation of amylose. Dextranase can only slowly hydrolyse the α (1 - 6) glycosidic bonds, in contrast with α (1 - 4) linkages of amylose, which are rapidly hydrolysed by α-amylose. For this reason, clinical dextrans normally have a high percentage (95%) of α (1 – 6) glycosidic linkages.

1.6 DEXTRINS.

Commercial dextrins are prepared by heating starch, which has been moistened with a

small quantity of dilute nitric acid, at 110 - 115°C. Three major reactions occur during this dextrinization of starch; hydrolysis, transglucosidation and repolymerisation[26].

In hydrolysis, scission of α (1 − 4) and α(1 − 6) glycosidic linkages between the glucose units occurs. In transglucosidation, glycosidic linkages are broken and reformed at other points to yield more highly branched molecules. Glucose is capable of repolymerising at high temperatures in the presence of catalytic amounts of acid.

Depending upon which of these reactions or combinations of reactions are involved, three major types of products are obtained, white dextrins, yellow dextrins or British gums. White dextrins are formed if hydrolysis is the dominant reaction. Moisture, high acidity and low converting temperatures are needed to make white dextrins, which have a wide range of viscosities and cold water solubility, depending upon the degree of conversion. Yellow dextrins are made using relatively higher amounts of acid, low moisture, and high converting temperatures. During the initial stages of the conversion, hydrolysis predominates, but during the latter stages, transglucosidation and repolymerisation reactions are dominant. Yellow dextrins are completely soluble in cold water. British gums are formed by heating starch at high temperatures, with little or no acid. While hydrolysis initially occurs, transglucosidation is the predominant reaction. The British gums are more soluble in cold water than the yellow dextrins.

Brimhall[27] has shown that dextrinization of amylose and amylopectin produces dextrins which are resistant to enzymic degradation. The dextrins were amorphous, branched and soluble in cold water.

1.7 SOLUBLE STARCH.

Starch is insoluble in cold water, but swells in hot water. Soluble starch is produced by controlled heating of potato or corn starch with dilute hydrochloric acid[28]. Linter soluble starch is prepared by treating raw starch with 7.5% hydrochloric acid for 7 days at room temperature[29]. This mild acid treatment destroys some of the hydrogen bonding in the starch molecule and causes slight hydrolysis. The resulting soluble starch is readily soluble in hot water, stains iodine blue-black, and can still be hydrolysed by α and β- amylases.

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