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Featured Article

A Review of Biodegradable Polymers: Uses, Current Developments in the Synthesis and Characterization of Biodegradable Polyesters, Blends of Biodegradable Polymers and Recent Advances in Biodegradation Studies

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Abstract: This review considers the uses of biodegradable polymers in terms of their relevance within current plastic waste management of packaging materials, biomedical applications and other uses; research papers and patents are catalogued. The chemical synthesis of polyesters and the microbial production of poly(hydroxyalkanoate)s, including recent publications in these areas, are covered and methods of characterization and structural analysis are outlined. Current research into two- and three-component blends is reviewed as a method of reducing overall costs and modifying both properties and biodegradation rates of materials. Finally, there is a summary of degradation processes. Both abiotic and biotic reactions are discussed, together with the development of biodegradation test methods, particularly with respect to composting. © 1998 Society of Chemical Industry

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Key words: biodegradation; biodegradable polyesters; packaging; synthesis; characterization; blends

1 INTRODUCTION

Within this review, biodegradation will be defined as by Albertsson and Karlsson[1] as an event which takes place through the action of enzymes and/or chemical decomposition associated with living organisms (bacteria, fungi, etc.) or their secretion products. However, it is necessary to consider abiotic reactions (e.g. photodegradation, oxidation and hydrolysis) that may also alter the polymer before, during or instead of biodegradation because of environmental factors. Albertsson and Karlsson[2] have also recognized that nature can be used

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as a model in the design of degradable polymeric materials, because it can combine polymeric materials with different degradation times into a hierarchical system that optimizes both energy and material properties. Naturally occurring biopolymers are susceptible to environmental degradation factors and their breakdown may be caused by a combination of these; for example, wood is both oxidized and hydrolysed. Tailoring the properties of polymers to a wide range of uses and developing a predetermined service life for the materials have become increasingly important and Albertsson and Karlsson suggest four different strategies:

(1) the use of cheap, synthetic, bulk polymers with the addition of a biodegradable or photo-oxidizable component;
(2) chemical modification of the main polymer chain of synthetic polymers by the introduction of hydrolysable or oxidizable groups;
(3) the use of biodegradable polymers and their derivatives, with poly(hydroxyalkanoate)s (PHAs) being the most studied;
(4) tailor make new hydrolysable structures, e.g. polyesters, polyanhydrides and polycarbonates.

All four approaches are considered within this review.

Current research interest in biodegradable polymers is connected with well-defined areas of use. Biodegradable plastics offer one solution to managing packaging waste. However, biomedical applications of biodegradable and biocompatible polymers generate an enormous amount of research and interest. Uses in this field range from wound dressings, drug delivery applications, surgical implants and other medical devices. There are also agricultural uses, e.g. for controlled release of fertilizers and pesticides, applications in the automotive industry and as surfactants.

As well as the uses of biodegradable polymers, this review will include sections on the chemical synthesis of polyesters, the microbial production of poly(hydroxyalkanoate)s (PHAs), blends of these biodegradable polymers, methods of characterization and structural analysis and, finally, recent research into the rates and mechanisms of the biodegradation of relevant polymeric materials. The latest published papers which have biomedical applications and relevant patents for the review are included as appendices.

2 USES OF BIODEGRADABLE POLYMERS

2.1 Biodegradable plastics for packaging

Synthetic polymers have become technologically significant since the 1940s and packaging is one industry that has been revolutionized by oil-based polymers such as polyethylene (PE), polypropylene (PP), polystyrene (PS), poly(ethylene terephthalate) (PET) and poly(vinyl chloride) (PVC). Despite the major advances in the synthesis, manufacture and processing of these materials, two major problems still confront the industry: the use of non-renewable, oil-based chemicals for the manufacture of commodity polymers and the ultimate fate of the waste materials.

2.1.1 Plastic waste management. The need to confront the problem of recycling packaging material of all types is recognized by governments and the European Community Waste Management Legislation is the first attempt to unify the approach to the problem by all the member states. The targets from the EU directive are shown in Table 1.[3]

In the UK, overall recovery targets for all packaging waste have been set for each year (1998–2001) with the obligation from 2001 onwards of over 50% recovery, of which at least half must be met by recycling, and up to 16% of each material, e.g. plastics, must be recycled. It is estimated that 200 000 t of post-consumer plastic waste were recovered in 1997 by incineration for energy or by recycling; as the consumption of plastics for packaging in the UK is about 2×10^6 t year^{-1}, this represents only 10% recovery.

In the UK, Valuplast is the organization responsible for implementing industry's obligations under the new recycling legislation. The aim is to increase the annual recycling capacity from the current 100 000 to 300 000 t by 2001, with upwards of 500 000 t year^{-1} of post-use plastics being recycled by 2005. Many companies have registered with Valpak, an organization that has undertaken to manage their recycling responsibilities. The implications for raw material producers and converters as well as packagers and retailers is considerable and a review of the legislation seems inevitable.

'Plastics Recovery' examining data from 1995 is the most recent report available on plastic consumption and recovery in Western Europe.[4] It is produced by the Association of Plastics Manufacturers in Europe (APME) and this is the seventh year that data from manufacturers, trade associations, waste management organizations and government bodies have been analysed in this way. The UK is shown to be the fourth

TABLE 1. Targets for EC waste management legislation

1	By 30/6/2001, between 50% and 65% by weight of packaging waste will be recovered
2	Within this general target and within the same time limits, between 25% and 45% by weight of packaging materials in packaging waste will be recycled
3	Within 2 above, a minimum of 15% of each material must be recycled, e.g. plastic packaging

largest consumer of plastics in Western Europe, using $3{\cdot}3 \times 10^6$ t in 1995 of the total $24{\cdot}3 \times 10^6$ t demand (UK demand in 1997 was 4×10^6 t). The consumption of plastics for packaging between 1993 and 1995 remained at about 40% of the total. Despite the huge demand for plastics in this and other industries, polymers account for only 0·6% by weight of total waste. It has been estimated that plastics make up about 8% of municipal solid waste (MSW)[4] and this represents the ultimate fate of about 63% of all plastic waste. Removing plastics from packaging would create problems, not solve them, as a German study has estimated the dramatic increase in terms of weight and volume of using non-plastic packaging and also the additional energy consumption required.[5] However, total plastic waste in Europe in 1995 was estimated at 16×10^6 t and, because of its low density and hence large volume, it has considerable environmental impact. Table 2 shows the figures for different disposal methods.

The three main strategies available for the management of plastic waste are incineration, recycling and landfill. Over 25% of the 16×10^6 t of plastic waste was recovered in 1995 either as energy ($\sim$17%) or by recycling (>9%). Plastic waste disposal to landfill represented a 14% fall from the $13{\cdot}3 \times 10^6$ t lost in 1994. Reviews of polymer waste management have been published by several authors.[6,7] The use of degradable plastics is, as yet, a minor factor in this overall strategy, but development of photo- and biodegradable polymers has considerable implications for medical, food and agricultural packaging.[8]

Incineration has the advantage that the plastics have high calorific value, but incineration plants have to be modified for efficient combustion of the polymers and regulation of the gas emissions to ensure that no toxic gases are released. Energy recovery through incineration is an essential part of an overall waste management scheme.

'In-house' recycling and interception of bulk waste at supermarkets, etc. are strategies that will have to be extended to meet the new British packaging regulations, which seek to double the amount of plastic packaging recycled by the year 2001, although a quadruple increase is more in line with EU targets. There are also emerging technologies for plastic feedstock recycling, which can take mixed plastic waste and turn it back into naphtha, monomers or other oil derivatives. A large-scale refinery-type operation is required to make these pyrolysis and hydrogenation procedures viable, but it is anticipated that the necessary economic climate will exist within the next 10 years.[9]

Some polymer manufacturers have their own recycling organizations, e.g. Petcore, the European PET container recycling organization. PET container collection for recycling in Europe has increased on average by 40% year-on-year from 13 000 t in 1991 to 86 000 t in 1997 although there has been a 10% annual growth in PET usage. However, the percentage increase in PET recycling in the UK was only 21% in 1997 and expected to be the same for 1998, because there is no funding mechanism to cover the cost of material collection as exists in other European countries. The new packaging legislation is bound to increase the UK totals, and recyclers are known to have excess capacity. However, the depressed price of virgin PET and all vinyl polymers makes it difficult for collectors and recyclers to run schemes profitably at the moment.

The non-statutory requirements given to local authorities by central government suggest a fivefold increase in materials collected from households in the next 4 years. Today about 40% of local authorities have packaging collection programmes, but it has been estimated that 80% of homes will have to participate in some form of kerbside collection by 2001. The two main challenges of plastic recycling are the separation and identification of the vast range of plastic items and the production of recycled material that has reproducible performance and is economically viable. The slight deterioration in the performance characteristics of a specific polymer only becomes significant after 5–8 cycles, but this closed loop recycling does not occur. Thus PET and PVC recyclates are used as fibres for thermal insulation (duvets, anoraks) and PS, PP and PE are reused for a range of office accessories, cassette cases, crates, pipes and refuse bags. Legislation prevents the use of recycled polymers in direct food contact packaging, and plastics with high technical specifications cannot be made from recyclates.

Plastic identification codes are one aid to separation that has been introduced, and mechanical sorting based on the specific gravity of the different polymers is well developed, although this will not effectively separate PVC and PET. Infrared technology is used to 'recognize' PVC from PET bottles, and both UV and surface dielectrics have been used to identify different polymers. Recently, Pira International has introduced a full-scale automated plant for the identification and separation of articles from mixed waste, using fluorescent tracers in

TABLE 2. European plastic waste disposal (1995)

Plastic fate	Amount (10^3 t)	%
Landfill	11354	70·7
Incineration without recovery	517	3·2
Waste exported from Western Europe for recovery	166	1·0
Energy recovery	2698	16·8
Feedstock recycling	99	0·6
Mechanical recycling	1222	7·6
Total	16056	100

various polymers which are then identified using ultraviolet light. Efficiencies of 95% have been achieved at sort speeds of six articles per second.

However, recycling from MSW is even more technically difficult and so far has proved uneconomic. Landfill sites are the main destination of plastic waste and the fate of even organic solids depends on the management of the site. The lack of air and water in the bulk of the material means that paper has been found intact even after 7 years. However, within these sites, temperatures up to 70°C can occur and anaerobic degradation can produce methane and other degradation products. Some bulk plastics undergo photochemical degradation, but most are not degraded.

Composting MSW has been attempted by some local authorities. This involves separating some of the landfill and, after preliminary screening, mechanically shredding the solid waste. The moisture content is then increased by the addition of sewage sludge and the mix is slowly agitated and aerated. After 5 days and screening, a large percentage of the material can be left to mature for use as compost. However, the plastic material is unchanged.

In the 1970s, work was started in the US and elsewhere to produce photodegradable and biodegradable plastics for the packaging industry. The requirements were:

(1) non-toxic materials with non-toxic degradation products that would not affect the drainage water from landfill sites;
(2) polymers with suitable mechanical properties for specific uses;
(3) economic viability;
(4) degradation control of the plastics via polymer modification;
(5) processability.

The source of these materials was of two main types: natural materials known to biodegrade and synthetic biodegradable polymers. Starch and cellulose are two natural materials that have been extensively investigated for use as packaging material and they have the added advantage of being renewable sources of polymeric material. The processability of the starch has been a major problem and the cellulose has to be significantly modified both to increase its biodegradability and to optimize its mechanical properties.

Poly(hydroxyalkanoate)s (PHAs), which are produced in plant cells and can be synthesized biochemically by fermentation, are another source of natural polymers. Poly(3-hydroxybutyrate) (PHB) and the copolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) are produced commercially by Monsanto and sold as Biopol®. PHBV copolymers were first manufactured by ICI in 1983 (see Section 3)[10] and were originally intended as biodegradable substitutes for oil-based polyolefins in plastic containers, films and bottles.[11] Their potential use was also seen for plastic items that could not easily be separated from other materials, e.g. nappies and sanitary towels. In 1990 the manufacture of blow-moulded bottles using Biopol for packaging shampoo was started in Germany by Wella AG, Darmstadt.[12] Hocking and Marchessault[13] have reviewed the actual and potential uses of PHB and PHBV for e.g. motor oil containers, film formation and paper-coating materials. The last two applications are based on biodegradable latex produced from PHAs with medium-length β-side chains.[14] The patents taken out over the last 10 years, which are summarized in Appendix II.1, give some indication of the range of possible uses.

Synthetic biodegradable poly(α-ester)s, e.g. poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and copolymers of these, have been manufactured for biomedical uses since the 1970s. These and other polyesters known to biodegrade, e.g. poly(caprolactone) (PCL), have been investigated as packaging materials. In order to optimize the properties of all these biodegradable materials and reduce costs, different research strategies have been adopted. Block and statistical copolymers have been synthesized and blending studies have been carried out to obtain compatible mixtures of components which still biodegrade. The use of plasticizers to increase the flexibility of polymers and the use of fillers to reduce the cost are two advantages of the blending approach. A further bonus comes from components that are photo- or biodegradable and induce this behaviour in components that do not degrade as pure materials.

The main constraint on the use of biodegradable polymers for bulk packaging is the difference in the price of these polymers compared with that of bulk-produced, oil-based plastics. The current cost of Biopol® is approximately £8000 per tonne, compared with the current UK prices of commodity polymers of between £500 per tonne (PVC and PP) and £600 per tonne (HDPE and high-impact PS). Current low oil prices, increased recycling capacity and improved technologies for the separation of plastics and their reuse make the use of biodegradable polymers for most packaging requirements uneconomic.[9]

There are several problems besides cost associated with certain biodegradable polymers as packaging. PGA and PLA are highly susceptible to bulk hydrolysis, so this limits the range of contents for the packaging, and rigorous storage conditions must be used. Microbial polyesters are relatively resistant to chemical hydrolysis but are susceptible to bacteriological attack, which restricts their use as packing for foodstuff. However, PHBV has excellent gas barrier properties[15] and Scherzer[16] has recently reported the development of barrier layers against oxygen transmission using radiation-cured methacrylated gelatin. The layers showed extremely low oxygen permeability, high resistance against boiling water and good adhesion characteristics. It is postulated that, using substrate foils of

biodegradable polymers, the methacrylated gelatins would be suited as barrier adhesives in laminates for completely biodegradable food packaging.

Another recent paper by Suominen *et al.*[17] reports their investigation into the migration of microbes in food-packaging paper and board coated with PE, mineral pigment or a biodegradable polymer. Confocal laser-scanning microscopy was used to examine the spatial distribution of microscopically observable bacterial cells. The paper and paperboard studied were an efficient barrier against translocation of microbes and it was concluded that there was limited access to free water within the cellulose. However, there were relatively high concentrations of microbes residing between the paperboard and its polymer coating, which on the coating facing the food is a potential site for leakage into the food. The need for high-hygienic-quality surface-sizing chemicals was emphasized and mineral-coating pigments were found to be a source of microbes and thus to be discouraged.

The relative merits of the different methods of managing plastic waste materials are eventually related to cost and legislation. This paper will review the current situation in the development of biodegradable blends of materials for uses including packaging.

2.1.2 Use of renewable resources. Renewable sources of polymeric materials offer an answer to maintaining sustainable development of economically and ecologically attractive technologies. The effect on the US economy of substituting production of corn-based polymer resins for petroleum-based polymers has been analysed by Beach *et al.*[18] and the use of agricultural materials and biomass has been reviewed by Mulhaupt.[19] Mulhaupt concluded that although Germany is at the forefront of green technology and a wide range of biodegradable pharmaceutical and novel surfactant materials can be made from renewable materials, it is only as components of packaging and as natural fibre composites that these materials are currently viable in terms of price and performance.

2.2 Biomedical uses

The most widely researched biodegradable polymers are the poly(α-ester)s (e.g. PLA, PGA) and the microbial poly(hydroxyalkanoate)s (e.g. P3HB, P3HV). These have been shown to be non-toxic and biocompatible both as polymers and as their degradation products, which in most cases occur naturally in the body. They have a range of pharmaceutical and biomedical uses based on these characteristics and their physicochemical properties.

The extremes of degradation rates between soluble macromolecules such as poly(vinyl alcohol) (PVA), which has a half-life of weight loss measured in hours, and the almost completely inert high-molecular-weight homopolymers such as polystyrene are more correctly defined as a measure of hydrolytic attack. Biodegradation occurs through the action of or in association with living organisms and its measurement is based on standardized tests developed over the last 30 years. PLA and PGA, which are used as absorbable suture material, are hydrolysed *in vitro* and *in vivo* in days, while PHAs are broken down much more slowly. Holland[20] produced the hydrolysis data for these polymers using physiological conditions and on which Table 3 is based. The uses of these polymers would be extended considerably if a wider range of degradation profiles were available. PHAs have the most potential, because although their rate of abiotic hydrolysis is relatively slow, microbial hydrolysis is more rapid and can be manipulated by variations in processing techniques, molecular weight of the polymer, copolymer composition and blending.[21] Although there have been considerable advances in controlled synthesis, the chemical synthesis techniques for these polymers have not yet produced tailor-made biopolymers with the fine structure (e.g. chirality, monomer sequence, tacticity) necessary to match enzyme specificity in degradation reactions in different microbial environments.

2.2.1 Speciality packaging. Speciality packing is potentially the largest use of biodegradable polymers. Polymeric materials and blends that have undergone toxicological and cytocompatibility testing for *in vivo* use and are biodegradable have obvious benefits as packaging materials for pharmaceutical products, drugs and wound dressings. The physiomechanical properties plus the gas and liquid barrier properties of these materials are obviously important, as is a knowledge of the conditions and rate of biodegradation. However, if processable biodegradable pure materials or blends could be manufactured, the economic imbalance between these materials and bulk-produced packaging materials would not be so great in this specialist packaging application.

2.2.2 Surgical fixation (*sutures, clips, bone pins and plates*). The development of the uses of PGA, PLA and PGLAs for surgical fixation has been summarized by Holland and Tighe.[21] These materials have been used increasingly from the late 1960s as absorbable, synthetic sutures because they could be produced as strong filaments and were shown to degrade rapidly. The most widely used absorbable sutures are Dexon®, a multifilament PGA material, Vicryl®, a copolymer with composition PLLA (8%)-*co*-PGA (92%) and PDS®, poly(*p*-dioxanone).

The use of biodegradable implants for the fixation of fractured bones and joints has been reviewed by Hofmann[22] and contrasted with the use of metal pins and clips. About 40 different biodegradable polymers and copolymers are currently being used as alternatives

TABLE 3. Comparative weight loss for different biodegradable polymers, given as time for 10% weight loss at 37°C and pH 7·4

Polymer	t_{10} (h)
Vicryl® absorbable suture (PLLA (8%)-*co*-PGA (92%))	450 (18·8 days)
Dexon® (PGA suture material)	550 (22·9 days)
PDS® (poly(*p*-dioxanone) suture material)	1200 (50·0 days)
PHBV (20% HV) ($M_w = 3 \times 10^5$)	4·7%@5500 (229·1 days)
PHBV (12% HV) ($M_w = 3{\cdot}5 \times 10^5$)	5·6%@5500 (229·1 days)
PHB (0% HV) ($M_w = 8 \times 10^5$)	18%@2500 (104·2 days)

to metal implants. The disadvantages of metal implants are cited as the need for 'stress protection', the sensitivity of patients to metals (particularly nickel) and the need for a removal operation. The use of self-reinforced PGA and polydioxanone, a polyester variation, as implants was also assessed and the main difficulties were listed as loss of stiffness in the material, in a time interval which is not long enough to guarantee bone healing, and the development of a sterile sinus over the implantation site. It is recognized that designs and assembling principles of metal implants in orthopaedic surgery cannot be directly transferred to biodegradable polymers. However, Hofmann states that these polymers can be used to treat osteochondral fractures and other defined injuries and a standard set of possible indicators for their use is given. Some specific examples of their use are ligating clips and bone pins produced from Lactomer®, P(LLA/GA70/30), and from various polydioxanones and polyoxalates, and high-strength bone implants of PGA, PLA and PGLA. Tunc and coworkers[23,24] have published a series of papers on the use of an 'Orientrusion' process for realigning PLA molecules to create a strong matrix with a fivefold increase in tensile strength over unprocessed PLA.

PHBV has piezoelectric properties similar to those of natural bone.[25] Electric stimulation can be used to repair and strengthen bone and there is a potential use for reinforced PHB composites, with bone-matching mechanical properties, as fracture fixative plates. Theoretically, growth and healing of the bone can be stimulated as the polymer slowly degrades, with no need of a replacement operation. The most recent developments that have been reported on using biodegradable materials for surgical implants are summarized in Appendix I.1.

2.2.3 Controlled drug delivery. This is the most important and versatile application of these polymers. Controlled drug delivery has applications not only in medicine but also in veterinary and agrochemical fields. Active ingredients from pesticides to contraceptives can be delivered by sustained release with the ultimate biodegradation of the carrier medium.

Drug delivery profiles of the concentration of the drug in plasma against time can be used to compare traditional methods of drug delivery (oral, intravenous) and controlled release. Figure 1 is based on a figure from the review of Holland and Tighe[21] and illustrates typical drug delivery profiles, showing the advantages of controlled drug delivery.

The initial drug release systems involved incorporation of the active substance into a polymer matrix, which was implanted into the patient in various ways,

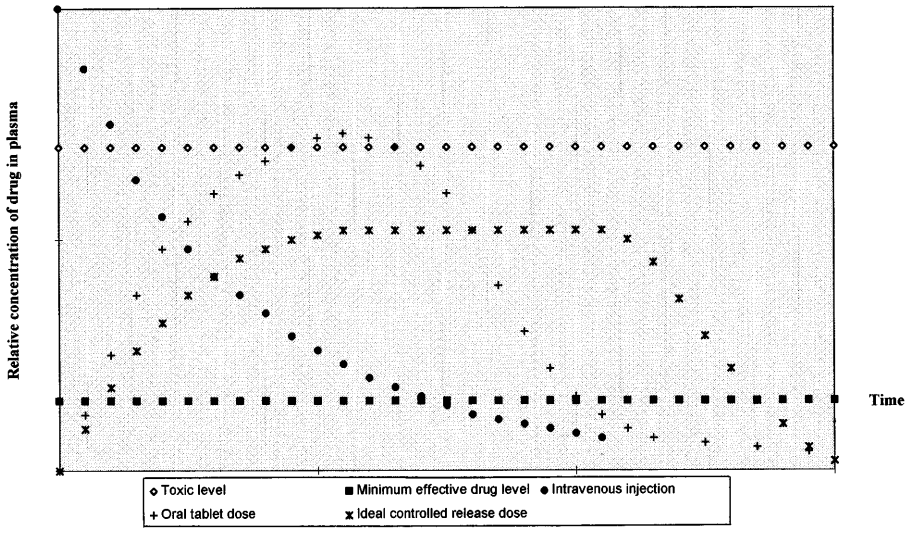


Fig. 1. Plasma–drug concentration profiles for various methods of administration.

and a diffusion mechanism produced the maintained drug release followed by bulk hydrolysis of the polymer. Controlled drug release has become the main application of (D,L)LA/GA copolymers and the best results, for relatively small drug molecules, have been obtained using PLGA copolymers with 20–50 mol% GA and molecular weights of approximately 25 000. However, modern techniques allow the incorporation of drugs which vary in nature and molecular size (from low-molecular-weight steroids to polypeptides (M_w = 22 000)). A diffusion mechanism is too slow for the release of large molecules, so an erosion release mechanism may be required for some polymer matrices. Currently it is claimed that drug loadings of upwards of 50% w/w and drug release rates from 170 mg day^{-1} to 20 μg day^{-1} can be achieved.

Polylactones such as PCL and PVL are also used for drug delivery. However, the most recent emphasis has been to blend PCL with PLLA and PGA or to produce block copolymers to control biodegradation and drug release characteristics. The slower hydrolysis rates of microbially produced polyesters suggest that PHB, PHV and their PHBV copolymers can be used to extend the range of drug delivery systems and Poulton and Aktar[26] have reviewed the potential of PHAs in this field. The relatively high melting temperature and rapid crystallization rate of PHB homopolymer make it difficult to entrap the drug. PHBV copolymers are also semicrystalline, but different solid matrices are produced by their slower crystallization rates. Release of low-molecular-weight drugs (drug loading $>5\%$) from these materials is shown to occur by water penetration and pore formation and is largely independent of erosion. However, surface erosion is particularly viable for drug delivery systems and with lower drug loading more effective entrapment occurs, and although it has been shown that porosity is initially poor, surface erosion does occur followed by greater water penetration. Other PHAs may provide drug delivery materials, as medium-chain PHAs have lower melting points and are more rubber-like than PHB. The various factors that control the rate of hydrolysis of PHAs have been extensively studied by Holland[20] and Yasin *et al.*[27] PHBV has been used for drug delivery both as tablets and as microcapsules, although the biodegradation mechanism has been found to be more complicated than the random chain ester hydrolysis of other polyesters.

Poly(orthoester)s, polyanhydrides and their blends are other groups of biodegradable polymers used for drug delivery and Thomas *et al.*[28] have studied microsphere production based on a blend of a soft, rapidly degrading polyanhydride, poly(carboxyphenoxyvaleric acid) (PCPV), and a rigid, slowly degrading polymer, poly(carboxyphenoxyhexane) (PCPH). Incorporating the soft polymer increased the density of the microspheres produced, but its rapid degradation led to a microporous structure from which small drug particles could diffuse rapidly, at rates that were independent of the blend composition. However, the rate of release of macromolecular particles of lysozyme was controlled by blend composition.

Park *et al.*[29] have recently reported on a new class of biodegradable compounds containing fatty acid dimer-based polyanhydrides, e.g. poly(fatty acid dimer–sebacic acid) (P(FAD–SA)). They were reported to have the right physicochemical and mechanical properties for drug delivery devices and to undergo pure surface erosion. The erosion characteristics of a polymer are very important and Park *et al.* investigated the degradation mechanism and the release profile and mechanism at various pH values. Disc-shaped devices, incorporating three model compounds with different hydrophilicity (mannitol, inulin and stearic acid), were fabricated and the release was studied in the range pH 1–9. All three model compounds were released faster in alkaline solution (release rate decreased pH 9 > pH 7·4 > pH 1–5). This was thought to be dependent on base-catalysed polyanhydride erosion, but it was found that erosion was not the sole controlling mechanism for drug release. In acidic solution the erosion of the polyanhydride was negligible but the highly water-soluble compounds were released rapidly. The release rate at all pH values mirrored hydrophilicity, decreasing from the very water-soluble mannitol to inulin to the lipophilic stearic acid. Pure erosion release profiles matched most closely the behaviour of the stearic acid. Molecular weight was also found to be a factor in the drug release. 100% of the low-molecular-weight mannitol was released within 6 weeks, but, after an initial fast release, only 60%–70% of the high-molecular-weight inulin was produced over this period and this was attributed to hindered diffusion through water-filled pores in the disc. No overall kinetic model was devised to fit the observed behaviour at all pH values and it was concluded that both bulk and surface erosion occurred but the balance depended on the pH of the medium and the nature of the incorporated drug.

Chiellini *et al.*[30,31] have reviewed the synthesis and semi-synthesis of multifunctional polymers and evaluated both their biodegradation and bioerosion. They have also assessed their potential for controlled drug release in pharmaceutical and agricultural areas and in particular focused on hydrophilic and/or water-soluble polymeric materials and their blends.

The most common methods of sustained drug delivery are as injectable micro- or nanospheres or as subcutaneous implant systems,[32,33] which involve some diffusion or erosion matrix. However, some novel methods have been devised recently. Giordano *et al.*[34] have manufactured and characterized non-porous, flexible, thin films made of PLLA and P(D,L)LGA (75 : 25 and 50 : 50) to which foetal human retinal pigment epithelium (RPE) cells were found to adhere when cultured *in vitro*. Films of controlled thickness and reproducible

surface morphology were produced. This technique has the potential to provide a means of transplanting allogenic RPE cells as a therapy for several ocular diseases related to RPE dysfunction.

Jeong *et al.*[35] have investigated the use of thermosensitive biodegradable hydrogels consisting of poly(ethylene oxide) and PLLA, aqueous solutions of which exhibit temperature-dependent reversible gel–sol transitions. The hydrogel can be loaded with bioactive molecules in water at approximately 45°C; a sol is formed, so subcutaneous injection can be carried out. Rapid cooling to body temperature occurs and the loaded copolymer forms a gel that can act as a sustained release matrix for the drug.

Akamatsu *et al.*[36] have synthesized a polymeric prodrug of prostaglandin E-1 (PGE(1)) using galaclosylated poly(L-glutamic acid) (Gal-PLGA) as a biodegradable and targetable carrier of liver parenchymal cells as polymeric conjugates. After intravenous injection into mice, conjugates rapidly accumulated in the liver with up to 65% of the dose, suggesting effective targeting.

Microspheres. The majority of recent papers on drug delivery involve the use of microspheres fabricated using single or multiple emulsion techniques followed by solvent evaporation, although there are several other standard procedures. Okada and Toguchi[37] have reviewed the preparation, characterization and biodegradation of PLA and PLGA microspheres, and sustained release depots and target drug delivery are also discussed. Recent developments in the production of microspheres of polyesters and their blends are summarized in Appendix I.2.

Nanoparticles. In certain circumstances the use of microparticles for drug delivery is either inappropriate or can be extended by reducing the particle size. The minimum microparticle size is about 10 μm, but nanoparticles with size under 100 nm can be fabricated. Research areas cover novel properties that have been developed for nanoparticles, increased efficiency of drug delivery, improved release profiles and drug targeting. Appendices I.3 and I.4 summarize the latest reported work using nanoparticles: the former highlights the preparation and characterization procedures and the latter deals with novel applications.

In vivo *and* in vitro *studies of effectiveness of drug release.* Monitoring sustained drug release can be carried out using a wide range of different techniques depending on the nature of the biodegradable material and the entrapped drug. The ideal drug delivery profile was illustrated in Fig. 1 and the purpose of *in vitro* and *in vivo* studies is to provide information about these profiles, including delivery dosage and release time. Recent developments are summarized in Appendix I.5.

Biodegradation, biocompatibility and cytology studies. Although various biodegradation studies using standard conditions have been set up to look at the rate and mechanism of the breakdown of individual polymers for non-medical use, the degradation of drug delivery systems represent a much more rigorous area of study. In Appendix I.6 the papers published in this area during 1994–1997 are reviewed. Some of the *in vivo* and *in vitro* studies covered in Appendix I.5 also relate to degradation of the polymeric carrier.

2.2.4 Other biomedical uses. Advances in tissue culture and tissue engineering have generated research into novel methods of producing biodegradable networks that are effective for a variety of applications both as hard and soft scaffolds. These latter networks have uses as wound dressings, as tubular conformations for intestine or vascular grafts and as skin substitutes. Advances in the use of biodegradable polymers for tissue engineering have been reviewed by Vacanti *et al.*[38] Although the use of biodegradable polymers for drug delivery has already been dealt with, there has been work carried out on the development of pastes that can be applied after surgery to inhibit tumour growth by the slow release of taxol. The latest papers and patents reporting work in these areas are summarized in Appendices I.7 and II.2.

2.3 Other uses

2.3.1 Detergent applications. The use of polymeric carboxylic acids (poly(acrylic acid) and its copolymers) in detergent powder formulas is reviewed by Paik *et al.*[39] They were initially introduced in the 1980s in combination with zeolites as partial replacements for polyphosphates. Wastewater treatment plants are unable to remove phosphates and, as these salts promote plant growth in rivers, this can lead to eutrophication and eventually environmental imbalance. The non-biodegradability of the original polymers eliminates the possibility of eutrophication, but acid accumulation eventually results from their use. Although the acids used are non-toxic, questions about their fate and long-term environmental impact remain. The review traces the history of the biodegradable polymer options that have been considered and the current state of the research. Freeman *et al.*[40] have identified poly(aspartic acid) as a biodegradable, synthetic polycarboxylate with acceptable detergent properties. Semi-continuous activated sludge (SCAS) removal tests and modified Sturm CO_2 production tests show that poly(α,β-D,L-aspartate)s prepared by acid-catalysed thermal polymerization are rapidly and completely removed by municipal treatment plant micro-organisms. However, polyaspartates prepared by other methods were only partially biodegraded. Structural analysis shows that a linear polyamide backbone is needed for total degradation.[41] Talaba *et al.*[42] have reported the modification of *O*-(2-sulphoethyl)cellulose (2-SEC) to produce alkylated SECs as cellulose-based biodegradable surfactants.

2.3.2 Agricultural and veterinary uses. Although the application of controlled drug release with respect to agricultural use has already been mentioned, it is worth emphasizing the fact that the bacterium *Alcaligenes eutrophus*, which can be used both to synthesize and degrade PHB and PHBV copolymers, was first isolated from soil. Holmes, in his original paper on the industrial production and potential uses of PHBV, recognized its potential for the release of insecticide into soil.[10] The PHBV/insecticide pellet would be sown with the seed and the pest activity would be directly connected with the bacterial activity, thus degrading the polymer and releasing the chemical at the appropriate time.

Veterinary controlled drug release also has specific applications. PHB and PHBV are broken down relatively slowly *in vivo* unless specific PHB depolymerases are present. The rumen in cattle has been found to produce good degradation conditions and so controlled release of medicine at preset intervals is achieved using a bolus of PHBV that remains in the rumen.[43] Appendix II.2 contains a summary of the patents taken out recently for agricultural uses.

2.3.3 Miscellaneous uses. Several alternative uses of biodegradable polymers have been postulated, e.g. the sorption of oil-based aromatic compounds from low-carbon sand by microbial polyesters, which has been investigated by Dohse and Lion,[44] and the application of these polymers in the field of oil pollution has been recognized.

A 1994 patient cited the use of poly(caprolactone) filaments blended with other biodegradable polymers as a biodegradable carrier for denitrifying bacteria in water purification. The recent patents identifying potential uses of these biodegradable polyesters are summarized in Appendices II.1 and II.2.

3 REVIEW OF BIODEGRADABLE POLYMERS

Many authors have reviewed biodegradable polymers, but, to give an overview of the range of materials available, Holland and Tighe[21] provide a concise summary on which Table 4 is based. Synthetic and microbial polyesters form the greatest number of reported types of biodegradable polymers and are the main focus for this review, so an outline of their preparation and properties follows. The processing of starch and cellulose is also covered because of the extensive use of these natural polymers in biodegradable blends.

3.1 Chemical synthesis of polyesters

Polyesters can be synthesized by polycondensation of the hydroxy acid or by ring-opening polymerization of the anhydrosulphite, the anhydrocarboxylate or the lactone/dilactone. The disadvantage of the stepwise polycondensation preparation is that water has to be removed continuously, requiring extreme conditions, and the polymerization requires long reaction times and produces chains with widely varying length. Ring-opening polymerization of the cyclic lactone/dilactone is the preferred method of producing high-molecular-weight polyesters of this type. However, the anhydrosulphites and anhydrocarboxylates can be synthesized from α-hydroxy acids and then the heterocycles

TABLE 4. Biodegradable polymers

Type	Comments	Examples
Polyesters	Formed by condensation, ring-opening polymerization or bacterial synthesis	Poly(α-ester)s, polylactones and poly(β/γ-ester)s
Polyamides	Only structurally modified synthetic polyamides are biodegradable	Hydroxylated nylon
Polyurethanes	Only structurally modified synthetic polyurethanes are biodegradable	Hydrophilic ether urethanes
Polyureas	Virtually 'non'-degradable	Urea formaldehyde
Polyethers	Dissolve if carbon chain is short; but also found to degrade	General formula $-(-O-(CH_2)_x-)_n$. Poly(ethylene oxide) (PEO), $x = 2$
Polyanhydrides	Degradation thought to be mainly by surface erosion	Poly(bis(*p*-carboxyphenoxy)alkane anhydride) or PCPX
Poly(orthoester)s	Degradation thought to be mainly by surface erosion	Poly(3,9-bis(ethylidene-2,4,8,10-tetraoxaspiro[5.5])undecane-*co*-hexane diol) or DETOSHU-HD
Polypeptides and proteins	Naturally occurring polyamides containing amino acid units	$-(-C(R)H-CO-NH-)_n$ with different R groups and chain lengths e.g. natural proteins collagen, gelatin
Polysaccharides	Basic sugar units joined by glycoside linkages; hydrolysed abiotically and by enzymes	Naturally occurring starches and different forms of cellulose

can be polymerized thermally or using nucleophilic- or base-catalysed reactions. The anhydrosulphites are more susceptible to ring-opening polymerization than the anhydrocarboxylates.

Table 5 summarizes the range of monomers used to produce polyesters, and polymerizations can be carried out either in the bulk or in solution. Ring-opening polymerization (ROP) of the dimeric cyclic glycolide or lactide produces the poly(α-ester) poly(glycolic acid) (PGA) or poly(lactic acid) (PLA) respectively. The conditions are sealed, dry containers under reduced pressure and at high temperature. A wide range of initiators can be used such as tin-, zinc- or aluminium-containing organo-compounds, e.g. tetraphenyl tin, stannous octanoate, aluminium iso-propoxide. Also anionic polymerization catalysts can be used, e.g. potassium methoxide, potassium *tert*-butoxide, butyl lithium and mechanisms for the polymerizations have been proposed.

The properties of PGA are related to its high molecular weight, highly crystalline structure and the fact that it is the most hydrophilic of the polyesters. It has a very high melting point (224–226°C) and much higher T_g (36°C) than poly(β-ester)s. Its use as a degradable suture material and its bulk, mainly abiotic, hydrolytic degradation are covered in other sections.

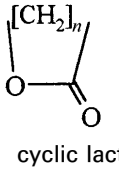
The conformations of both lactic acid ($HO-C(CH_3)H-COOH$) and dilactide monomers affect the resultant tacticity of the polymer and this has been the basis of many mechanistic studies. There is still some confusion about the naming of both the monomers and the polymers, as both the D,L and R,S systems appear in the literature. Some attempt to clarify the nomenclature seems to be required; the examples that follow are based on published work in which the tacticity is determined by application of Bernouillian statistics to monomer sequences found from the ^1H and ^{13}C NMR spectra of the polymers. Naturally occurring lactic acid is LLA and the pure enantiomeric polymers (PLLA and PDLA) are semicrystalline, but P(DL)LA is more amorphous, less mechanically strong and has a lower melting point (P(DL)LA, $T_m = 130–135°C$; PLLA, $T_m = 180°C$). P(DL)LA is more processable, as PLLA has a relatively high T_g (58°C). Both PGA and PLA undergo *in vitro* and *in vivo* degradation at similar rates. The hydrolytic scission of PLA is at a slightly slower rate than for the more hydrophilic PGA. Different copolymers of GA and LA are manufactured for a variety of biomedical applications. They are generally more amorphous than the pure polymers and are more resistant to hydrolysis if they contain high percentages of one polymer.

Stereo structure of lactic acid (LA)/lactide (L) monomers and the poly(lactic acid) or polylactide (PLA or PL) product

- D(−) lactic acid (or R(−) lactic acid) produces *isotactic* P(DLA).*

* Unless the mechanism for polymerization involves racemization[46] of the asymmetric centre or transesterification[48,49] occurs.

TABLE 5. Biodegradable polyesters

Type	Monomers	Examples of polymers
Poly(α-ester)s	α-Hydroxy acid e.g. HO—C(R)H—COOH. Dimeric cyclic lactide or glycolide undergoes ROP	Poly(glycolic acid) (PGA) (R = H). Poly(lactic acid) or polylactide (PLA or PL) ($R = CH_3$). Copolymers poly(lactic acid-*co*-glycolic acid) (PLGA)
Poly(β-ester)s or poly(3-hydroxy alkanoate)s (PHAs)	β-Hydroxy acid, e.g. HO—C(R)H—CH_2—COOH, or cyclic lactone	Poly(3-hydroxybutanoate) (P3HB) ($R = CH_3$). Poly(3-hydroxyvalerate) (PHV) ($R = C_2H_5$). Copolymers poly(hydroxybutyrate-*co*-hydroxyvalerate) mol% HV defined (PHBV (8% HV))
Poly(γ-ester)s	HO—C(R)H—$(CH_2)_2$—COOH or cyclic lactone	Poly(4-hydroxybutanoate) (P4HB) (R = H)
Other polyesters or polylactones	 cyclic lactone	Poly(ε-caprolactone), $n = 5$. Poly(valerolactone), $n = 4$

- L(+) lactic acid (or S(+) lactic acid) produces *isotactic* P(LLA).*
- Racemic mixture of D/L-lactic acid produces *atactic* P((D,L)LA).†
- D,D-dilactide or L,L-dilactide produces *isotactic* P(DLA) or P(LLA).*[45,46]
- Racemic mixture of D,D/L,L-dilactide produces predominantly *isotactic* P((D,L)LA).[47]
- Diastereoisomer of D,L-dilactide produces *syndiotactic* P((D,L)LA).[45]

3(or β)-Hydroxyalkanoic acids with β-alkyl side chains (HO—C(R)H—CH_2—COOH) also exhibit stereoisomerism. The simplest example is 3-hydroxybutyric acid (R = CH_3—) and again it is important to summarize the notation. Poly(β-ester)s (P3HAs) and poly(γ-ester)s (P4HAs) can be synthesized from the appropriate cyclic lactone or produced microbially, and recent research in both these areas will be considered separately.

Stereo structure of 3-hydroxybutyric acid (3-HBA)/3(β)-butyrolactone (3-BL) and poly(3-hydroxybutyrate) (P3-HB)

- All naturally occurring 3HAs have R (or D) configuration and microbial synthesis produces *isotactic* P(R)3-HAs.
- (R)-β-butyrolactone (R-3-BL) also produces *isotactic* P(R)3-HB.
- Racemic mixture ((R,S) or (D,L) 3-BL) produces P(3-HB) in which the tacticity depends on the catalyst used: *atactic*, *isotactic* and *syndiotactic* have been produced.[50–52]

Poly(ε-caprolactone) is synthesized by the ring-opening polymerization of the cyclic ε-caprolactone monomer using similar catalysts to those already mentioned. The semicrystalline polymer (<60% crystalline) has a low melting temperature ($T_m < 60°C$) even for relatively high-molecular-weight samples ($M_w = 80\,000$). The glass transition occurs well below ambient temperatures (−60°C) and the polymer has relatively low tensile strength (23 MPa) but extremely high elongation at break (>700%). The biodegradation of PCL in both soil burial and activated sludge tests was found to be relatively fast with rapid weight loss, indicating a bulk random chain scission mechanism, but abiotic hydrolysis occurs more slowly. Low-molecular-weight PCL is used as a processing elastomer for polyurethanes and for fabric coatings. The high-molecular-weight material is used as a thermoplastic processing aid for adhesives and a range of polymers.

† Unless the catalyst is stereo-selective, producing isotactic polymer from one entantiomer.

3.1.1 Recent developments in chemical synthesis of PHAs. Distannoxane complexes have been used to catalyse the ring-opening polymerization of optically active 3-butyrolactone (β-BL) to produce high-molecular-weight P3HB. Hori *et al.*[53] report that when 1-ethoxy-3-chlorotetrabutylstannoxane was used as a catalyst (catalyst/monomer ratio 1 : 8000, at 100°C for 4 h), a 99% yield of P(R)3HB was obtained. The process was extended to produce stereo-copolymers, by copolymerization of (R)β-BL and racemic-(β-BL), and statistical copolymers, from (R)-(β-BL) with ε-caprolactone, β-methyl-δ-valerolactone and L-lactide. These novel, optically active, biodegradable copolyesters were found to have high molecular weights and those with over 80 mol% (R)3HB have almost the same biodegradability as PHBV (11% HV) produced microbially. The reaction mechanism for (R)-(β-BL) catalysed by distannoxane complexes was shown to proceed by bond breaking between the carbonyl carbon and the oxygen atom of the lactone ring (acyl cleavage), with retention of the configuration, and little or no racemization. 1-Ethoxy-3-chlorotetrabutylstannoxane has also been reported[54] as the polymerization catalyst, under similar reaction conditions (100°C), for (R)-(β-BL) with other four-membered lactones, producing a series of P3HAs in good yields. Hori *et al.*[55] have also synthesized a copolymer of (R)-(β-BL) with cyclic carbonates to produce biodegradable poly(ester carbonate)s.

The usefulness of dimethyl, diethyl, dibutyl and dioctyl tin oxide (DMTO, DETO, DBTO and DOTO) for the preparation of syndiotactic poly(β-D,L-butyrolactone) was studied by Kricheldorf and Eggerstedt,[56] mostly in the bulk, but toluene or chlorobenzene was used in a few experiments. The reaction temperature had to be above 50°C to activate the catalysts, and temperatures between 50 and 100°C were used. DMTO proved to be the least reactive initiator, whereas DBTO produced high yields and the highest-molecular-weight polymers ($M_n > 80\,000$ and $M_w > 130\,000$). The highest-molecular-weight polymers were obtained at low monomer/initiator ratios (M : I = 50–400). Unexpectedly, high M : I ratios (>600) produced no conversion for any conditions used. The highest percentage of syndiotactic diads (72%) was found using DBTO at 50°C or with DETO at 100°C. Flexible, elastic, transparent films were cast from the 63% and 70% syndiotactic PBL samples, which may have an application as packaging materials.

Hirt *et al.*[57] have described the synthesis of microphase-segregated block copoly(ester-urethane)s from telechelic hydroxy-terminated poly(PHB-diol) (hard segments) and hydroxy-terminated PεCL or telechelic hydroxy-terminated poly(adipic acid)-alt-(1,4-butanediol; diethylene glycol; ethylene glycol) (Diorez®) (soft segments), with either 2,2,4-triethylhexamethylenediisocyanate (TMDI) or methyl (s)-2,6-diisocyanatohexanoate (LDI). High-molecular-weight

copolymers were obtained with or without a catalyst and the mechanical properties of the copolymer were investigated. The Young modulus was found to be directly dependent on the mol% of crystallizable PHB-diol in the copolymer, increasing with increase in the amount of PHB-diol, which produced a corresponding decrease in the percentage elongation at break. However, the amount of 'soft segment' or diisocyanate had only a minor influence. The chain length of the non-crystallizable (soft) segments indirectly incluenced the morphology and mechanical properties of the polymer by altering the phase segregation behaviour.

Reeve *et al.*[58] have prepared P(R)(3HB)–PεCL and P(R)(3HB)–PL diblock copolymers from low-molecular-weight (average DP = 26) poly(hydroxybutyrate) stereoisomers. These have been produced by degradative, acid methanolysis of high-molecular-weight PHB. Macroinitiators were then produced by the reaction of the purified, dry hydroxy-terminated units with aluminium triethyl to form PHB-O-$AlEt_2$ species, which were used to catalyse the ring-opening polymerization of ε-caprolactone or lactide. The A–B-type copolymers produced were characterized by VPO, GPC and ^{1}H NMR. DSC and X-ray diffraction studies showed that with short PCL (DP 12) and L-PL (DP 13) chain segments, PCL and PLA crystalline phases did not form. However, at longer chain length (PCL (DP 38 and 51) and L-PL (DP 23)), both segments produced superimposed X-ray patterns. PHB–L-PL diblock (PHB (DP 26) and L-PL (DP 23)), when melted and rapidly quenched, produced a kinetically frozen solid state mrophology with miscible chain segments and a new T_g (20°C), but this undergoes phase separation on annealing at 55°C for 24 h.

Transesterification has been carried out between several thermally stable polyesters.[59,60] Attempts by Vert[61] using PHB and PLA both in the melt in the presence of ethylene glycol and in solution using ethanol or dichloromethane with several different catalysts have been unsuccessful. However, Hirt *et al.*[62] report the preparation of telechelic OH-terminated P(R)-3-HB and P((R)-3-HB-*co*-(R)-3-HV) on a semi-preparative scale using transesterification from the high-molecular-weight polymers. The oligomers have well-defined reactive end groups and are well suited for the production of high-molecular-weight block copolymers by chain extension.

3.2 Microbial synthesis of poly(β-ester)s and poly(γ-ester)s

Poly(3-hydroxyalkanoate)s (PHAs) are biosynthesized as storage material in plants, from different organic substrates, using a wide range of bacteria. It has been shown that poly(3-hydroxybutyrate) P(3HB) is accumulated in the cells of many bacteria under unbalanced growth conditions when exposed to an excess of carbon.[63] In the 1970s ICI developed a large-scale fermentation process, based on a two-stage batch reactor, to produce P(R3HB) using sugar-based feedstock and the bacterium *Alcaligenes eutrophus*.[10] The oil crisis was the initial incentive for this investment, but as oil prices stabilized, the high cost of extraction and lack of processability of this plastic compared with polypropylene (PP) made its production uneconomic.

The properties of PHB, like those of PP, are of a thermoplastic material; Table 6 compares these properties based on the review of Hocking and Marchessault.[13] PHB is a stiffer, more brittle polymer than PP, but hot rolling can reduce the size of the spherulites in its structure and produce a more ductile material. However, a nucleating agent is often needed, as the rate of crystallization of all PHAs is very slow, which was recognized as a major disadvantage. Although PHB is insoluble in water, it has poor chemical resistance and undergoes isothermal degradation from 170 to 200°C. A drop in molecular weight to about half its original value was found when PHB was held at 190°C for 1 h. Packaging films, fibres and containers were produced in the ICI evaluation using moulding and extruding processes and the film was found to have excellent gas barrier properties.

Further advances in the ICI fermentation process using different feedstocks (glucose/propionic acid) resulted in the production of statistical copolymers of 3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV). The properties varied with the percentage of HV, the copolymers becoming less brittle with more PHV. As the mol% of PHV increased, the Young modulus (stiffness) decreased and the impact strength (toughness) increased. The copolymers had only slightly reduced percentage crystallinity, but lower melting points and similar thermal stability. The copolymers showed even lower crystallization rates with PHB. The biodegradability of these polymers, using bacteria in a variety of media, was an important consideration when in 1983 ICI, as Marlborough Biopolymers Ltd, started the first commercial production of Biopol®, a range of PHBV

TABLE 6. Comparison of properties of PHB and PP

Property	PHB	PP
Molecular weight (g mol^{-1})	5×10^5	2×10^5
Crystallinity (%)	80	70
Crystalline melting temperature (°C)	175	176
Glass transition temperature (°C)	4–0	−10
Density (g cm^{-3})	1·25	0·91
Tensile strength (MPa)	40	38
Extension at break (%)	6	400
Ultraviolet resistance	Good	Poor
Solvent resistance	Poor	Good

polymers. The advantage of a renewable feedstock was one of the attractions of the process, but the artificially high price of fermentation sugars within the EC made it impossible for the implementation of commodity-scale production, although a small 'high-added-value' business was envisaged.

Biopol is now manufactured by Monsanto and at about £8 per kilogram is about 10 times the price of PE. Biosynthesis of a range of homo- and copolymers of PHAs is now possible using a wide range of microbes and feedstocks in the fermentation broth. Recently, Monsanto and other workers have been investigating the production of PHAs from transgenic plant cells. A suspension culture of transgenic *Arabidopsis thaliana* plant cells has been shown to produce PHB with chemical structure identical to that produced by bacteria.[64] Genetic engineering of the DNA of both arabidopsis and oilseed rape, to introduce the genes known to produce PHB in microbes such as *Alcaligenes eutrophus*, has been investigated by a Monsanto/Durham University research collaboration.[65] Production of high-molecular-weight PHAs occurs in both the leaves and seeds of these plants. The aim is to obtain both the oil and polymer as cash crops from the oilseed rape. It has been estimated that 20% of the seed weight as PHA is needed for commercial viability and to achieve this workers have increased the production of the naturally occurring enzymes in the plant used to make the polymer. A method of turning off the production of the PHAs in the leaves has also been devised to concentrate the production in the seeds.

PHBV statistical copolymers with HV composition up to 90 mol% have been synthesized microbially and characterized by several workers.[63,66,67] Using *Alcaligenes eutrophus* in nitrogen-free conditions, with glucose and propionic acid as cosubstrates, PHBV with up to 47 mol% HV was produced.[13] A recent paper by Chung *et al.*[67] has suggested a method of overcoming the problem of the inhibited growth of *Alcaligenes eutrophus*, at concentrations of propionic acid above $0{\cdot}5\,g\,l^{-1}$ in the fermentation broth, by pH regulation. However, Doi *et al.*[68] have reported PHBV with up to 90 mol% HV using *Alcaligenes eutrophus* and feedstocks of different ratios of pentanoic acid and butyric acid.

Although in 1988 ICI was manufacturing PHBV (0%–25% HV) of different grades, from technical and standard (95%–96% purity) to specially purified grades for medical uses (>99·5% purity), the economic viability of the polymers was never really established. Hocking and Marchessault[13] have comprehensively reviewed the potential uses and the three main components of the cost of PHBV production (carbon source, fermentation processing/extraction and capital-related charges). As yet there has not been the necessary economic breakthrough for massively increased production of PHBV. The large-scale manufacture of Biopol by Monsanto now concentrates on a more limited range of PHBVs, with PHBV (8 mol% HV) as the major product. However, recent research publications indicate that there is still considerable interest in novel and economic methods for both the microbial and the chemical synthesis of PHAs.

3.2.1 Recent developments in microbial synthesis of PHAs. Two papers identify novel routes to the production of PHB and PHBV copolymers. Eschenlauer *et al.*[69] have produced a working model for the synthesis of PHBV from a single carbon source (glucose) via acetyl- and propionyl-coenzyme A, by expressing the PHA biosynthesis genes from *Alcaligenes eutrophus* in *Escherichia coli* strain K-12 under novel growth conditions. Maness and Weaver[70] reported a process to photoconvert low-grade organic material waste biomass into PHB and PHBV. Unique strains of photosynthetic bacteria were contained in thermally gasified dry biomass and under unbalanced culture conditions these produced up to 28% new cell mass of granular, high-molecular-weight PHAs. Extraction showed that the dominant monomeric unit was 3-HB, but the use of a green alga as coculture with the photosynthetic bacterium, in light–dark cycles, produced PHBV (30% HV) as 18% new cell mass.

Pure P3HV production was originally reported by Steinbüchel *et al.* in 1993[71] using the bacterium *Chromobacterium violaceum* and valeric acid as the sole carbon source in limited nitrogen. More recently, Steinbüchel and Schmack[72] have improved this process by using complex nutrients as supplements. A yield of 40 g of dry cell matter per litre of fermentation broth was obtained and 70% w/w of the cells were found to be P3HV.

Gross *et al.*[73] have carried out extensive research on the biosynthesis and characterization of PHAs using *Pseudomonas oleovorans* and sodium salts of *n*-alkanoic acids as the carbon source. PHAs containing at least two major (>5 mol%) monomer units were obtained and, depending on the carbon source, *n*-alkyl- pendant groups from methyl- to nonyl- were found. Maximum cellular yield and polymer content (percentage cellular dry weight) obtained were $1{\cdot}5\,g\,l^{-1}$ and 49% respectively using nonoate as the carbon source. GPC data indicated that the polymers had M_w between 160 000 and 360 000, whilst DSC and X-ray diffraction measurements showed that the polymers with longer side chains ($\geq$*n*-pentyl) were crystaline polymers (T_m from 45 to 61°C), with participation of both main and side chains in a layered packing order. Those with *n*-propyl- and *n*-butyl- side chains showed little tendency to crystallize. A more recent paper from the same group by Peres and Lenz[74] has reported the use of this microbial synthesis of optically pure copolymers. A copolymer containing 85 mol% β-hydroxyoctanoate units was converted into 97% optically pure (s)-β-pentyl-β-propiolactone (S-PPL) and then polymerized by a ring-opening process,

using aluminoxane and zinc alkyl catalysts, into stereoregular poly(β-hydroxyoctanoate). The diad stereochemical sequence in the polymer was determined using 50·3 MHz ^{13}C NMR spectroscopy and the polymers synthesized were also characterized by comparing their DSC profiles with those of racemic and optically active forms of poly(β-hydroxyoctanoate).

De Konig[75] has recognized two major problems with these PHAs: their tendency to soften and lose their coherence at temperatures as low as 40°C and their low crystallization rates limiting the practicability of many processing techniques. However, crosslinking was proposed as a solution and successfully implemented using a carbon source that introduced unsaturated bonds into the polymer. Crosslinking was achieved using electron beam irradiation and produced a true rubber with only chemical crosslinks. The properties were constant from T_g (30°C) to the decomposition temperature (170°C) and the degree of crosslinking was controlled by the fraction of unsaturated monomer units used and the radiation dose. The enzymatic degradation rate of these crosslinked PHAs was similar to that for the unmodified polymers and characterized by surface erosion. The application of these PHAs as latex to substrates such as paper and the use of heat, ultraviolet light or an electron beam source to establish crosslinking have been proposed.

Inoue *et al.*[76] have recently published a paper on an extension of Satoh and co-workers'[77] research into the use of activated sludge from wastewater treatment works, developed for anaerobic–aerobic biological phosphate removal, for PHA synthesis. Activated sludge is a valuable biomass source, but currently it is excessively produced and the majority is disposd of as landfill without reuse. Under optimum conditions it has been shown that it is possible to accumulate PHAs of more than 30% dried weight of sludge. Satoh *et al.*[78] have investigated an anaerobic take-up mechanism of acetate, propionate and lactate by the sludge developed in the phosphate removal system. The latest research[76] examines the microstructure of the PHAs produced from this sludge, using propionate as the carbon source, with ^{13}C NMR spectroscopy and identifies four types of monomer unit: 3HB, 3HV, 3H2MB (3-hydroxy-2-methylbutyrate) and 3H2MV (3-hydroxy-2-methylvalerate). It is suggested that these are essentially statistical copolymers, although some diad sequences could be allotted. It was concluded that there was no clear evidence for true four-monomer-component copolymers.

Neither of the two common bacteria used to produce PHAs (*Alcaligenes eutrophus* for PHB and PHBV production and *Pseudomonas oleovorans* for longer-side-chain PHAs) can be used to produce copolymers of hydroxybutyrate (HB) and other monomers with higher carbon number than valerate. However, Caballero *et al.*[79] have biosynthesized and characterized PHB-*co*-HC copolymers (2–4 mol% hydroxycaproate (HC)) and PHB-*co*-HC-*co*-HO terpolymers (hydroxyoctanoate (HO) units). The polymers were produced in good yield using *Comamonas testosteroni*, *bacillus cereus* and an unknown third organism when grown on caproate or octanoate. X-ray analysis and quantitative analysis of the melting point depression showed that the minor copolymer was rejected by the PHB crystallites. The reduced melting point but high degree of crystallinity shown by these materials leads the authors to suggest their use as melt-processable thermoplastics.

Doi[80] and co-workers have carried out extensive research on the synthesis and characterization of microbial polyesters. Most recently they have reported work on the microbial synthesis of copolymers of [R]-3-hydroxybutyrate (3HB) and 4-hydroxybutyrate (4HB) from various substrates by *Alcaligenes eutrophus*, *Alcaligenes lotus* and *Comamonas acidvorans*.[81] The P3HB-*co*-4HB copolymer composition varied from 0 to 100 mol% 4HB depending on the micro-organism and the carbon source mixture. The thermal and physical properties of the copolymers over the whole composition range were investigated and the results based on the table from the paper are summarized in Table 7. The copolymers exhibit a wide range of material properties, ranging from crystalline plastics to elastic rubbers, depending on composition. The crystallinity, shown by X-ray diffraction, decreased from 60% to 40% as the 4HB content increased from 0 to 27 mol% and the samples all had P3HB crysal lattices. However, the copolymers with >64 mol% 4HB showed increased crystallinity (from 15% to 34%) with increasing mol% 4HB and had P4HB crystal structure. The copolymers with high 4HB content (>64 mol% 4HB) exhibit the characteristics of thermoplastic elastomers, with tensile strength increasing from 17 to 104 MPa as the mol% of 4HB increases, whereas for low mol% 4HB the tensile strength decreases from that of pure P3HB (43 MPa) to 24–26 MPa for 10–16% 4HB in the copolymer. The increasing flexibility of these materials is reflected in the increase in elongation at break over the same 4HB range (0%–16%) from the value for the very brittle P3HB (5%) to 444% (16% 4HB copolymer). The remarkable biodegradation characteristics of the copolymers in different environments were also reported in this paper and will be reviewed later.

3.3 Natural biodegradable polymers

Abundant naturally occurring, biodegradable polymers can be processed into useful plastic materials and used to supplement blends of synthetic and microbial polymers. The polysaccharides starch and cellulose have been used most extensively. Cellulose is a constituent of woody plant material and is described as a complex three-dimensional, biopolymeric composite. It consists of repeating glucopyranosyl units. There are two groups

TABLE 7. Physical and thermal properties of P(3HB-*co*-4HB) copolymers at 23°C

Property	4HB fraction (mol%)										
	0	3	7	10	16	27	64	78	82	90	100
Melting temperature T_m (°C)	178		172		130		50	49	52	50	53
Glass transition temperature T_g (°C)	4		−2		−7		−35	−37	−39	−42	−48
Crystallinity (%)	60	55	50	45	45	40	15	17	18	28	34
Density (g cm^{-3})	1·25			1·23	1·23	1·23					
Water uptake (wt%)	0·32	34		0·20	0·14	0·45					
Stress at yield (MPa)		4		28	19						14
Elongation at yield (%)		28		5	7						17
Tensile strength (MPa)	43	45		24	26		17	42	58	65	104
Elongation at break (%)	5			242	444		591	1 120	1 320	1 080	1 000

of cellulose-based materials used on an industrial scale.[82]

- Regenerated cellulose, which is suitable only for fibre and film production and cannot be moulded.
- Chemically modified cellulose, e.g. cellulose esters (CEs) (cellulose acetate (CA), cellulose acetate butyrate (CAB)). These only biodegrade under certain circumstances as the three hydroxyl groups on the glucopyranosyl rings are replaced by the more hydrophobic ester groups, and it is important to know the degree of substitution (DS) of a CE. CEs are thermoplastics, but as hydrophilicity retards the rate of thermoplastic processing, these compounds are insoluble in water. A study of ether-substituted cellulose showed that biodegradation is associated with C-2 hydroxyl groups on contiguous repeating glucopyranosyl rings, with preferably a five- or six-segment run of C-2 unsubstitution.[83]

Research has been carried out on bacterial cellulose (BC) and water-soluble polymer (WSP) based on cellulose. Tajima *et al.*[84] report the production of bacterial cellulose from *Acetobacter xylinum* which has both good mechanical strength and biodegradability. This has been achieved by the incorporation of WSPs, e.g. carboxymethyl cellulose (CMC) and methyl cellulose (MC), in the incubation medium of the bacterium. The rapid biodegradation of the BC (CMC) product was shown both enzymatically, using cellulase, and in soil.

The biodegradation and non-toxicity of CMC have been tested by other workers,[85] as it can be found in wastewater treatment works. The continuous-flow activated sludge (CAS) test simulates sewage treatment and CMC (DS 0·7) added to raw sewage was partly degraded by micro-organisms in the bioreactor. Aquatic toxicity tests on the effluent and CMC intermediates produced by a pure culture were negative.

Citric esters have recently been introduced as biodegradable plasticizers by Gross and co-workers. They have reported[86] that cellulose acetate formed miscible blends (based on T_g) with both triethyl citrate and acetyl triethyl citrate. The effect of an increase in the amount of plasticizer was to reduce the tensile modulus, increase the elongation at break and increase the rate of biodegradation.

Starch is a homopolymer of glucopyranose units but consists of two α-types of polymer:

- α-(1,4) linked essentially linear amylose;
- α-(1,4) and α-(1,6) highly branched amylopectin.

The botanical and genetic background of the starch affects the branch chain lengths, chemical structure and functional properties. It is an enormous source of biomass and in most applications semicrystalline, native granule starch is either destroyed or reorganized. Water is frequently used as a plasticizer; the percentage of water has a great effect on the physical properties of the starch and therefore its uses. Recently, other plasticizers, low-molecular-weight alcohols, have been used for the production of thermoplastic starches.[87]

There are three ways starch can be used as a biodegradable material.

1. High-amylose starch is used directly as a biodegradable plastic and can be fabricated as strong film.
2. Small-granule starch can be used as a filler for PE film (6%–15%) with its original structure and granular form. If antioxidants are present in the

mixture, peroxides are produced and this cleaves the polymer chains, degrading the starch.

3. High-starch-containing mixtures (40%–60%) use a destructurized form of the polymer. Starch processing can involve different procedures, e.g. heat processing, pre-gelatinizing (this produces cold-water-soluble starches which can be dispersed without heat treatment) and chemical modification (to produce hydrophobic starch derivatives or dialdehyde starch, which can form crosslinks between starch and components with functional groups).[88]

4 BIODEGRADABLE POLYMER BLENDS

The blending of biodegradable polymers is a method of reducing the overall cost of the material and offers a method of modifying both properties and degradation rates. Miscibility is usually characterized by a single glass transition in the blend, which varies with composition and is measured by DSC or DMTA. The advantages of producing miscible blends include single-phase morphology in the melt and reproducible mechanical properties. However, forming a miscible blend, particularly with a non-biodegradable polymer, can reduce or even inhibit the degradation of the biodegradable component; e.g. PHB and poly(vinyl acetate) (PVAc) form miscible blends,[89] but even those containing a high wt% of PHB undergo only slight enzymatic degradation.[90] Immiscible blends have the disadvantage of having properties that are dependent on the blend morphology produced by processing and these are often not reproducible. However, some can show higher biodegradation rates than the unblended biodegradable polymer(s); e.g. PHB/PHBV (76 mol% HV) and PHB/poly(1,4-ethylene adipate) (PEA) blends are both immiscible, but the rate of enzymatic degradation of blend films was higher than for each single-component film.[91]

4.1 PHA-based blends

PHA blends have been reviewed by Verhoogt *et al.*[92] and Scandola[90] and some of their conclusions are included along with recent research by other workers.

4.1.1 PHA/synthetic non-biodegradable polymer blends. Hocking and Marchessault[13] identify a range of synthetic polymers which have been found to be miscible with PHB, including poly(vinyl acetate) (PVAc)[89] and poly(vinyl chloride) (PVC).[93] A recent study of PHBV/PVC blends[94] has produced DSC and DMTA results which show that PHBV (8 mol% HV) and PVC have separate T_g values for all compositions. However, PHBV (18 mol% HV)/PVC blends were miscible with a single T_g and a melting point depression for the whole composition range. FTIR studies revealed shifts in the absorbance for the C—O—C stretching vibration and the CH—Cl deformation, indicating specific intermolecular attractions between the two components. An increase in the PVC content of the blends increased the tensile strength and Young modulus of the blends but decreased the percentage elongation at break.

PHB/poly(methyl methacrylate) (PMMA) blends have been studied by Lotti *et al.*,[95] who have reported compatible blends of PHB/PMMA up to 20 wt% PHB when prepared by melt compounding followed by quenching to room temperature. Single-phase amorphous glasses with composition-dependent single T_gs are formed. Above 20 wt% PHB (the solubility limit for PHB in PMMA), partially crystalline PHB coexists with the constant-composition PHB/PMMA (20 : 80) mixture. Crystallization of PHB is retarded in these blends by increasing amounts of PMMA both from the melt and when heated from the rubbery state. The group found poly(cyclohexyl methacrylate) (PCHMA) to be immiscible with PHB.

More recent work by Scandola and co-workers[90,96] confirms these findings and also investigates the miscibility of other blends, including poly(epichlorohydrin) (PEC) which is a low-temperature rubber ($T_g = -21°C$), and PHB. The mixtures had to be solvent cast initially but were then melt processed, quenched in liquid nitrogen and stored at room temperature for at least 3 weeks before testing. The blends were shown by DSC and DMTA measurements to be miscible, with T_m and ΔH_m both increasing linearly with increasing wt% PHB. The single glass transition temperature of the blends is below room temperature for all compositions, so PHB partially crystallizes out at room temperature, and the increase in ΔH_m with increasing wt% PHB can be related to a constant fraction of this PHB crystallizing (58%). A space-filling spherulitic morphology develops from the melt, with the radial growth rate decreasing with PEC content.

Biodegradation studies, using wastewater activated sludge exposure for up to 1 year, were carried out on some of the miscible blends (PHB/PEC[90,96] and PHB/PVAc[91]) and showed a relationship between the T_g of the blend and its biodegradation rate. Kumagai and Doi[91] report that PVAc ($T_g = 38°C$) and PHB ($T_g \sim 0°C$) form miscible, non-biodegradable blends (i.e. even the biodegradation of the PHB is inhibited), but Scandola found that PEC ($T_g - 21°C$)/PHB (80 wt% PHB) blends showed progressive weight loss and surface erosion. SEM indicated that localized erosion spread over the sample surface with increased exposure time. He concluded that PHB/PEC blends, which have spherulitic morphology and lower T_g values than pure PHB, have sufficient mobility in the interlamellar amorphous mixed phase to allow degradation to be initiated, but in blends with higher T_g values insufficient mobility inhibits biodegradation.

4.1.2 PHA/synthetic biodegradable polymer blends. Several papers have been published on PHA blends with other biodegradable, synthetic polyesters. PHB/PEO blends are both miscible and show rapid rates of enzymatic degradation, but this is mainly caused by elution of the PEO into the aqueous buffer producing large holes in the surface of the blend, accelerating the enzymatic hydrolysis of the PHB.[97]

The effect of stereochemistry on the degradation and morphology of poly(lactic acid) has been mentioned previously: isotactic PLLA is a semicrystalline form which undergoes rapid non-enzymatic hydrolysis, although there have been recent reports[98] of some enzyme hydrolysis; atactic P(D,L)LA is amorphous and again is mainly hydrolysed abiotically. Two reports on the blending of PLLA with PHB[99] and PHBV[100] have produced interesting results. Blumm and Owen[99] investigated the spherulitic structure, growth rates and melting behaviour of PHB/PLLA (M_n 159 400 and 1759) blends using polarized light microscopy. Results indicated complete miscibility in the melt, over the whole composition range, for the PHB/low-molecular-weight PLLA blends, but with the high-molecular-weight PLLA, biphasic separation occurred in the melt and two types of spherulite formed on cooling. Some blends showed interpenetration of spherulitic growth. Iannace *et al.*[100] have concluded that PHBV/PLLA blends show partial miscibility based on calculations and their mechanical properties.

Koyama and Doi[101] have investigated the morphology and biodegradability of binary blends of P(R)-3HB (M_n 300 000) and P(R,S)LA (M_n 9000 and 21 000). Solvent-cast films were prepared and aged for at least 3 weeks at room temperature. Miscibility in the melt and in the amorphous state was demonstrated by single T_g values between that of pure PHB (4°C) and that of pure PLA (44°C) from DSC results for the PHB/P(R,S)LA (9000). Polarized optical microscopy was used to follow the spherulitic growth rate, which decreased as the PLA content increased; the spherulites were volume filled, indicating inclusion of the amorphous PLA within their structure. Biodegradation studies were based on biotic and abiotic conditions. Enzymatic hydrolysis using extracellular PHB depolymerase (*Alcaligenes faecalis*) was carried out at 37°C in phosphate buffer at pH 7·4 for 19 h. The abiotic hydrolysis was carried out for 150 days in buffer (pH 7·4) at 37°C.

The pure PLA showed no weight loss over 19 h in the enzyme medium, but all the other films showed mass loss (erosion) profiles which increase with time. The rate of mass loss increased with the wt% PHB in the blend. The non-enzymatic hydrolysis produced no weight loss in the pure PHB over 150 days, but pure P(R,S)LA weighed only 17% of the initial mass. The blends showed no initial mass loss, but after an induction period of 14–28 days they started to lose mass. The mass losses from the blends containing 25% PLA (23% loss) and 50% PLA (48% loss) are almost consistent with the initial mass of the PLA component, so it may be that only this component is being hydrolysed.

Molecular weight changes during the abiotic hydrolysis were recorded. The rate of decrease for PHB homopolymer was slower than for pure PLA. In the blends the M_n values decreased for both components more rapidly. The mechanism of the hydrolysis is discussed more fully later, but the acceleration of the chain scission of the PHB was attributed to the catalytic effect of PLA oligomers in the matrix.

The immiscibility of binary blends of PHB with PεCL has been established by Kumagai and Doi.[91] However, this group has reported more recently on the miscibility, morphology and biodegradability of binary blends of P(R)-3HB (M_n 300 000) and copolymers of PεCL-*co*-LA (M_n 1500–40 000).[102] The copolymers were synthesized using aluminium tri-iso-propoxide from mixtures of ε-CL and (*R,S*) lactide monomers. The statistical copolymers were analysed using ^{13}C NMR, and enzymatic hydrolysis was carried out in the presence of a lipase (*Rhizopus delemar*). It was found that enzymatic hydrolysis of the copolymers was faster than for PCL homopolymer. DSC measurements showed that miscible blends of PHB/PεCL-*co*-LA were prepared using amorphous PεCL-*co*-LA ranging from 30 to 100 mol% LA. The spherulites were volume filled in the miscible blends and the rate of spherulitic growth decreased with increasing wt% of copolymer. Enzymatic hydrolysis using extracellular PHB depolymerase (*Alcaligenes faecalis*) was carried out at 37°C in phosphate buffer at pH 7·4 for 19 h and, as with the studies using PHB/PLA blends, the rate of hydrolysis increased with increase in the PHB component.

Avella *et al.*[103] have carried out blend studies on PHB/poly[(D,L)lactide-*co*-poly(ethylene glycol)] (PELA). DSC measurements showed some shift in the T_m of the PHB and a linear decrease in ΔH_m as the wt% of PHB decreased. T_g values for the blends were found in the range 10–30°C (PEG, $T_g = 30$°C) and PHB spherulites were found in blends with ≥ 60 wt% PHB.

4.1.3 PHA/natural polymer blends. Blends of PHB and PHBV with cellulose that has been chemically treated to produce cellulose esters (CEs) are common. The CEs have thermoplastic properties, e.g. cellulose acetate (CA) and cellulose acetate butyrate (CAB), but are non-biodegradable. Scandola and co-workers[90,104] reported the formation of miscible blends of P(3HB) with CEs by melt compounding. Blends with ≤ 50 wt% PHB with either CAB or CAP produced transparent, stable, homogeneous, amorphous glasses, while blends with higher PHB content were partially crystalline and became opaque upon room temperature storage.[104] The data on the degree of substitution of the CEs and their T_gs in Table 8 are taken from Scandola's paper.[90] This paper also presents graphical evidence of the linear

TABLE 8. Composition and glass transition temperatures of polymers used in work by Scandola[90]

Polymer	Degree of substitution				T_g (°C)
	Bu-	Pr-	Ac-	Et-	
PHB					2–4
Cellulose tributyrate (CTB)	3·00				81
Cellulose acetate butyrate (CAB-1)	2·58		0·36		103
Cellulose acetate butyrate (CAB-2)	1·75		1·21		127
Cellulose acetate butyrate (CAB-3)	1·79		0·95		134
Cellulose acetate butyrate (CAB-4)	2·50		0·18		120
Cellulose acetate propionate (CAP)		2·39	0·37		146
Ethyl cellulose (EtC)				2·3	144

variation in glass transition, temperature, measured by DMTA, with composition of the blends of highly substituted CAP, CAB-1 and CTB with PHB (for 0–50 wt% PHB). This provides evidence, based on Wood's equation,[105] that the blends are miscible in the melt in these proportions, and as the T_gs for all these blends are higher than room temperature, the blends stay as stable, homogenous glasses. When the PHB content increases above 50 wt%, the decrease in T_g to below room temperature causes the PHB to partially crystallize out of the rubbery blend. Partially crystalline PHB/CE blends have spherulite morphology, but the crystallization rate of the PHB is reduced with increasing wt% CE because of the intimately mixed macromolecules. Scandola[90] also reports the non-biodegradability of PHB/CE blends even with high wt% PHB (no weight loss or surface erosion after 1 year in activated sludge for PHB/CAB-1 (80 : 20) blends; cf. PHB/PVAc blends reported by Kumagai and Doi[97]). The amorphous phase composition of partially crystalline 80 : 20 PHB/CAB-1 is 58 : 42 and this fills the interlamellar space between the pure PHB spherulites. The non-biodegradability of the PHB is explained as before by insufficient mobility in the amorphous phase, which prevents the known preferential hydrolysis that occurs with PHB degradation, and so subsequent enzyme attack on the lamellar PHB is prevented.

Unlike the cellulose esters, ethyl cellulose (EtC), with a T_g similar to that of CAP, forms only immiscible blends with PHB when they are melt blended at 195°C according to Scandola.[90] The PHB in low wt% PHB blends of PHB/EtC crystallizes out quantitatively on quenching and DSC curves on the second scan show the characteristic transitions of the two pure components. However, Zhang *et al.*[106] report that PHB/EtC blends appeared by solvent casting (60, 40 and 20 wt% PHB) and annealing (86, 60, 40 and 20 wt% PHB) showed composition-dependent glass transitions: T_g decreased with increasing wt% PHB. However, melt quenching or DSC cooling runs resulted in detection of only the T_g of pure PHB (5–9°C). Changes in the hydrogen bonding of the hydroxyl groups in the EtC were detected by changes in their FTIR absorption, as intermolecular hydrogen bonding between EtC and PHB carbonyl groups changes to intramolecular EtC hydrogen bonds.

Ceccoruli *et al.*[107] have also looked at the effect of the plasticizer di-*n*-butyl phthalate (DBP) on CAB/PHB blends. The DBP is miscible in all proportions with both CAB and PHB, showing variation in T_g with composition. Addition of a fixed amount of DBP plasticizer to CAB/PHB blends (0–100 wt% PHB) caused a significant decrease in the T_g of the binary polymer blends. The T_g depression increases with the DBP content in the ternary blends. In the two-component blends of PHB/CAB (<50 wt% PHB) the value of the single T_g decreases linearly with increasing wt% PHB (pure CAB-1, $T_g = 115$°C; 50 : 50 CAB/PHB, $T_g \sim 50$°C). However, there is also a characteristic relaxation in the DSC trace at lower temperatures which was associated with mobilization of the lower-T_g component (PHB). Concomitant with the expected 'plasticizing' effects of DBP on T_g, it also induces a decrease in the characteristic temperature for these additional low-temperature transitions.

Yasin and co-workers[108] have studied two- and three-component blends of Biopol with CEs or PCL or PLA and biodegradable plasticizers. Three-component phase diagrams were produced based on the optical properties of the blends and their glass transition characteristics. Compatibility was achieved over a limited composition range (30 wt% CAB, 40 wt% poly(ester glutarate) (P7092)) for PHBV/CAB/P7092 blends based on the optical clarity of these melt-pressed films and DSC measurements. However, for blends with low wt% CAB and high wt% PHBV, translucent and opaque films were formed and it was concluded that the blends were partially or totally immiscible. Increased crystallinity was found in films that were melt pressed compared with solvent-cast films and this affected their mechanical properties (melt-pressed films have greater tensile strength). High wt% CAB increased the tensile

strength but decreased the flexibility of the films. PCL/CAB/poly(ester glutarate) blends showed miscibility for all ratios of plasticizer with very high wt% CAB. However, there was little miscibility for blends with less than 50 wt% CAB. Blends of PHBV/PCL/poly(ester glutarate) showed no compatibility.[61]

Verhoogt *et al.*[109] have reported on PHBV (12% HV) blends with thermoplastic starch. Ramsay *et al.*[110] had previously established that blends of granular starch and PHBV (19% HV) had increased biodegradation rates but inferior mechanical properties compared with pure PHBV and formed a composite rather than a true blend. The Verhoogt group used thermoplastic starch plasticized with water and glycerol and processed in different ways. The possible advantages of gelatinized starch were that the starch can be deformed and distributed during blending and, by using glycerol/water instead of just water for gelatinization, reprocessing of the thermoplastic at elevated temperatures is possible. True blends were obtained, but they were brittle despite the flexibility of the starch. SEM studies showed irregular particles thought to be caused by viscosity differences.

4.1.4 Polymer blends of PHAs. The improved biodegradation of PHAs blended with other biodegradable polymers, particularly other PHAs, compared with that of pure PHAs has been established by several workers. Pearce and Marchessault[111] have studied the melting and crystallization of PHB/PHV and PHBV blends to compare phase separation with that in PHBV copolymers, which form isodimorphous crystals. They showed that in the melt, PHB/PHV blends are phase separated, which has been confirmed by Gassner and Owen.[112] The mechanical behaviour of random copolymers of PHBV was compared with that of blends of almost the same composition and found to be markedly different.

Blends of synthetic atactic PHB (a-PHB as 10–50 wt%) and bacterial isotactic PHBV (10 mol% HV) were prepared as solvent-cast films by Scandola *et al.*[113a] The blends were found to be miscible in the melt with decreasing crystallinity as the a-PHB content increased; this also increased the elongation at break of the 50 : 50 blend by a factor of 30 over pure PHBV. Abiotic and biotic hydrolysis studies were carried out. Enzyme hydrolysis was accelerated in the blends compared with PHBV. The pure a-PHB did not biodegrade. HPLC identified 3-HBA monomer and dimer as degradation products of the blends and this was thought to be a factor in their subsequent enzyme degradation. However, more recent work[113b] has indicated that the non-biodegradability of a-PHB is related to its amorphous character and the PHB depolymerase can only degrade PHB when a crystalline binding site is present.

Satoh *et al.*[114] have studied the hydrolysis (in phosphate buffer at pH 7·4 and 55°C) of PHB/PHBV (22 mol% HV) blends. DSC studies on the original polymer indicated that PHB was the main component of the crystalline phase, and the crystallinity decreases with increasing wt% PHBV in the blends. There was correlation between an accelerated degradation rate and a decrease in the degree of crystallinity in the blends.

Marchessault's group have also investigated the melting behaviour of blends of PHB with different tacticity and using solvent casting, melt crystallization and rapid co-precipitation procedures.[115,116] Solvent-cast films of isotactic PHB and atactic PHB were found not to be fully miscible prior to first melt for DSC scan; both annealing and recrystallization behaviour were identified. The work on bacterial and partially isotactic PHB blends showed that co-precipitation produced compatible blends, whereas atactic PHB was not compatible with bacterial PHB. However, blends with atactic PHB > 60 wt% exhibited a significant drop in the melting point of the bacterial PHB.

Abe *et al.*[117] related stereostructure to melting behaviour by studying a range of blends of microbial stereoregular P(R)-3HB and synthetic stereo-copolymers (P(R,S)HB), both atactic and syndiotactic forms. The T_g of the blends showed little variation (5 ± 2°C) but the melting temperature decreased from that of P(R)HB (191°C) with increasing wt% P(R,S)HB (174°C for 75 wt% P(R,S)HB), suggesting that the polymers are miscible in the melt and in the amorphous state and the degree of crystallinity decreases with increasing wt% P(R,S)HB. Enzymatic degradation of the blends in phosphate buffer at pH 7·4 and 37°C was carried out on the homopolymers and the blends. Surface erosion occurred on the bacterial PHB, but the synthetic polymer showed little surface hydrolysis. Blending of the two accelerated the hydrolysis, with the 50 : 50 wt% P(R)-3HB/(P(R,S)HB) showing the fastest erosion rate (similar to the results with a-PHB/PHBV blends investigated by Scandola and co-workers[113]). HPLC analysis of the degradation products suggested that synthetic PHB (atactic and syndiotactic) is hydrolysed by PHB depolymerase in the presence of P[(R)3HB] or its degradation products.

Abe *et al.*[118] have also reported the use of the block copolymer of atactic P(R,S)3HB and poly(6-hydroxyhexanoate) (PHH) to compatibilize blends of P(R)3HB/PHH. The AB block copolymer was prepared synthetically and the addition of small amounts to blends of the two homopolymers reduced the size of the PHH dispersed domains and improved their mechanical properties. Enzymatic hydrolysis results showed that PHH displayed least surface erosion, then pure PHB, and the ternary blend degraded more slowly than the binary (75 : 25) PHB/PHH blend.

4.2 Blends based on starch and cellulose derivatives

There has been some recent research on blends using other biodegradable polymeric materials, and those involving starch and cellulose are relevant to this

review. Pranamuda *et al.*[119] report on the feasibility of poly(ε-caprolactone) (PCL)/tropical starch blends, similar in type to PCL/corn starch blends, as biodegradable thermoplastics, but all these blends have low tensile properties compared with PE, although continuous phase dispersion of the starch in the PCL was observed in the films using SEM. The miscibility of blends of PCL/cellulose butyrate (DS $\geq$ 2·0) was observed using DSC measurements by Nishio *et al.*,[120] but the miscibility alters with the nature of the ester group and with the degree of substitution, and even butyl esters with DS < 1·5 showed poor miscibility with PCL. The fabrication of PLA/native starch blends with low-molecular-weight PEG as a plasticizer has been reported,[121] with DSC studies and thermogravimetric analysis carried out.

Mayer *et al.*[122] report that a corn starch (St)/cellulose acetate (CA, DS = 2·5)/propylene glycol (PG) blend with St/CA/PG wt% ratio 57 : 25 : 19 has mechanical properties similar to those of polystyrene, and these represent acceptable properties for injection-moulding applications. Increasing the wt% starch to PG reduces the tensile strength. The biodegradation of the starch and PG was observed in both soil and composting studies, but extended incubations were required to detect any weight loss from the CA in the blends. However, it was reported that in simulated municipal compost there was 90% weight loss from the pure CA after 90 days.

The properties of the blends of starch octanoates (OCST1·8 and OCST2·7, DS = 1·8 and 2·7 respectively) and starch dodecanoate (DODST2·7, DS = 2·7) with LDPE have been investigated.[123] The ester groups act as an internal plasticizer with increasing effect as the number and size of the grafted fatty acyl chains increase. The DODST2·7/LDPE blends showed generally better thermal stability and higher elongation but lower tensile strength and water absorption compared with the OCST/LDPE blends. However, the degradation rate of all blends was very low. Another group has reported different methods of modifying starch before blending with LDPE in order to maintain its thermoplastic properties but increase its biodegradability. Park and co-workers used Dupont Surlyn®,[124] acetic anhydride and three types of ionomers[125] to esterify the starch, with the aim of using the blends for packaging film. Ionomer-treated starches were reported to be more compatible with LDPE and to give better mechanical properties than other blends.

The production of biodegradable films for food packaging was the aim of a study by Arvanitoyannins *et al.*[126] The mechanical and gas/water permeabilities of extruded blends of LDPE/wheat starch/ethylene acrylic acid (EAA) or PCL were investigated after conditioning at various relative humidities. Both starch and PCL were found to have adverse effects on the mechanical properties of LDPE (particularly >30% starch) and both gas and water transmission rates increased with increasing amounts of starch/PCL in the blends. EAA acted as a compatibilizer and increased the percentage elongation at break of the blends.

The production of high-strength and high-Young-modulus PE/starch composite films has been reported by Nakashima and Matsuo[127] using ultrahigh-molecular-weight (6×10^6) PE and corn starch processed by gelation crystallization from dilute solutions. PE/starch compositions of 3 : 1, 1 : 1, 1 : 3 and 1 : 5 were tested and found to have high draw ratios at 135°C (1 : 5 composition reached 80) and good Young moduli (1 : 5 was 10 GPa up to 1 : 1 with 105 GPa, cf. 70 GPa for aluminium). The starch particles in most composites remained within the composite films even at high draw ratios. Enzymatic degradation with different fungal species on agar nutrients was investigated, and although the biodegradation of the starch and a decrease in the mechanical properties of the films were observed, no disruption of the PE fibrous texture was discernible by SEM.

5 CHARACTERIZATION AND STRUCTURAL ANALYSIS

In this section a range of the current methods used to characterize the properties and determine the structure of biodegradable polymers will be identified. Spectroscopic studies are used to characterize the composition of polymers, e.g. Fourier transform infrared (FTIR), Raman, electron spin resonance (ESR), ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and ultraviolet/visible light spectroscopy. Molecular weight characteristics can be determined by an extensive range of methods, e.g. colligative properties, as in vapour pressure osmometry (VPO), end group analysis, e.g. quantitative ^{31}P NMR,[128] as well as viscometry, light scattering, small-angle X-ray and neutron scattering (SAXS and SANS) and gel permeation chromatography (GPC) or size exclusion chromatography (SEC).

Erlandsson *et al.*[129] have recently published a paper that indicates that SEC of the degradation products of some polymers may not be very accurate. Thermally degraded and UV-aged PP containing recycled material was found to have a changing relationship between its molecular weight and retention volume. The Mark–Houwink–Sakaruda (MHS) equation offers a convenient means of determining the molecular weight of polymers soluble in organic solvents. For linear polymers the MHS parameters are constant over a wide range of molecular weights, but for highly branched polymers there are deviations because the hydrodynamic volume is not directly related to molecular weight. The observed deviation in the degraded polyolefins was related to the effects of chain branching and increased polydispersity with prolonged degradation.

Structural analysis mainly involves measurement of either bulk or surface properties of polymers in the solid phase. However, studies of crystallization from the melt and from solution, thermal testing and methods of analysis for degradation products in the liquid or gas phase are also important.

5.1 X-ray diffraction

Single-crystal X-ray goniophotometry has limited use in the study of semicrstalline polymers, whereas stretched fibre, thin film and powder X-ray diffraction do have applications. Gazzano *et al.*[130] report a recent study of bacterial P(3HB) films which were crystallized from the melt at different temperatures up to 120°C. The diffraction patterns at increasing crystallization temperatures (T_c) were seen to differ more and more from that of the original powder, and the quenching range also affected the relative intensity and peak width of the reflections. The differences were correlated with changes in average spherulite size (0·1–2 mm) in films of limited thickness. It was found that when the spherulites were large, the lamellar crystals were strongly oriented parallel to the film plane.

X-ray diffraction studies for examining the crystal structure of solids have been extended by the development of small-angle X-ray scattering (SAXS) and wide-angle X-ray scattering (WAXS) or diffraction (WAXD). A typical WAXS curve shows the intensity of X-ray scattering as a function of the diffraction angle and features relatively sharp peaks for crystalline scattering and broader 'humps' for the scattering from amorphous areas, allowing the degree of crystallinity to be assessed. SAXS is used to determine lamellar thickness, and better results are obtained from solution-grown crystals than from melt-crystallized polymers. These techniques are used with computer modelling to find out about crystal phases, lamellar thickness, degree of crystallization, as well as rates of crystallization, melting and spherulitic growth.

Orts *et al.*[131,132] have used SAXS and WAXD and Scandola *et al.*[133] have used WAXS to study the isodimorphic systems of PHBV random copolymers. More recently, Gross and co-workers[134] have used both X-ray diffraction and WAXS to study the crystallization behaviour of predominantly syndiotactic PHB.

X-ray diffraction studies are often used in conjunction with other characterization techniques, and Jerome and co-workers[135] have employed SAXS, WAXS, TEM, AFM and PCS to study a stable non-aqueous colloidal dispersion formed by poly(caprolactone-*co*-glycolide) (P(εCL-*co*-G)) block copolymers in toluene. The polymerization produced 'living' polymers, so there was control on both molecular weight and composition. A micelle model was proposed consisting of PG core and a PCL corona, with both constituents semicrystalline. SAXS observations indicated that the core is two concentric spheres, with the internal sphere containing crystalline PG.

5.2 NMR spectroscopy

5.2.1 Solid state CPMAS ^{13}C NMR. The advances in solid state ^{13}C NMR allow analysis of specific regions within the solid structure. The monomeric ratio for PHBV copolymers can be determined in the amorphous and crystalline regions, and Orts and co-workers[132,136] report different ratios of HV in the crystalline regions of PHBV related to the overall crystalline disorder. The HV incorporation into the PHB-type crystalline phases was shown to be over the composition range.

5.2.2 High-resolution ^{13}C NMR spectroscopy. This technique can be used to determine the tacticity of a wide range of polymers and is particularly useful for analysing P(3HB). Hocking and co-workers[137,138] have analysed a range of racemic synthetic polymers with different tacticity and interpreted the results based on several statistical models. Average isotactic and syndiotactic block lengths were calculated.

5.3 Microscopy techniques

A range of different microscopy-based techniques have been used to study the surface structure and erosion characteristics during biodegradation.

5.3.1 Optical microscopy. Isothermal crystallization kinetics in miscible blends can be examined using optical microscopy. Pearce *et al.*[139] have used the technique to study isotactic/atactic PHB blends and seen a reduction in the rate of spherulite growth in the isotactic polymer.

5.3.2 Transmission and scanning electron microscopy (TEM and SEM). Lauzier *et al.*[140] have examined the structure of PHB granules isolated from bacterial calls using SEM and identified the outer lamellar crystalline coating and non-crystalline core. Miyata and Masuko[141] reported the use of TEM to examine the morphology and structural features of PLLA grown from acetonitrile solution by an isothermal crystallization technique. Diffraction mode WAXS diffractometry and AFM were also used and the packing of the molecular chains within the crystals was investigated along with the orthorhombic unit cell parameters.

5.3.3 Atomic force microscopy (AFM). The surface erosion of biodegradable polymers in aqueous solutions has been followed *in situ* using AFM by Shakesheef *et al.*,[142] showing the versatility of the technique and its ability to achieve resolutions comparable with vacuum-based SEM. This group[143] has also achieved spontaneous acquisition of atomic force microscopy and surface plasmon resonance data with a combined AFM/SPR instrument which can be used to study the effect of morphology on erosion. This has particular

applications for the degradation of immiscible polymer blends. A study of the degradation of thin films of poly(sebacic anhydride) (PSA) and P(D,L)LA enabled dynamic changes in surface morphology during degradation to be related to SPR-recorded kinetics of film erosion. Three stages in the film erosion at pH 11 were demonstrated: initial rapid loss of PSA, slow loss of PLA in the final stage and an extended transition in the intermediate stage.

5.3.4 Scanning probe microscopy. The use and design of these microscopes, for investigating drug delivery systems, have been reviewed by Shakesheef *et al.*[144] These instruments should provide information about the molecular structure of polymeric surfaces, the conformation of target molecules, the influence of morphology on biodegradation, the adsorption of proteins onto synthetic surfaces and the structure and interactions of colloidal particles.

5.3.5 Confocal laser-scanning reflection microscopy (CLSM). Semler *et al.*[145] have used CLSM to visualize and quantify the microtopography of the surface of the porous matrix of PLA-*co*-GA copolymers after degradation. The surface matrix was reported to change significantly during degradation, with increased local clustering of textured regions.

5.4 X-ray photoelectron spectroscopy (XPS)

Shakesheef *et al.*[146] have shown that XPS provides a method of tailoring the surface properties of microparticle drug delivery systems so that the polymer chemistry and fabrication parameters can be related to the final surface chemistry of the microparticles. Significant variations in the surface chemistry of microparticles containing the polymers PLA and PLGA and the block copolymers PLA-*co*-PEG and PLGA-*co*-PEG were identified. These variations were related to the mechanism for the microparticle/water interface stabilization, which is controlled by the interfacial surface tensions of the polymers within the aqueous environment. Differences in the adsorption of a surfactant, e.g. poly(vinyl alcohol) (PVA), were connected to the composition of the polymer, with PEG copolymers adsorbing less PVA because the PEG segments stabilize the polymer/water interface.

Leadley *et al.*[147] have also reported the use of XPS analysis to correlate the experimental and theoretical chemical composition of the surface of drug delivery systems using biodegradable poly(β-malic acid) and its ester derivatives.

5.5 Differential scanning calorimetry (DSC)

DSC is a standard technique for studying thermal behaviour in semicrystalline samples of pure polymers, copolymers and blends to determine enthalpy changes during melting. The process can be used to identify glass transition and melting temperatures (T_g and T_m); melting endotherms can be used to calculate enthalpy changes (ΔH_m) and show annealing and recrystallization characteristics. The degree of crystallinity of semicrystalline polymers can be assessed by comparison with ΔH_m for completely crystalline samples. DSC is frequently used to determine the miscibility of polymer components when blended, i.e. single-phase morphology in the melt, as this is characterized by a single glass transition of the blend, which varies with composition and can be measured by DSC or DMTA.

A recent study by Kemnitzer *et al.*[134] looked at crystallization in predominantly syndiotactic, synthetic P(3HB) with different degrees of syndiotacticity. After melting and annealing, at least one and often two distinct melting transitions were seen over a broad temperature range (often at about 40°C). This was interpreted as evidence of different crystalline regions with different thermodynamic stabilities. The annealing temperature (T_c) was related to the crystallization behaviour, with maximum crystallization at $T_c = 30$°C and partial or complete exclusion of the syndioregular chain segments occurring at $T_c \geq 45$°C. From the dependence of the higher melting transition on T_c the equilibrium melting temperatures for the different samples were obtained. Avrami analysis of *syn*-PHB results showed similarities to those obtained for microbial PHB and so a two-dimensional crystal growth mechanism was proposed.

5.6 Dynamic mechanical testing and analysis (DMTA)

Dynamic mechanical testing involves a torsion pendulum in which polymer samples undergo sinusoidal oscillations at a fixed temperature and known testing frequency. The transition behaviour of polymers can be studied as a function of testing temperature, and information about changes in tan δ (where δ is the phase angle or lag for any viscoelastic material) and the shear modulus (G_1) gives clear evidence of T_g values (referred to as the α-transition). Variations in tan δ at temperatures below the glass/rubber transition of a polymer represent other secondary transitions, which can be difficult to assign but may be related to phenomena such as side chain rotation and co-operative motion of segments of the main chain.

Scandola's paper[90] presents a clear example of graphical evidence of the linear variation in glass transition temperature, measured by DMTA, with composition of the blends of highly substituted CAP, CAB-1 and CTB and PHB (for 0–50 wt% PHB). This provides evidence, based on Wood's equation,[105] that the blends are miscible in the melt in these proportions, and as the T_gs for all these blends are higher than room temperature, the blends stay as stable, homogenous glasses.

In a previous paper, Celli and Scandola[148] relate DSC and DMTA results to the aging of PLLA.

5.7 Mechanical testing

Standard tests for measuring tensile modulus and percentage elongation at yield and break are used to characterize polymers and to study bulk property changes during degradation.

Hiljanenvainio *et al.*[149] have synthesized copolymers of ε-CL/L-lactide and ε-CL/D,L-lactide with different compositions (80–40 wt%) of ε-CL in the monomer feed. The copolymers differed widely in their physical characteristics from weak elastomers to tougher thermoplastics. Their properties were compared with those of the homopolymers and the tensile modulus and tensile strength were higher for the homopolymers than for the copolymers. However, the maximum strain, which is very low for the pure PLA polymers and high for PCL, also showed large elongation for the copolymers.

Merloz *et al.*[150] have used static and dynamic mechanical testing *in vitro* for assessing the potential of PLLA for surgical implants. Traction and flexion measurements were carried out on bars of PLLA in different environments. Stress–strain results showed that the bars were fragile but unaffected by sterilization procedures. Bars placed in a physiological environment at 37°C became ductile, and after 1 month at this temperature the Young modulus and maximal strain before breaking were reduced by 50% of their original value.

5.8 High-performance liquid chromatography (HPLC)/gas chromatography (GC)

HPLC is used to isolate and analyse mixtures of polymer degradation products. The method can be used to give biodegradation product profiles and yield concentrations for different products, which are required to assess the toxicological nature of the polymers and their degradation products. Marcato *et al.*[151] have used the technique to identify some of the hydrolytic decomposition products of PαHAs. The hydrolysis of PLLA, P(D,L)LA, PGA and PLGA was carried out in a stirred, aqueous suspension for 12 h. Peak identification was carried out by comparison with pure standards and the optimum shape and separation of the peaks were achieved by preliminary studies on the composition and pH of the mobile phase (ratio (v/v) of acetonitrile/phosphate buffer was 75 : 25 and pH 5·8). PLLA was found to be quite stable, PGA degraded easily to GA, whereas P(D,L)LA and PLGA exhibited intermediate behaviour, producing not only the monomers but also dimer, trimers and higher oligomers.

Microbially produced PHBV with different compositions was analysed by Nedea *et al.*[152] using NMR spectroscopy and, after partial methanolysis or ammonolysis, HPLC and fast atom bombardment mass spectrometry (FAB-MS). Normalization of the oligomer peaks provided an estimation of both the copolymer composition and the monomeric sequence distribution. It was concluded that statistical copolymers had been produced.

Wang *et al.*[153] have investigated the *in vitro* breakdown products associated with the biological hydrolysis of a biomedical polyurethane. Ultrafiltration, freeze drying and solid extraction were used to remove protein contamination, and more than 20 degradation products were separated using gradient HPLC, optimized using a photodiode detector, and simultaneously identified using a tandem mass spectrometer (MS).

A qualitative method for determining the mechanism of hydrolytic scission in biodegradable polymers has been developed by Shih.[154] The molar fraction of the monomer was determined by ^{1}H NMR or HPLC and the degree of polymer degradation was also measured using proton NMR. Experimental data from different hydrolysis reactions were fitted to theoretical curves and the hydrolysis mechanism was then proposed.

Easily hydrolysable polyesters produce few degradation products, mainly monomer, dimer, etc., but these can be obtained by head space GC–MS and identified. For the degradation of PE, which cannot be carried out by hydrolysis, hundreds of products may be obtained according to the reaction conditions and the use of additives, e.g. pro-oxidants and starch. The same analytical technique can be used to fingerprint the polymer and the degradation conditions.[155]

In a quantitative study of intact ester linkages in P(D,L)LA and PLGA, Shih *et al.*[156] have converted the ester linkages into amides using *n*-butylamine, in ambient conditions, and then used gas chromatography (GC) to analyse the product mix. The amidation of both types of ester bond was found to be quantitative and was complete after 3 h for the glycolate and 8 h for the lactate. The values were within 5% of those obtained by ^{1}H NMR.

5.9 Other methods of structural analysis

The use of several novel methods of analysis has been described in the literature. Holland and Tighe[21] and Yasin and co-workers[108] report the use of goniophotometry to investigate the surfaces of films and have found the technique very sensitive to changes in surface rugosity. Changes in the relative gloss factor were used to characterize small changes in the roughness of the surface of PHBV (20% HV) films over a period of 72 h.

Foster and Tighe[157] used enzymatic assay of HBA monomer formation as a measure of the degradation of P(3HB). This technique has applications for forms of the polymer e.g. fibres and microcapsules, which cannot be monitored by standard surface or gravimetric methods. The potential use was demonstrated in the

enzymatic hydrolysis of gel-spun fibres of PHB. HBA dehydrogenase is used to hydrolyse the polymer in the presence of nicotinamide adenine dinucleotide (NAD). This is reduced to NADH and the conversion is associated with an increase in the absorption of light at 340 nm, which also serves to indicate the concentration of HBA monomer in the sample.

6 BIODEGRADATION CONDITIONS AND TEST PROCEDURES

The definition of biodegradation given by Albertsson and Karlsson[1] was quoted at the beginning of this review. These biotic changes must be considered alongside environmental factors which may produce chemical and other abiotic reactions, e.g. oxidation, photodegradation or hydrolysis.

6.1 Degradation processes

6.1.1 Oxidation. The initial step in the oxidation of polymer films may occur without any biodegradative attack, although both microbes and fungi may accelerate the process. The rate of initial oxidation of polymers is likely to decrease when air is limited, e.g. in the body of landfill sites (1–2 m below the surface). However, certain micro-organisms can utilize oxygen in chemically bound form (e.g. nitrate, sulphate, carbonate) to biodegrade the polymers in anaerobic conditions. A mechanism for the abiotic oxidation of polyethylene (PE) has been proposed using iron ions in anaerobic conditions.[158] A recent paper has also reported that the toxicity of oxygen free radicals is enhanced by iron. It suggests that the human body can therefore produce very toxic hydroxyl radicals, which may be one of the main causes of degradation in implanted polymeric devices.[159]

The mechanism for the biodegradation of PE was presented in 1987,[160] involving initial abiotic oxidation. Hydroperoxides are introduced into the polymer chain with a gradual increase in 'in-chain' keto groups, followed by a decrease as short-chain carboxylic acids are released; the final stage involves microbial biodegradation by a β-oxidation mechanism. To elucidate the initial mechanism further, LDPE films containing biodegradable starch and a pro-oxidation formulation were inoculated with bacteria or fungi and left in an aqueous medium at ambient temperatures for 1 year.[161] Two interactive mechanisms occurred: the water containing trace elements triggered autoxidation of the pro-oxidant and, with the synergistic biodegradation of the starch, this initiated the autoxidation of the LDPE, which was monitored by chemiluminescence, DSC and CSLM. There are several reasons why the initial degradation of PE has to be abiotic. The biotic degradation of long-chain alkanes by the β-oxidation mechanism, which is similar to that of fatty acids and alkanes in man and animals, cannot occur enzymatically beyond n-C_{44} because of steric hindrance. The initial step of C—C bond splitting cannot be carried out by any natural enzyme in either the side or main chain.[1]

LDPE, polystyrene (PS), poly(vinyl chloride) (PVC) and urea formaldehyde resin (UF) that had been buried in bioactive soil for over 32 years have been examined for biotic degradation.[162] Only the LDPE showed any signs of this, as the surface in contact with the soil had whitened and was severely degraded. The inside of the LDPE was still transparent and differed in its FTIR spectrum from the surface. The oxidation products in the clear part were carboxylic acids and ketones, whereas the whitened surface showed some carbonyl absorbance but increased C=C (double-bond) absorption. The usual oxidation mechanism was proposed for the clear regions and a more complex mechanism postulated for the whitened surface. Under biotic conditions the alkoxyl radical scission may proceed through a γ-scission mechanism, a form vinyl groups and volatile products, in addition to the β-scission mechanism. This group has published more recent results on attempts to accelerate the biodegradation of LDPE.[163] UV irradiation was used and several oxidizing agents (biodegradation-inducing agents) were added to the LDPE, which was left in bioactive soil for 12 months. It was found that UV radiation accelerated the biodegradation very effectively and that addition of vegetable oil, metal carboxylates (iron stearate) and small amounts of dicumyl peroxide (DCP) also promoted the degradation of LDPE.

Weiland *et al.*[164] have investigated the biodegradability of thermally oxidized PE using a wide range of biotic conditions, including different bacteria on solid agar, various composting units (varying in temperature, moisture content or composting medium) and different liquid media. There was some evidence of oxygen uptake in the respirometric method using liquid suspensions of compost micro-organisms and very small concentrations of substrate. Day *et al*[165] have carried out abiotic and biotic degradation testing on PE-based films. The results confirmed that they were susceptible to abiotic thermal oxidative degradation at 60°C, and although this temperature was achieved in the laboratory-scale, aerobic composting test, no degradation was obtained. It was concluded that the test time was insufficient compared with the induction period necessary for the desired thermal oxidation process.

LDPE and HDPE films have been filled with up to 20% by weight of starch and tested for biodegradation under a wide range of microbial conditions.[166] There is evidence of the removal of starch from the different films by massive colonization of various organisms, and this is accompanied by a small but significant drop in the average molecular weight and decrement in mecha-

nical strength of the films. The composting trial, which experienced prolonged severe temperature conditions, produced small but detectable spectroscopic evidence of oxidation of the PE matrix. The hydrophobicity of polyolefins is considered to be an obstacle to microbial attack. The incorporation of surfactant onto PE films has been shown in a different study to increase the rate of biodegradation.[167]

6.1.2 Effect of light and other forms of EMR. Polymers are susceptible to electromagnetic radiation, with both γ-irradiation and light having an adverse effect on polymer properties; e.g. poly(α-ester)s show reduced molecular weight. Photo-oxidation has a profound effect on the biodegradability of certain polymers. PE without photo-oxidation has been shown to degrade by only 0·2% over a 10 year period, but with 42 days prior oxidation a 2% weight loss was obtained after soil incubation for a similar time period.[1] Research has recently been presented on the use of a photodegradable coating on polymeric materials.[168] Three polymeric materials were prepared: starch/PE blend, photoactivator/starch/PE blend and photo/activator/starch/PE coated in gelatin. The results suggested that a more powerful photo-oxidizer would introduce control into the initial degradation. The gelatin coating (which contained iron(III) salts) extended the induction period, but after it had degraded, there was accelerated photo-oxidation.

As γ-radiation is a means of sterilization, it is an important consideration particularly in biomedical uses of these polymers. However, Collett *et al.*[169] have reported a method of minimizing the effects by careful reduction of the oxygen pressure during γ-irradiation. Mitomo *et al.*[170] have reported more recently on the effects of γ-radiation on PHB and PHBV in air or a vacuum. They found that polymer scission and crosslinking occurred. The samples irradiated in air showed greater relative decrease in T_m, T_g and number-average molecular weight, with less crosslinking. It was found possible to radiation graft both PHB and PHBV with MMA and hydroxyethyl methacrylate (HEMA).

6.1.3 Hydrolysis. Chemical and microbial hydrolysis mechanisms are the most important biodegradation reactions involving polyesters. Shih's graphical method for the determination of the mode of hydrolysis of biodegradable polymers has already been mentioned[154] and is just one mathematical treatment used to help determine the mechanism of hydrolysis. The method requires the determination of the mole fraction of the monomer (m_1) by ^{1}H NMR or HPLC and the degree of polymerization (α) by ^{1}H NMR. Kuhn predicted that if completely random scission of backbone bonds occurs, $m_1 = \alpha^2$, and if exclusive chain-end unzipping occurs, $m_1 = \alpha$. If the data fall between the two curves, chain-end scission is faster than random scission, and for data that fall below both curves, the chain-end bonds are more stable than the internal bonds. Shih showed that acid-catalysed hydrolysis of poly(orthoester)s and base hydrolysis of P(D,L)LA occur by random scission, whereas the acid-catalysed hydrolysis of PLA involves faster chain-end scission.

Two controlling mechanisms of polyester breakdown are recognized either at the surface (homogeneous) or within the bulk (heterogeneous). The relative contributions of each depend on the following factors:

(1) the nature of the polymer or copolymer (including their hydrophobicity/hydrophilicity, molecular weight and glass transition temperature) and the nature of additives, e.g. other polymers, plasticizers, fillers;
(2) the degree of crystallinity of the polymer and the morphology/miscibility of any other components;
(3) degradation products of degradable polymers;
(4) the degradation environment.

Hydrolysis of poly(α-ester)s. In vitro systems, in which abiotic hydrolytic processes are studied, are usually based on physiological conditions, i.e. iso-osmolar phosphate buffer at pH 7·4 and 37°C, whereas microbial systems are more varied both in the conditions and the nature of the inoculum. Data for the rate of hydrolysis of different biodegradable polyesters under physiological conditions were given in Table 3. Poly(α-ester)s (PLA, PGA, etc.) hydrolyse much more rapidly than PHAs and by an almost abiotic bulk hydrolysis mechanism because of their more hydrophilic character. The rate of abiotic hydrolysis is mainly controlled by their molecular weight, although it has been reported[171] that the rate of any enzyme hydrolyse of poly(α-ester)s is principally determined by crystallinity.

A two-stage hydrolysis mechanism for poly(α-ester)s was established in the early 1980s by Chu[172] using *in vitro* studies; diffusion of water into the amorphous regions of the polymer occurs, producing random hydrolytic scission at the susceptible ester linkage. When most of the amorphous regions have been eroded, the second stage commences, as hydrolysis of the crystalline regions, so during the first stage there is a characteristic increase in the percentage crystallinity of the polymer. The rate of hydrolysis of PLLA has been shown to be modified by using blends of monodisperse PLLA (M_w 7600 and 82 500). In physiological conditions for 28 days the rate of release of lactic acid was shown to increase with the percentage of low-molecular-weight PLLA in the blend.[173] The rate of hydrolysis of blends of amorphous P(D,L)LA (a-PLA) and isotactic crystalline PDLA or PLLA (c-PLA) as solvent-cast films was studied in the same *in vitro* conditions. The mass of blend films remaining after 20 months was greater for films with a higher percentage of c-PLA initially. The tensile properties remained

unchanged in the initial period of hydrolysis but then decreased rapidly, although the tensile strength was retained longer in blends with higher amounts of a-PLA.[174]

A recent investigation[175] looked at the effect of dimensions on the degradation rate of P(D,L)LA material. Compression-moulded plates, millimetric beads, microspheres and cast films were fabricated from the same batch of polymer and allowed to age in iso-osmolar phosphate buffer at pH 7·4 and 37°C. It had previously been recognized that large PLA devices degrade faster than smaller ones and this was related to the diffusion mechanism, in which the outer layer of material degrades less rapidly than the bulk internal polymer. In this study it was found that the plates and beads degraded more rapidly, with bulk disintegration, compared with the slower, surface only, hydrolysis of the films or microspheres. Park reported[176] that the *in vitro* degradation behaviour of a wide range of PLGA copolymers was investigated for up to 53 days. It was found that amorphous PLGA showed transient multiple crystallization behaviour of D- or L-lactic acid oligomers during degradation, indicating preferential hydrolytic scission of D- or L-lactic acid to GA linkages or of GA to GA linkages. DSC reveals two distinct T_gs when the crystallization occurs as these transient domains are produced.

The degradation rate of solution-cast films of PLA was studied at 37°C in NaOH solution to give accelerated hydrolysis.[177] Four different PLLA films with different morphologies and molecular weights ($3{\cdot}0 \times 10^5 < M_w < 3{\cdot}0 \times 10^6$) were tested and SEM revealed swelling particularly of the spherulites, which are then eroded from the centre. The highly amorphous films became more crystalline as degradation proceeded and the reduction in transparency measured at 570 nm, using a spectrophotometer was ascribed to increased spherulite density.

The hydrolytic degradation of PEG/PLLA block copolymers ($1000 < M_w < 6000$) has shown that initially there is preferential scission of the ester linkage between the PEG and PLLA blocks.[178] GPC analysis of the molecular weight characteristics of the copolymers showed the formation of bimodal material and there is NMR and FTIR evidence, in the first 200 h, of the formation of —OH-ended PEG blocks and —COOH-ended PLA.

The factors that control the enzyme hydrolysis of poly(α-ester)s have only recently been investigated. McCarthy and co-workers[171] have investigated the effects of the stereoisomeric content of PLA on the rate of degradation of solvent-cast films incubated with proteinase K. Surface changes in the film and weight loss were investigated and changes were found in both. The main controlling factor was the degree of crystallinity in the samples, although high mol% LLA was also important. It was concluded that initial degradation occurred at the surface and that the rate of degradation increased with high LLA content (92% optimum) and a high percentage of amorphous domains. There was little change in molecular weight over the period of the investigation. Torres *et al.*[179] have used a filamentous fungus (*Fusarium moniforme*) and a bacterium (*Pseudomonas putida*) to study the enzymatic degradation of lactide oligomers and dimers and lactic acid monomers with different stereostructure in liquid cultures using HPLC. The micro-organisms totally used the oligomers regardless of enantiomeric composition, although the L-dimer was consumed rapidly. The fungus was faster acting than the bacterium, and the racemic oligomers were slowly assimilated but the higher L-oligomers were biostable; the crystallinity of these latter species was thought to be the cause.

Hydrolysis of water-soluble polyethers. The microbial aspects of the degradation of PEO have been studied by Otal *et al.*[180] using a model, integrated, wet air oxidation/aerobic wastewater treatment. The partial wet air oxidation was found capable of converting the original polymers (M_n 10 000) into lower-molecular-weight compounds (oligomers and short-chain acids), and subsequent treatment with wastewater achieved an 80% total organic carbon (TOC) removal rate compared with almost no removal of the polymer using 0·5 day residence time with just the biological treatment. After a 4 day residence time, 60%–70% TOC removal was recorded with no pretreatment and >90% TOC removal was achieved with the integrated wet air oxidation step. Kawai[181] has also commented on the microbial degradation of water-soluble polymers (e.g. PEO) as a combination of oxidation and hydrolysis. In both these studies the effect of increased molecular weight of the PEO on the reduction in the rate of hydrolysis was noted. Penco *et al.*[182] have studied the degradation of multiblock PLGA and PEG copolymers using physiological conditions and found that the solubility of the block copolymers and the degradation rate increased with increasing length of the PEG segment; this is presumably connected with the subsequent ease of hydrolysis of the PLGA segments.

Hydrolysis of poly(ε-caprolactone). The biodegradation of poly(ε-caprolactone) (PCL) using soil burial, activated sludge tests[183] and composting[184] is reported to produce loss of mechanical properties and rapid weight loss.[183] A bulk random chain scission mechanism has been proposed, but other workers[185] have produced evidence that mixed micro-organisms from industrial compost of household refuse initiate the degradation by chain-end scission. Abiotic hydrolysis occurs more slowly because of the hydrophobicity of the polymer and in this way it is similar to PHB. Koenig and Huang[186] have investigated the use of crosslinked PCL and solution-cast PHBV (12% HV) as biodegradable hydrophobic coating materials for hydrophilic substrates (filter paper, Mater-Bi® (a corn

starch/vegetable seed oil thermoplastic) and a PCL/starch blend). The PCL was a better water barrier than the PHBV for the paper at 23°C but acted as a semipermeable membrane with the other substrates and increased the water absorption over untreated samples; also, the low melting point of PCL (60°C) was an additional problem.

Hydrolysis of cellulose derivatives and starch. The effect of the esterification of cellulose on its biodegradation has been mentioned and the production of a biodegradable form of cellulose has been approached by looking at the effect of substituent distribution in cellulose esters.[187] A range of acyl substituents were attached to the cellulose and DS between 0·1 and 3 was assayed. It was found that the rate of cellulolytic enzyme degradation decreased with increased DS and with increase in the number of carbons in the side chain. 75%–80% of the maximum rate of biodegradation was reached within approximately 7 days of incubation. The maximum degree of acylation which (a) could be tolerated before the polymer became undegradable or (b) resulted in more than 10% weight loss was related to ester type and varied from DS 0·5 to 1·0.

Gardner and co-workers[188,189] and Gross *et al.*[190] have investigated the biodegradation of CA films in bench-scale composting medium (at 53°C and 60% moisture content). Based on the disintegration of the films and weight loss, they concluded that CAs with DS $< 2{\cdot}20$ decomposed in compost at similar rates to PHBV and PCL films and were found to totally disappear after 14 days. There was NMR and GPC evidence of the preferential removal of low-molecular-weight fractions of CAs, leaving the more highly substituted material.[188] Little weight loss was observed using abiotic conditions with similar temperature and moisture conditions. The nature of the composting formulation was investigated and found to have little effect in five out of the seven types tested. However, moisture content had a significant effect and a reduction from 60% to 40% resulted in changes in the complete polymer degradation time of CA (DS 1·7) from 6 to 30 days. CA (DS 2·06) and triethylcitrate (TEC) were thermally compounded[189] and fabricated as both compression-moulded films and injection-moulded bars. The former degraded rapidly using the compost and 10%–12% weight loss was recorded for the bars. Miscible blends of cellulose acetate propionate (CAP) and both poly(ethylene glutarate) (PEG) and poly(tetramethylene glutarate) (PTG) were prepared and composted. The nature of the polyester was found to have no effect on the rate of degradation, but the ratio of CAP to polyester and the DS of CAP both affected the degradation. When the DS of the CAP was high, almost all the weight loss was from the polyester, but for blends with DS of CAP $< 2{\cdot}0$ both components degraded. The rate of degradation increased as the wt% of polyester in the blend increased.

Mino *et al.*[191] have studied the biodegradation of starch under anaerobic, anoxic and aerobic conditions as a model organic substrate using an analytical method based on starch–iodine complex formation. The tests show the range of bacterial media that will degrade the polysaccharide from pure α-amylase enzymatic degradation to hydrolysis by the complex mixture of inoculum found in activated sludge. This was found to follow surface-limited adsorption kinetics as it was independent of the biomass concentration. An *in vitro* study[192] of the biodegradation of starch-based materials was carried out using excess *Bacillus licheniformis* α-amylase and *Aspergillus niger* gluco-amylase at 37 and 80°C and there was preliminary evidence of biodegradation from weight loss, but the complexity of the materials tested did not allow the extent of biodegradation to be accurately assessed.

Studies of starch/PCL systems have shown that blends using high-amylose corn starch are the strongest, as the small size of the starch granules produces good dispersion in the PCL matrix, but, like PHBV (11·6 mol% HV)/starch blends, they are phase separated.[193] The biodegradation of starch/PCL systems has been studied[194] in a variety of biotic and abiotic conditions. The rate of biodegradation of the blend in compost was found to increase in the presence of starch.

Hydrolysis of poly(3-hydroxyalkanoate)s. PHAs undergo quite rapid enzymatic hydrolysis in soil, sewage sludge and seawater especially in the presence of extracellular P(3HB) depolymerases, but the polymers are hydrophobic and abiotic hydrolysis is relatively slow. It has been shown[195] that surface area and thickness of film have little effect on the rate of biodegradation of PHB or PHBV. The major factor controlling the microbial hydrolysis rate of the PHB or PHBV is the degree of crystallinity.

The enzymatic degradation of PHB, both intra- and extracellular, has been extensively investigated and Hocking and Marchessault[13] and Doi[80] have reviewed the role of extracellular P(3HB) depolymerases, which were found to degrade PHB by different mechanisms according to their bacterial source. Saito and co-workers[196] showed that *Pseudomonas lemoignei* depolymerase could be separated into four separate enzymes: the A pair and the B pair. The B pair produced a higher percentage of trimer during degradation and the final product ratio of monomer to dimer was higher than for the A enzymes (0·75 compared with 0·2 for the A pair). All the enzymes specifically hydrolyse the polymer and oligoesters of D(−)3-hydroxybutyrate but show no activity towards the dimer and have an affinity for the hydroxyl rather than the carboxyl end of the oligomers. Cleavage occurs at every second or third ester bond from the hydroxyl end by these exoenzymes. However, *Pseudomonas lemoignei* also produces intracellular D(−)3HB dimer hydrolase and this can absorb the dimer, hydrolyse it and then oxidize it into acetoacetate,

which leads to its ultimate biodegradation via acetyl-CoA and the citric acid cycle.

A strain of *Alcalignes faecalis* isolated from active sewage sludge has been found to excrete an effective P(3HB) depolymerase (optimum pH 7·5) and an extracellular D(−)3HB dimer hydrolase when grown in a medium with PHB as its only carbon source. The mechanism of the depolymerase enzyme's activity has been described as an endo-type hydrolase, with the second ester linkage from the —OH end being particularly susceptible to hydrolysis. It has been shown[197] that as well as its catalytic site the enzyme has a hydrophobic site, which is not essential for the hydrolysis of water-soluble oligomers but is necessary for the hydrolysis of hydrophobic substrates. The extracellular D(−)3HB dimer hydrolase excreted was found to be capable of hydrolysing the DD dimer and has some activity towards the LD and DL dimers, but not the LL dimer.

The rate of enzymatic hydrolysis of copolymers of PHAs was studied by Doi *et al.*[198] using the purified depolymerase from *A. faecalis* in a phosphate buffer (pH 7·4) at 37°C. The rate of erosion was measured by weight loss and found to be strongly dependent on the copolymer composition, with copolymers containing C_6 to C_{10} in the basic 3HA unit showing no hydrolysis. However, the copolymers P(3HB-*co*-4HB) 10–17 mol% 4HB) showed accelerated hydrolysis under these conditions. There was no correlation between the crystallinity or molecular weight of the microbial films and the rate of hydrolysis. This latter point supports the endo-type hydrolase mechanism, as the M_n value of the films remains almost unchanged during the degradation, indicating that P(3HB) depolymerase hydrolyses only the surface layer of polymer chains and polymer erosion occurs by surface dissolution. There is SEM evidence of surface erosion and no bulk hydrolysis is thought to occur.

Albertsson and co-workers[199] have established a series of biotic and abiotic test conditions to investigate the biodegradation of P(3HB-*co*-6% 3HV) copolymer films. Microbial degradation was investigated, with the films as the main carbon source, (a) in a stirred, complex aqueous, mineral medium inoculated with *Aspergillus fumigatus* at pH 5·5 and 25°C, (b) in a sterile control of this mineral medium and (c) in a composting facility, which could be turned and consisted of typical garden waste, with a maximum internal recorded temperature of 64°C and approximately 60 wt% moisture content.

Microbial degradation produced SEM evidence of rapid surface degradation, also observed by Doi *et al.*[198] and explained by Nobes *et al.*[200] as a splintering phenomenon. This was also seen in single-crystal degradation by a chain-end mechanism, causing no decrease in average molecular weight. Hocking *et al.*[201] have now proposed that *A. fumigatus* uses both endo- and exoenzymes in the degradation of PHB, explaining the fast rate of degradation. Doi has shown that PHB degrades faster enzymatically than PHBV and Albertsson has produced ATR–FTIR spectral evidence of structural changes in the surface layer of polymer during microbial degradation, which indicated that the PHB units were more easily degraded than the PHV and this increased the abundance of the latter in the surface layer.

The abiotic rate of hydrolysis of microbial polyesters in physiological conditions is slower than the enzymatic rate under similar conditions by a factor of two or three. Using phosphate buffer (pH 7·4) and temperatures ranging from 37 to 70°C, Doi[80] found that P(3HB), PHBV (68% HV), P(3HB-*co*-9% 4HB) and P(3HB-*co*-16% 4HB) showed no weight loss after 180 days at 37°C, although after a characteristic induction period, molecular weight loss was observed. The induction period varied from 80 days for PHB and PHBV to only 20 days for the P(3HB-*co*-4HB) copolymers and it was postulated that this may be the time for the water to permeate the polymer matrix. The molecular weight loss for the P(3HB-*co*-4HB) copolymers was also greater than for the others and increased with mol% 4HB.

Accelerated hydrolysis at elevated temperatures was investigated by Doi[80] (55 and 70°C) and Albertsson and co-workers[199] (at 60°C for 347 days) and found to involve a homogeneous process in two stages. There was no induction period above 60°C and the initial random scission of the ester groups occurs throughout the polymer (amorphous and crystalline regions), which leads to a decrease in molecular weight but little change in the polydispersity and almost no bulk mass loss. Albertsson used DSC changes to investigate further the multiple melting behaviour of PHBV during initial random scission hydrolysis in sterile water. When molecular weight values have decreased to about 13 000[80] and 8000–9000 (after 200 days),[199] the second stage occurs involving weight loss; the polydispersity rapidly increases to a value of 4 and then remains constant.

The kinetics during the second stage indicated a change to non-random chain scission and, based on data of Knowles and Hastings[202] on the effect of pH on hydrolysis, it was found that after 200 days the pH had fallen from 7 to 3 because of hydroxy acid production. ^{1}H NMR evidence showed that the mol% of HV in the polymer had decreased from its initial value and it was inferred from this that the final stage of the abiotic hydrolysis favours hydrolysis of HV units. Doi found that P(3HB-*co*-4HB) copolymers again show more rapid hydrolysis in abiotic conditions compared with P3HB and PHBV copolymers.

In Albertsson's study there were irregular changes in the average molecular weight of the films in compost but there was no general trend. The results were in

general agreement with those of Margaert *et al.*,[203] who observed no decrease in the average molecular weight in a compost over 150 days at temperatures ranging from 7 to 32°C and concluded that the degradation was microbial. A study at slightly higher temperatures (28–40°C) has produced both abiotic molecular weight loss and biotic mass loss. The variations in the nature of the compost, its water content and temperature make it difficult to generalize about the mechanism involved. The polymer film in the study by Albertsson became brittle in sterile water and in compost, indicating some abiotic hydrolysis, and Gilmore *et al.*[204] noted the same increased brittleness during composting studies on PHBV.

There have been several studies on the biodegradation of PHB with different stereoisomer composition and different tacticity.[205,206] The factors that control the rate of biodegradation of chemically synthesized PHB stereoisomers are the nature of the micro-organism, the morphology of the polymer, the tacticity of the polymer and its stereostructure.

6.2 Biodegradation testing

6.2.1 Test methods. Testing chemicals for their biodegradability has been carried out for over 30 years, although the development of scientifically acceptable methodologies in the field of polymers and plastics is still inadequate. Current standard test procedures either describe biodegradability inadequately or fail to provide meaningful practical data which can be used to make environmental assessments.

Definitions of biodegradability which specify 100% biodegradability are impracticable, but standards authorities have produced definitions, e.g. ISO 472:1988, ASTM (D20.96 proposal) and DIN103.2, which are all quoted by Seal.[207] These definitions, along with that given at the start of this review, are pragmatic as they concentrate on the biodegradation of the plastic material without assessment of its ultimate fate and with no attempt at classification in terms of rate of biodegradation.

A tiered approach is needed to assess chemicals for their effect on and fate in the environment and this has applications whether they are biodegradation or not. Tier testing for biodegradable polymers can be represented as follows.

1. The first tier is short-term screening under stringent conditions in a controlled laboratory environment. Chemicals which biodegrade under these conditions, with limited time for acclimatization, are described as *readily biodegradable*.
2. The second tier involves prolonged exposure to a natural inoculum of micro-organisms. Favourable test conditions, including an acclimatization period, are included, so the classification *inherently biodegradable* cannot be assumed to represent rapid and reliable biodegradation in a natural environment.
3. The third tier is a range of protocols designed to simulate repeated exposure of the material to a disposal route, e.g. sewage treatment, composting, The tests have to be long-term and require careful validation and justification, so they are expensive.

The degradation tests must incorporate realistic environmental disposal conditions which are either terrestrial (landfill, soil, composting) or aqueous (groundwater, marine, waterways, sewage or sediment). The development of some tests is quite advanced, but the standardization of other tests, e.g. composting, with variation in both composition and conditions, is very difficult. Five screening test protocols have been accepted by the EC and EPA and are described in the OECD guidelines, but two involve measurement of dissolved organic carbon (DOC), which cannot be used for biodegradable. The three appropriate tests for biodegradable polymers are the modified Sturm test, the modified MITI test are the closed bottle test (OECD 301B, C, D). They are all aquatic, aerobic tests and the test substance provides the sole carbon source and is exposed to a low level of inoculum for 28 days with stirring. The evolved CO_2 or consumed O_2 is determined and calculations based on the total carbon content of the polymer are used to find the percentage degradation. The criterion for a polymer to be *readily* biodegradable is to achieve 60% of the total theoretical oxygen demand (TOD) within 28 days and this should be reached within 10 days of the biodegradation reaching 10% TOD.

The next tier of testing involves *inherent* biodegradability and there are four test protocols recommended, which do not have pass/fail criteria, but at least 20% biodegradation within the 28 days suggested exposure time is recommended by the OECD. They are the modified SCAS (semi-continuous activated sludge) test, the modified Zahn–Wellens test, the modified MITI test and inherent biodegradability in the soil (OECD 302A, B, C and 304A). They allow for greater flexibility in exposure time, acclimatization procedures and microbial selection. Seal[207] has comprehensively reviewed these different test methods, the test parameters and the criteria for the pass level. He has also summarized the standards for resistance testing of plastics and the test strains of fungi and bacteria used in these tests. The degree of sophistication in measuring the degradation varies from visual examination of surface growth of fungi or bacteria and material weight loss to testing of mechanical properties.

Itavaara and Vikman[208] have also given an overview of methods of biodegradability testing used at the Tech-

nical Research Centre of Finland (VTT) for testing solid polymers and packaging materials. Aquatic screening tests include an automated, aerobic Sturm test (OECD 301B; ASTM D5209), the VTT head space test and an anaerobic test (ASTM D5210). Also reviewed are different composting systems and, in a later paper,[209] improvements in the VTT head space test for insoluble polymers are outlined, including the method of determining the CO_2 distribution between the gas and liquid phases.

A screening and long-term test procedure has been proposed by Puechner *et al.*[210] Aerobic and anaerobic aquatic and soil screening was carried out using carbon balances to evaluate the biodegradation of PHB, PHBV, PCL and other manufactured biodegradable polymers. However, polymers that were not fully degraded were then tested in an aquarium system for 1 year, monitoring biodegradation by a variety of methods, including DOC release into the water, mass loss and SEM.

Margaert *et al.*[211] have looked at the biodegradation of PHB and PHBV (10 and 20 mol% HV) copolymers in natural waters. After 358 days in a freshwater canal the mass loss was 34% for PHB, 77% for PHBV (10% HV) and the other copolymer completely disintegrated. In seawater after 270 days the PHB lost 31% and the copolymers 49%–52% of their initial mass. Temperature had an effect, as mass loss was greater in the summer. Degradation reduced the tensile properties of the copolymer samples, but no relevant changes in the molecular weight were observed indicating surface erosion. Over 90 micro-organisms were isolated and identified from the polymer surfaces. Mayer *et al.*[212] have also investigated the biodegradation of polymeric films in marine and soil environments. They have developed standardized accelerated test methods and quantified the degradation using weight loss/surface area measurements. A range of unblended polymers were tested, but the increased complexity of testing blends was recognized, in which leaching of plasticizers and other factors can affect the results.

6.2.2 Recent developments in composting test methods. The biodegradation of polymeric materials and the implications of this factor in the management of solid waste have been discussed. The standardization of composting tests and the correlation between biodegradation behaviour in miniaturized, laboratory-based composting facilities and that observed in actual composting conditions are problems that have to be addressed.

Pettigrew *et al.*[213] have compared a tiered testing strategy for assessing the compostability of PCL (OECD 301B and ASTM D5338 test methods) with realistic composting of ^{14}C-PCL. These tests confirmed that screening-level biodegradation tests can provide information about the inherent biodegradability of polymers, but the rate of degradation and the ultimate fate of polymers in environmental conditions must be determined using realistic tests.

Urstadt *et al.*[214] have shown that calculating the degree of biodegradation of PHB in a mixed culture compost could not be done accurately using CO_2 release alone. However, establishing carbon balances has the potential to be more accurate, with different analytical methods being adopted to determine the carbon fraction in particular test conditions. Quantitative determination of biomass was assessed theoretically and practically from the protein content and by selective oxidation with hypochlorite. Limitations in the methods were recognized; but reasonable assays were achieved within acceptable standard deviation range. Protein assay was the basis of another study of the carbon content of biomass by Spitzer *et al.*[215] The Lowry method was used and this showed that the ratio of protein to carbon content is not constant but depends on the composition of the microbial population, the growth phase and the substrate supply. Correlation of the absorbance with carbon content obtained during the Lowry test was used to estimate biomass/carbon content dependence during biodegradation by a mixed population of micro-organisms, and calibration curves were obtained.

The problem of quantifying biodegradation has also been approached in other ways. David *et al.*[216] investigated a manometric method of measuring oxygen consumption during biodegradation using various inocula ranging from a single bacterium to complex mixtures of micro-organisms in compost and sewage sludge. Pagga *et al.*[217] in Germany and Gross and co-workers[218] in the US have produced laboratory-controlled composting facilities which have the potential to become standard test facilities. Gross and co-workers have simulated municipal solid waste compost at a constant temperature of 55°C and 54% moisture content. It was proposed that the effect of acclimatization time and exposure time should be studied, as well as the rate of biodegradation of PHBV (20 mol% HV) and PHB samples at various points in the composting cycle. Some PHBV samples were exposed for 18 days continuously and weight loss was recorded every 3 days. Other samples were exposed to the compost for only 3 days but at different times in the composting cycle. The cycle was found to have three stages, with the maximum rate of degradation occurring between the 10th and 15th day. The continuously exposed samples degraded most and PHBV samples degraded more than pure PHB. Very recently, Itavaara *et al.*[219] have proposed a method of using steel frames to test the biodegradation of polymer packaging materials in windrow compost. The polylactide- and Biopol-coated cardboard samples completely degraded and there was no toxicity introduced into the compost.

6.2.3 Biocompatibility and toxicity screening for biodegradable polymers. The biocompatibility of any macromolecules that are used as implants or as drug delivery systems involves cytotoxicity testing using appropriate extraction protocols, which help to establish the presence of potential 'leachables' that are capable of inducing a measurable degree of systematic toxicity, localized tissue irritation sensitization or other biological response. Tissue culture testing is a rapid, economical, *in vitro* approach to biocompatibility testing, but because of its sensitivity it should always be used in conjunction with *in vivo* studies. Holland and Tighe[21] have compared two approaches to cytocompatibility testing: the semiquantitative agar overlay test and the quantitative cell growth inhibition test. Chaput *et al.*[220] have used *in vitro* direct contact and agar overlay cell cultures of mouse fibroplasts to test PHBV films and extracts in different media. Direct contact cultures with the films produced mild cell reactions and lyses, and agar overlay cultures did not give any significant cell death or changes. Recently, Dang *et al.*[221,222] have carried out toxicity screening tests on biodegradable polymers (CA, Biopol, PCL and others) using standard *in vitro* animal cell culture tests. Phase contrast light microscopy enhanced by neutral red staining was used to provide qualitative evaluation of cell morphology and lysis. The method was shown to be more sensitive and accurate than other comparable tests.

Saad *et al.*[223] have studied the biocompatibility of multiblock copolyesters. Cell adhesion, cell growth and cell activities of macrophages and fibroblasts cultured on the newly developed degradable copolymers were studied to establish whether the latter were suitable for biomedical applications. Their biocompatibility was established by subcutaneous implantation of the polymer foil into rats and their tissue compatibility and biodegradability were also demonstrated. It is beyond the scope of this review to give more than an indication of the range of toxicity and biocompatibility studies that need to be carried out on polymers for biomedical applications.

7 GENERAL SUMMARY OF FINDINGS

The thorough literature search that has been carried out has made it possible to draw a number of conclusions.

1. The use of plastics in packaging and the waste produced have had considerable environmental impact because of the low density and hence large amount of most commodity polymers. However, the removal of plastics from packaging is not a viable option, as there would be a dramatic increase in terms of weight and volume using non-plastic packaging and the additional energy consumption would be prohibitive. Recycling of materials and energy recovery are obviously primary strategies for reducing plastic waste, but the use of biodegradable polymers also has some advantages.
2. Renewable sources of polymeric materials and synthetic biodegradable polymers may provide ecologically attractive technologies, but even in countries that are at the forefront of green technology it is only as components of packaging and as natural fibre composites that these materials are currently viable in terms of price and performance. The main constraint on the use of biodegradable polymers for bulk packaging is the difference in the price of these polymers compared with that of bulk-produced, oil-based plastics. The current cost of Biopol® is approximately £8000 per tonne, compared with the current UK prices of commodity polymers of between £500 per tonne (PVC and PP) and £600 per tonne (HDPE and high-impact PS). Current low oil prices, increased recycling capacity and improved technologies for the separation of plastics and their reuse make the use of biodegradable polymers for most packaging requirements uneconomic.
3. Polymeric materials and blends that are biocompatible and biodegradable have obvious benefits as packaging materials for pharmaceutical products, drugs and would dressings. The physicomechanical properties plus the gas and liquid barrier properties of these materials are obviously important, as is a knowledge of the conditions and rate of biodegradation. If processable biodegradable pure materials or blends can be manufactured, the economic imbalance may be less for specialist packaging applications.
4. The production of biodegradable polymers with the necessary controlled structure (e.g. monomer sequence, tacticity, chirality) to match enzyme specificity in degradation reactions in different microbial environments is not possible with currently available techniques for chemical synthesis. However, some progress has been made in the synthesis of block copolymers with controlled molecular weight and tacticity. There is therefore potential to make materials with improved physicomechanical properties and varied degradation profiles.
5. The degradation of poly(α-ester)s is mainly by abiotic hydrolysis, but although the use of polymers with different molecular weight and tacticity has varied the biodegradation rates of these materials, the range is still quite limited. PHAs have the most potential, as although their rate of abiotic hydrolysis is relatively slow, microbial hydrolysis is more rapid and can be

manipulated by variations in processing techniques, molecular weight of the polymer, copolymer composition and blending.

6. Pharmaceutical uses of biodegradable polymers are well established, e.g. resorbable sutures, hard and soft tissue implants and controlled drug delivery systems, mainly because the unique properties of the materials override the costs involved. Drug release systems incorporate the active substance into a polymer matrix which is implanted into the patient. A diffusion mechanism produces maintained drug release followed by bulk hydrolysis of the polymer, which is ideal for the delivery of relatively small drug molecules. However, modern techniques require the incorporation of drugs which vary in nature and molecular size and a diffusion mechanism is too slow for the release of large molecules, so an erosion release mechanism must be used. The range of degradation profiles available is limited in terms of both degradation time and character. The balance between abiotic and biotic degradation, both *in vivo* and *in vitro*, is not fully understood, even for the most studied biodegradable polymers, namely poly(α-ester)s and poly(hydroxyalkanoate)s. More pure or blended materials with surface erosion characteristics are required for drug delivery.
7. The blending of biodegradable polymers is a method of reducing the overall cost of the material and offers a method of modifying both the properties and the degradation rates of the materials. Miscibility is usually characterized by a single glass transition in the blend, which varies with composition and can be measured by mechanical or thermal testing. The advantages of producing miscible blends include single-phase morphology in the melt and reproducible mechanical properties. However, forming a miscible blend, particularly with a non-biodegradable polymer, can reduce or even inhibit the degradation of the biodegradable component. Immiscible blends have the disadvantage of having properties that are dependent on the blend morphology produced by processing and these are often not reproducible. However, some can show higher biodegradation rates than the unblended biodegradable homopolymer(s).
8. The development of two- and three-component blends represents an area of considerable interest. The use of natural polymers and biodegradable polyesters has been studied by several groups, but there is still considerable potential, especially if compatible blends can be made using materials with relatively low glass transition temperatures. The use of low-molecular-weight plasticizers and photoactive materials in tertiary blends of biodegradable and non-biodegradable, synthetic and natural polymers is another potential field of study.
9. Pragmatic definitions of biodegradation concentrate on the degradation of the plastic material without assessment of its ultimate fate and with no attempt at classification in terms of rate. Standards authorities have produced definitions, e.g. ISO 472:1988, ASTM (D20.96 proposal) and DIN103.2. Tier testing for biodegradable polymers can represent a method of classifying polymers, and readily biodegradable, inherently biodegradable and a third tier have been identified.
10. Many current standard test procedures for biodegradable polymers either describe biodegradability inadequately or fail to provide meaningful practical data which can be used to make environmental assessments. The degradation tests must incorporate realistic environmental disposal conditions which are either terrestrial (landfill, soil, composting) or aqueous (groundwater, marine, waterways, sewage or sediment). The development of some tests is quite advanced, but the standardization of other tests, e.g. composting, with variation in both composition and conditions, is very difficult. The development of a laboratory-scale composting facility would provide valuable information about the optimum conditions needed for large-scale composting, and there is a need to improve the knowledge base of abiotic hydrolytic degradation rates and mechanisms of different biodegradable polymers, copolymers and blends.

APPENDIX I RELEVANT RESEARCH PAPERS

I.1 Summary of current research papers on use of biodegradable polymers as surgical implants (1994–1997)

Aim	Materials	Points	Ref.
The design and fabrication of biodegradable polymer devices to engineer tubular tissue and as a delivery vehicle for transplanting cells.	Porous films of P((D,L) LGA) formed into tubes capable of resisting compression forces *in vitro* and *in vivo*.	1. There is ingrowth of fibrovascular tissue after transplant of device. 2. Using intestinal epithelial cells, attachment was found and an organized epithelial layer.	224 1994
PHA polymers as culture surfaces for spinal ligament fibroblasts (HSF) from scoliotic patients.	PHBV (HV = 7, 14 and 22 mol%) surfaces tested with PS and type I collagen. The surfaces were used as culture substrates for HSF.	1. Proliferation rates were slow for PHBV 2. Surface chemistry and wettability PHBV had no effect. 3. Preferential growth on PS and collagen surfaces.	225 1995
A novel way to make porous P(D,L)LGA sponges without organic solvents. Applications: tissue engineering to transplant cells or growth factors and as tissue regeneration templates.	Macroporous sponges made by exposing P(D, L)LGA discs to high-pressure CO_2 for 72 h at room temperature, then fast pressure reduction to atmospheric level.	1. Polymer sponges with large pores and porosity were produced. 2. The porosity could be controlled by a preform technique. 3. Fibre-reinforced foams produced.	226 1996
Biodegradable and macroporous PL foam implants for cell transplantation. 1. The preparation of supports by solid–liquid phase separation.	Microcellular foams were produced by freeze drying PL solutions in 1,4-dioxane.	1. Thermally induced phase separation studied w.r.t. several variables, e.g. [polymer]. 2. Tubular macropores with porous substructure observed and variations in morphology with conditions followed.	227 1996
2. The preparation of polylactide macroporous foams by liquid–liquid phase separation as a porogen technique.	Amorphous P(D,L)L and semicrystalline P(L)L in 87 : 13 dioxane : water mixture producing liquid/liquid phase separations.	1. The possibility of gels discussed. 2. Freeze drying can produce flexible tough foams with isotropic morphology. 3. Potential for use established.	228 1996
A novel method for fabrication of biodegradable scaffolds with high compression moduli suitable for fibrocartilage regeneration in meniscal implants and prothesis. Excellent adhesion properties of polymer known to enhance healing of meniscal lesions.	Stiff porous materials of high-Mwt 50/50 P(L LA-*cc*-εCL). Porous microspheres agglutinated with NaCl crystals and mixed with solid solvent to give homogeneous solvent distribution	1. For a meniscal prosthesis the compression modulus has to be larger than 150 kPA to protect the articular cartilage; the compression modulus of this product could be varied over the range 40–1100 kPa. 2. The density could be varied from $0{\cdot}07\,g\,ml^{-1}$ to $0{\cdot}5\,g\,dl^{-1}$.	229 1997
Soft tissue and cancellous bone reaction to the implantation of novel biodegradable pins and plates in rabbits	Novel fibre-reinforced material based on lactide.	1. *In vitro* mechanical performance test found to be superior to former implants after 1, 6, 12 months. 2. *In vivo* test in a neutral environment showed biocompatibility.	230 1997
The influence of manufacturing procedure on the degradation of PLGA implants. 1. 85/15 PLGA. 2. 50/50 PLGA. Implants formed by compression of microcapsules and *in vitro* studies at pH 4·5, 7·4, 9·4 monitored by varied techniques.	Microcapsules prepared by non-solvent-induced phase separation of solvent/non-solvent. 1. Methylene chloride/hexane (non-polar). 2. Acetone/phosphate buffer (polar).	1. 85/15 and 50/50 PLGA prepared by non-polar method degrade faster. 2. SEM shows water uptake faster in more porous matrix of non-polar product. 3. Average degradation times: • 85/15 PLGA ~26 weeks; • 50/50 PLGA ~6–8 weeks.	231 1997

I.2 Summary of research papers on production of microspheres for sustained drug delivery systems using biodegradable polymers (1994–1997)

Aim	Material	Points	Ref.
Novel encapsulation technique by in-water drying method to produce a sustained drug delivery system and persistently suppress steroidogenesis for 1–3 months after a single injection.	PLGA and PLA microspheres containing leuprorelin, a potent LHRH agonist, were formed.	1. Particles with high trap ratio of drug and small initial burst were obtained. 2. The systems effectively reduced required dose compared with daily injection system, with more continuous receptor hits on the target organs.	232 1994
Microencapsulation of protein bovine serum albumin (BSA) using novel ternary blends. Inclusion of low-Mwt PCL-enhanced phase mixing by a reduction in the Mwt of the components. WOW multiple emulsion method was used to encapsulate BSA. The PO-181 was found to retard rate of crystallization and improve the sphericity and regularity of the particles.	The ternary blend was based on P(ε)CL (high and low Mwt) and poloxamer 181 (PO-181), a triblock copolymer (PEO–PPO–PEO). Various factors were varied to see their effect on the microsphere properties.	1. A mean particle size of 10–42 μm was achieved altering the internal phase volume of the primary emulsion 2. Protein entrapment (11% w/w) was possible with protein-to-polymer ratio of 1 : 4. 3. Native-PAGE analysis of the entrapped BSA indicated maintenance of bulk structural integrity.	233 1995
The production of peptide containing microspheres from low-Mwt and hydrophilic copolymer. Initial analysis for Mwt, T_g, bulk density and carboxylic acid content was carried out. The effect of these on the microspheres was investigated.	P(D,L)LA-*co*-GA (Mwt < 10 000) from different suppliers was synthesized in different ways. T_g increased with Mwt of PLGA. Bulk density and —COOH content of microspheres decreased with increased Mwt.	1. % yield of product decreased with increased water-soluble fraction polymer. 2. 75 : 25 PLGA showed decreased peptide incorporation with Mwt but the 50 : 50 PLGA did not. 3. Higher-Mwt microspheres show lower initial peptide release. 4. Peptide incorporation can be controlled by preparation modification with low-Mwt PLGA.	234 1996
The effect of the processing technique on the Mwt of the polymer in biodegradable multiphase microspheres. Produced by agitation, potentiometric dispersion or sonication.	P(D,L)LGA was investigated by GPC before and after processing. Two surfactants were used at different levels in the continuous phase.	1. Only microsphere produced by sonication (21% decrease) showed any significant Mwt change. 2. Potentiometric dispersion produced the narrowest MWD. 3. A decrease in the internal diameter of the infusion tube used in the potentiometric dispersion technique decreased product mean particle size. 4. Increased surfactant level gave increased mean particle size of product containing BSA in potentiometric dispersion method, attributed to increased conductivity in the continuous phase with increased surfactant level.	235 1996
Microparticle preparation by a salting-out process, which is mainly suited to the production of nano- or microparticles for parental drug delivery systems. Careful tailoring of the process parameters is needed, based on the nature of the polymer and the drug.	Size, size distribution and morphology are important parameters. *In vitro* properties of the drug must also be considered.	1. Water-insoluble and low soluble compounds are well encapsulated by this method. 2. The encapsulation efficiency for water-soluble compounds such as BSA is small.	236 1997

Aim	Material	Points	Ref.
The preparation of biodegradable polymer microspheres encapsulating protein with micron sizes. Solvent evaporation composite emulsion technique is used to produce particles with narrow size range.	P(D,L)LA–PEG of known Mwt synthesized using stannous octoate by cationic ROP. PVA aqueous solutions were used as dispersion medium for microsphere preparation.	1. The size of microspheres increased with increased Mwt of copolymer. 2. PLA–PEG (10%) produced microspheres of improved size control 3. [PVA] can affect the size of microspheres. 4. DSC data show protein efficiently encapsulated, with low crystallinity, improving ability to trap protein. 5. The amount of protein carried was related to the nature of the protein.	237 1997
Polymer alloys as a new preparation method for biodegradable microspheres. This multi-reservoir technique is based on phase separation of the two polymers when their concentrations exceed a critical value. Amino acid powders were added to the biphasal solutions.	PLA–PLGA–methylene chloride ternary systems were studied. Solubility parameters governed the powder distribution in the two phases. Cisplatin is all found in the PLGA-rich phase.	1. The critical [polymer] that produces phase separation is (i) independent of PLA : PLGA ratio, (ii) dependent on solvent, Mwt and L : G ratio in PLGA. 2. Particles of unique 'polymer alloy' with cisplatin distributed in internal PLGA-rich phase. 3. Encapsulation efficiency approximately 100% at 16% loading. 4. *In vitro* dissolution study showed release of cisplatin over 45 days with no initial burst.	238 1997
A novel method of encapsulation of a drug from microspheres using silicone oil-based phase separation process. Several different polymer blends were used and the encapsulation was characterized both *in vivo* and *in vitro*.	PLGA and P(D,L)LA (low-Mwt) microspheres were prepared to encapsulate granulocyte–macrophage colony stimulating factor (GM-CSF).	1. Steady release of GM-CSF over 1 week; no significant protein 'burst'. 2. GPC analysis of degradation kinetics shows low-Mwt PLA enhanced degradation of PLGA and affected release kinetics. 3. GM-CSF release biologically active and physically intact. 4. Formulation parameters affect encapsulation and controlled release of drug in PLGA/PLA. 5. There was an intense local tissue reaction in mice to one GM-CSF formulation.	239 1997
A review that covers (i) the techniques for the preparation of injectable monolithic microcapsules for prolonged drug release, (ii) a discussion of phase separation, solvent evaporation and spray-drying procedures. (iii) manufacturing techniques for efficient entrapment of highly water-soluble drugs.	PLA, PLGA and copoly (lactic/glycolic acid) are reviewed as the basis for the release of water-soluble drugs over different times.	1. Degradation of these polymers is altered by copolymer ratio and the Mwt. 2. The amount of released microencapsulated drug correlates almost linearly with polymer degradation, indicating that controlled release formulations can be designed using suitable PαHAs with different degradation rates.	240 1997
Production of biodegradable microparticles for drug delivery and vaccine formulation using an emulsion/evaporation technique on modified PLG. Surface attachment of the hydrophilic species of PEG was used to anchor segments, as PEG or dextran polymers alone did not produce microparticles. Zeta potential measurements and X-ray photoelectron spectroscopy confirmed PLG surface modification.	Poly ethylene glycol–dextran (PEG–DEX) conjugates were used as combined stabilizer and surface modifier to produce resorbable P(D,L)LG microparticles.	1. PLG particles modified by post-adsorbed PEG–DEX conjugates flocculated in 0·01 M salt solutions, but if PEG–DEX used as surfactant, PLG particles stable in $<0{\cdot}5$ M NaCl. 2. Immunological detection used to show surface-exposed dextran. 3. The findings support the model of PEG in the conjugate acting as an anchor with steric stability provided by DEX.	241 1997

Aim	Material	Points	Ref.
Microcapsule fabrication using blends, including PHBV, with BSA; surrogate protein-loaded agarose as the active ingredient. Factors affecting its release were identified. Single (SET) and double (DET) emulsion techniques with solvent evaporation were used to produce spherical reservoir-type microcapsules.	Biodegradable polymer blends of (i) 80 : 20 poly(ethylene adipate) (PEAD) : PεCL by both techniques, (ii) 80 : 20 PEAD : PHBV (10·8% HV) by DET, (iii) 40 : 40 : 20 PEAD : PHBV (10·8% HV) : PεCL by SET. A range of BSA loadings were used. All the polymers generated high yield of microspheres (>75%)	1. 80 : 20 PEAD : PεCL produced smooth particles by both methods and 80 : 20 PEAD : PHBV particles were smooth using DET. 2. 40 : 40 : 20 PEAD : PHBV : PεCL produced a mixture of smooth-surfaced microporous and macroporous microsphere fractions using SET. 3. BSA incorporation has no significant effect on particle size distribution. 4. Encapsulation efficiencies were low (<14·5% overall) 5. BSA release increased significantly with theoretical % loading but could not be confirmed. 6. Significant BSA release could be monitored up to 26 days.	242–243

I.3 Summary of research papers on production of nanospheres for sustained drug delivery systems using biodegradable polymers (1994–1997)

Aim	Material	Points	Ref.
To form and stabilize a polymeric colloidal suspension of nanoparticles by transfer from a good solvent (L1) to a non-solvent (L2). Photon correlation spectroscopy (PCS) is used to confirm the product.	Nanoparticles were made of P(ε)CL. Effect of [P(ε)CL], L1/L2 ratio and dielectric of final mixture on morphology tested.	1. Experimental model proposed 2. Account taken of flocculation concentration and L1/L2 ratio. 3. Model allows the optimum conditions for nanoparticle formation to be determined.	244 1995
The colloidal properties of surfactant-free nanospheres from biodegradable polyesters investigated.	A range of benzyl esters of poly(β-malic acid) (PMLA-BeH) and PLGA. Differences in colloidal behaviour of systems relating to copolymer composition.	1. PLGA/fully benzylated PMLABe(100) system less stable to electrolyte addition and coagulated near pH corresponding to K_θ of carboxy group. 2. PLGA/partly benzylated PMLABeH systems did not coagulate at relatively high [electrolyte] and low pH of suspending buffer. 3. Difference in the construction of nanospheres involving steric component postulated.	245 1995
The use of polyester nanospheres in the area of ophthalmy for optimizing the bioavailability of drugs depends on their stability during storage in sterile aqueous media, i.e. for 6 months at 25 and 40°C.	P(ε)CL nanospheres were made in sterile, isotonic solutions, with a preservative, and stored using different conditions. Physical stability judged by visual appearance and mean particle size.	1. PH and temperature have most effect on the degradation of the PCL. 2. Temperature of 25°C and pH 7 produce negligible variations in M wt over 6 months. 3. At 40°C and in pH 7 buffer there was 50% loss of initial Mwt over 6 months and 60% in unbuffered solution. 4. Mean particle size of all nanospheres unchanged over 6 months.	246 1996

Aim	Material	Points	Ref.
Mechanistic study of the formulation of polymer nanoparticles by the emulsification–diffusion technique. Results show each emulsion droplet forms several nanospheres and interfacial phenomena during solvent diffusion determine size of colloidal particles.	P(D,L)LA was the model polymer. It was evaluated with different preparation conditions and by turbidity measurements. Results cannot be entirely explained by convection effects caused by interfacial turbulence.	1. Nanoparticle formation thought to be caused only by diffusion. 2. 'Diffusion stranding' mechanism suggested for spontaneous emulsification, with local supersaturation near interface, phase transformation and polymer aggregation producing nanospheres. 3. Turbidity measurements support this interpretation.	247 1997
Physicochemical characterization of lipid nanoparticles, prepared by melt emulsification and evaluation of their drug-loading capacity and sustained release potential. Severe stability problems such as high gelatin tendency, excessive particle growth and drug expulsion occur.	The choice of stabilizer for the colloidal lipid suspension restricted because of lipid's tendency to recrystallize. Drug payload of crystalline particles is generally low, so amorphous, supercooled cooled melts made.	1. Characteristic signal of supercooled melts obtained by high-resolution LH-NMR. 2. Viscous carriers not able to immobilize the incorporated drug as well as a solid matrix, so sustained drug release over days or weeks difficult. 3. No success as yet in combining advantages of both systems.	248 1997
The formulation by emulsification/solvent technique and characterization of biodegradable nanoparticles for intravascular local drug delivery. Different emulsion systems were used and drug loading in particles from 10% to 30% was found. Typical particle size 60–200 nm and 85% in range 70–165 nm.	BSA-loaded PLGA model systems were investigated. Then Pharmacia and Upjohn drugs (U-86983, U-61431, U-74389G) and dexamethasone, which are related to the group's interest in the prevention of post-angioplasty restenosis.	1. Faster *in vitro* release rate of BSA in lower-Mwt PLGA over 7 weeks. 2. Crosslinking on particle surface reduced release rate. 3. Nanoparticle uptake by arterial wall evaluated using *ex vivo* model. 26% of infused particles initially retained. 4. Higher arterial uptake if particles have smaller mean size and lower drug loading. 5. Irradiation to sterilize the drug-loaded particles showed no adverse effect on properties.	249 1997
The long-term stability of cyclosporin-loaded freeze-dried samples of biodegradable polymer nanoparticles stored at 8 and 25°C. The simultaneous effect of technical factors temperature of aqueous phase, needle gauge) and formulation variables (volume of acetone, amount of polymer and surfactant) was measured. Additional experiments looked at effect of freezing temperature (−70 and −196°C) and of 5%, 10% and 15% cryoprotector on 100 nm particles.	Cyclosporin-A (CyA)-loaded PσCL nanoparticles obtained by a modified nanoprecipitation method. The effect of variables on the stability of 100–220 nm particles measured. The cryoprotectors used were mannitol, sorbitol, glucose and threalose.	1. % CyA in the particles stored at 8 and 25°C for at least 3 months was constant and no change in particle size. 2. After 4 months the physical stability was affected. Reconstituted freeze-dried material was observed by light microscopy and showed considerable increase in mean size and size distribution and there was a mean 1% increase in the trapped drug. 3. Addition of >10% glucose/threalose produced adequate reconstitution of the freeze-dried product with conservation of encapsulated CyA.	250 1997

I.4 Summary of research papers on novel uses of nanospheres for sustained drug delivery systems using biodegradable polymers (1994–1997)

Aim	Material	Points	Ref.
The elimination of conventional drug carriers from reticuloendothelial systems within minutes of their intravenous injection prevents their use for site-specific drug delivery or medical imaging This trial uses biodegradable polymeric nanospheres.	Monodisperse, biodegradable, long-circulating polymeric nanospheres were made from amphiphilic copolymers composed of two biocompatible blocks.	1. Particles had dramatically increased blood circulation times and reduced liver accumulation in mice 2. 45% by weight of the drug was trapped in the dense core produced in a one-step process, easily freeze dried and then redispersed without additives in aqueous solutions.	251 1994
Biodegradable nanoparticle DNA carrier which has sites for both polynucleotide adsorption and targeting ligand on the surface. Produced by either a solvent evaporation or a diafiltration method, the particle size for latter method controlled by varying the initial [graft copolymer].	Nanoparticles of P(D,L)LA and poly(L-lysine) (PL) graft polysaccharide copolymers formed. Graft copolymers (with high % polysac.) had minimum particle size of 60 nm but not from PL. Polysac. moieties on particle surface found, by aggregation assay, to interact with a specific lectin.	1. Increase in polysac. content of graft copolymer increased the polynucleotide adsorption capacity. 2. Suggested that adsorption conformation of PL moiety different in homo- and graft copolymer. 3. Graft copolymer nanoparticles are resistant to self-aggregation and non-specific protein serum adsorption. 4. Potential use of graft copolymer as good DNA carrier *in vivo*.	252 1997
Micro- and nanoparticles prepared from biodegradable and biocompatible copolymers successfully used as immunopotentiating antigen delivery systems. The study was used to improve polyclonal antibody production to clenbuterol (CBL), a modern hapten.	PLGA and PMMA nanoparticles were loaded with either CBL only or clenbuterol–transferrin conjugate (CBL–Tfn) and administered subcutaneously to mice. PLGA particles given with or without the saponin adjuvant Quil A. The current methods of raising antibodies to CBL were the positive control.	1. Anti-CBL titres in sera determined by enzyme immunoassay (ELISA). 2. CBL–Tfn-loaded PLGA particles with Quil A had obvious advantages immunologically over the positive control. 3. The combined adjuvanticity of Quil A and the PLGA nanoparticles gave increased positive response and higher antibody titles in all four mice tested (cf. positive control). 4. Sustained immunogen release from nanoparticles, so reduction in immunizing frequency over 15 week study	253 1997

I.5 Summary of research papers on sustained drug delivery profiles using biodegradable polymers (1993–1997)

Aim	Materials	Points	Ref.
The effect of incorporating gelatin, an interactive polymer (below its isoelectric point), on the matrix stability and release of fenthion from crosslinked matrices (with Cu^{2+} ions) of carboxymethyl cellulose. Formulations analysed to monitor physical integrity of matrices and release profile of fenthion in water.	Controlled release formulations of fenthion (10% and 20%) were prepared with sodium carboxymethyl cellulose and gelatin.	1. Matrix stability of up to 39 weeks enhanced to 55 weeks with gelatin incorporation. 2. Variation in average release rate 5·15–11·91 mg $week^{-1}$ 3. Variation in apparent diffusion coefficient $4{\cdot}00 \times 10^{-9}$ and $2{\cdot}01 \times 10^{-8}\ cm^2\ s^{-1}$. 4. Fenthion exhibits a Fickian release pattern, but the mixture containing gelatin shows more non-Fickian release character. 5. Postulated use in mosquito control programmes.	254 1993

Aim	Materials	Points	Ref.
Biodegradable microspheres prepared by either solvent extraction or solvent evaporation of WOW multiple emulsion system. Investigation of physical integrity and antigenicity of the protein treated under different processing conditions. Partial loss of antigenicity associated with lyophilization process and affected by the nature of organic solvent.	Purified tetanus toroid, a high-Mwt M8 protein, entrapped in PLLA and P(D,D)LGA microspheres. All types of particles (independent of Mwt of polymer) displayed high protein-loading efficiencies (>80%), but microparticle size related to Mwt of polymer.	1. Protein release patterns related to polymer type and Mwt. Rate was lower for PLLA than PLGA. 2. Low-Mwt (3000) PLGA produced burst then increased release rate. 3. Entrapped tetanus toroid significance more immunogenic than fluid toroid in mice, but antibody response not significantly different in magnitude and duration from similar dose of aluminium phosphate-adsorbed toroid.	255 1994
An *in vitro* study of release of a dye using spectrofluorophotometry. Cultured bovine RPE cells were treated with the microspheres to study phagocytosis of the particles by the RPE cells using fluorescent and transmission electron microscopy, and post-phagocytosis intracellular release of dye was evaluated. An *in vivo* study in rabbits was also carried out.	Microspheres of PLLA and PGA used to deliver substances to retinal pigment epithelial (RPE) cells directly. Fluorescent dye (rhodamine (6GX) was the drug marker involved.	1. The release rate of dye was controllable by changing the Mwt and monomer composition of the copolymers *in vitro*. 2. Phagocytosis by RPE cells and intracellular dye release occurred during incubation 3. *In vivo* studies showed degradation of microspheres in the RPE cytoplasm, but fragments seen up to 4 weeks. 4. Retinal architecture overlying delivery site well preserved.	256 1995
The biodegradability and antigen release characteristics of polyester blend microspheres using BSA. The kinetics of degradation were derived from the rate of formation of H^+ ions during ester linkage hydrolysis measured by pH changes in microsphere suspensions and the water uptake.	Microspheres were fabricated by blending high-Mwt (61 000) P(D,L)LA and low-Mwt (2000) PLA (PLA2000). The model protein antigen BSA was used.	1. PLA2000 microspheres undergo controlled degradation and produce continuous release profiles of the BSA. 2. Immunization of rabbits by subcutaneous injection of BSA-loaded particles enhanced antigenicity of BSA and increased significantly the duration of humoral immune.	257 1995
The influence of microcapsule formulation on the continuous release of proteins from biodegradable polymer blends. *In vitro* study of biodegradation and protein permeability. *In vivo* study in mice of potential vaccine adjuvant.	PLA/PGA blends were used with model proteins BSA, transferrin and trypsin to make microparticles. Control experiments and blank checks carried out.	1. Microparticles used all had continuous release profiles. 2. *In vivo* study showed continuous release for more than 142 days. Adjuvanticity superior to $Al(OH)_3$, comparable with Freund's incomplete adjuvant. 3. Immunization of animals with BSA-loaded microcapsules more effective than one prime and two booster injections of BSA/saline.	258 1995
Properties and drug release characteristics of PHB/PHBV microcapsules. Some were made incorporating a polyphosphate Ca^{2+} complex into the membrane. The morphology changed with the type of polymer, the drug introduction and the complex incorporation.	Solvent evaporation technique used to encapsulate model drug 2,7-dichlorofluorescein in PHBV or PHB microcapsules.	1. Drug release behaviour, encapsulation efficiency and loading loading influenced by polymer type. 2. DSC showed crystallinity of polymer decreased with HV incorporation. Hence increased segment mobility and increased loading and encapsulation efficiency 3. DSC showed the complex became an integral part of the membrane.	259 1995
Biodegradable PCL has been considered as unsuitable for protein delivery because of its poor permeability to macroparticles. An *in vivo* study was carried out in rats using a single injection of BSA-loaded PCL microspheres.	PεCL microspheres loaded with BSA were studied. Slow rate of degradation of the PCL was monitored and so no increased acidity.	1. The immune response compared in size and time kinetics with three conventional injections. 2. As PCL degrades slowly compared with PLA or PGA, low pH can adversely affect the vaccine antigenicity. Thus PCL has potential as a vaccine carrier.	260 1996

Aim	Materials	Points	Ref.
Microspheres of PHBV were fabricated using single (SET) and double (DET) emulsion techniques with solvent evaporation. >75% yield of microcapsules, % incorporation BSA had no effect on particle size. Size distribution (SET, 6–50 μm; DET, 21–200 μm) for 20% BSA-loaded microspheres obtained.	Spherical microporous reservoir-type PHBV (10·8% HV) (Mwt 330 000)/20% PCL microspheres prepared containing BSA-loaded agarose. BSA loss from (i) partitioning into the continuous aqueous phase, (ii) micropores during precipitation concomitant with solvent evaporation.	1. BSA loss produced loading efficiency <15% with SET and 12% loading efficiency with DET. 2. BSA release could be monitored for up to 24 days for both methods. 3. Total cumulative release of BSA as % of trapped drug showed release only marginally altered by theoretical % loading and was influenced as much by micropore numbers and diameter.	261/262 1996
The effects of drug properties, particle size, drug loading and method of encapsulation were examined. The release rate of the hydrophobic drug (lidocaine) and the hydrophilic drug (propranolol hydrochloride) were examined.	PLA and PLA/PEG microspheres were prepared with drug loading by coarcervation technique using solvent evaporation and emulsion methods	1. PLA/PEG copolymers are more hydrophilic than PLA homopolymers and have lower T_g values. 2. PLA/PEG microparticles rougher than those of PLA. 3. Drug release from PLA/PEG affected by copolymer properties. 4. In PLA/PEG release rate of both hydrophilic and hydrophobic drugs faster than in PLA. 5. The emulsion method produced porous particles with adequate drug permeability.	263 1997
Antibody responses in rabbits tested after single immunization with viral glycoprotein-loaded microspheres or immunity-stimulating complexes (ISCOMs) were studied in long-term (31 week) experiment.	Micropheres of linear copolymers of P(D,L)LGA (50/50) or branched oligoesters of (D,L)LA were compared with ISCOMs. All had incorporated glycoproteins of bovine herpesvirus-1 (BHV-1).	1. Similar antibody response from single injections. 2. Stronger, longer and markedly biphasic antibody responses observed when immunized with BHV-1-loaded branched chain oligo-LA microspheres. 3. Indirect evidence of protein incorporation into both microspheres and ISCOMs.	264 1997
The effect of the PLGA/PEG blend ratio on the release kinetics of the microspheres produced by double emulsion/solvent extraction technique with high efficiency *In vitro* studies showed initial burst effect dependent on PLGA/PEG blend ratios and release rate increased in direct relation to the PEG content for up to 28 days.	Microparticles of PLGA (50/50) and PEG blends with entrapped model drugs (FITC–IgG and FITC–dextran)	1. Linear release profiles and constant release rates were calculated for FITC–IgG-loaded particles with PEG wt% <5% 2. FITC–dextran-loaded particles had a biphasic release profile and rates varied with % PEG. 3. Feasibility of release profile modulation by PLGA/PEG blend ratio shown.	265 1997
Non-invasive *in vivo* monitoring of drug release and polymer erosion from biodegradable polymers by electron paramagnetic resonance (EPR) spectroscopy and NMR imaging (MRI).	PαHAs were made into sandwich-like tablets and implanted. Nitroxide radicals used as model drug-releasing substances containing ^{15}N and ^{14}N.	1. MRI makes it possible to monitor water content, tablet shape and response of the biological system. 2. MRI gives direct proof for *in vivo* mechanisms of polymer erosion.	266 1997
Biodegradable polymeric device for sustained intravitreal release of ganciclovir in pigmented rabbits. *In vivo* release of ganciclovir was evaluated by spectrophotometry and [ganciclovir] measured using HPLC.	A scleral plug made of biodegradable PLGA (Mwt 121 000) with 25% ganciclovir was implanted. Biocompatibility was determined by several techniques.	1. Ganciclovir was released throughout 10 week period. 2. [Ganciclovir] in vitreous maintained in therapeutic range to treat CMV retinitis for 12 weeks. 3. No significant retinal toxicity observed.	267 1997

Aim	Materials	Points	Ref.
Biodegradable microspheres were prepared by spray drying for an *in vitro* evaluation of the release profile of acycloguanosine and an *in vivo* study of humoral drug concentration after intravitreal administration in rabbits.	Polylactide (PL) and polylactide-*co*-glycolide (PLG) (with Mwt 109 000–3000). The process gave good. encapsulation of acycloguanosine.	1. Morphology of the particles was investigated. 2. *In vitro* dissolution varied with Mwt of the polymer. 3. *In vivo* study showed sustained drug release obtained.	268 1997
Microspheres were made using a double emulsion/evaporation method. The particles were characterized for size distribution, drug loading, release kinetics, surface morphology and hydrophobicity. The influence of these factors on the dynamics of immune response following i.m. administration was studied.	P(L)L, P(D,L)L, P(D,L)LG, PHB and PHBV polymers were used and BSA was incorporated in the microspheres.	1. The hydrophobicity of PHB microspheres compared with PL/PLG particles was confirmed and the generation of a significant immune response was delayed using PHAs. 2. The effect of the Mwt of the PHB was also investigated.	269 1997
The characterization and evaluation of *in vitro* and *in vivo* release mechanism of biodegradable microspheres containing an antihypertensive drug (L-158 809). *In vitro* study based on microspheres of blends of high- and low-Mwt polymer. *In vivo* study studies evaluated by (L-158 809) antagonist AT1 function against the shift of the normal dose–response curve of blood pressure induced by angiotensin II.	Microspheres of P(D,L)L blend formulation were made by spray-drying technique. Characterization of microspheres and polymer blends carried out using DSC, SEM, confocal laser microscopy and size analysis.	1. 95% average yield of L-158 809 microspheres (10% w/w). Average microsphere diameter 1–3 μm. 2. A significant 'burst' effect (<15%) was observed in all release experiments. Followed by almost zero-order release kinetics. 3. *In vivo* studies with two different formulations showed a strong shift of angiotensin II dose–response curve. 4. The system is described as a multiparameter controlled release system in which the drug is molecularly dispersed.	270 1997

I.6 Summary of research papers on biodegradation, biocompatibility and cytology studies of biodegradable polymers used for controlled drug delivery

Aim	Material	Points	Ref.
Microparticle/polymer degradation rates of PLG and *in vitro* release of model protein (ovalbumin) were assessed by three methods. 1. Surface morphology using SEM. 2. Weight loss. 3. Molecular mass using GPC.	Four commercially available PLG polymers of known composition and Mwt were used to prepare microspheres. Each method confirmed degradation increased with high glycolide content and low Mwt of PLGs.	1. Rate of release of protein dependent on (i) Mwt of polymer (ii) copolymer composition (iii) protein loading. 2. Generally the rate of polymer degradation and the rate of protein release showed good correlation.	271 1994
The effects of polymer degradation on drug release from the bulk were studied using (i) SEM to look at morphology changes, (ii) transport properties and mechanism (TM).	P(D,L)lactide-*co*-glycolide (50 : 50 PLGA) was used with the model drugs (i) 5-fluoro-uracil (5-FU), (ii) cyclosporin (CyA).	1. Initially non-porous PLGA became more porous with degradation and there were concomitant increases in permeability and transport parameters of drugs. 2. Sharp transitions at Mwt ~5000 and porosity 70–80% were attributed to changes in transport mechanism. 3. Change corresponds to degradation developing pore network, which becomes the dominant pathway. 4. Shift in TM cause of typical trimodal, final, rapid release profiles from bulk degradation polymers.	272 1995

Aim	Material	Points	Ref.
In vitro biodegradation study of BSA-loaded PHBV/PCL microspheres. Incubated in Hank's buffer (pH 7·4) (HB), newborn calf serum (NCS), 1·5% pancreatin (P) and synthetic gastric juices (SGJ) over 30 days and measuring %wt loss, gravimetric changes and surface morphology using SEM.	Spherical BSA-loaded microporous matrix-type microspheres of PHBV (11% HV)/20% PCL blend were made using an O/W emulsion/solvent evaporation technique. Greatest %wt loss was with newborn calf serum and decreased as follows: NCS > P > SGJ > HB. Increased theoretical % loading only increased %wt loss with SGJ and HB.	1. Surface erosion of microspheres. (a) Limited in HB with increase in Pore diameter, coalescence and pits forming. (b) Micropores disappeared in Pancreatin and diameter of Particles decreased. Fracturing of Surface then matrix break-up easier. (c) In SGJ, little surface erosion but flaking and bulk erosion broke up matrix. (d) In NCS, spherical shape kept but diameter reduced. Bulk erosion with macropores extending into spheres which appeared hollow. 2. Exogenous enzyme activity assumed to enhance biodegradation in NCS and P compared with simple ester hydrolysis.	273 1996
PL microspheres biodegradable in days can be used for chemoembolization purposes. Microspheres made by oil-in-water emulsion/evaporation. Final polymer content was compared with initial composition of the oil phase. DSC analysis was used to determine if a monophase blend was formed.	Two samples of P(D,L) lactide (PL) of different Mwt were combined PL (M_n 65 000) for high mechanical strength and PL (M_n 3500) for rapid biodegradability.	1. Varying the weight ratio of two PLs in Microspheres is an efficient way to control *in vitro* degradation kinetics. 2. Morphology changes seen with SEM. 3. Mwt data and increased acidity of incubation medium were used to determine kinetics of degradation and progress of ester group hydrolysis.	274 1996
In vitro release of LHRL agonist from injectable microcapsules of PLGA tested to clarify differences in the release with *in vivo* systems. pH, [salt] and osmolarity of dispersion medium were found to change drug release.	P(D,L)LGA microcapsules produced to optimize conditions for release of leuprorelin an LHRH agonist.	1. Using an *in vitro* test system, that successfully predicted *in vivo* drug release, PLGA weight loss and Mwt decrease delayed. 2. *In vivo* accumulation of PLGA degradation products and ratio of LA : GA in copolymer most effect on PLGA weight loss.	275 1996
Cell response of cultured macrophages, fibroblasts and co-cultures of rat Kupffer cells and rat hepatocytes on short-chain PHB (M_n 2300).	P(R)-3-HB is known to biodegrade. A study of a new block copolymer produced small crystalline, short-chain PHB, ideal for a biocompatibility study.	1. High-[PHB] ($>10\,\mu g\,ml^{-1}$) phagocytosis dose dependent, associated with cell damage in macrophages but not in fibroblasts. 2. Low-[PHB] macrophages not activated either, so biocompatible. 3. No cytotoxicity or changes in albumin secretion shown with co-cultures.	276 1996
In vitro biodegradation study of PHBV/PCL microspheres. Incubated in Hank's buffer (pH 7·4) (HB), newborn calf serum (NCS), 1·5% pancreatin and synthetic gastric juices containing 10% pepsin (SGJ) over 30 days and measuring %wt loss, gravimetric changes and surface morphology using SEM.	A PHBV (11% HV)/20% PCL blend loaded with BSA was made into microspheres using a W/O/W double emulsion/solvent evaporation technique. Greatest %wt loss was with newborn calf serum and decreased as follows: NCS > P > SGJ > HB	1. Only 5%, 10% and 15% BSA-loaded particles in SGJ and HB showed increased %wt loss with BSA loading. 2. Surface erosion of microspheres in each medium. (a) Limited in HB, with increase in pore diameter, coalescence and pits forming. (b) In pancreatin, increases degradation with loss of spherical shape and partial loss of structure to surface and bulk. (c) In SGJ, significant and bulk erosion but no loss of shape. (d) In NCS, surface and bulk erosion, total loss of shape and structure after 30 days.	277 1997

I.7 Summary of current research into other biomedical uses for biodegradable polymers

Aim	Material	Points	Ref.
The effect of wetting on P(L)LA and P(DL)LGA foams for tissue culture. Two-step immersion in ethanol for 1 h and then water was used to wet discs.	Porous discs of the polymer foams of P(L)LA and P(DL)LGA (85/15 and 50/50).	1. Prewetting with EtOH increased volume void filled after 48 h from 23% to 79% for PLLA and from 59% to 97% for PLGA (85/15) discs. 2. Water entry even after 1 h was close to plateau value for all prewet polymers tested. 3. Use in uniformly seeding 3D biodegradable polymer substrates for cell and tissue culture realized.	278 1994
Preparation and characterization of P(L)LA foams. Particulate-leaching method used to prepare very porous biodegradable polymer membranes using (i) casting of polymer/salt membrane (ii) dissolution of the salt.	1. Preparation of P(L)LA membranes of controlled porosity, surface/volume ratio and crystallinity. 2. Sieved particles of NaCl, Na tartrate and Na citrate salts were used.	1. Salt weight fractions (wt%). (a) 50–60 asymmetric membranes–particle size had no effect. (b) 70–90 homogeneous membranes—pores interconnected. Membrane properties independent of salt used but dependent on particle size. 2. Porosity increased with salt wt% 3. Median pore diameter increased as size of salt particle increased. 4. Polymer/salt composite membrane quenched or annealed to adapt crystallinity. 5. Foams 99·9% salt-free and with porosities up 0·93 obtained.	279 1994
Fabrication of pliable, porous, biodegradable foams to engineer soft tissue. The effects of four different processing parameters on pliability and pore morphology of biodegradable scaffolds.	PLGA/PEG blends were made by solvent-casting/particulate-leaching technique. Parameters: PLGA copolymer ratio, PLGA/PEG blend ratio, initial salt weight fraction, salt particle size.	1. A wide range of shear moduli, porosities and median pore diameters were obtained. 2. Initial salt weight fraction and blend ratio have most significant effect on physical/mechanical scaffold properties. 3. Enhanced pliability of 3D foams of PLGA/PEG shown by tube formation. 4. Potential uses: regeneration of soft tissue (skin), intestine/vascular grafts.	280 1996
Synthesis of polymer network scaffolds from L-lactide and PEG and their interaction with cells. Scaffolds with cell adhesion resistance, ligand immobilization and biodegradation character for use in tissue engineering. Non-toxic macromolecules used.	UV photopolymerization of triacrylated lactic acid oligomer from glycerol base (GL) plus monacrylated PEG GL–PEG cross-linked polymer networks obtained.	1. Glassy and transparent networks. 2. Gel content approximately 90%. DP of lactide and amount and Mwt of PEG had no effect. 3. All networks showed relatively low swelling in water because of crosslinking. 4. No melt endotherms but T_g indicated PEG phase mixing. 5. PEG shown in surface and if high-Mwt PEG incorporated more. More hydrophilicity in surface/less cell adhesion.	281 1997
In vivo biocompatibility study in rats of a biodegradable matrix used as a cell-seeded skin substitute for large-scale skin defects and skin substitute. N.B. Previous study on cell substrate properties and physicochemical character of matrix. Implant for large body surface area and study biocompatibility at 2, 4, 13, 26, 52 weeks.	Synthetic polymer matrix with dense top layer and porous underlayer of P-ether/P-ester (PEO : PBT) copolymer called Polyactive™ and also PLLA were investigated.	1. Early re-inflammation = surgical implantation trauma. 2. Neovascular and fibrous tissue ingrowth in porous underlayer within 2–4 weeks for all matrices. 3. Copolymer and PLLA show increased fragmentation and liquifaction. 4. After 1 year small polymer fragments retrieved from site. 5. No systematic effects on animal organs, so has potential for use as skin substitute.	282 1994

Aim	Material	Points	Ref.
Design and physicochemical properties of new biodegradable temporary wound dressings, used in the treatment of burns, which meet the objectives of skin substitute in application, safety and comfort.	Biodegradable PLA and PCA polymers produced from research programme BMFT/FRG, 01KG8809/7. Films were made of the copolymer and characterized and tested for degradation.	1. Opacity increased during hydrolysis. 2. Variation in water vapour permeance with method of measurement. 3. Mechanical properties characterized; maximum elongation (37°C) >2000% and very low elasticity modulus. 4. Aptitude for wound dressing established.	283 1995
Development of polymeric surgical paste formulation for local delivery of taxol (an inhibitor for tumour growth). *In vitro* release of taxol was carried out using HPLC assay for taxol. Antiangiogenic activity assessed using a chick CAM.	P-ε-CL or blends of P-ε-CL with methoxypoly(ethylene glycol) (MePEG) of Mwt 350 were incorporated with taxol and characterized. *In vitro* conditions: 37°C in phosphate-buffered saline at pH 7·4.	1. 30% MePEG in PCL: decreased T_m by 5°C, decreased tensile strength from 153 to 27 N cm^{-2}. increased PCL crystallinity from 42% to 51%. 2. Release profile of taxol from PCL unchanged. 3. Uptake of water increased with added MePEG but taxol release rate decreased. 4. CAM assay showed antiangiogenic activity.	284 1996
In vitro and *in vivo* study of biodegradable polymeric paste formulations for local delivery of taxol. Release of taxol into PBS albumin buffer was measured by HPLC.	PD, LLA–PEG-PD, LLA and blends of low-Mwt PD, LLA and PCL melted and mixed with taxol to produce pastes. Characterization of polymers and pastes carried out.	1. Sustained release of taxol from copolymer paste over 2 months by diffusion and erosion. 2. The blend released taxol mainly by erosion and there was burst followed by slow release. 3. Both pastes significantly reduced tumour growth in mice after 16 days. 4. Pastes with faster *in vitro* release rates more effective inhibitors of tumour growth.	285 1996

APPENDIX II PATENTS

A basic search was carried out using the World Patent Index (WPINDEX), Derwent Information Ltd, on the STM network for relevant patents on biodegradable polymers. The following appendices are based on applications, e.g. packaging, pharmaceutical and agricultural uses, and also developments in microbial and chemical synthesis, processing and blending.

II.1 Packaging, hygiene and non-pharmaceutical applications

Year	Reference	Title	Author(s)/organization(s)
1990	90-317488	Biodegradable film used as wrapping material for foods—obtained by applying aqueous emulsion of poly(hydroxybutyric/valeric acid) copolymer on glass plate and heating.	*Chuko Kasei Kogyo KK*
1991	91-261627	Biodegradable mouldable material for disposal by burying—comprises natural high-Mwt substances, e.g. starch thermoplastic resin and optional filler.	Koyama, M., Suzuki, M. & Tokiwa, Y. *Agency of Ind. Sc. & Tech. Chuo; Kagaku Ltd*
1991	91-354911	Non-woven fabric composite for hygiene articles, etc.—by extruding layer of molten polymer onto spun-bonded web or staple fibre web under reduced pressure.	Sturm, V. & Utz, K. *AOE Plastic GMBH; BP Chem. Plastec. GMBH*

Year	Reference	Title	Author(s)/organization(s)
1992	92-105979	Decoration, e.g. wreath, or packaging for short-term use—has ribbon made of biodegradable plastic for easy disposal.	Mans, L. H. A. *Mans, L. H. A. Holdings*
1992	92-222619	Novel microbiologically degradable plastic moulding for afforestation—comprises biodegradable aliphatic polyester and calcium and/or magnesium carbonate, for disposable outdoor goods including cutlery.	*Agency of Ind. Sc. & Tech. Chuo*; *Chem. Ind. Co. Ltd.*
1992	92-309206	Non-woven material for disposable nappies, bed underlays, etc.—comprises thermoplastic spun endless filaments, consists of at least 50 wt% biologically degradable poly(caprolactone) of Mwt 35 000–70 000.	Emirze, A., Eschwey, H., Giesen-Wiese, M., Grill, M., Kauschke, M., Klein, B. & Seidler, H. *Freudenberg FA Carl*
1993	93-036358	Biodegradable, liquid-impervious films used as back sheets for nappies, etc.—comprises blend of interpenetrated network of destructured starch and copolymer, preferably vinyl alcohol co polymer, and an aliphatic polyester.	Toms, D. & Wnuk, A. J. *Procter & Gamble Co.*
1993	93-059674	Paper economy with paper handkerchiefs—involves handkerchiefs stored in container that can always be refilled.	Weigele, R. *Weigele, R.*
1993	93-120869	Separable laminates used as wrapping materials for foods, etc.—comprise biodegradable plastic layer laminated between layers.	*Toppan Printing Co. Ltd*
1993	93-182613	Structure having controlled water resistance—comprises a core material having dry but not wet strength and a coating of a water-resistant biodegradable polymer.	Brunger, P. & Kemmish, D. J. *Zeneca Ltd; Monsanto Co.*
1993	93-345009	Liquid-impervious biodegradable films—comprising interpenetrated network of destructured starch with ethylene–acrylic acid or –vinyl alcohol copolymer, with aliphatic polyester.	Adams, J. M., Chang, P. I., McBride, R. K. & Ray, C. D. *Tredegar Ind. Inc.*
1994	94-000953	Biodegradable carrier for denitrifying bacteria in water purification—made of spun fleece of poly(caprolactone) filaments, optionally blended with other biodegradable polymer.	Groten, R., Heidecke, G., Mansbart, T. & Siekermann, V. *Freudenberg FA Carl*
1994	94-018030	Heat-moulding composition—contains starch component, biodegradable polyester and salt of hydroxycarboxylic acid, giving biodecomposable water-resistant articles.	Fleche, G., Gosset, S. & Videau, D. *Roquette Freres SA*
1994	94-114861	Biodegradable paper container—comprises paper laminate with biodegradable plastic layer	*Toppan Printing Co. Ltd*
1994	94-201936	Biodegradable packaging material especially for liquid or solid foodstuffs—having improved oxygen, aroma and/or water vapour blocking properties.	Kammerstetter, H. & Schroeter, J. *Buck Werke GMBH & Co.*
1994	94-249165	Poly(hydroxyalkanoate) film product—by melt extrusion onto chilled roller to form film, followed by heating thereof to crystallize the polymer.	Waddington, S. D. *Zeneca Ltd; Monsanto Co.*
1994	94-338741	Biodegradable fibre preparation used for agricultural and fishery material, etc.—by melt spinning a mixture of poly(beta-hydroxyalkanoate)	*Zeneca Ltd*
1994	94-338742	Biodegradable multifilament used for fishery material—composed of multifilament comprising poly(beta-hydroxyalkanoate), having good heat resistance.	*Zeneca KK*

Year	Reference	Title	Author(s)/organization(s)
1995	95-067312	Bonding articles together using poly(hydroxyalkanoate)s—which are biodegradable, useful in packaging, carton sealing, sanitary towels, disposable nappies, hospital equipment, etc.	Kemmish, D. J. *Zeneca Ltd; Monsanto Co*.
1995	95-092171	Laminate manufactured by vacuum deposition of functional layer between two films—using non-metallic transparent material acting as a barrier and bonding layer, eliminating need for laminating adhesive and allowing recycling.	Utz, H. *Fraunhofer Ges Foerderung Angewandten*
1995	95-117776	Toilet material for pets, giving good biodegradability—including deodorant and/or antibacterial agent, biodegradable resin and optional aromatic material.	*Suzuki Sogyo KK*
1995	95-215202	Manufacturing biodegradable cellulose and/or cellulose acetate film having good water barrier properties—comprises applying aqueous suspension of at least partly amorphous particles of poly(hydroxyalkanoate) to cellulose and/or cellulose acetate film and heating.	Kemmish, J. D., Montador, J. H. & Kemmish, D. J. *Zeneca Ltd; Monsanto Co*.
1995	95-162679	Increasing transparency of moulded articles or sheets of thermoplastic starch or starch–polymer blend—by treating granulate, articles or sheets with silicone oil, animal or plant fat or oils or solution of synthetic or natural polymer.	Bueler, F. S., Fanelli, R., Treutlein, R. & Zechner, T. *EMS Inventa AG*

II.2 Pharmaceutical and agricultural patents

Year	Reference	Title	Authors(s)/organization(s)
1979	79-15983B	Microcapsule and microspherule preparation for medicaments, etc.—by addition of phase separation agent to solution of polymer with solution or dispersion of active compound at −100 to −40°C.	*Sandoz SA*
1979	79-68042B	Microsphere production from particle dispersion in polymer solution—by adding phase separation agent at low temperature.	Fong, F. W. *Sandoz Inc*.
1986	86-049030	Microcapsules for controlled release of regulatory peptide(s)—containing poly(D-3-hydroxy butyric) acid as biodegradable carrier.	Sandow, J. & Seidel, H. R. *Hoechst AG*
1984	84-301706	Slow release composition for treating aquatic plants—containing herbicide, etc. in strands of polymeric, e.g. poly(lactic acid), fibre.	Dunn, R. L. & Lewis, D. H. *Stolle Res. & Dev*.
1986	86-226722	New composite materials useful as hard tissue prosthetics—comprise synthetic biodegradable polymers and unsintered calcium phosphate biomaterials, with optional pore-forming agent, and polymerized *in situ*.	Dorman, L. C. & Meyers, P. A. *Dow Chem. Co*.
1986	86-306804	Biodegradable polymer with low free acid content—and used in drug microencapsulation is e.g. hydroxy-acid ester or poly(cyano-acrylic-ester).	Miyaggawa, T., Ogawa, Y., Okada, H. & Yamamoto, M. *Takeda Chem. Ind. Ltd; Wako Pure Chem. Ind. Ltd*
1989	89-066688 89-150631	Polymer-encapsulated microsphere preparation—by adding core material to polymer solution in non-solvent for core, adding synthetic or vegetable oil and quenching with second non-solvent.	Lewis, D. & Sherman, J. D. *Stolle Res. Dev. Corp*.

Year	Reference	Title	Authors(s)/organization(s)
1989	89-146427	Microcapsule production containing soluble protein or peptide—using mixture of poly(hydroxybutyric acid) and poly(lactide-*co*-glycolide).	Sandow, J. K. & Schmeidel, R. *Hoechst AG*
1991	91-324928	Biocompatible microspheres for parenteral administration—containing biodegradable and biocompatible polymer and surfactant, used for treating inflammation, infections and cancer.	Spenlehauer, G., Veillard, M. & Verrechia, T. *Rhone Poulenc Rorer SA*
1992	92-060919	Auxiliary material for fixing artificial joint or filling bone defects—comprises biodegradable or bio-absorbable material and leaves structure to allow formation of fresh bone.	*Terumo Corp*.
1992	92-176586	Controlled release microparticles containing polysaccharide gelling agent, etc.—comprising biodegradable polymer, interfacial agent, amphiphilic polymer and active substance, especially calcitonin, etc.	Canal, T., Carli, F., Lovrecich, M. & Lourecich, M. L. *Vectorpharma Int. SPA*
1992	92-391146	Synthetic bone–tooth filler for artificial limb and denture material—comprises water-insoluble, biodegradable coating applied to substrate with porous surface layer.	Fuse, T., Machino, H., Matuura, K., Niijima, K., Otani, S. & Yanagisawa, S. *Mitsubishi Kasei Corp.; Res. Dev. Corp. Japan*
1993	93-153559	Dispenser, especially for controlled release of sexual pheromone(s)—used in plant protection, comprises pheromone-impermeable container sealed with pheromone-permeable foil.	Buschmann, E., Kiessling, U., Neumann, U. & Renz, G. *BASF AG*
1993	93-164265	Slow release fertiliser—has multiple alternate coatings of a biodegradable polymer (I) and a water-soluble polymer (II).	*Nippon Steel Chem. Co.; Nippon Steel Corp*.
1994	94-135531	Particles of crystallizable polymer coated with surfactant of phospholipid to maintain amorphous state—for making shaped articles, e.g. fibres, for controlled release of pharmaceutical or agrochemical or for removal of solvent, etc.	Clauss, J., George, N., Horowitz, D. M., Hunter, B. K. & Sanders, J. K. M. *Zeneca Ltd; Monsanto Co*.
1994	94-183125	Sustained release microspheres requiring no surgical implant—contain hydrophobic antipsychotic encapsulated in biodegradable polymer, allowing prolonged therapeutic effect by infrequent administration.	Kino, S., Mizuta, H. & Osajima, T. *Yoshimoto Pharm. Ind. KK*
1994	94-193872	Preparation of drug-releasing biodegradable compositions used for antibiotics, etc.—by ultrasonically melting biodegradable polymer matrix and pharmaceutical substance.	Miettinen-Laehde, S. S. & Toermaelae, P. O. *Orion-Yhtymae OY*
1995	95-041205	Sustained release preparation of water-soluble peptide hormone—comprising fine tube with specified diameter made of water-insoluble biodegradable, high-Mwt substance, containing core.	*Kirin Brewery KK*
1995	95-044929	Biodegradable urethral stent—substantially shorter than the urethra in which it is totally installed.	Viherksoski, E.
1995	95-351303	Polymer microspheres useful for drug delivery and targeting, etc.—prepared by mixing solution of water-soluble polymer and solution of conjugate of poly(ethylene glycol) and evaporating first solvent.	Coombes, A. G., Davis, S. S. & Schacht, E. *Univ. Ghent; Univ. Nottingham*
1995	95-122881	Absorbable polymer composition for surgical and medical devices, e.g. sutures—comprises absorbable poly(ester anhydride), polylactone or poly(iminocarbonate) and polysuccinimide as bio-absorbable reinforcing filler.	Arnold, S. C., Reilly, E. P. & Scopelianos, A. G. *Ethicon Inc.; Johnson & Johnson*

Year	Reference	Title	Authors(s)/organization(s)
1996	96-040269	Degradable multilayer melt-blown microfibre webs used for surgical dressings, etc.—comprise layers of polyolefin, PCL and degradable resins.	Joseph, E. G. & Rutherford, D. R. *Minnesota Mining & MFG Co*.
1996	96-201507	Long-term fertiliser rods—consist of a shell made of segments of polymer material which biodegrade at different rates and are filled with plant nutrients.	Pluder, K. *Buna GMBH*
1996	96-267854	Surgical device, e.g. staple or ligating clip—comprises absorbable matrix made of e.g. polyamide and discrete filler material comprising polysuccinimide to increase stiffness of polymer, and device can withstand heavy loads.	Arnold, S. C., Reilly, E. P. & Scopelianos, A. G. *Ethicon Inc*.
1996	96-350294	Biodegradable resin composites used as containers for medical products—comprise poly(3-hydroxybutyric acid) and poly(caprolactone) with specified capillary size.	*Mitsubishi Gas Chem. Co. Ltd*
1997	97-044600	Expandable intra-luminal stent—made from sheet materials curled into cylinder having over lapping edges, and incorporating external protrusions which engage apertures to hold the stent in the expanded condition.	Williams, M. S. *Advanced Cardiovascular Systems*
1997	97-459058	Membrane for tissue or bone regeneration—contains at least three layers and is flexible and biocompatible.	Hutmacher, D. & Kirsch, A. Kirsch, A.

II.3 Microbial and chemical methods, synthesis and degradation patents

Year	Reference	Title	Author(s)/organization(s)
1991	91-051341	Construction and modification of polyester bio polymers—by introduction of poly(hydroxy butyrate/alkanoate) genes into bacteria or plants.	Peoples, O. P., Sinskey, A. J. & Sinskey, A. L. *Massachusetts Inst. Technology*
1991	91-305113	Purification of biodegradable polyester(s)—by re precipitation from organic solvent with water to remove low-Mwt contaminants.	Heya, T., Ogawa, Y. & Yamada, M. *Takeda Chem. Ind. Ltd*
1992	92-224054	Block copolyester useful for biodegradable transparent film—prepared by reacting poly(3-hydroxybutyric acid) with 6-hexanolide in presence of acid catalyst for high purity.	*Nippon Kayaku KK*
1992	92-346704	Poly(hydroxybutyrate) production—by two-stage culturing of *Alcaligenes eutrophus* strain.	Kim, K. & Lim, K. *Korea Synthetic Textile Co*.
1992	92-398873	Transgenic plants for poly(hydroxyalkanoate) production—containing genes encoding enzymes catalysing poly(hydroxyalkanoate) production.	Bright, S. W. J., Byrom, D. & Fentem, P. A. *Zeneca Ltd; ICI plc*
1993	93-058785	Transgenic plants producing poly(hydroxyalkanoate) polymer(s)—obtained by transformation with DNA encoding 3-ketothiolase, acetoacetyl-coA reductase and PHE synthase.	Dennis, D. E., Poirier, Y. & Somerville, C. R. *Univ. Michigan State; Univ. Madison James*
1993	93-058791	Production of poly(hydroxyalkanoate) polymer(s)—by culturing *Chromatium vinosum* of transformants containing *C. vinosum* PHA biosynthetic genes.	Liebergesell M. & Steinbuchel, A. *Zeneca Ltd*

Year	Reference	Title	Author(s)/organization(s)
1993	93-404052	New hydroxyalkanoate polymer derivatives—with terminal alkenyl or alkyl group, and production of partially degraded hydroxy alkanoate polymer derivatives.	Yalpini, M.
1994	94-012388	Biodegradable block copolymer for packaging, agriculture or medical use—obtained by ring-opening polymerization of lactone(s) in organic solvents, giving good miscibility to poly(hydroxybutyrate) and poly(caprolactone).	Doi, Y. *Sumitomo Metal Ind. Ltd*
1994	94-199818	New fibre-producing plants expressing hetero logous bioplastic, especially poly(hydroxyalkanoate)—contain enzymes involved in bioplastic synthesis under control of fibre-specific cotton promoter.	Maliyakal, J. & John, M. *Monsanto Co*.
1994	94-265917	Poly(3-hydroxybutyric acid) production by ring-opening polymerization—of beta-butyrolactone in presence of tin compound catalyst.	Hori, Y., Nishishita, T. & Yamaguchi, A. *Tagasago Int. Corp.;* *Takasago Perfumery Co. Ltd*
1994	94-542560	Polyester composition.	Hammond, T., Liggat, J. J., Montador, J. H. & Webb, A. *Zeneca Ltd*
1995	95-012035	Biodegradable resin composition preparation for films or sheets—by blending biodegradable plastic with water-soluble thermoplastic resin.	*Sumitomo Seika Chem. Co. Ltd*
1995	95-075207	New block copolymers with hydrophilic and hydrophobic blocks—covalently bonded to multifunctional compound, used to make particles for controlled and optimal targeted delivery of therapeutic or diagnostic agents.	Domb, A. J., Gref, R., Langer, R., Minamitake, Y. & Peracchia, M. T. *Massachusetts Inst. Technology*
1995	95-162982	Genetically modified micro-organism producing poly(hydroxyalkanoate)—includes foreign gene for hexosyl transferase to allow use of in expensive sugar substrates, also producing specific polysaccharide.	Buttcher, V., Kossman, J. & Wesh, T. *Inst. Genbioligische Forschung* *Hoechst-Schering*
1995	95-311553	Production of biodegradable, e.g. poly(hydroxy butyrate), fibrils for non-wovens—by contacting melted or solvated liquid resin mixture with gas flow.	Lampe, R. A., Noda, I. & Satkowski, M. M. *Proctor and Gamble Co*.
1995	95-367684	Degradation of waste polymers, especially poly (hydroxy fatty acid)s—by incubating in aqueous medium in presence of micro-organisms; especially isloate SK37, and/or heating in aqueous alkaline medium.	Batz, H., Jendrossek, D., Sluka, P. & Steinbuechel, A. *Boehringer Mannheim GMBH*
1996	96-160943	Poly(hydroxy acid) production using recombinant bacteria—especially *Pseudomonas* or *Alcaligenes spp*, containing *Thiocapsa* poly(hydroxy acid) synthesase gene.	Liebergesell, M., Pries, A. Steinbuchel, A. & Valentin, H. *Monsanto Co*.
1996	96-486372	Thermoplastic biodegradable ether ester direct production from cellulose—uses quaternary ammonium base in etherification with oxiran before esterification with monocarboxylic acid, useful for moulding, film or fibre.	Engelhardt, J., Koch, W., Kramer, H., Meister, F. & Michels, C. *Wolff Walsrode AG*
1997	97-120248	Thermoplastic biodegradable poly(saccharide ether ester) for fibres—prepared by esterifi cation of the polysaccharide with anhydride and reaction of free carboxyl groups with an epoxide, for heat-stable products.	Engelhardt, J., Fink, H., Kalbe, J., Koch, R., Koch, W., Mueller, H. P., Szablikowski, K., Weber, G., Weigel, P. & Mueller, H. *Wolff Walsrode AG*

Year	*Reference*	*Title*	*Author(s)*/organization(s)
1997	97-226171	Surface-modified cellulose micro-fibrils for filler in composite—have hydroxyl functions on surface esterified by at least one organic compound, containing at least one function capable of reacting with hydroxyl groups.	Cavaille, J. Y. N., Chanzy, H. D., Fleury, E. & Sassi, J. F. *Rhone Poulenc Rhodia AG*

II.4 Blending, composition and plasticizer patents

Year	Reference	Title	Author(s)/organization(s)
1991	91-001725	Destructurized starch and hydrophobic, thermo plastic polymer blends—have improved dimensional stability, humidity resistance and toughness, are biodegradable and used in capsules, foams, films, etc.	Lentz, D. J., Sachetto, J. & Silbiger, J. *Warner Lambert Co*.
1992	92-024382	Hydroxyalkanoate polymer composition—contains ammonium chloride as nucleating agent, avoiding problems with opacity and improving biodegradability.	Barham, P. J., Organ, S. J. & Webb, A. *Zeneca Ltd; Monsanto Co*.
1992	92-064909	Biodegradable plastic composition—containing microbiologically produced copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate.	Carlson, A., Galvin, T. J. & Webb, A. *Zeneca Ltd; Monsanto Co*.
1993	93-093341	Biodegradable blend of microbiologically produced poly(hydroxybutyric acid)—with chemically synthesized random copolymer of hydroxybutyric acid optical isomers, for production of flexible film, sheet, etc.	Doi, Y. & Kumagai, Y. *Sumitomo Metal Ind. Ltd*
1993	93-200640	Biodegradable polymer composition—contains PVA and poly(3-hydroxybutyric acid).	*Denki Kagaku Kogyo KK*
1993	93-288377	Compatible for polymer blends—comprises A–B block copolymer where A is at least 50% alkylene side chains and B is at least 70% hydroxybutyric acid residue side chains.	Ballard, D. G. H & Buckmann, A. J. P. *Zeneca Ltd; Monsanto Co*.
1994	94-083141	Plasticizer for biodegradable, highly crystalline polyester(s)—comprising an esterified hydroxy carboxylic acid with multiple ester groups in the molecule.	Montador, J. H. & Webb, A. *Zeneca Ltd; Monsanto Co*.
1994	94-183458	Polymer composition—containing an oligomer of a polymer selected from poly(hydroxyalkanoate), polylactide, poly(caprolactone) and copolymers.	Bal, J. S. & Hammond, T. *Zeneca Ltd; Monsanto Co*.
1994	121:135566	Polyesters, e.g. PLA or 3-hydroxybutyric acid/3-valeric acid copolymer, are plasticized with an ester such as Ac tri-Bu citrate.	Montador, J. H. & Webb, A., *Zeneca Ltd; Monsanto Co*.
1995	95-022746	Polyester composition with improved impact strength and elongation—comprises a biodegradable polyester and a specific plasticizer.	Hammond, T., Liggatt, J. J., Montador, J. H. & Webb, A. *Zeneca Ltd; Monsanto Co*.
1995	95-022752	Polyester composition comprising two poly(hydroxy alkanoate) compounds—including one in (semi)crystalline form, as a nucleant, for paper, fabric, hygiene articles, sustained drug or agrochemical release system, adhesive, etc.	Liggatt, J. J. *Zeneca Ltd; Monsanto Co*.
1995	95-024214	Resin composition used to produce porous biodeg radable film—comprising resin mixture of copolymer of D-hydroxybutyrate and D-hydroxy valerate, poly(epsilon-caprolactone) and inorganic filler.	Hisanaka, T., Kuwaki, T., Matsumura, H., Muta, Y., Ochi, Y. & Shimada, T. *Zeneca Ltd*

Year	Reference	Title	Author(s)/organization(s)
1995	95-027727	Starch composition for biodegradable sheet—includes biodegradable aliphatic polyester, low-Mwt aliphatic polyester and reinforcing additives.	*Tsutsunaka Plastic Ind. Co. Ltd*
1995	95-076327	Biodegradable thermoplastic mixture of starch ester and poly(alkylene glycol)—using low-Mwt polyether(s) and polyester(s) derived from amylopectin starch.	Kakuschke, R., Rapthel, I., Reichwald, K., Hermann, C. & Runge, J. *Buna GMBH*
1996	96-343504	Biodegradable composition used as plasticizing or compatibilizing compound—containing poly(3-hydroxybutyric acid) or copolymer and block copolymer having a stereospecifically regular structure.	Hagiwara, T., Hongo, H., Hori, Y., Takahashi, Y. & Yamaguchi, A. *Takasago Int. Corp.; Takasago Perfumery Co. Ltd*
1997	97-414566 97-70811	Biodegradable emulsion binder for non-woven fibre stock—containing poly(hydroxybutyrate/hydroxyvalerate) copolymer, imparts good mechanical properties and is readily biodegradable.	Iovine, C. P. & Walton, J. H. *Nat. Starch & Chem. Investment Holding Corp*.

APPENDIX III LIST OF ABBREVIATIONS

The abbreviations of individual monomers, polymers and copolymers are based on those used by the original authors and are identified within the text. However, some more generally used abbreviations are as follows.

III.1 Chemical terms

Prefixes: R- = alkyl-, Me- = methyl-, Et- = ethyl-, Bu- = butyl-, Pr- = propyl-, Ac- = acetyl-

dimethyl tin oxide	DMTO
diethyl tin oxide	DETO
dibutyl tin oxide	DBTO
dioctyl tin oxide	DOTO
di-*n*-butylphthalate	DBP
propylene glycol	PG
ethylene acrylic acid	EAA
triethyl citrate	TEC
glycolic acid	GA
L(+) or S(+) lactic acid	LLA
D(−) or R(−) lactic acid	DLA
3(or β)-butyrolactone	3(or β)BL
3(or β)-hydroxybutyric acid	3-HBA
R-3-hydroxybutyric acid	R3HBA
4(or γ)-hydroxybutyric acid	4-HBA
3-hydroxyvaleric acid	3-HVA
methyl methacrylate	MMA
hydroxyethyl methacrylate	HEMA
R-3(or β)-butyrolactone	R3(or β)BL
RS-3(or β)-butyrolactone	RS3BL

III.2 Polymers and copolymers

poly(α- or 2-hydroxy acid)	PαHA
poly(glycolic acid)	PGA
poly(lactic acid)	PLA
poly(L-lactic acid)	PLLA
poly(D-lactic acid)	PDLA
poly(D,L- or R,S-lactic acid)	P(D,L or R,S)LA
polylactide	PL
poly(ε-caprolactone)	PεCL
poly(valerolactone)	PVL
poly(β-D,L-butyrolactone)	P(β(D,L)BL)
poly(hydroxyalkanoate)	PHA
poly(3- or β-hydroxyalkanoate)	P(3 or β)HA
poly(3-hydroxybutyrate)	P3HB
atactic poly(3-hydroxybutyrate)	a-PHB
poly(R-3-hydroxybutyrate)	PR3HB
poly(RS)-hydroxybutyrate	P(RS)HB
poly(3-hydroxyvalerate)	P3HV
poly(4-hydroxybutyrate)	P4HB
poly(6-hydroxyhexanoate)	PHH
polyethylene	PE
low-density polyethylene	LDPE
high-density polyethylene	HDPE
polystyrene	PS
poly(vinyl alcohol)	PVA
poly(ethylene oxide)	PEO
poly(vinyl acetate)	PVAc
polypropylene	PP
poly(ethylene terephthalate)	PET
poly(vinyl chloride)	PVC
poly(methyl methacrylate)	PMMA
poly(ethylene glycol)	PEG
poly(sebacic anhydride)	PSA
poly(*p*-dioxanone)	PDS
poly(carboxyphenoxyhexane)	PCPH
poly(1,4-ethylene adipate)	PEA
poly(cyclohexyl methacrylate)	PCHMA
poly(epichlorohydrin)	PEC
poly(carboxyphenoxyvaleric acid)	PCPV
poly(glycolic acid-*co*-lactic acid)	PGLA
poly(lactic acid-*co*-glycolic acid)	PLGA
poly(D,L-lactic acid-*co*-glycolic acid)	P(D,L)LGA
poly(3-hydroxybutyrate-*co*-3-valerate)	PHBV
poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)	P3HB-*co*-4HB
poly(R-3-hydroxybutyrate-*co*-ε-caprolactone)	P(R)(3HB)–PεCL
poly(R-3-hydroxybutyrate-*co*-L-lactone)	P(R)(3HB)–L-PL
poly(ε-caprolactone-*co*-lactone)	P(εCL-*co*-LA)
poly((ε-caprolactone-*co*-glycolide)	P(εCL-*co*-G)
poly(lactic acid-*co*-ethylene glycol)	P(LA-*co*-PEG)
poly(LGA-*co*-ethylene glycol)	P(LGA-*co*-PEG)
urea formaldehyde resin	UF

III.3 Starch/cellulose connected terms

starch	St
starch octanoate	OCST
starch dodecanoate	DODST
cellulose ester	CE
cellulose acetate	CA
cellulose acetate butyrate	CAB
cellulose tributyrate	CTB
cellulose acetate propionate	CAP
degree of substitution	DS
methyl cellulose	MC
ethyl cellulose	EtC
bacterial cellulose	BC
carboxymethyl cellulose	CMC

water-soluble polymer	WSP

III.4 Other terms

municipal solid waste	MSW
total organic carbon	TOC
dissolved organic carbon	DOC
theoretical oxygen demand	TOD
semi-continuous activated sludge	SCAS
continuous-flow activated sludge	CAS

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