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Formulation of a Biodegradable and Biosynthetic Latex Paint

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Appendix D - UNIVERSITY HONORS PROGRAM
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FORMULATION OF A BIODEGRADABLE AND BIOSYNTHETIC LATEX PAINT

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Formulation of a Biodegradable and Biosynthetic Latex Paint

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1. ABSTRACT

In this study, I developed a formula for a biosynthetic and biodegradable latex paint, which would lead to an easy, environmentally safe, and effective removal method by microorganisms as well as an environmentally benign manufacturing process. The key ingredient in the formula of a biodegradable/biosynthetic paint is the polymer emulsion. The emulsion used in this study is based on the copolymer PHBV, a combination of 3-hydroxybutyrate and 3-hydroxyvalerate which are thermoplastic polyesters that can be biologically synthesized by bacteria. The biodegradable latex formula is based upon a control latex paint containing an emulsion similar to the biodegradable polymer. The emulsion used in coating applications with the closest relevant characteristics to PHBV is based on a copolymer consisting of butyl acrylate and methyl methacrylate. By designing a control paint with this copolymer, I determined a formula and an experimental protocol for the production and testing of a biodegradable/ biosynthetic latex paint. The essential properties of the paint are tested through various treatments such as scrub and water tests, while the biodegradability of the paint is determined through tensile testing.

2. INTRODUCTION

Present paint removal techniques suffer from several disadvantages: inefficiency, high costs, environmental concerns, and explicit operating conditions. However, the existence of a paint which exhibits biodegradability under certain restricted environmental conditions could easily be removed in an environmentally safe manner using naturally occurring bacteria. To obtain a biodegradable latex formulation, it is first necessary to identify the ingredients of latex paint and decide what key ingredient(s) allows the coating to biodegrade. The next step is determining and locating the appropriate biodegradable key ingredient(s). The final step involves formulating a coating around the key ingredient(s), making sure the coating has essential properties such as adherence, ease of application, and consistent quality.

Several studies have already shown that there is potential for formulating a biodegradable latex, especially in the formation of paper coatings and other film formations. In paper coating applications, biodegradable biopolyesters applied by a powder coating approach showed good barrier properties and good adhesion between the polymer and the paper fibers (Marchessault 1993). Another study experimented with emulsion film suspensions on impervious substrates. Hot-pressing of the film converted the "cake like" structure suspension into a transparent, flexible film which biodegraded much faster than solvent cast films (Lauzier 1993). The general consensus among various researchers in the field is that coating and other film applications of biodegradable latex require blending and various treatments to obtain the desired properties.

The ingredients of a latex paint are the emulsion which provides continuity and adhesion through film formation, the solvent which provides low viscosity for application,

and the pigments and additives which add to or change the paint properties. The key ingredient in the formula of a biodegradable paint is the biodegradable polymer emulsion. An emulsion is a dispersion of solid polymeric particles in a continuous aqueous phase. As the paint dries, the solvent and the aqueous phase of the emulsion evaporate and the polymeric resin particles are crowded together. Gradual coalescence of the particles continues until equilibrium is reached. The result is an adhesive film of polymer with characteristic structure and mechanical properties. Since the polymer is the largest component of the dry paint and is also the source of adhesion, it is the ingredient which must be biodegradable.

There are two important types of biodegradable polymers, polylactic acid (PLA) and polyhydroxyalkanoates (PHAs). PLA is produced through the self-condensation of lactic acid and also breaks down in the environment to lactic acid. Recent research has shown that the initial monomer, lactic acid, can be produced from bacterially fermented starch obtained from food wastes (Fried 248). Another important class of biopolymer emulsions that has experienced extensive research and development is the PHAs. This class of thermoplastic polyesters is biologically produced by serving as an energy reserve by numerous organisms. PHA deposits accumulate as distinct granular inclusions in response to nutrient limitations, and therefore can be enzymatically degraded by microorganisms when the limitation is removed. In addition, extracellular PHA can also be utilized by organisms as a souree of energy. PHAs include a wide variety of monomers. The most appropriate emulsion to use in a biodegradable latex is the copolymer PHBV, a combination of 3-hydroxybutyrate and 3-hydroxyvalerate. The combination of these two polymers increases the extension to break and lowers the glass transition temperature, the

temperature at which the polymer changes from a hard glassy state to a tough rubbery state. This improved flexibility and toughness is an important characteristic of a latex emulsion.

The biodegradable latex formula is initially based upon a control latex paint containing an emulsion similar to the biodegradable polymer. By starting with this conventional latex formula, a good estimate of ingredients and amounts can be made to maintain the essential properties of an effective paint. An emulsion found in existing paints showing very similar characteristics to the PHBV emulsion needs to be found first. Many emulsions are suitable for design and formulation of paint, but the important binding ability of coatings is a function of particle size, molecular weight, and glass transition temperature (T_g). This temperature characterizes a polymer and predicts its end uses. Other properties of paint that can be effected by T_g include durability, tensile strength, elongation, and adhesive properties. Due to its importance in the characteristics of film formation, the glass transition temperature is the key property in finding a comparable polymer to the PHBV emulsion (T_g = 5°C). All PHA polymers have characteristics similar to polypropylene, with a melting temperature around 175°C and a crystallinity of about 80%. The emulsion used in coating applications with the closest characteristics to polypropylene and hence PHA is a combination of methyl-methacrylate and butyl-acrylate. The average T_g of this emulsion is 0°C, but can be modified by changing the weight percentage of the copolymers. Therefore, I chose methyl-methacrylate/butyl-acrylate as the emulsion used in the control formula.

A conventional latex paint formula involves the emulsion, a pigment slurry, water, thickener, defoamer, ammonia, and preservative. The water exists as the solvent, acting as

the vehicle for application and evaporates upon drying. The thickener gives the latex paint the desired viscosity, and the defoamer is added to the formula to keep fine bubbles from forming while mixing. The preservative is added to keep bacteria trapped in the can from growing and spoiling the paint, and ammonia is added to keep the paint at the desired pH.

3. BACKGROUND

3.1 Paint Removal Techniques

The are a number of reasons why the removal of paint is desired: to apply a new coating, to inspect the substrate surface, to clean the substrate surface, and if the paint could cause environmental difficulties at the time of disposal. There are several present paint removal techniques, but each of them can be associated with several disadvantages. Table 1 summarizes some of the advantages and disadvantages of several paint removal techniques.

Technique	Advantages	Disadvantages
Biodegradation	• Ambient operating conditions • Effective • Environmentally safe • Low costs • No damage to substrate	• Potential to biodegrade in normal conditions
Chemical Solvent	• Effective for currently available paints	• Surface preparation • VOCs, high emissions • Slow removal • High temperature often required
Lasers	• Environmentally safe • No damage to substrate	• High cost • Closed facility • Limited paint thickness removal
Blasting	• High removal rate	• Substrate damage • Surface preparation • Seal deterioration • High Waste Volume
Flashlamp	• Operational versatility • No damage to substrate	• High waste volume • Detrimental to composites

Table 1: Advantages and disadvantages of coating removal techniques

The use of chemical solvents to remove coatings is a well known and efficient procedure. There are two basic categories of this removal technique: hot and cold removal. Cold stripping simply involves subjecting the coated substrate to a chemical solution while hot stripping involves a chemical reaction to remove the coating. Both types of chemical stripping require the soaking of the substrate into the solvent over a period of time to break the bonds of the paint. Even though chemical solvent based methods are extremely effective, they involve several disadvantages: time consuming surface preparation, high VOC emissions, and slow removal (Reitz 1994). The main problem with chemical solvents is high emissions. Since most strippers contain methyl chloride and phenols, flammability, toxicity, and volatility are encountered. Even though chemicals that are now being used do not contain methyl chloride, they are not as effective.

The removal technique using lasers is relatively new. It is a viable surface removal technique in which the energy of the laser generates heat, removing the coating through thermal shock, melting, evaporation, or vaporization (Reitz 1994). This process is much more environmentally safe than chemical stripping and also has the advantage of not damaging the substrate. However, disadvantages include high costs, the requirement of a closed facility, and limited paint thickness removal.

Blasting is the oldest and most common removal process. It can involve the blasting of ice, pressurized water, sand, or plastic. Fracture of the coating is caused by the pressure of impact or melting of the solids or solution. The process easily removes coatings as chips or microscopic pieces. The main disadvantage of this process is the high

amount of waste volume produced. Other disadvantages include the possibility of substrate damage, surface preparation, and seal deterioration.

The newest removal method is the flashlamp. This concept combines the methods of lasers and blasting. Stripping occurs when a high energy pulse from a xenon bulb delivers a high temperature beam which destroys the molecular structure of the coating (DeMeis 1995). Blasting is then used to sweep the residue from the surface. This method allows for a much more efficient method than blasting and lasers alone, but is still accompanied by the disadvantage of high waste volume.

3.2 Paint Classification

There are two main ingredients in paint that determine its classification: film former and solvent. The film former or binder provides the continuous phase in the paint film and is responsible for the general properties of the film. Binders can be divided into two general classes: convertible and non-convertible. Convertible binders undergo a chemical reaction in the film. In an oxidizing film former, oxygen from the air enters the film and cross links the gel. Examples of this type include drying oils, varnishes, and alkyds. Another example of a convertible binder is a catalyzed film former in which a chemical agent blended into the coating causes cross linking in a solid polymer at room temperature. A heat activated catalyst can also be blended into the coating such as in many industrial finishes. The second type of film former is the non binders. These binders do not depend on any type of chemical reaction, but rather or the evaporation of solvents. Examples of this include lacquers, where a solvent based vehicle evaporates leaving a dry film without oxidation or polymerization, and emulsions, where the solvent evaporates leaving behind polymer particles which coalesce together. The second ingredient that

determines the classification of a paint is the type of vehicle or solvent. The first type of vehicle is an organic solvent while the second is water. These types of vehicles are the most common classification factor of paint since most consumers typically choose between a solvent based and a water based paint. All this simply means is that the vehicle is reducible by an organic solvent or water, respectively.

The combination of the two types of classification, film former and vehicle, leads to a variety of different types of paint. Not only can you choose between water based and solvent based, but also convertible and non-convertible. The most common type of paint is the latex paint which is a combination of an emulsion non-convertible binder and a water based vehicle. This is becoming the more widely used paint due to its more environmentally safe production, use, and disposal. Other types of paint include solvent based polymer emulsions, water based acrylic emulsions, solvent based resin lacquers, etc.

3.3 General Latex Paint Ingredients

All latex paints generally contain the same ingredients: emulsion, pigment, water, coalescent, thickener, surfactant, ammonia, defoamer, and preservative. The difference between them lies in the brand and type of each ingredient and the relative amount of each. Table 2 gives a brief explanation of each ingredient and lists the conventional amount in a latex paint formula.

Ingredient	Wt %	Effect
Emulsion	47.2	Polymer dispersion in water; provides continuity and adhesion through film forming
Pigment Slurry	31.1	Pigment that is put into solution through grinding
Water	18.0	Vehicle for ingredients; provides viscosity for application and evaporates upon drying
Coalescent	1.7	Solvent that lowers the glass transition temperature of the emulsion
Thickener	1.3	Alters the viscosity and flow properties and provides stabilization of the paint
Surfactant	0.2	Assists in pigment dispersion and stabilization
Ammonia	0.2	Keeps the paint at the desired pH
Defoamer	0.2	Solvent that collapses the air bubbles produced from vigorous mixing
Preservative	0.1	Protects against attack by bacteria in the paint can

Table 2: Conventional paint ingredients and respective liquid weight percentage

3.3.1 Emulsion

As discussed above, the most important ingredient in latex paint is the emulsion. An emulsion is a two phase system in which one phase exists as tiny droplets in a liquid phase. There are two basic types of this two phase system: a liquid-liquid emulsion and a solid-liquid dispersion. In latex paints, the solid-liquid dispersion is the type of interest. Even though this system is actually a dispersion, it is still commonly referred to as an emulsion. The solid phase can exist as a polymer emulsion or an acrylic emulsion. A polymer emulsion, the most conventional emulsion found in latex paints, is commonly referred to as latex or rubber. Examples found in paints of the monomer before

polymerization include: vinyl esters, acrylic and methacrylic esters, fumaric and maleic esters, hydrocarbons, and other miscellaneous types (Surface Coatings 165). The most common form of the liquid solution in which the first phase disperses is water. This is the case for a latex emulsion. However, organic compounds can also be used as the liquid phase, although this is rare.

A polymeric emulsion can be prepared by the emulsion of a monomer in water by agitation. Two key ingredients must exist for the dispersion to form: a surfactant and an initiator. The surfactant is a colloid, such as polyvinyl alcohol, which provides surface tension so that the different phases will remain stabilized and not separate. The initiator is a source of free radicals that initiates the monomer reaction to form the resulting polymer. The most common types of initiator used contain a peroxy linkage. Examples of this include hydrogen peroxide and potassium persulfate.

Once the emulsion is formed, it becomes the key ingredient in the paint. The main function of the emulsion in a latex paint is its ability to form a film which leads to subsequent adhesion. Key factors in determining the success of this function is monomer composition, particle size, and the type of solution. The way in which the polymer emulsion forms a film is a complex process that involves the loss of water and a coalescence of the polymer particles. When the emulsion is allowed to dry, the water phase evaporates and the remaining polymer particles crowd together. When this occurs, capillary forces overcome the repulsive forces between the particles, and polymer particles become locked into a gel state and coalesce together (Surface Coatings 175). It is through this film formation of the emulsion that allows the paint to form a coating which adheres to the substrate as it dries. Common emulsions used in latex paint include acrylic,

vinyl-acrlic, vinyl-acetate, styrene-butadiente, styrene-acrylic, ethyl-acrylate, butyl-acrylate, methyl-methacrylate, and vinyl-chloride

3.3.2 Pigment

The second main ingredient in latex paint is pigment. Pigments are organic or inorganic substances used in paint coatings to fulfill one or more of three functions: optical, protective, and reinforcement. The most important and most common of these is the optical property, which determines the color, opacity, and gloss of the coating. The next function is the protective function. This helps aid the paint in weathering, flexibility, corrosion resistance, and surface hardness. Finally, reinforcement of the coating occurs when the pigment adds to the film adhesiveness and elasticity. The performance of the pigment depends on its particle shape, size distribution, type, and optical characteristics. The most common and conventional type of pigment put into coatings is rutile (TiO_2). This talc like substance adds color to the paint and is added to the formula in the form of a slurry. A slurry is the product formed from mixing the titanium dioxide into solution through grinding (mixing the ingredients at a very high speed while avoiding the formation of a gel). Since getting the dry powder into solution is difficult, ingredients such as a surfactant and an initiator must be added to the mix.

3.3.3 Water

In a latex paint, water is used as the vehicle for application of the paint. Its primary function provides a means through which the emulsion, pigment, and other ingredients can be applied to the substrate. As the water evaporates after application, the paint dries and the desired product is achieved.

3.3.4 Coalescent

As discussed previously, one of the most important properties that effects the characteristics of paint is the emulsion's glass transition temperature, T_g. Since a key property of paint is elasticity at ambient temperatures, the T_g should be at least below room temperature. However, a lot of emulsions have a high T_g. Therefore, a coalescent is added to lower this critical temperature. It is most simply seen as a temporary plasticizer, promoting the coalescence of the polymer particles. A coalescent is a solvent which is absorbed into the polymer particles. This results in the softening of the polymer and the lowering the T_g (Morgans 346). Examples of coalescents include propylene glycol, Texanol (Eastman Chemical Company), and Dalpad A (Dow Chemical Company).

3.3.5 Thickener

Thickening agents are added to the formula of a latex paint for three purposes: paint consistency, prevention of pigment settling, and thickening of the paint. A thickener's main purpose is to aid in the desired viscosity of the coating. However, it is very useful in dispersing the pigment and for stabilizing the paint so that flocculation is prevented (Surface Coatings 374). Thickeners are protective colloids that add structure to the paint by reducing the mobility of the emulsion. Types of thickeners include: non-ionic cellulose derivatives, hydrophilic clays, protein thickeners, and water-soluble acrylic polymers. Besides the main functions of the thickener, other properties that are altered by its addition include flow, leveling, color acceptance, water resistance, stain removal, and ease of application (Surface Coatings 374).

3.3.6 Surfactant

Used in the formation of the polymer emulsion, surfactants are also added to the general formula of a paint to stabilize the ingredients and assist in pigment dispersion. Commonly referred to as soap, surfactants keep the paint from "wetting" the substrate surface, allowing the paint to form a uniform and stabilized film surface.

3.3.7 Ammonia

Ammonia is added to the latex paint formula to keep the pH at the desired range, 8-9. This caustic ingredient is important to the formula because it enables the polymer particles to maintain their charges so that they will repel each other and remain separated. The ammonia also neutralizes the thickener. Therefore, ammonia is usually added in conjunction with the thickener so that the pH reduction effect from the thickener is corrected.

3.3.8 Defoamer

Foam can be introduced into the system as mixing of the paint occurs. This foam consists of a gaseous phase dispersed throughout the solution. Since these bubbles can lead to the immobility and instability of the paint, they must be removed. A defoamer is an insoluble oil which spreads throughout the system, causing weaknesses in the bubble walls. These weaknesses result in the collapse of the bubble, and therefore the removal of foam (Morgans 346).

3.3.9 Preservative

Protective colloids are added to the paint formula to fight off bacteria attack . The amount of added preservative determines the extent of attack. Small amounts are added when molds and fungi are unwanted in the liquid paint. However, if sufficient amounts of

preservative are added, the dried film can be protected from bacteria attack. Therefore, the amount of preservative in a biodegradable paint is critical. Paints of the latter type are referred to as fungistatic emulsion paint. Several types of preservatives are available and include: chlorinated phenols, organotin compounds, chloracetamide, and other commercial materials (Morgans 1990).

3.4 Paint Formulation

The formulation of a paint begins with determining the exact type of each ingredient you plan to use. After this is set, the first value you must decide is the total mass of dry paint solid. This is done by understanding that the two ingredients in dry paint are emulsion and pigment slurry and these typically exist in a one to one proportion. A typical gallon of liquid paint leads to five pounds of dried paint and ten pounds of liquid paint. Knowing the percent solid of the emulsion and slurry solutions, the wet mass can be calculated for the two ingredients. The liquid volumes can then be determined by using the volumetric weights. The next liquid volume that can be determined is the defoamer. After setting the exact amount of liquid mass desired, the defoamer volume is calculated by knowing that it is typically two-tenths of one percent of the total liquid mass. Similarly, preservative is typically one-tenth of one percent of the total liquid mass. The next ingredients, thickener, ammonia, and coalescent, are always added according to the specific paint properties desired. However, an initial guess of the volumes of each can be made. Thickener normally exists in the formula as less than twenty pounds per one-hundred gallons; ammonia exists most commonly around two pounds per one-hundred gallons; coalescent usually exists around fifteen pounds per one-hundred gallon. After determining these initial masses, trial volumes can be calculated through their volumetric

weights. A trial and error procedure is then used to obtain the paint of the desired consistency, pH, and elasticity. Finally, the amount of water is determined by calculating the remaining mass needed to reach the desired liquid mass.

3.5 Biodegradable polymers

3.5.1 PLA

An important biodegradable polyester is polylactic acid (PLA), whose base monomer is shown is Figure 1.

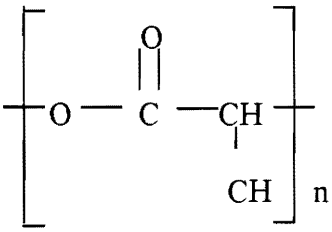


Figure 1: Chemical formula for Polylactic acid (PLA)

PLA is produced through the polymerization from the self-condensation of lactic acid, which is formed through the fermentation of potato waste. Not only is PLA formed from lactic acid, but it also breaks down in the environment to lactic acid which can be metabolized (Fried 248). PLA is relatively expensive compared to other commercial thermoplastics.

3.5.2 PHA Class

Another class of biologically derived, biodegradable polymers that has undergone extensive global research and development is PHA, polyhydroxyalkanoates. These linear, homochiral, thermoplastic polyesters are produced naturally by many bacteria and microorganisms. The simple homopolymer PHB, polyhydroxybutyrate, was the first of the class to be discovered around 1925 and is the most widespread in nature (Horowitz

1994). Around 1970 it became apparent that PHB was only one member of an entire class of polyesters. Since then, the variety of monomers that can be incorporated into PHA has increased, due to the discovery of PHBV, a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate, and PHO, polyhdroxyoctanoate. At least 91 different hydroxyalkanoic acids have now been identified as constituents of PHA (Williams 1996). PBA can be divided into two broad classes depending on the length of the pendent groups. The class with short groups (C_1 - C_3) tends to be very crystalline, while long pendant groups (C_3-C_{11}) make the compound more thermoplastic.

3.5.2.1 PHB

Polyhydroxybutyrate is a short pendant group polymer and has been found to have many properties similar to polypropylene. PHB has a melting temperature 180°C, heat resistance to about 130°C, and a crystallinity of around seventy percent. Not only does PHB have mechanical properties very similar to polystyrene and polypropylene, but it also maintains good water resistance. Other properties of PHB include: adequate fat and odor barrier properties, good resistance to ultraviolet light, and a low water vapor transmission rate. PHB can be produced in two bacteria, *Alcaligenes Eutrophus* and *Zoogloea Ramigera*, which are commonly found in soil and water. These bacteria produce PHB as a carbon source from sugar feedstocks. The biosynthesis of the polymer involves three enzymes: thiolase, reductase, and PHA synthesis. This synthesis process is shown schematically in Figure 2.

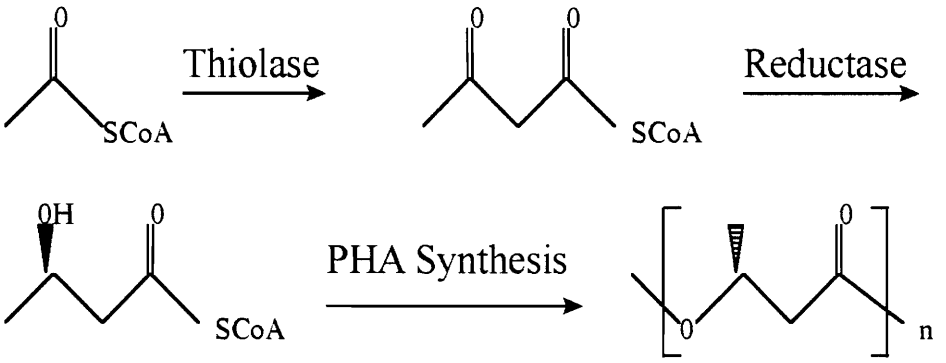


Figure 2: Biosynthetic pathway of PHB

PHB is derived from the coenzyme acetyl A (Coa). The first enzyme catalyzes a Claisen condensation of two acetyl A coenzymes to form acetoacetyl A. The reductase enzyme then reduces that intermediate to the chiral form of R-3-hydroxybutyrl. The PHB is formed when the chiral intermediate is polymerized by PHA synthesis (Poirier 1992, Williams 1996). After the enzymes break the feedstock into PHB, the polymer is transferred into the cells of the bacteria where they form into distinct granular inclusions. These granules are formed due to the insolubility of PHB in water. The polymer granules can compromise up to 80% of the dry weight of the cells (Horowitz 1994). The isolation and preservation of these granules can be obtained through three general separation procedures: solvent extraction, chemical digestion, and selective enzymolysis (Lauzier 1993). An example of PHB granules are shown in Figure 3 where they have been stained in an activated sludge bacteria.

Figure 3: PHB stained granules in activated sludge bacteria (*Manual on the Causes and Control of Activated Sludge. David Jenkins)*

3.5.2.2 PHBV

PHBV is a copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate (Figure 4). In addition to the basic properties already mentioned for PHB, PHBV has improved flexibility and toughness due to the increased size of the hydroxy acid pendant groups. The copolymer has a lower melt and glass transition temperature and a higher extension to break. According to the amount of valerate in the polymer, the Young's modulus can decrease five times from PHB. The biosynthesis for PHBV is the same as PHB and is derived for the feedstocks glucose and propionic acid. The glucose is metabolized by the bacteria to form Ac-CoA, with the propionic acid providing the C_3 unit for the Claisen condensation.

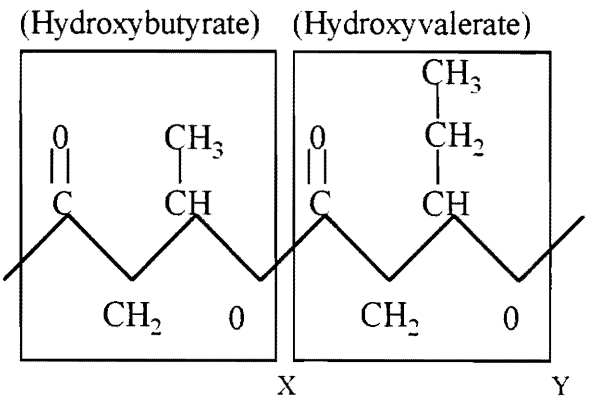


Figure 4: Chemical formula for PHBV

3.5.2.3 PHO

Polyhydroxyoctanoate is a long pendant group polymer and is, therefore, a thermoplastic elastomer. Due to its long pendant groups, PHO is only about 30% crystalline. It's melting temperature is 50-60°C and has a glass transition temperature of -35°C. Since this T_g is below room temperature and the polymer maintains low crystallinity, PHO is elastic at room temperature. Although PHO is structurally very different from other thermoplastics, it has much of the same stress and strain properties and maintains a high tensile strength (Williams 1996). PHO is produced by *Pseudomonas* bacteria. The biosynthesis is not as well understood as that of PHB and PHBV, but is believed to include oxidation and fatty acid synthase in the monomer production (Williams 1996).

3.5.2.4 Biodegradation

PHAs are very stable under normal conditions, but biodegrade readily when exposed to very active and dense active microbial communities. There is a range of environmental conditions that can biodegrade PHA: aerobic and anaerobic sewage, compost, landfill, and marine sediment. Over 300 bacteria have been isolated that can

biodegrade the material under specific conditions. Studies have shown that depending on the microbial activity and other conditions, biodegradation can take from three weeks to three months. The rate of degradation depends on four main factors: pH, temperature, surface area of the polymer, and microbial activity. The most important factor for biodegradation is microbial activity. The biodegradation of PHA begins when bacteria start to grow on the surface of the polymer and secrete enzymes. These enzymes break the polymers down into the monomer fragments, which are then transported into the cells of the bacteria where the carbon is used as a food source and carbon dioxide and water are produced. Photographs of biodegraded PHBV materials are shown in Figures 5 and 6 at a microscopic and macroscopic level, respectively.

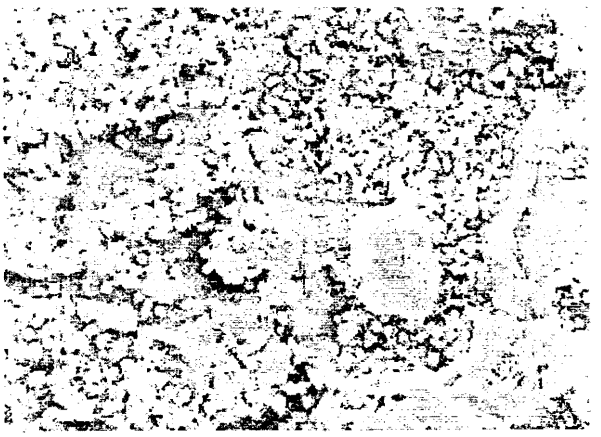


Figure 5: Electron micrographs of PHBV following 7 weeks burial in a marine sediment (*R.C. Thompson and C.H.. Dickinson*)

Figure 6: 'Biopol' (PHBV) molded bottles after biodegradation (*'Biopol' Biodegradation - Monsanto)*

4. Method

4.1 General Strategy

To obtain a biodegradable latex formulation, it was first necessary to identify the key ingredient(s) of latex paint which would allow the coating to biodegrade. The next step was determining and locating the appropriate biodegradable key ingredient(s). By analyzing the various properties and characteristics of the biodegradable ingredient(s), a similar non-biodegradable ingredient(s) used in conventional paint formulas was identified and used to formulate a control paint. Once the approximate formula for the non-biodegradable control paint was developed, it was mixed in the lab to get a precise formula and detailed mixing procedure. The final step involved testing the control formula for essential properties such as adherence, ease of application, and consistent quality.

4.2 Identification of the key biodegradable ingredient(s)

Latex paint ingredients include: emulsion, pigment, water, coalescent, thickener, surfactant, ammonia, defoamer, and preservative. The main and most important ingredient for latex paint is the emulsion, not only because it is present in the highest weight percentage of all of the ingredients, but it also provides the primary functions for paint formation, adhesion and film formation. When a paint is biodegraded, the paint loses its adhesion and/or film structure through the work of the microorganisms. Therefore, if the microorganisms consume the emulsion, the remaining ingredients of the paint (mainly pigment) will lose their adhesion and fall off the substrate. Almost all of the other ingredients in latex paint evaporate upon the drying of the paint. Therefore, they are not considered key biodegradable ingredients since the microorganisms will be attacking the dried paint.

4.3 Determination and source of the biodegradable emulsion

The biodegradable polymer that has undergone the most extensive global research and development is PHA. Since there is more information about its characteristics and properties and also more commercial sources, PHA was chosen as the polymer in the biodegradable emulsion. The specific emulsion identified for use in the latex paint formula was PHBV. This copolymer was chosen because the combination of 3-hydroxybutyrate and 3-hydroxyvalerate increases the extension to break and lowers the glass transition temperature, resulting in improved flexibility and toughness.

Several commercial sources of PHA were identified. A principle producer is Monsanto who produces the emulsion in Brussels, Belgium (*See Appendix 4: Contacts)*. Their proprietary line for PHBV is labeled 'Biopol' and is available in a varied grade range. The grade range results from different amounts of the 3-hydroxyvalerate in the copolymer and leads to granules suitable for injection moulding and extrusion processes. It is sold in the form of pellets, but it is in the emulsion form at one point in the production process. Some of their final 'Biopol' products include cosmetic packaging, food packaging, and coated paper bags. The rights to 'Biopol' were just recently purchased by Monsanto from Zeneca, a spin off of ICI Biologicals. It is because of this recent purchase that the emulsion was unavailable.

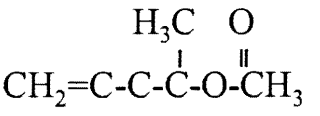
Another commercial source of PHAs is Metabolix of Cambridge (*Appendix 4)*. This company was founded in 1992 by Oliver Peoples, Anthony Sinskey, and Simon Williams to produce PHAs using the proprietary transgenic technology. Metabolix holds the exclusive license to the M.I.T patent portfolio of methods for producing the PHAs in

transgenic fermentation systems and crops (Williams). Because their work is currently in the exploratory stage, a PHA sample could not be obtained from this source.

4.4 Identification of the control emulsion

To formulate a control latex paint, an emulsion found in existing paints showing very similar characteristics to the biodegradable PHBV emulsion needed to be determined. Many emulsions are suitable for design and formulation of paint, but the important binding ability of coatings is a function of particle size, molecular weight, and glass transition temperature (T_g). Other properties of paint that can be effected by T_g include durability, tensile strength, elongation, and adhesive properties. Due to its importance in the characteristics of film formation, the glass transition temperature is the key property in finding a comparable polymer to the PHBV emulsion (T_g = 5°C). The emulsion used in coating applications with the closest characteristics to polypropylene and hence PHA is a combination of methyl-methacrylate and butyl-acrylate (Figure 7). The average T_g of this emulsion is 0°C, but can be modified by changing the weight percentage of the copolymers. Therefore, the copolymer Methyl-methacrylate/Butyl-acrylate was chosen as the emulsion used in the control formula.

Methyl methacrylate (MMA)



Butyl acrylate (BA)

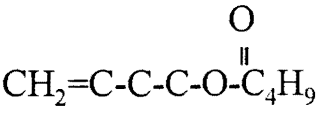


Figure 7: Chemical formulas for MMA and BA used in the control formula

4.5 Formulation of the control paint

Based on a three-hundred gram dry weight and a nine-hundred gram liquid weight, the calculations (*Section 3.3*) for the control formula were performed and are shown in Table 3. The use of the BA/MMA emulsion results in the formulation of a flat latex exterior paint.

Ingredient	**% solid**	**Weight (g)**	**Volumetric Weight (g/qt)**	**Volume (qt)**	**Volume (mL)**	**Solid weight (g)**
Emulsion Butyl acrylate/ Methyl methacrylate	53.5	280.4	1006.2	0.2787	263.7	150
Pigment Slurry Dupont "Ti-Pure"	76.5	196.1	2721.6	0.0720	68.1	150
Coalescent	-	-	-	-	-	-
Defoamer Dow Corning 1430 Emulsion	-	1.8	851.3	0.00211	1.9	-
Thickener	30.0	~10	998.8	0.01101	9.5	-
Preservative	-	0.9	1078.3	0.00176	1.7	-
Ammonium Hydroxide (37%)		~2	1021.5	0.00196	1.85	-
Water	-	408.8	945.0	0.433	409.8	
		900 g		**0.799 qt**	**756.6 mL**	**300 g**

Table 3: Initial formula for the BA/MMA control paint

4.6 Mixing procedure

The next step following the calculation of the approximate control formula was mixing the ingredients in the lab. The supplies and procedure are in Appendix 1.

4.7 Testing procedure

4.7.1 Tests for Paint Properties

There are many characteristics which can describe a good paint: durability, good adhesion, flexibility, good coverage on various substrates, ease of application, etc. However, there are a multitude of paint formulas each unique and most often formulated with desired properties for a specific application. One desirable characteristic for one application could be undesirable for another. Since each application has its own desired properties, there is no test or characteristic which can determine if a formula is a paint and whether or not it is a "good" paint. Therefore, the tests used in this research simulate situations that occur on a flat latex exterior paint and are based upon a comparison with a similar commercial source.

For the first two comparison tests, the control and commercial coatings were each painted onto a piece of wood and allowed to sit for a one week period to ensure complete drying. The first simulation was the scrub test in which the samples were scrubbed over a five minute period with a wide bristle brush to observe flaking. The second test involved the submersion of the samples in water for one week to observe the loss of adhesion. The third simulation was the coverability test where several substrates were coated with the control and commercial paints to compare the ability to cover.

4.7.2 Tests for Biodegradation

There are several options for determining how much of a material has biodegraded. One method is used by Monsanto in their studies on their 'Biopol' products in which they measure the carbon dioxide evolution relative to a control. They also use Scanning Electron Microscopy (SEM) photographs to actually observe how much has biodegraded through the pitting and erosion of the material surface. Another test that can determine biodegradation is simply the measurement of weight loss (Lauzier 1993). Another option involves measuring the loss of a mechanical property, such as tensile strength. This was the test that was chosen in this research.

The tensile strength of a material is measured with a tensile testing machine. The tensile test is used to evaluate the strength of a material by pulling a sample with definite volume to the point of failure in a relatively short time at a constant rate. The mechanical properties that can be obtained through a tensile test include: modulus of elasticity, yield strength, percent elongation at fracture, and energy to break point.

To obtain paint samples with a defined cross sectional area, a draw-down blade was first used to create coatings of equal thickness throughout (Figure 8). A cutter with known shape and area was then used to cut out the paint samples from the draw-down. The samples were then put under various loads to observe certain mechanical properties.

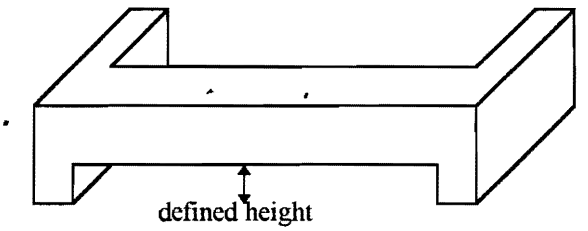


Figure 8: Draw-down blade

5. RESULTS

5.1 Control Paint Formulation

Starting with the calculated amounts (Table 3), the control paint was mixed with several adjustments made to the ingredient amounts due to the following observations:

1. It is very important that the pH remains around 8.0 in the can after mixing so that the polymer particles remain charged and repel each other to keep the paint stabilized and well mixed. The pH of the coating was very sensitive to time and certain ingredients.

	pH
a) After emulsion was added	8.84
b) First pH check after thickener was added	5.24
c) After the addition of 0.75 mL ammonium hydroxide	6.40
d) After the addition of 0.75 mL ammonium hydroxide	8.22
e) After sitting for 24 hours	8.04
f) More addition of 4 mL thickener	7.70
g) After the addition of 0.5 mL ammonium hydroxide	8.10

2. The viscosity of the coating was also sensitive to time and added ingredients. The addition of thickener changed the viscosity of the coating nonlinearly. On the first day, the 6 mL that was added resulted in no change of viscosity. The next addition of 3 mL resulted in slight changes in viscosity. The final 2.5 mL resulted in large changes in viscosity of the coating. Each addition of these final 0.5 mL increments was very critical. Longer delays in thickening response resulted as more thickener was added. This was partly due to inadequate mixing due to higher viscosity. Over a thirty minute period after the coating was mixed, the paint thickness decreased slightly. However,

after 2 hours of sitting, the paint thickened considerably. Upon the addition of 2 mL surfactant, the paint became very thin. It took 4 mL more of thickener to get it back to the desired viscosity.

3. After the first mixing of the control formula was made, a sample was produced using the draw down blade. Since the initial formula did not include surfactant, "spotting" occurred on the sample in which the surface tension was too high, keeping the coating from stabilizing and staying well dispersed. Therefore, 2 mL of surfactant was added to keep the pigment and emulsion well dispersed and stabilized.

The resulting final formula for the control paint is shown in Table 4:

Vol, mL	Ingredient
410	Water
265	Emulsion- Butyl-acrylate/Methyl-methacrylate
68	Titanium Dioxide Pigment Slurry- Tipure 746
15.5	Thickener
2.0	Defoamer
2.0	Surfactant
2.0	Ammonium Hydroxide - 29%
1.6	Preservative
766.1	

Table 4: Final control paint formula

The conventional way to express paint ingredients and amounts is in a one-hundred gallon formula (Table 5).

Vol, gal	Weight, lb	Ingredient
53.52	445.6	Water
34.59	306.6	Emulsion- Butyl-acrylate/Methyl-methacrylate
8.88	213.1	Titanium Dioxide Pigment Slurry- Tipure 746
2.02	17.8	Thickener
0.26	2.0	Defoamer
0.26	2.0	Surfactant
0.21	2.0	Preservative
0.26	2.3	Ammonium Hydroxide - 29%
100	**991.4**	

Table 5: Conventional 100 gal control formula

5.2 Tests for Paint Properties

The paint properties for the designed control formula were compared to a commercial flat exterior latex paint which was believed to be the most similar formula. Although these tests are qualitative and somewhat subjective, they showed how the control formula would hold up under certain exposure or conditions. After scrubbing the two paint samples over five minutes, the control paint showed better adhesion with very little flaking and just a general loss of overall color. The commercial paint broke down much sooner with the loss of adhesion of large paint flakes in the central area of the wood. The water submersion simulation over a one week time period had hardly no effect on either sample. There was a loss of paint on both samples at the water line. To test for coverability, both paints were coated onto wood, metal, and concrete. Both paints

covered all of these substrates very nicely. However, the control paint coated a little thinner, and brush marks were visible. Table 6 gives a brief summary of these results.

	Control Paint	Commercial Paint
Scrub Test	Overall loss of color	Flaking of paint in central area
Water submersion	Very small area of loss of adhesion after one week	Very small area of loss of adhesion after one week
Coverability	Good coverage on all substrates, brush marks visible, somewhat thin layer	Good, thick coverage on all substrates

Table 6: Paint property tests results

5.3 Biodegradation Test

Even though the control formula was not biodegradable and was not subjected to bacteria, the tensile test was still used to evaluate the test and to compare and observe some of the mechanical properties of the control to the commercial. These properties are summarized in Table 7. All of the tensile test results showed much lower tensile strength in the commercial source compared to the control formula. The control paint showed great flexibility and tensile strength as it stretched 117 mm under a 2 kgf load and returned to its initial size after the load was taken off. However, the commercial paint only stretched about 6 mm under the same load before it broke into two pieces. The plot of these two different results are shown in Figure 9. This large difference in ductility and elasticity is presumably due to the difference in formulas. Even though the commercial source was chosen due to its similarities in ingredients, its formula involves several other ingredients which are added for more complex properties. It also has ingredients which are added simply as fillers to cut down on costs due to the large production. These fillers

reduce the ductility and elasticity of the paint. Therefore, the control paint is a very simple and basic formula but has good mechanical properties due to the lack of the use of filler ingredients.

	Control Paint	Commercial Paint
Tensile Strength (kgf/mm^2)	0.237	0.160
Max Displacement (mm)	117	~6
Energy to Break Point (kgf-mm)	2.38	0.275

Table 7: Mechanical properties resulting from the tensile test

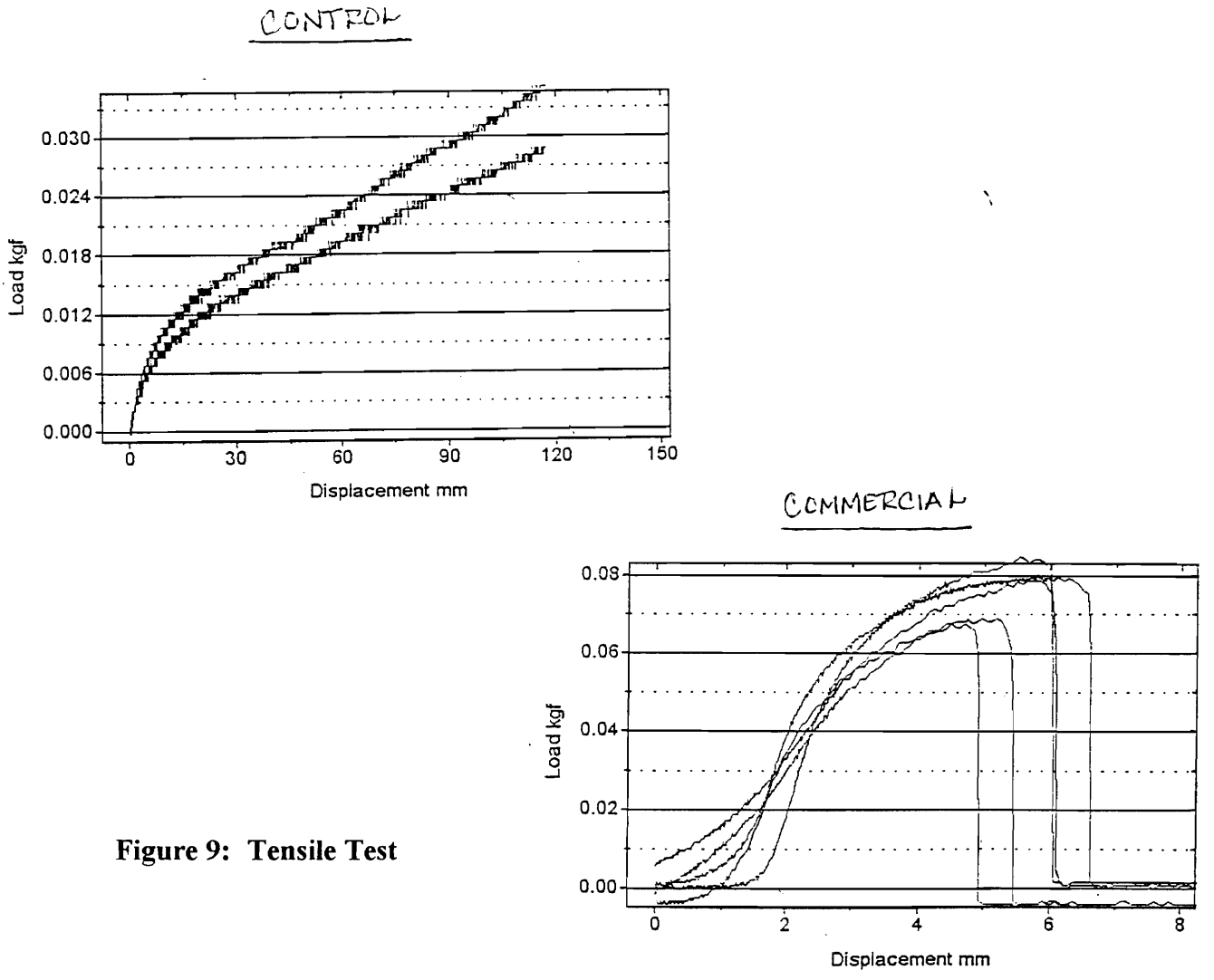


Figure 9: Tensile Test

6. Conclusions

This research focused on initial studies in the design of a biodegradable/ biosynthetic latex paint. These studies resulted in an initial paint formula as well as a mixing and testing protocol through the creation of a control latex paint. Further steps for the actual creation of the "biopaint" should follow the following:

1. Locate and obtain a source of the biodegradable/biosynthetic emulsion, PHBV, through two possible venues:
    - Commercially - Monsanto or Metabolix were willing to give a PHBV emulsion sample but were unable to due to current situations
    - Preparation in the lab - Grow a bacteria culture producing PHBV and isolate the polymer through solvent extraction, chemical digestion, or selective enzomolysis (Lauzier 1993)
2. Create and mix the "biopaint" using the paint formula and mixing procedure resulting from this research with the substitution of PHBV for the BA/MMA emulsion.
3. Test the "biopaint" paint for paint properties using the testing protocol from this research and compare the results to the control BA/MMA paint as well as a similar commercial source.
4. Subject dried "biopaint" and control paint to cultured bacteria and use the tensile to test to observe the extent of biodegradation.

Research in the area of biodegradable paper coatings and emulsion films has already shown the need for blending and heat treatments to obtain the desired properties.

Therefore, it will probably be necessary in the creation of the "biopaint" to blend the emulsion with other types and also apply heat treatments.

7. ACKNOWLEDGEMENTS

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***See Appendix 2**

9. Appendix 1: Mixing Procedure

9.1 Supplies

* Mixer
* 1000 mL beaker
* Graduated cylinder
* Pipette
* pH meter
* Water
* Titanium Dioxide Pigment Slurry- *Dupont "Ti-Pure"*
* Defoamer - *Dow Corning 1430 Emulsion*
* Emulsion - *Butyl acrylate/ Methyl methacrylate*
* Ammonium Hydrxide - 29%
* Preservative
* Surfactant - *Union Carbide Triton GR-5M*

9.2 Procedure

1. In a 1000 mL beaker, add 410 mL water and begin mixing at about 300 rpm or until a vortex is formed without allowing the mixer paddles to show.
2. Add 68 mL Pigment Slurry
3. Add 2 mL defoamer
4. Add 264 mL emulsion to the mixing solution, being aware that some emulsion will remain on the wall of the cylinder after emptying.
5. Add 2 mL of surfactant
6. Check pH of the solution. If the pH is below 8.0, add ammonium hydroxide in 0.5 mL increments until the desired pH between 8.0 and 9.0 is reached.

   While mixing continuously, add thickener in 1 mL increments until the desired viscosity is reached, being aware that there is about a 2 minute delay in the response of

thickening. The total amount of thickener should be around 10 mL. The mixing speed will have to be adjusted according to the resulting viscosity. The final speed should be around 500 rpm.

7. Add 1.65 mL of preservative.
8. Check and adjust the pH of the coating to 8.0 - 9.0 with ammonium hydroxide.
9. Allow the coating to mix for 15 minutes.
10. Let the coating set for two hours before any applications.

10. Appendix 2: Selected Articles

Novel Biodegradable Microbial Polymers

edited by

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University of Hull, Hull, U.K.

Kluwer Academic Publishers

Dordrecht / Boston / London

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PROPERTIES OF POLY-β-HYDROXYALKANOATE LATEX: NASCENT MORPHOLOGY, FILM FORMATION AND SURFACE CHEMISTRY

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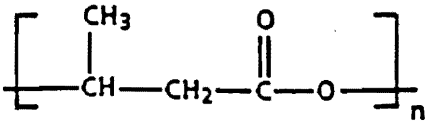
ABSTRACT. Poly-β-hydroxyalkanoates (PHA) occur as an intracellular storage material in a variety of bacteria. Statistically random copolyester chains of β-hydroxybutyrate (HB) and β-hydroxyvalerate (HV) units have been isolated from the bacteria in their nascent state as a colloidal suspension of submicron size particles. This latex or native granule suspension (NGS) of 20 to 30% solids is easily spreadable on porous or impervious substrates to produce under the proper drying conditions films or coatings that range from microporous and opaque to non-spherulitic and transparent. The preparation, processing, mechanical properties and surface characteristics of films made of this latex whose contact angle vs. water is 70°, will be discussed.

1. INTRODUCTION

The discovery of poly-β-hydroxyalkanoates (PHA) owes much to the keen observations of Maurice Lemoigne (1) while working at the Lille branch of the Institut Pasteur. He became aware of hydrophobic granules in certain bacteria which were not ether soluble, as expected for lipids. By careful experimentation he identified the material (2) as a crystalline, high molecular weight polyester corresponding to



E. A. Dawes (ed.), Novel Biodegradable Microbial Polymers, 97–112.

and named it "lipides β-hydroxybutyriques". The name poly-β-hydroxybutyrate (PHB) has now come into general usage. The ubiquity of PHB in microbes and the homologous molecular family with alkane sidechains led to the general classification: poly-β-hydroxyalkanoate (PHA) (3).

It is entirely fitting that this family should be a leading candidate to be the "clean" thermoplastic polymer which has the benign characteristics sought after by environmentalists, i.e. biodegrades and hydrolyses to biocompatible small molecules. Lemoigne's career as an agricultural/industrial microbiologist displayed a lifelong interest in the decomposition of organic matter in soils by microbial processes: a forerunner of today's environmental research activities.

1.1. NASCENT MORPHOLOGY

Although he did not use the term nascent morphology, Lemoigne's studies often dealt with the autohydrolysis of PHB granules (4). He and his colleagues related the drop in melting point and new solubility characteristics to the loss in molecular weight of the polymer as a result of autohydrolysis (5). Later progress in molecular biology and polymer morphology would identify the higher order structure that results from simultaneous polymerization and phase separation of water insoluble biopolymers as *nascent* (6). Subsequent solubilization and reprecipitation is unlikely to duplicate the unique thermodynamic and kinetic conditions which were present in this initial morphogenesis (7), hence the nascent state is unique. Nevertheless, polymer scientists have devoted much experimental effort to attempt to understand the nascent morphology of polymers in order to promote simultaneous polymerization and crystallization leading to functional shape and properties in solid polymers, "as polymerized" (8).

In the case of PHA, the native granule state has been the preoccupation of only a few biologists and physical chemists. To understand the biosynthesis of PHB in terms of a surface membrane around the native granule which may encompass potential biosynthetic and biodegradable enzymes, several biologists observed granules in the electron microscope after drying and shadowing (9). The most noteworthy characteristic of the "never dried" native granules would seem to be their lack of crystallinity *in vivo* (10) although the first drying induced x-ray crystallinity (11). A second important property is ductility as demonstrated by freeze fracture studies (12) which showed splaying of the fractured granules inside the cells into fibrils at temperatures far below the glass transition. Such observations suggest a nascent organization resembling a nematic state, albeit not three dimensionally crystalline. This was in keeping with prior electron microscopy on partly swollen nascent granules (11) which showed remarkable fibrillar and lath-like organization after mild swelling with acetone /water solutions.

1.2. SURVEY OF PHA PURIFICATIONS METHODS

Over the past fifty years numerous methods have been devised for the isolation of PHA in a relatively pure state. In early studies, the purified polyester molecule was sought. Later, the nascent bio-system was the object of isolation studies hence the least possible damage to the surface was important. More recently, competitive advantage is the driving force for

industrial biotechnologists who seek to minimize the cost of producing PHA for a given application. In broad terms, three purification approaches may be described:

- Solvent extraction
- Chemical digestion
- Selective enzymolysis

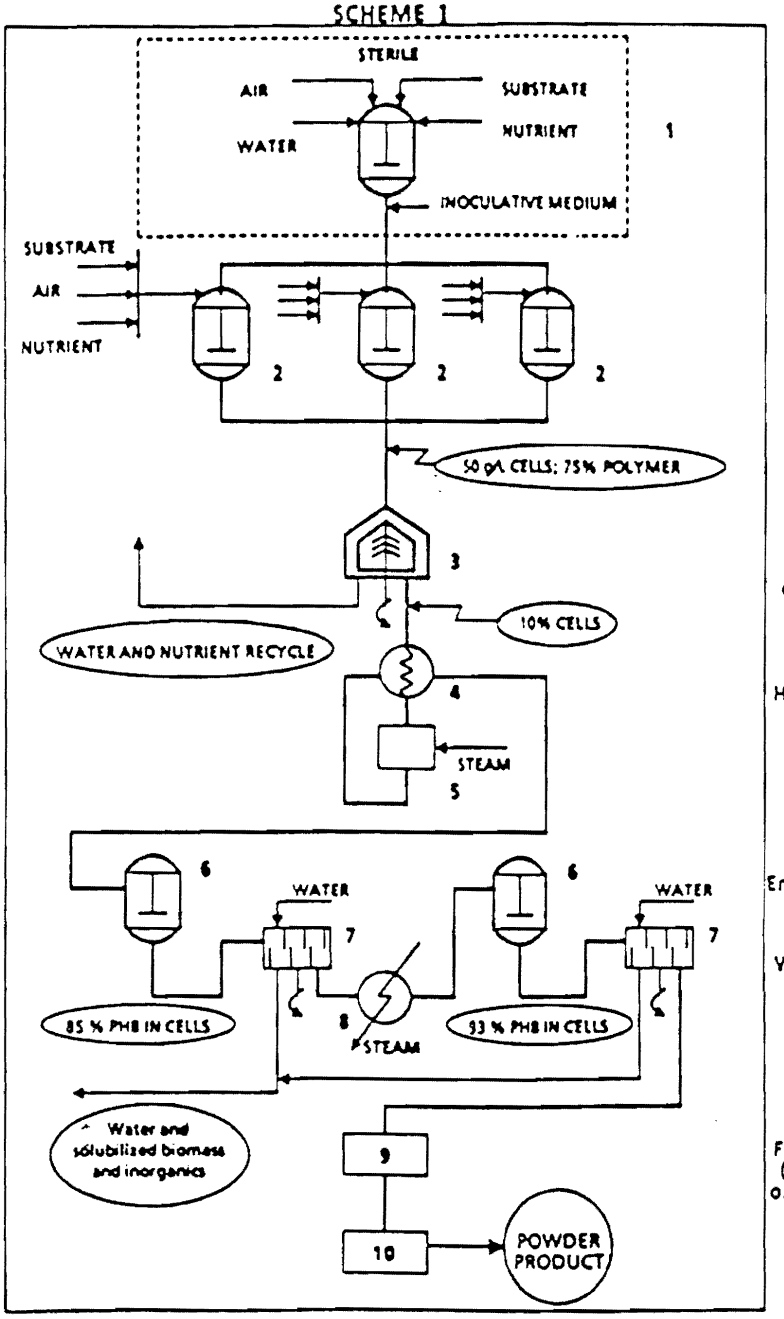
The three types of purification processes are summarized in the chart titled "Survey of PHA Purification Processes ". The "chemical digestion" and the "selective enzymolysis" approaches, aimed at eliminating all that is non-PHA in order to harvest the native granules (NG), are pertinent to this study.

SURVEY OF PHA PURIFICATION PROCESSES

Isolation Process	Procedure
Physical Approach: cell wall weakening and solvent extraction of PHA	a) Pretreatment with acetone to weaken cell wall and membranes b) Propylene carbonate @ 120 °C where PHA is soluble up to 200 g/L, precipitates at room temperature
Chemical approach: selective disruption and digestion of non-PHA components	a) Pretreatment with detergents b) Washing of residue with sodium hypochlorite at pH 13
Biochemical approach: granule isolation after selective enzymolysis of biomass	a) Shock disruption e.g. heat, ultrasound b) sequential enzyme treatments and washes

The most advanced version of the selective enzymolysis process is that described by Holmes *et. al.* (13) and shown in Scheme 1. The process aims at the highest level of biomass production with the highest possible weight percent of PHA per dry weight of biomass (% PHA). The purification then proceeds by washing and sequential enzyme/wash treatment cycles to bring

SCHEME 1


STERILE
AIR
SUBSTRATE
WATER
NUTRIENT
1
INOCULATIVE MEDIUM
SUBSTRATE
AIR
NUTRIENT
2
2
2
50 g/L CELLS; 75% POLYMER
3
10% CELLS
WATER AND NUTRIENT RECYCLE
4
STEAM
5
6
6
WATER
WATER
7
7
85 % PHB IN CELLS
8
STEAM
93 % PHB IN CELLS
Water and solubilized biomass and inorganics
9
10
POWDER PRODUCT
1 Continuous fermentor
2 Batch fermentors in phased operation
3 Cell concentrating
4 Heat exchanger
5 Cell lysing
6 Enzyme digestor
7 Water washing (centrifuge)
8 Heat exchanger
9 Final treatment (solvent wash, oxidizing agent)
10 Drying

the % PHA level in the dry product to ≈ 95%. Presumably, residual protein and cell wall residue make up most of the 5% impurities in the final sample which is normally spray dried in step 10 of Scheme 1.

For obvious economic reasons, the suspension of NG at step 9 in Scheme 1 is a 30 - 50% solids suspension. It is this NG suspension which we are referring to as a *latex* in this paper. Since the NG have undergone considerable exposure to physical and chemical factors, not all of which have been revealed by the manufacturer (Marlborough Biopolymers Ltd., Imperial Chemical Industries), our experimental control leaves something to be desired.

The latex state of PHA native granules can also be created via a "chemical digestion" process (14). In most respects the colloidal characteristics of the NSG produced in this way are similar to those derived from the NSG supplied to us by the manufacturer.

2. MATERIAL AND METHODS

2.1. QUALITATIVE DESCRIPTION

The sample "as received" (ICI, Marlborough Biopolymers Ltd. Biopol™) was a white, uniform suspension of pleasant fruity odour. At low temperatures (5 °C) the suspension was fluid and foamed easily when shaken; upon warming to room temperature the slurry set to a thick paste of chunky appearance. X-ray diffraction of the suspension "as received" revealed a material showing crystalline diffraction in the PHB lattice (15, 16). However, high resolution ^{13}C NMR on the slurry showed a spectrum characteristic PHB/V. These results suggest that the NG suspension derived from the process of Scheme 1 is a polyphasic material: crystalline domains and perhaps water hydrated mobile domains (10).

2.2. PHYSICAL CHARACTERIZATION

2.2.1. *Sample Composition and Solids Content.* Proton NMR (17) showed the sample to be composed only of butyrate and and valerate , the latter being 21 mole %. The sample was found to be 33% w/w solids. This material was easily spread to a smooth finish with a spatula, but when air dried at room temperature it lacked strength and disintegrated readily to a fine powder. Drying the sample in the oven at 100 °C produced a low level of fusion yielding a peelable film.

The slurry "as received" was easily diluted with water and readily rinsed off surfaces. The air dried slurry was crystalline and showed a typical PHB crystalline lattice as in the case of the wet slurry.

2.2.2. *Melting Temperature and Differential Scanning Calorimetry.* The melting point of the air dried slurry was found to be 128. 4 °C by DSC (peak maxima), at this temperature the sample went from opaque-white to transparent. Thermograms were also obtained from samples oven dried at 100, 125 and 150 °C , the melting points were found to be 128.4, 128.4 and 124.8 °C respectively. All thermograms displayed broad endotherms with shoulders or separate small peaks at either side of the main melting peak. This

is a common occurrence in the melting of native PHB/HV copolymer powders supplied by ICI.

2.2.3. *Molecular Weight and Intrinsic Viscosity.* The viscosity of the sample was measured in chloroform at 30 °C. The intrinsic viscosity was found to be 1.664 dl/g. The molecular weight of the material was calculated using the Mark-Houwink-Sakurada equation ($[\eta] = K' M^a$) with $K = 1.18 \times 10^{-4}$ and $a = 0.78$ (18). It was found to be 205.5×10^3 g/mole.

2.2.4. *Electrophoretic Mobility (E.M.).* The electrophoretic mobility (E.M.) of the sample was measured in very dilute dispersions (< 0.25%) at 25 °C in distilled water, acidic (HCl) and basic (NaOH) pH. The sample was found to have negative mobility in water (-1.25×10^{-8} m^2 sec^{-1} V^{-1}) and at pH 8 (-2.78×10^{-8} m^2 sec^{-1} V^{-1}). At pH 3 the charge of the dispersed particles was neutralized and flocculation occurred. The few suspended particles remaining in suspension after neutralization moved very slowly outside the measurable range in either direction, lowering the pH further (2 pH units) caused even more precipitation, not the hoped for charge reversal.

A summary of the results obtained in the characterization of the latex are shown in Table 1

TABLE 1

SUMMARY OF PHA LATEX CHARACTERIZATION

Physical parameter	Results
Composition	PH8/HV, 21% HV
Solids content	33%
Particle size (S.E.M.)	0.2 to 1.5 µm diameter
Electrophoretic Mobility (E.M.)	Distilled water* -1.25×10^{-8} m^2 sec^{-1} V^{-1} pH 8 -2.78×10^{-8} m^2 sec^{-1} V^{-1}
Melting temperature (T_m)	128.4 °C
Intrinsic Viscosity [η]	1.664 dl/g
Molecular Weight (M_v)	205.5×10^3 g/mole

* pH from 5.5 to 6.6 pH units

2.3. FILM FORMING

2.3.1. *Poured Films.* Films were made by leveling the "as received" latex (33% w/w) with a spatula or diluting it with distilled water (20-25% w/w). At that concentration it was not possible to spread the dispersion with a metering rod or bird applicator on an impervious surface. Films were made by carefully applying the diluted slurry with an eye dropper onto glass, teflon or mylar

strips. The films, protected from dust, were allowed to dry overnight at room temperature before any further treatment.

2.3.2. *Spread Films*. Films could not be made by spreading the diluted latex suspension (20-25% w/w) with metering rods or applicators on an impervious surface. The dispersion would not wet or adhere to the coating surface in a uniform manner, instead it would streak the surface in random patches. The applicator would sweep most of the suspension off the substrate. This wettability problem was solved by the addition of a surfactant, a commercial soap (MIR®). A couple of drops of a 50% w/w aqueous solution of this detergent added to 30-35 g of diluted suspension was enough to render the material spreadable.

On the other hand a 30% w/w latex suspension was spreadable on mylar. If a metering rod was used for spreading, the grooves were quite visible in the dry film, indicating that the suspension lacked self-leveling characteristics. Addition of dilute MIR® improved the leveling problem.

2.3.3. *Air Drying*. Once spread or poured, air dying produced films or coats with little or no strength. If handled, they disintegrated into a powder. Nevertheless, air dry coats were uniformly white and opaque as the wet latex.

2.4. FILM FUSION AND POST-TREATMENT

2.4.1. *Convection Heat*. Heating the air dried films in a convection oven produced a series of different films depending on the temperature and time of exposure. Heating the films below their melting temperature (T_m), at 100 °C for at least 10 min. induced moderate fusion. Partial fusion rendered the film flexible and peelable from the substrate. The films were as white and opaque as after air drying, peelability being their only new characteristic.

Heating the films at their T_m for at least 15 minutes enhanced crystallinity and strength, if exposed less time partial fusion as in films heated at 100 °C is achieved. Films heated at T_m or 150 °C were translucent and highly crystalline (PHB lattice structure) but only the one treated past the melting temperature was slightly birefringent, spherulites were not observed. These films are robust but not tough. Heating beyond T_m causes shrinkage and excessive cracking.

2.4.2. *Hot Pressing*. Films were also made by hot pressing air dried or partially fused films (100 °C drying oven, 10-20 min). A minimum temperature of 100 °C and a pressure of 1000 psi was necessary to produce complete fusion. The films were prefused, peeled and placed between mylar sheets, wrapped in aluminum foil and hot pressed at 100 °C and 5000 psi for a minute or two. The resulting films were transparent and easily peelable from the mylar. They are highly crystalline (x-ray diffraction), weakly birefringent but not spherulitic, probably due to local strain birefringence (polarizing microscopy).

2.4.3. *Liquid/Solvent Vapor Fusion*. The dry material (air or oven dried), is readily soluble in chloroform. Films were also made by solvent casting and vapour coalescing. In the later case, air dried films were exposed to liquid and solvent vapor atmospheres for a period of of 24 hours. Chloroform vapor produced extremely smooth, flexible and tough films that were very

transparent and conformable on drying. They show x-ray crystallinity but are not birefringent as their hot pressed equivalents.

Exposure to acetone or ethanol vapor does not produce fusion, instead the coats substantially shrink and shatters into pieces. This would appear to be due to stress cracking under the influence of liquids which slightly swell the PHB/HV material but are non-solvents.

Solvent mixtures were also used. The most promising one was found to be 1:1 ethanol/chloroform. The films were quite opaque but crystalline, flexible and plastic even though they showed appreciable shrinkage i.e., scrolling. Acetone/chloroform mixtures did not produce any fusion, the polymer films just shattered as with pure acetone.

2.5. SURFACE PROPERTIES

Three types of films were used for contact angle measurements, air dried, hot pressed and chloroform vapor fused. The liquids used were water, formamide and benzyl alcohol. According to the film characteristics, two methods were employed: capillary rise and goniometer measurements. The observations were made at room temperature.

2.5.1. *Contact Angle*. Air dried and preheated films (100 °C, 10-25 min) are extremely porous. Their contact angle could not be measured using a goniometer, liquids were quickly absorbed by the surface. Instead, a capillary rise method adapted from the one reported by Aberson (19, 20) was used. Polymer coated strips of mylar or glass microscope slides were suspended in a flask with a small amount of the desired liquids at the bottom. After an equilibration period of 15 minutes, the rate of capillary rise was measured by barely immersing the bottom of the coated substrate in the liquid and measuring the time of rise to a given distance (5 cm at 5 mm intervals). A plot of capillary height vs. the square root of time should be a straight line if the Lucas-Washburn equation is obeyed. Mylar or glass substrate did not significantly affect the capillary rise process of the liquids used in this study nor did partial fusion of the films with moderate heat.

Contact angle for hot pressed and vapor fused films were measured directly on the film with a goniometer.

2.5.2. *Surface Energy* . The surface energy (γ) of the material was calculated using the Young and Fowkes equations (21).

Young's equation:

$$\gamma_{lv} \cos\theta = \gamma_{sv} - \gamma_{sl} - \pi_e$$

where

γ_{lv} = free energy of the liquid against its saturated vapour

γ_{sv} = free energy of the solid against its saturated vapour

γ_{sl} = free energy of the interface between liquid and solid

π_e = equilibrium pressure of adsorbed vapour of the liquid on the solid

Fowkes' equation:

$$\gamma_{lv} = \gamma_l^d + \gamma_l^h$$

where

γ = free energy

d = dispersion force or non-polar component

h = hydrogen bonding or polar component

Assuming $\pi_e = 0$ and combining the two equations the total surface energy of the solid and its components can be calculated using the following equation:

$$\left(\gamma_s^d \gamma_l^d\right)^{\frac{1}{2}} + \left(\gamma_s^h \gamma_l^h\right)^{\frac{1}{2}} = \frac{(1 + \cos\theta)\gamma_{lv}}{2}$$

Table 2 summarizes the total free energy and its components of some common liquids used in surface energy studies.

TABLE 2

FREE ENERGY (γ_{lv}) OF COMMON LIQUIDS USED FOR SURFACE ENERGY STUDIES

Liquid	$\gamma_l^d \times 10^3$ (N/m)	$\gamma_l^h \times 10^3$ (N/m)	$\gamma_{lv} \times 10^3$ (N/m)
water	21.8 ± 0.7	51.0	72.8 (Ref.22)
methylene iodide	49.5	1.3	50.8 (Ref.22)
formamide	39.5 ± 7	18.7	58.2 (Ref.22)
glycerol	37.0 ± 4		63.4 (Ref.22)
benzyl alcohol	38.3	1.6	39.9 (Ref.23)
hexane			18.43 (Ref.24)

The actual results for the total free energy (γ_{sv}) of the polymer film and its components were obtained by solving the last equation for liquid pairs that have different polar and non-polar surface energy contributions: hydrogen bonding (γ_l^h) and dispersion component (γ_l^d), respectively.

3. RESULTS AND DISCUSSION

3.1 FILM FORMING AND POST-TREATMENT

The latex was coated or easily spread on either porous or impervious substrates. Room temperature drying of the resulting film seems a critical step in obtaining malleable and smooth films after the various treatments. Rapid evaporation of the solvent by heat hardens the material.

The first drying of the suspension into a film did not induce spherulite formation, not even when the film was heated past its melting temperature. Its percent crystallinity remained constant right after cooling i.e. it did not change with time.

Wide angle x-ray diffraction (WAX) patterns recorded on the latex "as received" and after different treatments showed the PHB crystal lattice. In the case of the wet slurry there was some crystalline order in the "as received" state, perhaps as a result of the various purification steps.

Spray drying of the latex to a powder form (ICI commercial process) irreversibly changed the nascent granular morphology and solid state characteristics even though their particulate nature remain unchanged (S.E.M.) (Fig. 1). The observed crystallinity of the granules in the "as received" suspension suggests that the original nascent morphology was induced to crystallize, to a degree, due to the isolation treatment.

Air dried films were partially fused when heated at 100 °C for a period of 10 minutes. These white opaque films are extremely porous as indicated by capillary rise measurements and scanning electron microscopy (Fig. 2). The latter technique clearly showed the particulate nature of the starting latex. These films consist of 0.5 to 1.5 µm particles forming an open network with some coalescence between the particles. Heating the air dried films at 50 °C did not produce fusion.

Hot pressing produced transparent and flexible films of uniform crystallinity. The particulate, porous nature of the partially fused film was completely lost. These tough films, if of sufficient thickness, neck and turn white and opaque when cold stretched. The elongation to break by hand stretching is about 30%. Thin films display brittle fracture, similar to paper (no necking). Since the continuity in these films is due to coalescence or surface fusion of the outer layer of the granules as opposed to chain entanglements in solvent cast films, this low elongation is not surprising. Chloroform vapour fused films do not show very high elongation to break either but their clarity is remarkable as is the absence of spherulites.

3.2. SURFACE PROPERTIES AND CAPILLARY RISE

The surface energy of a material and its polar and non polar components is useful in predicting interactions with liquids and its adhesion properties. Contact angle (θ) of a liquid against a solid is a measure of the interaction forces characteristics. As described in the experimental section two methods of surface characterization were used according to the films characteristics.

3.2.1. *Capillary Rise, Capillary Radius and Contact Angle.* The capillary rise results for air dry and partially fused microporous films are summarized in Table 3.

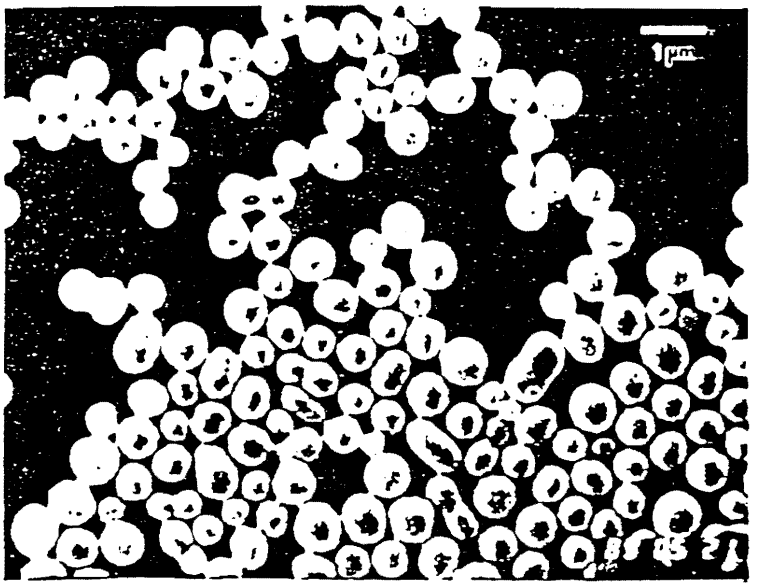


Fig. 1 Scanning electron micrographs of PHB granules (spray dried powder), ICI Biopol™ Marlborough Biopolymers Ltd..

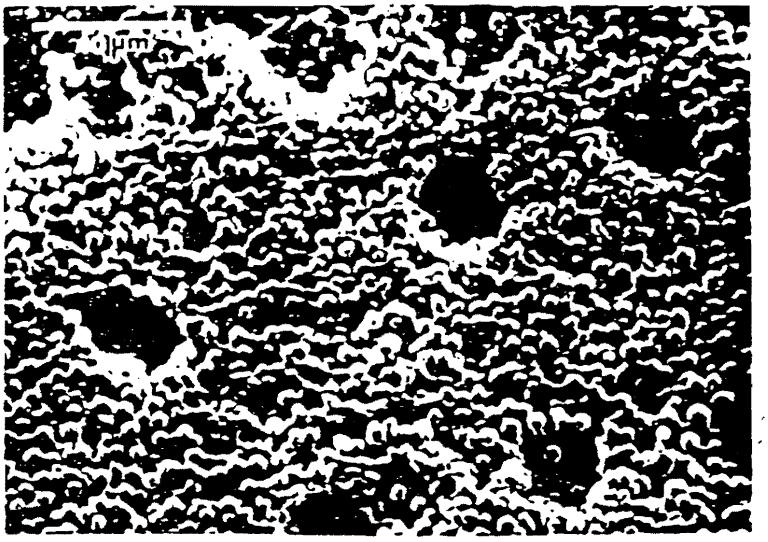


Fig. 2 PHB/HV film (21% HV), made by air drying a 20% w/w solids latex suspension followed by heating at 130 °C for 10 minutes.

TABLE 3
CALCULATED CONTACT ANGLE (θ) OF LIQUIDS
AGAINST AIR DRY AND PREFUSED
(POROUS) PHB/HV LATEX FILMS

Liquid	Contact Angle (θ) deg.	cosθ
Water	71.11	0.324
Formamide	70.00	0.342
Benzyl alcohol	89.68	0.006

Capillary rise of liquids was linear with respect to the square root of time. From the knowledge of the slope, solvent viscosity (η), surface tension (γ) using a liquid that has a zero contact angle (θ) with the surface (cos 0° = 1), the effective capillary radius of these films can be calculated (19). Hexane was chosen as it has approximately zero contact angle. The capillary radius was found to be 0.158 μm. This is in good agreement with electron microscopy observations.

Contact angle of liquids against hot pressed and chloroform vapour-fused films could be measured directly on the films with a goniometer. The results are shown in Table 4.

Chloroform vapor fused and hot pressed films have the same transparency but as seen in Table 4 they behave significantly different with liquids other than water. The chloroform film being more inert to formamide and benzyl alcohol. It should be noted that the chloroform vapor treated film surface was extremely smooth while the hot pressed film was not, as the small dents and roughness of the hot plate were transferred to the film. Contact angle values and liquid spreading may be influenced by microscopic surface roughness (24).

The hot pressed film reacted with benzyl alcohol, the film shrank and buckled under the liquid, no reliable measurement could be obtained.

3.2.2. *Surface energy*. The surface energy components of the various latex films are summarized in Table 5.

Hot pressing of the films produced a significant increase in the total surface energy of the material. It also changed the contribution of the dispersion or non polar component to a higher value. In the case of the chloroform vapor fused film each component contributes almost half total surface energy. For chloroform vapor fused films increased contact angle values may be due to the disappearance of the capillary structure as opposed to a decrease in the hydrophilic interaction contribution. Chloroform readily solubilizes the polymer.

TABLE 4

MEASURED CONTACT ANGLE (θ) OF HOT PRESSED AND CHLOROFORM VAPOR FUSED PHB/HV LATEX FILMS

Sample treatment	Liquid	Contact Angle (θ) deg.	cosθ
Hot pressed	Water	68.15	0.372
"	Formamide	44.68	0.711
"	Benzyl alcohol	-	-
Chloroform vapor fused	Water	68.65	0.364
	Formamide	59.28	0.511
	Benzyl alcohol	21.29	0.932

TABLE 5

FREE ENERGY (γ_{sv}) AND ITS COMPONENTS OF PHB/HV LATEX FILMS

Sample treatment	Liquid pair	$\gamma_{sv} \times 10^3$ (N/m)	$\gamma_s^d \times 10^3$ (N/m)	$\gamma_s^h \times 10^3$ (N/m)
air dry or partially fused films	water/benzyl alcohol	33.33	4.42	28.91
"	water/ formamide	31.06	8.82	22.24
hot pressed films	"	42.8	31.96	10.88
chloroform vapor fused films	"	34.86	16.16	18.70

In both cases, the segmental motion induced by heat or solvents in the absence of water tends to minimize free energy. Hot pressing of the films in the absence of water, forces the hydrophilic functional groups (carboxylated esters and hydroxy groups) to rotate inside the rest of the polymer chain (hydrocarbon, hydrophobic component) creating also an hydrophobic surface with a significant high dispersion component contribution.

For comparison purposes the surface energy of widely used synthetic and natural polymers are shown in Table 6 (26).

4. CONCLUSIONS

The morphology of the latex films which develops due to hot pressing or by exposure to solvents encourages the use of this material for novel film and coating applications. These results show that a different physical form of the PHA material, or its use at various stages of the extraction and purification process, yields products of different properties and characteristics. The post-treated air dried films made of the never dried latex are uniformly crystalline, non-spherulitic, transparent and very flexible. Furthermore, the degree of porosity is adjustable and the wettability characteristics suggest that the films could be loaded with medicaments or adjuvents for particular applications.

TABLE 6

SURFACE ENERGY (γ_{su}) OF SOME POLYMERS AT 20 °C

Polymer	$\gamma_{su} \times 10^3$ (N/m)
cellulose	34 - 42
hemicelluloses	33 - 36
lignin	33 - 37
polyvinylalcohol	37
polystyrene	33
polyvinyl chloride	40
polyethylene	31
polytetrafluoroethylene	18.5
starch	39

In particular, the ready sorption of the PHA latex by fibrous constructs such as paper or non wovens suggests applications such as binder, coating material or barrier. Techniques of spherical agglomeration of the latex should allow control of particle size beyond the natural occurrence of about 0.5 µm. Even asymmetry in particle shape is conceivable by suitable shear treatment of the

latex. These applications in fibrous constructs become particularly attractive when the natural biodegradability of PHA is considered.

From a polymer morphology perspective, the most notable result in this study is the non-spherulitic character of the films. They are flexible but their extension to break is almost like that of paper. This suggests that chain entanglement is limited to interparticle surface and that a strong element of nascent morphology is still present inside particle domains. The chloroform vapour treated films have improved toughness but still lack the large extension to break characteristics of thermoplastics that display necking on extension.

In a separate study it has been shown that the enzyme purification process and/or some HV content in the granules are not limiting requirements to produce non spherulitic, translucent, flexible polymer films from PHA latex. Films made out of granules purified by the hypochlorite process proved to be of comparable flexibility and crystallinity. All results seem to indicate the importance of processing the material in its never dried latex state.

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Amorphous, Biomimetic Granules of Polyhydroxybutyrate: Preparation, Characterization, and Biological Implications†

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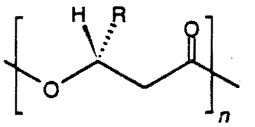
Abstract: It is a striking feature of the bacterial storage polymer poly-3-hydroxybutyrate (PHB) that, although the isolated polymer is highly crystalline, native PHB storage granules in cells are only found in an amorphous, mobile state. It has recently been proposed that the failure of PHB granules to crystallize is simply the result of slow nucleation kinetics that are operative for small particles. In support of this new model, we report here a straightforward procedure by which pure crystalline PHB can be reconstituted into submicron-size artificial granules. In the artificial granules, a synthetic surfactant has been substituted for the native granule coating. The artificial granules are amorphous and stable in suspension, and they are essentially indistinguishable from their native counterparts in terms of size, morphology, molecular mobility, and density. Furthermore, when the surfactant coating is removed from the artificial granules by dialysis, the granules crystallize, verifying the nucleation hypothesis. *In vivo*, the PHB granule surface is likely to consist of both protein and phospholipid; *in vitro* it is possible to prepare amorphous PHB granules which are stabilized solely by phospholipid. PHB artificial granule latexes crystallize well on drying and annealing, making them potentially useful in the preparation of polymer coatings. Artificial amorphous granules may also be prepared from other bacterial polyhydroxyalkanoates (PHAs) and from blends of incompatible polyesters.

Introduction

Poly-3-hydroxyalkanoates (PHAs, **1**) are linear, hydrophobic polyesters that occur naturally in a wide variety of bacteria and other organisms.[1] In bacteria the polymers function as energy and carbon storage materials. Poly-R-3-hydroxybutyrate (PHB, **2**) was the first of the PHAs to be discovered and is the most widespread in nature. PHAs have attracted substantial industrial interest as biologically derived, fully biodegradable thermoplastics; poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate (PHBV or Biopol, **3**) is now produced commercially by fermentation for use in packaging and other applications. Due to its absolute stereoregularity, isolated bacterial PHB crystallizes readily from the melt (T_m 180 °C) and achieves a high degree of crystallinity, typically about 70%.



1: PHA, R = various alkyl, alkenyl, and aryl groups (C_{1-11})
2: PHB, R = CH_3
3: PHBV (Biopol), R = CH_3, CH_2CH_3

As PHB is insoluble in water, the polymer *in vivo* occurs in the form of discrete inclusion bodies or "granules", which under

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† A preliminary account of this work has appeared: Horowitz, D. M.; Clauss, J.; Hunter, B. K.; Sanders, J. K. M. *Nature* **1993**, *363*, 23. Abbreviations used: PHB (poly-3-hydroxybutyrate), PHBV (poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate), PHO (poly-3-hydroxyoctanoate), PHA (poly-3-hydroxyalkanoate), CTAB (cetyltrimethylammonium bromide), SDS (sodium dodecyl sulfate), EDTA (ethylenediaminetetraacetate), BHT (butylated hydroxytoluene), WAXS (wide-angle X-ray scattering), O/W (oil-in-water).

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appropriate cultural conditions may comprise up to 80% of the dry weight of the cell. The granules vary in size and number among different organisms, but in the commercial bacterium *Alcaligenes eutrophus*, a typical PHB-rich cell contains 8–12 granules, averaging 0.25 μm in diameter.[2] PHB is by far the major constituent of the granules; native granules from *Bacillus megaterium* were reported to contain 97.7% PHB together with 1.9% protein and 0.4% lipid,[3] the latter two presumed to form a surface coating around an essentially pure PHB core. The physical state of the PHB within the granules has been much debated.[4] PHB granules were characterized initially in the 1960s by carbon-replica electron microscopy[5] and X-ray diffraction.[6] It was concluded that PHB *in vivo* was crystalline with the same structure as the isolated polymer. This finding remained unchallenged until the late 1980s when studies of whole, PHB-producing cells by ^{13}C-NMR spectroscopy,[7] wide-angle X-ray scattering (WAXS),[8] and transmission electron microscopy[9] revealed unequivocally that the polymer *in vivo* is completely amorphous. ^{13}C-NMR spectroscopy proved to be an especially suitable technique for resolving this issue, as mobile polymer could be detected directly in intact, living bacterial cells in aqueous suspension. Crystalline PHB granules would display no NMR spectrum using conventional solution-state techniques; however, mobile amorphous polymer would be expected to exhibit sharp lines. It was found that the polymer *in vivo* does indeed have a readily observable spectrum at temperatures 50 °C above T_g but some 130 °C below T_m. As T_g for the homopolymer is 5 °C, the polymer in living cells at 30 °C is in an elastomeric state.

Isolation of the PHB granules by all but the mildest techniques has been found to lead to apparently irreversible crystallization. In early studies, biochemical "inactivation" of native granules, now understood to involve crystallization of the polymer, was associated with a variety of treatments, including excessive centrifugation, repeated freezing and thawing, and exposure to organic solvents, proteolytic enzymes, and heat.[10] In recent years, numerous hypotheses have been advanced to explain the metastable amorphous state of the polymer *in vivo*. The involvement of labile protein or lipid factors,[11] the existence of natural plasticizers,[12] keto–enol tautomerism,[13] and the existence of a water-stabilized extended chain conformation[14] have all been suggested. None of these hypotheses, however, have gained much direct experimental support.

Two groups proposed recently that the amorphous state of PHB *in vivo* can be explained by a simple physical–kinetic mechanism.[15] The essence of the kinetic model is that polymer that is newly synthesized in an amorphous mobile form within the granule will only crystallize as a result of spontaneous nucleation. It has long been recognized that the macroscopic rate of crystallization of a liquid polymer or other substance can be significantly retarded by subdividing the substance into a large number of small, physically isolated droplets.[16] In such systems, crystallinity in one droplet does not seed crystallization in other droplets. When crystallization does occur in a droplet, its propagation within that droplet is rapid. Bulk crystallization may be slowed dramatically provided that (1) the droplets are sufficiently small that only a small proportion contain nucleating contaminants and (2) the frequency of spontaneous nucleation within individidual droplets is low. Slow crystallization has been observed experimentally at temperatures well below T_m for isolated droplets of a variety of metals and small molecules[16,17] as well as for several polyolefins, polyethers, and polyamides.[18] In such systems, the fraction of droplets nucleated spontaneously with time (n/n_0) is given by a simple exponential function:[19]

$$n/n_0 = 1 - e^{-Iv t}$$

where I is the nucleation rate constant and v is the droplet volume.

As PHB occurs in quite pure form within native granules, which are extremely small and are isolated from their environs by a protein/lipid coat, it is proposed that the rate of PHB crystallization *in vivo* is governed by the rate of spontaneous nucleation events within individual granules. Since this rate depends upon the granule volume, and therefore on the third power of radius, its predicted value is surprisingly low for the submicron-size polymer storage granules. The upper limit for the rate constant I of spontaneous crystal nucleation in isolated PHB at 30 °C (the temperature of bacterial growth) is 2.5 events mm^{-3} s^{-1}.[20] The corresponding rate of nucleation, Iv, for a typical storage granule of diameter 0.25 μm is 2.0×10^{-11} events s^{-1}. In the absence of any perturbation that causes granule coalescence or exposure of the polymer to heterogeneous nucleation catalysts, an ensemble of native granules should thus exhibit a crystallization half-life of at least 3.4×10^{10} s, or >1000 year.

It is a consequence of this model for the physical state of PHB *in vivo* that it might be possible to recreate the amorphous state of the polymer *in vitro* using pure PHB.[15a] Specifically, the model predicts that the native state could be duplicated if it were possible to generate PHB that was (1) amorphous at the outset; (2) contained in discrete, physically separate microscopic particles; and (3) either relatively free of nucleating contaminants or else coated in such a way that direct exposure of the polymer to the potential nucleants would be minimized.[21] We show here that a remarkably straightforward procedure can be used to prepare artificial PHB granules that are amorphous and generally indistinguishable from their native counterparts. Once again, as in the case of the native granules contained in whole cells, NMR has been used in a simple and noninvasive manner to verify the

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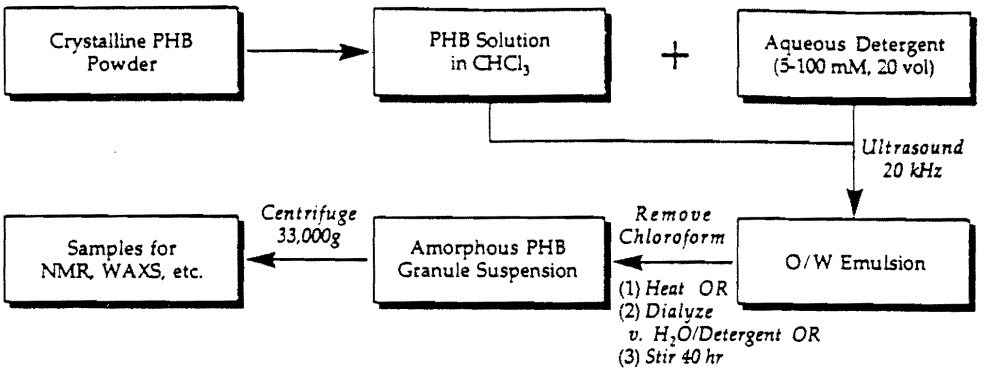
Scheme 1



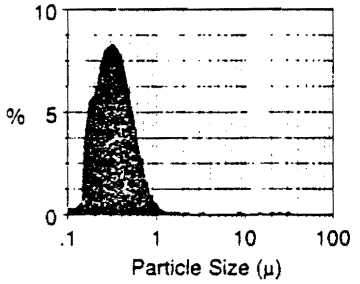


Figure 1. Particle size volume distribution for CTAB-coated artificial PHB granules, as determined by laser light scattering. The median of the distribution function was 0.3–0.4 μm.

presence of mobile, amorphous material within the artificial PHB particles. The crystallization properties of the artificial granules provide strong evidence for the competence of the nucleation model to explain PHB behavior *in vivo*.

Results

Preparation of Artificial PHB Granules. Artificial, amorphous PHB granules were prepared as shown in Scheme 1. First, a chloroform solution of PHB was emulsified ultrasonically with 20 volumes of an aqueous surfactant (e.g. 50 mM cetyltrimethylammonium bromide, CTAB). The resulting opaque emulsion contained PHB in chloroform solution, as confirmed by ^{13}C-NMR spectroscopy. The organic solvent was removed from the emulsion by (a) heating (75 °C for 90 min); (b) stirring in air (40 h); or (c) dialysis against additional aqueous surfactant. The translucent suspension that formed contained amorphous, surfactant-coated PHB particles, which were similar to native granules by a variety of criteria, as described below. This general procedure could be used to make artificial granule suspensions from PHB, PHBV copolymers (various compositions), poly-3-hydroxyoctanoate (PHO), and several other PHAs. Dichloromethane, but not 1,2-dichloroethane, could also be used as a solvent for PHB to produce amorphous granules; water is the only solvent identified thus far that may be used for the continuous phase. A variety of surfactants could be used to prepare granules, which by the criterion of ^{13}C-NMR spectroscopy, contained amorphous PHB (see Chart 1). These included a number of cationic detergents, of the quaternary amine family; several common anionic detergents, including sodium dodecyl sulfate (SDS) and the bile acid salts sodium cholate and deoxycholate; common fatty acid salts (soaps); and one nonionic detergent. Omission of the surfactant from the system resulted in rapid flocculation and crystallization of the PHB following removal of the organic solvent. While ultrasonic treatment was the preferred method of emulsification, a mechanical homogenizer was also partially effective. Ultrasonic treatment resulted in some reduction of the average polymer molecular weight (M_w), from 690 000 to 140 000–330 000.

Artificial granules could be concentrated by centrifugation, which was usually carried out in two rounds of 30 min each at

Chart 1

Cationic Detergents

Benzalkonium Cl
Benzethonium Cl
Benzyldimethyldodecylammonium Br
Benzyldimethylhexadecylammonium Br
Benzyldimethyltetradecylammonium Br
Cetyldimethylethylammonium Br
Cetylpyridinium Cl
Cetyltrimethylammonium Br
Dodecyltrimethylammonium Br
Methylbenzethonium Cl
Tetradecyltrimethylammonium Br

Anionic Detergents

Sodium Cholate
Sodium Deoxycholate
Sodium Dioctylsulfosuccinate
Sodium Dodecyl Sulfate
Sodium Sarkosyl

Soaps

Sodium Laurate
Sodium Myristate
Sodium Palmitate
Sodium Stearate

Nonionic Detergent

Sorbitan Monopalmitate

8000 and 33000*g*. Provided the granules were promptly resuspended in water, centrifugation did not appear to promote crystallization, in contrast to the behavior reported for native granules.[10a]

Artificial Granule Size and Morphology. Artificial granules were examined by several techniques to determine their size and morphological characteristics. Suspensions of granules prepared using CTAB and other surfactants were examined by laser light-scattering (Figure 1). The median particle diameter for the CTAB-coated granules estimated by this technique was 0.3–0.4 μm. Variables such as the surfactant concentration during emulsification, the duration of ultrasonic treatment, and the nature of the surfactant did not exercise any pronounced effect on the particle size distribution of the granules.

In addition, dilute suspensions of CTAB-coated granules were air-dried onto holey carbon films and examined by transmission electron microscopy (Figure 2). The granules appeared as somewhat deformable microspheres with typical diameter 0.1–0.3 μm. Similar results were obtained by fluorescence microscopy. Artificial granules were stained with the dye Nile Red,[22] a selective histological stain for PHB and other lipid inclusions. The granules were visible as red-orange microspheres, fluorescent at >580 nm (data not shown). Comparison with similarly stained native granules contained in whole *A. eutrophus* cells showed no significant difference in size or morphology. It has been reported that solid fatty inclusions do not color with this dye,[23] and thus the staining of artificial granules is consistent with their containing amorphous, elastomeric PHB.

Granule Structure and Molecular Mobility. Artificial granules prepared using CTAB were collected from aqueous suspension by centrifugation at 8000*g* and applied as a paste to a glass X-ray slide. Both whole, PHB-rich cells of *A. eutrophus* (Figure 3a) and artificial granules (Figure 3c) show only an amorphous halo when viewed by WAXS. When PHB is isolated from whole cells

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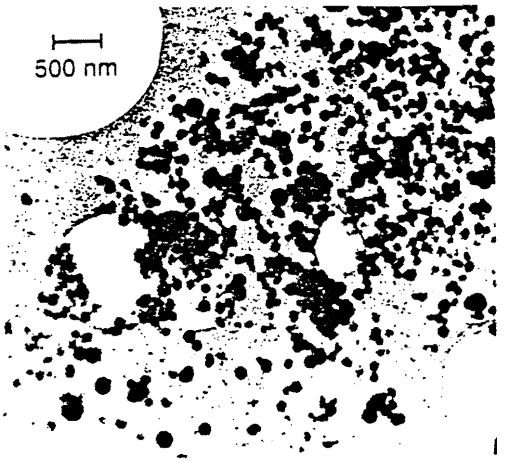

Figure 2. Transmission electron micrograph of CTAB-coated artificial PHB granules. Artificial granules appear as small dark spheres; large circles are holes in the carbon film support.

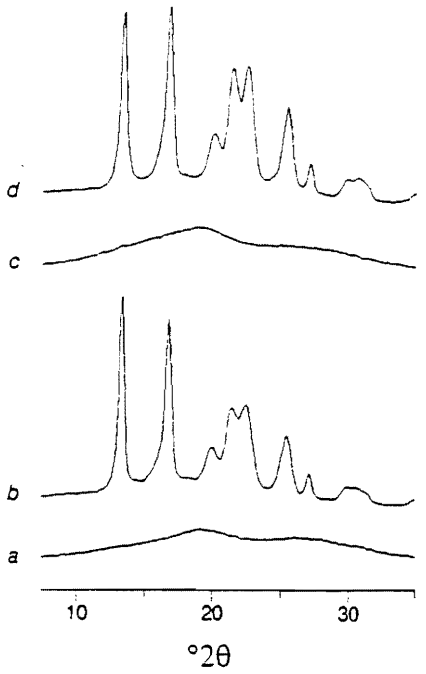

Figure 3. Wide-angle X-ray scattering (WAXS) patterns for (a) whole, PHB-rich cells of *A. eutrophus*; (b) isolated, crystalline PHB powder; (c) artificial, amorphous PHB granules; and (d) artificial granule paste dried and annealed overnight at 125 °C.

by the commercial aqueous method, the polymer undergoes ready crystallization. Thus the isolated PHB sample in Figure 3b shows a series of sharp peaks in its diffraction pattern, indicative of a crystallinity of 69%. Air drying of the granule paste sample in Figure 3c likewise results in the onset of crystallization, presumably due to the destabilization of the protective surfactant coating. On annealing overnight at 125 °C, the artificial granule paste forms a film of 73% crystallinity (Figure 3d), comparable to that of the initial powder form.

The elastomeric state of PHB granules *in vivo* was originally detected by ^{13}C-NMR spectroscopy. Elastomeric polymers at temperatures well above T_g show sharp NMR resonances, whereas the resonances derived from crystalline solids are extremely broad (>1000 Hz) and are thus invisible by conventional solution techniques. Aqueous suspensions of artificial, CTAB-coated granules and whole, PHB-rich cells of *A. eutrophus* were examined in parallel by NMR at various temperatures from 30–90 °C. The spectra obtained (Figure 4) show strikingly similar mobility properties across the entire temperature range. The NMR spectra also reveal the notable absence of residual chloroform from the

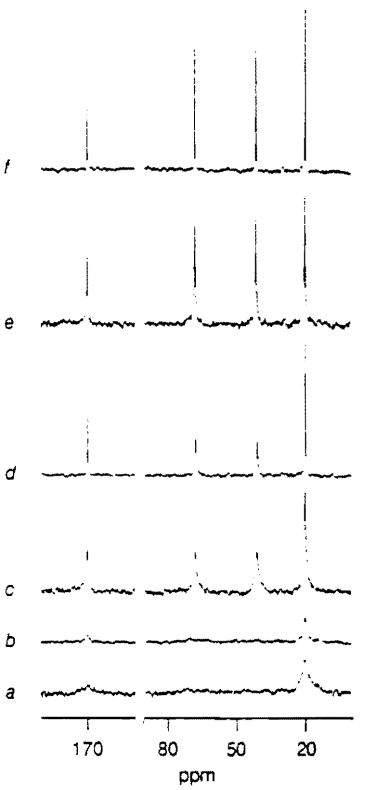

Figure 4. Natural abundance 100-MHz ^{13}C-NMR spectra of whole, PHB-rich cells a *A. eutrophus* and artificial, amorphous PHB granules. Cells or granules were collected by centrifugation and resuspended in D_2O. Samples a, c, and e are whole cells at 30, 50, and 90 °C, respectively; samples b, d, and f are artificial granules at 30, 50, and 90 °C. Signals at δ 170, 68, 41, and 20 ppm represent the PHB carbonyl, methine, methylene, and methyl resonances, respectively.

artificial granules;[24] furthermore, only a trace of surfactant can be seen. It should be noted that 70% crystalline PHB powder has no NMR spectrum at these temperatures, as the amorphous domains of the polymer are sufficiently small and constrained to prevent their giving rise to sharp lines. Determinations of the NMR relaxation time constants T_1 and T_2, described elsewhere,[25] also reveal a remarkable similarity between native and artificial granules over a range of temperatures.

Granule Density Analysis. The densities of native and artificial granules were determined in parallel by Nycodenz density gradient centrifugation. Crude native PHB granules were isolated by a gentle lysozyme/ultrasound treatment from *A. eutrophus* grown under nutrient limitation; the native granules were then purified on sucrose step gradients. Suspensions of purified native granules and SDS-coated artificial granules were applied to linear 30–50% (wt/v) Nycodenz gradients, which were developed at 110000*g*. Gradients were fractionated and the fractions assayed quantitatively for PHB (Figure 5). The average density of artificial granules was estimated to be 1.180 g/cm^3, compared to 1.170 g/cm^3 for native granules. Both values were similar to the density of 1.176 g/cm^3 reported for pure amorphous PHB quenched from the melt.[26] By contrast, crystalline PHB powder

(24) Artificial granule suspensions prepared by the heating method were examined by GC to quantify residual chloroform at various stages in the heating process. After 60 min at 75 °C, residual chloroform had been reduced to <13 ppm (i.e. <0.5% wt/wt compared to PHB). After 90 min at 75 °C, chloroform was no longer detectable in the suspension (detection limit 1–2 ppm), but extrapolation from earlier values would indicate the presence of 0.24 ppm (<100 ppm wt/wt on PHB).

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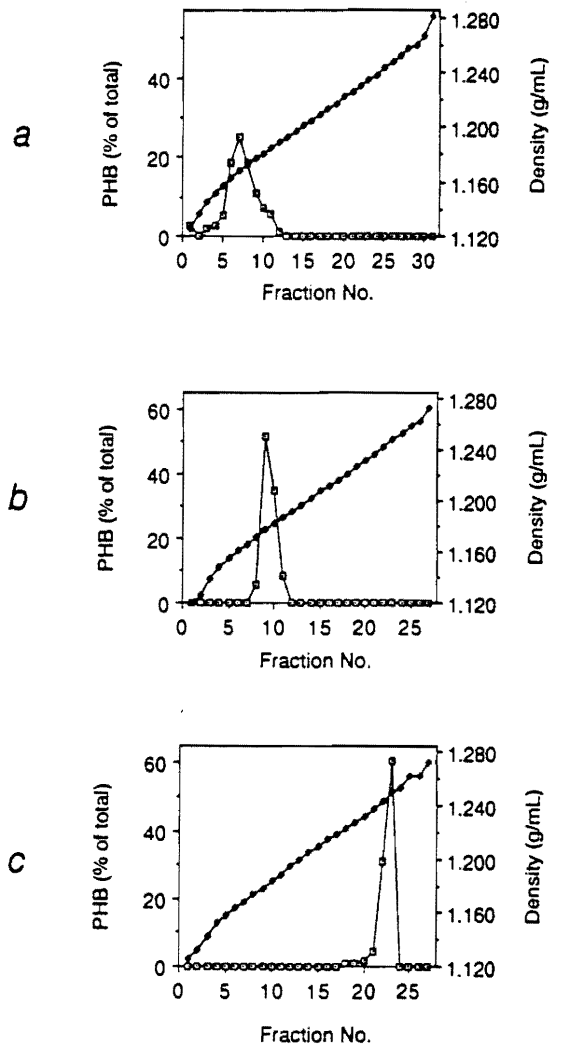

Figure 5. Nycodenz density gradient profiles for (a) isolated, native PHB storage granules from *A. eutrophus*; (b) artificial, amorphous PHB granules (SDS-coated); and (c) crystalline PHB powder. Linear Nycodenz gradients (prepared from 30–50% wt/v stock solutions) were loaded with sample, developed at 110000*g*, and fractionated. Fractions were analyzed by gas chromatography for PHB (open squares); density was determined by refractometry (closed diamonds).

exhibited a density of 1.246 g/cm^3 in Nycodenz (identical to the literature value[27]).

Artificial Granule Stability. A sample of CTAB-coated artificial granules was maintained at 30 °C for a prolonged period in a sealed NMR tube containing a benzene-d_6 capillary as internal reference (Figure 6a). The sample was examined regularly by ^{13}C-NMR spectroscopy (70 °C) to detect amorphous PHB. After a period of nearly one year, there was no measurable decrease in the intensity of the four polymer resonances relative to that of the benzene internal standard (Figure 6b). Similar stability was observed with granules prepared using the anionic surfactants SDS, sodium sarkosyl, sodium deoxycholate, and sodium dioctylsulfosuccinate (data not shown).

Although artificial amorphous granules appeared to be stable indefinitely when not perturbed, stability was absolutely dependent upon the presence of the surfactant coating. When a sample of CTAB-coated PHB granules was dialyzed against a large volume of water to remove the surfactant, flocculation and crystallization rapidly ensued. No polymer signals were visible in the ^{13}C-NMR spectrum of this sample following dialysis (Figure 6c). However, inclusion of 1 mM CTAB in the dialysis buffer was sufficient to protect the granules from denaturation (data not shown).

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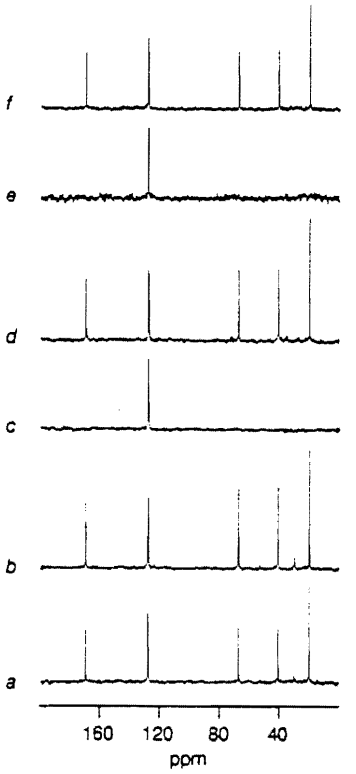

Figure 6. Natural abundance 100-MHz ^{13}C-NMR spectra, at 70 °C, of aqueous suspensions (D_2O or H_2O) of various artificial granule preparations. The peak in each spectrum at δ 128 ppm is derived from a sealed benzene-d_6 capillary, which served as an internal intensity standard. Spectrum a was obtained from a freshly prepared sample of CTAB-coated, artificial PHB granules; spectrum b was obtained from the identical sample after 11 months' incubation at 30 °C. Spectrum c was obtained from a sample of CTAB-coated, artificial amorphous granules dialyzed exhaustively against distilled water. Spectrum d was collected from a sample of artificial amorphous PHB granules prepared using the detergent sodium cholate. Dialysis of these granules against distilled water causes crystallization and loss of NMR signals (e). Addition of solubilized soy phospholipids prior to dialysis protects the granules from crystallization, leaving the NMR spectrum intact (f).

Phospholipid-Coated Granules. The readiness with which water-soluble surfactants could be removed from the granules suggested that the granule coating could be replaced by exchange with other amphipathic substances. Accordingly, artificial granules coated with sodium cholate (NMR spectrum, Figure 6d) were collected by centrifugation and resuspended in an aqueous buffer containing soy phospholipids solubilized by sonication in a buffer containing sodium cholate (2%). A variety of lipid concentrations, from 0 to 40 mg/mL, were used. Granules resuspended in lipid/detergent cocktail were then dialyzed exhaustively against phosphate buffer containing Amberlite XAD-2 to remove the cholate detergent. Inclusion of soy phospholipids at concentrations of 10 mg/mL or higher was found to protect PHB NMR signals from loss during dialysis (Figure 6f); in the absence of added lipid these signals were completely lost (Figure 6e), as with the CTAB-coated granules above.

Native-Granule-Associated Phospholipids. Lipids extracts were prepared from whole, PHB-rich cells of *A. eutrophus* and from sucrose density gradient-purified native granules. Analysis of the two samples by two-dimensional TLC revealed the same spectrum of four phospholipids in each. Three were apparently identical to phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The fourth minor component (R_{f1} – 0.33, R_{f2} – 0.34), which was ninhydrin-positive and stained with the Dittmer phosphate reagent, has not been identified.

Artificial Granules from Polymer Mixtures. Artificial granules were prepared as above from a chloroform solution containing 2.5% (wt/v) of PHB and 2.5% (wt/v) of poly-3-hydroxybutyrate-

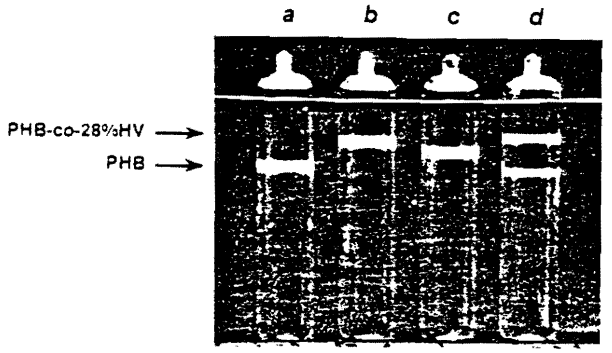

Figure 7. Nycodenz density gradients (prepared from 30–50% wt/v stock solutions) of various SDS-coated artificial amorphous granule samples. Gradient a was loaded with granules prepared from PHB homopolymer (ρ = 1.18 g/cm^3), while gradient b contained granules made from poly-3-hydroxybutyrate-*co*-28%-3-hydroxyvalerate (PHB-*co*-28%HV, ρ = 1.16 g/cm^3). Gradient c was loaded with granules made from a chloroform solution containing PHB and PHB-*co*-28%HV in equal proportions. Gradient d was loaded with a mixture of separately prepared PHB and PHB-*co*-28%HV granules. Note that in gradient c a single band of homogeneous density was observed ($\rho \approx$ 1.17 g/cm^3), indicating the coexistence of PHB and PHB-*co*-28%HV, two polymers that are immiscible in the melt, within individual artificial granules. In a mixture of PHB and PHBV granules (d), two distinct bands occur, showing that the results in gradient c are not simply due to aggregation of granules derived from two separate populations.

co-28%-hydroxyvalerate, two polymers that are incompatible in the melt.[28] Following organic solvent removal, the granules were concentrated by centrifugation and examined on Nycodenz density gradients, in parallel with samples of pure granules obtained from each polymer separately (Figure 7). It was found that the mixed polymer granules formed a single homogeneous population (Figure 7c), with density intermediate between that of the two pure polymer granule samples. In a control experiment to exclude the possibility of granule aggregation, it was shown (Figure 7d) that a mixture of separately prepared PHB and PHBV granules displayed a clearly bimodal density distribution. A paste of mixed polymer granules, which was completely amorphous by WAXS (data not shown), formed a 60% crystalline film on drying and annealing at 125 °C.

Discussion

Artificial Granules and the Nucleation Model. The foregoing results demonstrate that isolated, crystalline PHB can be simply reconstituted into an aqueous suspension of amorphous polymer particles, which are virtually indistinguishable from native PHB storage granules. The procedure used to prepare these artificial granules is effective apparently because (1) at the point at which the polymer is precipitated from chloroform solution, it is already in the form of particles that are too small to nucleate spontaneously and (2) the ionic detergent used in the emulsification provides a robust coating for the PHB, both protecting the granules from heterogeneous nucleation catalysts and preventing granule coalescence through electrostatic repulsion.

A variety of techniques was used to characterize the artificial granules. Electron micrographs indicate that the size range of the artificial granules, 0.1–0.3 μm, is essentially the same as that of native storage granules from *A. eutrophus*, a conclusion that is confirmed by light scattering and optical fluorescence microscopy. Wide-angle X-ray scattering and ^{13}C-NMR data further demonstrate that the granules contain PHB in a wholly amorphous state, with molecular mobility essentially identical to that of the native polymer. Buoyant density measurements provide further confirmation of the similar physical state of native and artificial granules.

(28) Hammond, T. Personal communication.

The PHB used in these experiments was of extremely high purity (99.9%), and thus models in which endogenous plasticizers or other auxiliary factors are required to keep the polymer amorphous *in vivo* are explicitly disproved. The only cofactor required to maintain the polymer in an amorphous state in the artificial system is an amphiphatic species capable of coating the polymer granules. Artificial granules may be prepared using any of a wide array of synthetic surfactants, indicating that no specific surfactant–polymer interaction is responsible for the amorphous state of the polymer.

In one recently proposed alternative to the nucleation model, a small amount of hydrogen-bonded water in the native granule interior maintains the polymer in a hypothetical β-conformation that is incapable of crystallizing.[14] Infrared spectroscopy and other measurements do support the presence of some water ($\leq$5%) associated with both native granules[14b] and artificial granules (data not shown). These methods cannot distinguish, however, between water entrapped in pockets within or between the granules and water that is actually PHB-bound. In any case, no model system has been reported to date in which the polymer remains amorphous through formation of a complex with water. On the other hand, our results strongly support the kinetic nucleation model. It is especially noteworthy that removal of the surfactant from the artificial granules by dialysis results in ready crystallization of the polymer. Surfactant removal allows the coalescence of the artificial granules into larger masses (which have higher nucleation frequencies) and/or allows the polymer access to nucleating contaminants in surfaces. Small amounts of surfactant added to the dialysis buffer completely protect the granules from crystallization. None of these observations can be accommodated by the thermodynamic water model.

The stability of the artificial granules is far greater than that of native granules or of most other polymer droplets studied. The extraordinary stability of the artificial amorphous granules at 30 °C is, however, entirely in keeping with the >1000 year half-life predicted by the kinetic model. Even at 50 °C, where the spontaneous nucleation rate constant for PHB is maximal,[20b] the predicted half-life for granule crystallization is still on the order of at least 100 years. Indeed, artificial granules that were repeatedly heated from 30 to 70 °C during the course of one year for NMR experiments showed no measurable loss of signal. Thus the bacterial polyesters appear to form a class of substances where no significant solidification of amorphous droplets occurs over the entire range of temperatures where crystallization could occur.

Although slow crystallization has previously been seen with other polymer droplets,[18] in all cases (with the possible exception of isotactic polybutene-1)[18e] the amorphous droplets could withstand only a finite degree of undercooling. Critical solidification usually occurred at a point 50–100 °C below the bulk T_m. The comparatively high stability of artificial amorphous PHB granules may be due to (1) the absence of solid catalyst residues or other nucleating impurities from the bacterial polyester, resulting in an apparently lower spontaneous nucleation rate; (2) the unique protection from solid surfaces, the suspending medium,[29] and other potential nucleants afforded by the surfactant coating utilized in the current work; and/or (3) the very small particle size achieved with the PHB granules. Experiments are underway to distinguish among these possibilities.

Native Granule Proteins. The nature of the protective surface coating of native PHB storage granules is the subject of continuing

(29) Interfacial nucleation by the suspending medium has been invoked to explain the crystallization behavior of droplets of several polymers and other materials and is a possibility in the PHB system. If water itself can serve as a nucleating agent for the polymer, it could explain why amorphous PHB particles are ever observed to crystallize, as in the dialysis experiment or in the commercial harvesting process. For other cases see ref 18d and also the following: Frensch, H.; Jungnickel, B.-J. *Colloid Polym. Sci.* **1989**, *267*, 16–27. Cordiez, J. P.; Grange, G.; Mutaftschiev, B. *J. Colloid Interface Sci.* **1982**, *85*, 431–441. Grange, G.; Lévis, A.; Mutaftschiev, B. *J. Colloid Interface Sci.* **1986**, *109*, 542–551. Rasmussen, D. H.; Javed, K.; Appleby, M.; Witowski, R. *Mater. Lett.* **1985**, *3*, 344–348.

terest. The presence of protein, in particular an active PHB olymerase, in association with PHB storage granules has been emonstrated in a number of organisms;[30] in *A. eutrophus* it as been demonstrated that the bound PHB polymerase esides exclusively at the granule surface.[31] Detailed analyses of ne granule-bound proteins in *Chromatium vinosum* D and *Pseudomonas oleovorans* have revealed the presence of one or ore polymerases along with lower molecular weight proteins of nknown function.[30d,32] In *A. eutrophus*, there is striking evidence at a low molecular weight, granule-associated protein (the *phaK* ene product) plays a surfactant-like role in granule formation nd/or stabilization.[33]

Role of Phospholipids. Data reported here represent the first etailed analysis of phospholipids associated with native PHB anules. The three phospholipids identified were found in mparable proportions in whole cell and granule lipid extracts. ellular phospholipids in *A. eutrophus* have been analyzed eviously with similar results.[34] Those authors also reported at a 1.8-fold increase in the concentration of total cellular lipids curs during the early stages of PHB accumulation, which they tributed to the synthesis of a granule membrane. As the same ospholipids were found here in whole cells and granules, the ssibility of contamination of the granules with fragments of e plasma membrane during isolation cannot be excluded. owever, it appears more likely that during granule biosynthesis, ospholipids are recruited in an adventitious fashion to the drophobic surface of the polymer.

The experimental results reported here demonstrate that ospholipids by themselves are a chemically competent coating the maintenance of the physical state of amorphous PHB nules. When detergent-coated granules were mixed with ergent-solubilized phospholipids and then dialyzed against a ergent-free buffer, little or no crystallization occurred. treated artificial granules were, on the contrary, completely ceptible to dialysis-induced crystallization. The most reason- e explanation is that, in the former case, the detergent coating eplaced during dialysis with a stabilizing phospholipid layer some type. As it is certain that *in vivo* proteins are major nponents of the granule coating, it might be supposed that the e of lipids in the native system is to fill in gaps that occur in coating between the various granule-associated polypeptides.

Applications. Notwithstanding the remarkable stability of the ificial granules in suspension, granule pastes crystallize easily drying and annealing to form films of high crystallinity. The fulness of these techniques to the preparation of biodegradable A coatings is being actively investigated. It is of additional rest that polymers that are otherwise incompatible in the t (such as PHB and PHB-*co*-28%HV) are capable of coexisting in amorphous state within individual artificial granules, as wn by density analysis, although there are likely to be phase ndaries within individual granules.[35] These mixed polymer nules may be useful in coatings or in studies of nucleation in ary polymer systems.

Conclusion. The preparation of amorphous, biomimetic nules of poly-3-hydroxybutyrate (PHB) reported here provides ng support for the recently proposed nucleation model for the physical state of polyhydroxyalkanoates (PHAs) *in vivo*. These artificial granules, which resemble native storage granules in many important respects, constitute a remarkably stable amorphous form of the polymer and have potentially significant practical applications. The results also suggest an *in vivo* model in which amphipathic lipids are recruited adventitiously to the granule surface during PHB biosynthesis and play a role in granule stabilization.

Experimental Section

General. Aqueously purified PHB (M_w 690 000; M_w/M_n 2.9; 99.9% pure) and PHB-*co*-HV[36] were obtained from Zeneca Bio Products, Billingham, England. *Alcaligenes eutrophus* H16 tron, a glucose-utilizing variant of the wild type, was obtained from Zeneca and cultured as described elsewhere to promote PHB accumulation.[25] Detergents, phospholipid standards, soy phosphatidylcholine (type II-S, containing 10-20% phosphatidylcholine, remainder other phospholipids), lysozyme, and Nycodenz (5-(*N*-(2,3-dihydroxypropyl)acetamido)-2,4,6-triiodo-*N,N'*-bis(2,3-dihydroxypropyl)isophthalimide) were all obtained from Sigma. Cellulose dialysis tubing was obtained from Medicell International (London) and had a molecular weight cutoff of 12 000–14 000. Nile Red was prepared from commercial Nile Blue A by acidic hydrolysis.[37] Sonications were performed at a frequency of 20 kHz using a Heat Systems-Ultrasonics W-375. Particle size measurements were made using a Malvern MS 20 laser particle sizer. Polymer molecular weight determinations by gel-permeation chromatography (GPC) and GC chloroform analyses were carried out by Zeneca Bio Products.

Native Granule Preparation. All manipulations were performed at 4 °C unless otherwise indicated. PHB-containing *A. eutrophus* cells grown under nutrient limitation (0.75-L culture) were centrifuged (3500*g*, 30 min) and resuspended in 15 mL of buffer A (50 mM sodium phosphate, pH 8.0) containing 1 mM EDTA. Hen egg-white lysozyme (20 mg) was added, and the suspension was incubated at 37 °C for 45 min. The suspension was then cooled to 4 °C and sonicated (50-W power) for 3 min on an ice–water bath. Sucrose gradients were prepared from 1.00, 1.25, 1.50, 1.75, and 2.00 M sucrose (35 mL total), and 4 mL of sonicated cell suspension was applied to each gradient. The gradients were centrifuged in a Beckman SW28 swinging bucket rotor at 100000*g* for 1 h (20 °C); native granules were collected at the 1.25–1.50 M interface (density 1.16–1.19 g/cm³). Native granules were removed from the gradients, dialyzed overnight against buffer A, and stored at 4 °C.

Artificial Granule Preparation. In a typical procedure, a solution of PHB in chloroform (1–10 mL, 5% wt/v) was placed in the bottom of a heavy-walled glass tube or beaker, and 20 volumes of an aqueous solution of cetyltrimethylammonium bromide or other detergent (5–100 mM) was added. The two layers were then emulsified using a probe-type ultrasonicator (1–3 min at 200-W power). Heating of the sample was controlled with an ice–water bath. Chloroform was then removed from the emulsion by one of three techniques: (a) heating; (b) stirring in an open vessel; or (c) dialysis against aqueous surfactant. All operations were carried out in a ventilated fume hood. In method A, the emulsion was maintained at 75 °C for 90 min in an uncovered, magnetically stirred flask. In method B, the emulsion was stirred at ambient temperature in a wide-mouth vessel for 40 h, adding water as necessary to maintain constant volume. In method C, the emulsion was dialyzed exhaustively over 48 h against additional aqueous detergent of concentration equal to that of the original solution. The resulting granule suspension was stored at room temperature. Granules could be concentrated by centrifugation, which was conveniently done in two rounds of 30 min each at 8000*g* and 33000*g*, respectively (20 °C).

NMR Studies. Artificial granules were collected by centrifugation as described above, immediately resuspended in D_2O (0.5 mL), and

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(35) We have also prepared artificial granules from a combination of PHB and poly-3-hydroxyoctanoate (PHO), another bacterial polyester. There was strong evidence for phase separation within these granules. As with the PHB/PHBV granules, density analysis showed that individual granules contained both polymers. In this case, however, the glass transition of the PHO, which occurs some 40 °C lower than that of PHB, could be observed directly by differential scanning calorimetry (DSC). The T_g of PHO contained in the PHB/PHO mixed polymer granules was found to be −36 °C, essentially the same as in bulk amorphous PHO, pure PHO artificial granules, or PHO contained in quenched co-melts with PHB (which showed two independent glass transitions at −36 and 5 °C): Horowitz, D. M.; Sanders, J. K. M. Unpublished results.

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EXPERIMENTAL

Biodegradable Latex

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Film formation and paper coating with poly (β–hydroxyalkanoate), a biodegradable latex

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ABSTRACT: An aqueous latex of a poly (β–hydroxyalkanoate) (PHA) coated on paper imparted water imperviousness without changing mechanical properties. Hot–pressed films biodegraded faster than solvent cast films. The PHA coating on paper degraded totally in activated sludge within 12 days, leaving the cellulose matrix relatively untouched. Blends of PHA latexes with sodium carboxymethyl cellulose, polystyrene latex, carboxylated styrene/ butadiene latex, natural rubber latex, and starch powders form satisfactory films at room temperature.

KEYWORDS: Biodegradability, coating, film, formation, latex, mixtures, paper, starch, water permeability.

The purpose of this work was to assess the film forming characteristics and biodegradability of poly (β–hydroxybutyrate) (PHB) and poly (β–hydroxybutyrate co–valerate) (PHB/HV) concentrated native granule suspensions or latexes. We demonstrate their use as films, coatings, and composites as well as blended with other polymers. There is particular emphasis on paper applications where synthetic, nonbiodegradable latex finds extensive use. Our findings have broad applications to the whole family of PHA. We will therefore refer to this family, although most of our work concentrated on PHB and PHB/HV latexes.

Preservation of the nascent state and granular morphology of the polymer depends on the separation procedure of PHA from bacterial cells. Table I shows the three major approaches to PHA isolation: solvent extraction, chemical digestion, and selective enzymolysis. Compared to solvent extraction, the other two methods preserve the granular morphology of PHA. They are therefore suitable for latex production. Both chemical and enzymological approaches consist of selective digestion on non–PHA components. The chemical procedure (*1*) involves pretreatment of the biomass with detergent and subsequent washing of the residue with sodium hypochlorite at pH 13. One must carefully control the process to avoid degradation of the PHA by hypochlorite oxidation.

Selective enzymolysis is similar to chemical digestion except that enzymes such as proteases and deoxyribonuclease hydrolyze the unwanted biomass components. Since these enzymes act with much greater specifity than chemicals such as hypochlorite, the recovered granules should bear a closer resemblance to their native state in the microbial cytoplasm. There are commercial applications of this method presently in operation (*2, 3*). Research laboratories have made extensive use of similar techniques to prepare "freshly isolated" granules with disruption of cell walls by lysozyme treatment followed by sonication. Centrifugation allowed subsequent recovery onto a bed of glycerol (*4*).

Enzymatic isolation of PHA granules partially preserves the original molecular organization of the polymer chains. Films from suspensions of such granules should possess properties different from those of solvent–extracted PHA as long as the film processing conditions are at temperatures less than the melting temperature. Starch is an example of a hydrocolloid where commercial isolation processes pre-

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serve the original structure. Starch undergoes reversible cycles to reform a crystalline hydrate after each dehydration. Starch gelatinization with steam irreversibly destroys its structure. By contrast, PHB and PHB/HV native granules are noncrystalline in their original form. Isolation and drying provokes crystallization and thereby changes irreversibly the film forming properties of the original particles (*5*).

Recent studies (*6, 7*) on the morphology of isolated granules have established that a crystalline shell invariably develops at the surface of the granules after isolation from the cytoplasm. The texture of this crystalline shell corresponds to single, lamellar crystals. This topotactic shell surrounds a noncrystalline core whose mobile properties are discernable from high-resolution nuclear magnetic resonance methodology (*8*).

I. Survey of PHA isolation processes

Isolation process	*Procedure*
Extractive solvents: solvent extraction of PHA	a. Pretreatment with acetone to remove water and polar lipids
	b. Chloroform extraction of PHA followed by precipitation into methanol
Chemical digestion: selective for non–PHA components	a. Pretreatment with detergent to solubilize lipids and protein
	b. Washing of residue with sodium hypochlorite at pH 13
Biochemical digestion: granule isolation after selective enzymolysis of biomass	a. Flash steaming to lyse cells and denature DNA
	b. Sequential enzyme treatments for removal of insoluble macromolecules

Experimental

Latex sources

We obtained latex samples from a commercial source or prepared them ourselves. The term latex refers to an aqueous suspension of PHA granules never subjected to drying. We isolated the original granules from *Alcaligenes eutrophus* by an enzymolysis process (*2*) or by chemical treatment of the biomass using a detergent–hypochlorite process (*1*). In both we did not dry the granules. We repeatedly washed the isolated granules with water and concentrated them to between 20% and 50% solids on a weight basis. Both the biochemical and chemical methods produced a latex of similar purity. They contained about 95% PHA and retained the granular morphology. The latex samples in this study were the following:

- PHB homopolymer
- PHB/HV copolymer isolated by the hypochlorite process containing 5.5%, 7.5%, and 11% HV
- Twenty-one percent and 27% HV copolymer samples obtained commercially

The copolyesters of PHB/HV indicated above are random copolymers (*9, 10*).

Film forming techniques

We cast the latex suspensions at 15–20% solids on an impervious substrate such as glass or polyester to prepare films. Drying the cast suspension at room temperature produced a "cake-like" structure with poor mechanical properties. Subsequent hot-pressing converted this structure into a transparent, flexible film. Hot–pressing used a laboratory press at 5000 psi for 1 min at a temperature always below the melting temperature of the dry polymer. Depending on the HV content, these temperatures ranged from 100°C to 140°C. This was the minimum requirement for complete formation of a dense film. Hot–pressed films were crystalline by X–ray diffraction but nonspherulitic in a polarizing microscope.

There was an intermediate state of coalescence when heating the air-dried latex "cake" of 21% and 27% HV materials at 130°C for 10 min. These films were white, opaque, porous, and peelable from the casting surface. Scanning electron microscope (SEM) views of these films, shown in **Fig. 1**, indicated a three-dimensional array of 0.5–1.5-μm spherical particles or granules partly coalesced with interparticle capillaries of 0.16-μm radius. **Figure 2** clearly shows the particulate nature of the starting latex (*5*).

Films cast from freshly isolated PHB granules (*4*) using hot–pressing at 5000 psi and 135°C were nonspherulitic but showed large domains of birefringence in the polarizing microscope as if shear stresses were frozen inside the films. Their degree of crystallinity, determined by powder X–ray diffraction seen in **Fig. 3**, was much higher than that of the films isolated chemically or from enzymolysis.

We dissolved PHB and PHB/HV powders in chloroform to make solvent cast films. We cast these solutions on glass or polytetrafluoroethylene (PTFE) at room temperature or 40°C to evaporate the solvent. These films showed the expected high extension to break for films with spherulitic texture (*11*). The films aged for two weeks to allow them to reach equilibrium crystallinity (*12*). Solvent casting destroys the original morphology of the granules.

We prepared films with clarity and toughness similar to plasticized cellophane by vapor coalescing air dried, cake–like latex films. We exposed them to solvent vapors for a period of 24 h in

a desiccator. Chloroform vapors produced extremely smooth, flexible, and tough films that were transparent and conformable on drying. They showed X-ray crystallinity but were not birefringent.

Latex coated paper

We made latex coated paper using unconditioned basestock paper strips of 40 g/m^2 basis weight with a 1:1 mixture of mechanical and chemical pulp. We used a 20% solids content latex dispersion to coat paper strips by hand with a set of contoured metering rods. The coatings were flexible, coherent, and adhesive to paper even when dried at room temperature. Adhesion of latex particles to paper substrates depends on conformability of the latex to drying stresses. This is quite favorable in the case of a latex which has not undergone drying. On the other hand using reconstituted latexes made by dispersing commercial spray-dried PHA powders in water to coat paper strips produced coatings which did not adhere to paper because of the higher crystallinity of the granules.

Hot-pressing between polyester sheets of coated paper samples used 5000 psi at temperatures ranging from 100°C to 140°C, depending on the copolymer composition, for 1–2 min. The latex coating fused and adhered to the paper. The coated surface was transparent and glossy after the hot-pressing.

Results and discussion

Mechanical properties

Table II lists the physical and mechanical characteristics of latex coated sheets according to standard paper tests. The tearing behavior of PHB and PHB/HV coated paper was almost the same as the basestock. Polyolefin coated papers using melt extrusion do not tear in the same manner. In that case the paper tears first, leaving a plastic film that trails and necks to 100% elongation or more. Hot-pressed PHA coatings were not peelable from PHB/HV coatings did not significantly change the mechanical properties of the basestock.

Wetting and barrier properties

Water is the most important liquid to consider for wetting of PHA films. The measured value of contact angle θ between PHA latex film and water (*5*) was about 72°. For comparison, the contact angles of polyester and nylon with water are 71° and 65°, respectively. Polyethylene and PTFE have values of 95° and 112°, respectively (*13*). PHB is therefore a polar material which water will wet. Indeed, PHB will wick water, if it is in the form of a microporous network.

We measured water vapor transmission rate (WVTR) on samples of PHA coated paper prepared by a direct electrostatic powder coating technique (*14*). This novel coating method deposits the spray dried powder directly onto paper with subsequent fusing and pressing under similar conditions to those described above. The WVTR values for the PHB/HV electrostatically coated paper were higher than the values of polypropylene (PP) coated paper prepared under the same conditions for comparison purposes. This is normal for a more polar polymer. Figure 4 provides the plot of WVTR at varying coating weights for PHB/HV with 8.1% HV and PP. It shows a pattern similar to those of binary composite laminates (*15*). Poor barrier properties at low coating weights are due to penetration of the cellulose fibers through the polymer film. After exposure to moisture, these fibers wick water through the polymer film. Increasing the coating weight gave a steady decrease of the permeability values. The observed permeability rate of coated paper approached the value of the polymer film for coating weights exceeding 40 g/m^2.

Microbial degradation

This study used hot-pressed PHB/HV latex films and PHB and PHB/HV latex coated hot-pressed paper samples. from 0.08–0.09 mm to 0.2 mm. The study also contained chloroform cast films made with bacterial PHB to assess the influence of film morphology upon the rate of degradation. Other researchers detail the procedure to evaluate the degradation of test specimens exposed to a microbial environment of simulated activated sewage sludge (*8*).

The PHB/HV with 21% HV hot-pressed latex films showed a steady weight decrease over a period of 12 days leading to the total weight loss of 83% shown in Fig. 5. The SEM of Fig. 6 showed the erosion of the film surface as a result of bacterial attack.

In a separate experiment, paper coated with PHB latex lost 35% of its initial weight after 12 days. This corresponded to almost the entire coating weight of the sample. SEM examination of the surface of PHB coated paper after 12 days of incubation (Fig. 7) showed that the weight loss was due to the biodegradation of the PHB layer, since the cellulosic fiber support remained intact.

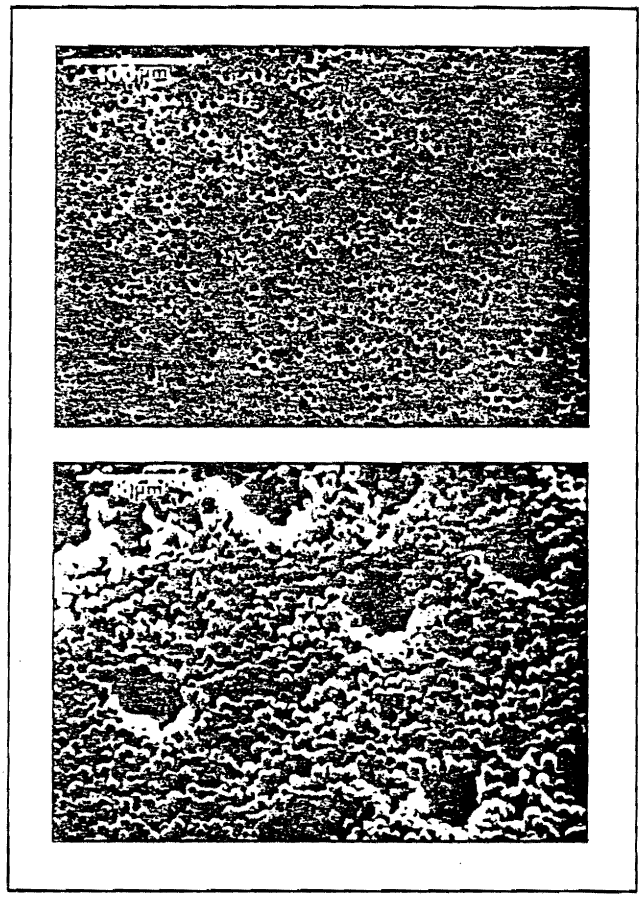
Filmmaking technique affects the rate of PHA biodegradation. Hot-pressed latex films degraded faster than solvent cast films. An explanation is the loss of the granular morphology of the original PHA granules due to the dissolution and casting of the polymer compared to the hot-pressing technique (*12*).

To assess the influence of the copolymer HV content upon film biodegradability, we determined the rate of degradation by activated sludge for two copolymer compositions of 5% and 11% HV after eight days exposure. There was no clear evidence that the degradation rate corresponded to the HV content in the copolymer PHB/HV.

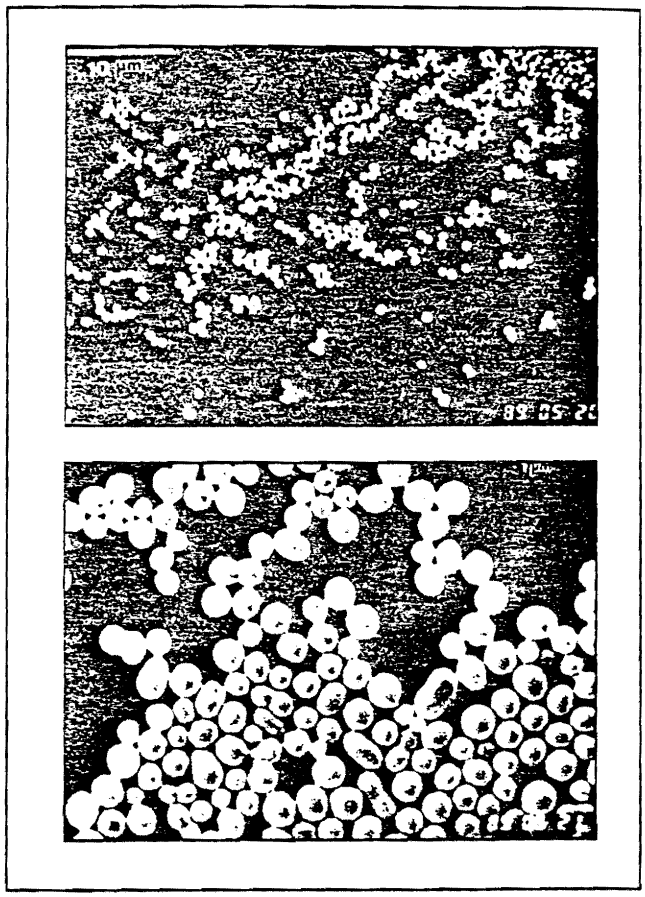
PHA latex formulation

Film forming and coating applications of PHA latex may require formulation and blending to obtain the necessary properties. Paper applications and the production of PHA composites require blending with inorganic pigments, plas-

1. Two views of PHB/HV film with 21% HV made by air drying a 20% solids latex suspension followed by heating at 130°C for 10 min



2. Two views of SEM of PHB granules using spray dried powder suspended in distilled water and treated by ultrasound for 1 min prior to deposition on the support for observation



tic pigments and binders made from synthetic polymer latexes, and other biopolymers such as starch or cellulose and its derivatives.

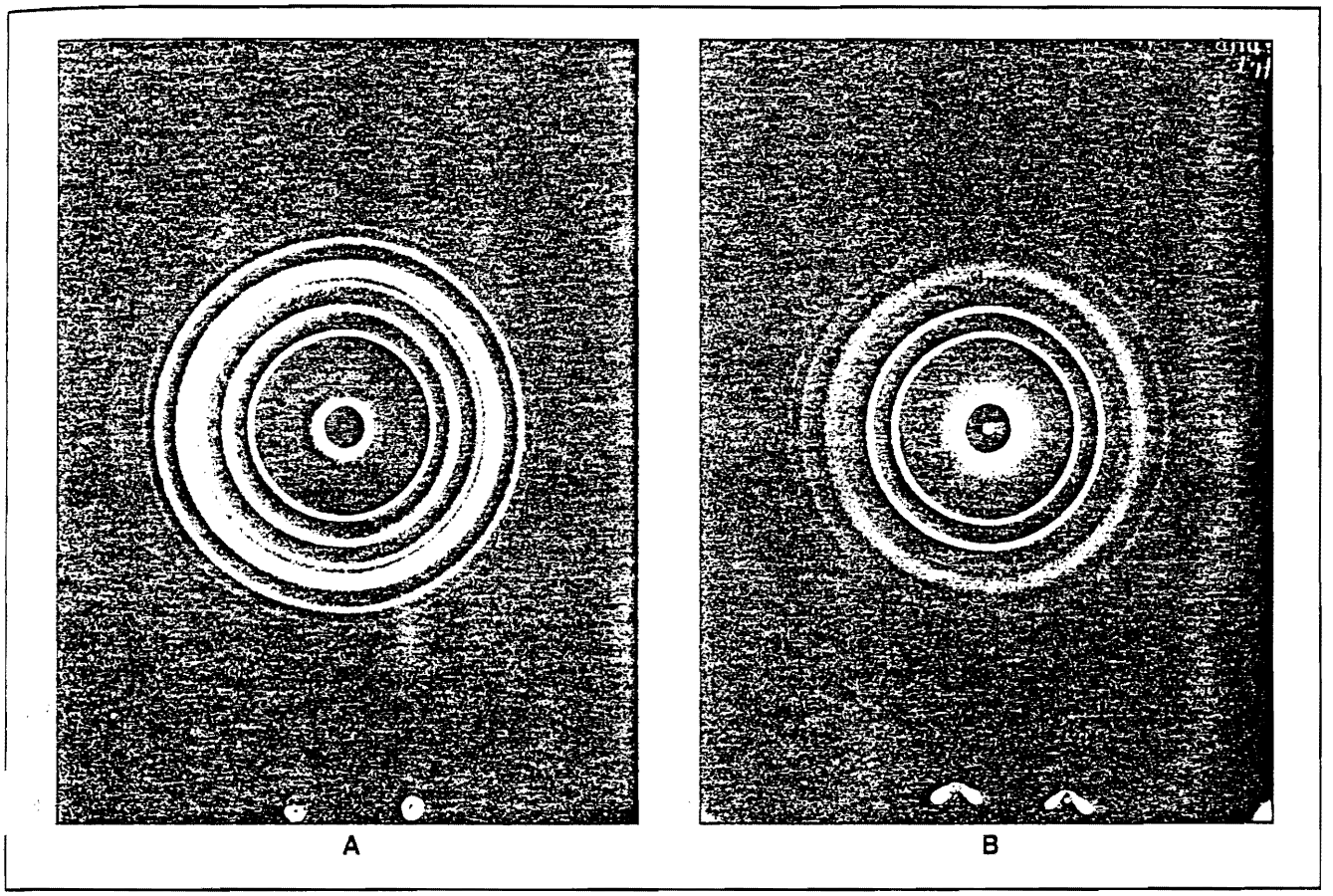
In a preliminary study, we blended PHB and PHB/HV latexes with sodium carboxymethyl cellulose (CMC), polystyrene latex, clay, carboxylated styrene/butadiene latex, natural rubber latex, hybrid starch with 70% amylose, and cross-linked potato starch. We prepared the blends by mixing the two components at 25%, 50%, and 75% weight ratios and stirring the resulting mixture at low shear to produce a uniformly dispersed phase. Some cases required water addition to improve dispersion. We cast the films as before, allowed them to dry at room temperature overnight, and subsequently hot-pressed them for 1 min at 5000 psi and at a temperature in the range necessary to fuse an unblended polymer film (100–140°C). It is possible to mix PHA latex never subjected to drying with all the materials mentioned above without phase separation at room temperature. In the case of PHA with natural rubber and PHA with carboxylated styrene/butadiene latex blends, colloidal compatibility did not translate into a uniform film after drying. The two components segregated to yield the PHA component on the casting substrate covered by a rubbery film. Hot-pressing of air-dried cast latex blends of PHA with carboxylated styrene/butadiene, using less than 25% carboxylated styrene butadiene, produced uniform films. This implies dispersion compatibility of the two components.

Mixing starch granules with PHA latex gave films with good characteristics. There were mixtures of PHB/HV latex (both 5.5% and 27% HV) and 65% amylose content starch powder in 25%, 50%, and 75% ratios. Hot-pressing air-dried cast blends of 1:1 PHA/starch produces films where

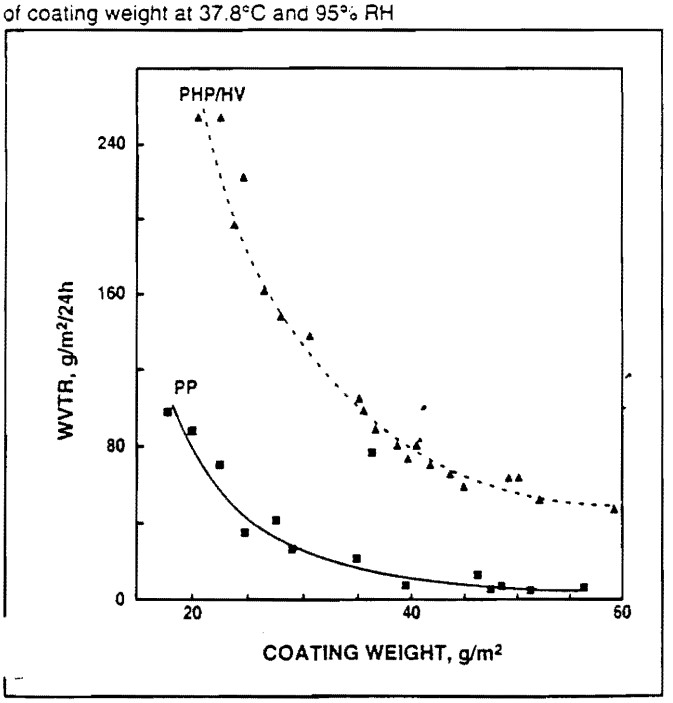
II. Physical and mecnanical characteristics of PHB and PHB/HV latex coated paper

Characteristic	*PHB*	*PHB/HV*	*Base stock*
Basis weight, g/m²	48.0	74.0	37.4
Burst index, kPa·m²/g	0.90	1.65	2.31
Breaking length, km	4.75	5.10	6.85
Stretch, %	0.95	2.10	1.59
Tensile index, N·m/g	46.30	50.20	67.16

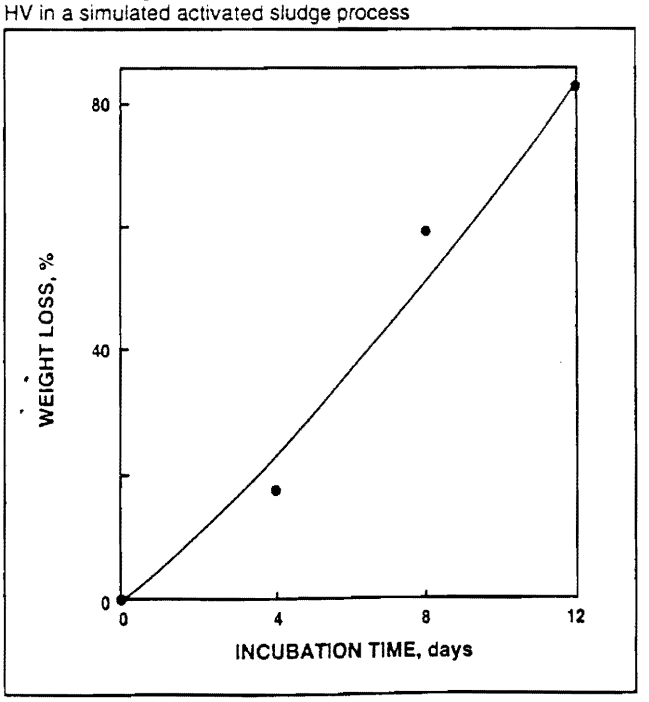
3. Wide-angle X-ray pattern of latex films made by hot pressing at 135°C and 5000 psi for 1 min from PHB granules isolated with enzymes in our oratory (A) and by a chemical approach (B)



4. WVTR of PHB/HV with 8.1% HV and PP coated paper as a function of coating weight at 37.8°C and 95% RH



5. Microbial degradation of hot-pressed PHB/HV latex film with 21% HV in a simulated activated sludge process



starch granules are embedded in a continuous PHA phase in such a way that overnight immersion in water at room temperature does not affect the resulting material. Experiments are under way to characterize further the exact morphology of such films. They appear to be a uniform dispersion of starch granules in a PHA matrix (*16*).

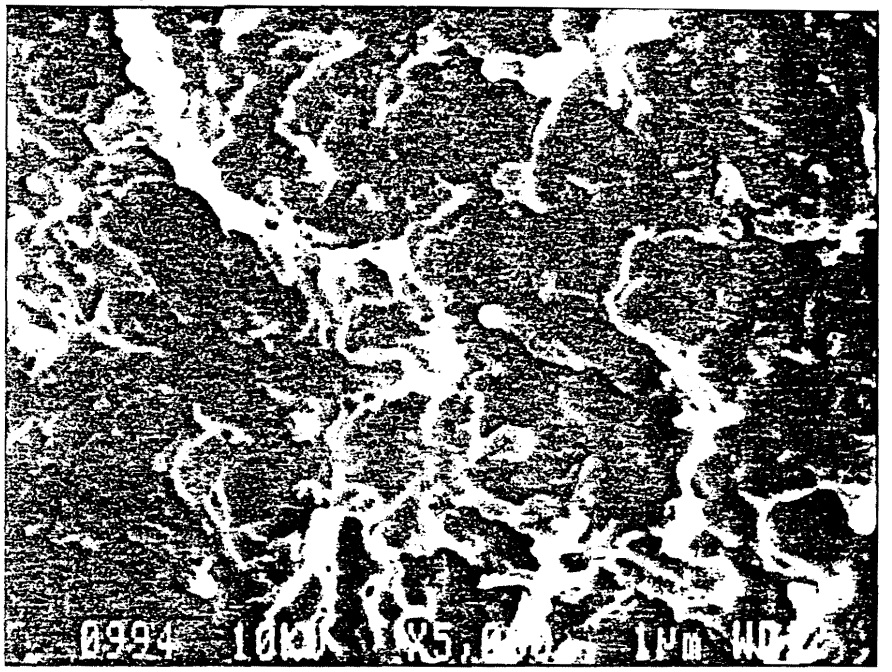
Conclusions

From a polymer morphology perspective, the most notable result in this study is the nonspherulitic character of the hot-pressed PHA films. They are flexible, but their extension to break is almost like that of paper. This suggests a limitation in chain entanglement of the interparticle surface. There also is a strong element of original morphology still present inside particle domains.

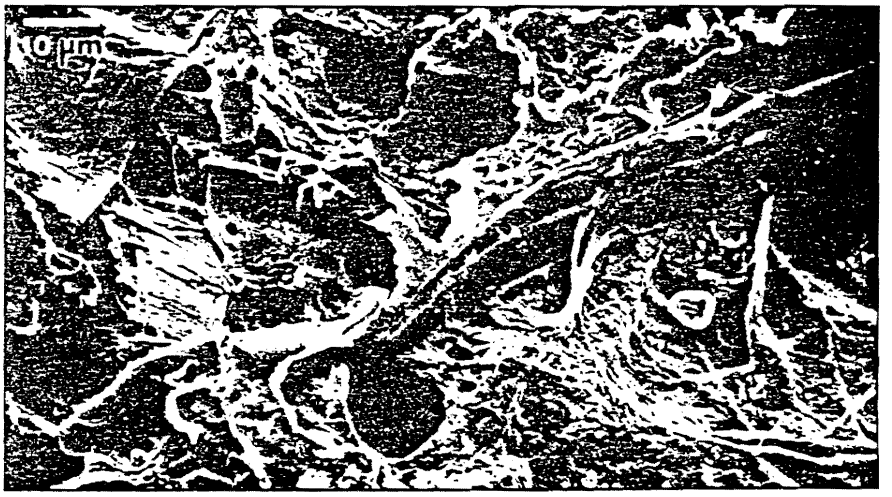
The lack of particle coalescence on drying at room temperature relates to the core and shell character of the granules in the dispersion (*6*). During isolation, surface crystallization occurs. Conformability to surface tension forces that draw the particles together does not occur unless drying temperatures are close to the melting temperature of the crystalline PHA. The crystalline nature of this shell and the rate and quality of the crystallinity that develops in the core on drying are under study. It is clear, however, that latex films of PHA require heat and pressure to produce a level of sintering to make an acceptable film from a barrier point of view.

The use of the enzymatic isolation process and the presence of HV in the polyesters are not limiting requirements to produce nonspherulitic, translucent, flexible polymer films from PHA latex. Films made of granules purified by the detergent–hypochlorite process had comparable toughness. The other desirable properties described above were not present in the solvent extracted or previously dried materials. All results seem to indicate the importance of processing the material in its never-dried latex state for film forming applications.

6. SEM of hot–pressed PHB/HV latex film with 21% HV after 12 days immersion in simulated activated sludge



7. SEM of hot–pressed PHB latex coated paper after 12 days immersion in simulated activated sludge



In particular, the ready sorption of the PHA latex by fibrous materials such as paper or nonwovens suggests applications such as binder, coating, or barrier. These applications in fibrous constructions become attractive when one considers the natural biodegradability of PHA (*17*).

From the applications point of view, PHA latex blends could offer advantages in the economics of PHA use. Blends with a synthetic film forming latex such as carboxylated styrene/butadiene could improve the toughness of PHA but with some sacrifice in total biodegradability. Alternately, blends with starch would maintain total biodegradability with substantial lowering of material costs. Clearly the latex state offers a unique opportunity for creating these particulate blends.

The particle coalescence character-

istics of these PHA latexes are poor ›ared to a natural rubber latex in sp..e of the ^{13}C nuclear magnetic resonance evidence that most of the granules of PHA are in a noncrystalline state before drying (*17, 18*). Drying the spread latex at temperatures close to the melting point of PHB or PHB/HV can overcome the lack of particle coalescence on drying at room temperature. Exposing the films to solvent vapor would further improve their optical homogeneity. TJ

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Producing high molecular weight biodegradable polyesters

Lipase-catalyzed polymerization is used to produce aliphatic polyesters. A second, two-step chemical polymerization of lactic acid followed by chain-linking produces poly(ester-urethanes).

Yu-Yen Linko
Jukka Seppälä

Interest in the production of biodegradable polymers and the applications of biocatalysis in organic syntheses has grown rapidly as a result of increased demand for environmentally "friendly" products and processes (*1–3*). Lipases (triacylglycerol acylhydrolase [EC 3.1.1.3]) have been studied extensively because of their utility in many processes (see box). These enzymes catalyze the hydrolysis of glycerol esters at lipid–water interfaces. In organic solvent systems, however, they catalyze ester synthesis (*4*) and have been used to modify fats and oils to impart improved characteristics to novel materials (*5, 6*).

We have demonstrated that lipase biocatalysis may be used to upgrade inexpensive renewable raw materials to valuable biodegradable products such as butyl oleate (a biodiesel additive to decrease viscosity in winter), 2-ethyl-1-hexyl esters from rapeseed (canola) oil (solvent in car shampoos) (*7*), and trimethylolpropane esters (hydraulic oil and lubricant) (*8*). The possibility of enzyme-catalyzed synthesis of biodegradable linear polyesters through polytransesterification and direct polyesterification has been widely studied (*9–17*). In this article we report two novel methods to produce biodegradable polyesters: enzymatic polyesterification and polytransesterification and chemical polycondensation followed by chain linking. We discuss several important aspects in the enzymatic polyester synthesis and demonstrate that a high degree of polymerization can be obtained with both derivatized and underivatized diacids and diols by a simple and straightforward biocatalysis.

Biodegradable lactic acid–based polyesters are already produced commercially on a relatively small scale for medical implants. For these applications, poly(L-lactic acid) is produced by lactone ring opening polymerization via lactides. Highly purified L(+)- or D(–)-lactic acid is required in the process to obtain biodegradable polymers of acceptable mechanical properties, inasmuch as poly(DL-lactic acid) is hard and brittle. Furthermore, it is difficult to obtain a sufficiently high molecular weight by conventional condensation polymerization (*18*). This makes the current process expensive and the conventional polylactides high-cost specialty chemicals. However, during the past few years, large-volume applications such as packaging and agriculture have been suggested for biodegradable polymers, including poly(L-lactic acid) (*19*), which can be produced from renewable raw materials. Consequently, alternative routes for polymerization have been investigated (*20*). We describe a novel and attractive chemical method to produce, in a high yield, high molecular weight lactic acid–based poly(ester-urethanes) without the need of the lactide step by chemical polycondensation followed by chain linking.

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Uses for lipases

Hydrolysis of fats and oils
- Production of glycerol, fatty acids, and mono- and diglycerides

Modification of fats and oils
- Production of cocoa butter substitutes
- Alteration of melting point

Synthesis
- Optical resolution and chiral synthesis
- Surfactants
- Aroma and flavor compounds
- Lubricant esters
- Polyesters

Biocatalytic production of aliphatic polyesters

Carrying out the polymerization reaction at close to ambient temperature and pressure markedly reduces unde-

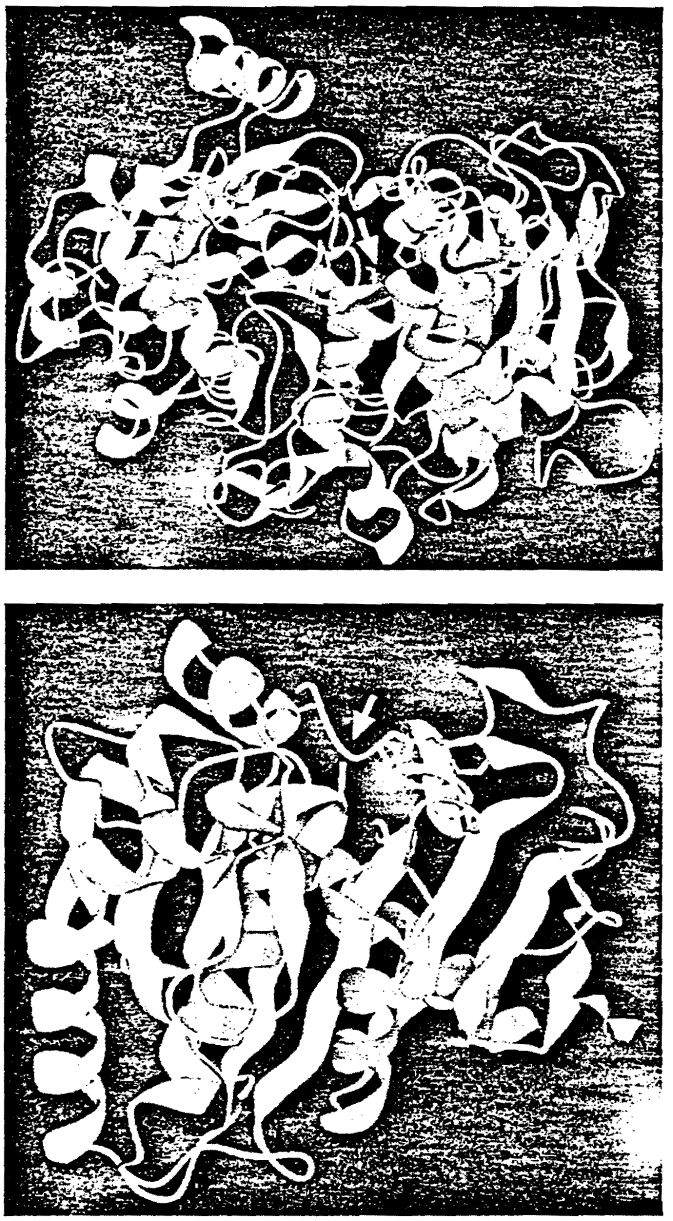


Figure 1. **The shallow, open active center on the** *Rhizomucor miehei* lipase allows the production of higher molecular weight polyesters. Shown are the active centers of *Candida rugosa* lipase (top), a deep, narrow tunnel, and *R. miehei* lipase (bottom), a shallow, open cleft on the surface. Under otherwise similar conditions, the weight-average molecular weight (M_w) obtained with the *R. miehei* lipase was at least 2–5 times higher than that obtained with *C. rugosa* lipase. Arrows indicate active centers with the amino acid triads Ser-209, Glu-341, His-449 (top; PDB entry 1LPS), and Ser-144, Asp-203, His-257 (bottom; PDB entry 4TGL).

the enzyme, interference by possible byproducts, and insolubility of the high molecular weight product limit the degree of polymerization (DP), and until quite recently, only oligomers of DP < 10 were reported in lipase-catalyzed polyester synthesis (*16*). Wallace and Morrow (*9*) were the first to obtain a relatively high weight-average molecular weight (M_w) polyester (14,900 g/mol) in porcine pancreatic lipase–catalyzed polymerization of bis(2,2,2-trichloroethyl) adipate with 1,4-butanediol or 1,6-hexanediol. These researchers introduced two marked improvements: polytransesterification instead of polyesterification, thus avoiding water formation during the reaction, and a halogenated acyl donor bis(2,2,2-trichloroethyl) adipate, minimizing the trend

Lipase. Of 25 lipases screened for high ester synthesis activity (*21*), *Rhizomucor miehei* (fungus), *Pseudomonas fluorescens* (bacterium), and *Candida rugosa* (yeast) were selected for further screening as biocatalysts for polytransesterification reactions in various organic solvents; porcine pancreatic lipase, already known to catalyze a number of esterification reactions, was also investigated. Lipases from the *R. miehei* and *P. fluorescens* gave the highest degree of conversion in aliphatic polyester synthesis from dicarboxylic acids and diols (*22*). Although the *C. rugosa* lipase exhibited high hydrolysis and high ester synthesis (*21*), it exhibited a poor polyesterification activity, apparently attributable to the different structure of its active center. Although the catalytic amino acid triads of serine–glutamine and aspartic acid–histidine of *C. rugosa* and *R. miehei* lipases, respectively, are similar, the active center of *C. rugosa* lipase (Figure 1, top) is a deep, narrow tunnel. That of *R. miehei* lipase (Figure 1, bottom), on the other hand, is a shallow, open cleft on the surface of the protein molecule. This morphology makes the synthesis of high molecular weight polyester possible. Under otherwise similar conditions, the M_w obtained with *R. miehei* lipase was at least 2–5 times higher than that obtained with *C. rugosa* lipase. Porcine pancreatic lipase, which is widely used experimentally, exhibited less polytransesterification activity under similar experimental conditions with any of the substrates tested. Consequently, most of our experiments were carried out with *R. miehei* lipase.

Polymerization reaction. Our approach to the lipase-catalyzed polymerization reaction was as follows. A suitable quantity of crude lipase powder was added to a reaction mixture of equimolar quantities of a dicarboxylic acid (or its activated derivative) and an aliphatic diol as substrates in an organic solvent in a round-bottomed flask equipped with a magnetic stirrer and a condenser. The equimolar substrate ratio used is essential for the AA + BB polycondensation to obtain a high molecular weight polyester. The mixture was stirred at 37–45 °C. Higher temperatures resulted in a partial inactivation of the enzyme and thus reduced molecular weight and yield. The byproduct formed was removed periodically by vacuum; care was taken not to remove the volatile small molecular substrates. After the first 20 h, the polymerization was completed in a 0.15-mmHg (20-Pa) vacuum. When the reaction was complete (~72 h or more), lipase was filtered off, and a white solid polyester was obtained on precipitation from methanol. Blank tests, without lipase, resulted in no observable polymerization.

Polytransesterification. The polymerization reaction of bis(2,2,2-trifluoroethyl) sebacate by transesterification with 1,4-butanediol to form poly(1,4-butyl sebacate) proceeds as follows:

$$n\ CF_3CH_2OCO(CH_2)_8OCOCH_2CF_3 + n\ HO(CH_2)_4OH \leftrightarrows [CO(CH_2)_8COO(CH_2)_4O]_n + 2n\ CF_3CH_2OH$$

When we used diphenyl ether as the solvent and *R. miehei* lipase as the biocatalyst for this polytransesterification, at ambient pressure and 37 °C, M_w (as determined by gel permeation chromatography [GPC]) of the polyester obtained in 96 h was only 8300 g/mol (DP = 33) or less, compared with 37,000 g/mol (DP = 147) obtained when the 2,2,2-trifluoroethanol formed during the transesterification was removed with a vacuum. Under optimal condi-

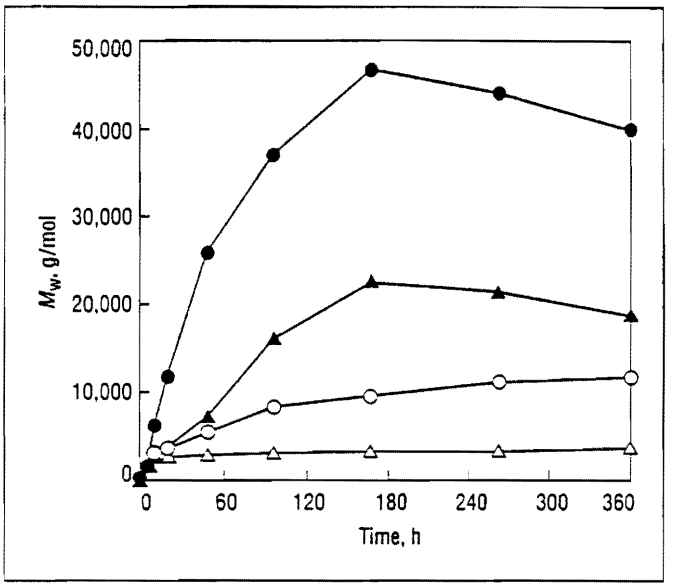


Figure 2. **The solvent choice and vacuum affect the product of the lipase-catalyzed polytransesterification with *Rhizomucor miehei* lipase.** M_w, weight-average molecular weight. △, in veratrole without vacuum; ▲, in veratrole with vacuum; ○, in diphenyl ether without vacuum; ●, in diphenyl ether with vacuum.

sebacate progressed with time up to 168 h (7 days), at which time a maximum M_w of 46,400 g/mol (DP = 184) was obtained (Figure 2). The high molecular weight polyester was purified by precipitation from methanol; using [^{13}C]NMR, we determined that the white solid precipitated was linear poly(1,4-butyl sebacate).

In all cases, the vacuum removal of the water or alcohol formed was an important step in obtaining a high M_w polyester. Nevertheless, during the early stages of polymerization, care should be taken to prevent the simultaneous removal of low molecular weight reactants. The relative lipase quantity also affects the M_w of the polyester obtained. The rate of the reaction and the degree of polymerization increased with increased quantity of lipase until the viscosity of the solution became so high as to cause difficulties in mixing and reduced mass transport, thus resulting in a decreased M_w. These results support the observation of Knani and Kohn (*14*) that mixing has little effect on the degree of polymerization in porcine pancreatic lipase–catalyzed polytransesterification of ω-hydroxyesters in hexane in dilute solutions but an adverse effect in concentrated solutions.

Solvent. Although the type of organic solvent used appears to be an important factor in lipase-catalyzed enzymatic synthesis and a considerable amount of work has been done to clarify the relationship between lipase activity and the properties of the organic solvent, solvent effects are still not well understood (*22*). Of the physical parameters studied, log*P* (the logarithm of the partition coefficient of a given component in the octanol–water two-phase system) has often been the most useful (*23*). Nevertheless, there is no general agreement on how to select a suitable solvent, and our own results have been somewhat contradictory with respect to the applicability of log*P*. The catalytic activity of lipases is expected to be low in hydrophilic solvents with log*P* < 2 (*22*). We have observed that solvents with similar log*P* values could behave quite differently (Table 1) and that one solvent can have different effects with different lipases (*24*).

Table 1. **Effect of solvent choice on M_w of poly(1,4-butyl sebacate)**

Solvent	log*P*	Boiling point, °C	M_w
Dodecane	6.6	214	5.9
Hexyl ether	5.0	228	9.8
Diphenyl ether	4.3	259	27.7
Isoamyl ether	4.3	173	9.0
Veratrol	2.2	206	17.4
Triglyme	−0.46	216	7.6

Reaction conditions: 1.5 mmol of substrate, 0.25 g *Mucor miehei* lipase, 37 °C, 400 rpm, 72 h, programmed vacuum profile. M_w, weight-average molecular weight; log*P*, the logarithm of the partition coefficient of a given component in the octanol–water two-phase system.

ered, particularly if vacuum is to be applied to the system. With halogenated esters as substrates for the polytransesterification, the boiling point of the solvent should be high enough that the solvent is retained in the reactor during removal of the alcohol formed from the reaction mixture. We tested several solvents with a sufficiently high boiling point and widely varying log*P* values and found that diphenyl ether and veratrole (1,2-dimethoxybenzene) are the most promising for *Mucor miehei* lipase–catalyzed polymerizations (Table 1). Again, there seemed to be no correlation between the log*P* value and the enzyme-catalyzed synthetic activity. Inasmuch as diphenyl ether was generally superior to veratrole (Figure 2), diphenyl ether was used in most subsequent experiments. It should be noted, however, that according to Morrow (*12*), 1,2- and 1,3-dimethoxybenzenes are better solvents than diphenyl ether in the polymerization reaction of bis(2,2,2-trifluoroethyl) glutarate and 1,4-butanediol catalyzed by porcine pancreatic lipase; Morrow obtained a M_w of ~40,000 g/mol in 432 h (18 days).

Substrates. Five diols ranging from C_2 to C_6 were polymerized with bis(2,2,2-trifluoroethyl) sebacate for 72 h. With 1,2-ethanediol, a low DP of 33 (M_w = 6730) was obtained. The DP increased with the chain length of the diol, and 1,5-pentanediol gave the maximum DP of 155 with M_w > 41,000 g/mol. We found that the M_w of the polyester increases with increased substrate concentration up to ~0.83 M bis(2,2,2-trifluoroethyl) sebacate and 0.83 M 1,4-butanediol. The GPC results showed that there were some low molecular weight oligomers in the product mixture when the substrate concentration was low.

Polyesterification for even higher molecular weights

Up to this point we had already reached an astonishingly high average molecular weight. However, the activated diacid released environmentally unacceptable chlorine or fluorine compounds on lipase-catalyzed polytransesterification. Only a few earlier reports had been published on the polyesterification of underivatized diacids with diols; the M_w of the oligomer obtained was on the order of 5000 g/mol or less. Okumura and colleagues (*25*) used *Aspergillus niger* lipase for the polyesterification of several diacids in the presence of an excess of a diol, and Ajima's group (*26*) used *Pseudomonas fluorescence* lipoprotein lipase solubilized with polyethylene glycol for the polymerization of hydroxydecanoic acid in benzene,

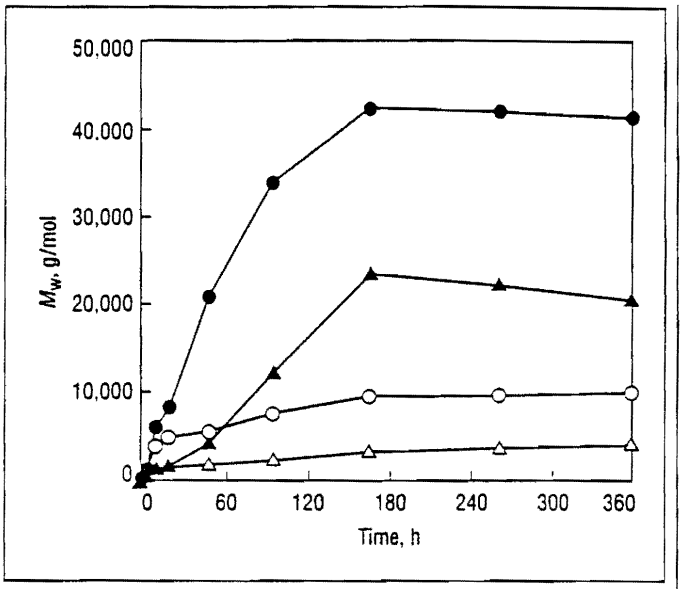


Figure 3. **The solvent choice and vacuum affect the product of the lipase-catalyzed polyesterification with *Rhizomucor miehei* lipase.** △, In veratrole without vacuum; ▲, in veratrole with vacuum; ○, in diphenyl ether without vacuum; ●, in diphenyl ether with vacuum.

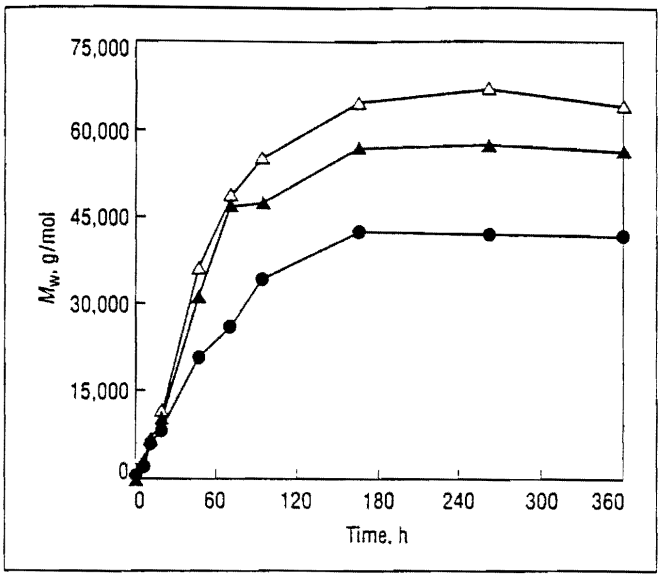


Figure 4. **Increasing the temperature to 45 °C increases the molecular weight of the product of the polyesterification.** ●, △, and ▲ are 37, 45, and 53 °C, respectively. At 53 °C, the enzyme was partially inactivated.

and co-workers (*15*) reported the highest M_w of 7100 g/mol and a M_w of 4600 g/mol with immobilized *M. miehei* lipase for polyesterification of adipic acid with 1,4-butanediol in hexane. Although these published trials of direct polyesterification between diacids and diols had more or less failed, we proceeded to find a possible solution.

The polyesterification reaction proceeds as follows:

$$n\ CHOCO(CH_2)_8COOH + n\ HO(CH_2)_4OH \leftrightarrows [CO(CH_2)_8COO(CH_2)_4O]_n + 2n\ H_2O$$

We noted that, in a vacuum, *R. miehei* lipase had a relatively high activity for the polyesterification of underivatized dicarboxylic acids with aliphatic diols in diphenyl ether. Even with the underivatized sebacic acid and 1,4-butanediol in vacuum at 37 °C, we obtained a M_w of 42,000 g/mol (DP = 167). With hexanedioic acid and hexanediol, a M_w of up to 70,400 g/mol (DP = 279) and a maximum M_w of 126,500 g/mol (DP = 501) were obtained. In general, we obtained at least a fourfold increase in the M_w with the polyesterification of underivatized diacids and diols in a vacuum compared with the polyesterification at atmospheric pressure (Figure 3). The M_w values obtained in a vacuum were of the same order of magnitude as those we obtained in the polytransesterification of the activated, derivatized diacid.

The effect of chain length of dicarboxylic acid and diol on the polyester obtained at 37 °C is shown in Table 2, and the effect of reaction temperature on polyesterification is illustrated in Figure 4. When we increased reaction temperature to 45 °C, the highest M_w increased to 64,600 g/mol (DP = 284); when the polymer was precipitated from methanol again, a yield of 82% was obtained, and the M_w of the partially purified poly(1,4-butyl sebacate) increased to 77,400 g/mol (DP = 340, polydispersity = 4.4; melting temperature = 66.8 °C as determined by DSC), and the maximum M_w was 131,000 g/mol (DP = 520). To our knowledge this is the highest molecular weight ever reported by lipase-catalyzed polyesterification between a diacid and a diol. Using [^{13}C]NMR, we determined that the partially purified white solid was linear poly(1,4-butyl sebacate).

We then scaled up the synthesis of poly(1,4-butyl sebacate) from derivatized and underivatized sebacic acid and 1,4-butanediol up to 100-fold to obtain enough polyester for further characterization purposes. The molecular weight distributions obtained in seven days of polyesterification or polytransesterification were nearly identical as confirmed by DSC analysis and [^{13}C]NMR. In a 100-g scale, the M_w from polyesterification was ~53,000 g/mol (DP = 210), number-average molecular weight (M_n) was 27,200 g/mol, and polydispersity (M_w /M_n) was 1.95.

***Table 2.* Effect of monomer carbon chain length on M_w with polyesterification**

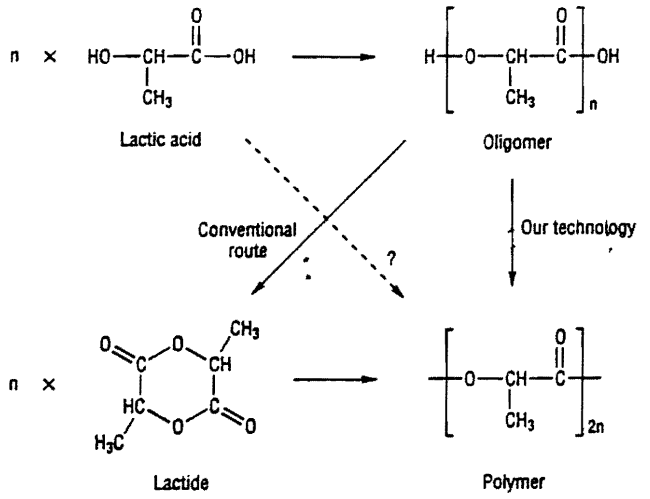
	Alkane diol				
Diacid	C_2 (Ethanediol)	C_3 (Propanediol)	C_4 (Butanediol)	C_5 (Pentanediol)	C_6 (Hexanediol)
C_4 (Succinic)	980	1150	2140	5290	3740
C_6 (Adipic)	6420	26,830	38,970	37,270	70,430
C_8 (Octanedioic)	2090	51,730	48,590	55,020	43,480
C_{10} (Sebacic)	9760	35,550	46,130	53,680	62,480
C_{12} (Dodecanedioic)	8960	26,070	21,450	28,320	16,220

DSC analysis (heating and cooling rates = 10 °C/min) (Figure 5) showed that the melting temperature was 64 °C (ΔH_{m_1} = 105 J/g), and crystallization temperature was 44 °C. No glass transition temperature could be observed by DSC, but dynamic mechanical thermoanalysis gave a barely noticeable peak at −50 °C, which is of the same order of magnitude as that of poly(ϵ-caprolactone). The polymer had good coating and water-repellent properties.

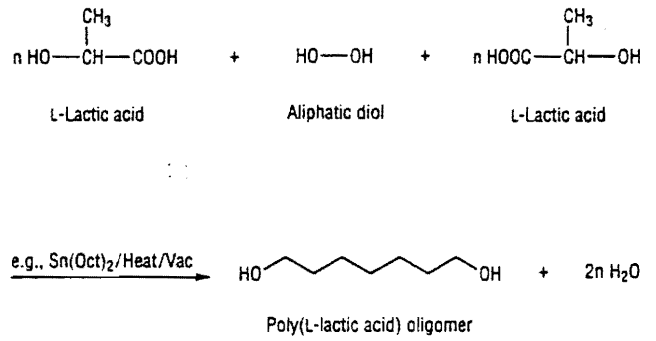
Chemical synthesis of poly(ester-urethanes) based on lactic acid

Conventional polyester synthesis is based on a step-growth mechanism with condensate removal. Such polymerizations are often carried out using acid-catalyzed esterification or transesterification. Chemical polycondensation usually takes a long time because it requires careful removal of the condensates and shearing of the copolymer melt to avoid equilibrium-limited chain growth. As in enzymatic polyesterification, an accurate stoichiometric ratio of the functional groups is essential to reach a high degree of polymerization. Even a slight deviation from the stoichiometric ratio may lead to a drastic decrease in the polymer's molecular weight. The duration of the polycondensation may exceed tens of hours, resulting in a highly viscous polymer melt that requires special equipment for handling.

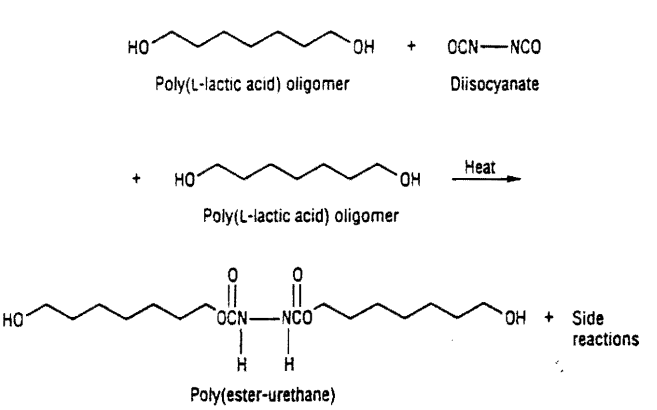
In the case of aliphatic polyesters, a direct polycondensation to controlled molecular structures of high molecular weight polyesters is difficult. To avoid problems in condensation polymerization of aliphatic polyesters, the extensively reported ring opening polymerization method is used (*20*, *27*, *28*). In this polymerization route, the polyester is formed through an ionic or coordination mechanism using cyclic lactones as monomers; problems associated with the condensate removal, equilibrium reactions, and long reaction times can be avoided. However, for large-scale applications, it is difficult to produce the lactone monomers in large quantities and high yields; especially in the polymerization of lactic acid, in which the polymerization through cyclic lactides dominates in the production of medical-grade, biodegradable homo- and copolymers. Different routes available to high molecular weight polymers starting from lactic acid are shown here.



We recently developed a novel two-step process for poly(ester-urethane) synthesis (*20*, *29*). First, 85% food-grade lactic acid is polymerized using a condensation reaction to a telechelic oligomer with a M_w of 2000–10,000 g/mol. An OH functionality is produced by adding a small amount of 1,4-butanediol to the system.



The telecheles are linked together immediately after the polycondensation step using urethane bonds.



This route is highly efficient; yields are >97% based on lactic acid conversion to the thermoplastic polymer. Consequently, the lactide step could be avoided, and it was possible to tailor the resulting M_w to up to ~300,000 g/mol. Furthermore, the average molecular weight and the molecular weight distribution could be separately controlled.

The properties of the poly(ester-urethanes) obtained via this route are similar to those of conventional polylactides—for example, tensile strength was in the range of 40–50 MPa. Nevertheless, the novel synthesis route

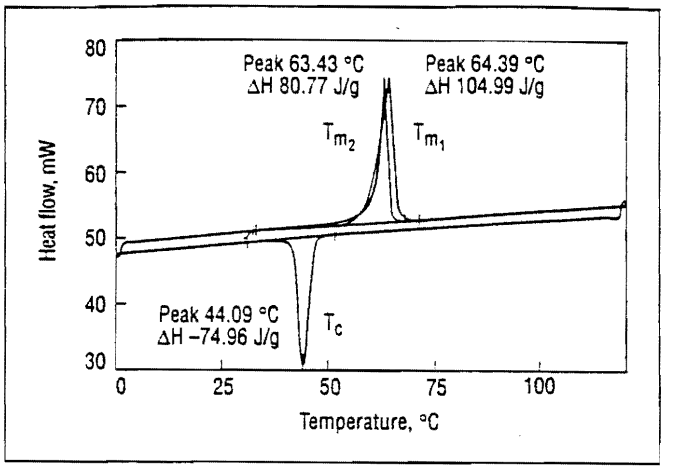


***Figure 5.* The polyester obtained from the derivatized sebacic acid substrate was shown by DSC analysis.** The heating and cooling rates were 10 °C/min. T_{m_1} and T_{m_2}, melting temperatures of the first and second heating cycle; T_c, crystallization temperature.

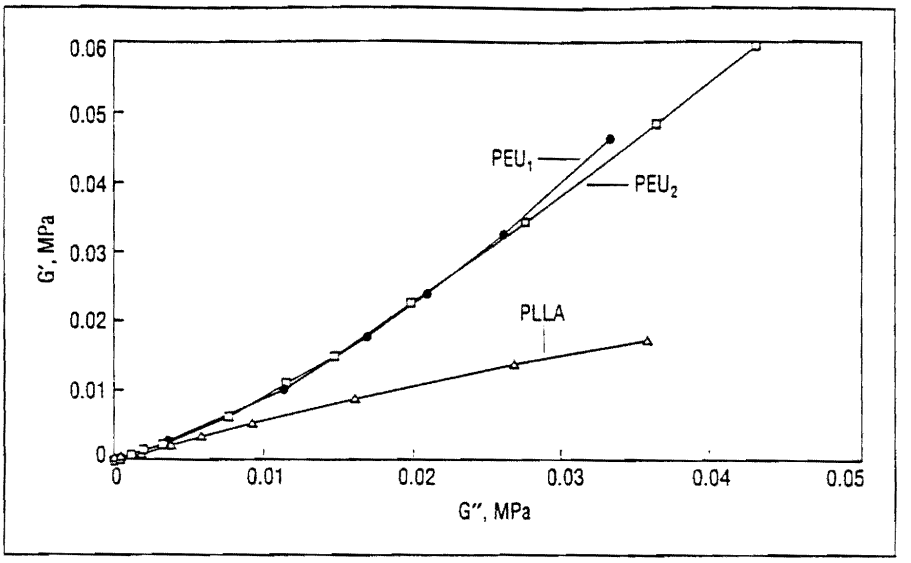


Figure 6. The poly(ester-urethanes) (PEU_1 and PEU_2) have greater melt elasticity than the linear polymeric structures (PLLA). The storage modulus (G') versus loss modulus (G'') is shown at 180 °C with rotational rheometry.

creates marked differences. Polylactides are essentially linear in structure, as are the aliphatic polyesters produced by lipase biocatalysis. In poly(ester-urethanes), however, the isocyanate end groups of the polymer chains allow the formation of urethane bonds, thus forming long chain branches and, in the worst case, cross-linking. Our synthesis route results in a broadened molecular weight distribution but also makes it possible to control molecular weight and chain branching.

Chain branching is an important property from the rheological point of view. It is also important in applications such as paper coating. The lactic acid–based poly(ester-urethanes) exhibit markedly different dynamic rheological properties and better paper coating characteristics than conventional polylactides. Poly(ester-urethanes) are compared with polylactides in Figure 6, which gives the storage modulus (G) as a function of the loss modulus as measured by dynamic rheometry. The ratio G'/G'' shows that poly(ester-urethanes) have greater melt elasticity than linear polymeric structures. The polydispersity of the poly(ester-urethanes) is markedly higher than that of poly(L-lactic acid) (*30*).

The biodegradability of various poly(ester-urethanes) was also investigated by using the so-called head-space method based on the evolution of carbon dioxide by the action of the degrading test microorganisms (*31*). Practically no degradation was observed at 25 °C compared with the polylactides, which degrade to some extent even at ambient temperature. However, when the temperature was increased to 55 °C, close to the glass transition temperature of poly(ester-urethanes), rapid degradation took place, and decomposition was completed in one month. If controllable, such temperature-programmed degradation is a definite advantage in some applications.

Conclusion

We have shown two ways to produce biodegradable aliphatic polyesters: lipase biocatalysis and a two-step chemical method. The enzymatic, lipase-catalyzed polymerization of aliphatic dicarboxylic acids and diols results in linear polyesters with M_w equal to 77,400 g/mol and a maximum M_w on the order of 130,000 g/mol. The two-step chemical production of poly(ester-urethanes) from lactic acid results in very high molecular weight polymers (M_w > 300,000 g/mol) at 97% conversion. Highly purified lactic acid is not needed for this process, and the polymerization proceeds without the lactide step. No monomer residuals were found in either case. Both routes are extremely interesting and raise the possibility of structural control of polyesters as a motivator for further research. The use of lipase as the biocatalyst in polyesterification reactions opens up an interesting alternative of producing biodegradable polymers under mild reaction conditions, and the novel route for food-grade, lactic acid–based poly(ester-urethane) production allows the bulk manufacture of biodegradable polymers with characteristics suitable for paper coating, for example.

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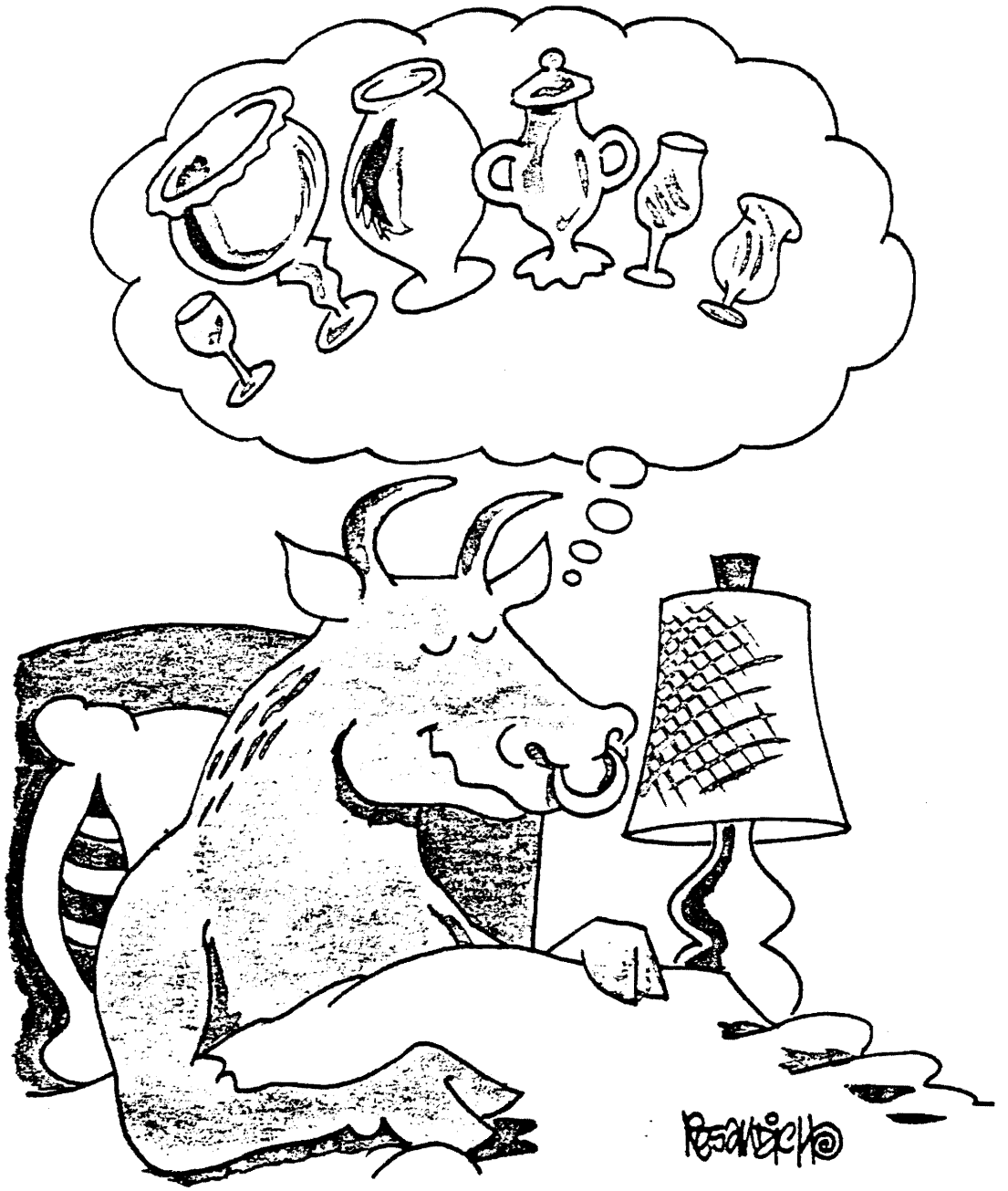

Direct electrostatic coating of paper

Robert H. Marchessault, Patrice Rioux and *Ilie Saracovan*, McGill University, Montreal, Canada

Keywords: Coated papers, Polyolefins, Poly-β-hydroxyalkanoates, Electrostatic coating, Corona discharge, Xerography, Toners, Adhesive strength, Barrier properties, Moisture transmission.

SUMMARY: Direct Electrostatic Coating (DEC) of paper uses "as polymerized" reactor polyolefin and an electrostatic spraying applicator. A glossy/transparent polymer coat was obtained after hot-pressing the electrostatically deposited powder layer on the paper surface. Paper coated using powders such as polypropylene and biopolyesters (poly-β-hydroxyalkanoates) showed good barrier properties and remarkable adhesion between the polymer film and the fibrous texture. Dry blends of polyolefin powder and common inorganic fillers (e.g., clay, $CaCO_3$) up to 40% (w/w) provided powder formulations suitable for electrostatic coating. DEC is environmentally friendly and potentially of low cost while being simple, flexible and adaptable to the end-use paper requirements.

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Electrostatic coating offers opportunities to meet today's environmental requirements (Azzam 1973). In this process, a fine dispersion of coating material is electrically charged (positively or negatively) and directed toward the charged substrate (opposite electrical sign). The electrostatic attraction between the charged particles and oppositely charged substrate controls the deposition and layering of the materials in the coating. Numerous applications of electrostatic coating, often involving elegant technology, have been described (Miller 1973).

Early applications employing electrostatic forces for coating fibrous substrates (e.g. paper and paper-related materials) are found in sandpaper manufacture (Melton et al. 1940) and in the flock coating process (Amstuz 1941). First mention of an electrostatic process for coating paper using dry polymer powders dates to 1955 (Reif 1955). The process was developed for the Bergstrom Paper Co., Neenah, Wis., in work sponsored at Battelle Memorial Institute, Columbus, Ohio. An outcome of this development was reported in 1961 as the commercial application for the production of a gummed web material (Holt et al. 1961). These electrostatics-based processes depended on complex equipment and expensive coating materials to meet the requirements of the process. Today it is the cost of the powder materials which limits development toward large scale application in the paper field.

An interesting use of electrostatic coating in the reprographic industry has been labeled "Direct Electrostatic Printing" (Schmidlin et al. 1990). It is a simple, inexpensive computer controlled means of creating images on paper directly from digital information. It involves direct projection of toner onto paper for temporary displays and may or may not involve fusing.

Although paper coating is based on a wide variety of technologies, wet-coating remains the "backbone" of the industry (Booth 1970). Generally, wet-coating creates an optimized layer at the surface of the sheet that smooths the contours of the pore-fibre interface. For certain speciality papers solvent coating is also used. To meet barrier requirements (e.g. moisture, O_2 barrier) laminating a pre-extruded hot polymer film onto the paper substrate has become an important paper technology. The solventless aspect of this approach is an attractive environmental feature. However, pre-extruded melt-coating does not allow formulation flexibility, as in wet-coating applications, and is energetically expensive.

This paper presents the results of our work in developing a solvent-free coating process for fibrous substrates (e.g. paper, non-wovens and the like) based on electrostatics. Our main objective is the use of inexpensive polymer powder for coating the substrate in a simple, direct process. The outcome will be a thin layer of polymer powder on the substrate which is subsequently fused and calendered to give a uniform coating. The final product will be an optimally designed coated paper with improved properties thanks to the formulation potential of blended powders to provide adhesion, opacity, porosity, wetting and sorption at the surface.

Materials and process

Three major elements retained our attention:

- source(s) of inexpensive polymer powder with thermoplastic characteristics corresponding to our purpose:

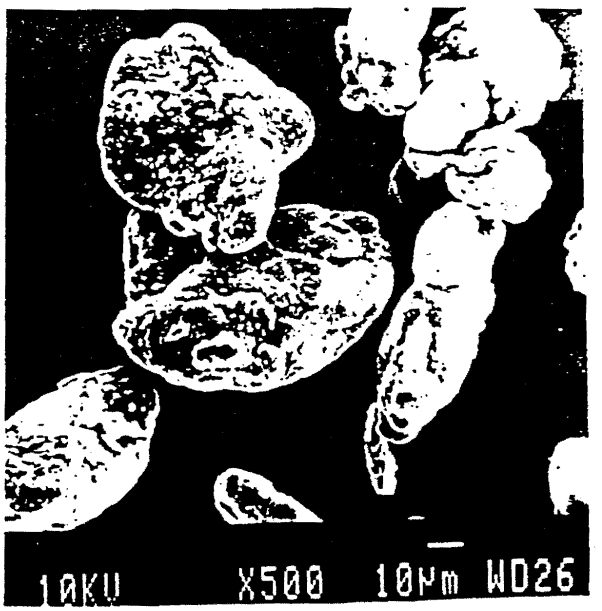

Fig. 1. Scanning electron micrograph of "as received" polypropylene powder (Himont) used for DEC experiments.

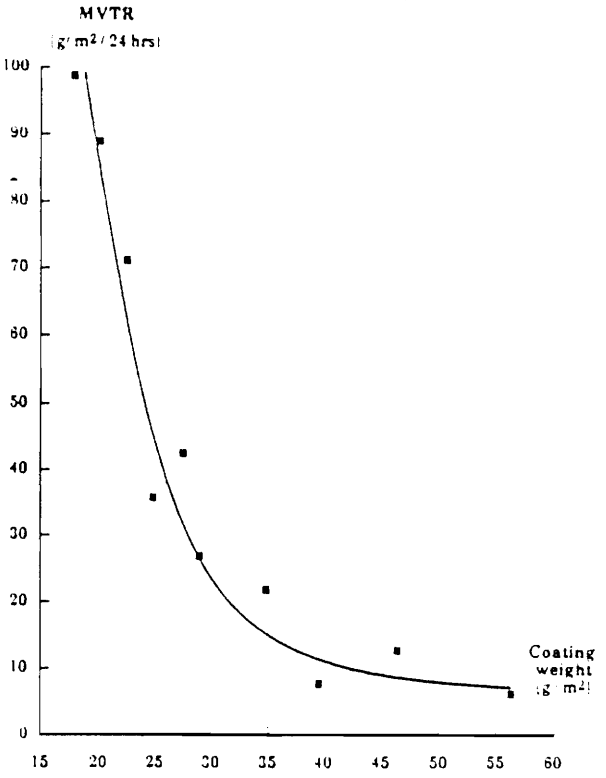

Fig. 5. Moisture vapor transmission rate (MVTR) of PP coated paper as a function of coating weight in DEC process. Measurements done on hot-pressed specimens at 162°C, 5000 psi (34 MPa), and 30 s.

has been shown to be similar to that of water on polyethylene terephtalate, i.e. about 75° (Marchessault et al. 1990).

The plot of MVTR versus "coating weight" for DEC coated paper (*fig. 5*) showed a pattern similar with those of binary composite laminates (Rogers 1967; Avery 1962; Galbraith, Kitchen 1962). Coating weights of less than 18 g/m² yielded poor barrier properties due to the cellulose fibers penetration through the PP film. When exposed to moisture these fibers wick water through the polymer film. Increasing the coating weight (i.e. thicker film) to 30 g/m² provoked an almost linear decrease of the permeability values. The observed permeability rate of DEC coated paper approached the value of the polypropylene film used as "control" (10.8 g/m² during 24 hrs) for coating weights exceeding 40 g/m².

Powder formulations. Blends of PP and inorganic fillers were made and experimentally tested from the point of view of charging, adherence and fusion on paper. Each formulation was examined through an optical and polarizing microscope prior to coating in order to visually assess the filler dispersion with the PP powder. Scanning electron micrographs of these formulations showed PP particles in close contact with filler ("anchored" to the polymer particle). At higher filler content, the PP particles were surrounded by filler particles attached to the surface (*fig. 6*).

A filler content of 40% appeared to be the upper limit in PP formulations to provide good charging/adherence of powder on paper substrates, and subsequently, a quality coating after hot-pressing. Above the 40% limit, the powder deposition on paper was uneven suggesting the

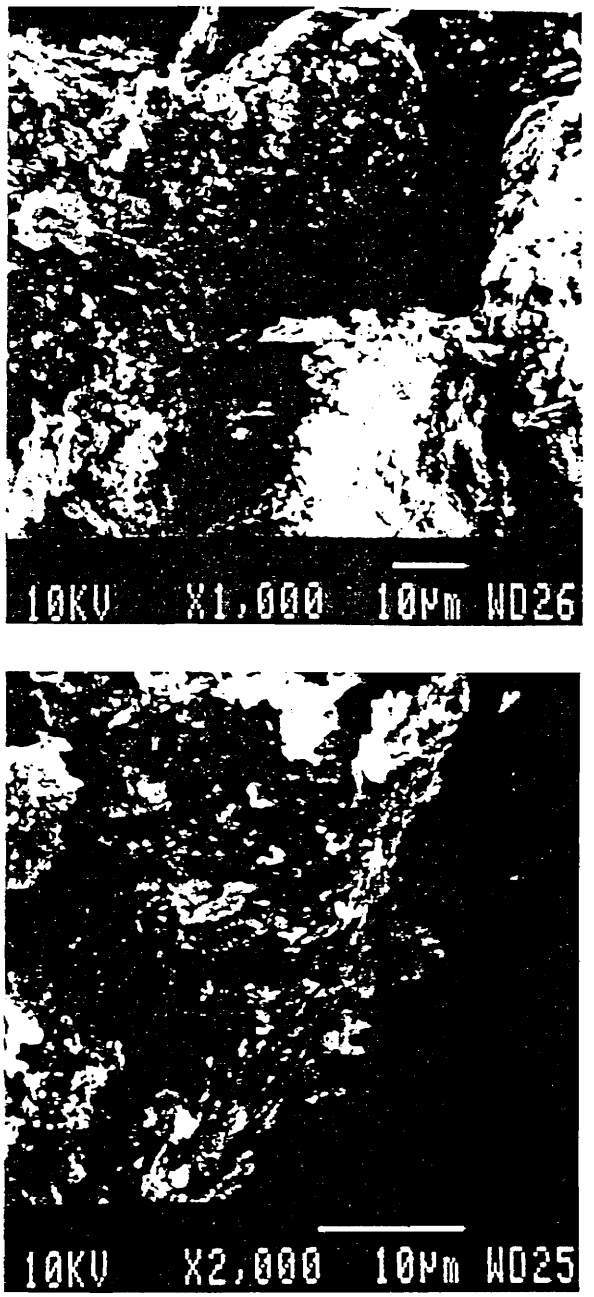

Fig. 6. Scanning electron micrographs of PP/clay formulation (30% w/w "Nuclay"). Top: overview showing filler particles attached to the PP particle surface; bottom: closeup of a PP particle practically covered by filler.

occurrence of the electrical discharges ("back-ionization" effect) related to the powder layer thickness and the charge characteristics of the polymer blend (Singh et al. 1978; Singh et al. 1979; Cross 1981). Moreover, a continuous film was impossible to obtain during hot-pressing. Apparently, PP particles play a double role:

- "carrier beads" for the inorganic particles during the charging–transport–adherence steps of the electrostatic coating process;
- "host-matrix" during hot-pressing, when filler particles are embedded in a molten polymer mass.

Formulations with a filler content up to 10% did not change the "plastic-like" aspect of the coated film on paper as compared to the PP powder. One of the important outcomes of the polymer-filler interaction in these

Mechanical properties of coated paper samples. Standard CPPA tests were performed on samples of PP and PHB coated paper at the Pulp and Paper Research Institute (Pointe Claire, Quebec). The results in *table 1* show little change in paper characteristics and overall mechanical properties after coating and hot-pressing. The relative increase of the apparent density after coating indicates that hot-pressing has collapsed the pore structure of the groundwood paper.

Adhesion of polymer film on paper. A qualitative test of adhesion between paper and polymer film was performed at the Canadian Pacific Forest Products Ltd., Paperboard & Packaging Development Laboratory (Montreal, Quebec). The coated paper specimen was exposed to 20% aqueous sodium hydroxide solution for a fixed period of time and the polymer film was delaminated from the fibrous substrate. DEC coated paper showed exceptionally good adherence between the two components even after 24 hours exposure to the caustic. For comparison, a polyethylene extrusion coating (lamination of a pre-extruded hot polymer film on paper substrate) delaminates after one hour exposure to the alkali solution.

The polymer/cellulose interface of DEC coated paper was observed using scanning electron microscopy. For SEM observations, specimens were prepared by multistep delaminating (transparent adhesive tape) the coated paper samples in order to observe the interface morphology. The appearance of the interface prepared using this technique is shown in *fig. 4*. Both specimens displayed a similar pattern: cellulose fibers embedded in a polymer mass which replicates the fibrous structure.

A quantitative test of bond strength on DEC coated paper provided a measure of adhesion between the polymer film and cellulosic substrate. The data in *table 2* substantiate the previously described qualitative observations: the bond strength between polymer/fiber and fiber/fiber are at least similar. This explains the good polymer film/paper adhesion exhibited by the electrostatically coated paper samples.

Barrier properties of PP and PHB coated paper. The moisture vapor permeability tests were performed at the Canadian Pacific Forest Products Ltd., Paperboard & Packaging Development Laboratory (Montreal, Quebec) following the standard procedure used for packaging products. The results of moisture vapor transmission rate (MVTR) tests are listed in *table 3*. Although these results showed considerable variability as compared with the control (25 µm extruded polypropylene film), the barrier properties of PP DEC coated paper were clearly demonstrated. PHB coated paper samples showed higher MVTR values than PP, as expected for a more polar polymer. Thus, the contact angle of water on PHB films

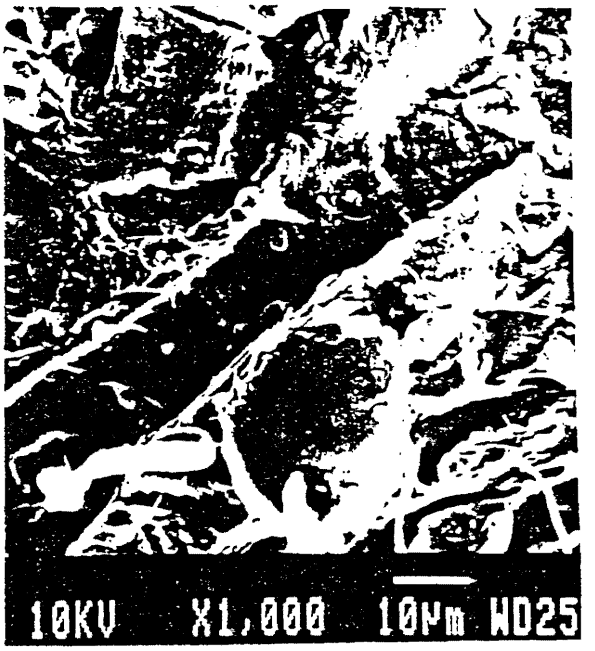

Fig. 4. Scanning electron micrographs of polymer/fiber interface: PP (top) and PHB (bottom) coated paper. Specimens prepared by dry delaminating the cellulosic fibers until the contact region polymer/cellulose was available for examination.

Table 2. Bond strength of polypropylene (PP) and poly-β-hydroxybutyrate (PHB) electrostatically coated paper.

Sample	Bond strength[a] [N/m]	
	MD	CD
PP	84.5	81.4
	78.3	80.7
Cellulose fibers (substrate for coating)	**83.7**	**94.5**
PHB	80.3	82.9
	80.7	81.4

[a] Tests done under standard conditions at Pulp and Paper Research Institute of Canada (Point Claire, Quebec).

Table 3. Moisture vapor transmission rate (MVTR) of polypropylene (PP) and poly-β-hydroxybutyrate (PHB) electrostatically coated paper.

Sample	Coating weight [g/m²]	MVTR[a] [g/m² during 24 h]
PP	39.4	8.2
	45.2	26.6
	46.3	12.8
	56.2	6.4
Control[b]	**23.0**	**10.8**
PHB	27.7	224.8
	30.2	237.2
	36.4	209.2

[a] Measured at 100°F (37.8°C) and 95% relative humidity.
[b] Control sample was 25 µm thick melt-extruded polypropylene film.

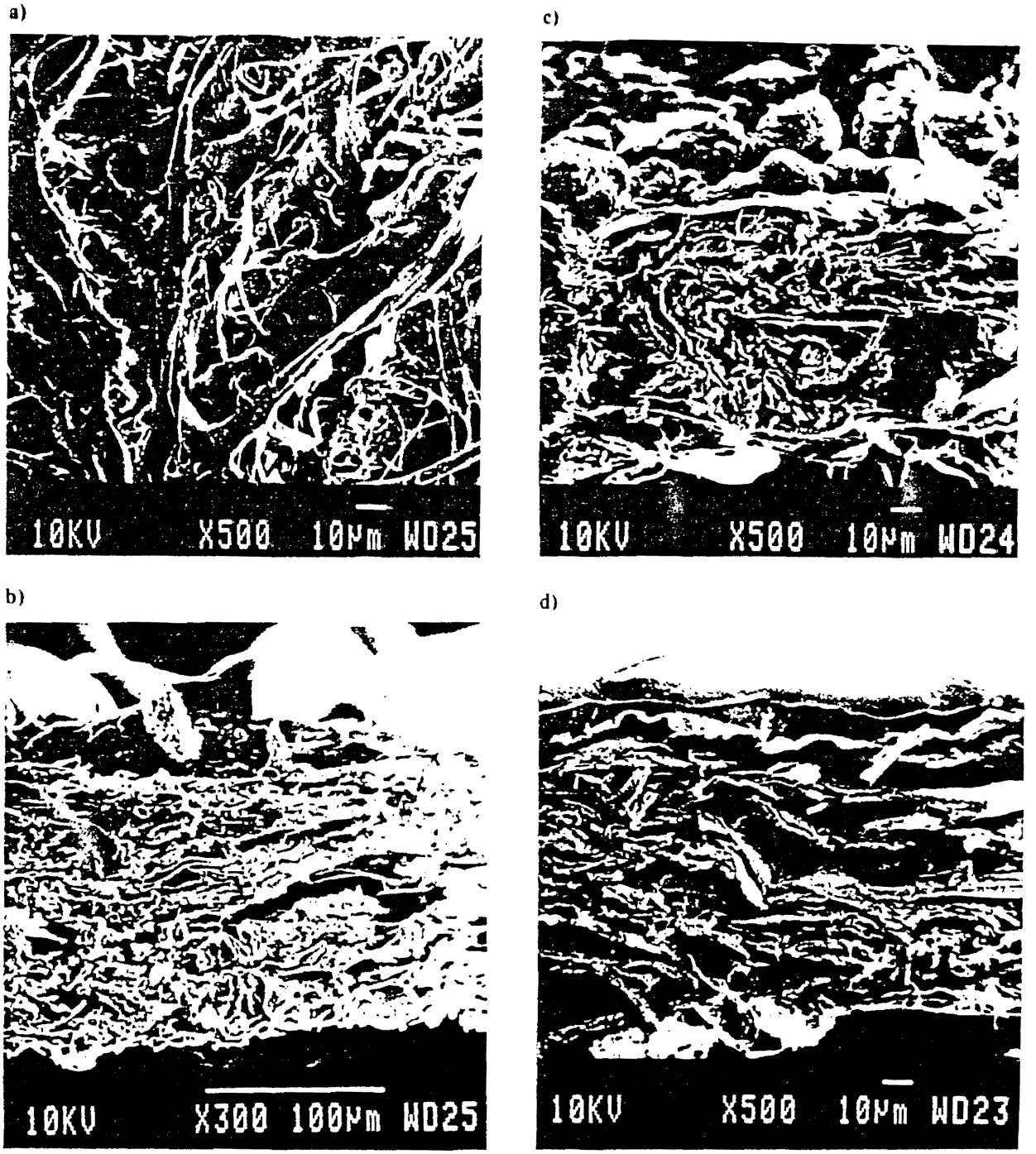

Fig. 3. Scanning electron micrographs illustrating the DEC process: ***a)*** *copier paper used as substrate (face view);* ***b)*** *PP particles deposited on paper (cross-section);* ***c)*** *PP powder layer fused on paper (cross-section);* ***d)*** *PP coated paper after hot-pressing (cross-section).*

lymerized" polyethylene and polypropylene. Each fraction was assayed for electrostatic charging and adhesion on selenium plate using a "Model D" Xerox Processor (Lauzier, Marchessault 1989). The results indicated that a polymer particle of average diameter less than 100 µm was acceptable for electrostatic charging and good adhesion on the paper surface.

Powder characteristics (particle diameter, melting point, melt viscosity) are the most important factors in electrostatic coating. *Fig. 3* presents the sequence "deposition–fusing–pressing" as observed through a scanning electron microscope. Scanning micrograph in fig. 3d shows a relatively thin (25-30 µm) polypropylene film adhering to paper.

Electrostatic deposition and subsequent hot-pressing of the powder is not substrate-dependent: dense and thick substrates (e.g., cardboard) as well as porous paper (e.g., paper towel) were coated with very good results. Remarkably, printed and color-printed paper and journal covers kept their graphic quality after hot-pressing.

A *sine qua non* condition for electrostatic deposition of the powder onto a dielectric substrate is the presence of the back electrode. Therefore, no powder deposition on paper was achieved when the back electrode was removed or was imagewise absent. Thus, a pattern of holes deliberately cut in the back electrode resulted in uncoated circles in the substrate.

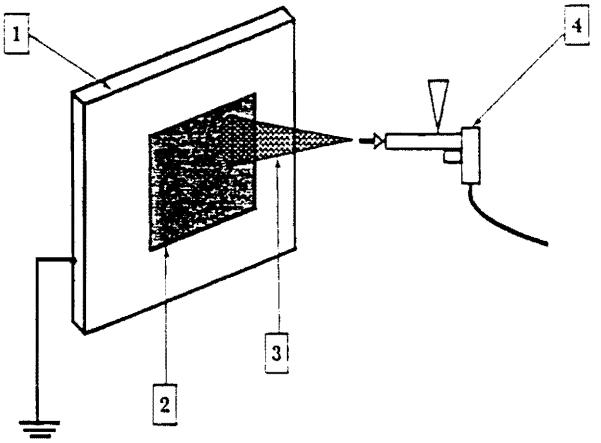

Fig. 2. Schematic of the experimental setup for DEC of paper using polymer powders: 1) metal plate (grounded), 2) paper sheet, 3) charged powder cloud, 4) electrostatic applicator (corona hand-gun).

- equipment and methodology for electrostatic charging and deposition of polymer powder on paper;
- powder formulations using conventional inorganic fillers with the polyolefin.

"As polymerized" polyolefin. Himont Canada (Varennes, Quebec) supplied commercial polypropylene (PP) homopolymer powder collected by cyclone fractionation of the reactor product. Scanning electron micrographs showed PP particles of irregular shape with average diameter of 50-60 μm and less (*fig. 1*, p. 211). The melting point of PP was 162°C as determined by differential scanning calorimetry (DSC). This powder was used throughout the experiments "as received" or in formulations prepared by us.

Other "as polymerized" polymer powders (e.g. polyvinyl chloride, polystyrene-butadiene copolymer) were also assayed. These latter materials were produced by proprietary suspension polymerization processes.

Biodegradable polymer. The idea of an electrostatically coated paper using a biodegradable polymer such as spray dried poly-β-hydroxybutyrate (PHB) ("Biopol™," ICI, Billingham, UK) is very attractive. PHB is a thermally unstable material and must contain additives to control degradation during normal melt-extrusion processing. The present process offers the potential of considerably reduced exposure to heat and undesirable development of crystalline morphologies which are often responsible for pinholes due to crack development on crystallization.

Coating substrates. Sheets of copier paper containing 85% bleached stoneground wood pulp, with controlled electrical conductivity, were the principal substrate. Other cellulose-based substrates (e.g. printed paper, cardboard, paper towels, etc.) were also used for coating studies.

Powder formulations. Blends of polypropylene with fillers were made by dry mixing at room temperature. Several types of commercial inorganic fillers were considered for blending:

- clay: "Hydrafine" (J.M. Huber Corp., Macon, Georgia)
  "Nuclay"-delaminated grades (Engelhard Corp., Edison, NJ)
- $CaCO_3$: "Hydrocarbon 65" (Omya Inc., Proctor, Vermont).

All fillers were in the range of 1-2 μm particle diameter and blends of 5 to 55% (w/w) filler content were prepared. To mix the components a "Plasti-Corder" Brabender (Brabender Instrument Inc., South Hackensack, New Jersey) equipped with a twin screw head was used. The following working conditions were observed during the blending process: mixing speed, 120 r/min; mixing time, 15 min; temperature, 23-25°C; and batch weight, 200 g.

Experimental setup. Sheets of paper, placed on a grounded metal plate (back electrode), were coated with polymer powder (*fig. 2*) using a commercial "Solidspray '90" corona gun unit (Volstatic Coatings Ltd, London, UK). The relative humidity in the room was not controlled. The polymer powder was gravity-fed into the gun barrel through a conical feeder attached to the gun. This allowed the use of a minimum amount of powder (about 30-50 g) instead of a large quantity (minimum 1 kg) required using the normal hopper. A voltage of 80 kV was used to negatively charge the polymer powder. The gas-carrier of 20 psi (0.14 MPa) was dry, oil-free air supplied from a cylinder. Layer thickness and uniformity of deposited powder on paper were visually assessed. Coating weights were accurately determined after fusing and pressing.

Fusing and calendering. Fusing and calendering were done in a single step by hot-pressing the powder-coated paper between mylar sheets in a laboratory Carver press. Preliminary hot-pressing trials demonstrated that 5000 psi (34 MPa) during 30 s and a temperature of 10°C below the melting point of the polymer are minimum requirements to convert the deposited powder layer to a transparent and visually smooth polymer film on paper. All samples were processed under the same conditions unless otherwise specified.

Results and discussion

Electrostatic charging and adhesion of powder on paper. One of the key-parameters of electrostatic charging and deposition of powder is the diameter of the polymer particle (Reif 1955; Singh 1978). Preliminary evaluation of particle charging was studied on fractions of "as po-

Table I. Physical properties of PP and PHB electrostatically coated paper.

Characteristic	Units	Ref.[a]	PP	PHB
Grammage (basis weight)	g/m^2 (OD)	**60.0**	91.1	85.9
Caliper (single sheet)	μm	**122.6**	124.6	111.2
Specific volume	cm^3/g	**2.04**	1.37	1.29
Apparent density	g/cm^3	**0.49**	0.73	0.77
Moisture content	%	**7.5**	4.8	4.8
Tear index	$mN \cdot m^2/g$	**3.76**	3.12	3.77
Breaking length	km (MD)	**5.70**	4.80	4.58
	(CD)	**2.94**	3.17	2.78
Tensile ratio	%	**1.94**	1.51	1.65
Stretch	% (MD)	**1.32**	1.65	1.48
	(CD)	**2.27**	2.44	2.15
Tensile index	N·m/g (MD)	**55.90**	47.03	44.89
	(CD)	**28.84**	31.11	27.21

[a] Reference was the uncoated substrate (copier paper).

dry blends is the uniform dispersion of inorganic component throughout the polymer matrix. As a result, changes in coated paper characteristics are expected, especially barrier properties and printability.

Conclusions

The feasibility of the Direct Electrostatic Coating (DEC) process using thermoplastic polymer powders has been demonstrated. Two key-elements enable this new approach:

- simple and efficient corona charging equipment to provide a high volume throughput of coating material;
- inexpensive polymer powder (e.g. "as polymerized" polyolefin) with suitable characteristics (i.e., particle diameter, electrostatic charging characteristics, melting point, etc.).

These results open new approaches to speciality paper with:

- barrier properties;
- adhesion/wetting characteristics;
- biodegradability;
- flexibility and simplicity of dry blending.

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Polyhydroxybutyrate, a Biodegradable Thermoplastic, Produced in Transgenic Plants

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Polyhydroxybutyrate (PHB), a high molecular weight polyester, is accumulated as a s age carbon in many species of bacteria and is a biodegradable thermoplastic. To proc PHB by genetic engineering in plants, genes from the bacterium *Alcaligenes eutrop* that encoded the two enzymes required to convert acetoacetyl–coenzyme A to PHB w placed under transcriptional control of the cauliflower mosaic virus 35S promoter introduced into *Arabidopsis thaliana*. Transgenic plant lines that contained both ge accumulated PHB as electron-lucent granules in the cytoplasm, nucleus, and vacuole: size and appearance of these granules were similar to the PHB granules that accumu in bacteria.

Poly-D-(−)-3-hydroxybutyrate (PHB) is an aliphatic polyester that is accumulated by many species of bacteria as storage material (*1*). Both PHB and related polyhydroxyalkanoates (PHA) are renewable sources of biodegradable thermoplastic materials (*2*). The cost of PHB produced by bacterial fermentation is substantially higher than that of other biomaterials, such as starch or lipids, that accumulate in many species of higher plants. Therefore, we investigated the feasibility of transferring the capability for PHB synthesis from bacteria to higher plants.

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In the bacterium *Alcaligenes eutrop* PHB is derived from acetyl–coenzym (CoA) by a sequence of three enzym reactions (*3*). The first enzyme of the p way, 3-ketothiolase (acetyl-CoA ac transferase; E.C. 2.3.1.9), catalyzes reversible condensation of two acetyl-C moieties to form acetoacetyl-CoA. toacetyl-CoA reductase (hydroxybut CoA dehydrogenase; E.C. 1.1.1.36) su quently reduces acetoacetyl-CoA to D- 3-hydroxybutyryl-CoA, which is then p merized by the action of PHB synthas form PHB. The genes encoding the t enzymes involved in PHB synthesis in *eutrophus* have been cloned, and expres of the genes in *Escherichia coli* is suffic for PHB production (*4–7*). Of the t enzymes involved in PHB synthesis in *eutrophus*, only the 3-ketothiolase is found in the cytoplasm of higher pla where it is involved in the synthesi mevalonate, the precursor to isoprenoi

Genes that encoded acetoacetyl-C reductase (*phbB*) and PHB synthase (*ph* were introduced into *Arabidopsis tha*

through Ti plasmid–mediated transformation. The coding sequences of the *phbB* and *phbC* genes were individually cloned into the binary Ti plasmid pBI121 so that the genes were under the transcriptional control of the constitutive cauliflower mosaic virus 35S promoter (*8*). Two series of transgenic *A. thaliana* plants, which contained either the *phbB* or *phbC* gene, were generated by transformation with *Agrobacterium tumefaciens* containing the Ti plasmid constructs (*9*).

Southern (DNA) blot and Northern (RNA) blot analysis of seven putative homozygous transgenic lines obtained by transformation with the *phbB* gene indicated the proper integration and transcription of the gene in the various lines (*10*). To assess whether the *phbB* mRNA was correctly translated and whether the polypeptide produced was functional, we assayed transgenic plants that had the *phbB* gene for acetoacetyl-CoA reductase activity (*11*). Enzyme activity was not detectable in leaf extracts of the untransformed wild-type plants. Leaf extracts of the seven transgenic lines exhibited specific activities ranging from 1.6 to 16.2 units per milligram of protein (*12*), compared to 1.4 units per milligram of protein for extracts of *Escherichia coli* DH5α that expressed the *phbB* gene present on the plasmid pTZ18U-PHB (*13*).

Southern blot and Northern blot analysis of three putative homozygous transgenic lines obtained by transformation with the *phbC* gene revealed proper integration and transcription of the gene in the various lines (*10*). However, transgenic plants that expressed *phbC* mRNA had no detectable PHB synthase activity. Similarly, expression of the *phbC* gene in *E. coli*, in the absence of *phbA* and *phbB* gene expression, did not result in significant PHB synthase activity (*7*). It has been postulated that in the absence of substrate synthesized by the *phbA* and *phbB* gene products, the PHB synthase may be unstable or inactive. Therefore, the absence of PHB synthase activity in transgenic plants that contained the *phbC* gene was not considered an accurate reflection of whether the enzyme would function in plants that contained the additional enzymes of the pathway.

To produce plants containing both *phbB* and *phbC* genes, we cross-pollinated homozygous transgenic lines that had the *phbB* gene (lines RedB-2A, -2C, -2D, -2G, and RedD-3A) with homozygous transgenic lines that had the *phbC* gene (lines S8-1-2A and S8-1-2C). Leaf samples of 2- to 3-week-old hybrid plants (F1) were analyzed for the presence of PHB by gas chromatography of transesterified ethylated derivatives of chloroform-soluble material (Fig. 1). Extracts of F1 hybrids that expressed both the bacterial acetoacetyl-CoA reductase and the PHB synthase genes contained a novel compound that eluted with the same retention time as ethylhydroxybutyrate. The compound was not detected in transgenic plants that expressed only one of the two *phb* genes or in untransformed *A. thaliana* plants. Analysis of the compound by gas chromatography–mass spectrometry (GC-MS) confirmed that it had the same mass fragmentation pattern as a reference sample of ethylhydroxybutyrate (Fig. 2). Ethylhydroxybutyrate was not detected in chloroform extracts of plant tissues in

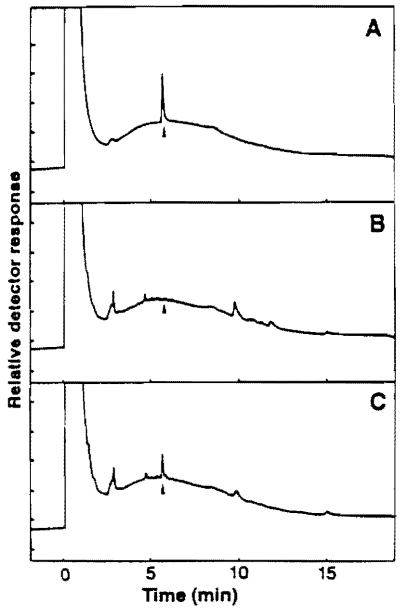

Fig. 1. Gas chromatographic evidence for PHB accumulation in transgenic plants. (**A**) Transesterified bacterial PHB (20 ng). (**B**) Transesterified chloroform extracts of leaves from wild-type *A. thaliana*. (**C**) Transesterified chloroform extracts of leaves from F1 hybrid between transgenic plants S8-1-2A (*phbC*$^+$) and RedB-2C (*phbB*$^+$). Arrowheads indicate elution time of ethylhydroxybutyrate. Between 5 to 50 mg of fresh or frozen plant shoot material was extracted in 1 to 2 ml of a chloroform and water mixture (1:1) for 16 hours at 65°C with constant agitation. This extract, which did not contain PHB, was discarded. The plant material was subsequently homogenized in water and reextracted in chloroform and water as described above. The chloroform phase was extracted twice with water. The products present in the organic phase were transesterified by acid ethanolysis, and one-hundredth of the final reaction mixture was analyzed by gas chromatography on a Hewlett-Packard 5890 series II GC (*18*). As a standard, bacterial PHB (Sigma) was used.

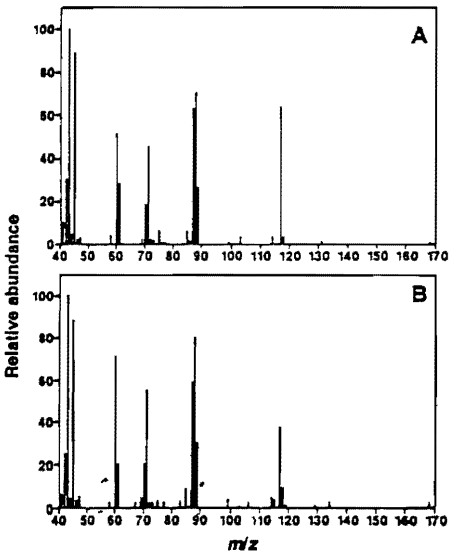

Fig. 2. Gas-chromatography–mass spectrometry analysis of PHB from bacteria and transgenic plants. (**A**) Mass spectrum of transesterified bacterial PHB. (**B**) Mass spectrum of the putative ethylhydroxybutyrate from F1 hybrid between S8-1-2A (*phbC*$^+$) and RedB-2C (*phbB*$^+$) (Fig. 1C). Electron impact mass spectral data was obtained on a JEOL JMS-AX505H mass spectrometer coupled with a Hewlett-Packard 5890 GC. The following parameters were used: source temperature, 200°C; ionization current, 100 μA; accelerating voltage, 3 keV (*19*); *m/z*, mass-to-charge ratio.

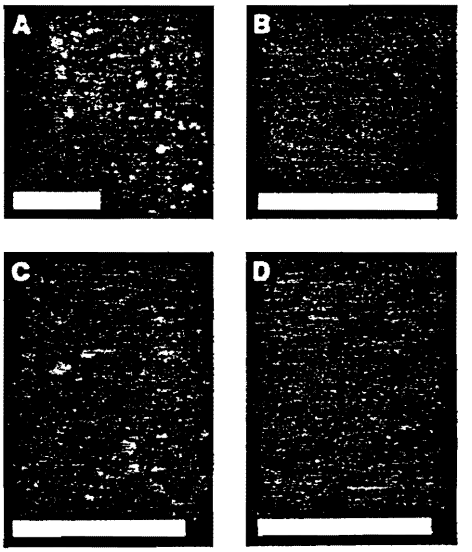

Fig. 3. Visualization of PHB granules by epifluorescence microscopy of tissues stained with Nile Blue A. Leaves (**A** and **B**) and roots (**C** and **D**) from PHB-producing F1 hybrids between RedB-2G (*phbB*$^+$) and S8-1-2C (*phbC*$^+$) (A and C) and from transgenic plants RedB-2G (*phbB*$^+$) that did not produce PHB. (B and D) were fixed with glutaraldehyde, stained with Nile Blue A, and viewed by epifluorescence microscopy (Axiophot, Zeiss) under an excitation wavelength of 546 nm. Bars represent 50 μm. Plants were grown aseptically on Murashige and Skoog basal media (Sigma) that contained 1% sucrose and 0.8% agar. Roots and leaves of 2-week-old plants were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 8.0) for 3 hours. Tissues were rinsed in water and stained for 5 min in 1% Nile Blue A. Tissues were rinsed several times in water and soaked 1 min in 8% acetic acid followed by a final rinse in water.

which the cell wall had not been disrupted by homogenization. However, if the chloroform-extracted tissue was then homogenized and reextracted under identical conditions, ethylhydroxybutyrate was detected (Fig. 1). Because the cell wall is permeable to molecules with a molecular mass below ~60,000 daltons (*14*), these results indicate that the ethylhydroxybutyrate was derived from a large molecular size precursor. Thus, we conclude that transgenic plants that expressed both the bacterial acetoacetyl-CoA reductase and PHB synthase genes accumulated PHB.

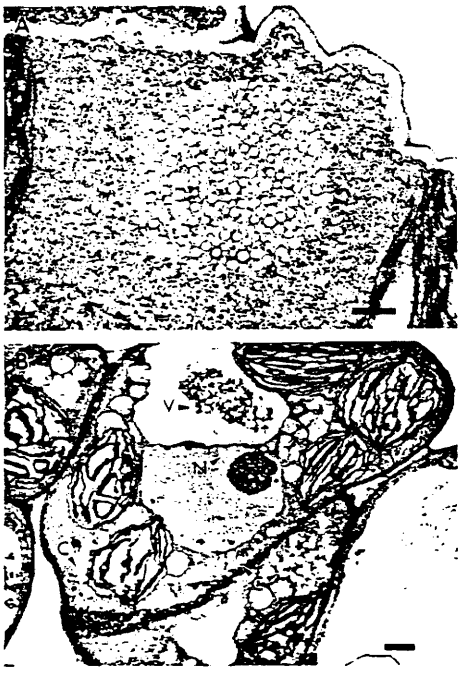

Fig. 4. Transmission electron micrographs of thin sections from PHB-positive transgenic *A. thaliana* plants. Transgenic line S8-1-2A (*phbC*$^+$) was pollinated with transgenic lines RedB-2D and RedB-2C (*phbB*$^+$). Tissue samples from 1- to 3-week-old F1 plants were analyzed by TEM. (**A**) Leaf mesophyll cells from a RedB-2D × S8-1-2A F1 hybrid with an agglomeration of granules in the nucleus. (**B**) Two adjacent mesophyll cells from a cotyledon of a RedB-2C × S8-1-2A F1 hybrid showing electron-lucent granules in the nucleus (N), vacuole (V), and cytoplasm (C). Arrows indicate agglomerations of electron-lucent granules. Bars represent 1 μm. Plant tissues were fixed with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 1.5 to 2 hours at room temperature. The samples were washed four times in 0.1 M phosphate buffer (pH 7.2) and fixed with 1% OsO_4 in phosphate buffer for 2 hours at room temperature. The tissues were then dehydrated in a graded ethanol series and embedded in Spurrs epoxy resin. Sections of 80 to 90 nm were cut, placed on copper grids, and stained with 5% uranyl acetate for 30 to 45 min, followed by staining with Reynolds lead citrate for 3 to 4 min. Sections were viewed in a JEOL 100CX II transmission electron microscope operated at 80 kV.

Similar results were obtained with the F1 progeny of four different crosses involving four independent *phbB* transgenic lines and two independent *phbC* lines. The amount of PHB accumulated in leaves ranged from approximately 20 μg per gram of fresh weight for F1 hybrids between RedD-3A and S8-1-2C to approximately 100 μg per gram of fresh weight for F1 hybrids between RedB-2C and S8-1-2A.

Bacteria accumulate PHB as electron-lucent granules of 0.2 to 0.5 μm in diameter surrounded by a 2-nm-thick layer of electron-dense material (*15*). Bacterial PHB granules stained with Nile Blue A emit orange or red fluorescence at excitation wavelengths of 460 and 546 nm, respectively (*16*). To determine if similar granules could be detected in F1 hybrids shown to be positive for PHB production by GC-MS analysis, we examined plant tissues with epifluorescence microscopy and transmission electron microscopy (TEM). In all tissues of PHB-producing plants stained with Nile Blue A, bright foci of red fluorescence with an approximate maximum diameter of 10 μm were observed (Fig. 3). Similar granular red fluorescence was never observed in untransformed *A. thaliana*, in transgenic plants that expressed only one of the *phbB* or *phbC* transgenes, or in PHB-producing tissues not stained with Nile Blue A. Insoluble material partially purified from tissues of PHB-producing plants was shown to contain PHB granules by GC-MS and epifluorescence microscopy (*10*, *17*).

Cells in the mature leaves, cotyledons, and roots of PHB-producing plants had agglomerations of electron-lucent granules (Fig. 4). These granules were detected in all analyzed F1 hybrids that expressed both *phbB* and *phbC* genes. Similar granules were never detected in the parental transgenic lines that expressed only one of the *phb* genes or in untransformed *A. thaliana*. The granules were detected in the nucleus, vacuole, and cytoplasm of the F1 hybrid tissues. No granules could be detected in the chloroplast. In the nucleus, individual granules were found to reach a maximum size of approximately 0.2 μm. In the vacuoles and cytoplasm, the granules were generally larger and reached a maximum diameter of approximately 0.5 μm. At higher magnification, the granules appeared to be surrounded by electron-dense material. Both the size and appearance of these granules were very similar to granules observed in bacteria accumulating PHB (*15*).

The polypeptide products of the *phbB* and *phbC* genes used here are expected to be located in the cytoplasm of *Arabidopsis* cells, as the genes lack sequences that encode organelle-specific targeting signals. The accumulation of PHB granules in the nucleus and vacuole of transgenic hybrid plants was unexpected. The nuclear lo ization of granules could result from entrapment of existing cytoplasmic gran during reassembly of nuclear membrane mitotic telophase. However, because vacuolar membrane does not break do during any stage of the cell cycle, this re does not explain how the granules accur lated in this organelle. An alternate po bility is that PHB granules may be capa of crossing the membranes of the nuc and vacuole.

Expression of large amounts of a toacetyl-CoA reductase in transgenic pla caused a significant reduction in growth seed production relative to wild-t plants. For example, for the transgenic l RedB-2G and RedB-2C, which expre approximately 5 and 9 units of acetoace CoA reductase activity per milligram protein, respectively, the fresh weigh 22-day-old shoots was reduced to 45% 19% of wild type, respectively. Seed duction was reduced in approximately same proportion. This phenotype coul the result of the diversion of a signific amount of acetyl-CoA or acetoacetyl-C away from an essential biochemical p way such as isoprenoid biosynthesis. pression of the PHB synthase, by itself, no apparent effect on the growth or vig transgenic plants. However, the F1 hyb that contained both genes were more verely stunted in growth than plants contained only the acetoacetyl-CoA rec tase gene, which could result from eith more severe depletion of substrate from mevalonate pathway or a noxious effec the PHB granules.

The present report of synthesis of P in plants represents a first step toward production of novel biopolymers in pl through genetic engineering. Productio a large quantity of PHB or PHA in pl will require additional genetic manip tions to divert reduced carbon away f endogenous metabolic pathways and to ulate the tissue specificity, timing of exp sion, and cellular localization of the zymes involved. It might be possible divert carbon from synthesis of storage ids toward PHB production in plast where accumulation of PHB granules not have deleterious effects.

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17. We produced a population of plants in which approximately half accumulated PHB by sowing F3 seeds derived by the self-fertilization of the progeny of a self-fertilized F1 hybrid between transgenics RedD-3A and S8-1-2A. Leaf tissue (1 g) was homogenized in 50 mM tris-HCl (pH 8.0), 5 mM EDTA, and 1% SDS. The homogenate was cleared of large debris by low-speed centrifugation, and the insoluble material was precipitated by repeated centrifugation at 4000*g* for 15 min. The pellet was resuspended in 200 μl of 10 mM tris-HCl (pH 8.0) and 1 mM EDTA. Nile Blue A was added to a fraction of the extract (0.01% final concentration) and examined under epifluorescence microscopy. A fraction of the extract was also analyzed for the presence of PHB by GC-MS. As controls, tissues from transgenics RedD-3A and S8-1-2A were also analyzed. Red fluorescent particles could be detected only in extracts of PHB-producing tissues. Extracts that contained these particles were shown by GC-MS analysis to contain PHB. Approximately 6 μg of PHB could be recovered by this procedure from 1 g of fresh tissue.
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X-ray Structure of T4 Endonuclease V: An Excision Repair Enzyme Specific for a Pyrimidine Dimer

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The x-ray structure of T4 endonuclease V, an enzyme responsible for the first step of a pyrimidine-dimer–specific excision-repair pathway, was determined at a 1.6-angstrom resolution. The enzyme consists of a single compact domain classified into an all-α structure. This single domain has two distinct catalytic activities: it functions as a pyrimidine dimer glycosylase and as an apurinic-apyrimidinic endonuclease. The amino-terminal segment penetrates between two major helices and prevents their direct contact. The refined structure suggests the residues involved in the substrate binding and the catalysis of the glycosylation reaction.

Ultraviolet (UV) irradiation causes formation of pyrimidine dimers within DNA that are lethal and mutagenic in vivo. The first step of the excision repair pathway of UV-damaged DNA is strand scission of the DNA in the vicinity of a pyrimidine dimer (*1*). The enzyme T4 endonuclease V, encoded by the *denV* gene of bacteriophage T4, is responsible for this step in bacteriophage-infected *Escherichia coli* (*2*, *3*). Although the enzyme is a rather small protein (138 amino acids), it has two distinct catalytic activities (*4*–*12*): it acts as a pyrimidine dimer glycosylase and as an apurinic-apyrimidinic endonuclease (Fig. 1). This latter reaction proceeds through the β-elimination of the 3′-phosphate of an abasic site rather than by the actual hydrolysis of the phosphodiester bond (*13*–*15*). Before binding to a pyrimidine dimer, the enzyme nonspecifically binds by electrostatic forces and scans the double-stranded DNA (*6*, *11*, *12*, *16*). Once the enzyme has specifically bound to a pyrimidine dimer, the DNA is incised at the 5′-glycosyl bond in the dimer, and, subsequently, scission of the phosphodiester bond occurs at the exposed backbone.

We report the three-dimensional (3-D) x-ray structure of the enzyme and discuss its functional implications. Combined with results from site-directed mutagenesis, the examination of the structural features allows the identification of residues participating in the substrate binding and the catalytic reaction.

Crystals of T4 endonuclease V (*17*) belonging to the space group $P2_1$, with unit cell parameters of $a = 41.4$ Å, $b = 40.1$ Å, $c = 37.5$ Å, and $\beta = 90.01°$, contain one molecule per asymmetric unit and diffract x-rays beyond 1.6 Å resolution.

An initial electron density map was calculated at 2.5 Å resolution with multiple isomorphous replacement (MIR) phases, which were obtained with five heavy-atom derivatives (Table 1). Consistent with the high value of the figure of merit, the electron densities were sufficiently well defined to allow the discernment of most residues even in a minimap, and thus an unambiguous chain tracing could be achieved. The $2F_o - F_c$ map after refinement with the restrained least-square program PROLSQ (Fig. 2) gave a final R value of 0.196 (*18*).

The enzyme T4 endonuclease V is composed of a single compact domain. The molecule has a roughly ellipsoidal shape with dimensions 50 by 42 by 40 Å. The enzyme consists of three α helices, five reverse turns, and extended chain segments and loops, but it contains no β structure (*19*) (Fig. 3). The enzyme should thus be classified into the all-α type of structure (*20*); 45% of its residues are located in α helices (Fig. 4). The first α helix (H1, residues 14 through 38) is centrally kinked at Pro^{25}, creating an inclination of 20°. All of the five reverse turns lie on the external surface of the molecule and in close proximity to NH_2- or COOH-terminal ends of the α helices, except for one reverse turn (Q98 to F100) (*21*).

The arrangement of α helices in this enzyme is unusual. The three helices, H1 (14 through 38), H2 (64 through 82), and H3 (108 through 124), stand side-by-side (Fig. 3), and their termini are covered by a caplike loop around the COOH-terminus.

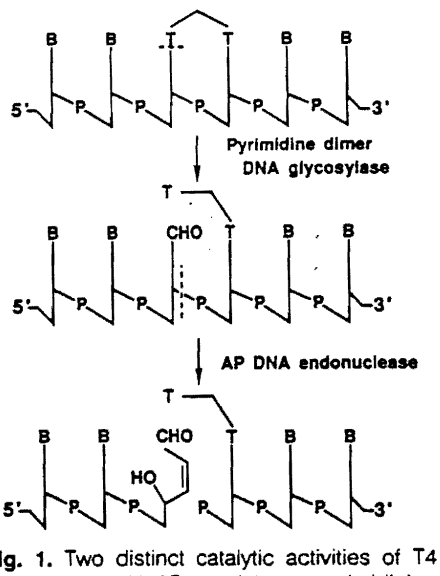

Fig. 1. Two distinct catalytic activities of T4 endonuclease V. AP, apurinic or apyrimidinic.

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Overview

Coating-Removal Techniques: Advantages and Disadvantages

Wayne E. Reitz

The removal of radioactive and nonradioactive coatings from various surfaces is a subject of increasing interest for a variety of reasons, including remaining life assessment, nondestructive evaluation of structural integrity, and life extension through the adoption of new surface-modification methods. This review summarizes the state of the art in coating-removal technologies, presenting their advantages and limitations. The methods covered include laser ablation, microwaves, flashlamps, ice, CO_2, and plastic blast media.

INTRODUCTION

Surface enhancement through the application of a coating is performed for either protection in a specific environment (e.g., wear, corrosion, or erosion resistance) or improvement of specific properties (e.g., electrical, magnetic, or optical characteristics). At some point, however, it may become necessary to remove such coatings. For example, depending on its composition, a coating could present environmental difficulties when the time comes to dispose of the material. In other cases, it may be necessary to remove a coating to inspect a surface or achieve a certain level of cleaning (e.g., removal of radionuclides to facilitate decontamination). Subsequent to coating removal, substrate quality is measured by surface condition, bulk integrity, applicability, and corrosion of the substrate.

For these reasons, there has been an increasing amount of work devoted to developing various techniques for removing surface coatings.

ISSUES IN COATING REMOVAL

The U.S. Navy spends more than $30 million annually to dispose of nearly 40 million liters of liquid waste generated from aircraft paint-stripping operations.[1] The U.S. Department of Energy (DOE) has budgeted $5 billion over the next 30 years to decontaminate and decommission (D&D) numerous government-owned nuclear installations that contain radioactive hot cells, uranium separation facilities, and gaseous diffusion plants (just a few of the critical manufacturing elements[2] and radioactively contaminated concrete structures).

Table I. Radioactively Contaminated Volume at Japan Power Demonstration Reactor

Area (%)	Depth (cm)
54	0–1
29	1–2
15	2–3
2	>3

Chemical solutions are routinely employed to remove paint from surfaces, and mechanical scabbling (e.g., mini-jackhammers) has been used to remove contaminated surfaces from concrete. However, these low-technology treatments do not effectively minimize waste volumes. The key issues for material disposal are volume reduction and separation by waste type. Generally, contamination is confined to surface layers (~2 cm or less),[3,4] and economical removal techniques are needed.

Contamination of concrete surfaces due to spills and leaks were surveyed as part of the dismantlement procedure plan for the Japan Power Demonstration Reactor.[4] Contamination was found in the reactor containment, liquid-waste-treatment, turbine, and spent-fuel-storage buildings. As shown in Table I, 83 percent of contamination was within 2 cm of the surface. Radionuclides penetrated more than 2 cm when scratches or cracks were present. The deepest penetration was 11 cm.

Paint is another "hot" item when it contains lead. Previously, industrial and marine structures were painted with red-lead primer,[5] which, at that time, was the most cost-effective method of corrosion protection. When repainting, however, surface preparation using the traditional methods of abrasive blasting is no longer acceptable due to legislation covering waste disposal and lead contamination of the atmosphere during blasting.

Just as red-lead primer is outdated, paint-removal technologies have not kept pace with the rapid advances of new polymeric resins in the coating industry. When alkyd primers and alkyd enamel topcoats or acrylic nitrocellulose topcoats are used as coating materials, their removal is easily accomplished with solvent-based strippers, predominately methylene chloride. However, as coatings have evolved from alkyds and nitrocellulose to epoxies, polyurethanes, and fluoropolymers, traditional solvent-type strippers are no longer effective removers. The alkyds and acrylic nitrocellulose are prone to rapid erosion, which makes them functional for only one to two years; in some applications (e.g., aircraft), they are aggressively attacked by fluids.[6] Current epoxy and polyurethane coatings, however, can last five to seven years because of their excellent environmental, erosion, and fluid resistance. At the same time, these coatings have become progressively resistant to chemical strippers.

Routine removal of primer and topcoats are required prior to inspection and repainting. A number of technologies exists for the paint-removal aspect of this process, and their relative benefits are fairly well understood for standard metal surfaces.[7] In recent years, however, there has been a growing trend towards utilizing advanced composites in aircraft,[8] and this small group of parts will soon expand to include a larger percentage of the total aircraft structure.[7] The effect of paint removal on composite surfaces has not been studied to the extent that metal surfaces have been characterized. Still, composites are nonetheless susceptible to damage by both chemical and mechanical techniques.[9] As such, coating removal must be accomplished without introducing structural damage and in a manner that is economical, efficient, and environmentally acceptable.

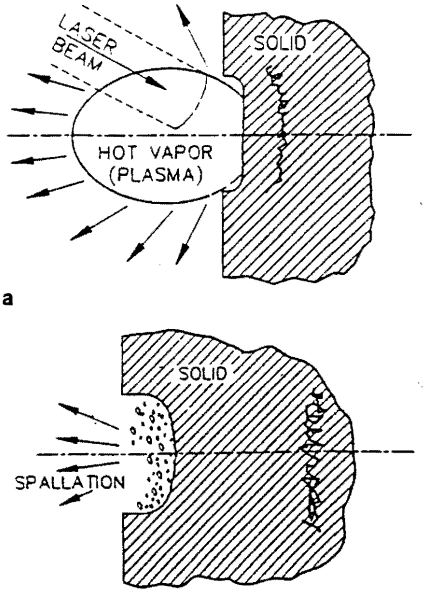

Figure 1. The (a) initial and (b) subsequent interaction of laser pulse radiation with a solid surface.[12]

Wastes

The waste streams that result from coating removal fall into three categories—airborne, liquid, and solid.

In the physical sense, solid wastes are probably the easiest to deal with since they are readily accountable and visible. Generally, solid wastes have not been reduced in volume, except to have been separated from the gross structure. Liquid wastes, however, are one of the more insidious forms of by-products from D&D and paint-stripping operations. Actually, it is the liquid in combination with gravity that aggravates the surface-removal process. Liquids seek and find all available cracks, holes, and cavities, thus making large quantities inaccessible. If the liquid is confined to piping, its presence is more amenable, but it is still in a form to be addressed with caution. Liquid wastes require further processing to refine and consolidate the fractions that are radioactive or hazardous. On the other hand, airborne wastes create a severe health hazard unless special precautions are taken. Airborne wastes permeate the available atmosphere and settle on all available surfaces, vertical as well as horizonal. Extra care is required when generating airborne wastes to minimize further contamination or accidental releases to previously clean areas.

Although it is relatively easy to identify various surface-removal technologies, removal rates, and operational costs, one of the subtler aspects of coatings removal is recognition of the existence, type, and quantity of the secondary waste generated by the surface-removal process. The ultimate goals of surface and coating removal are minimizing waste, minimizing the creation of secondary waste, and minimizing the existence and quantity of mixed waste. The "3 Rs"—reducing, reusing, and recycling—are emphasized in industry.[9]

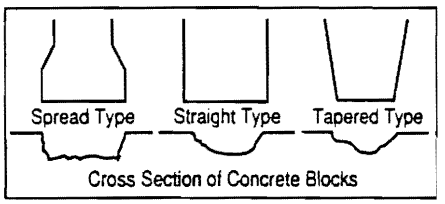

Figure 2. Shapes of microwave irradiating heads.[4]

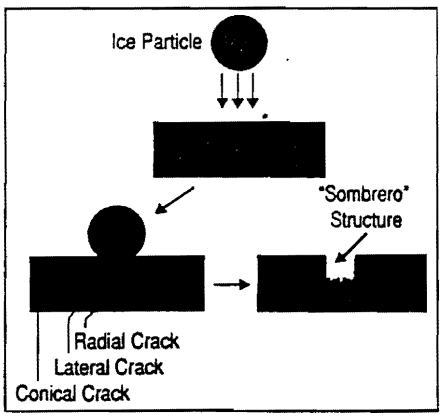

Figure 3. Surface erosion via ice blasting.[34]

Technique Characteristics

Surface-removal techniques can be classified by three important characteristics: strip rates, waste production, and interaction with substrates.

Strip rates are measured in two ways: area removal of nominal thickness (e.g., paint) and volumetric removal (e.g., radioactively contaminated surfaces).

Waste products range from the extremely toxic to the relatively benign. For example, chemical strippers produce a by-product laden with hazardous chemicals (methylene chloride is a known carcinogen).[10] The best removal techniques would convert the waste products into nontoxic forms (e.g., innocuous wastes such as water and CO_2) or disperse them as omnipresent basic elements such as nitrogen and carbon.

It is also critical to understand the effect that coating-removal techniques exert on the substrates.[11] The various types of substrate materials include composites, concrete, and metals. Concrete is of the least concern, since it usually involves radioactively contaminated areas requiring volumetric removal prior to dismantling. However, painted metal and composite surfaces are routinely encountered. Lead paint exists on metal and composite (wood) surfaces that are destined for D&D work. Removal of lead paint from structures is required prior to demolition or refurbishment of various structures (e.g., bridges, buildings, metal structures, army bases, and other facilities). The aircraft industry has stringent requirements on substrate quality, especially subsequent to coating removal. Some of the criteria include surface roughness, masking of existing cracks, and degradation of substrate physical properties, such as fiber breakage and increased residual stress.

COATING-REMOVAL METHODS

Lasers

Laser ablation is a viable surface-removal technique. The laser energy interacts with subatomic particles, transferring photon energy to vibrational energy, which generates heat. The process removes surface materials by thermal shock, melting, evaporation, or vaporization.[12-15] Figure 1[13] shows the material-laser interaction when the material is ablated. The vaporized material begins to absorb the laser-beam energy and becomes ionized, shielding the substrate. As the pulse ends, the surface vaporization halts, and a microscopic expansion occurs on the surface, causing a thin surface layer to spall.

Situations requiring surface removal have routinely employed lasers. Specific applications include cleaning debris from electronic components[16,17] and museum artifacts,[13,18] removing paint from aircraft without damaging the substrate,[19-21] and removing radioactive species from aluminum ductwork.[22] Since the ablation process destroys organics, it is possible that the generated wastes will not have any hazardous constituents, thus making this technique superior to conventional cleaning technologies.

During the development of the laser, it has been shown that the beam does not interact with the atmosphere. This characteristic—combined with narrow, low-power-loss beam characteristics—allows the work piece to be positioned at a significant distance from the laser source. However, this requires a line-of-sight between the laser beam and the work piece, which is becoming restrictive for special applications, such as D&D work. In the past, fiber optics have been employed with the shorter wavelength lasers, such as the excimer and Nd:YAG types, which tend to be lower power than CO_2 lasers. CO_2 lasers less than 3 kW have been successfully transmitted by hollow metallic waveguides. Large diameter tubes and gentle bends lead to minimal attenuation (i.e., maximum transmission).[23-25] This aspect promotes remote decontamination and cleaning and helps reduce maintenance problems. Typical lasers employed include the KrF excimer, 35 mJ/cm², 10 Hertz, 248 nm;[17] the CO_2, 10 J/cm², 100 ns pulse;[16] and the 5 kW continuous wave CO_2.[26]

Microwaves

Concrete structures are able to absorb microwave energy efficiently. Microwave energy is absorbed within a few centimeters of the surface and heating occurs via internal friction produced inside a dielectric material when its molecules vibrate in response to an oscillating microwave field.[1] The dielectric constant is the ratio of electric flux density produced by an electric field in a me-

Table III. Surface Roughness on Al 2024-T3

Pressure (MPa)	Pass	Roughness (μm)
165	1	0.58
	2	0.91
	3	0.69
	4	3.18
198	1	0.93
	2	2.44
	3	6.13
	4	6.33

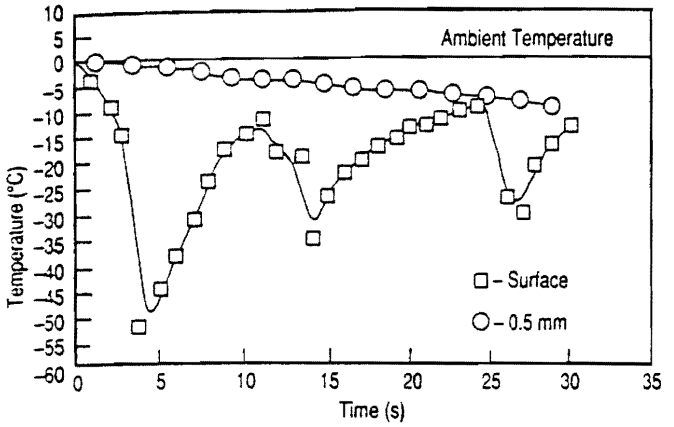

Figure 4. Temperature changes at the surface and at 0.5 mm deep for bare plate during CO_2 blasting.[37]

dium similar to that produced in a vacuum by the same field.[27,28] The microwave energy is directed at the concrete surface using a specialized waveguide applicator, producing heat in the concrete and the free water present in the concrete matrix. The removal depth is proportional to the moisture content.[4] Even though the presence of steel reinforcements has a minor effect on the depth of penetration,[3] metal surfaces have a profound negative effect on energy absorption.

Penetration into concrete is a function of the shape of the waveguide tube head. The three types of heads are shown in Figure 2—spread, straight, and tapered.[4] Microwave heads are connected to the end of the waveguide tube, and the shape of the head affects the beam-surface interaction. Heads that spread the beam enhance the removal rate by providing a uniform depth of removal. The straight and tapered heads result in removal profiles that are concentrated in the center of the head. The peripheries show minimal volume removal. Spread-type heads produce uniform removal and require a minimal number of overlapping passes. As the dimensions of the heated area increase, the depth of penetration decreases. Therefore, the head design and the area of application can be optimized to remove the required volume of contaminated concrete.[3] Microwave equipment is capable of removing as much as 3 cm of concrete in a single pass; a maximum removal rate of 11 cm^3/sec has been achieved[4] on concrete. The process generates little dust, avoids mechanical impacts, and is dry.

Flashlamps

Stripping begins when a high-energy pulse, about 0.001 seconds in duration, is delivered to the xenon bulb from the power supply. This pulse ionizes the xenon gas into a hot radiating plasma at several thousand degrees. The intense light emitted from the plasma is then focused on to the surface, producing a high-energy-density beam, typically 10 J/cm^2.[20,29] Surface temperatures rapidly rise as this energy is absorbed into the surface. If the surface temperature is increased sufficiently, a thin layer is pyrolyzed or ablated.

Pyrolysis is a heat-induced breakdown of the material surface into lower molecular weight components. The resultant brittle surface still must be fractured away from the substrate in solid form, whereas the higher-temperature process of ablation entails explosive combustion of the surface into hot gases and soot. The flashlamp-induced ablation process typically removes a thin layer with each light pulse, less than 25 μm.

Application of this technique has been studied on aluminum skin (0.8 mm thick). The back surface of the aluminum skin was monitored with thermocouples to characterize the heat transfer through the thickness of the skin. After a single pass, the backside temperature increased by 15°C; multiple passes resulted in a temperature increase of 89°C.[29,30]

Ice Blasting

This technology was originally developed to remove coatings from the interior of submarines as it was safe in a confined environment with minimal air flow.[31] Ice blasting is not an abrasive technique—the pellets melt upon impact and the removal mechanism is fracture.[7,32] Impact thresholds exist for various blasting conditions. The blast stream is usually normal to the surface as opposed to a 30–40° impact angle for abrasive removal techniques.[33,34] When impact occurs at sufficiently high energies, three types of cracks are produced (Figure 3):[34] conical, radial, and lateral. Large ice particles initiate the cracks, while smaller ice particles are responsible for crack propagation. A direct consequence of this particle interaction with the coating is that an optimal size distribution exists for a particular coating-substrate combination. Debonding readily occurs when adhesive strength is less than 1.47 MPa, such as rust and paint.[35,36]

Cryogenic and CO_2 Blasting

Shearing is the principle mode of operation for this technology. Thermal shock occurs when the surface is blasted with CO_2 pellets, making it much colder than the substrate and resulting in a stress differential.[7,33,37] If the resulting stress is greater than the tensile strength of the surface material, cracks will develop. Surface tension causes the cracks to curl upward at the edges, which is known as fracking.[7]

Liquid CO_2 (–20°C, 2.1 MPa [gauge]) expands through a nozzle to 0 MPa, causing the temperature of the CO_2 to drop to –80°C.[32] The process is a nonimpact, heat-transfer phenomenon. The CO_2 sublimes prior to impact, causing the cold atmosphere to chill the surface. The resulting temperature drop causes chemical and physical bonds to relax and, thus, spall. This thermal shock is confined to thin surface layers (<0.5 mm),[38,39] as shown in Figure 4. This process was patented by Lockheed in 1977[40] and 1983.[41] The latter patent addresses improvements to particle velocity, particle shape uniformity and breakage, insufficient particle feed, and particle freezing (clogging) in nozzles.

Paint stripping of an F-15 aircraft yields about 120 kg of waste since the blast media sublimes.[39] Supercooling the transport air to –85°C and using small-diameter pellets (1 mm) resulted in successful paint removal from 0.8 mm thick aluminum aircraft skin.[42,43] Table II shows the time required to strip the paint from an F-15 aircraft using several methods.

Pressurized Water

The pressurized-water process employs high-pressure, low-volumetric flow rates to remove coatings;[44] it is similar to rain erosion, except removal by pressurized water can be controlled. The process easily removes laminar planes of coating so that the coating is removed in chips rather than microscopic debris.[45] Typically, pressures of 165 MPa and volumetric flow rates of less than 0.3 l/s are used.[32,46] By controlling the water pressure, delivery, and flow rate, the stripping efficiency can be increased and the resulting surface roughness can be minimized. This is accomplished by delivering the water at the threshold energy level sufficient to remove the coatings without roughening the sub-

Table IV. Physical Properties of Selected Blast Media[52]

Property	Plastic	Walnut	Glass
Density (g/cm^2)	1.5	1.3	2.5
Hardness (Mohs)	4	3	5.5
Impact Strength (Scale of 1 to 10)	7+	4	2
Water Absorption (24 hrs, 25°C)	0.25%	100%	100%
Explosibility Index	<0.2	10	0
Ignition Temp. (°C)	>530	430	NA
Chemical Nature	Inert	Degrade	Inert

NA—not applicable.

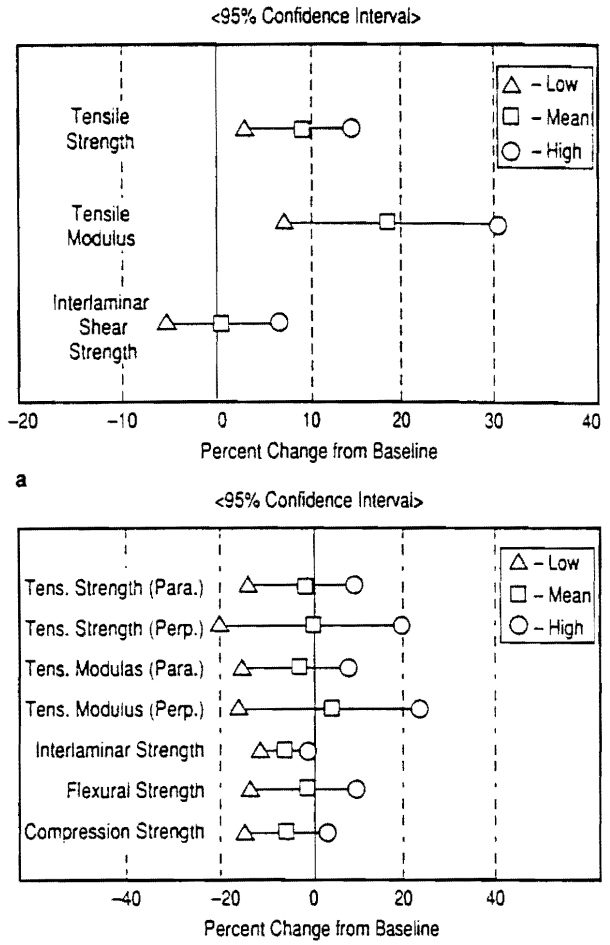

Figure 5. The stripping of (a) quasi-isotropic graphite epoxy and (b) unidirectional graphite epoxy via plastic blast media.[36]

strate.[46,47] Table III shows the effect of pressure and number of passes on surface roughness.

Plastic Blast Media

Plastic blast media, known as dry stripping,[48] is an environmentally responsible coating-removal technique. It is similar to sand blasting but uses specially designed equipment to propel and recover nontoxic, reusable plastic granules. The lightweight sharp-edged plastic particles have proven to be an effective abrasive at pressures ranging from 0.07 MPa to 0.28 MPa.[49] The low operating pressure, combined with the relative softness of the media, permits rapid removal of paint and other coatings from many types of substrates without damaging surfaces or warping panels. The granular surfaces incorporate sharp, angular edges that contribute to the precise coating removal qualities.[49]

The U.S. Air Force[50] has studied the effect of plastic blast media on graphite-epoxy panels and have found that no degradation of the composite panels occurs. For isotropic panels, the tensile strength and modulus actually increased. Figures 5a and 5b show the 95 percent confidence intervals for tensile strengths, tensile modulus, and interlaminar shear values.

Table IV lists several attributes for typical plastic blast media as compared to walnut shells and glass beads. Soft media may transfer less kinetic energy upon impact, but correspondingly longer dwell times are required. Therefore, it does not necessarily cause less damage than the harder media.[31,51]

Powder coatings, electrocoatings, and chemically resistant coatings have been stripped from a variety of substrates, including glass,[45,48] rubber,[48,49] chrome surfaces,[48,49] graphite/epoxy,[50] graphite,[51,52] fiberglass,[52] and kevlar.[52] Plastic blast media are typically 1.2–1.8 mm mesh to 425–600 µm mesh, produce less dust than walnut shells, and are capable of removing paint at a rate of 5.4 cm^2/s from aluminum.[53]

Chemical Strippers

The use of chemical paint strippers to remove organic coatings from substrates is a well-known procedure in metal finishing. Paint strippers are used to strip coatings from rejects and to clean components and products. Chemical stripping is divided into two categories:[54,55] hot and cold. Cold stripping is relatively easy to operate and maintain; the parts enter a solution and are stripped. Hot stripping removes the coating from the substrate via a caustic chemical reaction. Chelates are usually present in hot stripping systems; chelates can remove metal ions from sludges and deposits and can separate the waste constituents.[56,57]

Chemical strippers are applied to the surface and allowed to soak for a short time to break the chemical bond between the coating and substrate.[54,55,58] It is then removed by rinsing. Chemical strippers currently used in production contain methylene chloride and/or phenols, which attack the resin binders and gel coats that are in composites[53] and cause irreparable destruction of corrosion-prevention putties, elastics, and other synthetic materials.[59]

All chemical methylene chloride substitutes have their downside. They are flammable or volatile or not as efficient as methylene chloride.[31,33,58] The new strippers do contain organic solvents with low toxicity and low vapor pressure; they are also capable of removing polyurethane and epoxy primers.[60] Although these strippers perform as well as methylene chloride, they are slower.

CONCLUSION

When considering coating adhesiveness, wastes generated, and final substrate quality, an optimum surface-removal technique exists for each application. In removing a coating from concrete, for example, microwave and laser techniques are generally most attractive. For metals, lasers, flashlamps, cryogenics, ice blastings, plastic blast media, and chemical strippers are commonly used. For composites, lasers, ice blasting, and plastic blast media are particularly applicable. Table V provides a qualitative summary of the removal techniques.

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Degradable Polymers

Monsanto Takes a Chance on Zeneca's Biopol Technology

MONSANTO SAYS IT HAS BOUGHT Zeneca's Biopol biodegradable plastics business unit. The companies say the agreement covers the Biopol technology and marketing operations but does not include Zeneca's small-scale fermentation unit at Billingham, U.K. Specific financial terms of the deal were not disclosed.

Monsanto says it will continue manufacturing the polyhydroxyalkanoic acid (PHA)-based polymers using the bacterial fermentation process. But the company also says it is developing a plant-based production process, using soybeans or canola plants, that could eventually provide a lower-cost route to PHA and that could be ready for commercialization after 2000. Monsanto points to attractive markets for the biopolymers, including food packaging, disposable plates, garbage bags, fibers, and bottles for personal care products.

The deal reflects the continuing reshuffling of players in the biodegradable polymers business, as companies adjust to the sluggish development of markets for the plastics. In addition to Zeneca's exit, in January BASF announced it was curtailing development of the plastics (*CW, Jan. 31, p. 24*).

Meanwhile, Bayer reports developing a polyester amide biodegradable polymer. In Japan, Showa Highpolymer and Showa Denko recently formed an agreement with Cargill to market degradable plastics.

Monsanto says it is bullish on the markets for biodegradable plastics. "More companies are getting in than are getting out. There's a lot of interest by a lot of companies," says Mark Paster, manager/process technology. "We view [degradable plastics] as a significant piece of the solution to the plastics waste problem for certain applications." The European market, he says, is most advanced, with Japan second, and the U.S. a "distant third."

CLEAR LEADER. Paster says the PHA-based polymers have significant advantages over other degradable plastics. The PHA polymers are "the clear leader in inherent biodegradability," says Paster. PHA plastics can potentially achieve performance characteristics comparable to conventional thermoplastics, he adds.

While sales of Biopol have been hurt by the relatively high price of the polymers, Paster says costs will drop as volumes increase. "One of the reasons for the high costs is the scale of operations. As volumes and scale increase, prices will come down for products made using the fermentation process. It will come down to even lower levels using the plant-based route."

A spokesperson for Zeneca says the company sold the business because Biopol—once one of the company's star R&D projects—was not "being given significant focus. We believe [Biopol] is better placed with a company where polymers from renewable resources is a core business."

—DAVID ROTMAN

Additives

Ethyl Settles Suit for $4.75 Million

THE U.S. JUSTICE DEPARTMENT SAYS ETHYL has agreed to pay $4.75 million to the U.S. government to settle claims that the company sold petroleum additives to it that failed to meet defense specifications for use in military vehicles.

"This settlement should send a strong message that the government is serious about its testing requirements," says Frank Hunger, the Justice Department's assistant attorney general for the civil division. He says the agreement settles a 1994 lawsuit filed in U.S. District Court in St. Louis.

According to the Justice Department, from 1986 through 1991 the government bought additives that Ethyl had "falsely certified" met specifications. The Justice Department says Ethyl submitted false documents and information to the Lubricants Research Institute to qualify the products for military use.

A spokesperson for Ethyl says the agreement with the Justice Department is "old news." The company accounted for the anticipated settlement in its third-quarter 1995 financial statement. At the time, Ethyl said it believed the penalty was excessive and that it voluntarily disclosed the situation to the government in 1991. —DR

Biodegradable plastics from plants

Polyhydroxyalkanoates are naturally occurring materials produced in some bacteria. With the use of metabolic engineering, these thermoplastic polyesters can be expressed in Escherichia coli *and in sunflower crops.*

Simon F. Williams
Oliver P. Peoples

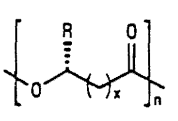
Agricultural biotechnology seems to be finally bearing fruit after all the investments in plant gene technology made during the past 15 years. Most of these efforts have focused on agronomic traits such as herbicide and pesticide resistance, which tend to be single-gene traits. However, with increased understanding of plant modification technology, many major chemical companies are becoming increasingly interested in developing technologies for the production of chemicals from crops. Organic acids, amino acids, oleochemicals, and biopolymers are chemicals amenable to crop production using approaches similar to those currently used to modify bacterial fermentation strains. For example, significant steps have already been taken to modify the amino acid content of soybeans to improve their nutritional content (*1*). The principal technology applied in these efforts—metabolic engineering—usually involves the application of recombinant gene technology to modify and direct the biochemical pathways toward the target products.

During the past 10 years the class of biopolymers that has experienced the greatest increase in research and development efforts globally is the polyhydroxyalkanoates (PHAs). PHAs are linear homochiral thermoplastic polyesters produced as intracellular energy reserves—in effect, fat deposits—by numerous microorganisms. These biopolymers accumulate as distinct granular inclusions in response to nutrient limitation. The microorganisms can also enzymatically degrade these granules when the limitation is removed. Nature has indeed provided us with a source of natural, renewable, biodegradable polyesters. The key to the successful commercial development of PHAs is to learn to assist nature through recombinant metabolic engineering, to reduce the cost of PHA manufacture, and to add to the PHAs' already significant performance characteristics. Recent developments in our understanding of the biology of PHA biosynthetic systems have already removed many of the major technical barriers that previously prevented achievement of these goals.

Natural plastics

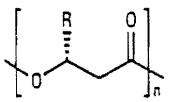
Much of the early work on the PHAs focused on the simple homopolymer polyhydroxybutyrate (PHB), which was discovered around 1925. It first became apparent that PHB was only one member of an entire family of polyesters in the 1970s. Examination of environmental samples from a CPC International sludge facility indicated the presence of PHAs containing 3-hydroxybutyrate and 3-hydroxyvalerate, which had a melting temperature below that of the PHB homopolymer (*2*). Interestingly, these PHAs accumulated during a specific period of high aerobic activity, indicating that they were microbially produced. Since then, the production of the copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV) and, subsequently, polyhydroxyoctanoate (PHO) by controlled fermentation has greatly increased the variety of monomers that can be incorporated into PHAs. The ability to incorporate 3-hydroxy acids with different pendant groups was just the beginning; a second avenue to variation was provided by the incorporation of 4-, 5-, and (most recently) 6-hydroxy acids into the polymer backbone (*3*). At last count, a total of 91 different hydroxyalkanoic acids had been identified as constituents of microbial PHAs (*3*).

It is useful to divide the different PHA types into two broad categories based on their monomer compositions. Those with short pendant groups—PHB, for example—are highly crystalline,



R = H, C_1–C_3
X = 1–3

whereas those with longer pendant groups, such as PHO,



R = C_3–C_{11}

These diverse properties provide tremendous versatility in terms of end-use applications.

PHB has melting points similar to those of polypropylene, better oxygen barrier properties than polyethylene terephthalate and polypropylene, mechanical properties resembling those of polystyrene and polypropylene (*4*), and a water vapor transmission rate (measured as a laminate) about threefold lower than that of polypropylene. In addition to these properties, PHB has adequate fat and odor barrier properties for applications with short-term storage requirements (*5*), better resistance to ultraviolet light than polypropylene, good water resistance, and heat resistance to ~130 °C (*5*). PHB is, of course, inherently biodegradable and is also biocompatible (*6*).

Incorporation of longer pendant group hydroxy acid units into the PHB backbone can improve PHB's flexibility and toughness. For example, in PHBV, improved flexibility is apparent from a fivefold decrease in Young's modulus to 0.7 GPa for a PHBV copolymer containing 25% valerate (*4*). Incorporation of comonomers into the PHB backbone also decreases the melt and glass transition temperatures of the resulting polymer: in some cases it also increases extension to break. For example, a copolymer of 3-hydroxybutyrate with 16% 4-hydroxybutyrate has an extension to break of 444%, compared with 3–10% for the PHB homopolymer (*4, 7*).

The most studied long pendant group PHA is PHO, which contains a large percentage of 3-hydroxyoctanoate and smaller amounts of other 3-hydroxyalkanoates. The melt and glass transition temperatures of a PHO sample are ~55–60 °C and –35 °C, respectively, and molecular weights are 1–2 × 10^5. Because the polymer is only ~30% crystalline and the glass transition temperature is below room temperature, PHO is elastomeric at room temperature; the crystalline regions in the polymer act as physical cross-links (*8*). Although PHOs are structurally very different from other thermoplastic elastomers (TPEs), their stress–strain properties and hardness are in the same range as commercial TPEs. PHOs exhibit a relatively high tensile set (35% after 100% elongation), but this value is well within the range of commercial TPEs (*8*).

Synthesis in the cell

Each PHA is produced by a distinct metabolic pathway. These pathways can be divided into two stages: the biosynthesis of the hydroxyacyl coenzyme A monomers and the actual head-to-tail polymerization of the monomers to form the polymer chains. These chains can exceed 10,000 units in length. The best characterized pathway is that for PHB, in which three enzymes are involved (Figure 1). Thiolase catalyzes a Claisen condensation of two molecules of acetyl coenzyme A (Ac-CoA) in a carbon–carbon bond-forming step to give acetoacetyl coenzyme A, which is then reduced to the chiral intermediate *R*-3-hydroxybutyryl coenzyme A by the reductase and subsequently polymerized by a PHA synthase enzyme. PHA synthase has a substrate specificity that allows it to polymerize C_3–C_5 hydroxy acid monomers including both 4-hydroxy and 5-hydroxy acid units. This biosynthetic pathway, found in bacteria including *Alcaligenes eutrophus* and *Zoogloea ramigera*, is in fact the same pathway used to produce PHBV (also known under the trade name Biopol) from the feedstocks glucose and propionic acid. In this instance, the glucose is metabolized by the bacteria to Ac-CoA, and the propionic acid provides the C_3 unit (propionyl coenzyme A) for the Claisen condensation with Ac-CoA. The PHA synthase enzyme, which acts as a catalyst, is also thought to play a key role in the assembly of the PHA granules inside the cell (*12*).

Biodegradability

One of the properties of PHAs that distinguishes these biopolymers from petrochemical-derived polymers is biodegradability. Produced naturally by soil bacteria, PHAs are degraded on exposure to bacteria or fungi in soil, compost, or marine sediment. Degradation depends on several factors, including the microbial activity of the environment and the surface area of the item. Temperature, pH, molecular weight, and crystallinity are also important factors. Biodegradation starts when microorganisms begin growing on the surface of the plastic and secrete enzymes that break down the polymer into hydroxy acid monomeric units. In aerobic environments, the polymers are degraded to carbon dioxide and water; in anaerobic environments, the degradation products are carbon dioxide and methane (*9*).

Several reports have described PHA composting. In one report, PHBV was compostable over a range of temperatures and moisture levels (*10*). The maximum biodegradation rates were observed at moisture levels of 55% and temperatures of ~60 °C—conditions similar to those used in most large-scale composting plants. Up to 85% of the samples degraded within 7 weeks, and PHA-coated paper was rapidly degraded and incorporated into the compost. In another study, the quality of PHA compost was determined by measuring seedling growth relative to a control. Seedling growth of ~125% of the control was found for a 25% PHBV compost, indicating that the PHA compost can support a relatively high level of growth (*9*).

PHA biodegradation has also been tested in various aquatic environments. In one study in Lake Lugano, Switzerland, items were placed at different depths of water and on the sediment surface (*11*). A life span of 5–10 years was calculated for bottles under these conditions (assuming no increase in surface area); however, PHA films were completely degraded in the top 20 cm of sediment within 254 days at temperatures not exceeding 6 °C.

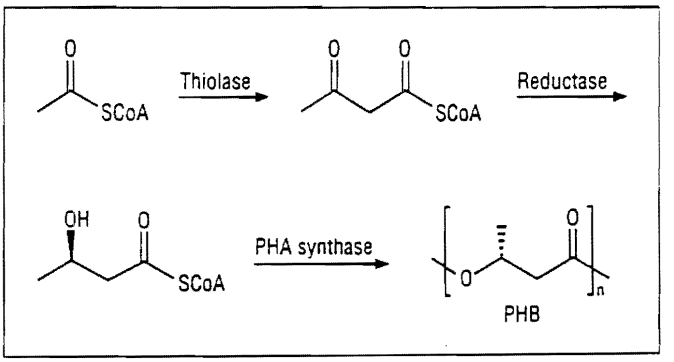


***Figure 1.* The biosynthesis of polyhydroxybutyrate (PHB) involves three enzymes.** Thiolase catalyzes a Claisen condensation of two molecules of acetyl coenzyme A (Ac-CoA). Reductase then reduces the product to the chiral intermediate *R*-3-hydroxybutyryl CoA, and PHA synthase polymerizes the compound and assists in forming the intracellular granules.

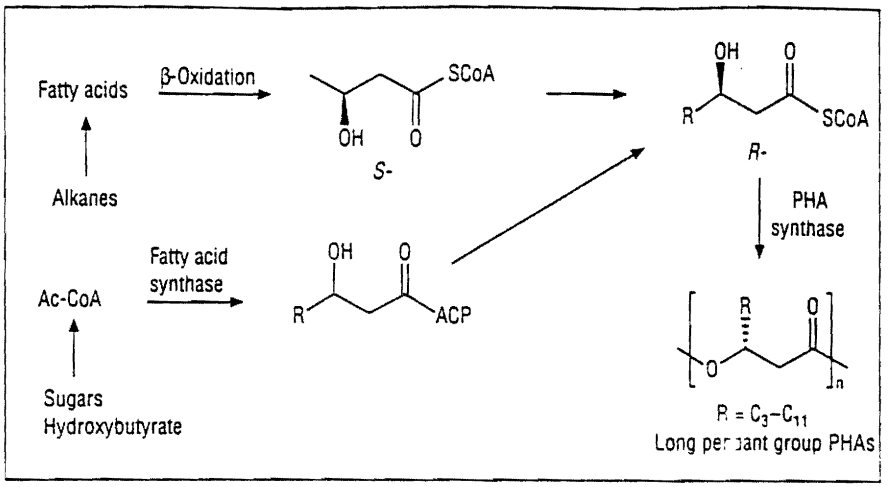


Figure 2. **The biosynthesis of long pendant group polyhydroxyalkanoates (PHAs) is less well understood.** Fermentation and physiology studies indicate that the degradation of fatty acids by both ß-oxidation and fatty acid synthase pathways is involved in monomer production.

Long pendant group PHAs are produced by *Pseudomonas* bacteria, a class of bacteria that can metabolize many organic substrates. The biosynthesis is less well understood with respect to the synthesis of the hydroxyacyl coenzyme A monomers. Fermentation and physiology studies indicate that the degradation of fatty acids by ß-oxidation and fatty acid synthase pathways are involved in monomer production (Figure 2). These units are then polymerized into PHA granules by PHA synthases with substrate specificities favoring the larger (C_6–C_{14}) monomers (*13*).

The first PHA gene was isolated because of interest in the mechanism of the carbon–carbon bond formation catalyzed by the thiolase enzyme (*14*). Subsequently, the study of PHAs was advanced because of industrial interest in biodegradable plastics in the mid-1980s. Genes and gene products responsible for the production of both categories of PHAs were isolated by several research groups at that time (*14–20*). It was found that PHA synthase, reductase, and thiolase, which produce the short pendant group PHAs in *A. eutrophus*, are coded by an operon made up of the *phb*C-*phb*A-*phb*B genes (Figure 3) (*16–18*). Researchers continue to examine the mechanism of the polymerization reaction (*21, 22*) and the role of this protein in the assembly of the PHA granules (*12, 22*). Particularly notable is the ability of the isolated PHA synthase enzyme to rapidly synthesize ultrahigh molecular weight ($>10^7$) PHB on exposure to purified substrate in a test tube (*22*).

In the *Pseudomonas* bacteria, the PHA synthases responsible for production of the long pendant group PHAs were found to be encoded on the *pha* locus, specifically, by the *pha*A and *pha*C genes, now also referred to as *pha*C1 and *pha*C2, respectively (*23, 24*). Since those initial reports, numerous PHA genes have been identified and characterized from a range of bacteria, thus providing a library of gene cassettes for engineering transgenic PHA production systems.

Transgenic approaches

The key concepts describing the role of recombinant engineering for the production of PHAs were described by Peoples and Sinskey (*14*). The approach was straightforward. Recombinant gene technology allows the transfer of genetic information across the species barrier. By isolating and characterizing the genes and gene products responsible for PHA biosynthesis, it is possible to engineer entirely new PHA production systems. In these systems, basis of their substrate specificity to produce a desired PHA polymer product. Given the variety of PHA monomers, applying combinatorial techniques to PHAs could provide an abundance of PHA polymer types.

The breakthrough, however, was the engineering of a transgenic organism by simply transferring the *A. eutrophus phb* operon into a strain of *Escherichia coli* that could efficiently accumulate PHA granules (*16–20*). These pioneering experiments established and defined the basis for transgenic PHA production in microbial and crop systems (*25*).

Transgenic systems can produce several PHA materials and can be adjusted to fine tune polymer properties to end-user needs. We are currently pursuing two transgenic routes to PHAs that use the same proprietary technology base. One route involves transgenic fermentation; the other uses transgenic crops and is expected to provide PHAs at prices competitive with petrochemical polymers.

Fermentation. Transgenic fermentation will precede crop production of PHAs and will meet target pricing for biodegradable and compostable plastics in European markets. At the same time, this short-term route will allow other end users in more price-sensitive markets to source material and begin product development. The latter is important given the fast rate at which crops can be scaled up. Moreover, because the same proprietary PHA genes are used in transgenic fermentation and crop systems, it is reasonable to assume that good continuity can be achieved between the PHAs derived from these two systems. This is a clear benefit to end users.

Using genetic engineering techniques, Metabolix has been able to transfer suitable gene cassettes required for PHA production into *E. coli* K12. This particular strain is the workhorse of the biotechnology industry and is already the source of many injectable therapeutic proteins, food enzymes, and food additives. The raw materials used for the transgenic fermentation are widely available sugars (e.g., dextrose). Relative to nontransgenic PHA production, there are several benefits of these new transgenic systems. First—and most significant from an economics point of view—is that *E. coli* K12 is a fast-growing organism. *E. coli* production systems can significantly reduce fermentation times. In the best cases, fermentation time can be reduced from 3 days in a nontransgenic PHA producer to 1 day in a comparable transgenic system. Additionally, the ability to select a specific host can be important if food contact is anticipated or if certain physical forms of PHA are desired. Host selection can also

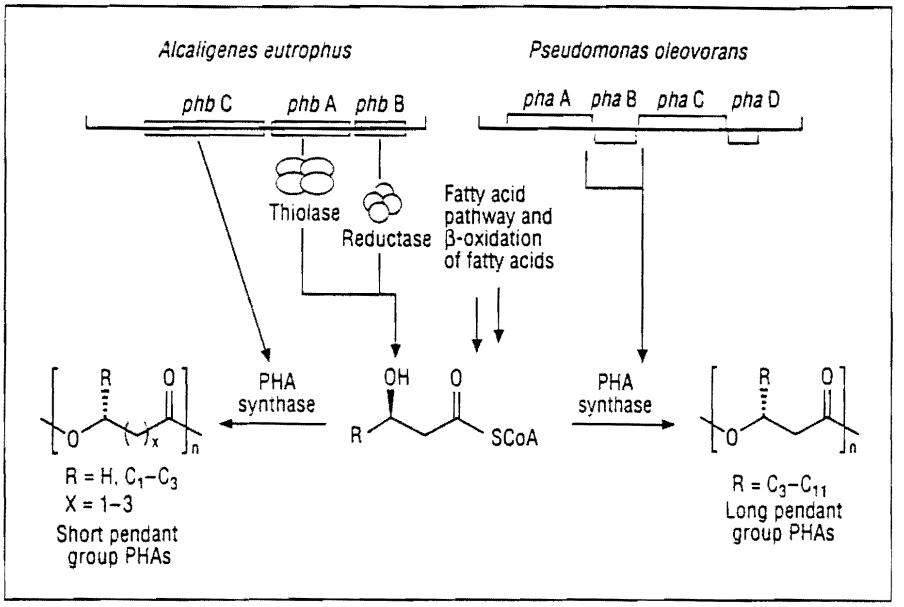


Figure 3. **PHA synthase, reductase, and thiolase—which produce the short pendant group PHAs in *Alcaligenes eutrophus*—are coded by an operon consisting of the *phbC-phbA-phbB* genes.** Similar genes that code for long pendant group PHAs were found in *Pseudomonas oleovorans.*

affect downstream recovery. For example, *A. eutrophus* used in the PHBV production process is difficult to lyse and adds additional cost in PHA recovery. *E. coli*, on the other hand, is relatively easy to lyse. The second advantage of the new transgenic systems is the ability to improve the yield of polymer relative to cellular biomass. Clearly, the greater the proportion of PHA in the cell relative to other biomass, the more cost-effective the downstream recovery. Purities of >99% can be achieved from transgenic PHA production systems in just a few routine steps. A third benefit of the transgenics is the option to obtain PHA material of high molecular weight and, potentially, to control PHA particle size. These properties can be important in PHA processing.

Depending on structure, the final form and grade, and the scale of production, PHA prices for the short pendant group materials are projected to be $1–$2/lb at production levels of 10–100 million lb/year. By commercial standards, these capacities would not be unusual. Some of the processes used for making amino acid feed supplements run at hundreds of millions of pounds per year. Notably, genetic engineering is also being applied to some of these processes to reduce costs. For these amino acids, the cost structure of traditional fermentation is already relatively low; prices of amino acid supplements are $1–$1.50/lb. Nonetheless, small improvements made using genetic engineering can make large differences in the profitability of the amino acid business.

Prices for the elastomeric long pendant group PHA materials are expected to be somewhat higher than for the short pendant group materials, mainly because the feedstocks are more expensive. However, processes using cheaper, alternative feedstocks can be developed, and in time, prices for these elastomeric materials could also come down. Current short-term projections are $4–$10/lb.

Crops. Low-cost PHAs priced competitively for use in global commodity markets, particularly for applications in single-use disposable markets, will come from transgenic crops. This approach, which involves transferring suitable microbial gene cassettes into crops, was first described in a patent application filed by M.I.T. in 1989 (*23*) and then later in a *Science* editorial (*26*). Results obtained by Metabolix in collaboration with others and by other groups

Metabolix Inc.

Metabolix Inc. was founded in 1992 by Oliver Peoples, Anthony Sinskey, and Simon Williams to produce PHAs at an attractive cost and scale using the proprietary transgenic technology, which was pioneered by Peoples and Sinskey at the Massachusetts Institute of Technology (M.I.T.) in the 1980s. Edward Muller (formerly of Halcon International) joined Metabolix as president and CEO, and Edward Giles of The Vertical Group serves on the board of directors. Metabolix holds the exclusive license to the M.I.T. patent portfolio of methods for producing the PHAs in transgenic fermentation systems and transgenic crops. To date, six U.S. patents *(29)* have been issued, and more U.S. and foreign patent applications are pending. Metabolix is continuing to develop its proprietary position on transgenic production of PHAs and is also collaborating with other companies to commercialize the technology.

In addition to focusing on PHA production by transgenic fermentation and crops, Metabolix is supplying PHAs to a select group of companies for applications development in commodity, specialty, and biomedical markets. Materials under development include PHA latexes for coating applications, including PHAs for the manufacture of single-use disposable products such as fast food serviceware, disposable diapers, packaging, and trash bags. Extrusion-grade PHA materials, including semicrystalline and elastomeric types, are also being developed for end-use applications.

Compared with many other new crop products that could be produced by transgenic techniques, the PHAs present a particularly promising opportunity. First, the utility of PHAs has been well proven, primarily by Zeneca (*6*), and many large end users are willing to move production of single-use disposables to biodegradable and renewable materials, as long as they are cost competitive. Second, the size of the target markets for PHAs in single-use disposable applications is in the billions of pounds per year. These volumes are large enough to be attractive to farmers, yet not so large that they affect food production. Third, PHAs are relatively inert materials despite their inherent biodegradability. After PHAs are produced inside a living cell, they appear to be well tolerated as storage

Chemical intermediates

As well as being industrially useful polymers, PHAs are a valuable source of hydroxy acids. PHB, for instance, can be readily depolymerized to 3-hydroxybutyric acid, a highly versatile chemical intermediate *(4)*. 3-Hydroxybutyric acid can be converted to 1,3-butanediol, crotonic acid, β-amino acids, butyl esters, lactones, and other compounds. Although the route is somewhat roundabout, hydroxy acids derived from plant PHAs could ultimately be cheap enough to displace more traditional routes to certain chemical intermediates. One obvious chemical target would be 1,3-butanediol, which currently sells for ~$1.25/lb. World capacity for this diol was ~50 million pounds in 1986 *(35)*. A still larger opportunity exists among butyl derivatives. The western block currently produces several billion pounds of butanols, about one-half of which is used directly or after esterification, as solvents *(35)*.

In the near term, fermentation can be used to produce hydroxy acids but not to compete with the low-cost bulk markets. Instead, these intermediates can be sold as chiral chemicals. PHAs are, after all, chiral polymers comprised of *R*-hydroxy acids. The monomer derived from PHB is already used on a scale of several metric tons as a starting point in the synthesis of Merck's antiglaucoma drug Trusopt *(36)*.

cellular pH or swell in the presence of water. Perhaps equally important is the fact that PHAs are naturally occurring materials with no known toxicity. Fourth, the infrastructure needed to isolate PHAs from the crops at reasonable cost can be added to current processing equipment. Finally, the tools necessary for the commercial development of transgenic PHA crops are currently available. The PHA genes have been cloned, sequenced, and characterized. Assays and diagnostics exist for the enzymes, and numerous techniques are available to analyze the PHA polymer products. These pieces have all been brought together at Metabolix.

Development of commercial transgenic crops still requires a significant development effort, estimated at 4–7 years. Unknown variables that make precise calculations difficult include the crop, crop price, scale, location, level of PHA produced in the transgenic crop, and ease of extraction. By considering each of these variables individually, however, it is possible to determine the sensitivity of each of these factors to the final cost. Given certain reasonable assumptions, the most sensitive factor is the amount of PHA in the crop. Typical crops yield 10–50% oil, and the oils sell for ~25–60¢/lb. We believe it is reasonable to expect to produce similar levels of PHAs. The PHAs would replace some or the majority of the natural plant product. In the 20–50% range, it should be possible to produce PHAs at prices competitive with petrochemical polymers. Clearly, the higher the PHA content, the lower the cost of PHA production. The analogy, of course, between the cost of plant oils (25–60¢/lb) and the estimated prices of crop PHAs serves as a good check on price estimates. It is also important to note that the estimates for crop PHA production do not take into account the value of any byproduct or any qualifying subsidies and credits.

For industrial use, we can imagine the magnitude of the new PHA farming opportunities by estimating the amount of farmland that would be needed to grow enough of a particular crop to yield commodity volumes of PHA. At a 30% PHA content, three million acres of farmland would be needed to produce a billion pounds of PHA. To put these numbers into perspective, a billion pounds of polymer represents ~6% of the U.S. packaging market, and three million acres of, for example, rapeseed/canola represents ~8% of the total worldwide acreage used to grow this crop *(30)*. Of course, Metabolix's technology is not limited to rapeseed/canola. Similar estimates can be made for other oil and starch crops.

Transgenic fermentation and transgenic crop production will coexist. Transgenic fermentation can be used as a near-term production route to test markets and thereby identify specific PHAs suitable for large-scale production in transgenic crops. This route is appropriate for production of smaller volumes for specialty applications and of some of the more functionalized PHAs that cannot be produced in crops. Identification of PHAs for crop production is not a small task. Even though PHBV is well known and at first glance might appear a good target for crop production, close to 100 different types of PHAs have been identified. Clearly, not all of these PHAs will be commercially useful, and the production of PHAs in crops is not amenable to large numbers of different PHA types. Rather, crop production of PHAs is more likely to focus on about four different PHAs selected from crystalline and elastomeric types. If necessary, these materials can then be blended with other PHAs derived from transgenic fermentation. This approach should provide materials suitable for use in most types of conventional polymer processing and end-use applications.

Short-term prospects

In the near term, the largest applications for PHAs are likely to be in the single-use disposable markets. In these types of end uses, plastics normally become heavily contaminated during use, making them difficult to recycle. In the food industry, plastic packaging and fast food serviceware in the waste stream contain significant quantities of food. It has been our experience that used diapers are usually contaminated. Collection of yard waste represents another large potential market. In the latter case, use of compostable bags for waste collection eliminates the bag-emptying step before composting. Such potential uses, which are well matched with PHA properties, raise questions regarding composting infrastructure, food contact approval, and price tolerance.

Composting infrastructure. For biodegradability to be an effective solution for waste management of plastics—particularly for single-use items—there must also be a widely developed composting infrastructure. This type of infrastructure is already highly developed in Germany and the Netherlands, where a significant proportion of waste is sent to composting facilities. Other European countries seem to be following this lead. Belgium, for example, recently approved a logo for compostable materials, and it is estimated that by the end of 1996 ~45% of the population will be connected to composting systems *(31)*. In the United States, legislation banning the disposal of yard waste in landfill sites has helped to establish a composting infrastructure, and this is likely to continue to grow as landfill sites continue to close across the nation.

Food contact approval. Despite the fact that the PHAs are natural materials and that the monomer 3-hydroxybutyric acid is a natural constituent of human blood, PHA polymers must be approved by regulatory agencies if they are to be used in contact with food. Approval for food contact varies by case but involves preparing toxicity data for the entire polymer product, including any additives, and measuring migration rates of any components that

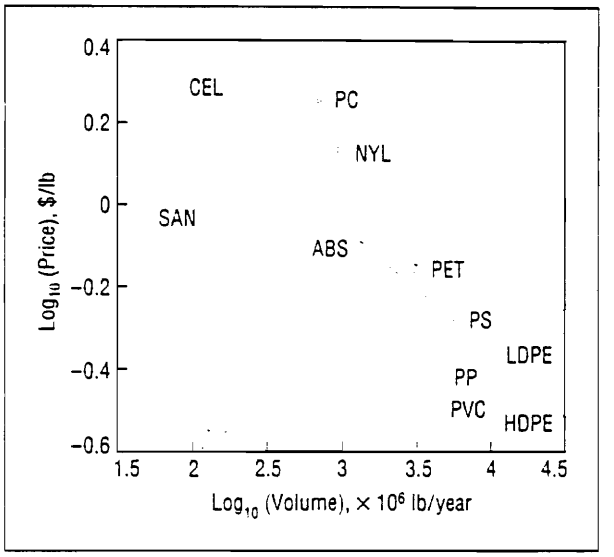
Market forecasts

Various sources have estimated the market sizes for biodegradable plastics, an important entry market for the PHAs. BASF recently published a 1993 estimate for the potential market for biodegradable plastics in western Europe based on the prerequisites of a price ceiling of DM 5/kg ($1.50/lb) for the materials and development of a composting infrastructure. The total market was estimated at 374 million lb/year *(37)*.

Application	Resins, $\times 10^6$ lb/year	
	Conventional	Biodegradable
Waste disposal bags for composting	NA	66
Disposable fast food utensils	220	110
Hygiene films	242	44
Paper coatings	924	88
Agricultural sector	143	66
Total	1529	374

When the costs of PHAs come down enough that the materials are priced competitively with petrochemical polymers, PHA biodegradability will likely remain a key selling feature for these materials—but not a costly or necessary one. One way to estimate the future market size for PHAs under these circumstances is to use price exclusion curves for commodity and engineering thermoplastics. The price exclusion curve is a logarithmic plot of the prices of thermoplastic resins against their total volumes for a given market; this curve is given in the figure below for U.S. thermoplastic consumption. The rationale behind this approach is that the market will select the lowest cost resin suitable for a given application. Sales of a new material such as a PHA can grow initially without the benefit of price reductions, and this growth will result in a horizontal movement across the graph. Once near the curve, however, volume can only continue to grow if the price declines or if a new, unique set of circumstances justifies a premium—such as a requirement for biodegradability.

At a price of $2–$2.50/lb, examination of the curve indicates that a PHA resin meeting the standard requirements described above could have an initial market of 10–100 million lb/year. A larger volume at this price could be attained if a premium is paid for biodegradability or some other unique feature. In the long term, when PHAs are produced by crops, the situation forecast by the exclusion curve is very different. At prices competitive with petrochemical-derived thermoplastics, the potential market sizes for PHAs are 1–10 billion lb/year for the United States alone. Thus, switching from fermentation to crop production can potentially make PHAs a billion-dollar component of the chemical industry.



CEL, cellulose; SAN, styrene/acrylonitrile copolymer; PC, polycarbonate; NYL, nylon; ABS, acrylonitrile/butadiene/styrene copolymer; PET, polyethylene terephthalate; PS, polystyrene; PP, polypropylene; LDPE, low-density polyethylene; PVC, polyvinyl chloride; HDPE, high-density polyethylene.

transfer from the polymer to the food. By carefully selecting processing aids already approved for food contact use, some of this approval process can be eased. Notably, ICI/Zeneca initiated the approval process for PHBV products with the U.S. Food and Drug Administration (*32*).

Price tolerance. Clear environmental advantages exist whenever nondegradable single-use disposable products can be replaced with biodegradable plastics. Despite the advantages, however, it is still questionable how much of a premium users are willing to pay. Many end users would like to simply substitute a biodegradable product for the petrochemical product, but they do not want to pay more than a modest premium for the substitution. In certain parts of the world, however, strict legislation has been or is being introduced that levies a punitive tax on nondegradable materials relative to natural materials. For example, the Duales System Deutschland fees imposed in Germany levy weight-related charges of around 90¢/lb on plastics versus 5.5¢/lb on natural materials. Currently, biodegradable materials get no break in this system, yet the government promotes green waste composting (*33*). Should PHAs qualify favorably in this system or any other equivalent, the short-term gap that exists between the selling price of a PHA resin and a nondegradable plastic could

In the absence of legislation favoring the use of compostable plastics, PHA-laminated products suitable for use in applications such as packaging, trash bags, and fast food serviceware provide good opportunities for commercial development because of the dilution in costs that occurs along the manufacturing chain. For instance, a packager may purchase a PHA laminate at a 50% premium over a polyethylene laminate but pass on only a 25% premium to the customer, because the packager's costs are split about 50/50 between raw material and processing costs. As a result, some converters appear willing to tolerate short-term prices of $2–$3/lb for PHAs.

It is also worth mentioning that in markets such as toiletries and cosmetics, natural materials can command a significant premium. This relates to not only the product but also the packaging. A more expensive packaging resin may add a small amount to the final product cost, yet offers a marketing edge in a well-established industry. In this regard, it is perhaps no coincidence that some of the first PHBV products were shampoo bottles and cosmetics containers.

Long-term opportunities

If the current rate of progress in developing transgenic PHA systems is sustained, during the next decade we will

the large-volume commodity markets. Under these circumstances, PHA polymers produced by transgenic crops will compete directly with petrochemical-derived plastics on price. By nature of the production route, the volumes of PHA material that can be produced could rival or even exceed production levels of the most common and widely used petrochemical plastics. This is not difficult to comprehend given that worldwide production of synthetic plastics is about one-half that of worldwide plant oil output (*34*).

In addition to their use as plastics, the PHAs also represent a potential source of hydroxy acid feedstocks for the chemical industry. In contrast to the introduction of new polymers, the PHA hydroxy acids and related derivatives can be readily integrated into existing chemical markets.

Ultimately, the long-term opportunities for the PHAs are enormous, spanning and benefiting many industries. The key to success depends primarily on the ability to engineer transgenic systems for efficient and selective PHA production.

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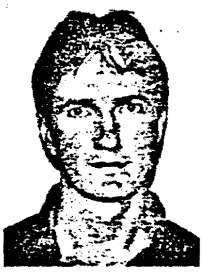

Simon F. Williams is a vice president and director of Metabolix Inc. (303 Third St., Cambridge, MA 02142; 617-492-0505). He cofounded Metabolix in 1992 and is currently involved in the company's corporate development activities. Previously, he held postdoctoral positions at the Departments of Chemistry and Biology at the Massachusetts Institute of Technology (M.I.T.) and a lectureship at Manchester University (U.K.). He holds a Ph.D. in organic chemistry from Cambridge University (U.K.)

Oliver P. Peoples is a vice president and director of Metabolix Inc. He cofounded Metabolix in 1992. Previously, he was a research scientist at M.I.T., where he pioneered the transgenic approach to engineering biopolymers. He holds several patents in this area, including key patents on transgenic methods for PHA production. His interests include metabolic engineering and industrial bioprocessing. He holds a Ph.D. in molecular biology from the University of Aberdeen (U.K.).

REVERSING DIRECTION

Yet the fundamental forces at work cannot be ignored. They are the consequence of a reversal of the historic direction of information flow. In the past people came to the information, which was stored at the university. In the future information will come to the people wherever they are. What then is the role of the university? Will it be more than a collection of ... the science laboratory and the football team? Will the impact of electronics on the university be like that of printing on the medieval cathedral, ending its central role in information transfer? ... Can we self-reform the university or must things get much worse first?

Eli Naom
Science

"I should have fired him years ago, before he became my boss. ..."

11. Appendix 3: MSDS for Paint Ingredients

DuPont Chemicals

6160CR | Revised 8-NOV-1996 | Printed 6-DEC-1996

"TI-PURE" TITANIUM DIOXIDE (R-706 and R-746)

CHEMICAL PRODUCT/COMPANY IDENTIFICATION

Material Identification

"TI-PURE" is a registered trademark of DuPont.

Corporate MSDS Number	DU008083
Formula	TiO2
Grade	See Tradenames and Synonyms (Remarks)

Tradenames and Synonyms

TITANIA
TiO2
RUTILE
TITANIUM DIOXIDE

Tradenames and Synonyms (Remarks)

GRADES COVERED BY THIS MSDS INCLUDE:

R-706 (Dry Pigment) and R-746 (Slurry)

Company Identification

MANUFACTURER/DISTRIBUTOR

DuPont
1007 Market Street
Wilmington, DE 19898

PHONE NUMBERS

Product Information	1-800-441-9485
Transport Emergency	CHEMTREC: 1-800-424-9300
Medical Emergency	1-800-441-3637

COMPOSITION/INFORMATION ON INGREDIENTS

Components

Material	CAS Number	%
TITANIUM DIOXIDE	13463-67-7	>93

(Continued)

FIRST AID MEASURES

First Aid

INHALATION

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SKIN CONTACT

The compound is not likely to be hazardous by skin contact, but cleansing the skin after use is advisable.

EYE CONTACT

In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

INGESTION

No specific intervention is indicated as compound is not likely to be hazardous by ingestion. However, if symptoms occur, consult a physician.

FIRE FIGHTING MEASURES

Flammable Properties

Will not burn.

Extinguishing Media

Any media as appropriate for combustibles in area.

Fire Fighting Instructions

None.

ACCIDENTAL RELEASE MEASURES

Safeguards (Personnel)

NOTE: Review FIRE FIGHTING MEASURES and HANDLING (PERSONNEL) sections before proceeding with clean-up. Use appropriate PERSONAL PROTECTIVE EQUIPMENT during clean-up.

Accidental Release Measures

For dry product, shovel into covered container for disposal. Flush residue to wastewater treatment system.

For slurry product, flush to wastewater treatment plant or settling basin, or soak up with sand or other absorbent and shovel into covered metal container for disposal.

(Continued)

PHYSICAL AND CHEMICAL PROPERTIES

Physical Data

Physical Data (dry product)

Boiling Point	: Not applicable
Vapor Pressure	: Not volatile
Vapor Density	: Not volatile
Melting Point	: Not applicable
Evaporation Rate	: Not volatile
Solubility in Water	: Insoluble
pH	: 6-9 (water extract)
Odor	: None
Form	: Powder, solid
Color	: White
Specific Gravity	: 3.8-4.3

Physical Data (slurry)

Boiling Point	: 100 C (212 F) @ 760 mm Hg
Vapor Pressure	: Same as water (Liquid component is water.)
Vapor Density	: Not applicable
Freezing Point	: 0 C (32 F)
Evaporation Rate	: Not available
Solubility in Water	: Solids are insoluble
pH	: 7.5-9.5
Odor	: Slight amine
Form	: Opaque dispersion, liquid
Color	: White
Specific Gravity	: 2.2

STABILITY AND REACTIVITY

Chemical Stability

Stable.

Incompatibility with Other Materials

None reasonably foreseeable.

Decomposition

Decomposition will not occur.

Polymerization

Polymerization will not occur.

TOXICOLOGICAL INFORMATION

Animal Data

"TI-PURE" Titanium Dioxide

Inhalation 4-hour ALC: >6,820 mg/m3 in rats (~96% TiO2)
Skin LD50 : >10,000 mg/m3 in rabbits

(Continued)

TRANSPORTATION INFORMATION

Shipping Information
Shipping Containers

Tank Cars.
Tank Trucks.

NOT REGULATED AS A HAZARDOUS MATERIAL BY DOT OR IMO.

REGULATORY INFORMATION

U.S. Federal Regulations
TSCA Inventory Status Reported/Included.

TITLE III HAZARD CLASSIFICATIONS SECTIONS 311, 312

Acute	: No
Chronic	: No
Fire	: No
Reactivity	: No
Pressure	: No

LISTS:

SARA Extremely Hazardous Substance	-No
CERCLA Hazardous Material	-No
SARA Toxic Chemical	-No

CANADIAN WHMIS CLASSIFICATION:

Not regulated.

OTHER INFORMATION

NFPA, NPCA-HMIS
NPCA-HMIS Rating

Health	0
Flammability	0
Reactivity	0

Personal Protection rating to be supplied by user depending on use conditions.

Additional Information
Caution: Do not use in medical device applications (including products which are implanted, contact internal tissues or body fluids, etc.) unless specific non-objection has been obtained from DuPont.

These grades are not recommended for use in food contact applications.

For more specific information on composition and properties, see DuPont "Ti-Pure" literature.

(Continued)

TRANSPORTATION INFORMATION

Shipping Information
Shipping Containers

Tank Cars.
Tank Trucks.

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Not regulated.

OTHER INFORMATION

NFPA, NPCA-HMIS
NPCA-HMIS Rating

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Flammability	0
Reactivity	0

Personal Protection rating to be supplied by user depending on use conditions.

Additional Information
Caution: Do not use in medical device applications (including products which are implanted, contact internal tissues or body fluids, etc.) unless specific non-objection has been obtained from DuPont.

These grades are not recommended for use in food contact applications.

For more specific information on composition and properties, see DuPont "Ti-Pure" literature.

(Continued)

UNION CARBIDE CORPORATION
MATERIAL SAFETY DATA SHEET

EFFECTIVE DATE 06/21/96

Union Carbide urges each customer or recipient of this MSDS to study it carefully to become aware of and understand the hazards associated with the product. The reader should consider consulting reference works or individuals who are experts in ventilation, toxicology, and fire prevention, as necessary or appropriate to use and understand the data contained in this MSDS.

To promote safe handling, each customer or recipient should: (1) notify its employees, agents, contractors and others whom it knows or believes will use this material or the information in this MSDS and any other information regarding hazards or safety; (2) furnish this same information to each of its customers for the product; and (3) request its customers to notify their employees, customers, and other users of the product of this information.

I. IDENTIFICATION

PRODUCT NAME:	TRITON GR-5M SURFACTANT
CHEMICAL NAME:	Dioctyl sodium sulfosuccinate
CHEMICAL FAMILY:	Not Applicable (mixture)
FORMULA:	Not Applicable (mixture)
MOLECULAR WEIGHT:	Not Applicable (mixture)
SYNONYMS:	None
CAS # AND NAME:	See Section III, "Ingredients"

II. PHYSICAL DATA (Determined on Typical Material)

BOILING POINT, 760 mm Hg:	87 C (188 F)
SPECIFIC GRAVITY(H2O = 1):	1.028
FREEZING POINT:	-48 C (-54 F)
VAPOR PRESSURE AT 20°C:	30.4 mmHg

EMERGENCY PHONE NUMBERS: 1-800-UCC-HELP (NUMBER AVAILABLE AT ALL TIMES) OR (304) 744-3487

UNION CARBIDE CORPORATION
39 Old Ridgebury Road, Danbury, CT 06817-0001

PRODUCT NAME: TRITON GR-5M SURFACTANT

EVAPORATION RATE (Butyl Acetate = 1): 2.68

VAPOR DENSITY (AIR = 1): 2.19

SOLUBILITY IN WATER by wt: 100%

APPEARANCE: Transparent yellow

ODOR: Pungent

PHYSICAL STATE: Liquid

PERCENT VOLATILES (by weight): 40.0

III. INGREDIENTS

%	MATERIAL	CAS#	EXPOSURE LIMIT
60	Di-2-etnylhexyl sodium sulfosuccinate	577-11-7	None established
20	Isopropanol	67-63-0	See Section V
20	Water	7732-18-5	Not Applicable

IV. FIRE AND EXPLOSION HAZARD DATA

FLASH POINT(test method(s)):

72 F (22 C)
Tag Closed Cup ASTM D 56

96 F (35 C)
Tag Open Cup ASTM D 1310

FLAMMABLE LIMITS IN AIR % by volume: LOWER: 2.0% (LFL of Most Volatile Ingredient)
UPPER: 12.7 (UFL of Most Volatile Ingredient)

SPECIAL FIRE FIGHTING PROCEDURES:

Use water spray to cool fire-exposed containers and structures.
Do not direct a solid stream of water or foam into hot, burning pools; this may cause frothing and increase fire intensity.
Use water spray to disperse vapors; re-ignition is possible.
Use self-contained breathing apparatus and protective clothing.

EXTINGUISHING MEDIA: Apply alcohol-type or all-purpose-type foam by manufacturer's recommended techniques for large fires. Use carbon dioxide or dry chemical media for small fires.

UNUSUAL FIRE AND EXPLOSION HAZARDS: Vapors form from this product and may travel or be moved by air currents and ignited by pilot lights, other flames, smoking, sparks, heaters, electrical equipment, static discharges or other ignition sources at locations distant from product handling point.
Vapors from this material may settle in low or confined areas or travel a long distance to an ignition source and flash back explosively.
During a fire, oxides of sulfur may be produced.
This material may produce a floating fire hazard.

Static ignition hazard can result from handling and use. Electrically bond and ground all containers and equipment before transfer or use of material. Use proper bonding and grounding during product transfer as described in National Fire Protection Association Document NFPA 77.

See "Other Precautions" in Section IX.

V. HEALTH HAZARD DATA

EXPOSURE LIMIT(S): Isopropanol: 400 ppm TWA, OSHA & ACGIH
500 ppm STEL, OSHA & ACGIH

EFFECTS OF SINGLE OVEREXPOSURE:

SWALLOWING: Moderately toxic.
May cause abdominal discomfort, nausea, vomiting and diarrhea.
Aspiration into the lungs may occur during ingestion or vomiting, resulting in lung injury.

SKIN ABSORPTION: No evidence of harmful effects from available information.

INHALATION: High concentrations of vapor or mist cause irritation of the respiratory tract, experienced as nasal discomfort and discharge, with chest pain and coughing.
Dizziness and drowsiness may occur.
Headache may occur.

SKIN CONTACT: Causes irritation with discomfort, local redness, and possible swelling.

EYE CONTACT: Causes moderate to severe irritation, experienced as discomfort or pain, excess blinking and tear production, with marked excess redness and swelling of the conjunctiva.

EFFECTS OF REPEATED OVEREXPOSURE:
Repeated skin contact may cause a dermatitis.

MEDICAL CONDITIONS AGGRAVATED BY OVEREXPOSURE:
A knowledge of the available toxicology information and of the physical and chemical properties of the material suggests that overexposure is unlikely to

aggravate existing medical conditions.

SIGNIFICANT LABORATORY DATA WITH POSSIBLE RELEVANCE TO HUMAN HEALTH HAZARD EVALUATION:

Contains surfactant which, based on studies with rabbits involving the sustained occluded contact of the undiluted surfactant with skin, indicate that such conditions may result in the development of inflammatory changes in the lung.

OTHER EFFECTS OF OVEREXPOSURE:

None currently known.

EMERGENCY AND FIRST AID PROCEDURES:

SWALLOWING:	If patient is fully conscious, give two glasses of water. DO NOT INDUCE VOMITING Obtain medical attention.
SKIN:	Remove contaminated clothing. Wash skin with soap and water. If irritation persists or if contact has been prolonged, obtain medical attention.
INHALATION:	Remove to fresh air. Obtain medical attention if symptoms persist.
EYES:	Immediately flush eyes with water and continue washing for several minutes. Remove contact lenses, if worn. Obtain medical attention.
NOTES TO PHYSICIAN:	There is no specific antidote. Treatment of overexposure should be directed at the control of symptoms and the clinical condition of the patient. Any material aspirated during vomiting may cause lung injury. Therefore, emesis should not be induced mechanically or pharmacologically. If it is considered necessary to evacuate the stomach contents, this should be done by means least likely to cause aspiration (e.g., gastric lavage after endotracheal intubation).

VI. REACTIVITY DATA

STABILITY: Stable

CONDITIONS TO AVOID: Prolonged excessive heat may cause product decomposition.

INCOMPATIBILITY (materials to avoid):

Contact with strong oxidizing and/or reducing agents may result in rapid energy release. Avoid strong bases at high temperatures, strong acids, and materials reactive with hydroxyl compounds.

HAZARDOUS COMBUSTION OR DECOMPOSITION PRODUCTS:

Burning can produce the following combustion products:
Carbon monoxide and/or carbon dioxide.
Oxides of sulfur.
Carbon monoxide is highly toxic if inhaled; carbon dioxide in sufficient concentrations can act as an asphyxiant.
Acute overexposure to the products of combustion may result in irritation of the respiratory tract.

PRODUCT NAME: TRITON GR-5M SURFACTANT

HAZARDOUS POLYMERIZATION: Will Not Occur

CONDITIONS TO AVOID: None known.

VII. SPILL OR LEAK PROCEDURES

STEPS TO BE TAKEN IF MATERIAL IS RELEASED OR SPILLED:

Eliminate sources of ignition.
Wear eye and skin protection. Floor may be slippery; use care to avoid falling. Contain spills immediately with inert materials (eg. sand, earth). Avoid discharge to natural waters. Transfer liquids and solid diking material to suitable containers for recovery or disposal. To avoid gelling and foaming problems, do not use water to flush away spills.

ACUATIC EFFECTS:
Like most surfactants, this product is expected to be relatively toxic to fish.

WASTE DISPOSAL METHOD:

FOR DISPOSAL OF AQUEOUS SURFACTANT SOLUTIONS: Aerobic biological wastewater treatment systems are effective in treating aqueous solutions of surfactants. Removal efficiency will depend upon treatment plant conditions. As with any wastewater, consultation with local treatment plant staff is recommended (and may be required by law) before disposal.

FOR DISPOSAL OF NEAT, UNUSED SURFACTANT: Incinerate in a furnace where permitted under Federal, State and local regulations.

VIII. SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (specify type):
None required if airborne concentrations are maintained below the TWA/TLV listed for this material. However, where misting may occur, wear a MSHA/NIOSH approved (or equivalent) half-mask air purifying respirator.

VENTILATION: This product should be confined within closed equipment, in which case general (mechanical) room ventilation should be satisfactory. Special ventilation is suggested at points where vapors can be expected to escape to workplace air.

PROTECTIVE GLOVES: Nitrile (NBR)

EYE PROTECTION: Monogoggles

OTHER PROTECTIVE EQUIPMENT:
Eye Bath, Safety Shower
Full Protective Clothing

IX. SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE:

WARNING!
FLAMMABLE.
CAUSES EYE AND SKIN IRRITATION.

ASPIRATION MAY CAUSE LUNG DAMAGE.

Keep away from heat, sparks and flame.
Avoid contact with eyes, skin and clothing.
Do not swallow.
Keep container closed.
Use with adequate ventilation.
Vapors form from this product and may travel or be moved by air currents and ignited by pilot lights, other flames, smoking, sparks, heaters, electrical equipment, static discharges or other ignition sources at locations distant from product handling point and may flashback explosively.
Wash thoroughly after handling.

FOR INDUSTRY USE ONLY

OTHER PRECAUTIONS:

Surfactants can cause foaming problems in biological wastewater treatment plants and other high shear operations.

Vapors may settle in low or confined areas, or travel a long distance to an ignition source and flash back explosively.

ADDITIONAL INFORMATION: Additional product safety information on this product may be obtained by calling your Union Carbide Corporation Sales or Customer Service contact.

PROCESS HAZARD: Sudden release of hot organic chemical vapors or mists from process equipment operating at elevated temperature and pressure, or sudden ingress of air into vacuum equipment, may result in ignitions without the presence of obvious ignition sources. Published "autoignition" or "ignition" temperature values cannot be treated as safe operating temperatures in chemical processes without analysis of the actual process conditions.

Any use of this product in elevated-temperature processes should be thoroughly evaluated to establish and maintain safe operating conditions. Further information is available in a technical bulletin entitled "Ignition Hazards of Organic Chemical Vapors."

X. REGULATORY INFORMATION

STATUS ON SUBSTANCE LISTS:

The concentrations shown are maximum or ceiling levels (weight %) to be used for calculations for regulations. Trade Secrets are indicated by "TS".

FEDERAL EPA

Comprehensive Environmental Response Compensation, and Liability Act of 1980 (CERCLA) requires notification of the National Response Center of release of quantities of Hazardous Substances equal to or greater than the reportable quantities (RQs) in 40 CFR 302.4.

Components present in this product at a level which could require reporting under the statute are:

*** NONE ***

PRODUCT NAME: TRITON GR-5M SURFACTANT

Superfund Amendments and Reauthorization Act of 1986 (SARA) Title III
requires emergency planning based on Threshold Planning Quantities (TPQs) and release reporting based on Reportable Quantities (RQs) in 40 CFR 355 (used for SARA 302, 304, 311 and 312).

Components present in this product at a level which could require reporting under the statute are:

*** NONE ***

Superfund Amendments and Reauthorization Act of 1986 (SARA) Title III
requires submission of annual reports of release of toxic chemicals that appear in 40 CFR 372 (for SARA 313). This information must be included in all MSDSs that are copied and distributed for this material.

Components present in this product at a level which could require reporting under the statute are:

Toxic Substances Control Act (TSCA) STATUS:

The ingredients of this product are on the TSCA inventory.

STATE RIGHT-TO-KNOW

CALIFORNIA Proposition 65

This product contains no listed substances known to the State of California to cause cancer, birth defects or other reproductive harm, at levels which would require a warning under the statute.

MASSACHUSETTS Right-To-Know, Substance List (MSL) Hazardous Substances and Extraordinarily Hazardous Substances on the MSL must be identified when present in products.

Components present in this product at a level which could require reporting under the statute are:

HAZARDOUS SUBSTANCES (= > 1%)

CHEMICAL	CAS NUMBER	UPPER BOUND CONCENTRATION %
Isopropanol	67-63-0	20

PENNSYLVANIA Right-to-Know, Hazardous Substance List Hazardous Substances and Special Hazardous Substances on the List must be identified when present in products.

Components present in this product at a level which could require reporting under the statute are:

HAZARDOUS SUBSTANCES (= > 1%)

CHEMICAL	CAS NUMBER	UPPER BOUND CONCENTRATION %
Isopropanol	67-63-0	20

CALIFORNIA SCAQMD RULE 443.1 VOC'S:

Vapor Pressure 30.4 mmHg at 20°C
VOC 205.24 g/L
VOC 258.36 g/L less exempted compounds

OTHER REGULATORY INFORMATION:

EPA HAZARD CATEGORIES: Immediate health, Delayed health, Fire

NOTE ----

The opinions expressed herein are those of qualified experts within Union Carbide. We believe that the information

contained herein is current as of the date of this Material Safety Data Sheet. Since the use of this information and the conditions of the use of the product are not under the control of Union Carbide, it is the user's obligation to determine conditions of safe use of the product.

REVISED SECTIONS:

Revisions in this MSDS occurred in the following sections:

Section VII : SPILL OR LEAK PROCEDURES
Waste Disposal Method
Section X : REGULATORY INFORMATION

PRODUCT: 89539
F NUMBER: N0583C

12. Appendix 4: Contacts

ÉCOLE
MONTRÉAL
Le

Bruce Ramsay (professeur agrégé)

Département: Génie chimique

local et téléphone Télécopieur: (514) 340-5913
Courrier électronique: bruce.ramsay@mailsrv.polymtl.ca

Principaux domaines de recherche:

1. Compostage
2. Microbiologie appliquée
3. Biorestauration des sols
4. Technologies de fermentation
5. Plastiques biodégradables

Unité(s) de recherche:

1. Centre de recherche en ingénierie de l'environnement et des biotechnologies (BIOPRO)
2. Chaire industrielle CRSNG en bioprocédés d'assainissement des sites

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Index A, B, C, ... Z des domaines d'expertise (un fichier de 10K par lettre)
Index A-Z des domaines d'expertise (un fichier de 120K avec recherche plein texte)
Index A-Z des professeurs et chercheurs
[page RECHERCHE]

Ces informations sont compilées par le **Bureau de la recherche**.

Adresse: C.P. 6079, succursale Centre-ville. MONTRÉAL (Québec) CANADA H3C 3A7

URL = http : // www . polymtl . ca / p232 . htm
Mise à jour: 1996-12-16 par Pierre Lavigne

Date: Wed, 19 Feb 1997 09:53:52 -0500
From: Bruce Ramsay <Bruce.Ramsay@mail.polymtl.ca>
To: "[Katie Lehnert]" <lehnert@utkux.utcc.utk.edu>
Subject: Re: research interest in biodeg.latex

[The following text is in the "ISO-8859-1" character set]
[Your display is set for the "US-ASCII" character set]
[Some characters may be displayed incorrectly]

I doubt that I can help you. I have a small quantity of latex here but it is very old and has undoubtedly lost much of its amorphous character. I have been trying to contact someone else who may have a supply (or may not) but he is not answering his phone.

As far as I know, there is no real commercial commercial source of the latex at present. As you probably know, Monsanto has purchased the rights to Biopol from Zeneca (formerly ICI Biologicals) I believe that Biopol is still produced by Zeneca) in England for Monsanto. Biopol is sold in the form of pellets but at one point in the

production process, it is in the form of a latex. If you asked the producer for a small amount of this latex, they may oblige you, but they may ask you to sign some sort of research agreement. All this could take a long time.

I will continue to search for the person I know who may have some of this material but even if he has some, he may not be allowed to give you any because of some research agreement that he may have signed. Sorry to be of so little help and good luck in your work. The latex paper coating really works quite well, whereas, the traditional approach does not, even with plasma-treated PHAs.

Yours sincerely,

Bruce Ramsay

Date: Mon, 24 Feb 1997 18:04:01 -0600
From: SPSOBL@monsanto.com
To: MALCOLM W FORSYTH <MWFORS@monsanto.com>,
"lehnert(a)utkux.utcc.utk.edu" <lehnert@utkux.utcc.utk.edu>
Subject: Re: Biopol questions

Katie,

Thanks for your interest in Biopol ... I am sending you a packet of literature and forwarding your response for a product sample to Malcolm Forsyth in Brussels.

Stacey Soble

From: MWFORS@monsanto.com
To: STACEY P SOBLE <SPSOBL@monsanto.com>,
"lehnert(a)utkux.utcc.utk.edu" <lehnert@utkux.utcc.utk.edu>
Subject: Re[2]: Biopol questions

Katie,

I am afraid we cannot help with a sample of BIOPOL emulsion - this product is still under development with another company, and we do not have material that we can make available to outside parties yet. If you have any further questions after you have read the literature, please feel free to send them to me at either :

fax : +32-10-471-232
email : MWFORS @ ccmail.monsanto.com

I wish you well with your research,

Malcolm Forsyth
BIOPOL Marketing Manager
Louvain-la-Neuve
Belgium

From: MWFORS@monsanto.com
To: "lehnert(a)utkux.utcc.utk.edu" <lehnert@utkux.utcc.utk.edu>
Subject: Re[4]: Biopol questions

Malcolm being out travelling I would like to help you with this request please advise what kind of product you would like and the deadline for reception, What I can offer you is following :

- COATED CUPS
- PLANTS CONTAINERS
- MOULDED BOTTLES LOW CAPACITY
- POSTERS AND PHOTOGRAPHS

Please advise

Regards,

C^Bciel Sprimont
Marketing Assistant

ate: Tue, 01 Apr 1997 14:21:03 -0800
From: Roger Hewitt <roger.r.hewitt@ukbla71.zeneca.com>
To: lehnert@utkux.utcc.utk.edu
Subject: Your Internet message to Zeneca

Thank you for your enquiry, as you state, we have sold the Biopol business to Montanso and the contact name and address is:
Malcolm Forsyth,
Marketing Manager,
Monsanto Ltd.,
The Technical Centre,
Rue Laid Burniat,
B1348 Louvain-La Nauve,
Belgium.

Tel: (Belgium) 1047 1336
Fax: (Belgium) 1047 1232

Nutrition and Food Science Centre

Nutrition and Food Science Centre
Director: E.B. Marliss
687 Pine Avenue West
Montreal, Quebec
H3A 1A1
Tel: (514) 842-1231
Fax: (514) 982-0893

The Centre coordinates the activities of the Faculty of Agricultural and Environmental Sciences, the Faculty of Medicine, McGill's teaching hospitals and other faculties concerned. It promotes the development of basic and clinical nutrition and food science research; provides postgraduate training for nutritionists; teaches nutrition to undergraduate students and postgraduate trainees in medicine; develops nutrition consulting services within the teaching hospitals; and disseminates information to all nutrition and food science professionals both within the University and in the community at large.

Pulp and Paper Research Centre

Pulp and Paper Research Centre
Director: T.G.M. van de Ven
3420 University Street
Montreal, Quebec
H3A 2A7
Tel: (514) 398-6177
Fax: (514) 398-8254

A university-industry collaboration focused on postgraduate research and education, including a non-thesis Master's level programs in pulp and paper engineering and chemistry. The collaborating centre is the Pulp and Paper Research Institute of Canada (PAPRICAN), a partnership of university (McGill and U.B.C.), industry and government (federal). Principal fields of research, in cooperation with appropriate academic departments, encompass organic, physical and polymer chemistry, chemical engineering and biotechnology. Thesis topics are generally related to the interests of the Canadian pulp and paper industry.

Royal Victoria Hospital Research Institute

Royal Victoria Hospital Research Institute
Director: H.M. Shizgal
687 Pine Avenue West, H4.17

McGill

Pulp and Paper Research Centre

3420 University Street
Montreal, Quebec
Canada H3A 2A7

Tel.: (514) 398-6180
Fax: (514) 398-8254

February 17, 1997

Ms. Katie Lehnert
310 Cheshire Drive
Apt. 159
Knoxville, TN 37919
U.S.A.

Dear Ms. Lehnert:

Reference is made to your recent e-mail. I am enclosing herewith reprints of the papers to which you refer. I am also including one which is entitled "Direct Electrostatic Coating" and other "Novel Biodegradable Microbial Polymers". These should give you extra background on the subject of "powder coating".

The coating could also be done using a "PHA latex" which would require special preparation in a microbial fermentation laboratory. The powder coating approach is much simpler and would give you a pretty good imitation of a "biodegradable paint".

I am not clear what you plan to do with the biodegradable paint? We used an electrostatic spray gun to deposit the powder on paper and subsequently fused it with heat and pressure.

Sincerely,

R.H. Marchessault

R.H. Marchessault
E.B. Eddy Professor
Tel: (514)398-6276
Fax: (514)398-7249
E-mail: ch21@musica.mcgill.ca

RHM/sa

>Marchessault, R.H. Dr. <Pulp and Paper Research Centre> Tel:6276
Pulp and Paper Research Centre
3420 University Street
Email: CH21@MUSICA.MCGILL.CA

July 22, 1996 -- New Biodegradable Plastic

A Cambridge company working with scientists are M-I-T are a big step closer to producing plastic that quickly biodegrades.

Metabolix of Cambridge has been awarded a patent to produce a bioengineered plastic called "polyhydroxyalkanoates" (P.H.A.s)...that could decompose in just a few months...compared to 200 hundred years for many plastics today.

I asked Metabolix Vice President Dr. Simon Williams to explain the major ingredients in that new plastic:

"P.H.A.'s are produced from glucose, typically starting with corn, the corn is converted into the glucose and the glucose is fermented to make the P.H.A. in bacteria." And does Dr. Williams agree that this new product could revolutionize the plastics industry around the world:

"Yes. I think that is a very true statement that there is no doubt that this technology could be a large component of the chemical industry. And the ability to change that industry is fairly significant." Dr Williams says the new biogradable plastic could be on the market in the form of diapers, trash bags and food containers in just a few years.
Inquiries should be addressed to: Metabolix, Inc., 303 Third Street, Cambridge, MA 02142 USA. Telephone: 617-492-0505, Fax: 617-492-1996.

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Biopol™ resin

nature's plastic

Born from nature. Back to nature.

Properties and Processing

Monsanto

'BIOPOL'* resin is a unique range of truly biodegradable polymers produced from agricultural feedstocks. They are thermoplastic polyesters composed of hydroxybutyrate (HB) units with hydroxyvalerate (HV) units incorporated randomly throughout the polymer chain. The chemical structure of PHB V copolymers is shown in Figure 1.

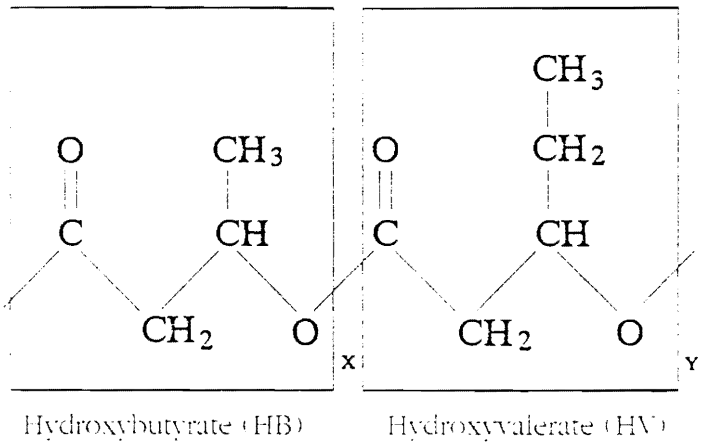


***Figure 1** – Chemical Structure of PHB V Copolymers*

Produced by the fermentation of a sugar feedstock by naturally occurring micro-organisms, these polymers have many of the properties of traditional plastics and can be processed using conventional techniques. They are stable, durable and moisture resistant in use.

Physical properties vary with the HV content of the polymer. The homopolymer (PHB) is relatively stiff and brittle but flexibility and toughness can be introduced by increasing the HV content. This allows a variety of copolymers to be produced with flexibilities and tensile strengths in a range encompassing those of polyethylene and polypropylene.

In addition to being compatible with conventional waste disposal systems such as recycling, incineration and landfill they are fully biodegradable and compostable. 'BIOPOL' resin provides customers and industry with a new choice, a polymer which combines many of the properties and characteristics of traditional thermoplastics with the added values of full biodegradability and manufacture from renewable resources.

KEY POINTS

'BIOPOL' resin – Nature's Plastic

- is fully biodegradable and compostable on disposal
- is adaptable to a variety of waste disposal systems
- is made from renewable resources
- is stable, durable and moisture resistant in use
- can be processed using conventional techniques

** 'BIOPOL' is a trademark, the property of Monsanto plc.*

THE 'BIOPOL' CYCLE

STRIKING A BALANCE WITH OUR ENVIRONMENT

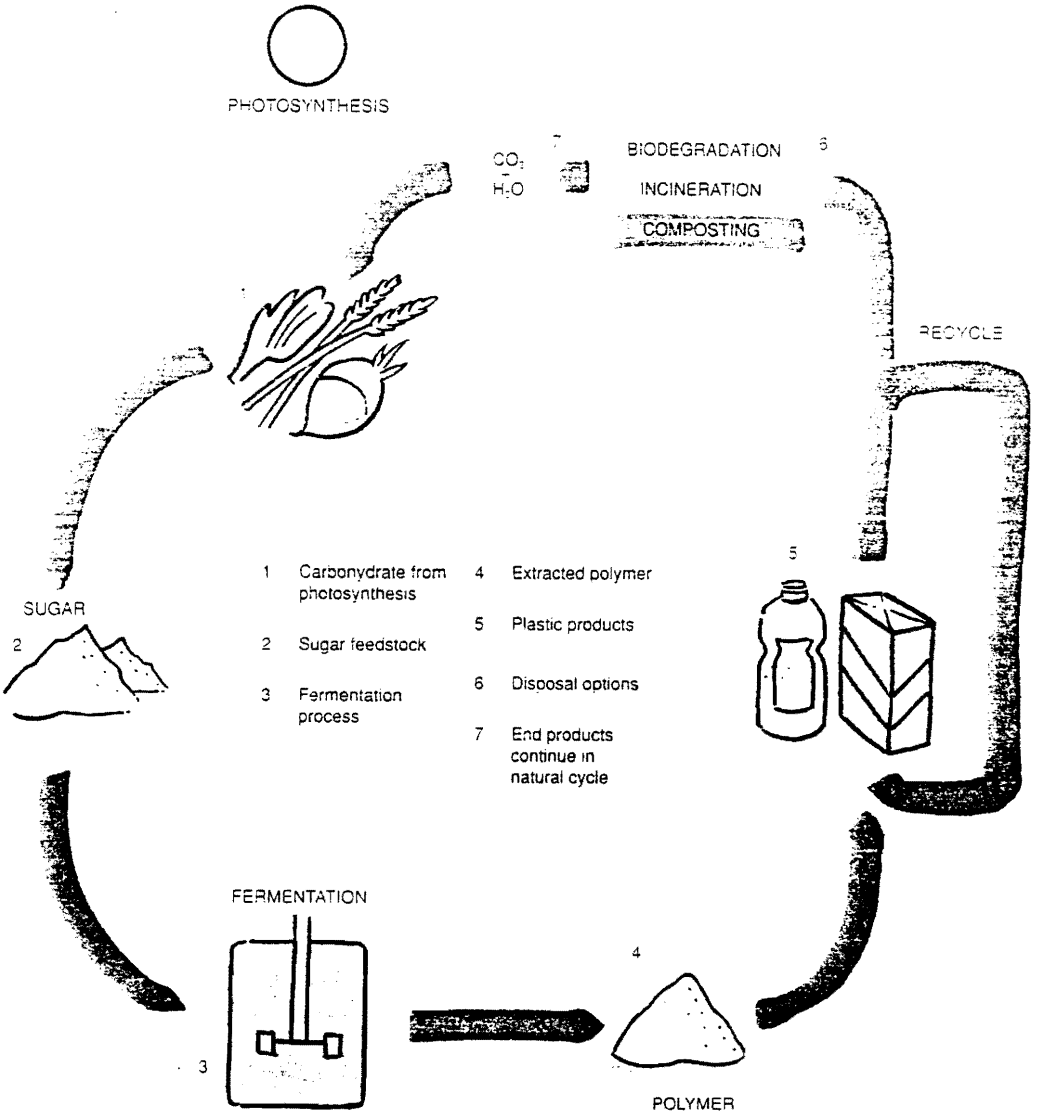


***Figure 2** – Illustrates the 'BIOPOL' Cycle.*

'BIOPOL' Production – Working With Nature

The manufacturing process for 'BIOPOL' resin starts with sunlight. During photosynthesis in crops such as sugar beet and cereal crops, carbon dioxide (CO_2) from the atmosphere is converted into carbohydrates. These carbohydrates are the raw materials from which 'BIOPOL' resin is made.

This innovative fermentation process utilises the natural formation of these polymers. The carbohydrate (currently glucose) is converted into 'BIOPOL' resin by a specific micro-organism **Alcaligenes eutrophus**, which occurs widely in soil and water. The HV content of the polymer can be varied by adding controlled amounts of a simple organic acid.

At the end of the fermentation, the micro-organisms have accumulated up to 80% of their dry weight as 'BIOPOL' resin. It is then harvested by breaking open the cells and extracting and purifying the polymer.

At this stage the polymer powder can be granulated prior to conversion into plastic articles such as bottles, mouldings, fibres and films, all of which are stable and durable under normal conditions of storage and use.

Back to Nature

A fundamental property of 'BIOPOL' resin is that while stable under normal conditions of storage and use, it can biodegrade when deposited in microbially active environments. Just as naturally occurring micro-organisms produce PHB as an energy food store, a wide variety of living organisms in the environment can consume 'BIOPOL' resin as a source of carbon. The material erodes progressively as these micro-organisms metabolise the polymer (Figure 3).

The final products of degradation in aerobic conditions are carbon dioxide (CO_2) and water, exactly equivalent to the final decomposition products of any other organic matter. The material will also degrade fully under anaerobic conditions.

'BIOPOL' polymers can be incinerated safely, yielding an energy value comparable with traditional plastic materials. Whether biodegraded or incinerated, the amount of carbon dioxide released on disposal is the same as that fixed during photosynthesis right at the beginning of the cycle.

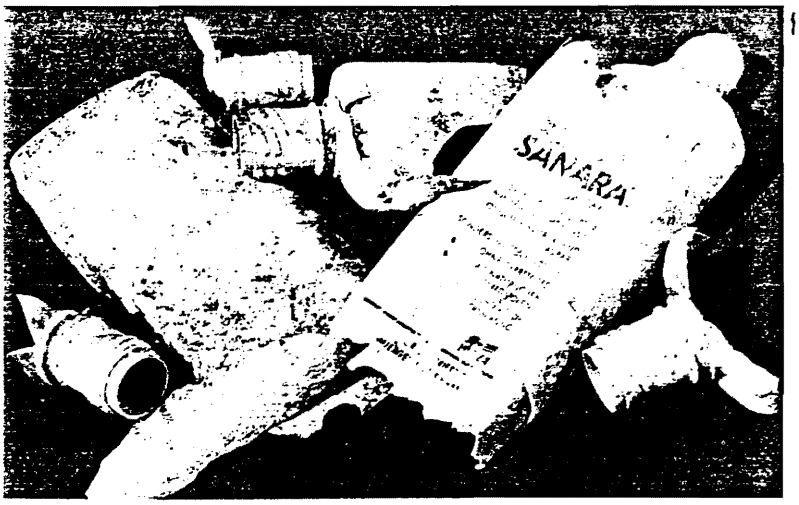


***Figure 3** – Biodegradation After Disposal*

'BIOPOL' resin is available in a range of grades with various physical properties and is supplied as granules (pellets). All grades contain a fully compounded nucleant to give faster processing cycles. Grades with low HV content are relatively stiff and have high tensile strengths, but have lower impact resistance and ductility. Grades with higher HV contents are more flexible, have greater impact resistance and ductility, lower tensile strengths and lower crystallisation rates. The addition of plasticiser to the copolymers improves flexibility, ductility and impact strength, particularly at lower temperatures, by lowering the glass transition temperature. The addition of plasticiser results in a decrease in tensile strength and a reduction in the melt viscosity of the polymer during processing.

'BIOPOL' Grade Range

Grade Code	Description
D300G	Low HV copolymer granules suitable for applications requiring high stiffness and strength
D311G	Plasticised low HV copolymer granules suitable for general injection moulding applications
D400G	Medium HV copolymer granules suitable for injection mouldings requiring greater toughness and for extrusion blow moulding
D411G	Plasticised medium HV copolymer granules suitable for general extrusion blow moulding
D600G	High HV copolymer granules suitable for extrusion processes
D611G	Plasticised high HV copolymer granules suitable for extrusion processes

Grade codes are subject to modification in line with our policy of continuous product improvement.

Typical Physical Properties of 'BIOPOL' resin are shown in Table 1; comparative data with other materials is shown in Table 2.

Table 1 – Typical Physical Properties of 'BIOPOL' resin

PROPERTY	UNITS	D300G	D311G	D400G	D411G	D600G	D611G
Melting Point (Tm)	°C	162	151	153	144	144	136
	°F	323	304	307	291	291	277
MFI (ASTM Method No 1238-906 2.16 kg load at 190°C)	g 10 mins	8	–	9	–	12	–
MFI (ASTM Method No 1238-906 5 kg load at 170°C)	g 10 mins	–	5	–	–	–	8
Youngs Modulus	GPa	1.0	0.8	0.9	0.7	0.5	0.4
	Kpsi	145	116	131	102	73	58
Tensile Strength	MPa	31	28	28	25	23	20
	psi	4500	4100	4100	3600	3300	2900
Elongation at Break	%	8	16	15	20	35	42
Flexural Modulus	GPa	2.7	1.8	2	1.0	1.4	0.8
	Kpsi	392	261	290	145	203	116
Izod Impact Strength	J m	60	161	98	198	200	360
	ft-lb inch	1.12	3.02	1.84	3.71	3.75	6.74

Specific Gravity	g cc	1.25
Thermal Conductivity	W m °C	0.156
Specific Heat	J g/°C 30°C 80°C 180°C	 1.42 1.97 2.0
Shrinkage	%	1.6

Table 2 – Comparison of 'BIOPOL' Properties with those of other Thermoplastic Materials

PROPERTY	UNITS	'BIOPOL'	LDPE	PP
Melting Point (Tm)	°C	136 -162	105-110	160 -168
	°F	277 -323	121-230	320 -334
MFI	g/10 mins	5-8 170°C/5kg	0.1 -22 190°C/2.16kg	0.3 -40 230°C/2.16kg
Youngs Modulus	Gpa	0.4-1.0	0.1-0.2	1.4 -1.8
	Kpsi	58-145	15-29	203 -260
Tensile Strength	Mpa	20-31	8-10	25 -35
	psi	2900 -4500	1200 -1400	3600 -5100
Elongation at break	%	8 -42	150 -600	400 -900

3.1 Melting Point

'BIOPOL' homopolymer (PHB) is a highly crystalline polymer with a melting point (Tm) in the range of 173-180°C (340-355°F) and a glass transition temperature (Tg) of 5°C (40°F). The melting point of the copolymers decreases with increasing HV content as shown in Figure 4.

Effect of HV Content on Melting Point

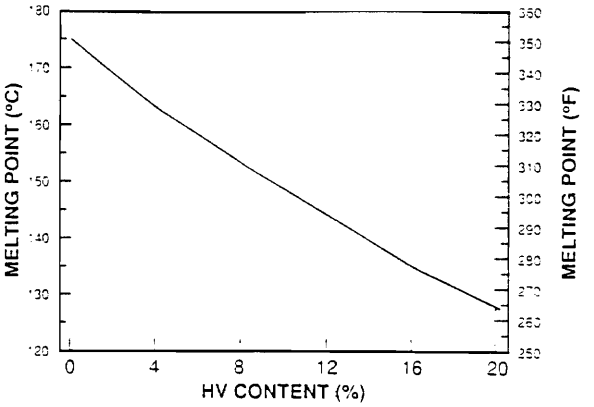


Figure 4

3.2 Crystallisation

'BIOPOL's crystallisation rate is dependent on temperature. The maximum rate of crystallisation is in the range of 55-60°C (130-140°F). It is essential, therefore, that the temperature of the mould or water bath used to crystallise the polymer is within this range. This will ensure shorter cycle times and superior mechanical properties.

Effect of HV Content on Flexibility and Toughness

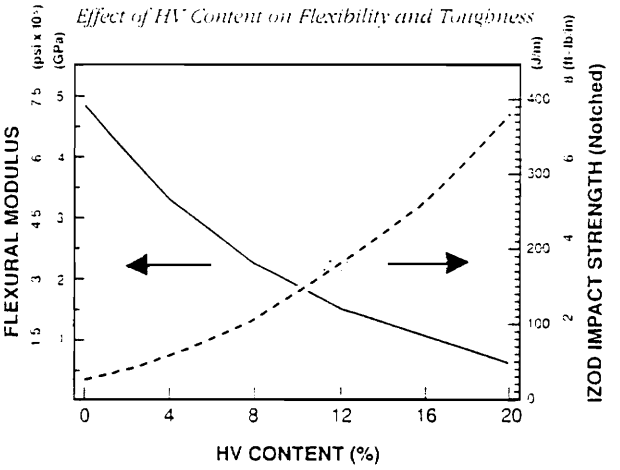


Figure 5

3.3 Stiffness/Toughness

'BIOPOL' resin is flexible and tough, toughness increasing with increasing HV content as shown in Figure 5. The properties of 'BIOPOL' resin can be modified by the addition of plasticisers, impact modifiers, reinforcing fillers and nucleating agents.

Tensile Strength

Tensile strength decreases with increasing HV content (Figure 6).

Effect of HV Content on Tensile Strength

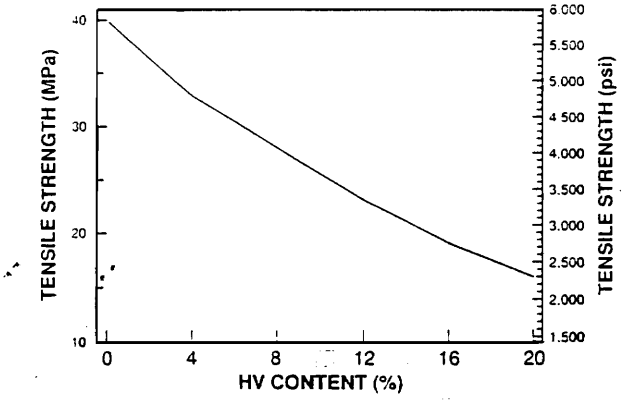


Figure 6

3.4 Chemical Resistance

The 'BIOPOL' range of polymers exhibit good oxygen, moisture and aroma barrier properties.

'BIOPOL' resin is stable in oils, can be slowly hydrolysed in some aqueous environments and is rapidly hydrolysed in some acids and bases.

Table 3 presents the chemical resistance of injection moulded copolymer when evaluated in an accelerated laboratory test carried out at 40°C (105°F) for 12 weeks, intended to simulate one years storage at 20°C (70°F). The mechanical properties were determined at 2 week intervals.

Little change in mechanical properties was observed for bars stored in chemicals such as oils, selected organic solvents, detergents, water and buffer (pH 3-10). However, bars stored in some acids and bases did show a reduction in mechanical properties. The results shown in table 3 are for guidance only and compatibility testing is recommended for specific applications.

Table 3 – Chemical Resistance of Injection Moulded Copolymer after 12 weeks at 40°C (105°F)

	Youngs Modulus		Maximum Stress		Extension at Break (%)	Weight Change (%)	
Chemical	(GPa)	(kpsi)	(MPa)	(psi)		Wiped dry	Oven dried
Control (0 weeks)	0.92	133	34	4,900	17		
Motor Oil	0.75	109	32	4,600	20	+0.06	-0.05
Turpentine	0.76	110	32	4,600	21	+0.14	-0.18
Acetone	0.63	91	27	3,900	18	+5.96	-0.03
Vegetable Oil	0.79	113	33	4,800	17	-0.06	-0.09
Heptane	0.75	109	32	4,600	22	+0.15	-0.15
Olive Oil	0.72	104	32	4,600	20	+0.07	-0.01
Water	0.88	128	34	4,900	21	+0.9	-0.15
Citric Acid	0.79	113	33	4,800	19	+0.88	-0.16
Detergent	0.68	99	32	4,600	19	+1.25	+0.02
2% Na_2CO_3	0.81	117	33	4,800	18	+0.63	-0.37
1% Soap Solution	0.84	121	33	4,800	19	+0.79	-0.28
pH3	0.74	107	32	4,800	18	+0.95	-0.13
pH5	0.75	109	33	4,800	14	+0.95	-0.05
pH7	0.75	109	33	4,800	18	+0.95	-0.06
pH8	0.81	117	32	4,600	19	+0.83	-0.17
pH9	0.77	112	32	4,600	18	+0.9	-0.18
5% CH_3COOH	0.72	104	31	4,500	13	+1.68	+0.18
10% HCl	0.68	99	29	4,200	11	-0.18	-1.02
3% H_2O_2	0.76	110	31	4,500	12	+0.96	-0.64

'BIOPOL' resin is fully biodegradable in a wide range of microbially active environments. The rate of degradation is influenced by a range of environmental and material parameters and is particularly dependent on the microbial activity of the environment and the surface area of the product. Other factors influencing the rate of biodegradation are temperature, molecular weight, crystallinity and pH.

Biodegradation is initiated by the action of micro-organisms growing on the surface of the polymer and secreting enzymes which degrade 'BIOPOL' resin into individual molecular fragments of HB and HV. These fragments are immediately transported into the colonising cells where they are used as a carbon source for growth. Under aerobic conditions, the polymer chain is broken down into CO_2 and water and under anaerobic conditions into CO_2 and methane.

The following researchers have demonstrated the biodegradation of 'BIOPOL' resin under a range of environmental conditions occurring in a variety of waste disposal systems.

ENVIRONMENT	DEMONSTRATED BY
Anaerobic Sewage	ICI (Brixham)
Aerobic Sewage	PIRA International Chemicals Inspection and Testing Institute, Japan ICI (Brixham)
Estuarine Marine Sediment	University of Newcastle upon Tyne, UK ICI (Brixham)
Lake Pond Water	Gent University, Belgium University of Stuttgart, Germany
Sea Water	Gent University, Belgium Tokyo Institute of Technology, Japan ICI (Brixham)
Soil	University of Newcastle upon Tyne,UK Minnesota University, USA Gent University, Belgium ICI (Ag Division & Plant Protection)
Compost	Gent University, Belgium University of Stuttgart, Germany Hechingen Compost Works, Germany
Simulated Managed Landfill	University of Stuttgart, Germany

NOTE: Gent University have isolated 270 Bacteria, 120 Streptomycetes and 100 moulds which degrade 'BIOPOL' resin.

The following results were observed:

4.1 Tests in Sewage

Degradation studies were performed on 'BIOPOL' resin according to ASTM D5209 in activated sewage sludge under aerobic conditions. The rate of % degradation was determined by measuring CO_2 evolution relative to a control at 25± 0.5 C (80 F). After 40 days, gas evolution was found to be equivalent to 85% of the theoretical value (Figure 7(a)). Values obtained from negative controls have been deducted from the results. In a separate experiment performed under anaerobic conditions, gas evolution was found to be equivalent to 78% of the theoretical value after 30 days (Figure 7(b)).

Percentage of Theoretical CO_2 Evolved During Degradation in Activated Sewage Sludge

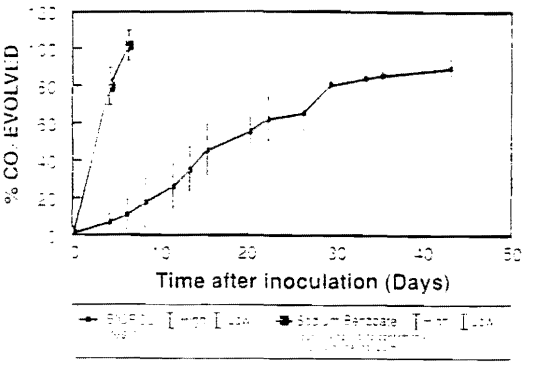


Figure 7 (a)

Gas Evolution During Anaerobic Degradation in Activated Sewage Sludge

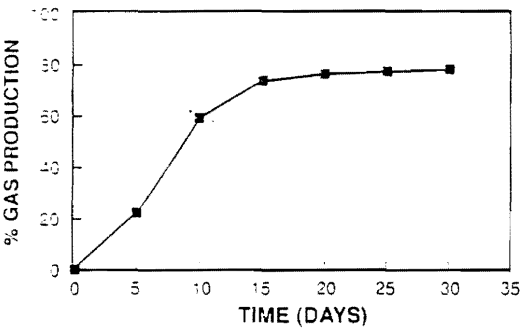


Figure 7 (b)

4.2 Tests in Soil

Biodegradation tests were performed under aerobic conditions at room temperature using two varieties of soil: a sandy loam soil with neutral pH (pear tree) and a more alkaline soil with a high content of organic matter (gore).

When samples of radioactively labelled 'BIOPOL' were buried in soil, up to 90% biodegradation was observed at 7–8 months (measured by CO_2 evolution). The samples did not biodegrade in humid air alone (see figure 8).

In a separate test in a slightly acidic loam soil, 'BIOPOL' film was buried for 72 days at 25°C (77°F). Scanning Electron Microscopy (SEM) photographs show how the surface has become pitted and eroded as a result of degradation. High numbers of Actinomycetes and rod bacteria which are associated with polymer degradation are visible on the surface. (See figures 9 and 10).

Gas Evolution During Aerobic Degradation of 'BIOPOL' resin in Various Soils

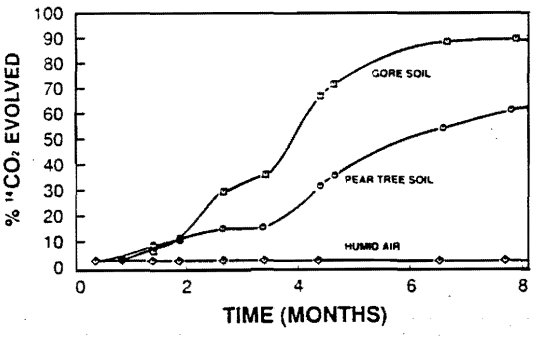


Figure 8

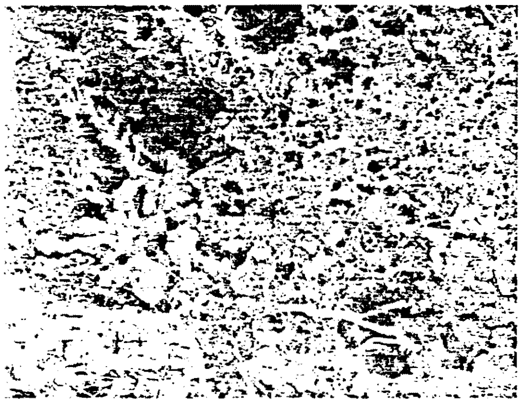


Figure 9 – 'BIOPOL' film after 72 days at 25 [illegible] (80°F) in soil (water holding capacity = 38 [illegible])

Figure 10 – 'BIOPOL' film after 72 days at 25 [illegible] (80°F) in soil (water holding capacity = 72 [illegible])

Photographs prepared by Trevor Booth and James Honeybourne, and reproduced [illegible] Dickinson, University of Newcastle upon Tyne.

4.3 Tests in Waste Water

'BIOPOL' bottles and strips of material cut from bottles were tested in microbially active waste water under aerobic conditions. An aerated aquarium with a mixture of biologically treated waste water and river water was used.

At room temperature the bottle degraded over a period of weeks such that after 25 weeks a 50% weight loss was observed (see Figure 11).

Degradation of 'BIOPOL' Bottles in Waste Water under Aerobic Conditions

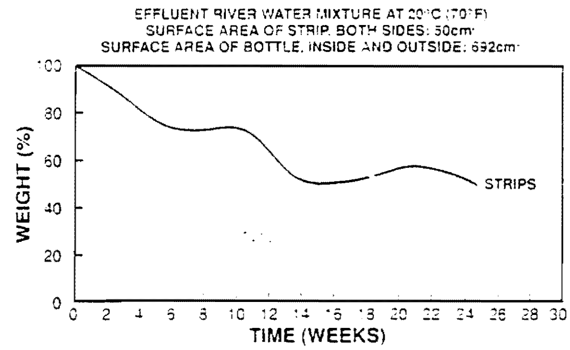


Figure 11
Graph reproduced courtesy of the University of Stuttgart.

4.4 Behaviour in Compost

Tests were carried out on 'BIOPOL' bottles at an industrial composting plant under aerobic conditions at a temperature of 70°C (158°F).

At this temperature, the bottles became very brittle and broke up. This was further enhanced by the turning of the compost heap, which mechanically broke up the bottles, thereby increasing the rate of microbial attack by increasing surface area. Weight losses of up to 80% were observed over 15 weeks.

4.5 Behaviour in Managed Landfill

'BIOPOL' bottles were tested under simulated managed landfill conditions at a temperature of 35°C (95°F). A weight loss of approximately 50% was observed over a period of 40 weeks (see Figure 12).

Degradation of 'BIOPOL' Bottles in Managed Landfill Simulation

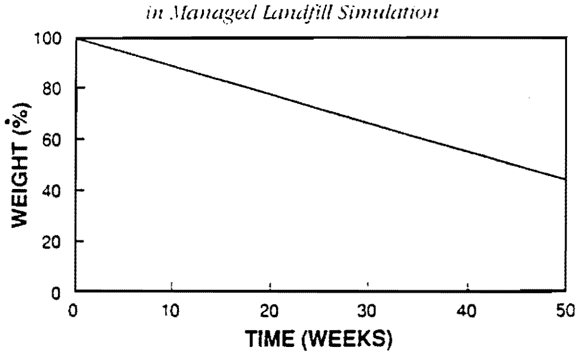


Figure 12
Graph reproduced courtesy of the University of Stuttgart.

5.1 Bottle Blow Moulding

Conventional equipment can be used for the extrusion blow moulding of 'BIOPOL' bottles. The following processing guidelines are recommended:

Setting Up

In order to minimise residence time in the melt, it is best to use the smallest suitable machine. Try to match the screw output to the size of the bottle to be produced. The use of a polyethylene type screw and head is recommended. The head should have a compression zone after the spider to ensure that the melt rejoins to avoid weak seams in the bottle. The die gap should be at a wider setting than that used for polyethylene. If possible, start with a clean machine, or purge out the machine with low density polyethylene before use. The polyethylene can then be purged out with 'BIOPOL'. It is essential that the blow pin and mould can be temperature controlled at 60 ± 5°C (140 ± 9°F).

Moulding

'BIOPOL' processing requires careful temperature control. The extruded tube should be examined: if the surface is very shiny and smoking, the melt temperature is too high; if the surface is matt and lumpy, the melt temperature is too low. Usually, only small adjustments to the temperature are necessary to get a good melt which is homogeneous with a semi-gloss surface. It is important that the melt temperature is correct to ensure that mouldings can be produced with reasonable cycle times. To produce good quality bottles it is better to have no time delay for the blow pin entry and blow. The blow should be in the downward direction. High blow pressures (6-9 bar) tend to produce a better surface finish than low blow pressures. Slightly longer cooling times may be required than with conventional thermoplastics. It is essential that the mould and blow pin are heated to 60 ± 5°C (140 ± 9°F). Reducing the mould temperature will increase the cycle time and lead to the blow pin sticking.

Process Control Requirements

'BIOPOL' resin should be processed at the lowest possible temperature and residence time in the machine kept at a minimum. If the machine is stopped for a short time (5-15 minutes), the degraded 'BIOPOL' resin must be purged out with fresh material before moulding can be started again. If the machine is to be shut down for longer periods, it is better to purge out with low density polyethylene. Regrind material can be incorporated at up to 20%. It should be thoroughly mixed with virgin material of the same grade.

Recommended Blow Moulding Processing Conditions

For standard blow moulding, the following conditions are recommended:

Grade	Temp	Zone 1	Zone 2	Zone 3	Die	Blow Pin	Mould
D411G	°C	140	160	170	170	60 ± 5	60 ± 5
D411G	°F	285	320	340	340	140 ± 9	140 ± 9

The minimum screw speed compatible with the cycle time should be employed.

Processing temperatures should not exceed 180°C (355°F). These settings should be used as a guideline only and may require adjustment.

5.2 Injection Moulding

A wide range of conventional injection moulding equipment can be used to process 'BIOPOL' resin. The following processing guidelines are recommended:

Setting Up

Standard machines with polyethylene type screws (L/D = 20:1) are suitable for moulding 'BIOPOL' resin. It is advisable to try to match the shot size of the machine to the weight of the part to be moulded in order to reduce the time that the polymer is in the melt. If possible, start with a clean machine, or purge out with low density polyethylene before use. The polyethylene can then be easily purged out with 'BIOPOL' resin. It is not necessary to use a shut off nozzle when moulding 'BIOPOL' resin as drooling can be prevented by using decompression between cycles. It is essential that the mould temperature is controlled at 60 ± 5°C (140 ± 9°F).

Moulding

The temperature window for 'BIOPOL' resin is relatively narrow. The melt should be examined to establish if the correct temperatures are being used. If the melt has a very low viscosity, has a shiny surface and is smoking, then the melt temperature is too high. If the surface is matt and lumpy, the melt temperature is too low. Usually, only small adjustments to the temperature are necessary to produce a good melt with reasonable viscosity. It is important that the melt temperatures are correct to ensure that mouldings can be produced with reasonable cycle times.

If multi-stage injection moulding is available, a high first injection pressure to rapidly fill the mould is recommended. This should be followed by low first hold pressure to prevent flashing then an increase to a higher second stage pressure to prevent sinking. Very high injection pressures and injection speeds should be avoided to minimise flash. High screw speeds should also be avoided to minimise degradation. There is usually no need to retract the nozzle after decompression as sprue break is usually achieved by mould opening. The length of the cooling time will depend upon the thickness of the part being moulded and the grade of 'BIOPOL'. It is essential that the mould is heated to 60 ± 5°C (140 ± 9°F); reducing the temperature will reduce the rate of crystallisation. This will lead to the parts sticking to the mould or longer cycle times.

Process Control Requirements

'BIOPOL' resin should be processed at the lowest possible temperatures and residence time in the machine kept at a minimum. If the machine is stopped for a short time (5-15 minutes), the degraded 'BIOPOL' resin must be purged out with fresh material before moulding can be started again. If the machine is to be shut down for longer periods, it is better to purge out with low density polyethylene. Regrind material can be incorporated at up to 20%. It should be thoroughly mixed with virgin material of the same grade.

Recommended Processing Conditions

The following processing temperatures are recommended:

Grade	Zone 1		Zone 2		Zone 3		Nozzle	
	°C	°F	°C	°F	°C	°F	°C	°F
D300G	150	300	170	340	180	355	180	355
D311G	140	285	160	320	170	340	170	340
D400G	150	300	170	340	180	355	180	355
D411G	140	285	160	320	170	340	170	340
D600G	140	285	160	320	175	345	175	345
D611G	140	285	160	320	170	340	170	340

In all cases it is recommended that the mould temperature is maintained at 60 ± 5°C (140 ± 9°F).

These settings should be used as a guideline only and may require adjustment.

General Process Control Requirements

Like most polyesters, 'BIOPOL' resin is not particularly melt stable at high temperatures. At temperatures above 195°C (385°F) the polymer can degrade rapidly. However, as HV content increases, melting point decreases, allowing higher HV content materials to be processed at lower temperatures with less risk of thermal degradation. Care should be taken to minimise the time in the melt, especially if it is intended to regrind scrap material. Temperatures of 170°C (340°F) or less and residence times of less than 3 minutes are therefore preferable for most applications.

5.3 Other Processes

The following processes are currently under development:

Film Processing
Extrusion Coating and Lamination
Fibre Making
Thermoforming

Further information, including Health and Safety Data, may be obtained from:

Monsanto Europe S.A.
Technical Center
Parc Scientifique
Rue Laid Burniat
B-1348 Louvain-la-Neuve
Belgium
Tel: - 32 - 10 - 471 - 404
Fax: - 32 - 10 - 471 - 232

Monsanto Company
800 N. Lindbergh Boulevard
St Louis
Missouri 63167
USA
Tel: - 001 - 314 - 694 - 5274
Fax: - 001 - 314 - 694 - 4228

Monsanto (Deutschland) GmbH
Immermannstrasse 3
D-40210 Düsseldorf
Germany
Tel: - 49 - 211 - 3675 - 266
Fax: - 49 - 211 - 3675 - 215

Monsanto Japan Ltd
Nihonbashi Daini Building
41-12 Nihonbashi
Hakozaki-cho
Chuo-ku
Tokyo (103)
Japan
Tel: - 81 - 3 - 5644 - 1648
Fax: - 81 - 3 - 5644 - 1631

13. Appendix 5: Tensile Test Results

Tensile Test 550

Tensile 550
For 550 class 1997
Using 2000 Gm load cell

Test type: Tensile
Operator name: Haibin Tang
Sample Identification: PAINTB
Interface Type: 1120

Instron Corporation
Series IX Automated Materials Testing System 7.27.00
Test Date: Wednesday, March 19, 1997

Sample Rate (pts/secs): 2.2758
Crosshead Speed: 2.0000 mm/min
2nd Crosshead Speed: 0.0000 mm/min
Full Scale Load Range: 2.000 kgf

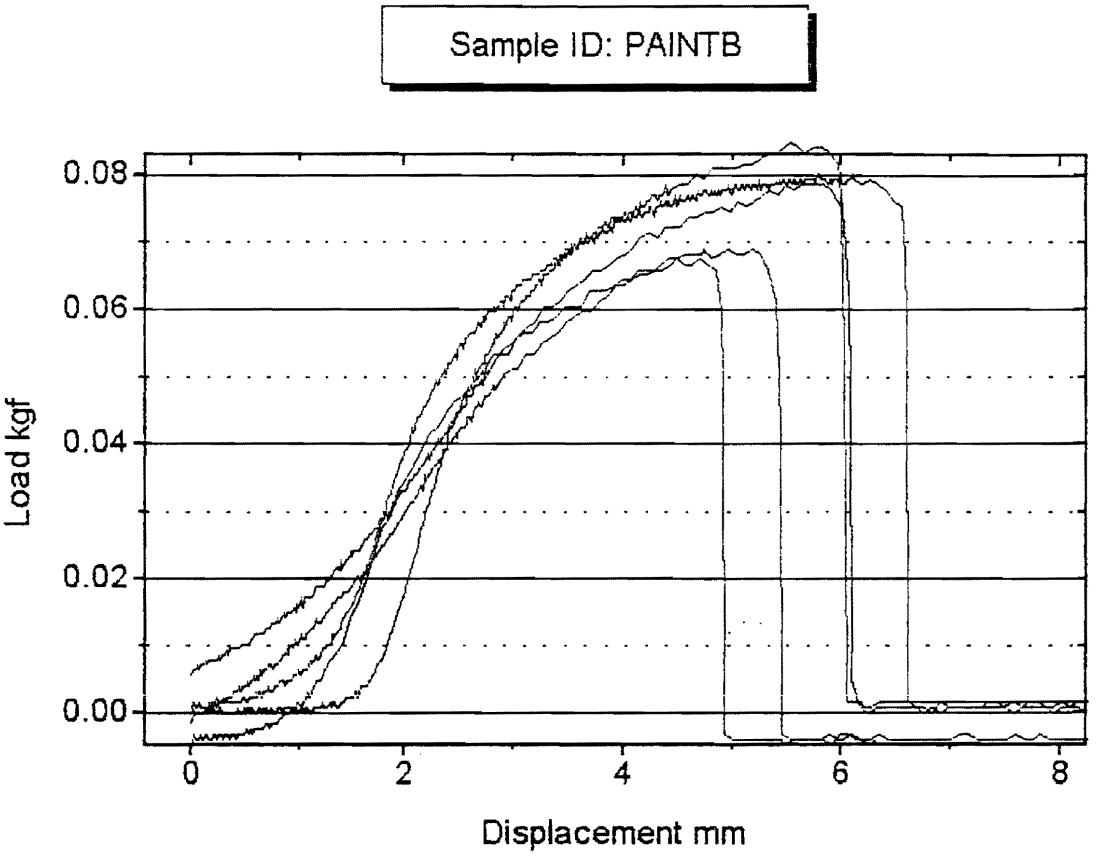
Humidity (%): 50
Temperature: 73 F

S.W. Commercial

Sample comments: Paint Sample B (Flat Paint) brittle

	Maximum Percent Strain (%)	Tensile Strength (UTS) (kgf/mm^2)	Energy to Break Point (kgf-mm)	Displcment at user Break (mm)	Stress at user Break (kgf/mm^2)	Strain at user Break (mm/mm)	Stress at offset Yield 1 (kgf/mm^2)	Displcment at offset Yield 1 (mm)	Strain at offset Yield 1 (mm/mm)
1	12.655	0.238	0.215	5.448	0	0.109	0.016	1.201	0.024
2	28.151	-0.011	0.183	4.920	0	0.098	0.068	1.582	0.032
3	27.536	0.005	0.306	13.768	0.005	0.275	0.122	2.109	0.042
4	61.135	0.276	0.374	30.568	0.005	0.611	0.062	1.567	0.031
5	44.321	0.292	0.298	22.160	0.005	0.443	0.059	1.992	0.040
Mean	34.759	0.160	0.275	15.373	0.003	0.307	0.065	1.690	0.034
S.D.	18.517	0.150	0.076	11.037	0.003	0.221	0.038	0.365	0.007

	Maximum Displcment (mm)	Yield Point Elongation (mm)	Grip Distance (mm)
1	6.327	--	50.000
2	14.075	--	50.000
3	13.768	--	50.000
4	30.568	--	50.000
5	22.160	--	50.000
Mean	17.380	0	50.000
S.D.	9.259	0	0


Sample ID: PAINTB
0.08
0.06
0.04
0.02
0.00
Load kgf
0
2
4
6
8
Displacement mm

Tensile Test 550

Tensile 550
For 550 class 1997
Using 2000 Gm load cell

Test type: Tensile
Operator name: Haibin Tang
Sample Identification: PAINTA
Interface Type: 1120

Instron Corporation
Series IX Automated Materials Testing System 7.27.00
Test Date: Wednesday, March 19, 1997

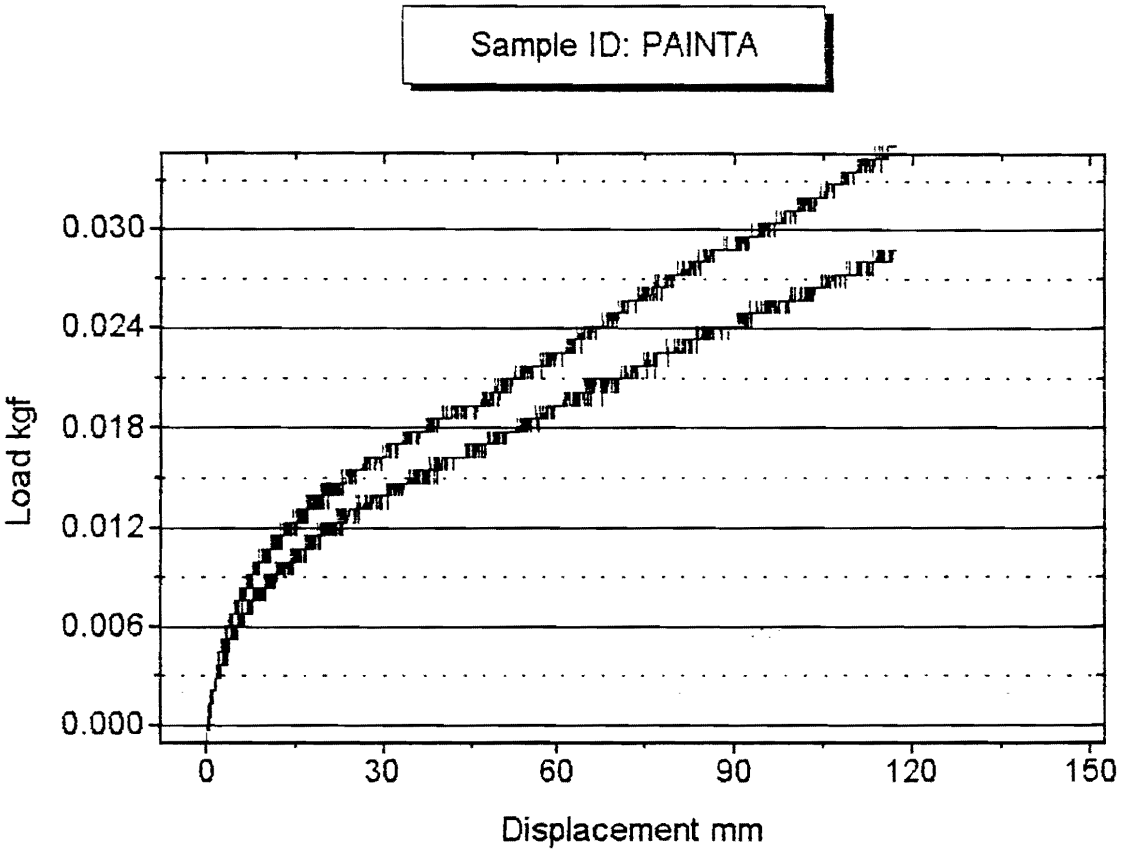
Sample Rate (pts/secs): 2.2758
Crosshead Speed: 2.0000 mm/min
2nd Crosshead Speed: 0.0000 mm/min
Full Scale Load Range: 2.000 kgf

Humidity (%): 50
Temperature: 73 F

Sample comments: Paint A (Semi Gloss) Very elastic Near 100% Recovery

	Maximum Percent Strain (%)	Tensile Strength (UTS) (kgf/mm^2)	Energy to Break Point (kgf-mm)	Displcment at user Break (mm)	Stress at user Break (kgf/mm^2)	Strain at user Break (mm/mm)	Stress at offset Yield 1 (kgf/mm^2)	Displcment at offset Yield 1 (mm)	Strain at offset Yield 1 (mm/mm)
1	234.288	0.237	2.166	117.144	0.237	2.343	0.004	0.015	0.000
2	234.288	0.289	2.586	117.144	0.289	2.343	-0.002	0.337	0.007
Mean	234.288	0.263	2.376	117.144	0.263	2.343	0.001	0.176	0.004
S.D.	0.000	0.037	0.297	0.000	0.037	0	0.005	0.228	0.005

	Maximum Displcment (mm)	Yield Point Elongation (mm)	Grip Distance (mm)
1	117.144	7.919	50.000
2	117.144	8.166	50.000
Mean	117.144	8.043	50.000
S.D.	0.000	0.175	0


Sample ID: PAINTA
0.030
0.024
0.018
0.012
0.006
0.000
Load kgf
0
30
60
90
120
150
Displacement mm