Biosynthetic Processes for Linear Polymers

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ABSTRACT: Biosynthetic processes for production of linear proteins, polysaccharides, and polyhydroxyalkanoates (PHAs) are discussed. Processes for converting the linear biopolymers into useful products are described. Techniques and additives for improving the processability of PHAs and potential applications of PHAs are reviewed. © 2002 John Wiley & Sons, Inc. J Appl Polym Sci 83: 457–483, 2002

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INTRODUCTION

Linear polymers formed by biosynthetic processes include proteins (polyamides), polysaccharides (polyglycosides), DNA, RNA, and polyhydroxyalkanoates (PHAs) (polyesters). These biopolymers are formed in plants, animals, and microorganisms where they serve as structural components, genetic machinery, energy storage materials, etc., and seem to be essential for all forms of life. Many of these materials are also used in commercial applications. The proteins and polysaccharides are reviewed briefly followed by a more complete review of PHAs.

PROTEINS (POLYAMIDES)

Living cells produce linear protein polymers by adding amino acid monomers one at a time to a lengthening chain. There are only 20 natural amino acids used in making these proteins. The genetic information for protein synthesis is stored in the DNA of the cell and the order of addition is directed by RNA, serving as a template.[1] DNA and RNA themselves are linear polyesters derived from sugars (D-ribose or D-2-deoxyribose), phosphoric acid, and a heterocyclic base (i.e., adenine, uracil, cytosine, guanine, thymine, 5-methylcytosine) attached to the sugar moiety.[2] The biological genetic system synthesizes protein chains with absolute control over molecular weight, composition, sequence, and stereochemistry. This control produces fibrous proteins with extraordinary mechanical properties, such as the strength and toughness of silk, a linear polyamide, and the elasticity and resilience of elastin and collagen, which are branched polyamides.[3]

Silk

The exceptional properties of silk are attributed to the spring-like microscopic mechanical response of alpha-helical polymers having a reinforcing intramolecular hydrogen bonding network. Because of the resistance to axial compressive deformation, microscopic evaluations of knotted single fibers have shown no evidence of kink-band failure on the compressive side of a knot curve.[3] Synthetic high-performance fibers fail by this mode even at relatively low strain levels. This is a principal limitation of synthetic fibers in some structural applications.[3]

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Table I Properties of Silk Compound with Other Fibers

Fiber	Elongation (%)	Modulus (GPa)	Strength (GPa)	Energy to Break (J/kg)	Species
Silkworm	15–35	5	0.6	7 × 10e4	*Bombyx mori*
Spider[a]	10–32	1–30	0.3–1.8	3–10 × 10e4	*Nephila clavipes*
Cotton	5–7	6–11	0.3–0.7	5–15 × 10e3	
Nylon	18–26	3	0.5	8 × 10e4	
Kevlar	4	100	4	3 × 10e4	
Steel	8	200	2	2 × 10e3	

[a] Quasistatic, Instron tensile test rates of 10%/s.

Silkworm silk has been used in textile applications for centuries. Spider silks have gained attention recently because of their extraordinary mechanical properties. Zemlin completed one of the most comprehensive studies on the mechanical properties of spider silks.[4,5] He reported initial modulus values for some spider silks of up to 288.2 g/denier (d) (34 GPa) and elongation values of up to 39%. *Nephila clavipes* had the highest average tenacity 7.93 g/d (0.9 GPa) of the five spider species studied and the highest initial modulus (12 GPa). In general, spider silks are comparable to silkworm silks in terms of extensibility, but higher in modulus and strength. Comparisons are made of silkworm silk, spider silk, and a variety of other fibers in Table I.[3]

Other Linear Protein Polymers

A number of other linear protein polymers have been isolated, treated (usually crosslinked) to improve properties, and in some instances, offered as commercial products. Examples of the polymers are given in Table II.

The production of Lanital involved extrusion of casein fibers, collecting the fibers as tow, treating with CH2O to harden, and cutting the fibers into staple for blending with wool. Aralac is similar to Lanital, but acetylated for greater stability to boiling water. Aralac is composed of 20- and 30-micron diameter fibers similar to 70s and 50s wool, respectively, and with water absorption ~ 14%, Merinova, which reached annual production of ~ 8 million pounds in the 1960s, was resistant to boiling dilute H2SO4 and shrinkproof. Fibrolane BX was stretched and hardened and insolubilized with CH2O.[6]

Vicara was produced by extracting zein, a corn maize protein, using 70% iPrOH, recovering the powder, which was then dissolved in caustic, ripened (uncoil globular protein molecules), and spun in an acid–CH2O bath. The crosslinked fiber was stretched to give a product more stable than Ardil, but less wool-like. The fibers were commercialized by the Va-Carolina Chem Corp. (1948–1957) and used in blends with nylon, cotton, and rayon. The price was ~ 50% of cross-bred wool.[6]

Table II Properties of Protein Fibers Derived from Milk, Corn, and Soybean

Protein	Treatment	Tenacity (g/denier)	Elongation at Break (%)	Commercial Name	Company
Casein	CH2O	—	—	Lanital	SNIA Viscosa
(Milk)	CH2O	1.1 (dry)	50 (dry)	Merinova	SNIA Viscosa
		0.6 (wet)	60 (wet)		
	Acetylation	0.8–1.0	15	Aralac	Aralac, Inc.
	CH2O			Fibrolane BX	Courtaulds
Zein	CH2O	1.2 (dry)	32	Vicara	Va-Carolina Co.
(Corn)		0.75 (wet)	35		
Soybean	CH2O	0.8 (dry)	50		Ford Motor
		0.25 (wet)	—	Silkool	Japanese Co.
Groundnut	CH2O	—	50	Ardil	ICI

Soybean protein spun from caustic solution was stretched and hardened to give fibers with a moisture regain of 11%. The fibers were made and used by the Ford Motor Co. for car upholstery for a short time. A Japanese company also made a similar product with the name Silkool.[6]

Ardil, a fiber commercialized by Imperial chemical industries (ICI) (1938–1957), was prepared by dissolving groundnut protein in dilute caustic, spinning into an acid bath, hardening with CH2O, and cutting the tow into staple for blending with wool. Ardil had outstanding hand, nearest to wool, a moisture content of 14%, a potential price ~ 50% of wool, but consumption never exceeded 3 million pounds.[6]

Generally, these fibers have a poorly ordered structure with low strength, which has led to a lack of commercial success.[7a]

Genetic Engineering of Proteins

Sequence-controlled protein polymers of high MW, consisting of repeating blocks of amino acid sequences modeled from silk and elastin, have been prepared by scientists at Amgen, PA Technologies, Syntro, and Protein Polymer Technologies.[4] Also, McGrath (University of Massachusetts) and Urry (University of Alabama) have been active with varying results.[4] The blocks were utilized in various lengths and dispersities such that a set of protein polymers was produced that could be systematically varied in composition from completely silk-like to primarily elastin-like. Several of the polymers were spun into fibers, but the methods used did not result in fibers with appreciable molecular orientation.[7b]

POLYSACCHARIDES

The information required for biosynthesis of starting components and polysaccharides is stored in DNA. The information is translated into enzymes which catalyze all reactions that occur in a cell. Sugars (monosaccharides) are formed by photosynthesis in plants. Polysaccharides (also called complex carbohydrates) consist of sugar units that have been enzymatically joined with the elimination of water. Polysaccharides give structure to the cell walls of land plants and seaweeds (cellulose), insects, and some microorganisms (chitin), and also store energy in plants (starch) and animals (glycogen).[8,9] In precise chemical nomenclature, polysaccharides are glycans and are described as being composed of glycosyl units. They may be composed of a single type of glycosyl unit, a homoglycan, or from two to six different glycosyl units, a heteroglycan. Of the heteroglycans, only the bacterial polysaccharides have regular repeating-unit structures because of a different pathway of biosynthesis as compared with plant and animal polysaccharides.[8]

Table III Linear Polysaccharides and Their Sources

Source	Linear Polysaccharides
Plants	Cellulose, gellans
Seaweed	Carrageenans, algins, furcellaran, laminarans
Plants	Amyloses, pectins
Microbial	Algins, cellulose, gellans, mannans, colominic acid, curdlan, pullulan
Fungal	Pullulan
Higher eukayotes	Chitins, chitosans
Glycosan	Heparin, hyaluronic acid, chrondroitins, dermatan sulfate, keratan
Glycans	Sulfate

Microbial polysaccharides are synthesized most often by the Leloir pathways: sugar → nucleoside of diphosphate sugar (cellular) → cofactors (oxidn, redn, polymn &/or substitution) → enzymes (polymn) → polysaccharide.

A second pathway of biosynthesis involves only disaccharides as starting materials. Biosynthesis of dextrans and levans from sucrose, a glucose-fructose dimer, is the most important example of this important, but more limited, type of biosynthesis. Manipulation of sugars is more difficult than manipulation of proteins and peptides.

Examples of linear polysaccharides and their sources are shown Table III.[8] Cellulose, chitin, hyaluronic acid, and pullulan can be either recovered as fibers or processed into fibers.

Cellulose

Cellulose is a linear polysaccharide consisting of glucose residues linked together by beta(1–4) glycosidic bonds. The beta(1–4) linkages cause the molecule to form straight chains. Because of their linearity and stereoregular nature, cellulose molecules pack together in extended regions forming polycrystalline fibrous bundles. The micelles are

stabilized by hydrogen bonds between the cellulose molecules. Micelles are packed into microfibrils, which are each about 10 nm in diameter and up to several micrometers in length. In turn, about 10 microfibrils are packed together to form macrofibrils, which are about 50 nm in diameter. The macrofibrils can be seen in a light microscope.[138] Cellulose is generally found in nature in close association with xylans, other polysaccharides, and polymers such as lignin.[10] Native cellulose is fibrous and rather insoluble. Its fibers are relatively strong, having break strengths of up to 1 GPa and moduli of elasticity ranging from 70–137 GPa.[11]

Natural fibers of vegetable origin are composed of cellulose, lignin, and various amounts of other natural materials. Vegetable fibers are classified according to the part of the plant in which they occur. Leaf fibers include abaca (Manila hemp), sisal, and henequen, and are hard fibers used for cordage. Bast fibers include flax, hemp, jute, and ramie, and are soft fibers used for textiles, thread, yarn, and twine. Seed hair fibers include cotton, kapok, and the flosses, and are short and single-celled.[12]

Polysaccharides, including cellulose, have the following disadvantages compared with synthetic commercial polymers. In general, they are less stable to thermal and biological degradation than petroleum-derived materials. Modification is more difficult because the structures are more complicated. They are generally insoluble in organic solvents, and relationships between structures and properties are poorly understood.

Efforts to improve the properties of cellulose began in the mid 1800s, and some milestones are summarized below.[6] Audemars' silk (1855) was drawn as threads using a steel needle from a mixture of highly inflammable cellulose nitrate and caoutchouc in ether/alcohol. The process is described in BP 283. Hughes's silk (1857) was spun from a mixture of starch, glue, resins, tannins, etc. into something resembling silk. The process is described in BP 67. Swan's silk (1883) was made by squirting a solution of cellulose nitrate in glacial acetic acid through holes to produce threads. In 1885, crocheted net fabrics made from denitrated filaments were shown at the Exhibition of Inventions in London. The highly inflammable cellulose nitrate had been converted into cellulose hydrate, which is harmless. Swan's filaments were used in early electric lamps. Soie de France was invented by du Vivier. The process used three solutions: cellulose acetate in glacial acetic acid, fish glue in glacial acetic acid, and gutta-percha in carbon disulfide which were mixed and spun. The product was marketed as "Soie de France." Chardonnet silk was the first process and silk-like product to avoid complex mixtures. The cellulose nitrate was later denitrated to give the product fibers. Count Hilaire de Chardonnet is credited with inventing the first practical rayon process. The process was patented in 1885, and samples of Chardonnet silk were shown at the Paris Exhibition in 1889. In the viscose rayon process, wood cellulose is purified, treated with base to convert it to alkali cellulose, reacted with carbon disulfide to form sodium cellulose xanthate, and spun into an acid bath to form regenerated cellulose viscose filament.

Bacterial Cellulose

Bacterial cellulose is formed as tangled extracellular masses called pellicles in liquid cultures of *Acetobacter xylinum.* Threads of bacterial cellulose grow to a length of a meter, compared with a few centimeters for cotton.[11] The pellicle, composed of microfibrils of cellulose, after squeezing and drying, forms tough membranes with a typical Young's modulus of 15 GPa across the plane of the sheet. Treatment with alkali and/or oxidative solutions increase the Young's modulus to as high as 30 GPa across the plane.[14] The good mechanical properties are attributed to the unique fibrillar morphology in which microfibrils are tightly bound by interfibrillar H bonds. Sheets made from bacterial cellulose have good acoustic characteristics and are used in speaker diaphragms for personal stereo headphones which are reported to have excellent acoustical properties.[13] The raw material is also processed into a pulp useful for making strong papers and reinforcing ordinary pulp papers and mats of other fibrous materials.[14] For example, handsheets made from hardwood Kraft pulp and bacterial cellulose showed an increase in tensile strength, folding endurance, Young's modulus, and dimensional stability, although opacity decreased with increasing bacterial cellulose content. However, paper sheets made from hardwood Kraft pulp and algal cellulose showed almost no improvement in tensile strength and folding endurance, although they had improved dimensional stability and high Young's modulus. The differences in physical properties, despite similar structure of the fine fibrils, can be explained by morphological differences between them. The microfibrils of algal cel-

lulose seem stiff and straight, whereas those of bacterial cellulose had a flexible twisting ribbon-like form. It was concluded that bacterial cellulose microfibrils are more likely to entangle with the Kraft pulp and contribute to improvements in mechanical properties.[15]

Pullulan

Pullulan is a water-soluble extracellular polysaccharide synthesized by *Aureobasidium pullulans,* a common fungus. Pullulan can be produced by fermentation with MW ranging from 1 × 10e5 to 4 × 10e6 by varying the conditions. Three sets of culture conditions have been defined for formation of low (<5 × 10e5), medium (1–2 × 10e6), and high (4 × 10e6) molecular weight polymers for producing films and fibers.[16]

Water-soluble pullulan fibers were prepared by spinning a 40% aqueous solution of pullulan (MW 150 k). The resulting unstretched fiber had a diameter of 20 microns, tenacity of 2.1 g/d, 20% elongation, and a Young's modulus of 1.5 GPa. The fiber dissolved instantly upon immersion in water.[17]

Hygroscopic pullulan nonwoven fabrics can be dry-spun from aqueous solution at 250 m/s with hot air drying to give 10 g/m^2 nonwoven web of fibers with average diameter of 5 microns, showing moisture absorption of 130, 230, and 640% after 30, 120, and 340 h, respectively, at 25°C and 100% relative humidity (R.H.).[18]

Hyaluronic Acid

A highly oriented Na hyaluronate prepared by wet-spinning showed hydration similar to other fibrous biopolymers.[19] Hyaluronic acid can be made to have low solubility in water by crosslinking with a carbodiimide[189] or with poly(ethylene glycol) diglycidyl ether. The crosslinked products have reduced water content (~ 60 wt % or higher) and degrade slowly when brought in contact with water.[191] Crosslinking of hyaluronic acid has been reviewed recently.[190]

Alginates

Calcium alginate (alginic acid from brown seaweed) can be spun as fine as 2 filament denier; it has dry strength comparable to viscose rayon, but wet strength is low. Tenacity and elongation at break of the fibers are 2.20 and 10% at 0% R.H., 1.14 g/d and 14% at 65% R.H., and 0.29 g/d and 26% at 100% R.H. The fibers are flameproof but dissolve in slightly alkaline soap solutions. Chromium alginate, a coarse green monofilament, was manufactured during World War II and used for camouflage purposes.[6]

Alginic acid, which possesses hydroxyl groups similar to hyaluronic acid, can also be crosslinked with poly(ethylene glycol) diglycidyl ether.[191] Evidence was obtained by infrared that intermolecular formation of ether bonds between the different polysaccharide molecules had taken place.[191]

Chitin

Natural chitin fibers with good mechanical strength and elasticity can be obtained from fungi (*Aphyllophorales* group). The fibers are 3–5 microns in diameter and up to several millimeters in length. The fibers are hollow with wall thickness in the 0.2- to 1-micron range. It is possible to vary the chemical composition and mechanical properties of the fibers by selection of fungi and the treatment method. The content of chitin may range from 60–95%, glucans from 5–35%, and melanins from 0–10%. Materials named Mycoton, have high sorptive properties for heavy metals and radionuclides, are useful in medical applications, and can be formed into nonwoven materials.[20]

PHAS

Polyhydroxybutyrate (PHB) was first isolated and characterized in 1925 by Lemoigne[21] at the Pasteur Institute in Paris. Since then, it has been studied extensively by biochemists who have generally concluded that bacteria store PHB as an energy reserve in much the same way that mammals accumulate fat. It remained an academic curiosity until W. R. Grace in the United States produced small quantities for commercial evaluation in the late 1950s and early 1960s. Several patents were issued as a result of that effort.[22] Commercial interest lay dormant for over a decade until ICI began a research and development program. This project followed their single-cell protein animal feed project. Thus, ICI had the skills in place to run large-scale fermentation processes, and polymer processing know-how was available in their plastics division.[23] In the late 1980s, ICI began worldwide commercialization of a family of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymers, PHBV, named Biopol®. In

1990, the agricultural and pharmaceutical businesses of ICI, including BIOPOL were spun-off as Zeneca Ltd. In 1996, Monsanto acquired the BIOPOL business from Zeneca Ltd. Since the acquisition, emphasis at Monsanto has been on producing the polymers in plants and improving their properties for different end-use applications.

Biosynthesis of PHAs

The exact nature of the enzymes involved in the synthesis and subsequent utilization of the energy reserve polymers is known to vary between microorganisms. The general pathway in the biosynthesis in *Alcaligenes eutrophus* is shown by the scheme below. The polymer accumulates in discrete, membrane bound granules in the bacterial cell.[24,25]

Carbohydrate → pyruvate → 2 acetyl-SCoA CO2

→ acetoacetyl-SCoA β-ketothiolase

→ reductase D(−)-3-hydroxybutyryl-SCoA

→ PHA synthase

The scheme involves the condensation of two molecules of acetyl-SCoA to form acetoacetyl-SCoA which subsequently becomes structurally modified by acetoacetyl-CoA reductase to form D-(−)-3-hydroxybutyryl-SCoA. This monomeric form of the polymer is then polymerized to form PHB by a synthase enzyme (PHB synthase, HB-CoA polymerase). This polymerization does not occur directly but is thought to require a protein fraction (A1) located in the granule membrane. A detailed mechanism of the polymerization has been proposed by Kawaguchi and Doi[26] and supporting evidence provided by Gerngross et al.[27] The PHB synthase enzyme is also thought to be physically located in the external membrane of the granule and is regarded as the key enzyme able to make different PHAs depending on the substrate used.[28] The explanation may lie in the nonspecificity of the synthase and other enzymes (such as ketothiolase) involved in the pathway, but the exact mechanism is not yet fully understood. This may not be possible until the structure of the synthase enzyme is elucidated.[29]

PHAs are produced biologically in a plant or microbial organism. Most typically, it is a fermentation product where a microorganism lays down PHA during normal or manipulated growth. Manipulation may be achieved by removing, or failing to produce, one or more nutrients necessary for cell multiplication. The percentage of PHB in bacterial cells is normally low, from 1 to 30%, but under controlled fermentation conditions of carbon excess and nitrogen limitation, overproduction of polymer can be encouraged to produce yields of up to 80% of dry cell weight.[30,31] Numerous microbiological species are known to be suitable for the production of PHAs.[32] The microorganisms may be of the wild type or mutated, or may have the necessary genetic material introduced into it, for example, by any of the methods of recombinant DNA technology.

In *A. eutrophus,* glucose is the common substrate for production of PHB.[23] Other substrates such as methanol, sucrose, ethanol, and acetic acid can be used by other microorganisms to produce the homopolymer.[33] Using various intracellular pathways, such as the hexose-monophosphate (HM) pathway, the organism is able to convert these substrates to the required acetyl-CoA. A process has been reported that contacts a gaseous mixture of H2 and CO with *Rhodospirillales* which metabolize CO in light, and exposing the photosynthetic bacteria to radiant energy to produce PHBV.[34,203]

A two-stage culture method has also been reported in which *A. eutrophus* was grown in an organic medium under heterotrophic conditions for exponential growth and the cells were cultivated for PHB accumulation under autotrophic conditions in which the O2 concentration in the substrate gas (H2 + CO2) was below the explosion limit of 6.9%. PHB was obtained at a high production rate and concentration.[35]

By modifying the carbon source of *A. eutrophus,* other PHAs can be produced. PHBV copolymers are produced using a combination of glucose and propionate.[23] By adjusting the composition of the carbon sources, copolymers with up to 95 mol % HV content have been produced.[23,36,37] P3HB4HB can be produced by *A. eutrophus* when fed on nitrogen-free cultures of either butyrolactone and butyric acid or 4-HB and 4-chlorobutyric acid.[38] Using *Pseudomonas oleovorans* and a range of *n*-alkanoic acids, PHAs containing up to 12 C atoms have been produced.[39,40] Although all the PHAs were heteropolymers containing up to six different monomers, the major monomer unit always had the same number of C atoms as the *n*-alkanoate substrate used.

The genes encoding for the first two enzymes in the biosynthetic pathway for both *Zoogloea*

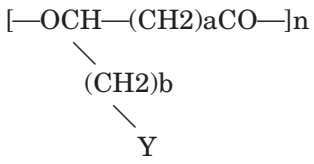
ramigera and *A. eutrophus* have been isolated, characterized, and overproduction systems developed.[41] The genes have been cloned and expressed in *Escherichia coli.*[42] *E. coli,* which does not normally synthesize the polymer, has been reported to accumulate PHB to levels approaching 80% of the dry cell weight when transduced with plasmids bearing appropriate genes.[43] The major significance of synthesizing PHB in *E. coli* is that all the genetic engineering principles that apply to this organism can now be utilized in optimizing the production of PHB and other PHAs.[29] High molecular weights, e.g., 4–5*M,* can be achieved because *E. coli* does not have the depolymerase enzymes. There are also potential advantages in terms of ease of extraction of the polymer and purity of the product.[29] A PHB synthesizing mutant of *E. coli* has been developed from which the polymer can be extracted by mild heat treatment rather than by chemical extraction techniques.[44] This strain is thought to release the accumulated polymer by thermally induced cell lysis at a low temperature of only 42°C.[29]

The enzyme encoding genes from *A. eutrophus* have also been introduced into cells of oil-producing plants (e.g., oilseed rape, canola, soya, or sunflower), which are consequently able to produce PHB.[45,46,202] Expression is directed to the developing oil storage organ (e.g., the embryo of oilseed rape) or the cytosol, mitochondria, or plastids.[202] The size distribution of the plant cell polymer is reported to be broader than the typical bacterial product. This advance raises the prospect of growing PHB as an arable crop sometime in the future, which could enable this biodegradable polymer to be produced cheaply enough for the material to become a genuine commodity plastic.[29]

Endogenous β-ketothiolase (phaA) activity is present in cotton fibers. Cotton has been transformed with engineered acetoacetyl-CoA reductase (phaB) and PHA synthase (phaC) genes by particle bombardment. High molecular weight PHB has been produced in the transgenic fibers. The transgenic fibers exhibited better insulating characteristics. The rate of heat uptake and cooling was slower in the transgenic fibers, resulting in higher heat capacity. Thus, metabolic pathway engineering in cotton has potential for producing new fibers for the textile industry.[47,48]

Biosynthetic PHA Compositions

PHAs can be produced as homopolymers or copolymers having the general structure shown below.



The nonspecificity of the biosynthetic enzymes in polymer-accumulating organisms has proven to be an advantage offering the potential of synthesizing unusual polymers not readily synthesized chemically.[29] Thus, the microbial PHA synthase enzymes have broad substrate ranges and are capable of incorporating a large number of hydroxyalkanoic acid (HA) monomers as constituents of biosynthetic PHA depending on growth conditions, precursor substrate availability, and the source of the PHA synthase enzyme. Examples[49] of the diversity of repeat units incorporated into PHAs include β-hydroxypropionates with beta-substituents that contain vinyl,[50] nitrile,[51–53] phenyl,[54,55] cyanophenoxy and nitrophenoxy,[53] and halogen[56,57] functionalities. Also, 3-hydroxypropionate (3HP) and 4-hydroxybutyrate (4HB) repeat units have been found in PHAs produced using the bacterium *A. eutrophus.*[58,59] The diversity in composition of biosynthetic PHA polymers is underscored by the fact that at least 91 HA monomers have been identified as substrates for PHA synthases.[60]

Morphology of PHAs

PHB and other PHAs accumulate in discrete spherical granules in the cell cytoplasm. Granules have a diameter that ranges from 100 to 800 nm[30,61] and are enclosed in a unit membrane about 2–4-nm thick.[61] The granules are typically composed of about 98% polymer and the rest is protein and phospholipid. C13 Nuclear magnetic resonance (NMR) studies[62,63] on live cells suggest that PHB is predominantly in a mobile state, but not in solution, in the granules. Water was shown to be an integral part of the granule and seems to act as a plasticizer for the polymer, although the exact conformation within which water is included to plasticize the polymer is not entirely clear. It is now proposed that the enzymes involved in the PHA biosynthetic pathway operate only on a mobile hydrated material and that the solid granules characteristic of dried cells are an artifact of the drying process. The crystallization of the polymer is thought to be under kinetic control and is inhibited by the submicron size of

the particles and their protein and phospholipid coat.[63] Recently, a method for extraction of amorphous granules has been reported.[64] It has also been possible to produce kinetically stable amorphous granules *in vitro* from crystalline PHB and PHAs, using surfactant to stabilize the submicron-size granules.[65] These studies may well have some future impact on the processing of PHB and other PHAs into articles of commercial use.[29] From a biological viewpoint, the work suggests that *in vivo* PHB is much more flexible and has an effective glass transition temperature (T_g) that is much lower than that measured for PHB in the solid state.[29]

Composition and Molecular Structure of PHAs

PHB is isotactic and similar to isotactic polypropylene (PP) as both have pendant methyl groups attached to the main chain in a single conformation.[66] PHB is a compact right-handed helix with a twofold screw axis and a fiber repeat of 5.95 A. The helix conformation is stabilized by carbonyl-methyl group interactions and represents one of the few exceptions of a helix found in nature that does not depend on H-bonding for its formation and stability. PHB can have a weight average molecular weight of 0.1–3*M,* although for processing, the molecular weights are usually in the range of 200 to 800 k. The polydispersity is in the range of 2.2 to 3.[66] Because PHB is made biologically as a stored energy source in cell walls, it can be separated from cell material in very high purity. The major impurities in PHB are inorganic nitrogen, phosphorous, and sulfur-containing compounds which are present at concentrations less than 200 ppm.[67] There are no catalyst residues to be concerned about in the polymer.

PHB*x*V copolymers have been shown to exhibit isodimorphism[68,69] with the HB and hydroxyvalerate (HV) cocrystallizing. As the HV content of PHBV is increased, the melting point decreases (from ~ 178°C for PHB) and passes through a minimum of ~ 75°C at ca. 40 mol % HV content and increases to ~ 110°C for PHBV containing 97 mol % HV. A change from the PHB lattice to the PHV lattice takes place at ~ 40% HB. At <40 mol % HV, HV units can crystallize in the PHB lattice and at >40 mol % HV, HB units can crystallize in the PHV lattice. Thus, isodimorphism makes it possible to achieve relatively high levels of crystallinity in these copolymers, retaining the useful hydrolysis and chemical resistance exhibited by PHB. Also, the same nucleants can be used for PHB and lower HV content PHBV. PHBV satisfies the physical requirements for isodimorphism in that the two monomer units have approximately the same shape and occupy similar volumes, and the polymer chain conformations of both homopolymers are compatible with either crystalline lattice. Like PHB, the PHV polymer chain has a 21 helix conformation, an orthorhombic unit cell, and space group P212121 with unit cell parameters, a = 9.32 A, b = 10.02 A, c = 5.56 A.[70] NMR evidence indicates that, at HV levels of 20–40 mol %, HV units are partially excluded from the PHB lattice.[71] A recent study suggests that co-crystallization of the two monomer units certainly does occur, but that the molar ratio of HV within crystals is approximately two-thirds of the total molar ratio present in the copolymer.[29]

Crystallinity of copolyesters of 3-HB and 4-HB has been reported to decrease with increasing 4-HB content.[38] PHAs containing longer side chains (C5 and greater) produced by *P. oleovorans* are less crystalline (heat of fusion up to 8.3 cal/g) and have lower melting points (e.g., 45–61°C).[40] Also, a copolymer of ~ 30% 3-hydroxyoctanoate/ ~ 70% 3-hydroxydecanoate produced by *P. putida,* exhibits a low melting point of ~ 47°C, with a low heat of fusion (~ 20 J/g), suggesting a lower level of crystallinity than observed in PHB. (The M_w was relatively low, ~ 120 k, with a typical polydispersity of ~ 2.3.)[72] Solid state C13-NMR characterization of PHBHH copolymers containing 5–25 mol % 3HH made by feeding alkanoic acids to *Aeromonas caviae* suggested the 3HH is excluded from the PHB crystalline phase.[73]

Physical and Mechanical Properties of PHAs

PHB can range from a predominantly amorphous material (<30% crystallinity) to a highly crystalline (>80%) material, depending on its history. The density in the amorphous state is 1.177 g/cc[74] and in the crystalline state it is in the range of 1.23–1.26 g/cc.[66,74,75] The mechanical properties of PHB decrease significantly below an M_w ~ 400 k, and the material is quite brittle at ~ 200 k.[72]

PHB*x*V crystallinity is typically in the 39–69% range.[76] The density in the amorphous state is 1.16 g/cc[77] and in the crystalline state is ca. 1.2 g/cc.[76] The crystallinity and mechanical properties of PHB*x*V copolymer can be varied by changing the percentages of the respective monomers. The higher the percentage of HV, the less crystal-

Table IV Physical and Mechanical Properties of PHB and Its Copolymers with HV

Polymer	M_w (k)	T_g (°C)	T_m (°C)	T_d (°C)	Hf (J/g)	Tensile Strength (MPa)	Tensile Modulus (MPa)	Flexural Modulus (MPa)	Elongation at Yield (%)	Elongation at Break (%)
PHB	370	1	171	252	51	36	2500	2850	2.2	2.5
PHB7HV	450	−1	160	243	32	24	1400	1600	2.3	2.8
PHB11HV	529	2	145	235	12	20	1100	1300	5.5	17
PHB22HV	227	−5	137	251	7	16	620	750	8.5	36

line and the more elastic the polymer becomes. Some thermal and mechanical properties are presented in Table IV.[80] A study of the thermal characteristics *in vivo* has been published,[78] and a mechanical evaluation *in vivo* and *in vitro* has also been published.[79]

Barrier properties are generally measured in terms of moisture vapor transmission rate (MVTR) and oxygen transmission rate (OTR). The MVTR seems to be fairly constant as a function of HV content for PHBV copolymers, and the OTR may increase somewhat with increasing HV level. Introducing orientation decreases OTR significantly, improving the barrier to oxygen. In general, the OTR for cast (un-oriented) PHBV is less than (i.e., superior to) that for low-density polyethylene (LDPE), PP, and polyvinyl chloride (PVC) with MVTR similar to PVC and approaching LDPE.

Medium chain length PHAs have been classified as thermoplastic elastomers.[81,82] However, their low T_m and low crystallization rate limit their applicability. A medium chain length PHA grown on 75 vol % n-octane and 25 vol % 1-octene contained some unsaturated bonds corresponding to 3-hydroxyoctenoic acid and 3-hydroxyhexenoic acid. The PHA, after crosslinking by irradiation, became a true rubber with constant properties over a wide temperature range from T_g (~ -15 to -27°C depending on dose) up to the degradation temperature. The polymer is completely biodegradable[83] and is thought to be the first microbially produced biodegradable rubber.[84]

Another property of note is that PHB is piezoelectric. The polymer molecules are arranged as 2/1 helices in orthorhombic crystals with *P*212121 space group which does not have a center of symmetry. Thus, if the crystals are deformed in a particular way, the direction of average dipole moment will change and a polarization will be produced. The particular symmetry of the PHB crystals is such that a surface charge will be generated if they are deformed in shear modes but not in simple tension, under hydrostatic load, or in response to thermal expansion. The size of the piezoelectric effect is typical of many biological polymers such as poly(*g*-methyl-L-glutamate) but is up to an order of magnitude less than that of polyvinylidene fluoride. PHB does have piezoelectric properties similar to natural bone. Moreover, bone is known to be strengthened and repaired by electrical stimulation. Thus, bone fracture fixation plates made from PHB composite may stimulate bone growth and healing, and such a bone plate would be slowly resorbed by the body, obviating the need for a second operation to remove it.[23]

Thermal Properties of PHAs

PHB is a thermoplastic that melts, T_m, in the range of 171–182°C,[1,6–8] has a T_g in the range of 4–15°C,[66,74–76] and a heat distortion temperature, HDT, of 157°C. The temperature for the onset of decomposition, T_d, with fast heat is in the 225–252°C range,[80,85] but is lower with longer exposure. PHB's beta-substituted structure makes it thermally unstable at temperatures >140°C. PHB*x*V copolymers are also thermoplastics with melting points, T_ms, in the range of 75 to 170°C,[76] depending on the value of x (the mol % HV). Increasing the amount of HV in the copolymer reduces the T_m from $\sim$ 180°C for PHB to ca. 75°C for a copolymer containing 30–40% HV. The heat distortion temperature of the copolymers decreases from 140 to 92°C as the HV content increases. The temperature for the onset of decomposition, T_d, with fast heat is in the 232–244°C range,[85] but is lower with longer exposure. Beta-substituted aliphatic polyesters are unstable at >170°C. The thermal degradation mechanism of PHB has been studied and is considered to be primarily a random chain scission process by a beta-elimination reaction via a six-membered cy-

clic transition state.[86,87] Activation energies between 109 ± 13 kJ/mol for PHB and 126 ± 13 kJ for PHBHV have been reported.[88] The major pyrolysis product of PHB isocrotonic acid produced by the beta-elimination reaction.[89–91] Significant quantities of PHB oligomers and small quantities of isocrotonic acid were also evolved on heating the polymer to approximately 300°C. Secondary products such as propylene, ketene, CO2, and acetaldehyde were also formed by further decomposition of the primary products. Conventional thermal stabilizers have been shown to have little effect in preventing thermal degradation of PHB.[91]

The thermal degradation mechanism of PHBV copolymers has been reported to be analogous to that for PHB, with a similar activation energy and degradation kinetics.[87] The significant benefit of the PHBHV copolymers is their lower melting points that enable them to be melt processed at lower temperatures than PHB, generally in the range of ~ 140–180°C, significantly reducing thermal degradation.[72]

Crystallization Behavior of PHAs

Table V shows the relationship between spherulite growth rate and spherulite size for PHB at different temperatures.[92] PHB and PHBV have also been shown to embrittle upon aging. For copolymers, the crystallization rate drops off rapidly with increasing HV content, as shown in Table VI.[92]

The embrittlement of PHB and its copolymers on storage at room temperature is not associated with relaxation of the amorphous regions, but rather with the development of interlamellar secondary crystallization. The small crystallites produced underpin the amorphous regions and reduce the mobility of the chain segments thus raising the modulus and embrittling the material. Crystallizing the copolymer at high temperature leads to rejection of the HV units into the amorphous regions and reduces the extent of secondary crystallization which can develop at room temperature, such that these materials do not embrittle on storage. Dynamic mechanical thermal analysis and dielectric thermal analysis were found to be useful techniques for measuring the mobility of the amorphous regions in partially crystalline samples, under conditions where differential scanning calorimetry (DSC) was unable to detect any glass transition.[93] A patent has been filed on PHB and high HB-containing PHAs in which aging had occurred and the original properties were restored by a heat treatment and subsequent aging was retarded.[94] A patent has been filed on cooling the polyester after preparation to <90°C and then heating to 90 to 160°C within 24 h of preparation to retard aging.[95] Other patents were filed on PHA–plasticizer compositions[96] and PHBV copolymers,[97] claiming to retard the aging process.

Table V Effect of Temperature on the Growth Rate and the Size of Spherulite in PHB

Spherulite Growth Rate		Spherulite Size	
Temperature (°C)	Rate (microns/s)	Temperature (°C)	Size (microns)
51	1	50	0.07
65	2	65	0.3
77	3	75	0.5
92	4	85	0.67
100	3		
106	2		
113	1		

Table VI Effect of HV Content on the Maximum Crystallization Rate of PHBV

Polymer	Maximum Crystallization Rate (microns, s)	T_c (°C)	T_m (°C)
PHB	4.5	88	197
PHB6HV	1.4	80	186
PHB12HV	0.43	78	173
PHB16HV	0.23	70	167

Rheology of PHAs

The rheological properties of PHB and PHBV have been studied and compared using both a rotational rheometer with parallel plate geometry and a capillary rheometer.[98] One of the key melt flow properties of a molten polymer is the extensional viscosity of the melt. In the case of polycaprolactone (PCL), the extensional viscosity increases with strain rate above the linear viscoelastic limit. This behavior is known as "extension thickening" or "strain hardening." This extension thickening melt viscosity behavior of PCL is thought to stabilize the polymer pro-

Table VII Biodegradation of PHB in Different Microbial Environments

Environment	Degradation Time for 1-mm Film (Weeks)	Average Rate of Surface Erosion (Microns/Week)	Time for 100% Weight Loss for 100-Micron Film (Weeks)
Seawater at 15°C	350	1	50
Soil at 25°C	75	5	10
Aerobic sewage	60	7	7
Estuarine sediment	40	10	5
Anaerobic sewage	6	100	0.5

cessing operations involving stretching or melts such as film blowing and fiber spinning.

Rheological characterization of PHBV indicates the viscosity decreases dramatically with increasing shear rate (shear thinning). The zero shear viscosity ($N0$) is estimated to be about 7.2 $\times$ 10e6 pascal-s which is much higher than most polymers that are typically easy to process, such as ABS. The extreme shear thinning nature of this material may indicate it would be easy to process in terms of extrusion and general molding operations which are high shear rate processes, but parts may have poor surfaces resulting from incomplete relaxation at the mold wall which is a low shear rate process.

Rheological characterization of PHB4HV of various molecular weights (400 to 800 k) and containing a plasticizer and two different classes of nucleating agents [boron nitride (BN) and cydohexylposponic acid (DZB)] showed that the main parameter for determining the rheological properties is the molecular weight. An increasing molecular weight results in an increasing shear viscosity and extensional viscosity. In the deformation range studied, the effect of molecular weight on the extensional viscosity is larger than on shear viscosity. Melt strength can be related to the extensional viscosity of a material. Thus, the results predict that increased molecular weight should result in enhanced stability of a bubble during blown film processing.[99] Use of a torque rheometer on a melt of PHBV showed that the degradation kinetics follow Arrhenius behavior with temperature, the degradation rate increased with increasing shear, and the activation energy for random chain scission is independent of shear rate. The effect of shear on the degradation process may be a result of viscous heating. The degradation rate is increased because of the increased internal energy of the polymer chains as a result of mechanical deformation. There was no evidence of direct mechanical degradation.[199]

Biodegradation of PHAs

PHAs biodegrade on disposal into a microbially active environment. Biodegradation has been demonstrated in aerobic and anaerobic sewage, estuarine and marine sediment, lake, pond, and sea water, soil compost, and simulated managed landfill. Many bacteria, streptomycetes, and molds have been isolated that degrade PHAs.[100] The rate of biodegradation depends on environmental factors such as moisture level, nutrient supply, temperature, and pH, and on material parameters such as comonomer content, initial molecular weight, degree of crystallinity, surface, and formulation (e.g., plasticizers).[101] These material parameters can be controlled/modified within certain limits to optimize biodegradation rates for different applications.[102]

The time for complete degradation of PHB films 1-mm thick and 100-microns thick in various environments are given in Table VII.[103] The biodegradation rate of PHBV copolymers tends to be faster than the PHB homopolymer (e.g., $\sim$ 2 versus 6 weeks in anaerobic sewage) but the relative rate depends on the environment.[101,104]

The rate of biodegradation of PHBV has been reported not to be affected significantly by the surface area or thickness of the samples, but is affected significantly by the crystallinity.[105] Crystalline domains are needed for binding of the depolymerase enzymes. However, highly crystalline PHAs biodegrade very slowly, whereas amorphous PHAs do not biodegrade at all.

Biodegradation seems to occur by colonization of the PHA surface by bacteria, which secrete an extracellular depolymerase that degrades the polymer to primarily monomer units in the vicin-

ity of the cell. The soluble degradation products are then absorbed through the cell wall and metabolized. Apart from a small amount of biological material, the final products of biodegradation are CO2 and water in aerobic conditions and methane and CO2 in anaerobic conditions. The surface erosion mechanism is supported by observations of a gradual decrease in sample thickness with time and constant molecular weight of the residual sample during biodegradation.[101]

The kinetics and mechanism of surface hydrolysis of PHB film were studied at 25–37°C and pH 6–8 with an extracellular PHB depolymerase purified from *A. faecalis* T1. The primary product of enzymatic hydrolysis was the dimer of hydroxybutyric acid (HBA), which was subsequently hydrolyzed to monomer. The heat of enzyme adsorption on the PHB surface was 43 kJ/mol, indicating a strong interaction between the enzyme binding domain and the hydrophobic surface of PHB. The activation energy of the enzyme-catalyzed hydrolysis was found to be 82 kJ/mol.[106]

Enzymatic degradation of PHAs with different chain lengths (C4–C10) produced from various carbon substrates by *A. eutrophus* or *P. oleovorans* was studied at 37°C in a phosphate buffer (pH 7.4) containing the PHA depolymerase from *A. faecalis* T1. The rate of degradation as determined by time-dependent changes in weight loss of films was strongly dependent on the composition of the polyesters and markedly decreased with an increase in the side chain length of the 3-HA monomeric units. The polyester chains were finally degraded into the monomers and dimers of 3-HA acids by the PHA depolymerase.[107]

Biocompatibility of PHAs

The degradation of PHB produces D-3-hydroxybutyric acid which is a normal constituent of human blood. Work at the Middlesex Hospital in England has shown that this material, which can be obtained by simple hydrolysis of the polymer, can be used as an intravenous or oral carbon supply and has a number of clinical advantages over the more commonly used glucose drip. It is reported that obese patients in this study, who were undergoing therapeutic starvation for 14 days, never complained of hunger.[23] There is evidence for both enzymatic and hydrolytic degradation *in vivo*.[78] *In vitro* studies[108,109] suggest that PHB and PHB*x*V copolymers degrade by hydrolysis in a multistage process in which the majority of the molecular weight loss occurs before any significant mass loss. As HV increases in PHBV copolymers, the percentage of amorphous regions that are readily attacked by hydrolytic degradation increases, thereby increasing degradation rates. In addition, elevated temperatures and alkaline conditions have been shown to increase degradation rates.

Processing of PHAs

PHAs, as isolated from natural organisms, are difficult to process because of their relatively low decomposition temperatures (140–170°C), their slow crystallization rates near their melting points, low melt strength, etc. Because of thermal degradation in the melt, the melt flow index (MFI) can change rapidly with time, and volatile decomposition products must be handled safely. The slow crystallization rates lead to tacky products (e.g., fibers, films, molded articles) that adhere to themselves and the process equipment. Products produced by thermal processing undergo embrittlement with time (aging). Various approaches, such as use of nucleating agents, plasticizers, crosslinking agents, and blends, have been taken to overcome the processing and product difficulties and shortcomings.

Effect of Nucleating Agents

PHB from biological sources is of very high purity. As a result, PHB melts may undergo homogeneous nucleation, but the nucleation density for PHB is orders of magnitude less than for polyethylene (PE) and PP. However, the growth rate, once nucleated, is similar to PP, nylon 6 and polyethylene terephthalate (PET). The low rate of nucleation in PHB leads to development of large spherulites within the PHB. The large spherulites can significantly reduce the physical and mechanical properties of the polymers.

The overall rate of crystallization can be increased by the use of nucleating agents which promote crystallization of the molten or glassy resin by increasing the number of nuclei within the PHB. The increased number of nuclei leads to smaller-diameter spherulites, a more rapid loss of tackiness and concurrent increase in mechanical strength, and reduced cycle times in thermal processes. Potential nucleating agents can be tested using DSC. As the molten polymer is cooled at a constant rate, an exotherm is produced as the polymer crystallizes. The temperature range over which crystallization occurs, the area of the peak,

and the peak sharpness give an indication of the crystallization behavior of the material. The addition of a nucleating agent generally causes an increase in the crystallization peak and the peak area. Effective nucleating agents include saccharin and particulates such as talc, micronized mica, boron nitride, chalk, calcium hydroxyapatite, and calcium carbonate.[110] Good dispersion of particulate nucleating agents is often difficult, leading to agglomeration and inhomogeneity in molding. The presence of agglomerates may give rise to regions of stress concentration, impairing the mechanical and barrier properties. The particulates can also impart an undesirable opacity to products formed from the PHB.[111]

BN is commonly used as the nucleant for PHB and other PHAs. With PHB containing 1 wt % BN, the T_c was at 109.6°C and the heat of crystallization, H_c, was 78.4 J/g versus 87.9°C and 64.0 J/g without BN.

Saccharin (m.p. 226°C) can nucleate PHB when added to the melt because of epitaxial growth on its surface.[67] The nucleating effect of saccharin is attributed to adsorption of molecules onto the surface in what is close to their crystallographic form. The key feature of this nucleating agent is the 1.179-nm repeat spacing between successive protruding oxygens on the (001) surface. The protruding oxygens will carry a negative charge which can interact favorably with the positively charged carbonyl carbons in the PHB molecule.[53] Talc is not effective when added to the melt, but it does nucleate the PHB if the polymer is crystallized, reheated, and finally recrystallized. Based on rate studies, it was concluded that the nucleating effect of talc is caused by the reduction in entropy of partially adsorbed molecules.[67]

Technical grade NH4Cl incorporated as an aqueous solution gives better dispersion and is a more effective nucleating agent than solid technical grade or high-purity NH4Cl. NH4Cl undergoes a transition between bcc crystal habit (3.86 A lattice parameter) and fcc habit (6.53 A lattice parameter at approximately 184°C. The lattice parameter of the fcc form of NH4Cl matches the C-axis span of PHB. High-purity crystals are in the bcc form, technical grade solution is in the fcc form, and technical grade crystals are a mixture with the major portion in the bcc crystal habit.[112]

PHB seed crystals prepared by heating PHB (m.p. 173.9 C, 54% crystallinity) for 7 h at 170°C at 0.2 bar, cooling under vacuum, and pulverizing, had an m.p. of 180.5°C and were 80% crystalline. The seed crystals were used to nucleate crystallization of PHB12HV (m.p. 149.9°C). At 5 phr of PHB seed crystals, the T_c was 88.7°C and H_c was 45.7 J/g. At 1 phr of PHB seed crystals, the T_c was 70.3°C and H_c was 41.9 J/g. Without the PHB seed crystals, no T_c was observed.[113]

Cyclohexyl phosphonic acid (CPA) and zinc stearate (ZS) mixtures are more effective nucleating agents than BN with PHBV polymers with high HV content. For example, when 0.10 phr CPA and 0.385 phr ZS were added to a PHB22HV polymer and compared with PHB22HV containing 1 phr BN at a T_c of 80°C, the $\frac{1}{2}$ crystallization time and the $\frac{1}{2}$ heat of crystallization peak were similar for CPA/ZS and BN—1.26 and 1.39 min and 19.85 and 19.26 J/g, respectively. At a T_c of 50°C, the $\frac{1}{2}$ crystallization time for CPA/ZS was 0.40 min versus 3.91 min for BN and the $\frac{1}{2}$ heat peak was 47.09 J/g versus 11.73 J/g. Thus, at lower T_cs, CPA/ZS yields significantly faster crystallization rates with improved energies of crystallization. With high-HV-containing polymers, it should be possible to use lower mold temperatures and shorter cycle times with CPA/ZS. However, for low-HV-containing polymers, both types of nucleant yield similar crystallization rates.[114] More recently, organophosphorous compounds having at least two phosphonic acid groups such as 1-hydroxyethylidene diphosphonic acid (HEDP) have been shown to serve as nucleating agents and give products with excellent clarity. For example, a PHB3HV containing 0.2 phr HEDP showed a T_c of 78°C and an H_c of 55 J/g. A combination of HEDP with Ca or Mg stearate has also been shown to be an effective nucleating agent. A PHB8HV containing 0.1 phr HEDP and 0.3 phr Ca or Mg stearate had a T_c of 72°C and an H_c of 51 and 50 J/g, respectively, compared with a T_c of 56°C and an H_c of 15 J/g for PHB8HV alone.[115]

Plasticizers

The use of monomeric and polymeric plasticizers in PHAs to impart properties and impede loss of properties by secondary crystallization has been described. The difference between the solubility parameters of the plasticizer and the PHA is a useful indicator of compatibility, but not an absolute predictor of compatibility. Such calculations do not take into account chemical features such as polar character, dielectric constants, dipole moments, and hydrophilic–lipophilic balance.

Plasticizers for PHB include a.) high-boiling esters of polybasic acids such as phthalates,

Table VIII Effect of Plasticizers on the Physical and Mechanical Properties of PHB

Plasticizer	wt %	T_g (°C)	T_c (°C)	T_m (°C)	H_c J/g	Young's Modulus (MPa)	Tensile Strength (MPa)	Elongation at Break (%)
GTA	10					1020	27	12
GTA	20	−22	24	164	73.2	680	20	64
GTB	20	−29	14	165	75.2	630	20	26
GMS	20	−22	22	162	74.6	820	20	20
GTP	20	−29	15	164	67.6	960	25	10

isophthalates, citrates, fumarates, glutamate, phosphates, or phosphites; b.) high-boiling esters and part esters of polyhydric alcohols, especially glycols, polyglycols, and glycerol; and c.) aromatic sulphonamides. For producing rigid, but not too brittle articles, 6 to 12 phr w/w is generally suitable.

Glycerol triacetate (GTA) (triacetin) and glycerol tributyrate (GTB) are effective plasticizers for PHB and show good compatibility. Glycerol monostearate (GMS) is also an effective plasticizer. Solid state C13-NMR showed that the mobility of the PHB chain is enhanced by the addition of GTA. Unfortunately, GTA is too volatile during melt processing or even storage at high ambient temperatures and the monomeric plasticizers are eluted with time with a corresponding decline in product properties. Data are shown in Table VIII.[116] Acetyl tri-*n*-butyl citrate (Estaflex) is also used as a plasticizer for PHAs and is considered an improvement over GTA.[121]

Di-*n*-butyl phthalate (DBP) displays a plasticizing effect toward PHB very similar to that found with PVC.[118] A near linear relationship exists for T_g and DBP weight fraction from the T_g of PHB of 8°C to the T_g of DBP of −90°C. The amount of DBP needed to lower the T_g of PHB to −40°C is about 30%. PHB is able to crystallize closer to room temperature in the presence of DBP as the temperature of the crystallization exotherm decreases, increasing DBP content. This is not surprising and is attributed to a concomitant decrease of T_g, above which the macromolecules acquire enough mobility to rearrange and crystallize. The enthalpy (H_c), correlates with the decrease of PHB in the polymer–DBP mixture; therefore, the ability of PHB to crystallize does not decrease in the presence of plasticizer. A level of 39% DBP lowers the m.p. of PHB from 175 to ca. 150°C.

The use of high molecular weight polymers as nonexuding plasticizers has been reported.[117] PCL and polybutylene adipate (PBA) have been used with PHB, but the mixtures are not compatible, and films made from the mixtures exhibit rapid aging and loss in tensile properties.[118,119] Based on the mechanical properties and scanning electron microscopy (SEM) of the immiscible blends, it was suggested that the PHB/PCL blend has a macro-phase separated structure and the PHB/PBA blend has a modulated structure with a micro-phase separation.[119] Polyethylene oxide (PEO), which has a T_g of −59°C, behaves like a high molecular weight plasticizer with PHB. As the PEO content is increased, a parallel lowering of T_g of PHB and a decrease in T_c are observed.[120] Polymeric esters and epoxidized soybean oils have given very good results as plasticizers for PHAs.[121]

Branching of PHAs

Certain polymers are difficult to process by conventional melt processes (e.g., cast film extrusion, blown film extrusion, and melt spinning processes) into films, fibers, or other forms having physical integrity. Generally, these polymers do not have the melt strength and/or set time required. A polymer having low melt strength is unable to withstand the minimum strain required to draw the polymer to the desired dimension and will exhibit instabilities such as breakage, sagging, or draw resonance.

The melt strength can be improved by branching. A PHA melted in the presence of a free radical initiator at a temperature above the decomposition temperature of the free radical initiator for a sufficient length of time undergoes crosslinking. The thermally induced decomposition of the free radical initiator results in the production of reactive radicals which produce interchain crosslink formation between PHA chains. The branched PHA compositions can be made by reactive extrusion, where the extrusion tempera-

tures and residence times are sufficient for melting the PHA and for causing the decomposition of the peroxide. When producing branched PHAs by this method, there are competing reactions occurring in the melt. Thermal decomposition of the PHA results in a decrease in its M_w whereas the branching reaction produces an increase in its M_w. The choice of extruder temperature, free radical initiator, and initiator concentration can be optimized to give control of resulting molecular weight and degree of branching. The most effective concentrations of peroxide seem to be in the range of 0.05–0.1 up to 0.3 wt %.[122a] At <0.05%, there is no appreciable effect, and at concentrations >0.5 wt %, the extruded materials become brittle. In fact, PHBV containing 0.5 wt % dicumyl peroxide (DCPO) after heating for 10 min at 160°C becomes completely insoluble in CHCl3, indicating that significant crosslinking had occurred.[122b] The m.p. increased from 159 to 172°C, T_c increased from 63 to 109°C, the tensile modulus decreased from 1.95 to 1.57 MPa, and the strength to break increased from 0.22 to 0.32 MPa. The DCPO-treated PHB4HV not only crystallizes at a much higher temperature than the untreated PHB4HV but also at a much faster rate. The DCPO-treated PHB4HV seems to be more nucleated and the decrease in tensile modulus indicates smaller spherulites.[123] Branching also slows down the age-related embrittlement of articles produced from PHAs.

Blending of PHAs

Totally miscible polymers are microscopically homogeneous (i.e., mixed on a molecular scale), and partially miscible blends display microscopic heterogeneity. Immiscible blends are macroscopically heterogeneous and display multiphase behavior. It would be desirable to impart "extension thickening" or "strain hardening" to blends of immiscible or partially miscible polymers composed of at least one polymer that does not exhibit "strain hardening" and at least one polymer that does exhibit "strain hardening" by using suitable plasticizers/compatibilizers.

As mentioned above, PHB and PCL are not compatible and will not form miscible blends. However, by using a block copolymer of PHB–PCL as a plasticizing and compatibilizing agent, stable, miscible blends can be obtained. The block copolymer was made by reacting PHB with e-caprolactone (e-CL). A blend composed of PHB5HV/PCL/iso-PHB-*b*-PCL in a 70 : 25 : 5 weight ratio, had an elongation of 29%. When the weight ratio of the blend was changed to 45 : 45 : 10, the elongation increased to 855%.[126]

Another patent claims compositions of at least 70 wt % of a blend of PHB and PCL and 5 to 30 wt % of a copolymer of CL and HA. The patent also claims an alternate method for making blends by forming the compatibilizing block copolymer *in situ*. For example, PHB and PCL were mixed with a catalyst [e.g., Zn(OAc)2] for the time and temperature required to form up to 30% copolymer by transesterification after which the melt was shaped into the desired form including fibers and nets.[127]

A synthetic block copolymer of P[*R*,*S*)3HB-*b*-PHH)] (M_n ranged from 43 to 81 k) was used as compatibilizer for PHB (M_n 650 k) and PHH (M_n 59 k). The PHB film had an elongation at break of 5%. A 75 : 25 blend of PHB/PHH had an elongation at break of 10%. A 71 : 24 : 5 blend of PHB/PHH/P[(*R*,*S*)3HB-*b*-PHH)] had an elongation at break of 62%.[129,130]

Block copolymers were also prepared by exposing PHB to alcoholysis to produce low molecular weight prepolymer ($M_w \sim$ 1 to 10 k) having a hydroxyl endgroup and an ester endgroup and reacting the hydroxyl endgroup with lactone or lactide. The block copolymers were used as compatibilizers to prepare blends of biodegradable polymers (e.g., PHB) and hydrodegradable polymers (e.g., PLA).[131] Oligomeric esters, used as plasticizer for PHBs, are also found to compatibilize blends of PHA with PCL and provide materials with excellent ductility.[132]

PHB has been blended with a copolyester prepared from adipic acid, ethylene glycol, and lactic acid. The blend showed a single T_g, which increased with the copolyester content. The growth rate of PHB spherulites, the crystallization temperature, and the melting temperature all decreased as the copolyester content increased. However, the degree of crystallinity, and the enthalpies of crystallization and fusion of PHB in the blend remained almost constant with compositional change.[196] Blends (80 : 20) of PHB and Bionolle 1010, an aliphatic polyester, have been prepared.[197]

Reactive Blending

PHBV and PCL are compatible in the melt at 160°C. DCPO (0.5 wt %) was added to the melt which was held at 160°C for 10 min followed by quenching with compressed air in an attempt to

achieve some grafting of PHBHV and PCL to get compatibilization of the PHBV and PCL phases on cooling. It was believed that the organic peroxides added during melt processing induced chemical interactions between PHBV and PCL to form a compatibilizing graft polymer.[122b]

Transesterification

Transesterification is another approach to forming a compatibilizing copolymer *in situ* from a PHA and another polyester. This must be done carefully because of the thermal sensitivity of PHAs and the high temperatures normally required for ester–ester interchange.[122a,b] Some examples of transesterification experiments are given below. Mixtures of equal weights of PHB with PHB27HV or PHB20HV containing 1 phr of additive [Ti tetra-*n*-propoxide (TNP), Ti tetra-*n*-butoxide (TNB), or Sb2O3 (AO)] were extruded into chips and then injection molded into tensile and impact test bars. The additives lowered the melting temperature by up to 9°C and increased the crystallization rates with little change in mechanical properties. The additives also cut the MFI of the blend in half and seemed to reduce thermal degradation of the blends by reducing the bond scission frequency by about 33%.[124]

Mixtures of PCL (M_n 27 k), PHB (M_n 780 k), and Zn(OAc)2 were heated at 180°C for 3 h in sealed tubes to form copolymers. When the CL incorporation is <15%, the m.p. of the copolymer is > 150°C; when CL rises to 22%, the m.p. of the copolymer drops to <65°C.[128] Equal weights of PCL (M_n 70 k) and PHB (M_n 100 k) with varying levels of Zn acetate (0, 0.1, 0.5, 1.0, and 3.0 parts by weight) were extruded at 200°C. The amount of copolymer formed was 0, 2, 15, 28, and 32 wt %, respectively. The percent elongation of extruded films was 50, 100, 350, 400, and 400%, respectively.[127]

A mixture of 80% PHB6HV and 20% PCL (Tone 787) containing 1 phr BN and 1 phr catalyst was extruded at 170°C and granulated into polymer chip. The granules were injection molded into tensile test bars. The samples were 6 weeks old when tested. Elongation to break test results are given as the percent increase compared with the control sample (no catalyst). The results provide evidence that TNB and TNP are catalyzing a transesterification reaction.[114]

Attempts to prepare equal weight blends of PLA and PHB27HV with 1 phr additive (TNB or AO) by extrusion through a 5-mm circular die at 208°C were mainly unsuccessful. The PLA and PHBV were mutually incompatible in the melt and amorphous phases as evident from the multiple T_g corresponding to PHB27HV (−5.9°C) and PLA (58.5°C). However, with AO, a third T_g did appear at 24°C, which is the T_g calculated for the mixture by the Fox equation. Therefore, heating provided a chemical change within a portion of the composition and produced a compatible polymer system.[114]

Equal weights of PET (M_n 15 k) and PHB (M_n 37 k) in nitrobenzene and a Bu2SnO catalyst heated at 170°C for 24 h gave a polymer that melted at 205°C, had an M_n of 22.5 k, and was reported to have good biodegradability.[193]

Block Copolymers Using Hydroxy-terminated PHAs

PHB can be degraded by acid-catalyzed methanolysis to form low molecular weight [average degree of polymerization (DP) = 26] stereoisomerically pure PHB chains. The hydroxyl terminus of these polymers was reacted with AlEt3 to form a PHB-*O*-AlEt2 macroinitiator species. These macroinitiators were then used to perform the ring-opening polymerizations of e-CL and lactide monomers to prepare PHB–PCL, PHB-D,L-PLA, and PHB-L-PLA A-B diblock copolymers of variable chain segment lengths. It was shown that for diblocks (PHB DP = 26 in all cases) with short PCL (DP = 12) and L-PLA (DP = 13) chain segments, PCL and PLA crystalline phases did not form. With increased chain length of the B chain segment (PCL DP = 38 and 51, L-PLA DP = 23), the crystallization of both components of the diblock was observed as a superposition of the respective X-ray diffraction patterns. The PHB-L-PLA diblock (PHB DP = 26, L-PLA DP = 23) when melted and rapidly quenched from the melt was kinetically frozen into a solid-state morphology such that miscibility of the two component chain segments resulted (a new T_g at approximately 20°C was observed). Phase separation occurred when the diblock was annealed at 55°C for 24 h before DSC analysis. Study of a blend of PHB and L-PLA which simulated the chain lengths and weight fractions of the PHB-L-PLA (DP = 23) diblock components clearly showed the effects of the covalent linkage between the respective chain segments of the diblock in decreasing the rates of crystallization and phase separation.[181]

To a solution of PHB4HV in diglyme was added a tenfold excess of ethylene glycol (EG) and dibutyltin dilaurate catalyst after which it was heated

at 140°C for 7.5 h with four additions of catalyst during the heating period. The macrodiol isolated in 74% yield had an M_n of ~ 2300 and an m.p. of 140°C, which was considered the "safe" limiting temperature, above which thermal degradation of PHB4HV takes place. A T_g for the amorphous fraction could be detected at −14°C by DSC. The M_w/M_n ratio was ~ 2, based on gel permeation chromatography calibrated with PS standards. The endgroup frequency was 39.1% primary hydroxyl, 58.5% secondary hydroxyl, and 2.4% crotonic acid ester. The oligomers were considered well suited for the preparation of high molecular weight block copolymers by chain extension.[182]

The PHB4HV-diol just described[114] was used as the "hard segments" and PCL-diol or a PAd-*alt*-1,4-BD-DEG-EG-diol used as "soft segments" with TMDI or LDI for synthesis of high molecular weight micro-phase-segregated block-copolyesterurethanes.[183] The Young's modulus (between 40 MPa and 1.3 GPa) was found to depend directly on the fraction of crystallizable PHB4HV-diol in the block copolymer whereas the type of noncrystallizable segment or diisocyanate had only a minor influence. Generally, the tensile strength increases and the elongation at break decreases with increasing content of the PHB4HV-diol. The chain length of the noncrystallizable segment indirectly influences the morphology and the mechanical properties of the polymers through changes in phase-segregation behavior. The block copolymers can be used for degradable implant materials that can be sterilized and its time in use varied between several days and several years by selection of the "soft segment."[183] The random hydrolytic cleavage of the amorphous part of these polymers might result, *in vivo,* in the production of small crystalline particles of low molecular weight PHB that could then undergo phagocytosis and biodegradation inside phagosomes. It was shown that macrophages are able, *in vitro,* to phagocytize and degrade the PHB-diol segment polymers as degradation products were found in extracts of cell supernatants after 8 days of incubation.[184]

The ability of polyethylene glycols (PEGs) to control molecular weight of PHB during the microbial fermentation polymerization process using *A. eutrophus* with fructose as the sole carbon source has been reported.[185] The interaction between PEG and the PHA production system leading to molecular weight reduction was enhanced for lower molecular weight PEGs and required at least one PEG chain end functionality which may be a hydroxyl group. It is believed that PEG interacts with the *A. eutrophus* synthase in such a way as to increase the rate of chain termination by water relative to chain propagation reactions. Analysis of all PHB products by ^{1}H-NMR showed that they did not contain PEG terminal groups. Thus, for the *A. eutrophus* strain used, molecular weight reductions of P3HB by PEG are not caused by chain termination reactions by PEG.[186] This is in contrast to an earlier study in which PEG-200 was found covalently linked to the carboxyl chain terminal end of high 4HB containing chains formed by *A. eutrophus.*[185]

Solution Spinning of Fibers

Monofilament

PHB fibers have been prepared by spinning 10–20% solutions of PHB in ethylene or propylene carbonate at 8 m/min into a 50 : 50 water-alkylene carbonate precipitating bath.[125]

Nonwovens

A 10% solution of PHB8HV in chloroform is poured through a 3-mm-diameter tip into a precipitation bath of methanol which is stirred gently; the polymer solution mixed with the circular flow of methanol instantaneously starts the precipitation and produces a steady stream of a suspension of fine highly elongated fibrils having a diameter between 10 to 500 microns and a length from 3 to about 30 mm. The fibrils are collected to form a continuous nonwoven fabric by filtering on a mesh screen. The resulting fabric is dried and heat cured in an oven at 120°C for 1 h. The fibrils are collected on a cardboard mat until the thickness is about 0.5 cm. More than 50% of the fibrils are 5- to 20-mm long with the longest up to 100 mm. The mat is pressed at 1000 lbs. of force in a Carver Press for 10 min at 5°C below the melting temperature of the PHBV. The resulting nonwoven fabric can be used as a topsheet in a compostable disposable diaper. Compostable scrub suits for use by surgical staff can be made by sewing the nonwoven fabric in the design of a pullover shirt and pants with a waist drawstring.[133]

Dry Spinning of Fibers

Centrifugal Dry Spinning of Fibers

Solutions of PHB (10–20% w/v) in a solvent such as CHCl3 and CH2Cl2 can be dry spun. To change

the hydrophobic PHB fibers to hydrophilic fibers, 1–2% w/v of a surfactant such as Empilan CDE, a coconut oil derivative, can be added to the solution. The solution, at 60°C, is pumped into a down-flow centrifugal spinning rig in which continuous filaments are carried by a blast of hot air onto a porous conveyor. Solvent evaporates as the fibers fall. Fusion can be controlled by operating at conditions in which filaments have some level of solvent and/or are contacted under varying amounts of compaction. The product can vary from a fleece of entangled long fibers, where entanglement of long fibers gives a low degree of cohesion, to, at the other end of the scale, a highly melded gauze having dimensional stability, determined by the strength of the fibers themselves.

Air-Blown Spinning of Fibers

A 10% solution of PHBV in solvent is poured through a 3-mm-diameter tip to flow vertically in air without any support for about 5 cm to attain a steady thin continuous strand of the solution and subjecting it to a sudden downward jet flow of air. The air jet applied parallel to the downward free flow of the solution instantaneously evaporates the solvent and produces a rapid steady downward stream of fine highly elongated fibrils of about 10 microns in diameter and length from 3 to 25 mm. The fibrils are collected to form a continuous nonwoven fabric over a mesh screen located 70 cm away from the point the air jet is applied. A 0.5-cm-thick fibril mat is placed in a Carver Press and pressed at 1000-lb. force for 10 min at a temperature 5°C below the melting temperature of the PHBV. The nonwoven is used as a topsheet in a compostable disposable diaper and also to make surgical scrubs.[134]

Gel Spinning of Fibers

A free-draining gel is formed from a solution of crystallizable high molecular weight polymer under conditions that cause crystallization of the polymer, and is subjected to nonrandom deformation to expel the solvent and form a shaped article.[135] Levels of cell attachment on gel-spun PHB fibers was extremely low. Cell viability pre- and post-testing was 90%. On alkali-treated fibers, cells exhibited fully spread morphologies and high level of attachment and subsequent adhesion possibly because of polymer degradation and creation of new surface.[204]

Melt Spinning of Fibers

Successful melt spinning of PHB and PHB*x*V requires careful control of crystallinity during the drawing step. The polymer and copolymers do not strain crystallize, so fibers cannot be drawn from the amorphous melt. Neither can fibers be drawn from a highly crystallized state because the preform is brittle and can only be drawn to a very small extent before it breaks; i.e., the elongation to break is low. If, however, at the time of drawing, the PHB polymer has a degree of crystallinity in the range below that at which it becomes brittle, then the preform can be drawn without breaking, but it is rubbery and on release of the drawing force, the product relaxes almost completely. Relaxation can be prevented by effecting crystallization of the polymers while maintaining the drawing force until the polymers become nonrubbery. If the crystallinity is too low, then the polymer tends to be sticky, creating handling problems. Thus, careful control of time and temperature to produce the correct level of crystallinity for successful orientation has enabled oriented fibers to be produced with increased tenacity and extension to break. The orientation achieved depends on the level of crystallinity (i.e., a window for orientation exists), the HV content (in general a higher HV content material that crystallizes more slowly will pass through the orientation window more slowly facilitating orientation), and draw speeds.[136] Nucleating agents can be added to the PHB to increase the crystallization rate and decrease residence time in processing steps.[137]

Monofilament

PHB pellets containing 1 phr BN were melt extruded at 185°C through a circular orifice 1.585 mm in diameter into a water bath (quench) at 60°C. The monofilament was taken by a haul-off roller at 0.026 m/s, passed around a pin at 120°C, and from the pin drawn into fiber by a second haul-off roller. The drawn fiber was then passed over a plate heated at 60°C (conditioning zone) and onto a windup roll. With a quench time of 1 s, the polymer stuck to the haul-off roller and pin, preventing drawing. With a quench time of 6 s and a conditioning time of 10 s, a maximum draw ratio of 8 was achieved to give fiber of 346 denier with a tensile strength of 209 MPa, a tenacity of 1.98 g/d, and extension to break of 40%. With a quench time of 20 s, the maximum draw rate was minimal and good fibers were not produced.[137]

Table IX Properties of Multifilaments of PHBV Containing Different Levels of HV and the Plasticizer

Polymer	BN (wt %)	GTA (wt %)	Draw (Ratio)	Tensile Strength (MPa)	Tenacity (g/d)	Biodegradability
PHB6HV	1	0	2	381	3.6	Good
PHB6HV	1	10	2.8	444	4.2	Good
PHB15HV	1	0	2.9	338	3.2	Good
PHB15HV	1	10	3.5	402	3.8	Good
PHB25HV	1	0	4.2	444	4.2	Good

Monofilament of 3 g/d tenacity (317 MPa tensile strength) has been reported by Kogyo Shinbum.[139]

PHB8HV was melt spun, quenched in water at 50°C, wound, treated at 150°C, drawn 7×, and stretched 1.1-fold at 100°C to give a biodegradable fiber with breaking strength 310 MPa and knot strength 290 MPa.[141,142] Wide-angle X-ray scattering studies showed that the fibers possess bimodal chain orientation with two populations of crystals. In the first population, the chain axes are oriented primarily along the fiber axis and in the second population the chain axes are oriented in the transverse direction to the fiber axis. At the annealing temperature of 100°C, the relative population of the crystals oriented normal to the fiber axis was found to be large, and their proportion decreases with an increase of annealing temperature. The tensile strength of these fibers improved by the increase of the proportion of the transversely oriented chains.[141]

PHAs (e.g., PHBV) are melt extruded at 100–250°C, crystallized in a water bath at 20–80°C, preheated to 100–200°C, drawn at 40–130°C, then at 60–150°C to give high-strength biodegradable fibers of *c*-axis-oriented crystals combined with other crystals.[200]

P3HB(13)4HB was melt spun at 162–180°C to give fibers with improved impact and heat resistance, with tensile strength of 0.16 g/d and elongation at rupture of 119%.[144]

PCL (m.p. 70°C) was blended with an equal amount of PHBHV (m.p. 164°C) which increased the m.p. of the blend to 165°C and was melt spun and stretched 7× at room temperature to give fibers with a diameter of 0.27 mm, a tensile strength of 17.8 MPa, and a knot strength of 12.0 MPa.[145]

PCL and PHB (80 : 20) were blended, melt spun, wound in water at 8.4°C, drawn 4× in water at 30°C, drawn 1.17× in air at 50°C to give a 161-d monofilament fiber with a tensile strength of 646 MPa at 23°C. The fibers are useful for wearing apparel, fishnets, etc.[195]

PCL and PHBV were cospun in a 1 : 1 weight ratio, cooled in water at 15°C, drawn 6× in water at 60°C, drawn 1.3× in an oven at 100°C, and relaxed at 100°C to give a 0.31-mm bicomponent monofilament with a tensile strength of 703 MPa, breakage temperature of 112°C, and good biodegradability.[143]

Biodegradable composite fibers can be prepared from a core of PHBV and a sheath of polybutylene succinate or polyethylene succinate (m.p. 70°C).[147]

Hydrolytic degradation of PHBV monofilament fibers was studied at pH 7.4 and 37, 60, and 70°C. The monofilament weights remained constant at 37 and 60°C, whereas onset of weight loss was observed at 70°C after 60 days, at which time the tensile strength fell to 0 (from 1300 MPa). The tensile strength followed a two-stage curve, where the tensile strength began to decrease at M_n of ~ 70 k and reached 0 at M_n of ~ 17 k.[146]

Multifilament

Biodegradable multifilaments of PHB*x*V with tensile strength >2.0 g/d have been prepared by melt spinning at 140–220°C, cooling by air to 40–80°C, and drawing >1.2× in one or more steps. For example, PHB6HV containing 1 part BN, 10 parts glycerol triacetate (optional) was melt spun at 180°C through a 0.3 × 36 mm spinning nozzle, cooled to 60°C, lubricant added, and drawn between rollers at 100°C and unheated rollers to give multifilament with 200 denier/36 filaments at 10 to 500 m/min and good biodegradability. Biodegradability was determined by burying a sample in soil for 2 months; if the multifilament-filament lost its shape or 50% of its original tensile strength, biodegradability was considered good.[139] Results are shown in Table IX.

In a similar set of experiments, PHB6HV (M_w 750 k) blended with varying amounts of PCL (M_w 80 k) was spun to give 200 denier/36 multifilaments with good biodegradability. At 20% PCL, 1 wt % BN, 10 wt % GTA, and a draw ratio of 7.0, the tenacity was 5.8 g/d.[148]

Nonwovens

Heat-adherable biodegradable bicomponent fibers were prepared by co-spinning a 1 : 1 mixture of PCL and PHBV at 260°C, winding, drawing twice, crimping, lubricating, drying, and cutting to give 5-denier staple fibers with a tensile strength of 473 MPa. The fibers were formed into a web, heated at 70°C to give a nonwoven with a tensile strength of 2800 g/cc, and good biodegradability.[149]

Ultraviolet-degradable, biodegradable composites have been made consisting of a nonwoven blend fabric of 50% crimped composite fibers with PHBV as sheath and PET containing 3.5% TiO2 as core at sheath/core of 50 : 50 and 50% rayon; the rayon decomposed extensively when left in the sun for 1 month and then buried in soil for 4 months.[150]

Centrifugal melt spinning of fibers. For melt spinning, PHB can be fed from an extruder or a pressurized heated vessel into a down-flow centrifugal spinning rig as described above. The melt-spun fibers can be fused together by bringing them together before they have cooled sufficiently to prevent fusion.

Extrusion melt-blown fibers. PHB or PHB12HV is extruded by using a twin-screw extruder equipped with a capillary die with a 0.5-mm diameter. The extruder speed is set to 5 rpm, and the extrusion temperature is 180°C. The extradite is allowed to flow down vertically in air without any support for about 50 cm to attain steady, continuous strands of molten resin before being subjected to a sudden downward jet flow of air. The air jet applied parallel to the downward free flow of molten resin produces a rapid steady downward stream of fine highly elongated fibrils having a diameter between 10 to 100 microns and a length from about 5 to 50 mm. The fibrils are collected to form a continuous nonwoven fabric over a mesh screen located 30 cm away from the point the air jet is applied.

Coated nonwovens. Biodegradable fiber webs can be produced from biodegradable fibers and biodegradable thermoplastic binders. For example, spreading 3% of a biodegradable PHBV powder (m.p. 160°C) on a rayon fleece (10 g/m^2) and hot-roll pressing at 170°C and 50 kg/cm gave a biodegradable nonwoven.[151]

Biodegradable paper-based laminates can be produced by coating a paper substrate with PCL, drying, extrusion coating with PHBV, and aging for 1 week to give a laminate with good interlayer adhesion for making cups with good biodegradability.[152] Laminates useful for cups, trays, and cartons may also be prepared by coating a paper substrate with a polyester adhesive (e.g., THW 3257), laminating with PHBV, and aging for 1 week to give a laminate with good adhesion that is degradable in soil.[153]

The use of PHB as a matrix in the preparation of natural-fiber composites to produce a completely biodegradable material has been reported.[154] For example, lightweight disposable 1-L containers for drinking water can be made from paperboard walls containing 30% PHB uniformly dispersed and with the inner surface covered with a film of PHB. The sterilizable package had a surface weight of 325 g/m^2, whereas a 1-L container made of laminated material of comparable strength had a surface weight of 400 g/m^2.[155]

Cellulose fibers processed with molten PHB showed a dramatic fiber-size reduction during processing. The size reduction was related to the degree of processing and could be correlated with the amount of crotonic acid produced during the thermal degradation of PHB. Samples processed with and without cellulose were analyzed, and the processing of cellulose was found to contribute to a greater amount of chain scission perhaps by local overheating as a result of shear forces developed during processing and hydrolysis of cellulose fibers by the acid during compounding.[156]

Paper and paperboard laminated with PHBV are manufactured by continuously casting a melt of PHBV to the surface of paper or board while attaching a release film to the polyester surface which can be removed after cooling.[157]

Biodegradable cards in which PHBV is incorporated into paper as a finishing agent (e.g., size) or used as lamination sheets, for improving service strength, have been produced.[158]

Other Melt Processing

PHAs can be melt processed on conventional equipment because their melting points cover a range similar to other thermoplastics including

PE and PP. A range of fabrication processes has been demonstrated including extrusion, injection molding, extrusion blow molding, thermoforming, oriented and unoriented cast and blown films, mono and multifilament spinning, coating, calendering, and foaming.[138]

Solid-state extrusion of PHBV has been performed at temperatures below the melting temperature (e.g., 135–150°C), depending on the HV content. The solid-state extrudates showed an extra melting endotherm about 15–20°C above the normal melting temperature, which became increasingly dominant at lower extrusion temperatures. The solid-state extruded samples did not show significant chain orientation along the extrusion direction. When the extrusion temperature was raised closer to the melting temperature, the quality of the extrudates improved, as reflected in the mechanical properties.[198]

Extrusion

Free-standing PHBV films have been obtained by coextruding PHBV between two layers of sacrificial polymer (e.g., polyolefins), stretching and orienting the multilayer film, and then stripping away the polyolefin layers after the PHBV has had time to crystallize. The PHBV film can be laminated to other films (e.g., water-soluble films) for applications.[160]

In another approach, multilayer films are coextruded in which PHBV is the internal layer surrounded by outer layers of biodegradable films for use as diaper films. The external layers are not stripped away, but remain as an integral part of the biodegradable multilayer film.[161]

In another process, PHBV compounded with plasticizers, nucleating agents, and/or other additives is extruded on a preformed supportive bubble of ethylene vinyl acetate resin or LDPE.[162]

Molding

Injection molding can be performed by using a conventional injection-molding machine with a PE type screw of length/diameter (L/D) ratio of 20 : 1. The shot capacity should be close to the weight of the molding to avoid long residence time in the barrel and consequent thermal degradation. Mold heating of 60°C is required for rapid crystallization and acceptable cycle time.[138]

Extrusion blow molding can be performed on a conventional single or multi-head machine, with a PE type screw of L/D ratio of 24 : 1, and mixing tip. A compression zone after the spider avoids problems such as weld lines. Mold and blow pin head temperatures of 60°C are required for rapid crystallization and acceptable cycle time.[138]

Applications of PHAs

Fishing Lines and Nets

Monofilaments useful for fishing nets, ropes, and marine agriculture are produced from PHBV having a $M_w \sim 400$ k by a process in which resin is molten for <6 min at $T_m = T$ m.p. + 15, spun at a shear rate >2 × 10e−2/s above the filter layer in the spinneret pack, and has a residence time in the pack <2 min to give monofilaments, which are solidified at $T_c \pm 10$°C for 5–10 s, and stretched.[140]

In another example, drawn fibers of PHB8HV were twisted to obtain ropes and used in nets for crab cages. The nets showed good strength and biodegradability in the sea.[159]

The tensile strength of PHBVs increases with decreasing HV content. PHBVs with (0–10%) HV exhibit tensile strength comparable to LDPE and PP. Orientation further increases this property. Fishing nets require good tensile strength. The relatively slow biodegradation rate of PHBVs in sea water permits a useful lifetime of articles such as fishing nets. On disposal, or loss, the PHBV net (PHBV density ~ 1.2) sinks to the sea bed where more rapid biodegradation is expected to occur in the more microbially active silt.[72]

Studies of PHA films, both in sea and freshwater by SEM show rapid colonization by bacteria and microalgae, including several species of diatoms. Very few hyphae were seen on the films even after >30 days in water, which contrasts with previous studies by the author (1992) of PHA degradation in soil where fungal hyphae were far more numerous. Signs of decomposition, such as etched areas associated with bacteria were found. Microalgae, although frequently observed, appeared to use the PHA films as a growth support rather than degrading it. Degradation was faster in aquatic medium than in soil.[163]

To take advantage of their respective properties, high-density polyethylene as the core and PHBV as the sheath were melt spun at a 60 : 40 vol ratio to give fibers with a tenacity of 5.4 g/d and exhibiting a very small amount of adhesion of marine substances on immersion of a net of the fibers in seawater for 6 months.[164]

Other Marine Applications

A biodegradable net of spun monofilaments of Biopol coated with polyvinyl alcohol (PVOH) was

used as a host for growing seaweed from seedling. Seaweed survival and tensile strength retention were 60% and 70%, respectively, after 3 months outdoors versus 15% and 32%, respectively, for the uncoated Biopol.[165]

Blends of PCL and PHBV have good anti-algae properties and are useful as nets for seafood cultivation.[192]

Medical Applications

Dry and melt-spun nonwoven PHB fibers are suitable for medical applications. PHB and PHBV can be steam sterilized without losing properties and are resistant to alcohol.[166] If sterilized, they may be left in place to aid blood-clotting without the rejection problems associated with cotton materials. Swabs, pads, or other articles made from PHB and left in the body by design or by accident will not cause toxemia and are slowly absorbed by the body. Being hydrophilic, they will take up aqueous liquids. They differ from cotton wool in having little or no tendency to break off small fibers, but even if small pieces were to enter a wound, they would be safe. There is no need to enclose them in a retaining gauze, and they can be readily tailored to size at the point of use.[167]

PHB is very biocompatible, producing an exceptionally mild foreign body response. A monofilament surgical suture degrades very slowly[169] and could require several years to be totally resorbed by the body. The time required for biodegradation is related to surface area and multifilament sutures resorb much more quickly.[23]

Monofilaments of PHB and PHBV were studied *in vivo* and *in vitro* and assessed for changes in mechanical properties and topography. *In vivo* biodegradation was observed only with PHB when pre-degraded by 10 Mrad of gamma-irradiation before implantation. High-temperature *in vitro* hydrolysis suggested that HV copolymer addition retarded the rate of degradation of PHB. Hydration reactions had the most effect on the ultimate properties of the materials. In contrast, the elastic properties appeared to be relatively unaffected.[169] Thus, it was concluded that neither PHB nor PHBV in monofilament form is very biodegradable, although susceptibility to degradation may be increased by exposure to gamma radiation.[170]

Levels of cell attachment on gel-spun PHB fibers were extremely low. Cell viability pre- and post-testing was 90%. On alkali-treated fibers, cells exhibited full spread morphologies and high level attachment and subsequent adhesion possibly because of polymer degradation and creation of new surface.[173]

PHB has been gel-spun into a novel form with one possible application as a wound scaffolding device, designed to support and protect a wound against further damage, while promoting healing by encouraging cellular growth on and within the device from the wound surface. The nonwoven combines a large volume with a low mass and is called "wool" because of its similarity in appearance to "cotton wool." The hydrolytic degradation of the wool was investigated in an accelerated model of pH 10.6 and 70°C. The PHB wool gradually collapsed during degradation which was characterized by a reduction in T_g, m.p., and a fusion enthalpy peak of maximum crystallinity (88%) which coincided with the point of matrix collapse.[174] An enzymatic assay of HBA monomers (HBA dehydrogenase converts NAD to NADH which is associated with an increase in light absorption at 340 nm) was used to monitor hydrolysis of gel-spun PHB fibers.[175]

Adsorbable polymeric scaffolds for cell culture and transplantation, tissular reconstruction, and *in vivo* drug or protein release have been proposed by salt-leaching/solvent-casting a PHBV. Electron microscopy and mercury porosimetry show highly porous, well interconnected microstructures with a porosity level of 0.85 and a mean pore diameter of 122 microns. The weight loss of the porous structures is about 50% for a 140-day period of hydrolytic degradation in phosphate buffered solutions, pH 7.4 at 70°C. Incubations from 1 to 35 days of canine anterior cruciate ligaments (ACLs) fibroblasts in scaffolds have shown a limited proliferation rate ($150 \times 10e6$ cells/g maximal d.) but high protein synthesis ($2.4 \pm 0.1 \times 10e{-}2$ ng/cell day at day 28). Additional work is underway on potential applications for orthopedic reconstructions.[176]

The hemocompatibility of a material can be evaluated through the study of adsorption of proteins stimulating the thrombus formation. Films of PHBV were treated with perfluorohexane and H2 plasmas. It was shown that hydrophobic polymer films adsorb more albumin than fibrogen. Moreover, the amounts of protein adsorbed seem to be mainly a function of the surface roughness of the films, the highest amounts being adsorbed on the rougher plasma-treated films, whereas the smoother perfluorohexane-treated surfaces adsorbed less proteins.[177]

PHBV films (7, 14, and 22% HV) were analyzed for *in vitro* cytotoxicity and aqueous accelerated degradation, *in vivo* degradation, and tissue reactions. The PHBV materials and extracts were found to elicit few or mild toxic responses, did not lead to *in vivo* tissue necrosis or abscess formation, but provoked acute inflammatory reactions slightly decreasing with time. PHBV shows low rates of degradation *in vitro* as well as *in vivo.* The weight loss rate generally increases with the copolymer (HV) content and ranges from 0.15 to 0.30 (*in vitro*) to 0.25%/day (*in vivo*). Compositional and physicochemical changes in PHBV materials were rapidly detected during the accelerated hydrolysis, but were much slower to appear *in vivo.* The structural and mechanical integrity on the PHBV materials tend to disappear early *in vitro* as well as *in vivo.* After 90 weeks in dorsal muscular tissue of adult sheep, 50–60% of the initial weight of the PHBV polymer still remained.[179]

Biocompatibility and degradation mechanisms of block co-polyurethanes containing telechelic PHB segments have found growing interest for possible biomedical applications for these materials. The random hydrolytic cleavage of the amorphous part of these polymers might result, *in vivo,* in the production of small crystalline particles of low molecular weight PHB that could undergo phagocytosis and biodegradation inside phagosomes. Studies suggested the macrophages could degrade the low molecular weight PHB.[201]

Resorption of PHB, PLA, PGA/C, and PDS sutures in sheep and rats was studied versus implantation site and species.[159] PHBV resorbs in 6–12 months versus 2–3 weeks for PLA. Sutures of P3HB4HB have been prepared and their properties studied.[171,172]

A review on synthesis and uses of PHB and PHBV as biodegradable thermoplastics and biodegradable fibers and textiles as well as their potential use in medicine has been published.[178]

Other Applications

A spun fiber felt composed of 50 wt % nylon-6 endless filaments and 50 wt % of PHBV is used as carbon and hydrogen source in anoxic water and is coated with spontaneously growing denitrificants.[168]

Biodegradable paper substrates for magnetic identification cards comprise a biodegradable plastic fiber (PHBV) and a natural fiber (e.g., cellulose).[180]

Non-fiber Applications

The combination of biodegradation, toughness, chemical resistance, useful barrier properties, and good storage performance facilitate PHBV use as a packaging material. Either 100% PHBV (e.g., as a film) or a PHBV coating (e.g., on paper, board) can be used.[102]

Recycling

Biopol can be recycled as regrind. Recycling PHAs by composting offers an additional waste management option where material recycling is not a viable option for technical, economic, or environmental reasons. Composting PHAs generates a useful product, valuable as a soil conditioner.[187]

Microbial degradation of tensile test pieces made of PHB, PHB10HV, and PHB20HV has been studied in small household compost heaps. It was concluded on the basis of weight loss and loss of mechanical properties that PHB20HV degraded much faster than PHB10HV and PHB and that there were 109 microbial strains capable of degrading the polymers in the composts. No decrease in M_w could be detected during the degradation process.[188]

A scheme for recycling has been described recently in which PHB is subjected to catalytic depolymerization in solution using Bu2Sn(OMe)2 or *p*-toluenesulfonic acid as catalyst to yield the cyclic oligomer, (*R*,*R*,*R*)-4,8,12-trimethyl-1,5,9-trioxacyclododeca-2,6,10-trione (TBL). Polymerization of TBL in the melt with Bu2Sn(OMe)2 as initiator produced poly[*R*-3-hydroxybutyrate] of low molecular weight ($M_n = 5 \times 10e3$).[194]

CONCLUSIONS

A number of linear polymers made by biosynthetic processes have unique and commercially useful properties. The major classes are the proteins (polyamides), polysaccharides (polyglycosides), and polyhydroxyalkanoates (polyesters).

Various spider silks (proteins) have extraordinary mechanical properties. Genetically engineered silk-like proteins can be produced and the composition varied to enhance desired properties.

A number of linear polysaccharides, especially cellulose, are commercially important. Bacterial cellulose, because of its unique morphology has been used to prepare speaker diaphragms with excellent acoustical properties.

Significant advances have been made in the biosynthesis of PHAs. At least 91 HA monomers have been identified as substrates for PHA synthases. Progress has also been made on melt processing of PHAs, PHA blends, and PHA block copolymers into fibers, films, and molded articles.

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