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Elastomers based on polyesters produced by the bacterium Pseudomonas oleovorans/

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ELASTOMERS BASED ON POLYESTERS PRODUCED BY THE BACTERIUM *PSEUDOMONAS OLEOVORANS*

A Dissertation Presented

by

KARLA DREW GAGNON

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Polymer Science and Engineering Department

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Both my research groups have been a constant source of stimulating ideas and knowledge. I cannot possibly name all the people who have made graduate school such a rewarding experience for me. All I can say is thank you.

Special mention to my parents and family for their support, love, and encouragement. And finally thanks to my husband, Eric Shaw, a person I unexpectedly came across here. He is one of the reasons deciding to return to school for a graduate degree became a very good decision.

ABSTRACT

ELASTOMERS BASED ON POLYESTERS

PRODUCED BY THE BACTERIUM *PSEUDOMONAS OLEOVORANS*

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This dissertation has explored the structure property relationships of as extracted and chemically modified polyesters produced by the bacteria *Pseudomonas oleovorans*. The as extracted polymers are considered biodegradable thermoplastic elastomers. The two polymers studied were: poly(β-hydroxyoctanoate), PHO, and poly(β-hydroxyoctanoate-co-β-hydroxyundecylenic acid), PHOU.

A fed batch biosynthesis was developed and the polymer yield was increased tenfold by optimizing feeding times of the carbon source(s). Batch-to-batch consistency was evaluated using GC, GPC, DSC, and TGA techniques.

Mechanical property evaluations included the determination of tensile properties, tensile set, and hardness. The physical network structure was elucidated through equilibrium modulus determination coupled with rubber elasticity theory. The testing results revealed a material that compared well to other commercially available thermoplastic elastomers (also tested) but the elastic recovery was relatively poor. A thermal analysis of stretched samples provided possible explanations for the moderate elastic response exhibited by the material. The adhesive properties of PHO to paper were also evaluated and compared to several commercial tapes.

Crystallization studies were conducted to understand the kinetics of crystallization and the effect of thermal history on the material properties. Several nucleating agents were evaluated to try and increase the rate of crystallization.

The bacterial polyesters were chemically crosslinked in order to improve the elastic recovery. PHO, a totally saturated polymer, was crosslinked with a variety of peroxides both with and without multifunctional coagents. A controlled level of unsaturation was incorporated into the polymer by growing the bacteria on a mixture of carbon sources yielding PHOU. Using these reactive moieties, various crosslinking reactions were evaluated including sulfur vulcanization in addition to the peroxide crosslinking reactions. The modified polymers displayed better elastic response than the as extracted thermoplastic elastomer. The network structure was evaluated using a dynamic mechanical analysis technique called impulse viscoelasticity.

Preliminary results of a study aimed at determining if PHO and PHOU biodegrade and the effect of crosslinking on the biodegradation of these materials are included. The study is being conducted jointly with the Microbiology Department at the University of Massachusetts, Amherst.

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CHAPTER 1

INTRODUCTION AND BACKGROUND

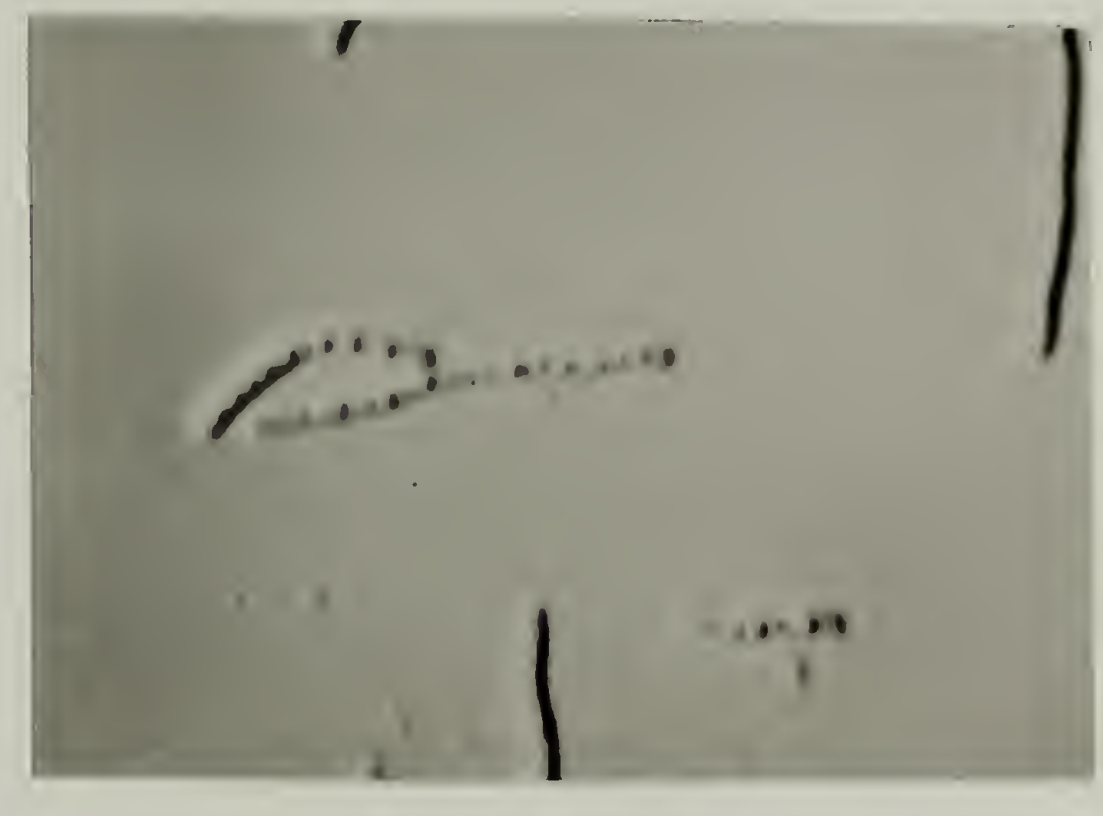
1.1 Introduction

The existence of elastomers in plants has been known since the 1500's.[1] Natural rubber continues as a main source of elastomeric material even with the advent of many synthetic elastomers due in part to the renewable resource, plants.

Since the 1920's polyesters, generally known as poly(β-hydroxyalkanoates), (PHAs), have been known to accumulate in various bacteria.[2] The polymer accumulates in intracellular inclusion bodies as seen in Figure 1.1. The polymer has been shown to be a reserve carbon and energy source for the microorganisms [3] much like fat is for animals or starch is for plants. These researchers found that polymer accumulation was triggered when the environment of the bacteria either lacked, or was limited in, an essential nutrient (such as nitrogen, in the form of ammonium) but had an excess of a carbon food source (such as glucose). The phenomenon is similar to a bear accumulating fat because of the environmental stress caused by the approach of winter.

Bacterial *thermoplastic* polyesters have been studied for approximately 70 years. The most widely recognized is poly(β-hydroxybutyrate), PHB, which was shown to be a brittle thermoplastic.[4] Since 1981 two PHAs, PHB and the more useful P(HB/HV), have been commercially available from ICI under the tradename Biopol.[5] The copolymer poly(β-hydroxybutyrate-co-β-hydroxyvalerate), P(HB/HV), is produced by the bacterium, *Alcaligenes eutrophus*, when grown on a mixture of glucose and propionic acid. The processed material has thermal and mechanical properties similar to polypropylene.[5, 6] The material is presently being marketed in Europe in the form of a shampoo bottle.[7]

a) computer enhanced optical micrograph of *Pseudomonas oleovorans* living cells, 2000X, bulging with polymer inclusion bodies.



b) thin section of another PHA accumulating bacteria, *Rhodospirilus rubrum*, 30,000X.

Figure 1.1 Bacteria with accumulated PHA.

What nature makes, nature destroys is the logic behind investigating naturally produced biopolymers as a source of truly biodegradable materials. Biodegradation is a real property advantage at a time when landfills are near full and plastic packaging is being blamed, fairly or not, [8] for a large volume of the waste stream.

Because PHAs are naturally produced and degraded by bacteria in response to environmental changes, the polymer is inherently biodegradable. When PHB or P(HB/HV) is extracted and processed, these polymers have been shown to biodegrade in various environments.[6-7, 9-14] Studies have indicated certain bacteria excrete polymer degrading enzymes (depolymerases) which enable the bacteria to use the processed polymer as their sole exogenous carbon source [15, 16] and fungus colonate the materials extensively.[17]

Besides being biodegradable, the polyesters produced by bacteria have the advantage of using renewable food sources such as glucose. Sometimes a food source is found which is considered part of a commercial waste stream; such as the use of whey, a waste product in cheese manufacturing.[18]

But, what if carbon food sources were used that are not normally found in the environment yet the bacteria could be coaxed to use these substrates for polymer production? Or, what if chemical modification was done on the extracted polymers to enhance the material's mechanical properties? Could nature still destroy these unusual or modified materials?

The concept of chemical modification brings up the example of vulcanized natural rubber. Unfortunately, few biodegradation studies have been conducted on unmodified natural rubber, the most prevalent elastomeric biopolymer. One review article [19] reported that thin strips of dried natural rubber were completely decomposed when buried in soil for 3 to 4 weeks by an organism identified as a species of the genus *Streptomyces*. Several species of *Actinomycetes* have also been identified as able to biodegrade unadulterated natural rubber.

Insufficient biodegradation studies on modified natural rubber have been conducted. One microorganism, *Nocardia asteroides*, has been identified as able to 'attack' vulcanized rubber but it was not clear if the rubber itself or additives of vegetable origin were attacked.[20] Mainly the degrading effects of ozone, heat, and ultraviolet radiation have been studied with the focus on identifying chemical compounds which could be added to slow down the degradative processes.[21] An interesting article tells of a downed airplane that was found with a natural rubber tire still inflated after 44 years at the bottom of Loch Ness under 70 meters of fresh water. [22] This finding implies that at least in that water environment, organisms capable of degrading vulcanized, carbon black filled natural rubber were not present.

Therefore, biodegradation studies of all new and modified PHAs must be considered an essential part of the material evaluation process. Understanding of the mechanism of enzymatic attack might also guide the type of modifications that can be done to a material without destroying the capacity of the material to undergo enzymatic degradation.

This dissertation has focused on the bacterium, *Pseudomonas oleovorans*, which produces polymers exhibiting elastomeric behavior [23] when grown on various long chain carbon sources. [23-39] The goal of this research is to enhance the understanding of the structure-property relationship behind the elastomeric behavior. Both as extracted and chemically modified PHAs were studied. Chemical modification was conducted in an attempt to improve the elastic response of the polymer.

1.2 Background on Elastomeric Polymers

Elastomeric behavior of solids which can be described as the ability of a material to be extended up to several hundred percent and then immediately recover upon release of the deforming stress is unique to polymers. For a material to behave in this way, three

general conditions are necessary. The material must be composed of long chain molecules which are essentially freely jointed, only weak secondary forces between molecules are present, and a three dimensional network of interconnected chains is present due to an interlocking of the molecules at a few places along their length.[40] The nature of the junction points subdivides the materials into thermoplastic elastomers (physical crosslinks) or vulcanized elastomers (chemical crosslinks).

1.2.1 Crosslinked or Vulcanized Elastomers

Vulcanized elastomers are the more prolific elastomers. This class is also known as chemically crosslinked or thermoset rubbers. Vulcanized natural rubber is the first and still most important example in this class. Several books expound upon the interesting history of natural rubber and the vulcanization process first discovered in 1836 by Goodyear.[1,21,41] The interlocking points which produce a network are formed by covalent bonds during the curing step of crosslinking. Figure 1.2 depicts an idealized structure of a vulcanized elastomer.

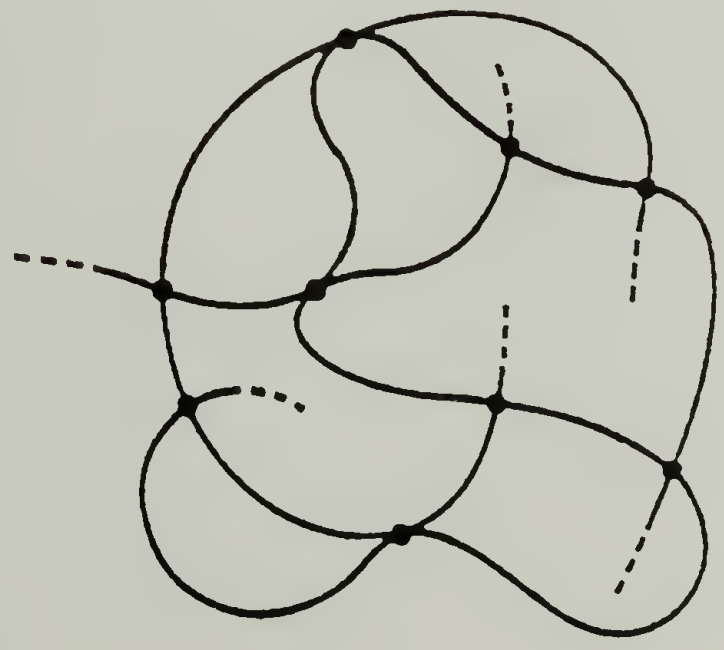


Fig. 1.2 Idealized structure of a vulcanized elastomer.

The materials of this class generally exhibit low tensile set and can withstand high temperatures during use. A high use temperature is possible because the crosslinks are permanent covalent bonds and heating will not produced a polymer capable of flow. Excessive heat will simply decompose the covalent bonds and destroy the network and basic polymer. Another feature of crosslinked elastomers is that the materials will not dissolve but simply swell when in contact with solvents. Also, very soft rubbers are achievable since crosslinking can be controlled to produce materials with very large molecular weights between crosslinks.

The raw materials are relatively inexpensive (although the synthetic rubber stocks are from petrochemicals), but processing these materials requires several time consuming steps which add cost to the final product. The material must be compounded with appropriate curing agents, formed into the desired shape, then heat cured. The presence of a covalent network in the cured material precludes recycling of scrap and finished goods. Automobile tires are produced from vulcanized rubbers and illustrate the problem associated with the disposal of crosslinked materials.

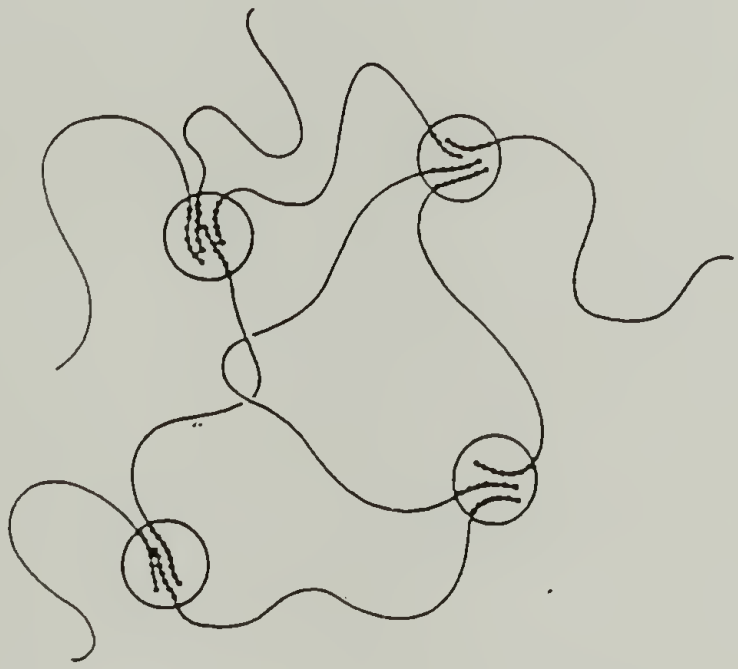
1.2.2 Thermoplastic Elastomers (TPEs)

Thermoplastic elastomers are a more recent development. A good history of TPE development can be found in the book edited by Legge *et. al.*.[42] The network of TPEs are formed from reversible physical crosslinks. These physical crosslinks can be ionic bonds, hydrogen bonds or can form during solidification of block copolymers. The most common TPEs are those composed of block copolymers where chemically different blocks form a two phase morphology during solidification. Typically one block called the hard block either vitrifies or crystallizes forming the network of physical crosslinks while the soft blocks remain amorphous and above their glass transition temperature. Either

triblock or multiblock copolymers have been found useful as TPEs. The morphology of the triblock or multiblock copolymers are shown in Figure 1.3.

There are six classes of commercial TPEs: styrenics, urethanes, copolyesters, amides, olefinics, and elastomeric alloys with the first four listed being block copolymers. The hard blocks can be glassy, as for styrenics and some urethanes, or crystallites as in copolyesters, amides, and some urethanes. Table 1.1 summarizes the types of commercial (and one non-commercial) TPEs.

a) long block TPE



b) short block TPE

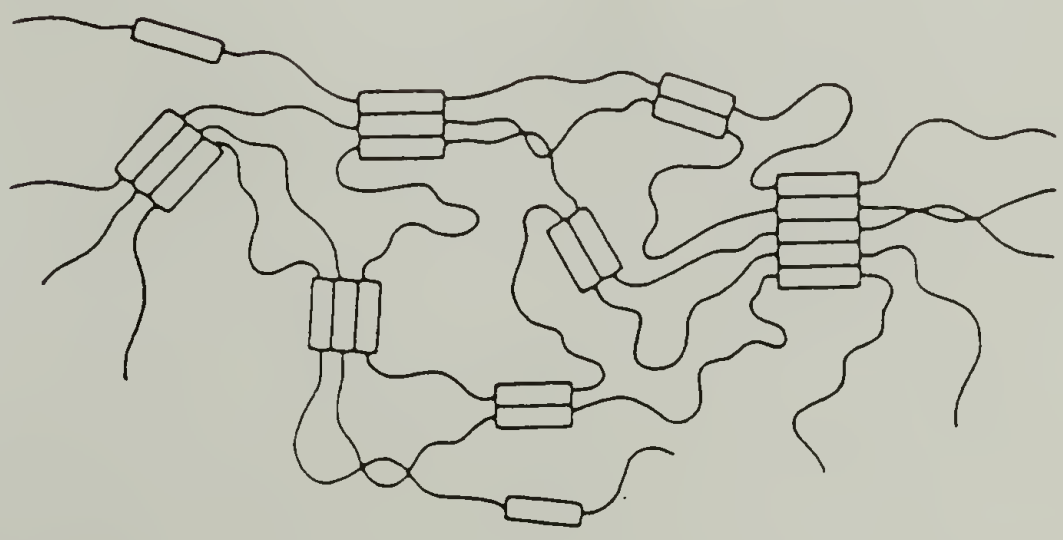


Figure 1.3 Representation of different TPE morphologies.

Table 1.1 Summary of the different types of thermoplastic elastomers.

CLASS OF TPE	CHEMICAL STRUCTURE OR MORPHOLOGY	DESCRIPTION
amide	multiblock	crystallizable hard segment
copolyester	multiblock	crystallizable hard segment
elastomeric alloy	miscible blend	MPR - melt processable rubber thermoplastic matrix with compatible polymeric plasticizers
	immiscible blend	TPV - thermoplastic vulcanizate thermoplastic matrix (minor component) dispersed thermoset rubber (major component)
styrenic	triblock	glassy hard segment
olefinic	multiblock	stereoblock homopolymer: alternating crystallizable (isotactic) and amorphous (atactic) sections
	immiscible blend	thermoplastic matrix (minor component) dispersed thermoset rubber (major component)
	graft copolymer	crystallizable grafts on amorphous backbone
urethane	multiblock	glassy or crystallizable hard segments

TPEs do not have as high a use temperature as vulcanized rubbers because the elastic network can be disrupted by heating the hard segments above their softening or melting temperature or by dissolving the material in a solvent. This feature, however, makes recycling of scrap and finished products possible.

In general TPEs exhibit high tensile or compression set. Very soft thermoplastic elastomeric materials cannot be produced because a minimum amount of hard segments must be incorporated to insure phase separation occurs. The starting polymers are more expensive but these materials can be processed on conventional injection molding and extrusion equipment and no cure cycle is required.

1.3 Background on Poly(ß-hydroxyalkanoates), PHAs

1.3.1 History of PHAs

In the mid-1920's, Lemoigne discovered that the lipid containing inclusion bodies he observed in *Bacillus megaterium* were actually a polymer of ß-hydroxybutyrate, (PHB) [2, 43, 44] with the chemical structure shown in Figure 1.4. During the 1950's and 1960's, many different bacteria were found to accumulate PHB. A list of genera known to accumulate PHB is contained in the 1973 review by Dawes and Senior.[3]

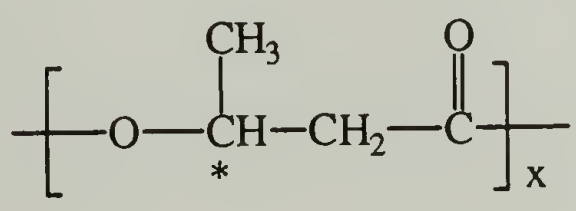


Figure 1.4 Chemical structure of poly(β-hydroxybutyrate), (PHB). The asterisk indicates the chiral carbon which is in the absolute [R] stereoconfiguration..

Workers experimenting with polymer extracted from activated sewage sludge identified another major hydroxyacid repeat unit in the polymer, hydroxyvalerate. [45] The name PHA, poly(β-hydroxyalkanoate), was used to describe this copolymer. See Figure 1.5 for the chemical structure of the P(HB/HV) copolymer.

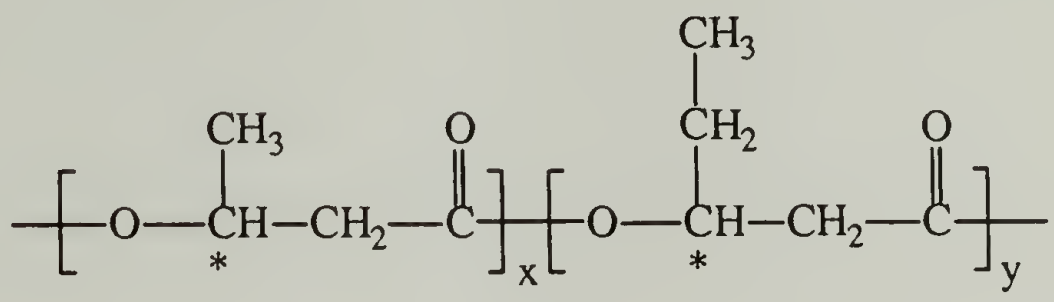


Figure 1.5 Chemical structure of the copolymer P(HB/HV). The asterisk indicates the chiral carbon which is in the absolute [R] stereoconfiguration.

Analysis of sewage sludge in 1983 by a capillary gas-liquid chromatographic technique [46] resulted in finding the presence of at least 11 different beta-hydroxyacids.[47] These findings indicated that polymers accumulated by bacteria were not limited to PHB or PHV. The name PHA was adopted in 1983 to describe bacterially produced polymers with the general repeat unit chemical structure depicted in Figure 1.6.

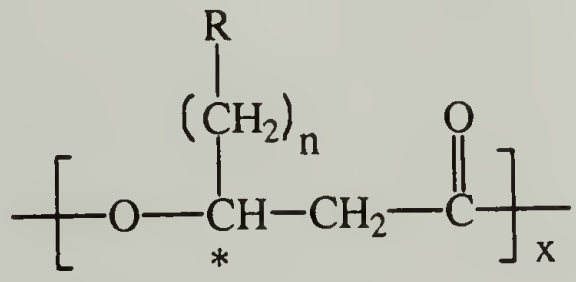


Figure 1.6 General chemical structure of the repeat unit for PHAs. The asterisk indicates the chiral carbon in the absolute [R] stereoconfiguration.

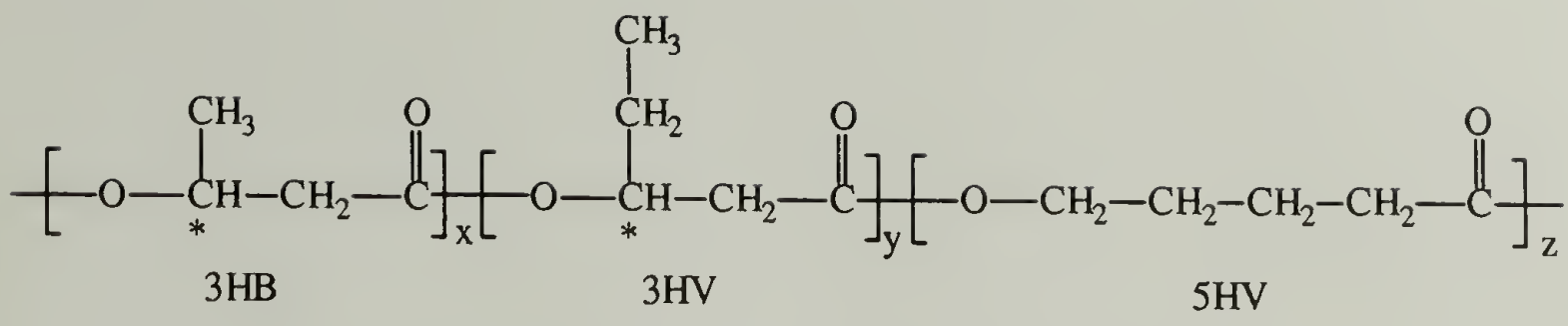
Further investigation revealed different repeat unit structures could be attained in *Alcaligenes eutrophus* when the bacteria was grown on certain substrates. [47-49] This necessitated a change to the repeat unit designation. A number was now included which designates the number of carbons in the backbone of the repeat unit. See Fig. 1.7 for structures of these different PHAs.

Many different aerobic bacteria have been found to accumulate PHAs. The list of PHB accumulating bacteria from Dawes and Senior [3] has been updated by Brandl [12] to include all presently known PHA producing bacteria.

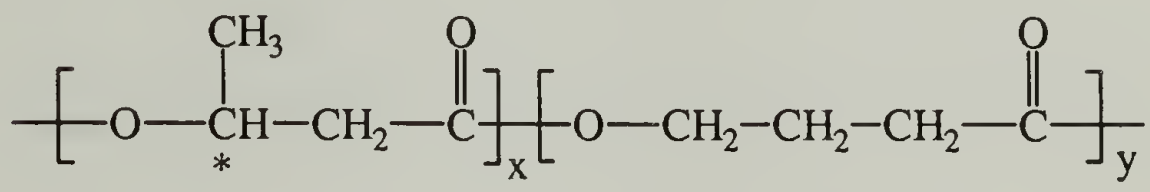
The most widely studied species that accumulate PHAs have been *Alcaligenes eutrophus* and *Pseudomonas oleovorans*. These two species exemplify an interesting divergence. *A. eutrophus* produces PHAs with relatively short chain substituents whether grown on short or long chain carbon sources. *P. oleovorans* on the other hand can only produce polymer when grown on carbon sources with at least 6 carbons. The PHAs produced contain relatively long pendant groups. [32] This difference in pendant group length results in different material behavior. The next section will focus on the polymers produced by *Pseudomonas oleovorans*.

1.3.2 PHAs produced by *Pseudomonas oleovorans*

In 1941, investigators discovered a bacterium capable of surviving in and on an emulsion of cutting/lubricating oil and water in a machine shop. [33] The bacteria was named *Pseudomonas oleovorans* since the bacteria resembled another type of bacteria called a monas (hence 'pseudo' monas) and the bacteria survived on oil or fat ('oleo' - fat; 'vorans' - eater).



substrates: 5-chloropentanoic and pentanoic acids



substrates: 4-hydroxybutyric acid or γ-butyrolactone

Fig. 1.7 Structure of PHAs with unusual repeat units produced by *Alcaligenes eutrophus* grown on the substrates indicated. Polymers have been determined to be random copolymers.

In 1983, while researchers were trying to optimize the production of 1,2 epoxyoctane by *Pseudomonas oleovorans* when grown on octane, intracellular inclusions which looked like the commonly known reserve material PHB, were observed. [34] Upon analysis, the material was found to be PHO, poly(β-hydroxyoctanoate). [24]

The polymers produced by *Pseudomonas oleovorans* have been shown to have the basic chemical structure as depicted in Figure 1.6. [24, 25] The R group and n length have been found to depend on the carbon source used as the growth substrate. The polymer was found to be optically active [35] with all chiral carbons in the absolute [R] stereoconfiguration [25] which indicates the polymer is 100% isotactic.

The study of this organism and the PHAs produced have been investigated when the bacteria was grown on C_6 to C_{12} n-alkanes [23-27], C_8 to C_{10} n-alkenes [25, 26], C_1 to C_{16} n-alkanoic acids [30], C_{18} alkenoic acids [30], C_1 to C_{10} sodium salts of n-alkanoic acids [28, 29], and C_8 to C_{10} alcohols. [30, 31] A compilation of the results from the different investigations including polymer characterization results are given by Lenz *et. al.*. [32] The main findings are summarized below.

1) Polymer was produced only on substrates with at least 6 carbons.

2) Except for hexane and heptane, when *P. oleovorans* is grown on a single carbon source, a copolymer with at least 2 and up to 8 different repeat units was produced.

3) The copolymers are random. [51]

4) The chiral carbon in the backbone is in the absolute [R] stereoconfiguration.[25]

5) For C_6 to C_9 substrates, the dominant repeat unit in the polymer contained the same number of repeat units as the carbon source. The remaining repeat units were usually multiples of two carbons more or less than the growth substrate. This incorporation of several different repeat units has been explained in terms of the beta-oxidation metabolism of the organism.[25, 26] Sometimes, the repeat units contained one carbon less or more than the substrate. An explanation in terms of metabolic decarboxylation was given. [27]

6) For C_{10} to C_{16} substrates, the even substrates showed preferential accumulation of C_8 units while odd substrates showed a preferential accumulation of C_9 repeat units.

7) The oxidation state of the substrate appeared not to effect the polymer composition in one study [30] but another study indicated otherwise.[32]

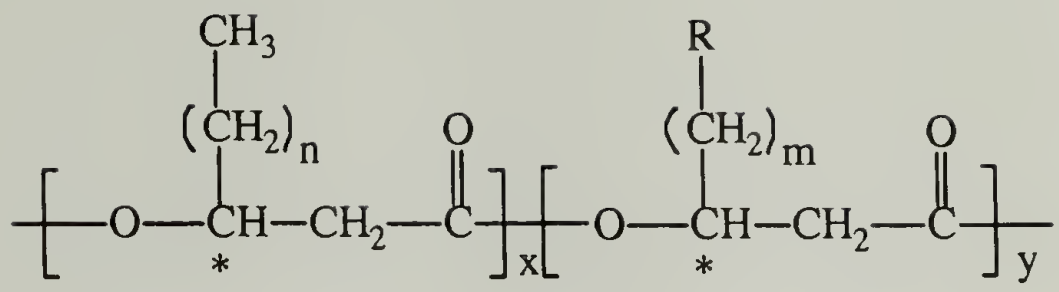
8) Alkene substrates produced a mixture of alkyl pendant groups and pendant groups with terminal double bonds.

The flexibility of the organism to grow and produce unusual polymers on novel substrates or co-substrates has been demonstrated. Co-substrates can be used to coax the bacteria to incorporate repeat units from a non-polymer producing substrate if mixed with a polymer producing substrate. [36, 38, 39] Polymers with various R groups including: an unsaturated group [25-27, 36, 37], a methyl branched group [38], a phenyl group [35, 36], a cyano group [36], a methyl ester group [36], a benzyl ester group [36], a cyclohexyl group [36], a hydroxyl group [36], a bromo group [36, 39], a chloro group [14], and a fluoro group [14]. See Figure 1.8 for the chemical structures of these unusual polymers.

As the experiments with the organism continue and more exotic substrates and co-substrates are tested, more unique polymers will undoubtedly be produced. The range of material properties will also change because of the differing structures.

Poly(β-hydroxyoctanoate), PHO, the polymer produced when *Pseudomonas oleovorans* is fed octanoic acid or sodium octanoate, was chosen for further investigation because of the high polymer yield and high percentage of one type of repeat unit. The chemical structure of PHO is shown in Figure 1.9.

Poly(β-hydroxyoctanoate-co-β-hydroxyundecylenic acid), PHOU, produced when *Pseudomonas oleovorans* is fed a mixture of octanoic acid and 10-undecylenic acid, was also included in this study because of the reactive vinyl group located as the terminus of the pendant chain as shown in Figure 1.10. Although alkenes have been shown to produce repeat units with olefin terminated pendant groups, [25, 26] neat 10-undecylenic acid produced 100% olefin containing repeat units [36] whereas other alkenes did not. The 10-undecylenic acid would facilitate the incorporation of a small number of olefin containing repeat units.



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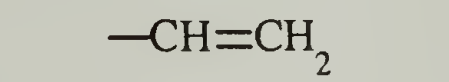

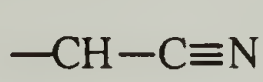

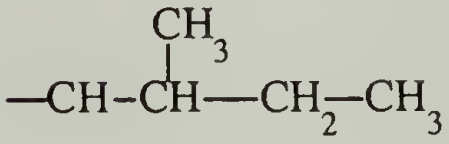

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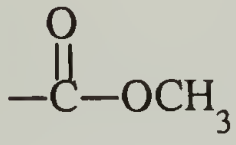

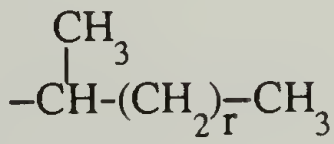

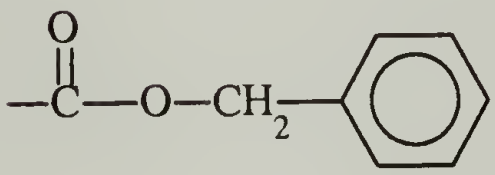

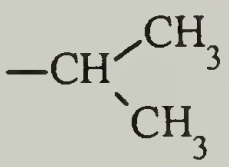

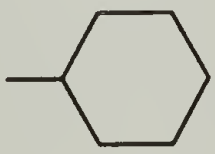

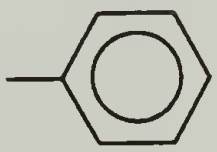

Figure 1.8 Chemical structures of PHAs produced by *Pseudomonas oleovorans* with unusual R groups. The iso-alkyl group and phenyl group polymers did not contain the first repeat unit depicted in the chemical structure.

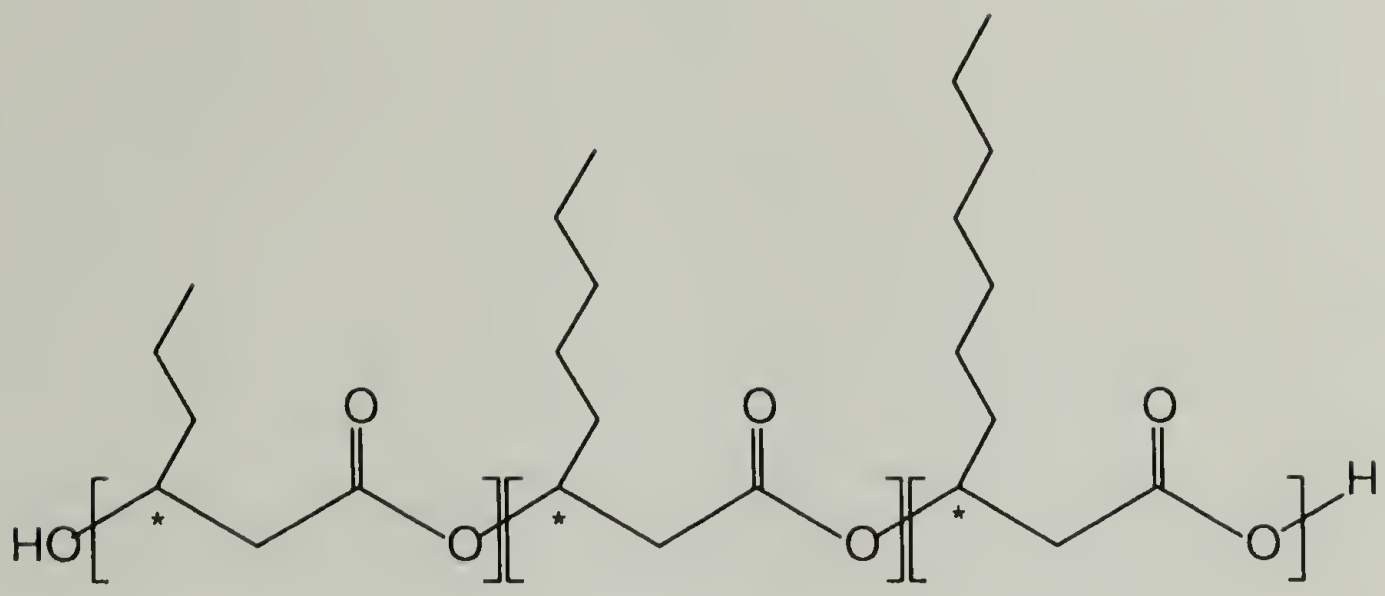

Figure 1.9 Chemical structure of PHO with the percent composition of repeat units indicated. C6 β-hydroxyhexanoate, C8 β-hydroxyoctanoate, C10 β-hydroxydecanoate.

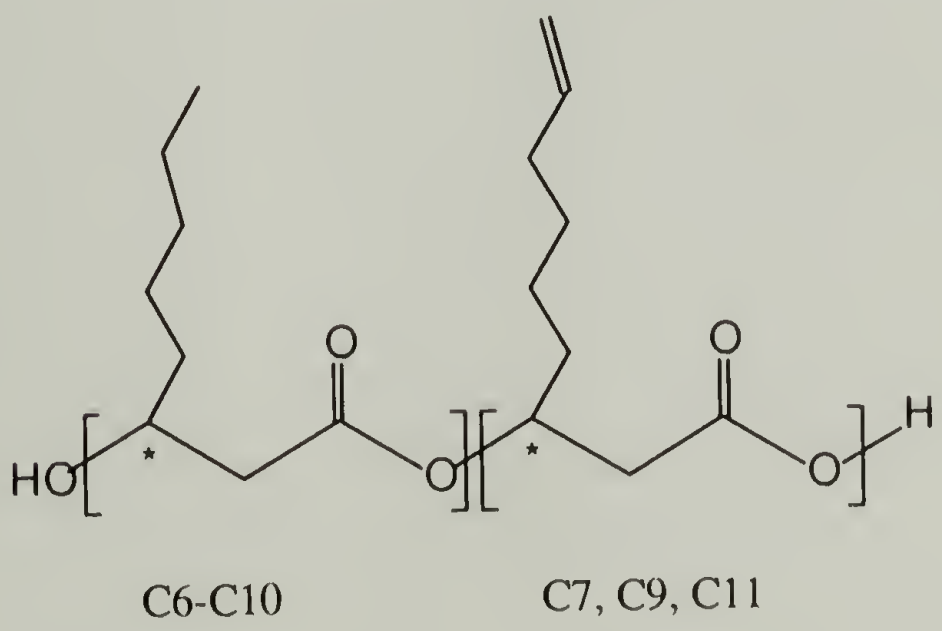

Figure 1.10 Chemical structure of PHOU. C(#) indicates the number of carbon atoms in the repeat unit.

1.3.3 Uses for PHAs

Investigators have realized the potential uses for bacterially produced thermoplastic polymers such as PHB and P(HB/HV). In the late 1950's and early 1960's, W. R. Grace and Co. produced small quantities of PHB for commercial evaluation [6] with ideas for the polymer including films, molded articles, surface coatings, fibers, and medical uses such as sutures and films to support injured arteries as outlined in 2 patents. [52, 53] ICI, however, was the first to commercially develop the biopolymers in 1981 [5, 54, 55] and have expanded the ideas for the uses of the material to include film moisture barriers for diapers, seedling containers, and sheaths for protecting saplings. A myriad of medical uses have been envisioned including controlled drug release for animals and humans, surgical swabs, wound dressings, powdered glove lubricant, vascular grafts and bone fracture fixation plates. If PHB is hydrolyzed to form beta-hydroxybutyrate, a common blood constituent, the monomers could be used as an intravenous or oral carbon supply, or as a stereoregular organic building block for drugs. [6, 56] Recent ideas include controlled pyrolysis of PHB to obtain optically pure vinyl compounds. [57] and a summary of proposed uses is included in the review by Brandl *et. al.* and a book by Doi. [12, 14]

Pseudomonas oleovorans, which produces elastomeric polymers, has opened the door to new uses for bacterially produced polymers which could take advantage of a whole new set of material properties. The chemical modification which is aimed at improving the elastic properties may result in a biodegradable crosslinked rubber.

1.4 Dissertation Objectives and Overview

This dissertation is concerned with the structure-property relationships of as extracted PHO and chemically modified PHO and PHOU.

Chapter 2 describes the PHO yield study conducted to increase polymer production and provide sufficient quantities of polymer for testing. The technique and results of PHOU biosynthesis are also discussed.

Material property testing is outlined in Chapter 3. The morphology of as extracted PHO was modeled, the tensile, hardness, and tensile set properties were determined and all results compared to commercial TPEs. A thermal analysis was conducted to understand the possible mechanisms of tensile set for PHO. Adhesive properties of PHO were evaluated and compared to two commercial tapes. Other properties reported include thermal expansion, optical rotation, and the solubility parameter estimated from studies with several solvents.

Since the physical crosslinks for as extracted PHO are crystalline regions, several studies on PHO crystallization were conducted and are discussed in Chapter 4. The studies included short term kinetics, possible nucleating agents, and long term crystallite development including the effect on mechanical properties. These studies were done to understand the kinetics of crystallization, to try and increase the rate of crystallization, and to insure preparation of consistent samples for mechanical evaluations. The effect of olefin content on the thermal transitions temperatures is also included.

A change to the structure of the polymers was accomplished by chemical crosslinking. Chapter 5 discusses the chemical crosslinking of both PHO and PHOU. Several crosslinking chemistries were evaluated. Peroxide crosslinking was used for both PHO and PHOU both with and without multifunctional coagents. Crosslinking success was evaluated using a sol-gel analysis and the results analyzed using a peroxide efficiency model. Optimization of several sulfur chemistries is discussed along with the methods employed to monitor the vulcanization process. Two other chemistries including the use of a tetrafunctional sulfur compound and epoxidation of the olefin followed by crosslinking were also attempted with PHOU. The properties of the modified polymers

were evaluated including tensile properties, tensile set, thermal properties, and network evaluation.

The studies on the biodegradation of PHO both as extracted and chemically modified are discussed in Chapter 6.

The conclusions found by this study and questions raised are discussed in Chapter 7 along with possible future work aimed at answering the new questions raised by this dissertation and expanding the knowledge on these bacterial polyesters.

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CHAPTER 2

POLYMER BIOSYNTHESIS AND CHARACTERIZATION

2.1 Background

The accumulation of PHA in bacteria was shown to be associated with unbalanced growth conditions where there was an excess of the carbon food source but a deficiency in an essential nutrient.

Many studies have been done to identify and control the limiting nutrients to enable extensive accumulation of polymer, up to 80% of the dry biomass. The specific nutrients that trigger PHA accumulation appear to be different for different genera of bacteria. For *Pseudomonas oleovorans* the nutrients that trigger PHA production include ammonia, magnesium, phosphate, and potassium sulfate.[1] Oxygen limitation has recently been shown to induce PHA accumulation in *Pseudomonas oleovorans*.[2] The ease in which nitrogen limitation can be accomplished, simply omitting ammonia in the growth media, has made this type of fermentation more widely used and studied [3, 4] although oxygen limitation greatly improved the yield in the ICI P(HB/HV) biosynthesis process.[5]

The control of fermentation and all the nutrient levels can be quite elaborate but can result in high polymer yields. Using *Protomonas extorquens*, PHB production was maximized to 149 grams of PHB per liter.[6]

A batch culture was the growth technique used in early studies.[3] The process involved inoculating a sterile growth media with the bacteria of choice and monitoring bacterial growth through optical density (OD) measurements. Optical density correlates

to media turbidity and indicates bacterial concentration. [7] Batch cultures, with some type of limiting substance, showed a typical growth curve as depicted in Figure 2.1.

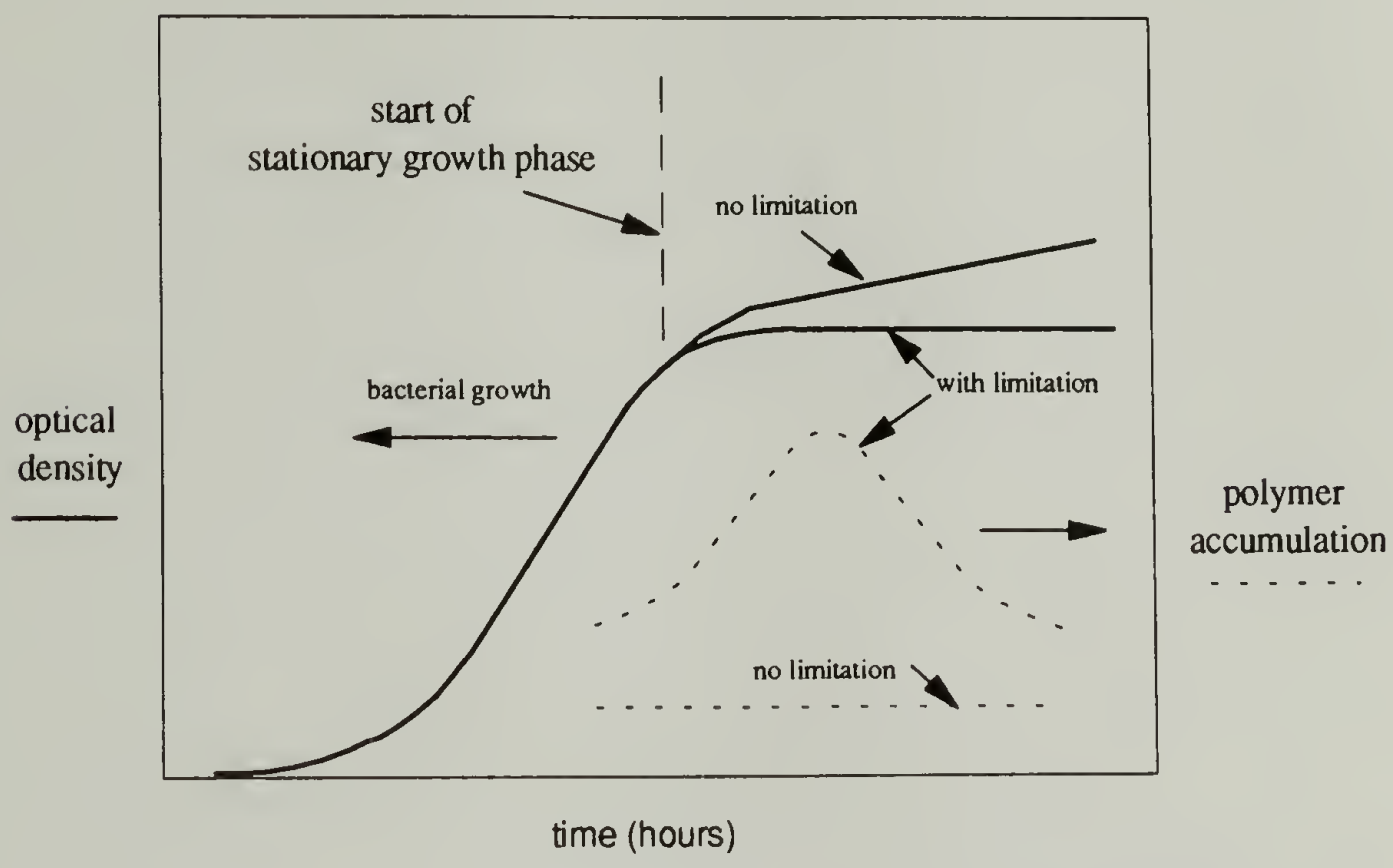

Figure 2.1 Typical bacterial growth and polymer production curve for a PHA producing bacteria under nutrient limitation. Adapted from reference 1.

Note the polymer accumulation peaked during the stationary growth phase which coincided with nutrient limitation and then the polymer was degraded, sometimes very quickly, as in the case of phosphate limitation in *P. oleovorans*.[1] The optimum harvesting time for maximum polymer yield was difficult to determine. Harvesting was accomplished by centrifuging the bacteria out of the media then lyophilizing (freeze drying) the biomass. Polymer extraction and purification are the next steps. The different extraction techniques will be discussed later.

Another type of batch culture is the two stage version, the technique used commercially.[8] First, the bacteria of choice is grown to a high concentration in a non-

limiting media. Then either the cells are centrifuged and resuspended into a nutrient deficient media [9, 10] or the media becomes depleted by not continuing the feeding of an essential nutrient.[6] The cells are now triggered into accumulating polymer. The centrifugation/resuspension technique has the advantage of not using an expensive carbon source for bacterial growth but only for polymer production.

Continuous culture experiments were begun in 1967 [3] by employing a chemostat. This technique affords the study on the effects of different stimuli or nutrient limitations. A fixed concentration of bacteria are maintained by diluting the culture with fresh media at a fixed rate. The technique is being investigated as a means to produce polymer on a continuous basis.[11,12]

A fully automated continuous fed batch culture technique has been developed by Suzuki using *Protomonas extorquens*.[6] Nitrogen limitation is induced after high cell concentrations are achieved by stopping the supply of ammonia. Oxygen concentrations are kept at non-limiting levels by monitoring the dissolved oxygen content and using an air/oxygen variable flowrate and stir speed system. All other trace nutrients are kept non-limiting by continually monitoring and feeding.

Solvent extraction with chloroform was the first technique used to isolate the material.[13, 14] A quicker extraction technique was developed which involved the digestion of the cells with an alkaline hypochlorite solution leaving behind the polymer granules.[15] The method was later shown to be extremely destructive to the polymer.[16-18] Recently, the hypochlorite digestion technique has been improved to limit polymer degradation.[19]

The literature is full of slight modifications to the basic solvent extraction technique and purification by precipitation in a non-solvent. Various solvents have been used including chloroform [13], pyridine [20], methylene chloride [21], 1,1,2 trichloroethane [22], and propylene or ethylene carbonate [23] to name a few. The use of most of these solvents was patented.

Another approach to extracting polymer, was the extraction then purification of the granules by enzymatic digestion of the other cell constituents followed by sonic disruption, centrifugation, and purification of the material by dialysis.[24] An enzymatic granule isolation technique was optimized and patented by ICI.[8]

The biosynthesis technique used in our research group was standardized but not optimized by a previous researcher in order to compare polymer production using different substrates. [25] The standard conditions for a 12 liter batch reactor included a high ammonium phosphate buffer growth media, 10 mM carbon source concentration, 2 liter per minute air flow, and 100 rpm stir speed. Typically, 3 grams of polymer were produced in a 24 hour batch biosynthesis. The stationary growth phase was believed to be triggered by a self-induced oxygen limitation. The environmental stress was considered self-induced because at some point all the cells would not get all the oxygen required to sustain unrestricted growth since the oxygen input was limited by the air flow rate and stir rate of the reactor. Once the stationary phase was reached, the bacteria were harvested immediately to insure the accumulated polymer was not degraded.

Polymer yield can be improved by increasing the number of cells present (perceived as a higher OD), by increasing the amount of polymer accumulated in the cells, by optimizing the harvesting time of the cells, and/or by preventing the internal degradation, or utilization, of the polymer by the bacteria. It should be noted that simply increasing the carbon source concentration was not viable because at high concentrations, the carbon source becomes toxic to the bacteria.

A multiple fed batch biosynthesis method was developed with the goal of increasing the number of cells produced and preventing polymer utilization by the organism thus negating the need to optimize the harvesting time. Sodium octanoate was chosen as the carbon source because of solubility in the aqueous media, the resulting polymer contained a large percent of one repeating unit, and the polymer was produced in high yield. [4]

Certain assumptions were utilized to determine the optimum feeding schedule of the carbon source. The assumptions were:

(1) Physiological Data -approximately 50% of the carbon source was used to produce biomass (cell dry weight.) as for similar bacterial systems.

(2) OD versus Dry Cell Weight - previous experimental evidence indicated that the OD and dry cell weight are linearly related.

(3) Sodium Octanoate Concentration - previous cell growth versus sodium octanoate concentration experiments indicated cell growth was optimized when the sodium octanoate concentration was between 10 mM and 30 mM, and concentrations above or below this range resulted in a sharp decrease in cell growth.

(4) Oxygen Limitation - To postpone this stress until higher cell concentrations, the air flow rate and stir rate of the reactor were increased to 5 l/min and 200 rpm (from 2 l/min and 100 rpm, respectively). These values could not be increased further because of foaming problems.

(5) Nitrogen Limitation - early nitrogen (ammonia) limitation critically hindered cell growth [4] but if nitrogen limitation could be coincided with the start of the stationary phase, the percent polymer accumulation could be increased. Calculations were done to determine when limitation occurred based on the assumptions that 10% to 15% of the biomass was made up of nitrogen and limitation occurred when the nitrogen concentration fell below 6 mM.

The first two assumptions gave a means to correlate OD, an easily measured parameter, with the carbon source usage. The third assumption coupled with the first two gave an easily measurable means (OD) to determine when the carbon source concentration fell below the optimum range. Therefore, feeding times were established when the OD increased by 2 i.e. 0 to 2, 2 to 4, etc.. Once experimental data was obtained to verify these assumptions, a flow rate was determined for use on a continuous feed apparatus using a peristaltic pump.

The chloroform solvent extraction technique was optimized for the higher production of biomass expected with the fed batch fermentation..

The polymer from different batches was thoroughly characterized using several techniques to insure a high batch-to-batch consistency was present. The techniques included GC of methanolysized polymer for composition [4], GPC for molecular weight determination, DSC for thermal transition temperatures, and TGA for decomposition temperature.

The biosynthesis of PHOU with controlled olefin content was accomplished by proportional mixing of two carbon sources: octanoic acid and 10-undecylenic acid. Previous studies indicated that although pure undecylenic acid requires longer biosynthesis times, a mixture of octanoic and undecylenic acids followed growth curves similar to the pure octanoic acid.[25] The designation used to describe such polymers is, for example, PHOU(95/5). The 95 indicates 95 mole % of the repeat units are saturated and 5 indicates 5 mole % of the repeat units contain an olefin group.

2.2 Experimental

2.2.1 Biosynthesis

A New Brunswick Microfermentor using 12 liter glass fermentation tanks was used for all biosynthesis. A stir rate of 200 rpm, 5 l/min air flowrate, and 31 °C were the standard growing conditions. Multiple or continuous feeding was achieved by either manually pouring in a concentrated feed solution or using a peristaltic pump and programmable timer.

The optical density of the culture was determined using a Bausch and Lomb Spectronic 20 instrument.

2.2.2 Gas Chromatography

The composition of the polymer was determined using a Perkin Elmer Model 8500 Gas Chromatograph equipped with DB-Wax megabore column and a flame ionization detector. Polymer or biomass samples were prepared using a methanolysis procedure developed in our lab [4]. The results are compared against known standards.

2.2.3 Gel Permeation Chromatography

Molecular weight data was obtained using either of two systems. One system consisted of a Waters Model 6000A solvent delivery system with a Model R401 differential refractometer detector and a Waters Ultrastyragel Linear column. A 1.0 ml/min chloroform flow rate was used with a 1,2-dichlorobenzene flowmarker. The other system consisted of a Rabbit Model solvent delivery system with a Waters Model R401 differential refractometer detector and three Polymer Labs PL 5 μ gel columns with mean pore diameters of 10^5Å, 10^4Å, and 10^3Å. A 2 ml/min tetrahydrofuran flow rate was used with a toluene flow marker. The molecular weight was based on polystyrene standards. All molecular weights were based on polystyrene standards.

2.2.4 Differential Scanning Calorimetry

Differential scanning calorimetry was conducted using a TA Instruments DSC Model 2910. Samples were tested from -85 to 100 °C at a 20 °C/min heating rate. The glass transition temperature reported is at the inflection point. The melting temperature reported is the peak temperature.

2.2.5 Thermogravimetric Analysis

Thermogravimetric analysis was conducted using a TA Instruments TGA Model 2950. TGA was conducted in both an air and nitrogen atmosphere from 30 °C to 500 °C at a 20 °C/min heating rate. Degradation temperatures are reported as the onset temperature of the weight loss.

2.2.6 Proton Nuclear Magnetic Resonance

The ^{1}H NMR spectra were obtained using either a Bruker 200 MHz or Varian 300 MHz ^{1}H NMR with 10 mg polymer/1 ml deuterated chloroform samples.

2.3 Results and Discussion

A schematic of the entire process of polymer production used in this study is shown in Figure 2.2. This schematic includes the modified solvent extraction technique which worked the best for large amounts of biomass.

2.3.1 PHO

Figure 2.3 compares the bacterial growth and polymer production curves for both batch and the new fed batch biosynthesis. The goals of increasing bacteria production (higher OD) and preventing polymer degradation were successful in increasing polymer yield approximately 10 fold as shown in Figure 2.4.

Different extraction techniques were tested during the polymer yield studies. These experiments account for some of the variation in polymer yield noted in Figure 2.4. Soxhlet extraction which can typically handle 15 grams of biomass was abandoned since

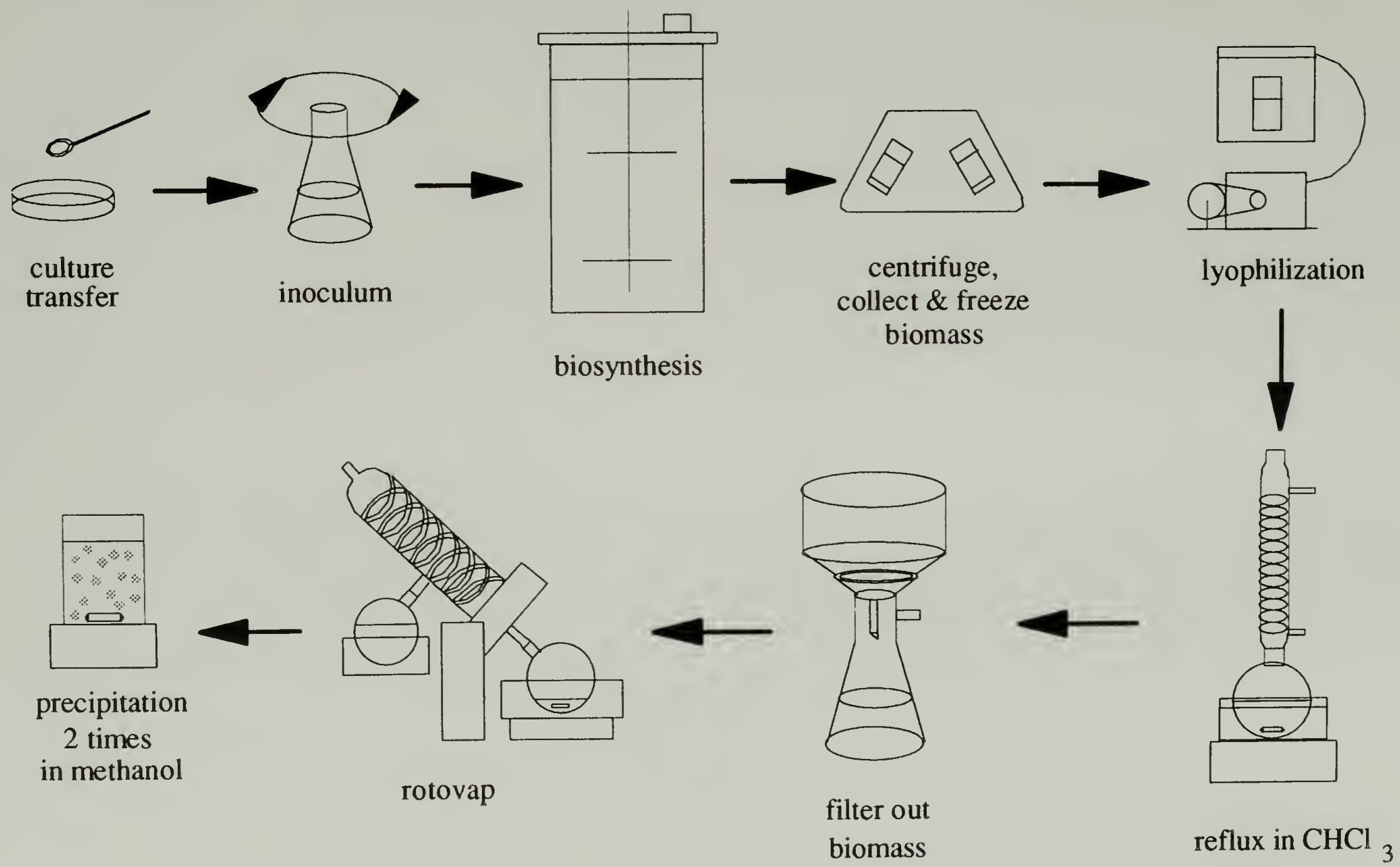

Figure 2.2 Schematic of the process used to obtain polymer from microorganisms.

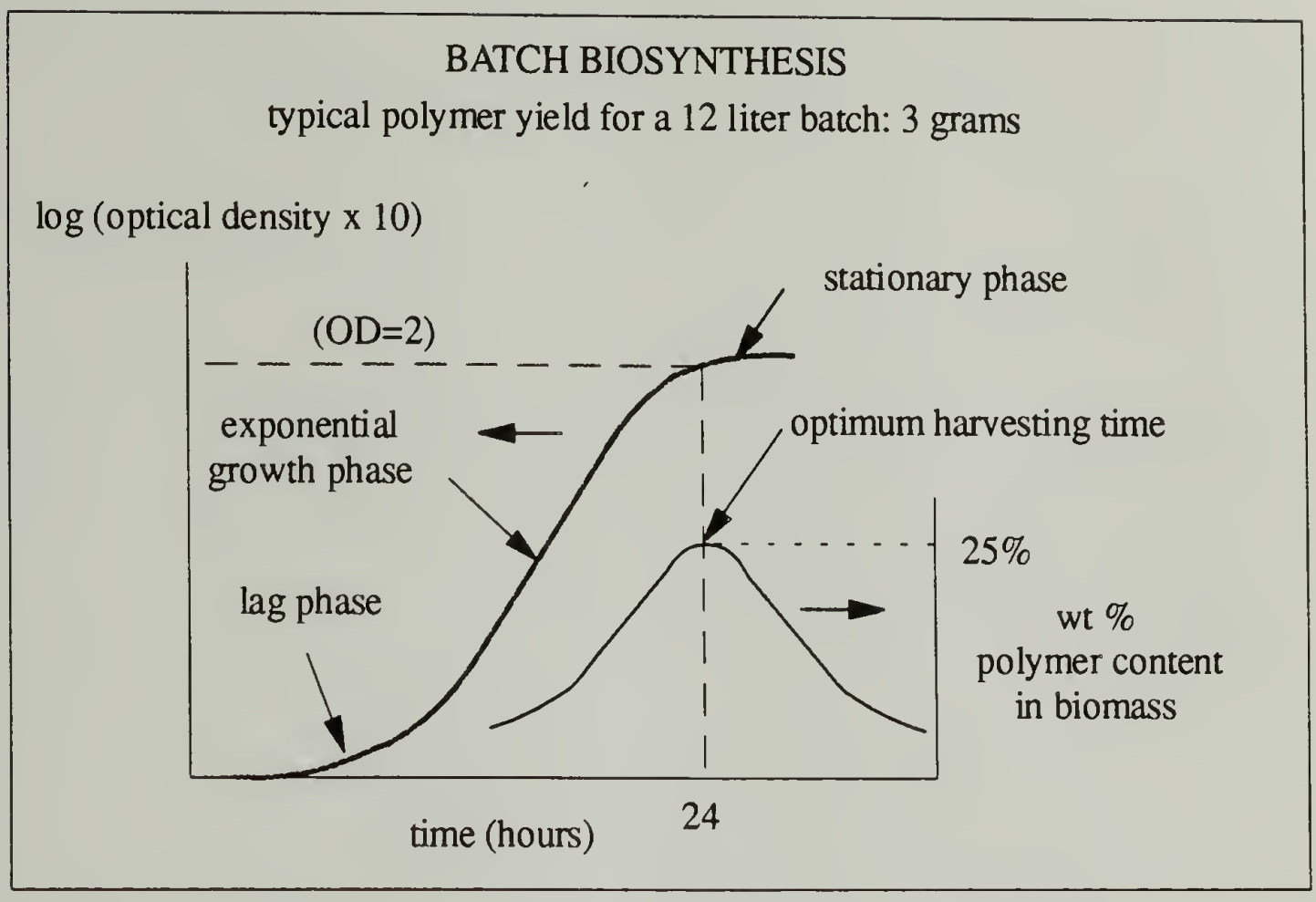

Figure 2.3 Comparison of bacterial growth and polymer production curves for both a batch and fed batch biosynthesis of *P. oleovorans* with sodium octanoate as the sole carbon source.

biomass yields started climbing towards 100 grams. Refluxing the wet biomass in chloroform was attempted. Unfortunately, the small amount of water present caused the released DNA and RNA of the cells to gel the biomass. The polymer could not be extracted from the gelled biomass. Partial recovery of polymer was achieved by sprinkling DNAse enzyme on the biomass which eliminated the DNA enough to de-gel the biomass and enable extraction of polymer. The best extraction method found was after lyophilization, the biomass was directly refluxed in chloroform for 48 hours. The biomass could easily be filtered off and the polymer solution recovered, reduced, and purified by the methods previously developed.

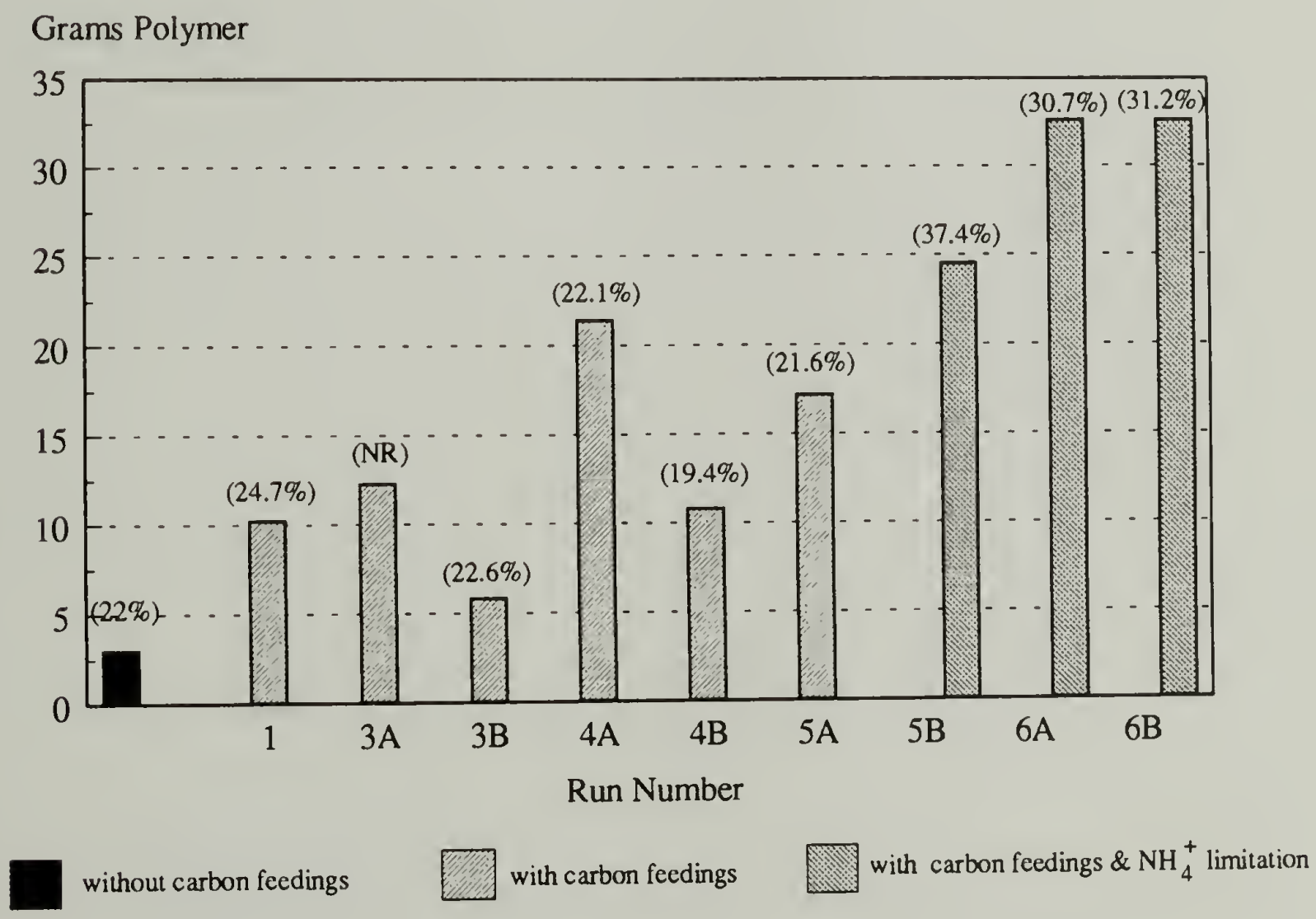

Figure 2.4 Summary of polymer production yield study of *P. oleovorans* grown with sodium octanoate as the sole carbon source. Numbers in parenthesis indicate % polymer of total biomass.

Figure 2.5 shows a representative analysis of the composition of the polymer during the biosynthesis. A change in the polymer composition over time was observed, but a steady state composition was attained after the culture reached the stationary growth phase which was the time when polymer accumulation had maximized and harvesting of the biomass was initiated.

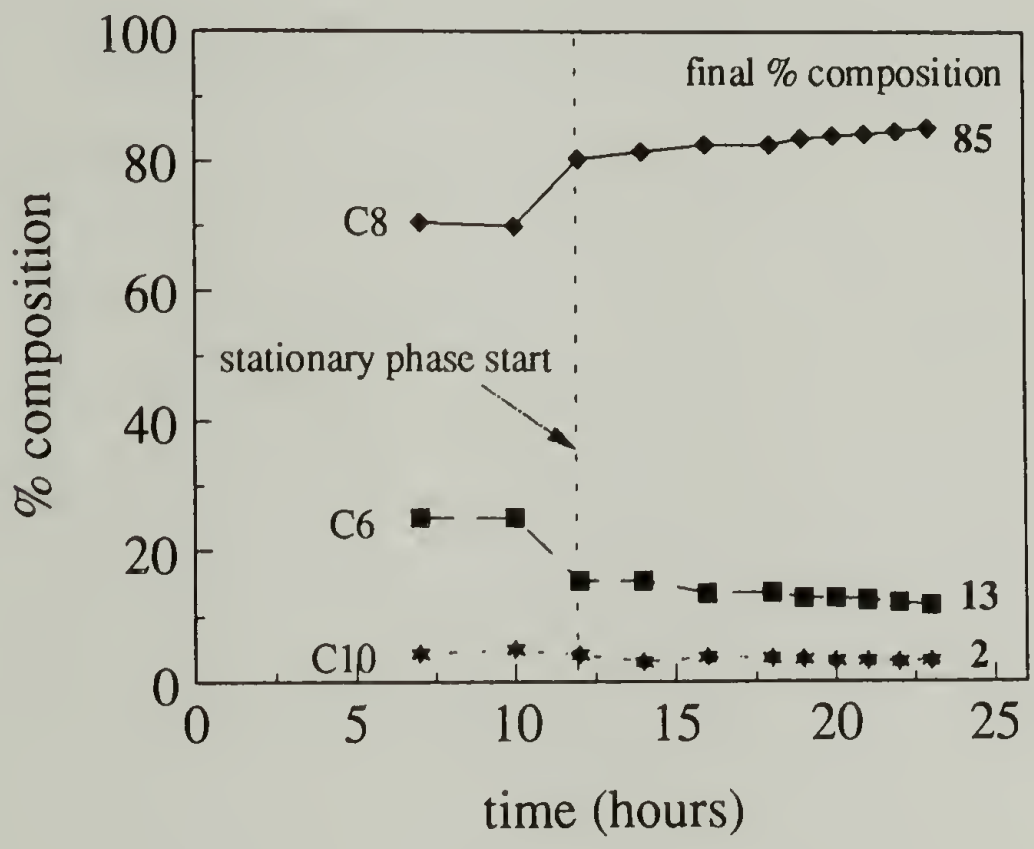

Figure 2.5 Typical variation in copolymer composition with biosynthesis time for *P. oleovorans* grown on sodium octanoate.

A fed batch biosynthesis which attempted to coincide self-induced oxygen limitation with nitrogen limitation was successful in increasing the percent polymer accumulated by the bacteria as shown in Figure 2.3 Runs 5B, 6A, and 6B. A recent study, however, has indicated that nitrogen limitation was not the cause of an increase in the

percentage of polymer accumulation. [11] The increase was simply an artifact caused by a decrease in the cell growth rate.

The adaptation from multiple fed batch biosynthesis to continuous fed batch biosynthesis was successful. However, the time to start the feed was difficult to determine without constant monitoring of the culture. The start of feedings could be correlated to the end of the lag phase but the lag time varied significantly from batch to batch. A correlation between lag time and inoculum OD was not found.

Batch-to-batch consistency of PHO was good as shown by the GC, GPC, DSC, and TGA results in Table 2.1. A typical TGA thermogram is shown in Figure 2.6.

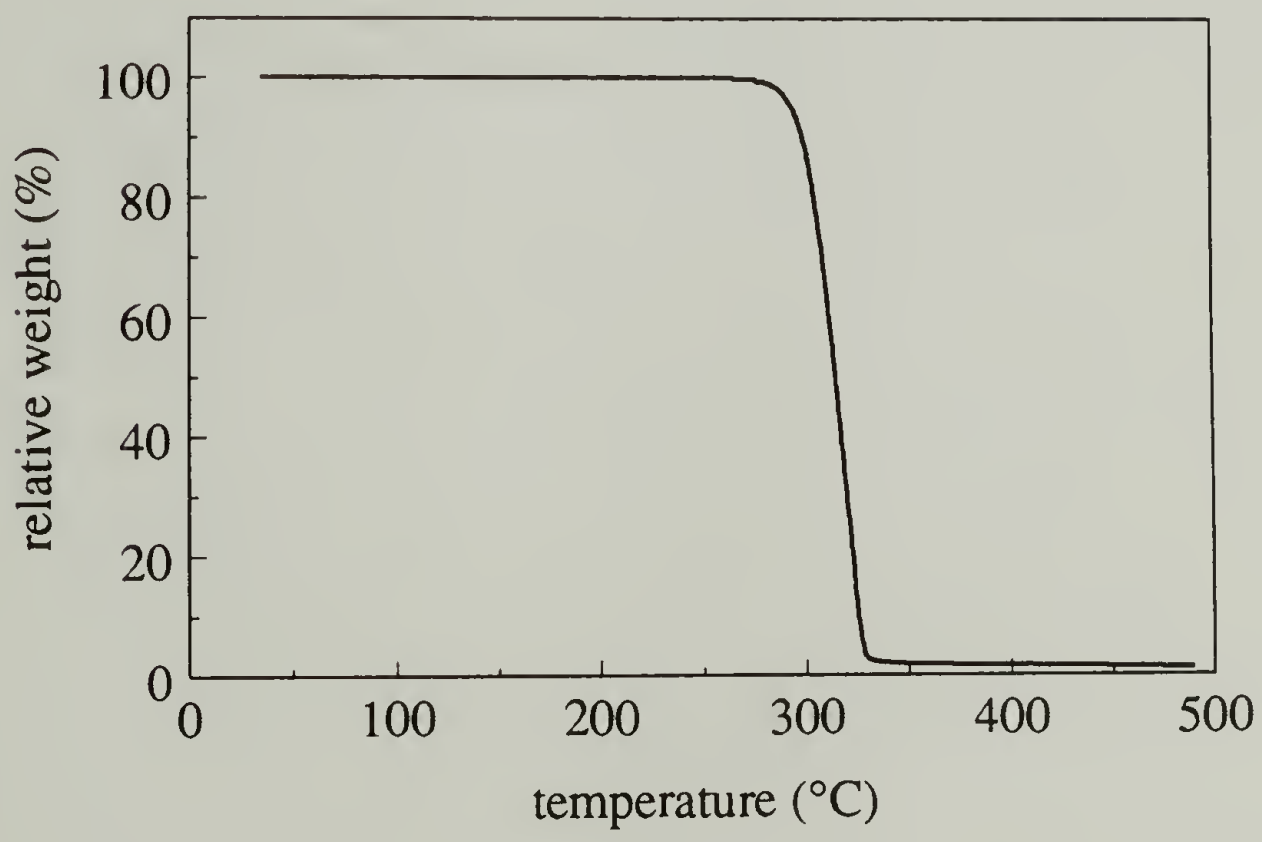

Figure 2.6 Typical thermogram of PHO in an air or nitrogen environment. Heating rate: 20°C/min.

Table 2.1 Indicative parameters used as a measure of batch-to-batch consistency of PHO from several fed batch biosynthesis.

Technique/Parameter	mean	coefficient of variation
GC-repeat unit composition[a]		
C6	12.0%	12.6%
C8	86.1%	1.5%
C10	1.9%	44.4%
GPC-molecular weight distribution		
M_n	84,000 (g/mol)	8.3%
M_w	135,000 (g/mol)	8.1%
PDI	1.6	3.1%
DSC-Thermal Transitions		
T_g[b]	238 K (-35 °C)	1.2%
T_m[c]	334 K (61 °C)	0.6%
TGA-Thermal Decomposition[d]		
T_{onset} in N_2	570 K (297 °C)	0.5%
T_{onset} in air	566 K (293 °C)	0.4%

[a] Values given in mole %.

[b] Inflection point on second heating.

[c] Peak value on first heating

[d] Onset temperature taken as the temperature where the intersection of the baseline with the tangent to the weight loss curve occurs.

2.3.2 PHOU

The production of an olefin containing polymer was successful as shown by the polymer yields in Figure 2.7. The polymer olefin content was established by ^{1}H NMR analysis of the polymer as seen in Figure 2.8. The production of PHOU with a controlled olefin content was possible. The amount of olefin in the mixed carbon source feed was found to directly correspond with the amount of olefin in the polymer as shown in Figure 2.9.

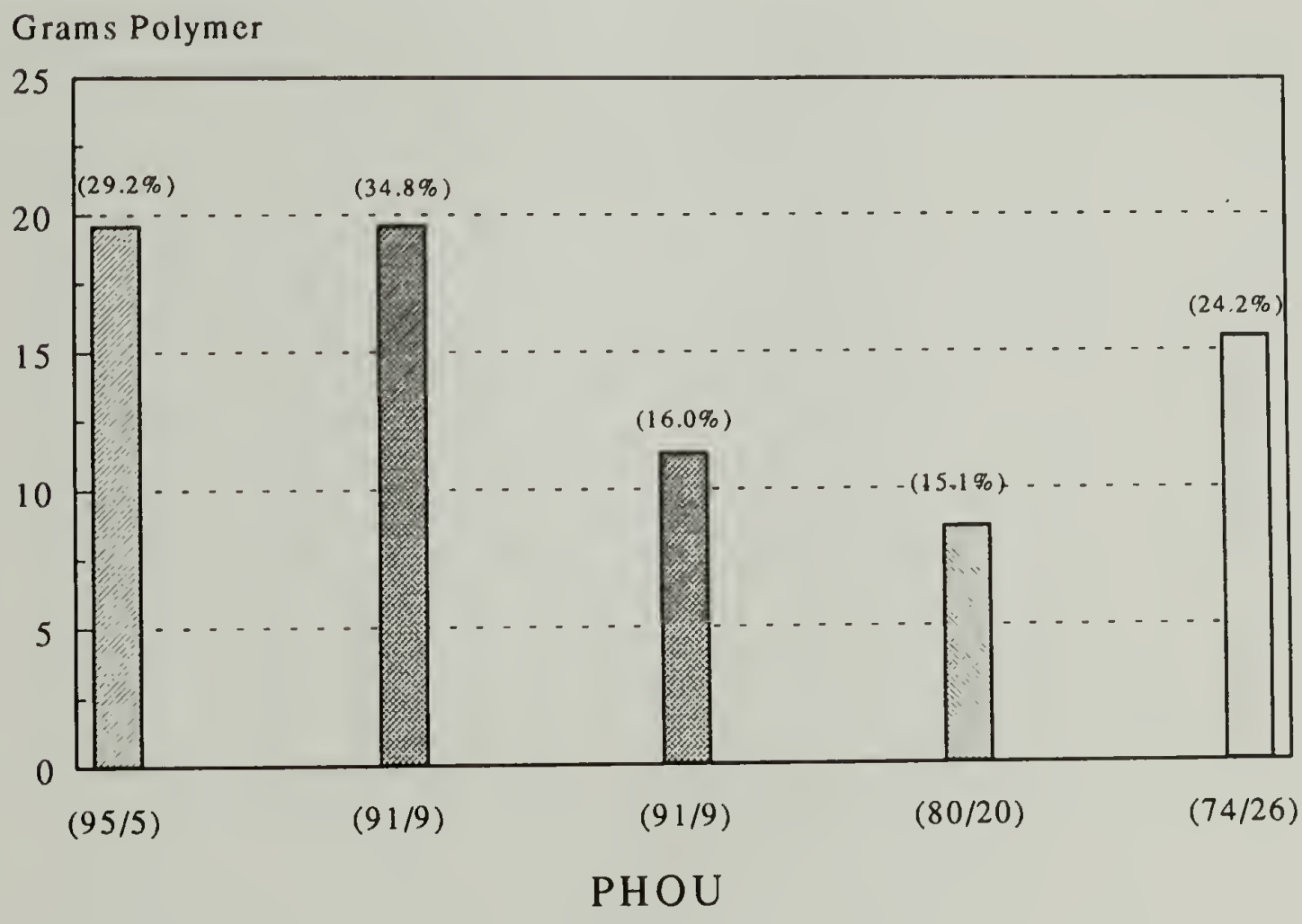

Figure 2.7 Summary of polymer production yield study of *P. oleovorans* grown with a mixture of octanoic and 10-undecylenic acids as the carbon sources. Numbers in parenthesis indicate % polymer of total biomass.

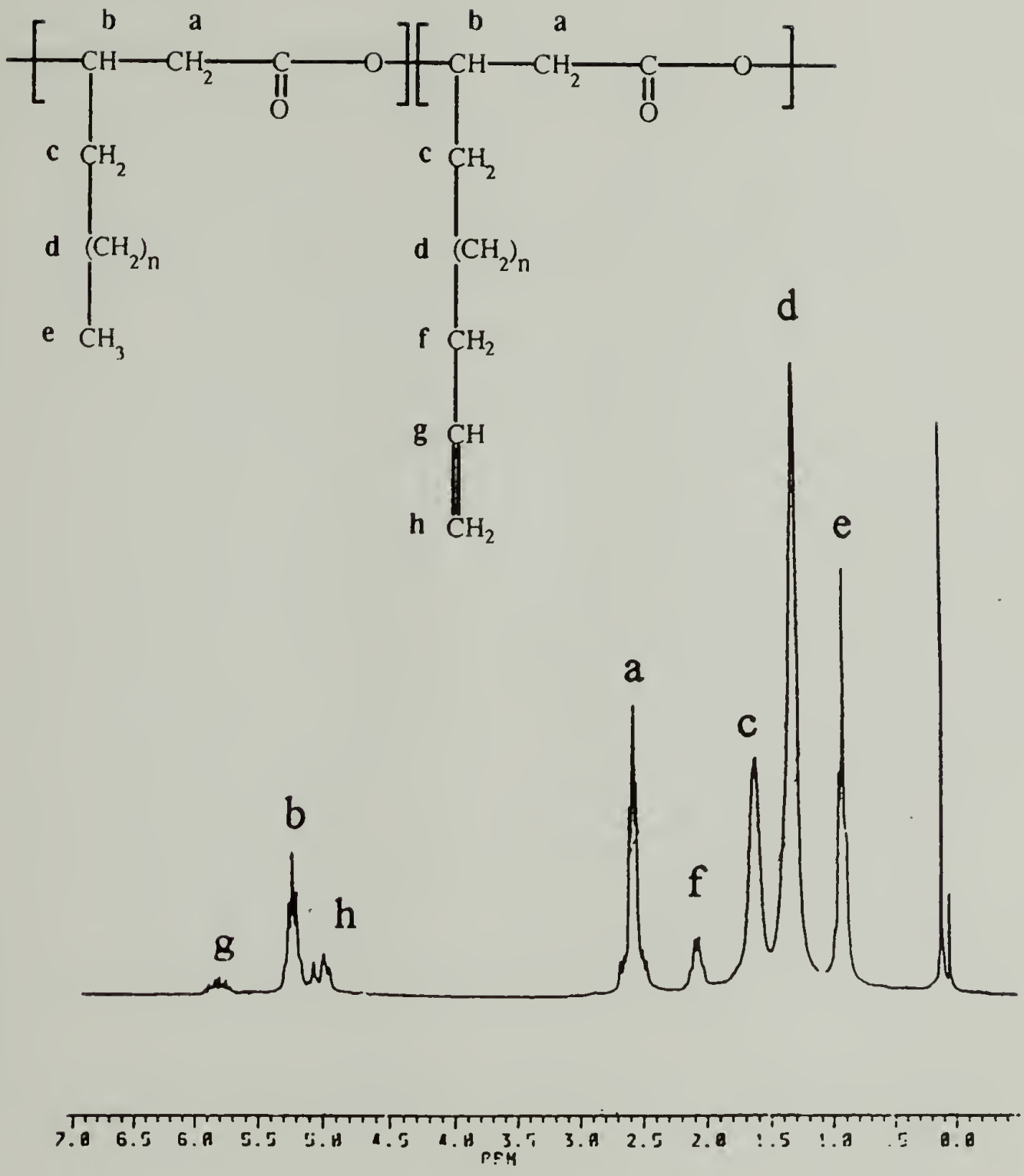

Figure 2.8 Typical ^{1}H NMR of the copolymer PHOU with peak identification.

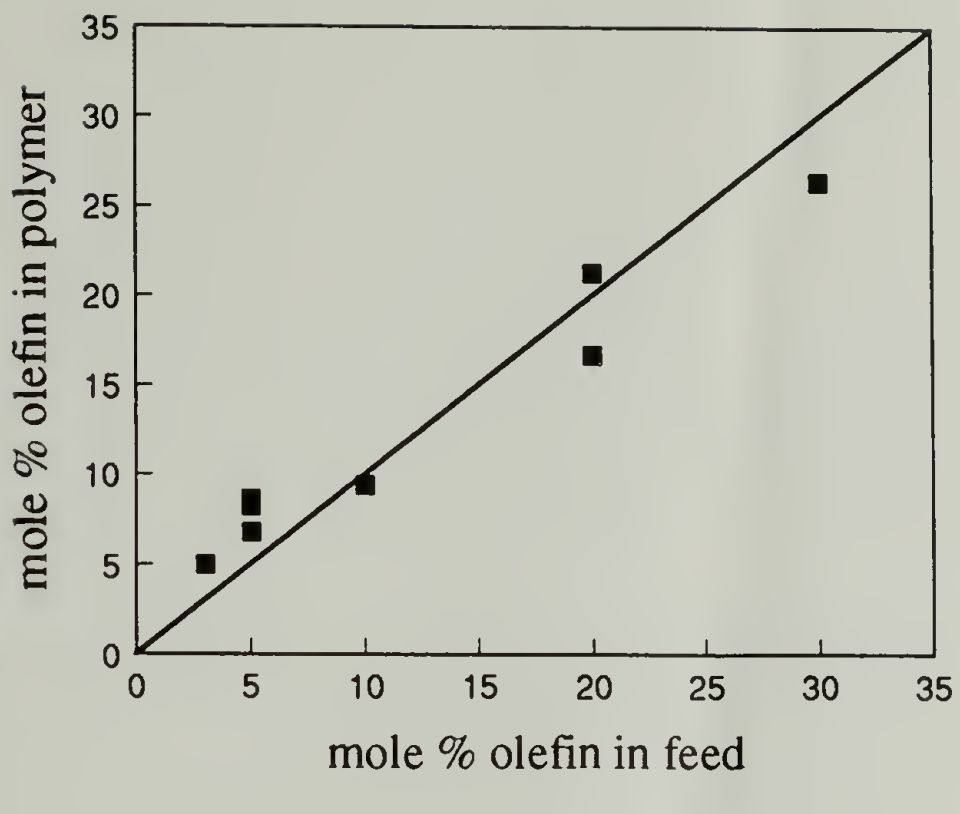

Figure 2.9 PHOU olefin content as a function of feed composition for *P. oleovorans* grown on a mixture of octanoic and 10-undecylenic acids.

Table 2.2 summarizes the characterization results for the different olefin containing polymers. The glass transition temperature, melting temperature, and heat of fusion all show a steady decrease as the amount of olefin in the copolymer was increased. As more olefin repeat units were incorporated, the number of repeat units with varying length pendant groups would also increase. These changes in structure would disrupt the ability of the copolymer to crystallize, lowering the heat of fusion. A lower melting temperature would occur if the crystallites that did form were smaller. The T_g would decrease since the length of the pendant groups generated by the 10-undecylenic acid are longer than octanoate. As was shown by Marchessault,[22] longer pendant groups decrease the Tg. Neither the thermal decomposition temperature nor the molecular weight were affected by the inclusion of olefin repeat units.

Table 2.2 Characterization results for several PHOUs. PHO included for comparison.

	PHO	(93/7)	(91/9)	(80/20)	(74/26)
DSC					
T_g (°C)	-35	-34	-33	-38	-40
T_m (°C)	61	59	58	53	49
ΔH_m (J/g)	15	22	20	14	13
TGA					
T_{onset} air (°C)	297	—	285	—	—
T_{onset} N_2 (°C)	290	—	280	—	—
GPC[a]					
Mw (g/mol)	161,000	165,000	148,000	168,000	172,000
Mn (g/mol)	86,000	62,000	64,000	80,000	68,000
PDI	1.9	2.7	2.3	2.1	2.5

[a] data obtained with THF solvent system.

2.4 Conclusions

The correlation developed between OD and carbon source usage coupled with the knowledge of optimum carbon source concentration was successful in increasing polymer yield approximately tenfold. Continuous fed batch biosynthesis was successful and eased the burden of constantly monitoring the biosynthesis once the growth phase was reached. Recently, the monitoring of dissolved oxygen content of the culture has proved useful as a means to determine when carbon source feedings should occur. This method has also eliminated the problem of determining when the first feeding should take place.

PHO production is consistent batch to batch with regards to composition, molecular weight, thermal transition, and decomposition temperatures as indicated by the low coefficient of variation for all these parameters.

A copolymer could be biosynthesized with a controlled amount of olefin repeat units using a mixed carbon source in the feed with the same ratio as the desired polymer composition.

Improvements in biotechnology and the identification of species which accumulate large amounts of PHA are needed in order to provide a more economical means of producing these materials. Ultimately, the use of molecular biology may have the best chance of reducing costs. The use of molecular biology has already been accomplished for the production of PHB [26] and P(HB/HV) [27] in *Escherichia coli*. The genes responsible for the production of the polymerases were identified and inserted into *Escherichia coli*. This species was chosen because of the wealth of knowledge on growth conditions and the fast generation time of the cells. The results were remarkable, with PHB accounting for up to 95% of the cell dry weight. In addition, the extraction of the accumulated polymer was facilitated by genetically incorporating another enzyme which lysed the cell wall.[28]

Another possible source of PHAs may be plants. Preliminary experiments have shown that a transgenic plant species, *Arabidopsis thaliana*, could be genetically engineered to accumulate PHB.[29] Directing the accumulation of PHA to the tuber of a plant such as a potato, may indeed result in the harvesting of 'plastic' potatoes. [30]

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CHAPTER 3

MATERIAL PROPERTIES

3.1 Background

PHB, the most prevalent PHA, was found to be a brittle thermoplastic.[1] Adjusting the mixture of carbon sources during biosynthesis of *Alcaligenes eutrophus* enabled controlled amounts of another repeat unit , PHV, to be incorporated into the polymer which substantially lowered the amount of crystallinity.[2] The resulting mechanical properties of P(HB/HV) where similar to polypropylene. [2, 3], a more useful material. The longer ethyl pendant group also decreased the glass transition and altered the melting temperature of the polymer.[4, 5]

Due to the metabolic pathway of PHA production in *Pseudomonas oleovorans*, even when grown on a single carbon source, a random [6] copolymer containing from 2 to 8 different repeat units is typically produced rather than a homopolymer. [7] All chiral carbons in the backbone are in the absolute [R] stereoconfiguration [8] making the polymer 100% isotactic. Crystallization occurs in PHO because the polymer is isotactic but the overall crystallinity is significantly reduced because PHO is a copolymer and not a homopolymer. Wide angle X-ray diffraction studies, WAXS, have indicated PHO is approximately 30% crystalline. [9]

The thermal properties of several different PHAs produced by *Pseudomonas oleovorans* have been reported by various workers. [9-13] The thermal properties were found to vary with the length of the side chain with a smoothly decreasing T_g as the alkyl pendant group increased in length. [9] Interestingly, the PHA with a three carbon pendant

group did not show evidence of crystallinity. [10] Thermal analysis of PHO revealed a glass transition temperature of -36 °C and a melting temperature of 61 °C. [9, 10]

Initial mechanical property evaluations of several PHAs from *P. oleovorans* including PHO, revealed a stress strain curve shape typically observed for elastomers; no yield stress or 'knee' appeared in the curve. A tensile modulus of 17 MPa and elongation of 250% to 350% were reported. [9] The material properties were compared to a S-B-S thermoplastic elastomer. The study also suggested that the coupling of high (side chain) and low (backbone) motional freedom components as determined by ^{13}C NMR was an explanation for the elastic material properties.

Phase-separated morphology is the general rule for thermoplastic elastomers, TPEs, and occurs because the majority of TPEs are block copolymer or immiscible blends as discussed in Section 1.2.2. The elastomeric properties of PHO are envisioned by this author to be due to a combination of a T_g below room temperature and a low level of crystallinity. The resulting two phase morphology enables the crystalline regions to act as physical crosslinks for the rubbery amorphous regions. PHO being a random copolymer, has a unique chemical structure for a TPE. A full material evaluation of PHO was undertaken to provide more details on the network structure of the physical crosslinks, tensile properties, hardness and to quantify the elastic response of the polymer.

For comparative purposes, examples from each of the six different classes (styrenics, urethanes, copolyesters, amides, olefinics, elastomeric alloys) of commercially available thermoplastic elastomers, both block copolymers and polymer blends, were prepared and evaluated alongside PHO. A comparison of these specific mechanical properties to commercial TPEs assesses the validity of classifying PHO as a TPE in light of the unique chemical structure of PHO compared to all other TPEs. Two olefin containing PHOUs were also evaluated.

While working with PHO, the use of the material as an adhesive became an obvious possibility. To quantify this assessment, the adhesive properties of PHO to paper

were evaluated using a rudimentary 180° peel test. Because fundamental adhesive properties cannot be gleaned from peel tests, two commercial tapes were included in a side by side comparison. The potential usefulness of a biodegradable adhesive for envelopes and other such paper products was the impetus for testing the adhesive strength to paper. Using such a polymer would make the entire envelope more environmentally friendly when discarded.

Several other properties were measured including contact angle, thermal expansion coefficient, optical rotation, and solubility in a number of organic solvents.

3.2 Experimental

3.2.1 Sample Preparation

The PHO used was biosynthesized and analyzed as described in Chapter 2. A 1.6 mm thick film of PHO was prepared by melt blending a portion of polymer from several different biosynthesis batches. The melt was stirred after first becoming molten to thoroughly mix the various pieces. The film was cooled slowly and allowed to crystallize at room temperature for 17 days prior to testing.

The commercially available TPEs included in this study are listed in Table 3.1. At least one sample from each of the six different classes of TPEs was represented in the various mechanical tests. Films, 1.6 mm thick, were formed using a Carver Hot Press Model C equipped with a vacuum chamber. The molding temperature was chosen according to published literature received from the different companies. The film was removed from the press and allowed to slowly cool to room temperature. A sample of a urethane TPE could not be found in a compression moldable grade. Therefore, a 1.6 mm thick film was formed from a solution processable grade cast from a 2% THF solution.

Table 3.1 Commercially available thermoplastic elastomers (TPEs) evaluated in this study.

TPE Category	Company	Material Tradename	Product Code
amide	EMS	Grilon	ELX23NZ
amide	Atochem	Pebax	2533SN00
elastomeric alloy	AES[a]	Santoprene	201-55
elastomeric alloy	DuPont	Alcryn	3155-NC
olefinic	AES[a]	TPR	9201-65
copolyester	DuPont	Hytrel	5556
styrenic	Shell	KratonD	4141
styrenic	Shell	KratonG	1726
urethane	BFGoodrich	Estane	5703-P

[a] Originally obtained from Monsanto, but products now produced by Advanced Elastomers Systems.

3.2.2 Tensile Testing

All testing was conducted using an Instron Universal Testing Instrument, Model TTBM. Testing was based on the procedures described in ASTM D638 [14]. Dumbbell samples used in tensile testing were die cut from the polymer films using a Type V die.

A strain rate of 2 min^{-1} was used for all tensile tests. All stress calculations are based on the narrow cross-sectional area of the undeformed dumbbell. Grip slippage was a problem even when pneumatic grips with sandpaper were used to hold the samples. No extensiometer was available so to measure elongation, a 25 mm gage length was marked on a sample of each material and a ruler held next to the sample during testing. A mark

was made on the chart paper when various known elongations of the gage length were reached. A linear correlation was found between the actual and recorded elongation and a correction could then be made to the recorded elongation data for the remaining samples of that material. Values reported are the average values from seven samples.

3.2.3 Tensile Set/Stress Relaxation Testing

All testing was conducted using an Instron Universal Testing Instrument, Model TTBM. Dumbbell samples were die cut from the polymer films using a Type V die for large deformation tensile set testing. Strips 4 mm to 5 mm wide were used in the small strain stress relaxation experiments. Testing was based on the procedures described in ASTM D412 [15]. The samples were extended and held for 10 minutes at the intended elongation then released at the same strain rate. The samples were then allowed to recover for 10 minutes prior to final measurement of the gage length. For large deformation testing (> 50%), different strain rates were used to reach the desired elongation within approximately 15 seconds and three dumbbell samples were tested at each elongation. The recovery after 13 hours was also measured for the large deformation samples. For small deformation testing, a 2 min^{-1} strain rate was used for all strip samples.

3.2.4 Hardness Measurements

The hardness measurements were based on the procedures outlined in ASTM D2240 [16]. Hardness measurements were taken within one second after indentation using either a Shore Durometer Type A or Type D-2 Tester.

3.2.5 Contact Angle Determination

Both advancing and receding dynamic contact angles were measured with water as the working fluid. The film tested had been crystallized from the melt at room temperature in a glass casting dish for many months. Both sides of the film were tested due to differences in film smoothness observed with the naked eye. The surface in contact with air had noticeable pits and irregularities. The film surface which was in contact with the glass casting dish was smooth.

3.2.6 Adhesive Property Evaluation

Coating rods were used to lay down controlled thicknesses of a known concentration of a chloroform or hexane solution of PHO onto either a 25μ thick PET or 41μ thick cellulose acetate backing material. A piece of white copy paper was firmly pressed onto the coated backing. One centimeter wide sample strips were cut and a 180° peel test was conducted within 1 hour of coating. Two commercial tapes, Scotch Magic Tape® which has a cellulose acetate backing and Scotch Transparent Tape® which has a PET backing, were tested alongside PHO for comparison. The 180° peel test was based on procedures outlined in ASTM [17].

3.2.7 Thermal Expansion Evaluation

The thermal expansion coefficient of PHO was determined using a TA Instruments thermal mechanical analyzer (TMA). A film sample was loaded with a minimal 0.005 N force and the change in length was monitored as a function of temperature between -25 °C and 20 °C. The thermal expansion coefficient was determined from the slope of the dimensional change versus temperature curve.

3.2.8 Optical Activity Determination

The optical activity of PHO in chloroform was measured by Mitch Johnson, an analytical chemist, at one wavelength using a home-built optical polarimeter with laser source and Faraday rotator. The source wavelength was fixed at 670 nm. The output was calibrated by rotating the analyzer.

3.2.9 Solubility Parameter Determination

PHO was screened for solubility in various solvents at room temperature. Three different solvent groups were included: strong, moderately, and poorly hydrogen bonding solvents. Solvents with a range of solubility parameters were included in each group. A small sample of PHO, typically 0.5 g, was placed in a test tube containing 5 ml of the solvent. The polymer was considered soluble if a one phase, non-cloudy solution was observed after 24 hours. In some cases the sample was heated slightly to try and induce solubility, but observations were made after the sample cooled to room temperature. A solubility parameter range for PHO was determined for each solvent group.

3.3 Results and Discussion

3.3.1 Morphology - Network Structure

From stress relaxation experiments at small strains (< 50%) and the statistical theory of rubber elasticity [18], the equilibrium shear modulus was determined graphically from equation 3.1 as shown in Figure 3.1.

$$\sigma = G\left(\lambda - \frac{1}{\lambda^2}\right) \tag{3.1}$$

where G = equilibrium shear modulus [MPa]

λ = extension ratio, L/Lo

σ = engineering stress [MPa]

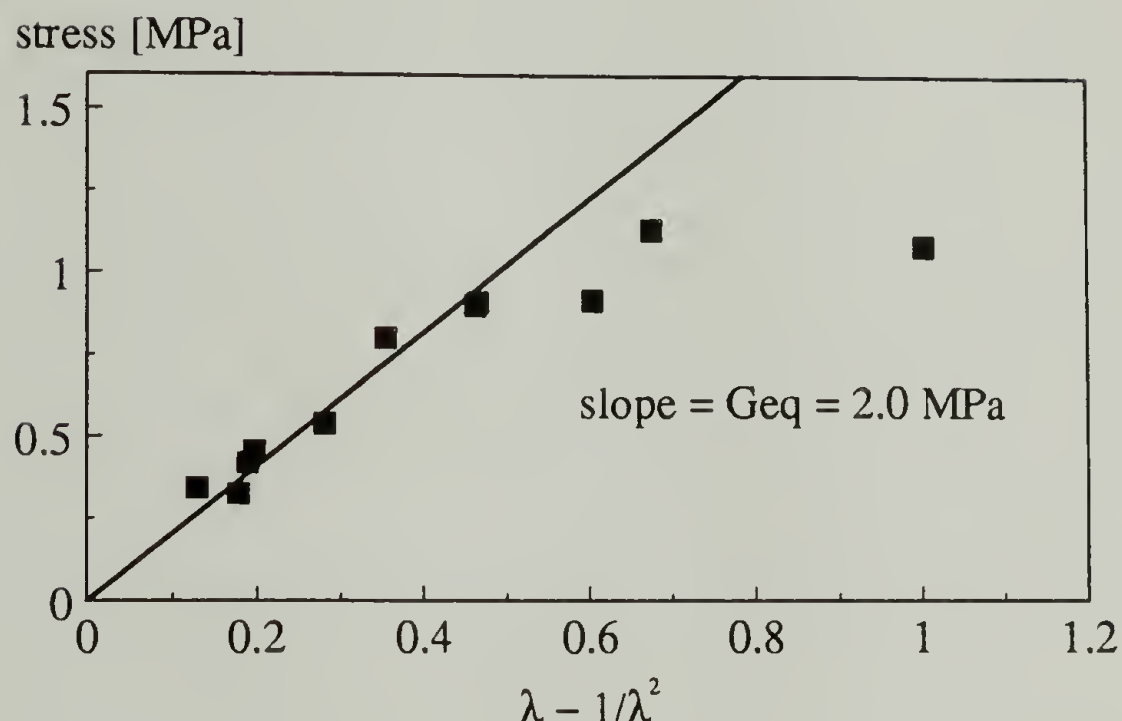

Figure 3.1 Graphical determination of the equilibrium shear modulus, G_{eq}, from the slope of the equilibrium stress vs $(\lambda - \frac{1}{\lambda^2})$ plot obtained from stress relaxation data at small strains.

The magnitude of the shear modulus in rubber theory is based on an ideal network of point crosslinks. In physically crosslinked systems, the crystalline regions would also act as filler particles due to their finite size which would increase the modulus substantially. This filler effect can be estimated using the Guth-Smallwood equation[18]

(3.2)

$$\frac{E_f}{E_o} = 1 + 2.5\ V_f + 14.1\ V_f^2 \qquad (3.2)$$

where E = tensile modulus

V_f = volume fraction of filler

o subscript refers to unfilled material

f subscript refers to filled material

The equilibrium shear modulus can then be modified to eliminate the filler effect of the crystalline regions. Assuming PHO is 30% crystalline [9] by volume ($V_f = 0.30$), $E_f / E_o = 3$ and the equilibrium shear modulus reduces to 0.67 MPa. The molecular weight between crosslinks (M_c) can then be calculated using equation 3.3.

$$M_c = \frac{\rho RT}{G_{eq}} = 3600 \text{ g/mole} \qquad (3.3)$$

where ρ = polymer density (1.019 g/cm^3)[9]

$R = 8.21 \times 10^6$ cm^3-Pa/mol-K

T = 298 K

The number of physical crosslinks per chain is then

$$\frac{\overline{M_n}}{M_c} = \frac{84000}{3600} = \text{approximately 10 to 20 crosslinks/chain}$$

As an interesting comparison, the soft segments of a segmented polyurethane typically have a M_n of 600-6000 which is analogous to the M_c.[19] The hard segments of

segmented polyurethanes have a M_n ranging between 600 to 3000. For a typical polyurethane with a M_n of 40,000, approximately 10 to 60 hard segments per chain would exist, which is analogous to the number of physical crosslinks per chain. This comparison indicates the network structure of PHO interpreted through the M_c and number of physical crosslinks per chain appears similar to that of another TPE, the segmented polyurethane. Figure 3.2 is a representation of such a morphology.

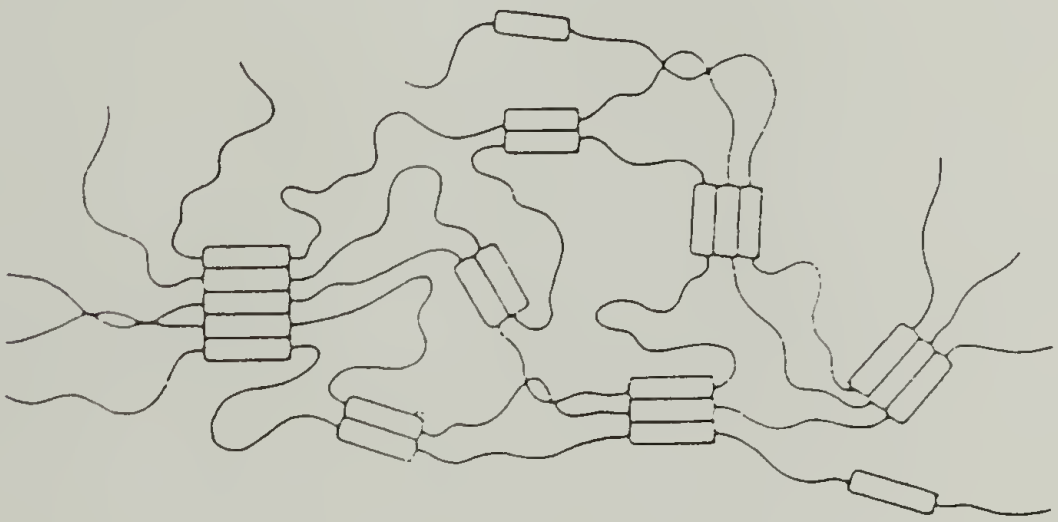

Figure 3.2 Graphical representation of the network structure of PHO. Each stack of rectangles represents a crystalline region. Adapted from reference 19.

3.3.2 Tensile Properties

The stress-strain curve and calculated parameters for PHO crystallized from the melt at room temperature are depicted in Figure 3.3. Figure 3.4 indicates the sensitivity of the tensile modulus of PHO to strain rate.

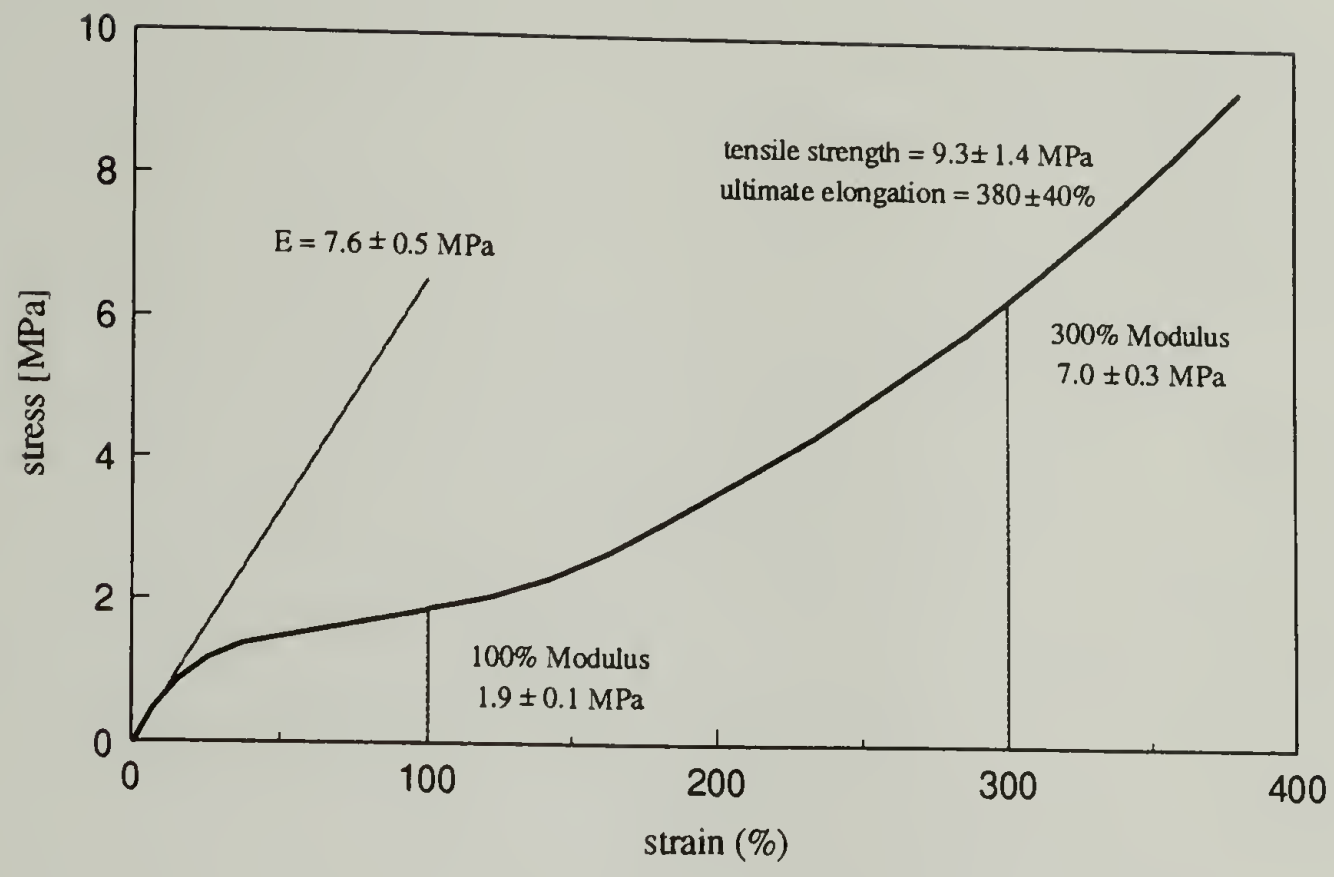

Figure 3.3 Stress-strain curve for PHO crystallized at room temperature from the melt. Average values and standard deviations from seven samples are given. Strain rate: 2 min^{-1}

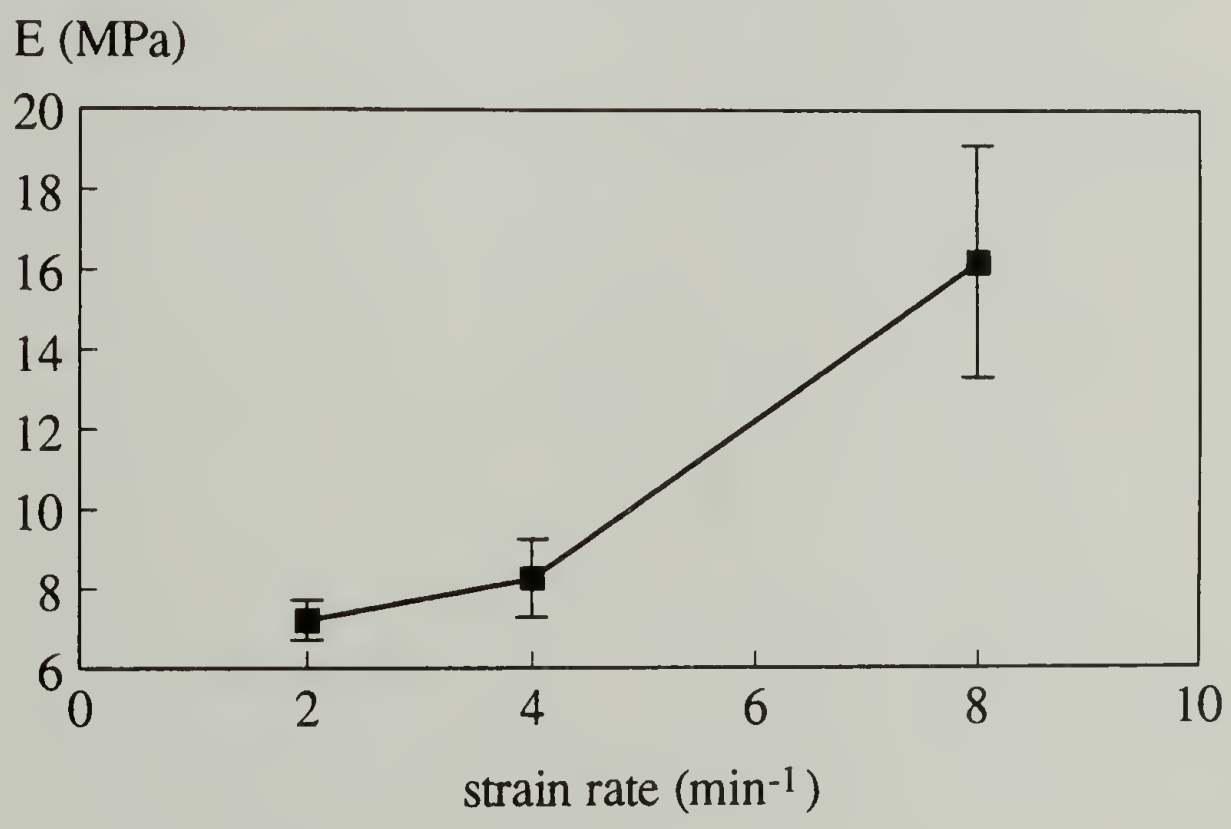

Figure 3.4 Young's modulus of PHO as a function of strain rate; sample geometry, dumbbell.

The values of Young's Modulus, 100% and 300% moduli, tensile strength, and ultimate elongation for the commercially available TPEs, PHO, and several PHOUs are charted in Table 3.2. PHO fell approximately in the middle of the range in most categories except for ultimate elongation where PHO ranked near the low end. The classification of PHO as a thermoplastic elastomer appears accurate based on the stress-strain parameter values exhibited by the commercial TPEs.

3.3.3 Tensile Set

Tensile set quantifies the deviation of a material from ideal elastic behavior. A high tensile set indicates poor elasticity and is an important consideration for any material considered to be an elastomer.

The tensile set obtained for PHO at various elongations is depicted in Figure 3.5. A substantial increase in tensile set was noted as the elongation was increased. No difference was obtained between samples allowed to relax for 10 minutes or 13 hours (not included on graph), which indicated that no additional relaxation occurred in the samples after 10 minutes. For comparison purposes, the tensile sets of all the TPEs evaluated after 100% elongation are listed in Table 3.3. PHO had a very substantial tensile set compared to the other commercially available TPEs evaluated, only fairing better than the copolyester TPE.

Tensile set can result from many sources such as:

1) irreversible orientation or permanent displacement (flow) of the physical crosslinks in the amorphous matrix
2) strain induced crystallization which does not melt upon release of the deforming stress
3) deformation induced break-up or rearrangement of the physical crosslinks

Table 3.2 Summary of tensile testing of PHO, two PHOUs, and the commercial TPEs included in this study[a].

TPE Category	Material Tradename	E, MPa	100% Modulus, MPa	300% Modulus, MPa	Tensile Strength, MPa	Ultimate Elongation, %
polyester	Hytrel	170	18	25	46	670
styrenic	Kraton G	78	2	2	3	430
amide	Grilon	71	14	27	42	530
styrenic	Kraton D	16	2	4	9	1070
amide	Pebax	11	4	5	21	850
bacterial polyester[c]	PHOU(83/17)	9	3	12	12	300
bacterial polyester[c]	PHOU(91/9)	8	2	9	10	330
bacterial polyester	PHO	8	2	7	9	380
elastomeric alloy	Santoprene	7	3	7	7	340
olefinic	TPR	7	1	ND[b]	2	180
elastomeric alloy	Alcryn	5	4	9	10	370
urethane	Estane	5	1	2	16	960

[a] Average value for 5 to 10 samples reported. Strain rate of 2 min^{-1} used for all materials.

[b] ND - no data - material ultimate elongation is less than 300%.

[c] Average from 3 ring samples.

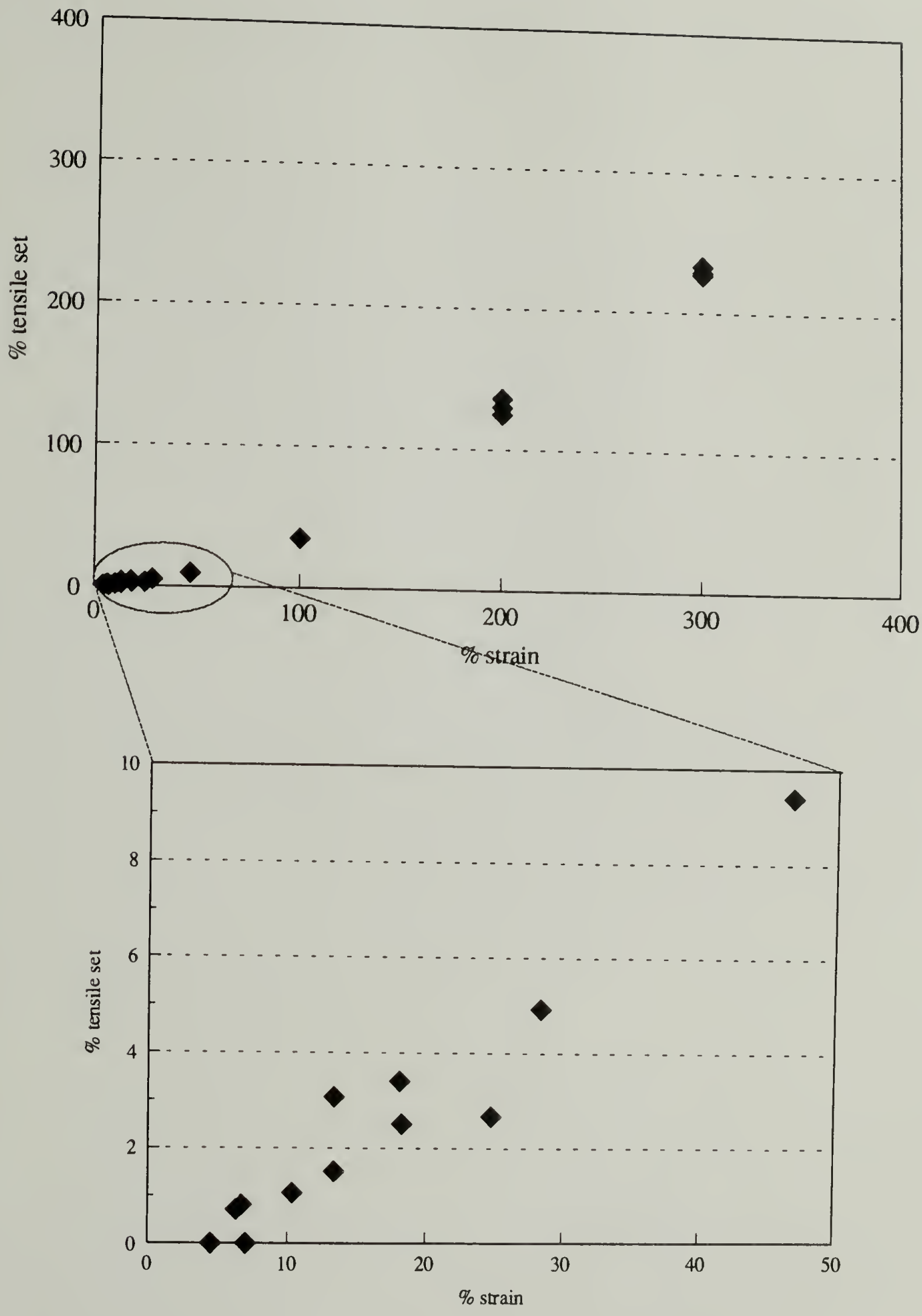

Figure 3.5 Tensile set of PHO as a function of elongation.

Table 3.3 Summary of tensile set results for PHO, two PHOUs, and the commercially available TPEs.

TPE Category	Material Tradename	% Tensile Set after 100% elongation
styrenic	Kraton D	2
styrenic	Kraton G	6
elastomeric alloy	Santoprene	8
elastomeric alloy	Alcryn	8
olefinic	TPR	10
urethane	Estane	12
amide	Pebax	12
amide	Grilon	31
bacterial polyester	PHO	35
bacterial polyester	PHOU(83/17)	43
polyester	Hytrel	56
bacterial polyester	PHOU(91/9)	57

In all cases, a permanent change to the undeformed reference state of the material would occur. Tensile set will be one consequence of this permanent change.

To investigate the possible sources of tensile set in PHO, a thermal analysis of the stretched samples was conducted. The results are shown in Figures 3.6 through 3.8.

Figure 3.6 shows that at small deformations (≤ 50% strain) no change occurred in the parameters T_g, T_m, or ΔH_m, and no change in the shape of the melting endotherm was noted in the stretched material (not shown). These observations indicate that no change to the size, distribution, or amount of crystallinity occurred upon small deformation. Some change did occur in the undeformed reference state since a small amount of tensile set was observed at these small strains as shown in Figure 3.5. These observations support the possibility that irreversible orientation or permanent displacement (flow) of the physical crosslinks in the amorphous matrix was a source of tensile set.

Figures 3.7 and 3.8 show the thermal analysis results of the samples after undergoing large deformations (100% to 300% strain). The heat of fusion, ΔH_m, a measure of the amount of crystallinity, increased by 60% after 300% elongation. This result supports strain induced crystallization, which does not melt upon release of the stress, as a source of tensile set.

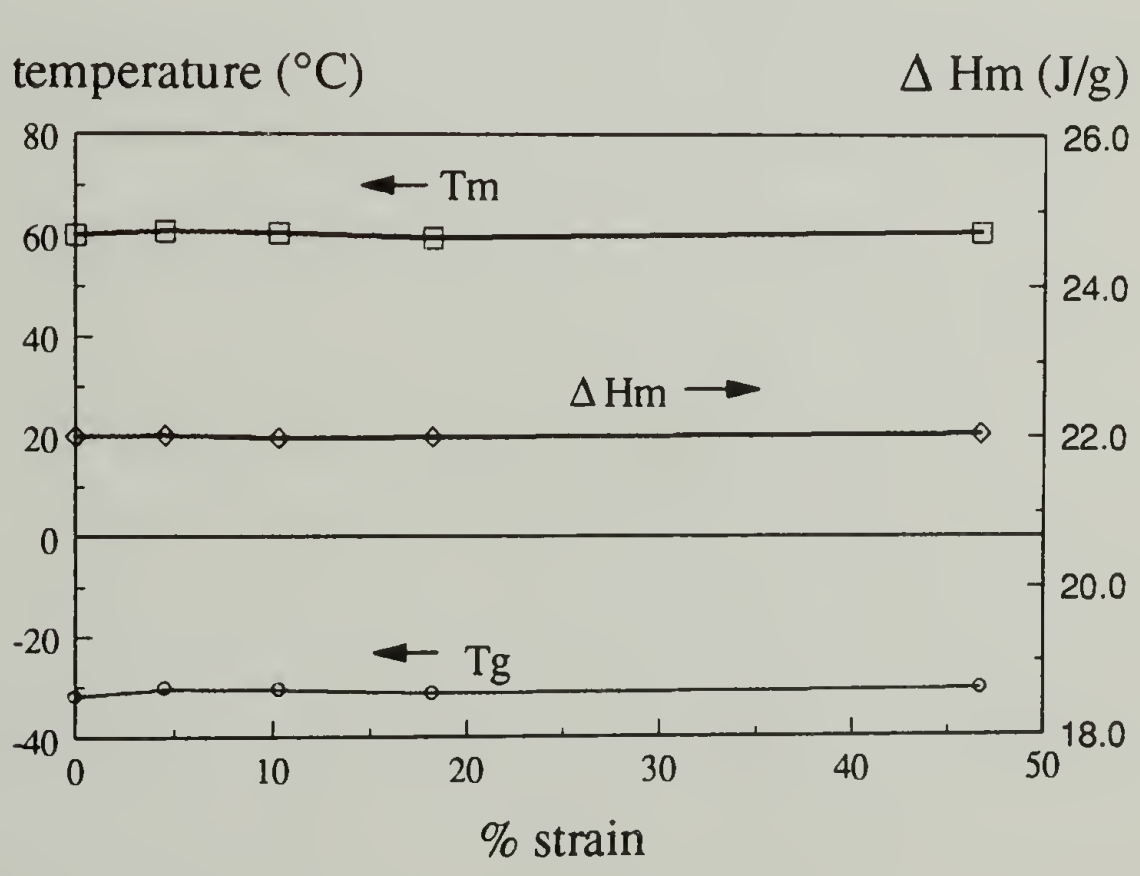

Figure 3.6 DSC parameters for PHO samples after exposure to small strains.

Figure 3.8 shows that the peak melting point of PHO decreased as the elongation increased. In addition, a change in the endotherm peak shape was observed as the elongation increased; the melting endotherm peak became more narrow as seen in Figure 3.9. These observations imply that overall, the size of the crystalline regions was decreasing (lower T_m) while the purity or perfection was increasing (peak narrowing). These observations support deformation induced break-up or rearrangement of the crystalline regions was a source of tensile set.

Figure 3.8 shows that as expected, the T_g did not change significantly with elongation. The glass transition temperature reflects the properties of the amorphous regions. The material, even after extension, would still possess a relatively low degree of crystallinity. Therefore, no appreciable change in the T_g was expected or observed.

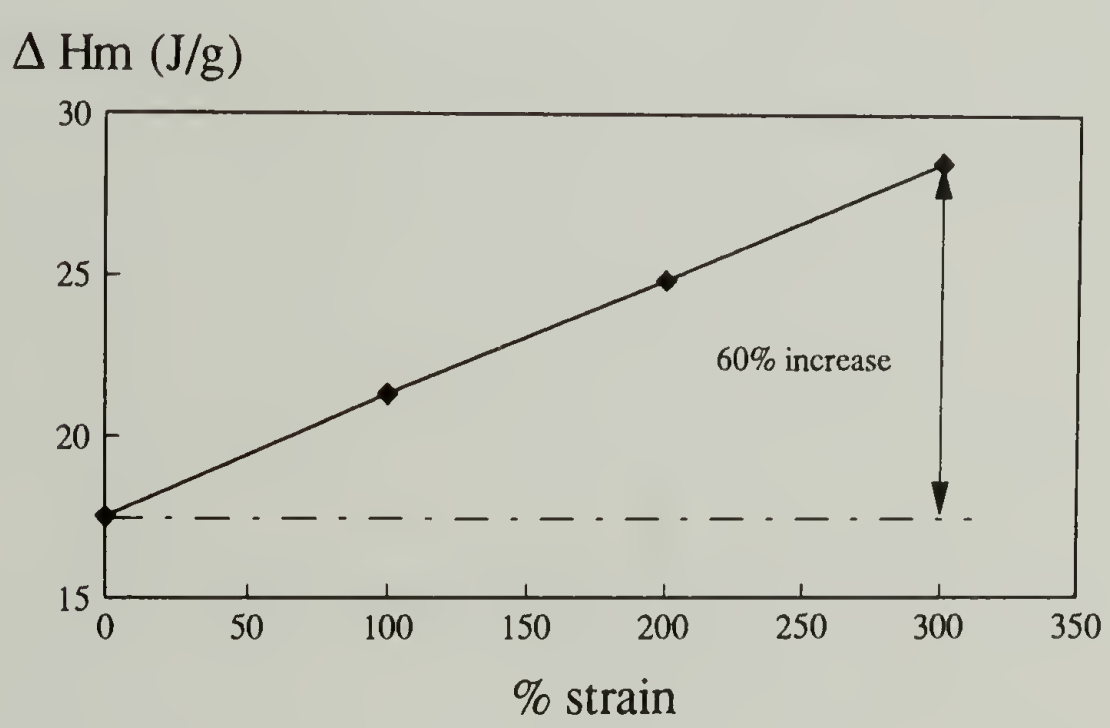

Figure 3.7 Changes in the heat of fusion, ΔH_m, for PHO as a function of elongation; large strains.

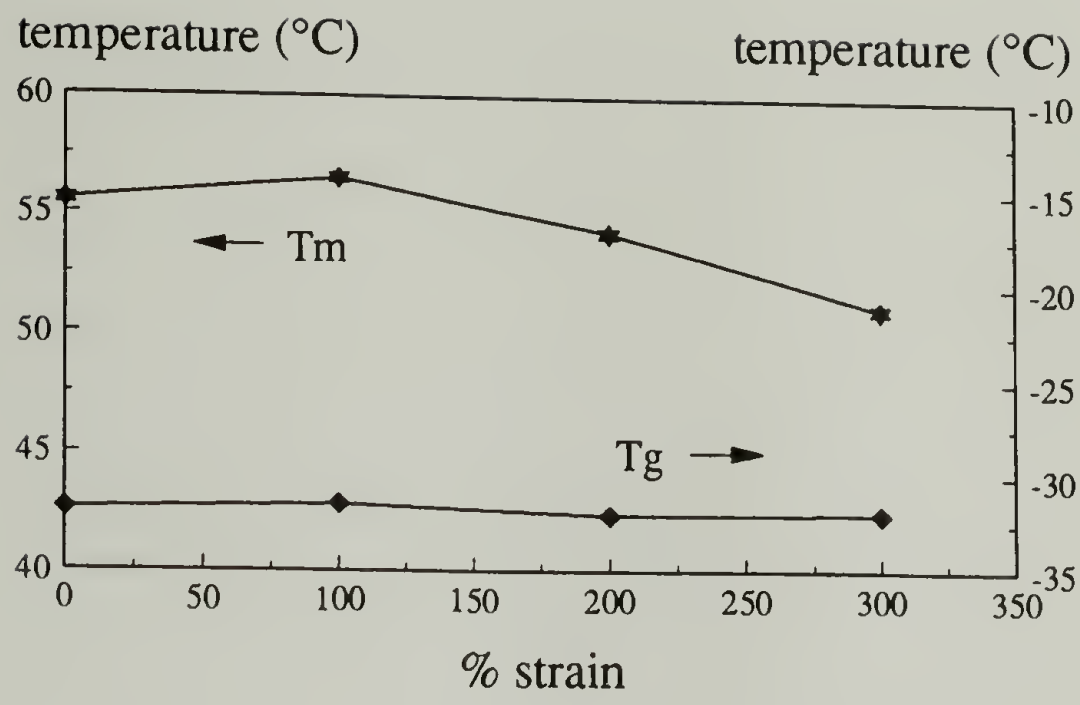

Figure 3.8 Thermal transition temperatures of PHO as a function of elongation; large strains.

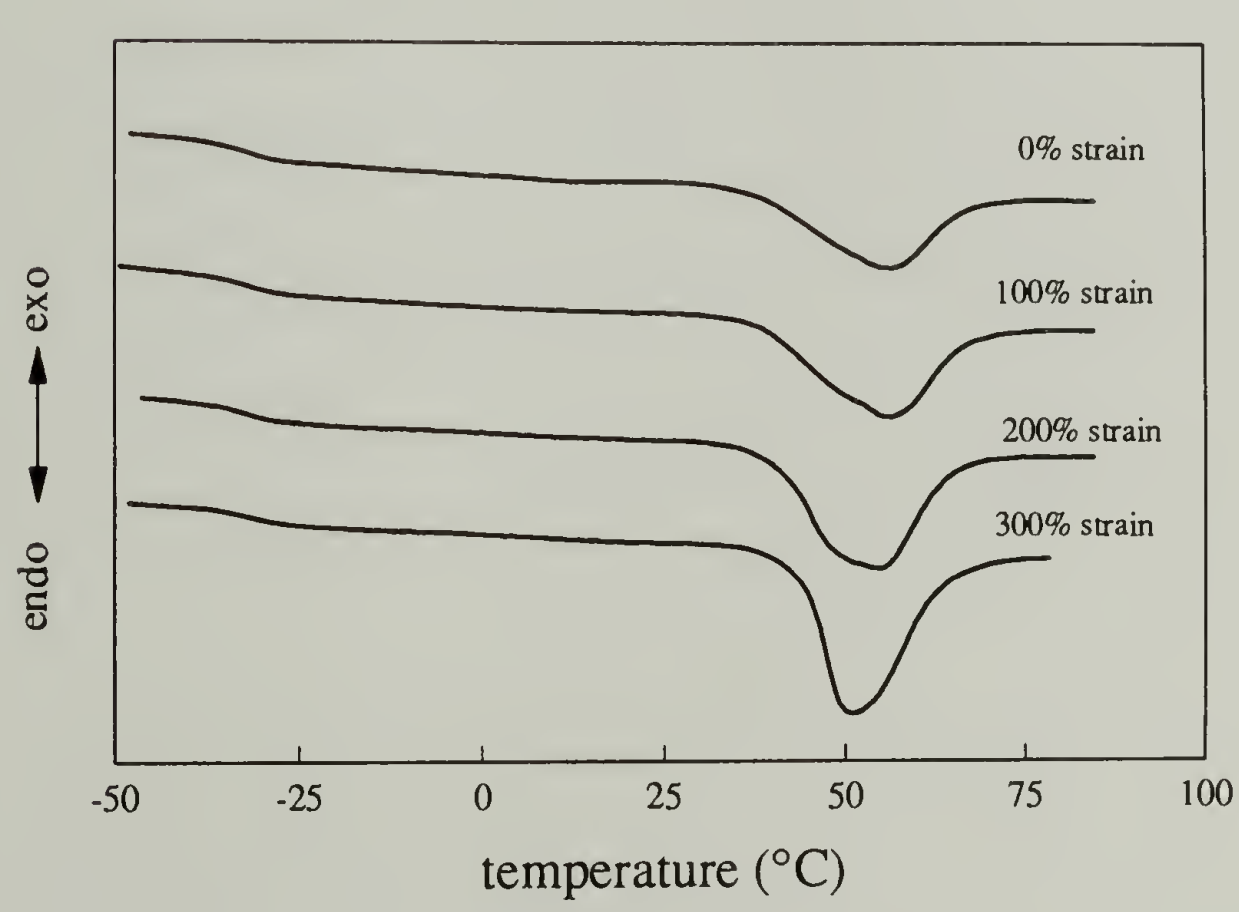

Figure 3.9 DSC thermograms for PHO stretched to various elongations.

In general, TPEs are block copolymers where one block, known as the soft segment, phase separates from the other block, known as the hard segment and the hard phases form a physically crosslinked network. For some polyurethane TPEs, strain induced crystallization of the soft segments can occur upon stretching. If the melting temperature of the soft segments is above room temperature, full recovery of the stretched material to the undeformed dimensions will not occur at room temperature. However, increasing the temperature above the melting point of the soft segments but below the melting or softening point of the hard segment will result in full recovery of the material. Recovery will occur because the original network of physical crosslinks formed by the hard segments was not affected by the strain or application of heat.

For PHO, where the physical crosslinks are crystalline regions, strain induced crystallization directly affects the physically crosslinked network by either forming new crosslinks or altering original ones, or both.

To demonstrate that for PHO strain induced crystallization resulted in a permanent change in the network structure and full recovery would not occur by the application of heat, one stretched sample was heated in a vacuum oven slowly and incrementally. A total recovery of 17% was observed after the heating treatment. This experiment showed that some of the strain induced crystallization which was preventing the material from recovering, could be removed (melted) by raising the temperature. However, full recovery could not be achieved because heating will eventually affect all crystalline regions whether part of the original network or strain induced.

Overall, various changes to the crystalline regions were occurring during deformation resulting in permanent alteration of the material which produced significant tensile set.

3.3.4 Hardness

Hardness and modulus vary proportionally, however no simple relationship bridges the gap between modulus and durometer, the common units for hardness. Since durometer is often used as a design criteria and a way to distinguish one grade of elastomer from another, hardness testing was conducted on PHO and the commercial TPEs. The results of hardness testing for PHO and the commercially available TPEs are listed in Table 3.4. As the level of crystallization increased over time, the hardness of PHO also increased. For PHO crystallized at room temperature for approximately six months, the hardness had increased to Shore 79A.

The hardness scales with the location of the different classes of TPEs and the value measured for PHO are shown on Figure 3.10. PHO, at Shore A 60, is a relatively soft elastomer compared to the other TPEs.

3.3.5 Contact Angle

The dynamic advancing/receding contact angles of PHO determined with water as the working fluid were: film surface formed in contact with air (irregularities observed), 90/7; film surface formed against the glass casting dish (smooth side) 89/13. A large contact angle indicates hydrophobic quality while a low angle indicates hydrophilic quality. The large advancing contact angle indicates PHO has a hydrophobic quality. The large difference between the advancing and receding angles indicates the surface molecules may possibly be rearranging during water contact in such a way as to expose a more hydrophilic quality.[22] A rearrangement that exposes the hydroxy or carboxylic acid chains ends could be envisioned as providing the more hydrophilic surface. The lower receding angle for the film with the irregular surface was expected. The fluid would tend to get caught by the irregularities and reduce the receding contact angle.

Table 3.4 Summary of hardness testing results for PHO and the commercial TPEs included in this study.

TPE Category	Material Tradename	Hardness, Shore Durometer
styrenic	Kraton D	52A
urethane	Estane	56A
elastomeric alloy	Santoprene	58A
bacterial polyester	PHO	60A
elastomeric alloy	Alcryn	61A
olefinic	TPR	64A
styrenic	Kraton G	78A
amide	Pebax	22D
amide	Grilon	47D
polyester	Hytrel	55D

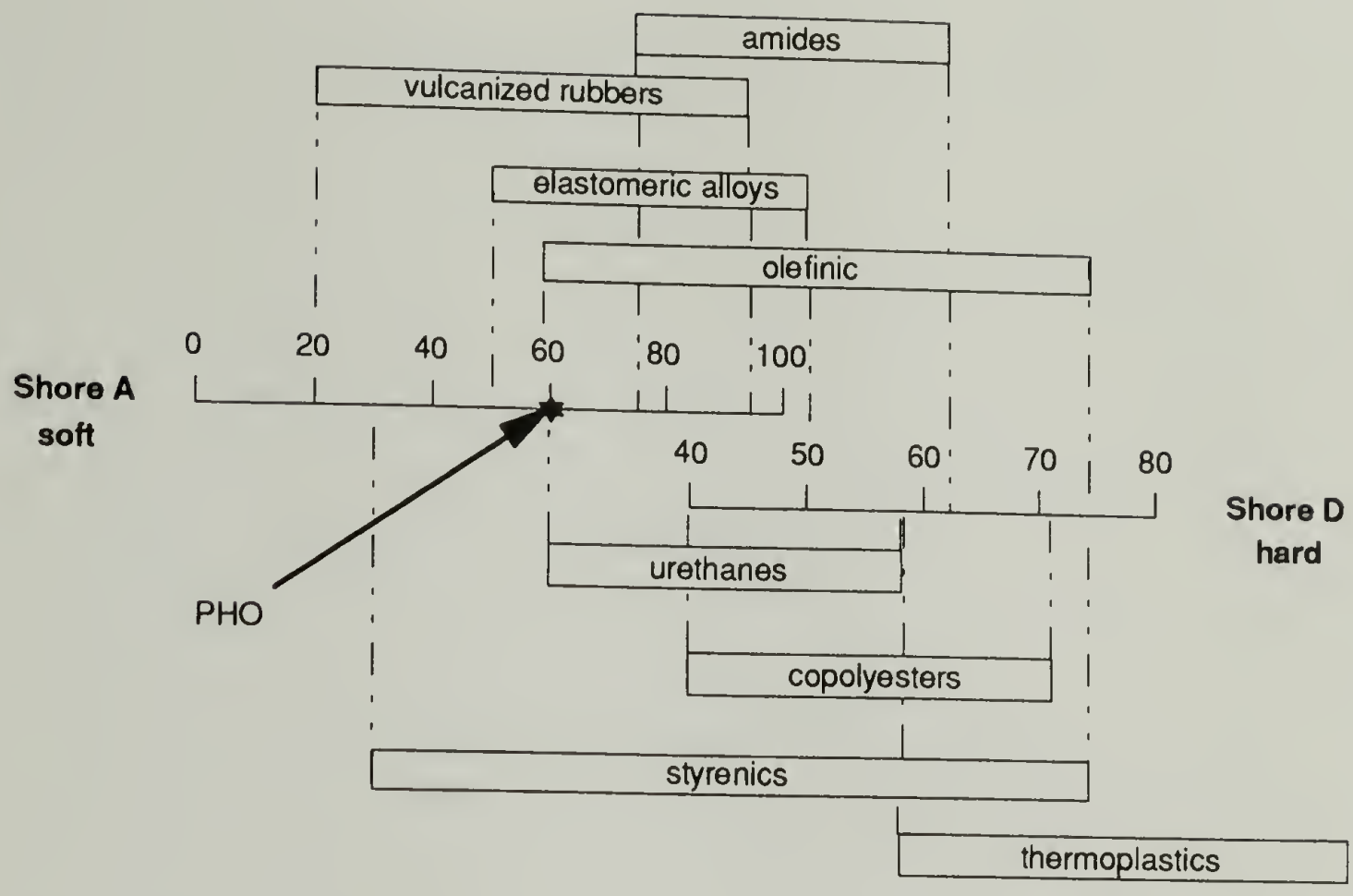

Figure 3.10 Hardness scales showing hardness ranges of commercially available TPEs and the position of PHO. Adapted from references 20 and 21.

3.3.6 Adhesive Properties

Previous studies conducted by our laboratory have revealed the peel test does not necessarily reveal the true adhesive strength of the material tested because the deformation of the plastic backing can significantly affect the measured peel strength. [23] However, the peel test can be used as a comparative test to determine adhesive strength if similar backings are used.

The peel strength of PHO and the commercial tapes are shown on Figure 3.11 as a function of separation speed. A slight increase of peel strength with separation speed was observed for all the samples. The peel strength of PHO increased significantly with increasing layer thickness but the difference in backing materials was not important. PHO appeared to fail cohesively since the polymer was detected on both surfaces. The

commercial tapes appeared to fail mainly by adhesive failure since no polymer was observed on the paper. However, the peeled tape did have small white spots of embedded paper . This indicates partial cohesive failure of the paper had occurred with the commercial tapes.

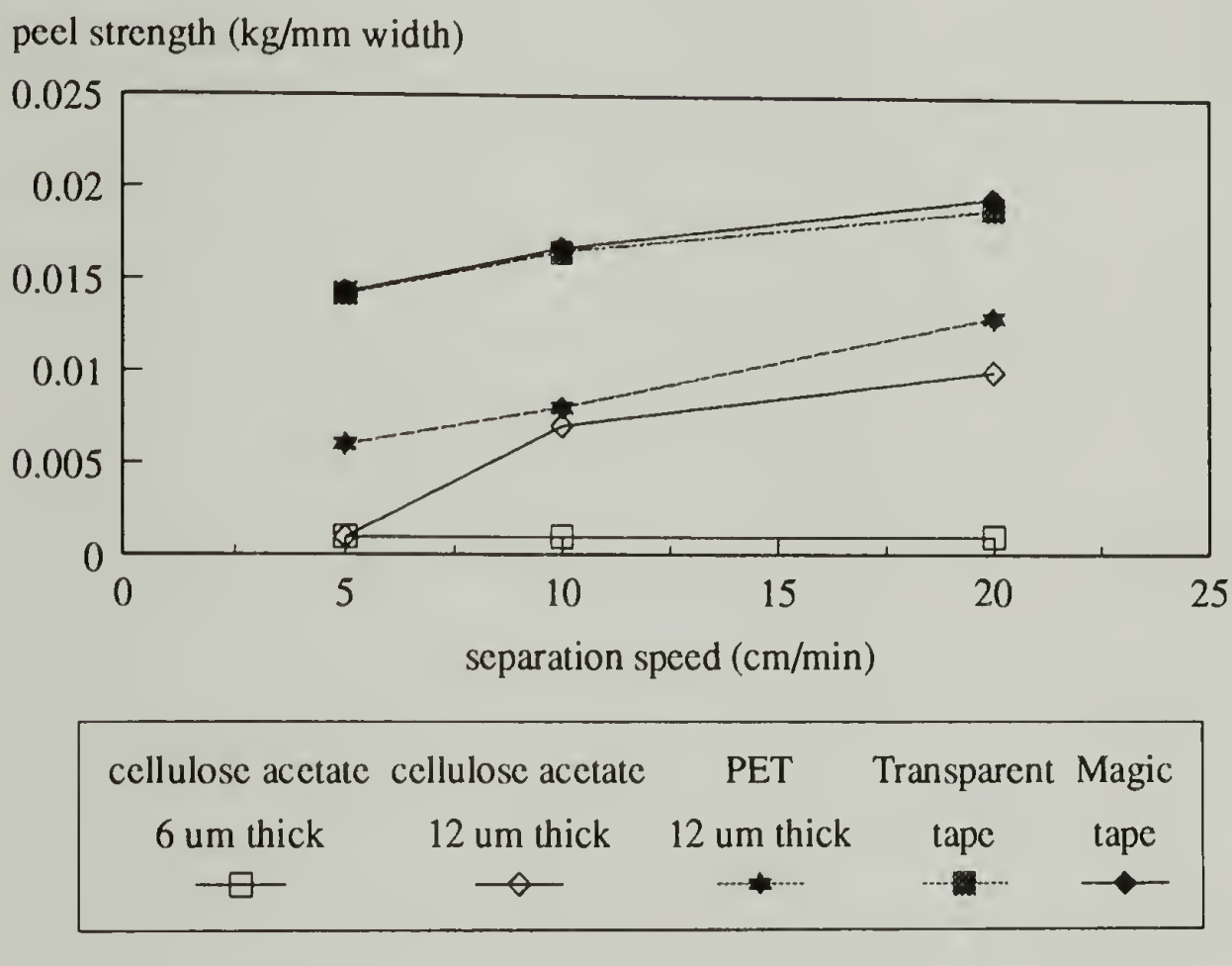

Figure 3.11 Peel test results for PHO of several film thicknesses and on two different backing materials including 41μ thick cellulose acetate and 25μ thick PET. Commercial tapes are included for comparison.

PHO has a relatively good adhesive strength as compared to the two commercial tapes. A thicker PHO layer and proper compounding with tackifiers and plasticizers would certainly improve the adhesive performance.

3.3.7 Thermal Expansion Coefficient

The thermal expansion coefficient of PHO was calculated to be α = 195 μm/m°C. This value was determined from the slope of the dimension change versus temperature curve shown in Figure 3.12. The thermal expansion coefficient was slightly higher than other polymers such as polybutylene (α = 130) and polyethylene (α = 100 to 180).

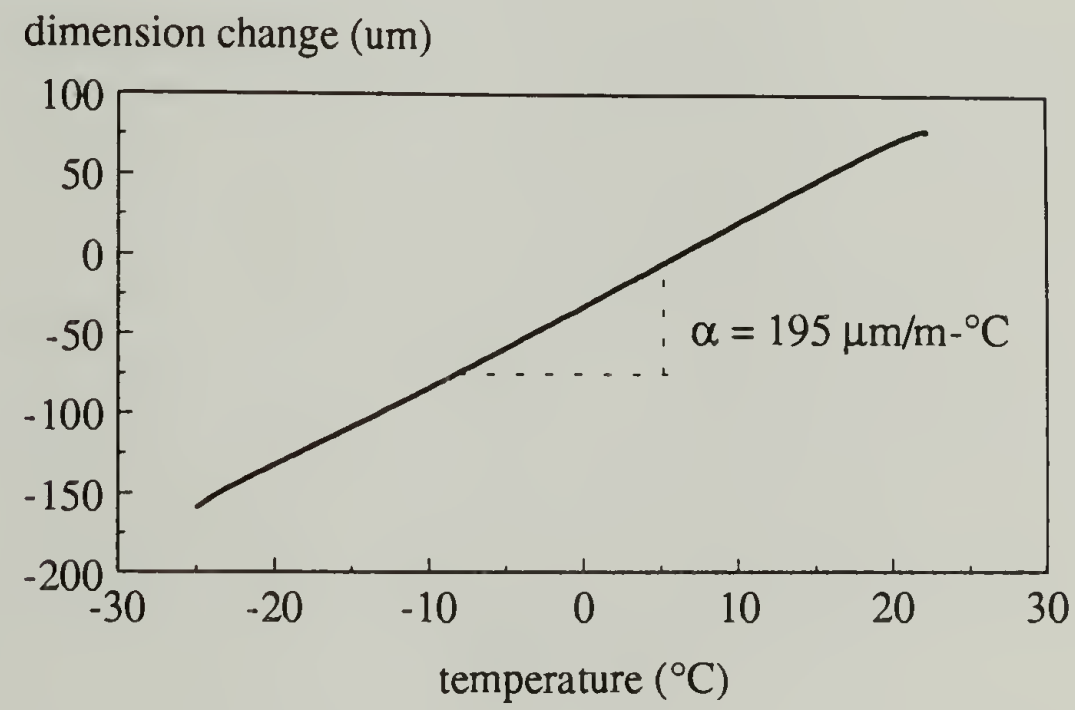

Figure 3.12 Thermal expansion of PHO determined from the slope of the length versus temperature experiment.

3.3.8 Optical Activity

A PHA produced by *P. oleovorans* with a phenyl containing pendant group was reported to have an optical rotation of $[\alpha]^{25}_{D} = -24.5° \pm 0.2°$.[12] PHO is also expected to

exhibit optical activity because of the stereoregularity of the pendant groups. The optical rotation, [α], in standard units was determined to be -1.2°/dm per unit concentration with a 95% confidence interval of 0.014. Due to the instrument design, rotations 180° apart are indistinguishable. Therefore, the actual [α] for PHO could be -181.2° or -361.2°. Firm conclusions regarding the optical activity of PHO were difficult to make because of the relatively low value for optical rotation. Measurement of the optical rotation as a function of wavelength from about 200 nm to 700 nm would be a more useful experiment but was not possible on the available equipment.

The conformation in solution can affect the resulting measured optical activity. The presence of both a right-handed and left-handed helix for instance could mask the optical activity of the polymer. Many studies on the conformation of PHB in solution were conducted often with conflicting results.[24 - 26] The present understanding is that PHB does exhibit a helical conformation in solution although whether a right or left-handed helix or both are present is uncertain. [26]

3.3.9 Solubility

PHO was screened for solubility in various organic solvents. The results of the solubility experiments are shown in Table 3.5. The goal of this screening was to determine the range of cohesive energy densities or solubility parameters, δ, in $(cal/cm^3)^{1/2}$ of solvents in each group capable of dissolving PHO.[27] However, the study did not result in a range of solvents capable of dissolving PHO bracketed by solvents incapable of dissolving PHO. Either a wide enough range of solvents was not tried in each group or solvents were found within a postive range that had produced negative results.

Table 3.5 Results of solubility screening of PHO in various solvents.

Type of Solvent [27]	Solvent	Solubility Parameter [27] δ [(cal/cm^3)$^{1/2}$]	Soluble (+ or −)
Strongly Hydrogen Bonded	methanol	14.5	−
	ethanol	12.7	−
	n-butanol	11.4	−
	pyridine	10.7	+
	propionic acid	8.1	+
Moderately Hydrogen Bonded	DMF	12.1	+
	acetone	10.0	+
	MEK	9.3	+
	THF	9.1	+
	ethyl acetate	9.1	+
	diethyl ether	7.4	+
Poorly Hydrogen Bonded	acetonitrile	11.9	−
	methylene chloride	9.7	+
	chloroform	9.3	+
	benzene	9.2	+
	toluene	8.9	+
	xylene	8.8	+
	carbon tetrachloride	8.6	−
	cyclohexane	8.2	+
	n-heptane	7.4	−
	hexane	7.3	+[a]
	n-pentane	7.0	+[a]
	pentene	6.9	+

[a] requires warming for total dissolution

3.4 Conclusions

PHO can be considered a thermoplastic elastomer unique in its source, bacteria. Because of this natural source, PHO has a unique chemical structure for a TPE, a stereoregular, random copolymer. The polymer is semicrystalline (30%) and because of a low T_g (-36 °C) and a high T_m (61 °C), the polymer exhibits elastomeric behavior within this temperature range. The commercial TPEs involved in this study were structurally different being either block copolymers (amides, copolyesters, olefinics, styrenics, urethanes,) or blended dissimilar polymers (elastomeric alloys). Yet the stress-strain properties and hardness of PHO were found to be in the range of these structurally different, commercial TPEs.

The statistical rubber elasticity theory with a correction for the filler effect of the crystalline physical crosslinks indicates a physically crosslinked network forms when PHO is crystallized from the melt at room temperature with a molecular weight between crosslinks of approximately 4000 g/mol and with approximately 10 to 20 physical crosslink regions per chain. This network structure is similar to the segmented polyurethanes.

As an elastomer PHO has a shortcoming, a relatively high tensile set, approximately 35% after 100% elongation. However, the commercial copolyester evaluated exhibited an even higher tensile set, approximately 55%, and is still classified as a thermoplastic elastomer. The occurrence of tensile set in PHO appears to be due to the many changes that occur in the crystalline regions upon deformation.

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CHAPTER 4

CRYSTALLIZATION

4.1 Background

Thermal history greatly affects the mechanical properties of semi-crystalline polymers mainly due to the changes in crystallinity. Thermal conditions can affect the crystalline growth rate, resulting crystallite size, and final degree of crystallinity. Annealing polymers to increase lamellar thickness, increase the purity or perfection of the crystalline regions and increase the overall degree of crystallinity is well known.[1-3] PHO has been described as a thermoplastic elastomer due to a glass transition temperature of -35 °C [4] , a melting temperature at 61 °C [4] , and approximately a 30% degree of crystallization.[5] Rubbery behavior is observed at room temperature where the crystalline regions act as physical crosslinks.

Because the crystalline regions of PHO act as physical crosslinks, an investigation into crystallization behavior as a function of temperature was conducted. The rate of crystallization was investigated over a range of temperatures to determine the temperature at which the crystallization rate is at a maximum and to estimate the theoretical maximum melting temperature (equilibrium melting temperature). Several possible nucleating agents [6, 7] were evaluated to see if the rate of crystallization could be increased for PHO crystallized at room temperature from the melt. A long term crystallization study was conducted on PHO films to determine the time required to reach a maximum level of crystallinity, to measure the levels of crystallinity obtainable at the different crystallization temperatures, and to measure the effect of different thermal histories on the mechanical properties of this material. Tensile set, an indication of the inelastic nature of the material

was also evaluated. A thermal analysis of these stretched samples was conducted to observe any changes to the crystalline regions after extension. GPC analysis was employed to evaluate any changes to the molecular weight distribution after deformation.

4.2 Experimental

4.2.1 Sample Preparation

In general, PHO films were cast from a 10% to 15% chloroform solution. Except for the samples used in the nucleation study, the solutions were filtered through a 10 micron filter into glass casting dishes. After solvent evaporation, DSC samples were taken from the films and melted at 80 °C for 3 to 5 minutes. Sample handling then diverges for each test and is described separately below. The remaining films were kept in the casting dishes and melted at 80 °C overnight in a vacuum oven and placed in the appropriate temperature bath and allowed to crystallize.

4.2.2 Crystallization Kinetic Study

PHO does not exhibit spherulitic texture so in order to determine crystallization rates, the melted samples were allowed to crystallize at different temperatures for 24 hours. After 24 hours a DSC thermogram was obtained to determine the heat of fusion (a proportional indication of the level of crystallinity) which had developed in the 24 hour period. Because all samples were compared after 24 hours and because crystallization was not complete in that period of time, relative rates of crystallization could be determined. Three to six samples were used for each data point.

4.2.3 Nucleating Agent Study

Several possible nucleating agents including talc, saccharin, boron nitride, and poly(β-hydroxybutyrate), PHB, were added at 0.5%, 1.0% and 5% by weight to chloroform solutions of PHO prior to casting the films. The melted samples were allowed to crystallize at room temperature for specific times. The samples were then re-evaluated in the DSC to determine the ΔH_m (a proportional indication to the amount of crystallinity) that had developed during the allotted time.

4.2.4 Long Term Crystallization Study

PHO films crystallized at three different temperatures were evaluated for crystallization development and mechanical properties. The temperatures used were, -20 °C, 5 °C, and 20 °C, which represent slow, fast and medium crystallization rates, respectively, as determined by the short term crystallization study. One of the two films crystallized at -20 °C contained a possible nucleating agent, talc, which was included to compare the effect of the possible nucleating agent on crystallization rate and final level of crystallinity reached after a long time. The melted DSC samples were placed in the appropriate temperature bath for various times then run in the DSC to determine the ΔH_m.

4.2.5 Tensile Properties

After 24 weeks the films were removed from the temperatures baths and allowed to equilibrate to room temperature. All tensile testing was conducted using an Instron Universal Testing Instrument, Model TTBM with an Interface SM-50 load cell. Ring samples per ASTM D 412 [8], Type 2, were punched from the films using a steel rule die and Carver Hot Press Model C at room temperature. Special grips were made to hold the

ring specimens. The ring was supported at each end by a pin, approximately 5 mm in diameter. Each pin was engaged at both ends in the inner track of a ball bearing to insure the stress and strain remained equal on both sides of the ring during testing. The pins are removable for loading the sample. The initial gage length was based on half the average circumference of the ring die. A strain rate of 1 min^{-1} was used for all tensile tests.

4.2.6 Tensile Set

After 28 weeks of crystallization, strip samples, 50 mm wide, were cut from the films and evaluated for tensile set. All tensile set testing was conducted using an Instron Universal Testing Instrument, Model TTBM with an Interface SM-50 load cell. Tensile set testing procedures were based on the procedures described in ASTM D412.[8] Grip slippage was a problem for the elastomers and since no extensiometer was available, a ruler was held next to the sample to insure the marked gage length reached the desired elongation. Different crosshead speeds were used to reach the desired elongation within approximately 15 seconds. The samples were held for 10 minutes at the intended elongation then released at the same crosshead speed. The samples were then allowed to recover for 10 minutes prior to final measurement of the gage length. Tensile set was determined as the % change in the marked gage length of the samples. Tensile set at break was also determined from the ring samples which had undergone tensile testing and was calculated as the percent change in the mean circumference of the samples. DSC samples were taken from stretched areas of samples crystallized at 20 °C for long times to evaluate any changes in crystallinity upon deformation.

4.2.7 Gel Permeation Chromatography

All molecular weight data was obtained using a Rabbit Model solvent delivery system with a Waters Model R401 differential refractometer detector and three Polymer Labs PL 5 μ gel columns with mean pore diameters of 10^5Å, 10^4Å, and 10^3Å. A 2 ml/min tetrahydrofuran flow rate was used with a toluene flow marker. The molecular weight was based on polystyrene standards.

4.3 Results and Discussion

4.3.1 Crystallization Kinetic Study

The results of the experiment are depicted in Figures 4.1, 4.2, and 4.3. Figure 4.1 shows the variation in heat of fusion, ΔH_m, with crystallization temperature, T_c. As expected, a bell shape curve resulted with the rate reaching a maximum at approximately the median between the glass transition temperature at -35 °C and the melting temperature at 61 °C. The location of the peak indicates that the fastest crystallization rate occurred between 0° and 5 °C.

At temperatures above 25 °C, scatter in the data was noted. The three open symbols indicate data points from single samples. The solid symbols indicate an average of the values from 3 to 6 samples. The extent of crystallinity in the majority of samples crystallized from 25 °C to 37 °C was higher than expected. Impurities can act as nucleating agents and even though filtering of the polymer solutions prior to film casting was conducted, all impurities may not have been removed. These undesired nucleating agents would have a more profound effect at the higher temperatures where nucleation can limit the rate of crystallization. These nuclei would enable a higher than expected extent of crystallization to occur during the 24 hours, thereby contributing to the data

scatter. Annealing effects are not significant, neither during the DSC scan nor during the initial 24 hours of crystallization, as will be shown in the long term crystallization study results. Crystallization at lower temperatures is generally limited by chain mobility and not by nucleation, so impurities acting as nucleation sites would not have as much effect on the extent of crystallization in 24 hours. In this case data scatter would not be expected, and in fact, was not observed.

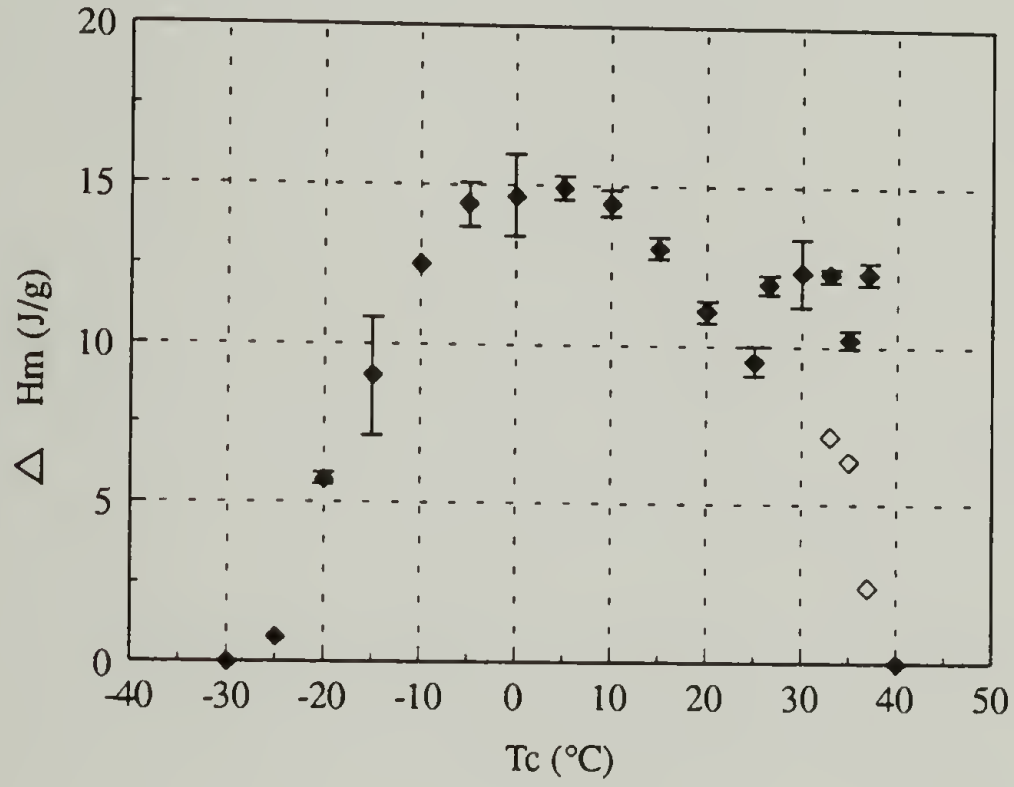

Figure 4.1 The variation in heat of fusion, ΔH_m, with the crystallization temperature, T_c, for PHO crystallized for 24 hours from the melt. Solid symbols represent average values from 3 to 6 samples. Open symbols represent single data points. Error bars indicate ± one standard deviation.

Figure 4.2 depicts the change in melting temperature, T_m, as a function of crystallization temperature, T_c. The melting temperature varied with crystallization temperature in a manner common for all polymers. The line shown through the data was determined by linear regression, and the intersection of this line with the theoretical

T_m=T_c line was used to estimate the equilibrium melting point. The equilibrium melting point of PHO was determined to be approximately 68 °C using this construction. Figure 4.3 shows that as expected, the glass transition temperature, T_g, did not vary significantly with crystallization temperature, T_c.

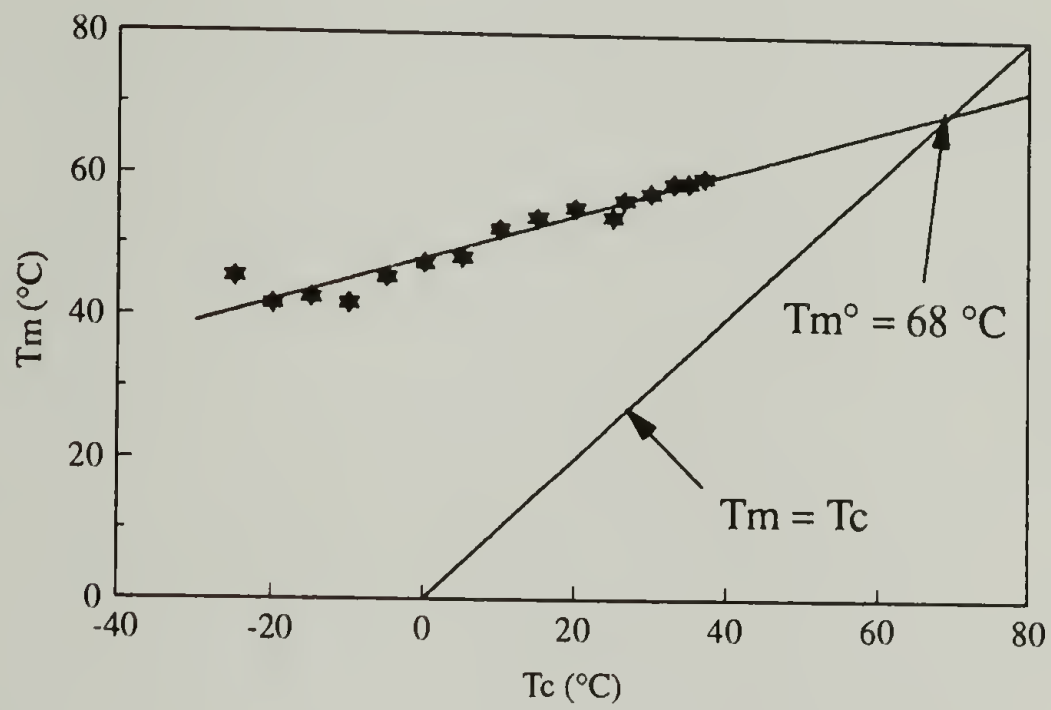

Figure 4.2 The melting temperature, T_m, as a function of the crystallization temperature, T_c, for PHO crystallized for 24 hours from the melt.

4.3.2 Nucleating Agent Study

Two potential nucleating agents, saccharin and PHB, were soluble in chloroform. The other two potential nucleating agents, talc and boron nitride, formed suspensions. Heterogeneous nucleation appears to have occurred in all systems upon crystallization from the melt. Upon cooling from the melt, the initially soluble nucleating agents formed

films with obvious irregularities believed to be regions where phase separation of the potential

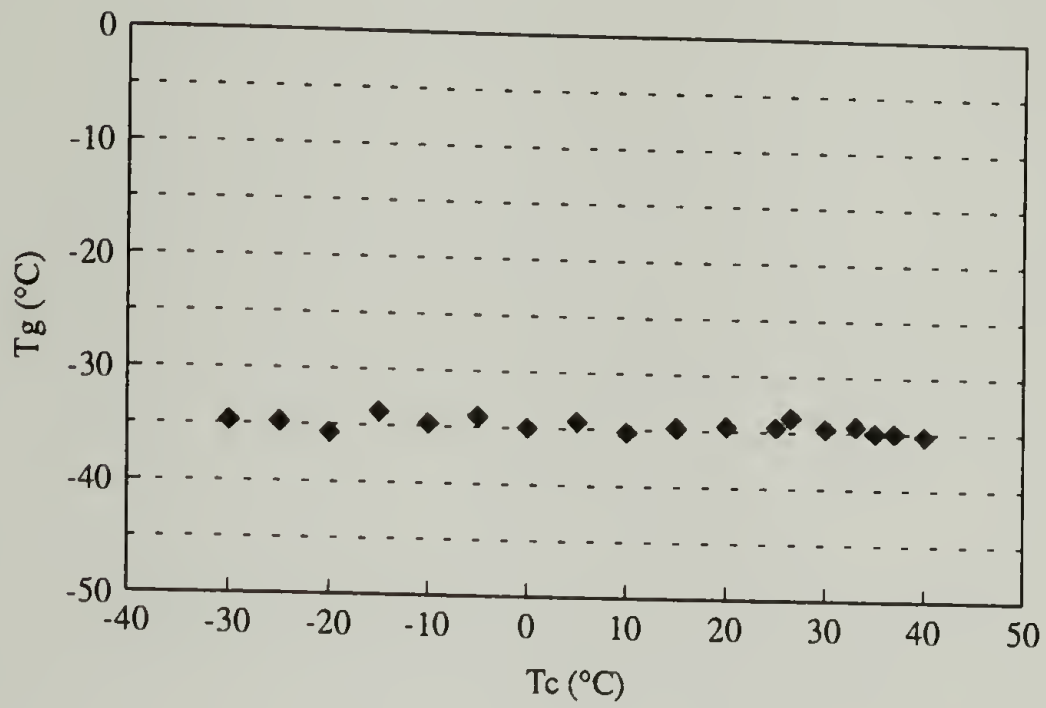

Figure 4.3 The glass transition temperature, T_g, as a function of the crystallization temperature, T_c, for PHO crystallized for 24 hours from the melt.

nucleating agents had taken place. These regions formed sites for heterogeneous nucleation.

Figures 4.4, 4.5, and 4.6 show the development of crystallinity in PHO as a function of time. Crystallinity was inferred from the heat of fusion, ΔH_m, which is proportional to the amount of crystallinity. Three different potential nucleating agent loading levels were used, 0.5 weight %, 1.0 weight %, and 5.0 weight %. All samples were crystallized at room temperature from the melt. Also included on each graph are the results for ΔH_m development at the fastest crystallization rate, 5 °C and at room temperature, 20 °C. None of the nucleating agents increased the development of crystallinity above the fastest rate obtained at the subambient temperature. At 0.5 weight % loading levels, no potential nucleating agent significantly altered the crystallization rate. At the 1.0 weight % loading level, saccharin increased the crystallization rate above that of

neat PHO crystallized at the same temperature. At the highest loading level of 5 weight %, no potential nucleating agent altered the rate of crystallization significantly. It is interesting to note that the nucleating agent with the largest size, saccharin, was the only nucleating agent which effected the crystallization rate significantly. These results are unexpected since a very fine compound such as talc, usually affords the best results.

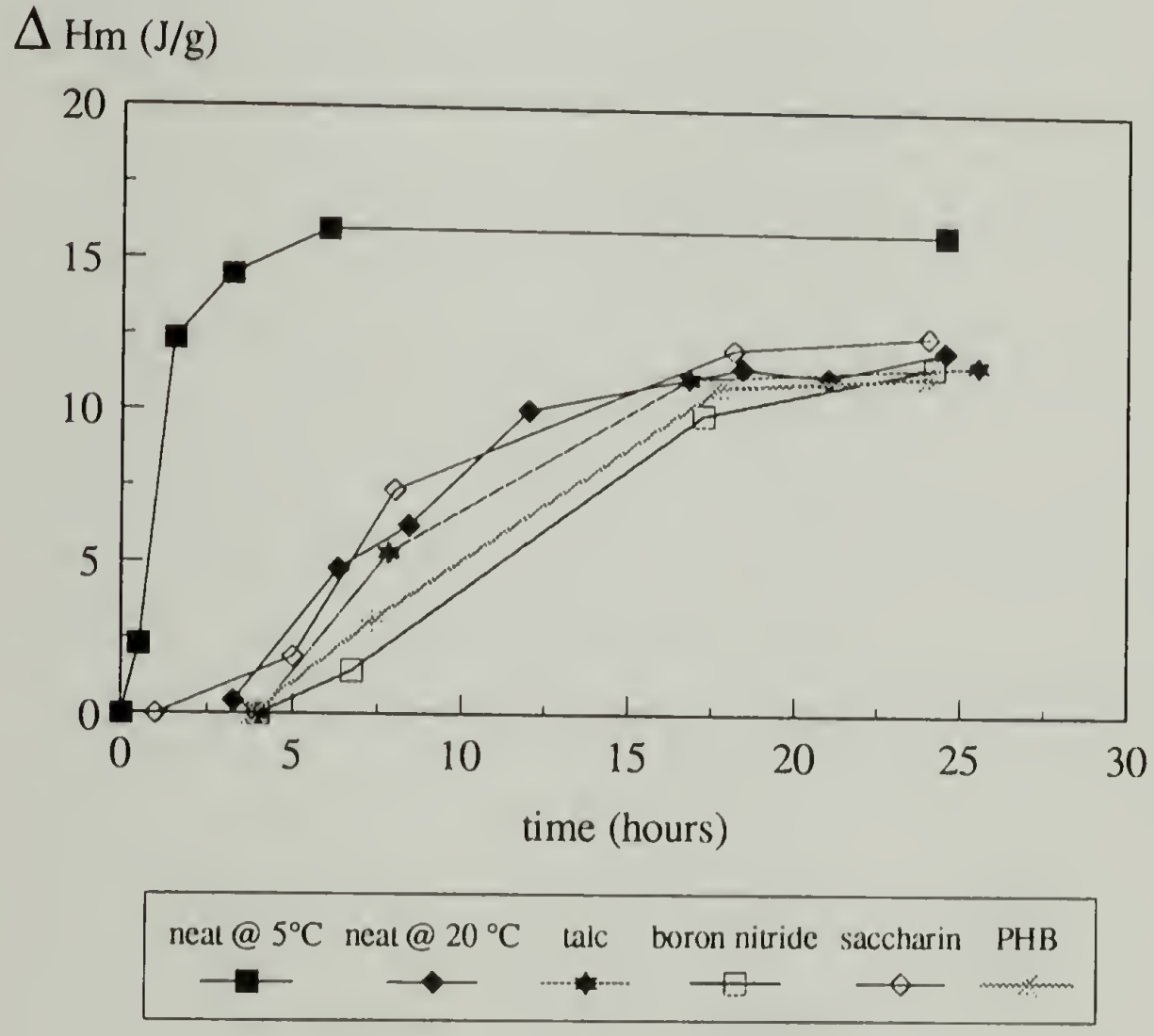

Figure 4.4 The heat of fusion, ΔH_m, as a function of time for a 0.5 weight % loading level of potential nucleating agents.

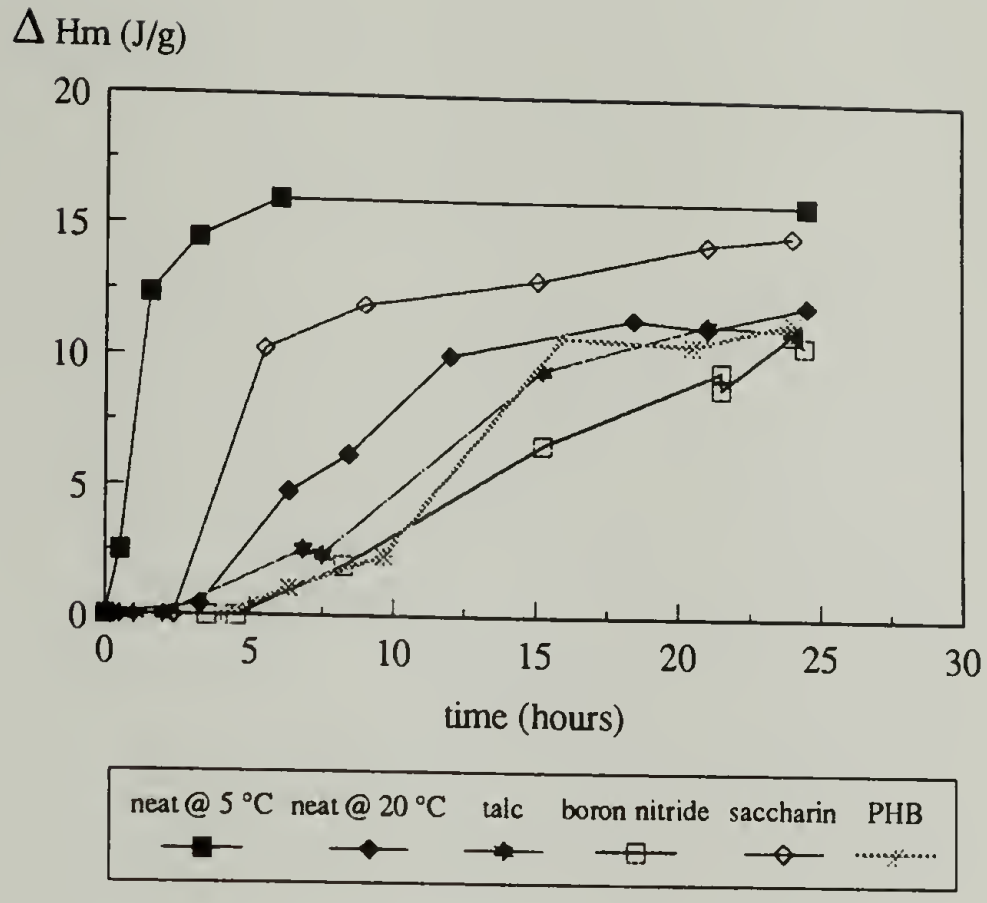

Figure 4.5 The heat of fusion, ΔH_m, as a function of time for a 1.0 weight % loading level of potential nucleating agents.

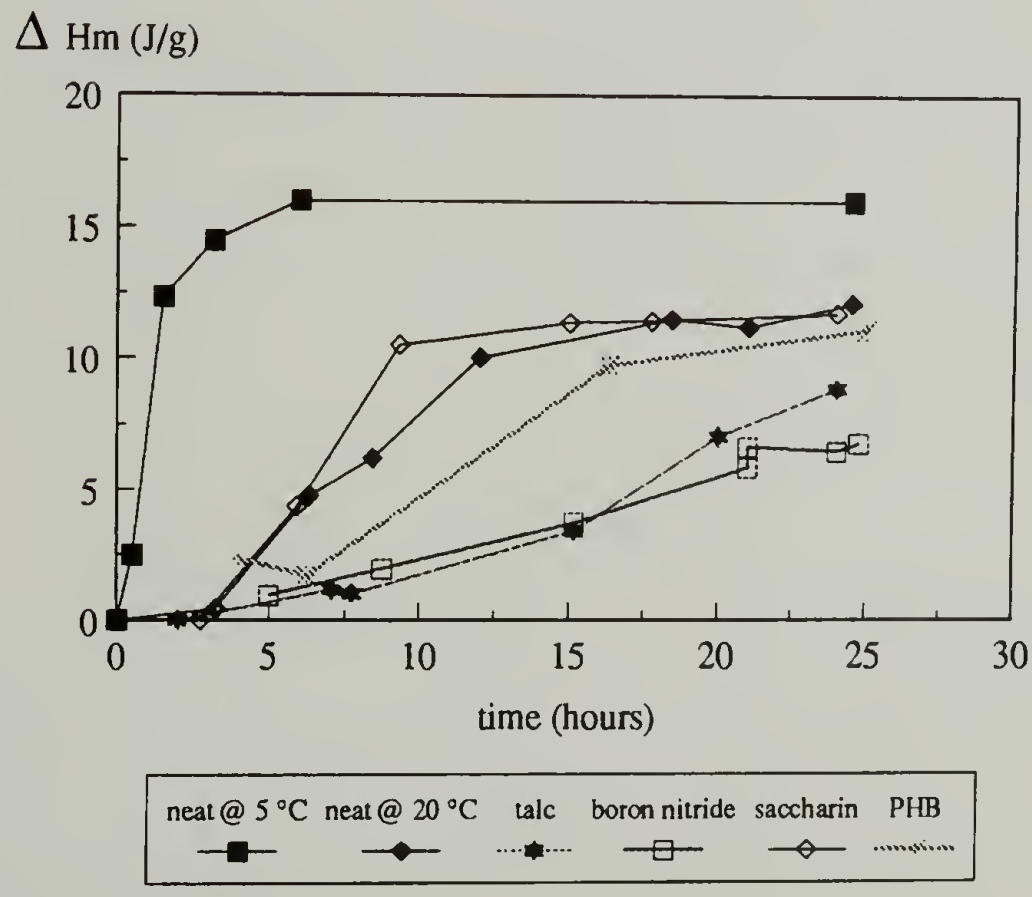

Figure 4.6 The heat of fusion, ΔH_m, as a function of time for a 5.0 weight % loading level of potential nucleating agents.

4.3.3 Long Term Crystallization Study

Figure 4.7 shows the heat of fusion, ΔH_m, as a function of time over 24 weeks of crystallization for all four films. Different maximum levels of crystallinity were reached in each film during the 24 weeks of crystallization, reaching constant levels after approximately 10 weeks. The maximum heat of fusion obtained in the films was directly proportional to the crystallization temperature; larger heats of fusion occurred in films crystallized at higher temperatures.

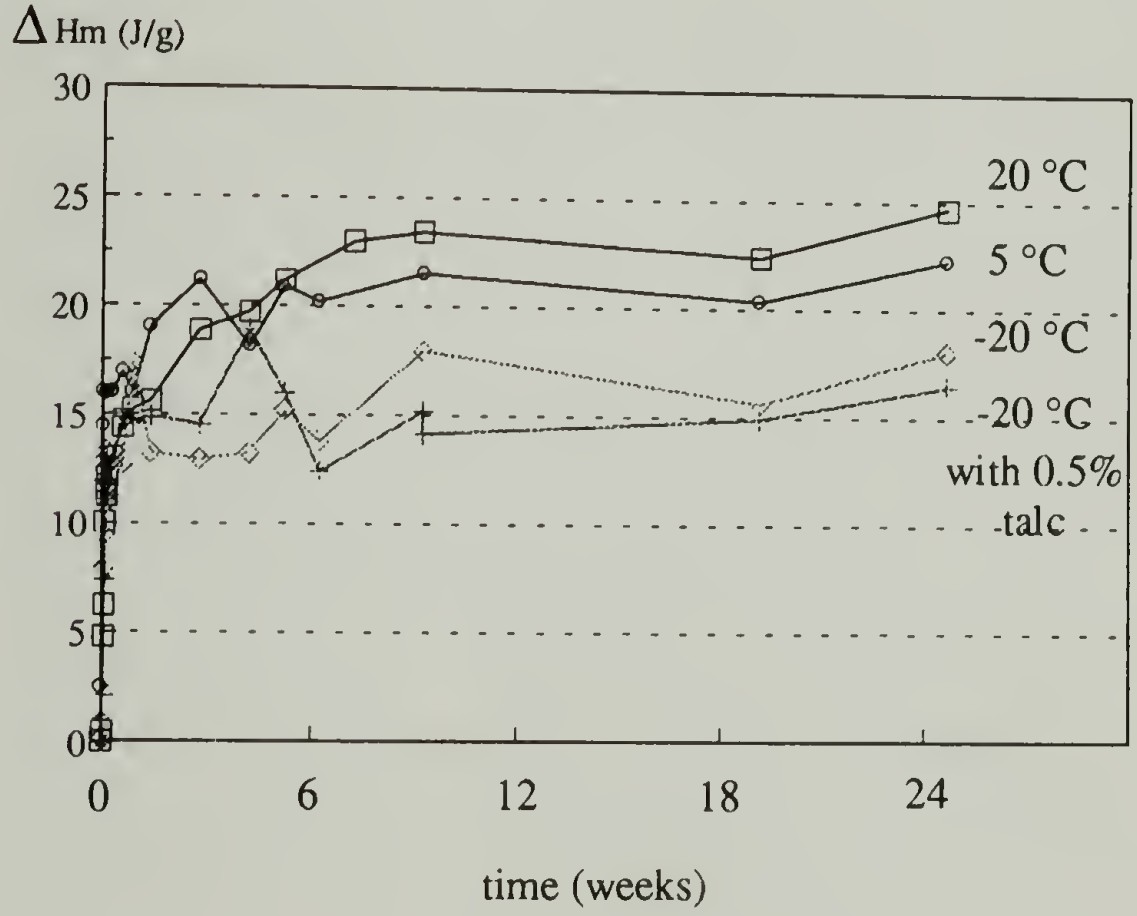

Figure 4.7 The heat of fusion, ΔH_m, as a function of the 24 week crystallization time for PHO crystallized from the melt at the indicated temperatures including one film containing a potential nucleating agent (0.5 weight % talc).

Results from the initial 50 hours are shown more clearly in Figure 4.8. From this figure, constant levels in the heats of fusion appeared in all the samples. For the film held at 5 °C, this level in the extent of crystallinity was reached after approximately 6 hours but took approximately 20 hours for the other films. In all cases, the levels were temporary with a substantial increase in the extent of crystallinity, a 30% to 100% increase, observed after the entire 24 weeks as shown in Figure 4.7.

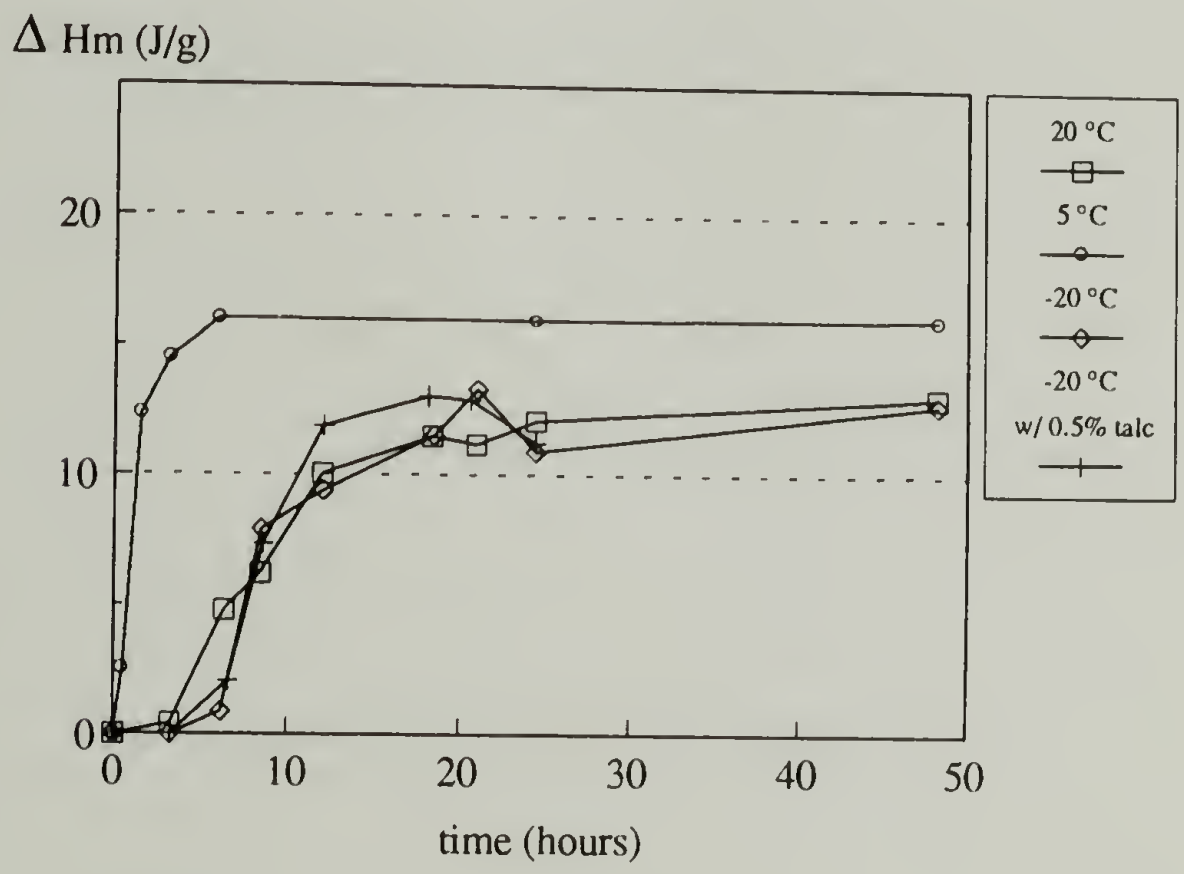

Figure 4.8 The heat of fusion, ΔH_m, as a function of the first 50 hours of crystallization time for PHO crystallized from the melt at the indicated temperatures including one film containing a possible nucleating agent, 0.5 weight % talc.

Both films crystallized at -20 °C showed nearly the same final level of crystallinity with only a slightly lower level of crystallinity observed in the film containing talc. In addition, no significant difference was noted in the time required to develop crystallinity for these two samples (Figures 4.7 and 4.8). These observations indicate the talc did not

behave as an effective nucleating agent on PHO crystallized from the melt at -20 °C. Perhaps the talc would have affected the crystallization rate more dramatically if the film had been crystallized at a higher temperature where nucleation is limited. In addition, the polymer solution containing the talc had been filtered prior to casting in an attempt to limit the talc particle size which may have significantly decreased the amount of talc incorporated into the film.

Annealing effects are expected over the 24 weeks because the films were maintained at temperatures above the T_g and below the T_m. Annealing effects observable from DSC experiments are manifested as changes in the shape of the melting endotherm, in location of the peak melting temperature, and in the area under the endotherm peak.

Monitoring the change in the peak melting temperature over time gave an indication of when annealing effects became significant. From Figure 4.9, no increase in the melting temperature was observed at PHO crystallized at any crystallization temperature until approximately 250 hours. Annealing effects were, therefore, not considered significant during the 10 minute DSC scans, or during the 24 hour crystallization study.

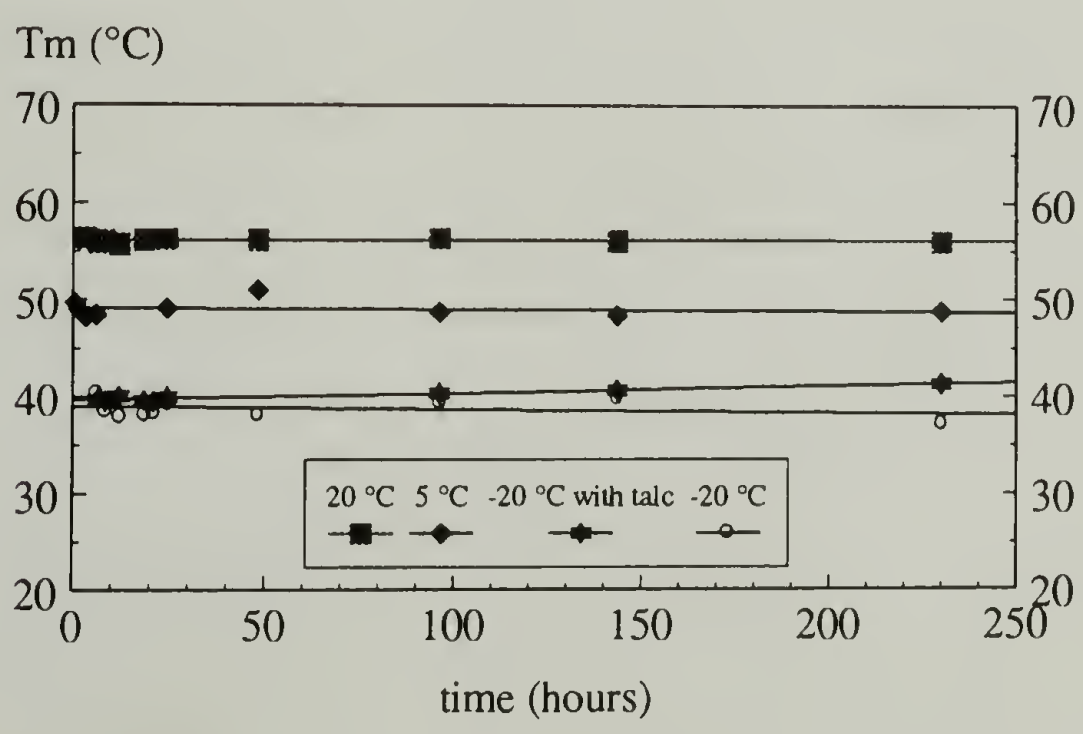

Figure 4.9 Changes in the peak melting temperature as a function of time.

The thermograms in Figure 4.10 illustrate the changes that occurred in the shape of the melting endotherm peaks which were typically observed for all films due to combined crystallization and annealing effects. In general, a broad melting endotherm with an obvious shoulder was at first observed. As crystallization continued, this shoulder

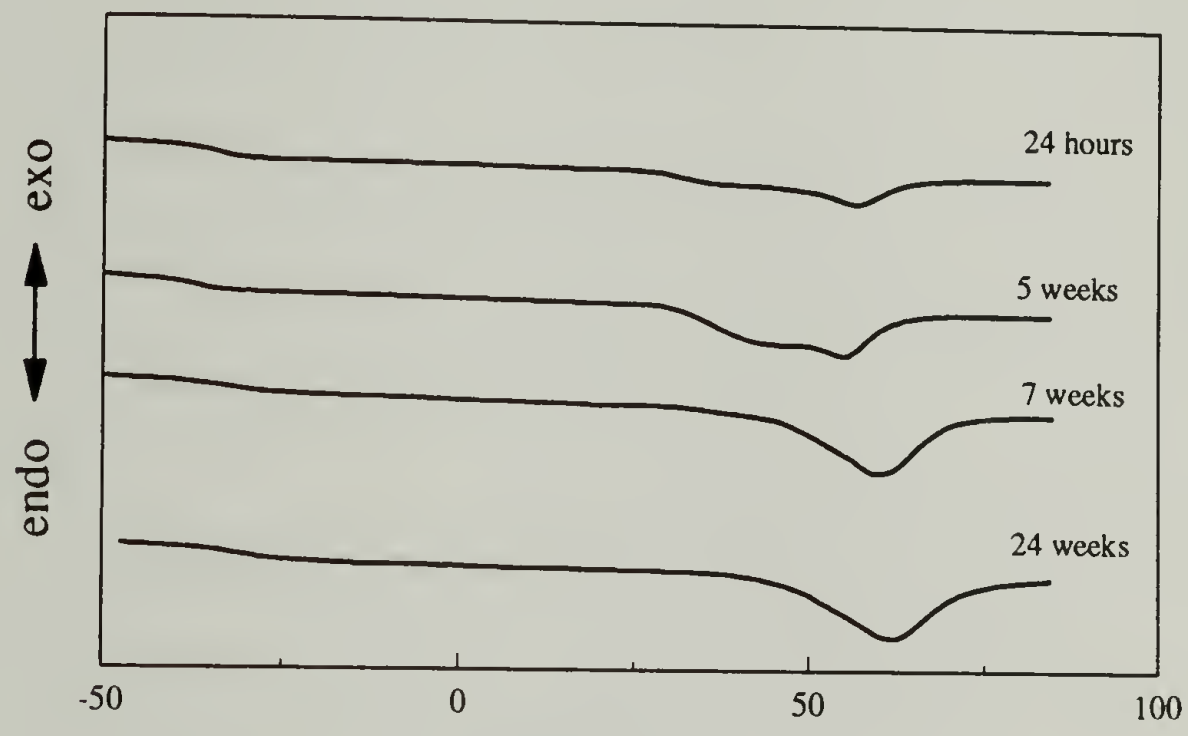

Figure 4.10 DSC thermograms of PHO crystallized at 20 °C from the melt at various times. These thermograms elucidate changes found for all films over time.

became more pronounced and it eventually coalesced into the main peak. The endotherm shown after 24 weeks illustrates the final state of the films prior to mechanical testing.

Table 4.1 lists the times taken to maximize the extent and stabilize the melting endotherm shape at the different temperatures. The time required for these changes depended on the crystallization temperature and crystallization rate. Overall, the minimum amount of time taken to reach both a stable endotherm peak shape and extent of

crystallinity was achieved with a medium crystallization rate and high temperature equivalent to a slow and steady combination of crystallization and annealing.

Table 4.1 Variability associated with certain crystallization parameters.

crystallization temperature (°C)	weeks to maximum extent of crystallization	weeks to stable melting endotherm peak shape
20	7	7
5	3	16
-20	8	19
-20 with talc	8	9

The T_g also increased slightly in all films, by an average of 3°C to 5 °C, with the increase observable only after approximately 250 hours. Crystallinity is known to increase the T_g of semicrystalline polymers due to the physical crosslinking effect these crystalline regions impart on the material.

Monitoring the level of crystallinity was continued and Figure 4.11 shows the ΔH_m as a function of log time. It appears there continues to be a gradual increase in crystallinity over very long times.

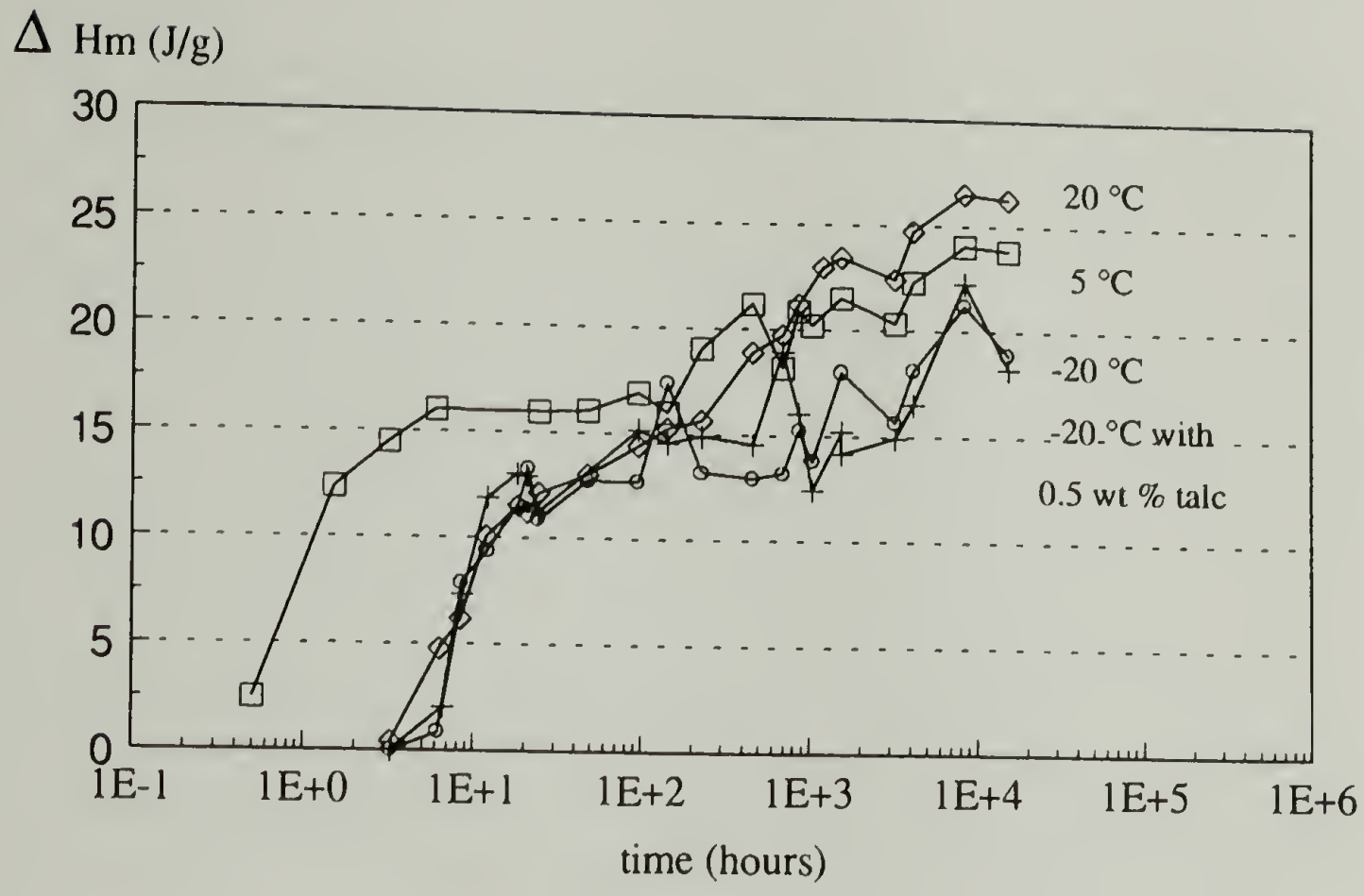

Figure 4.11 The heat of fusion, ΔH_m, as a function of log time for PHO crystallized from the melt at the indicated temperatures including one film containing a possible nucleating agent, 0.5% talc.

4.3.4 Tensile Properties

The results of the effect of crystallization temperature on mechanical properties are shown in Figure 4.12 where the Young's Modulus, tensile strength at break, and ultimate elongation are plotted as a function of crystallization temperature. The range of values obtained was large, with Young's Modulus showing a 200% increase, tensile strength at break a 60% increase, and ultimate elongation a 50% decrease over the crystallization temperature range evaluated.

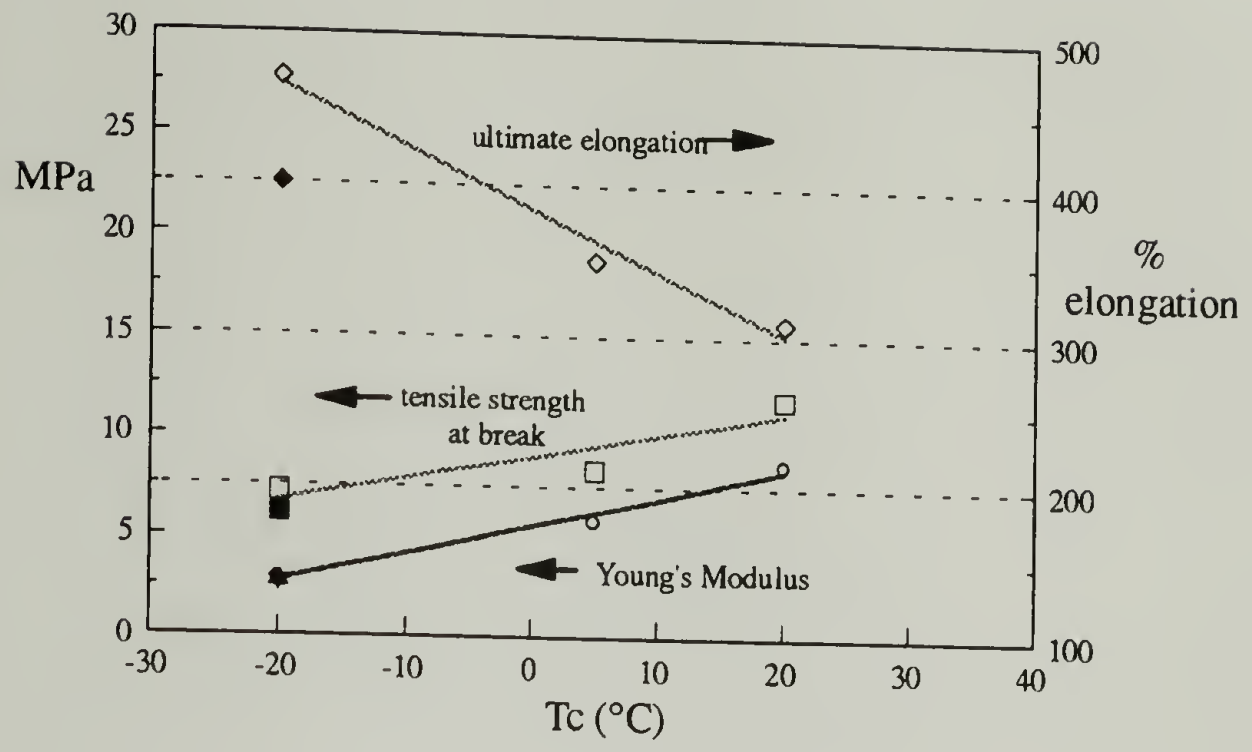

Figure 4.12 Young's Modulus, tensile strength at break, and ultimate elongation as a function of crystallization temperature. Open symbols represent PHO, closed symbols represent PHO containing 0.5 weight % talc. Results are average values from ring samples tested at a 1 min^{-1} strain rate.

The trends in the modulus and ultimate elongation can be explained in terms of the differences in the maximum extent of crystallinity. Higher moduli are expected as the amount of crystallinity increases due to the physical crosslinking and filler effect crystalline regions have on the material. The lower ultimate elongation maybe a result of the lower extensibility of crystalline regions and thus the whole material will become less extensible as the extent of crystallinity increases.

After two years the material still behaved elastomerically as indicated by the stress strain curve illustrated in Figure 4.13. The results are typical for all films independent of crystallization temperature. Table 4.2 summarizes the effect of crystallization temperature and time on the mechanical properties of PHO. Perhaps the elastomeric behavior of the material would eventually be replaced by thermoplastic behavior when some critical level of crystallinity is reached, however this would take very extended periods of time.

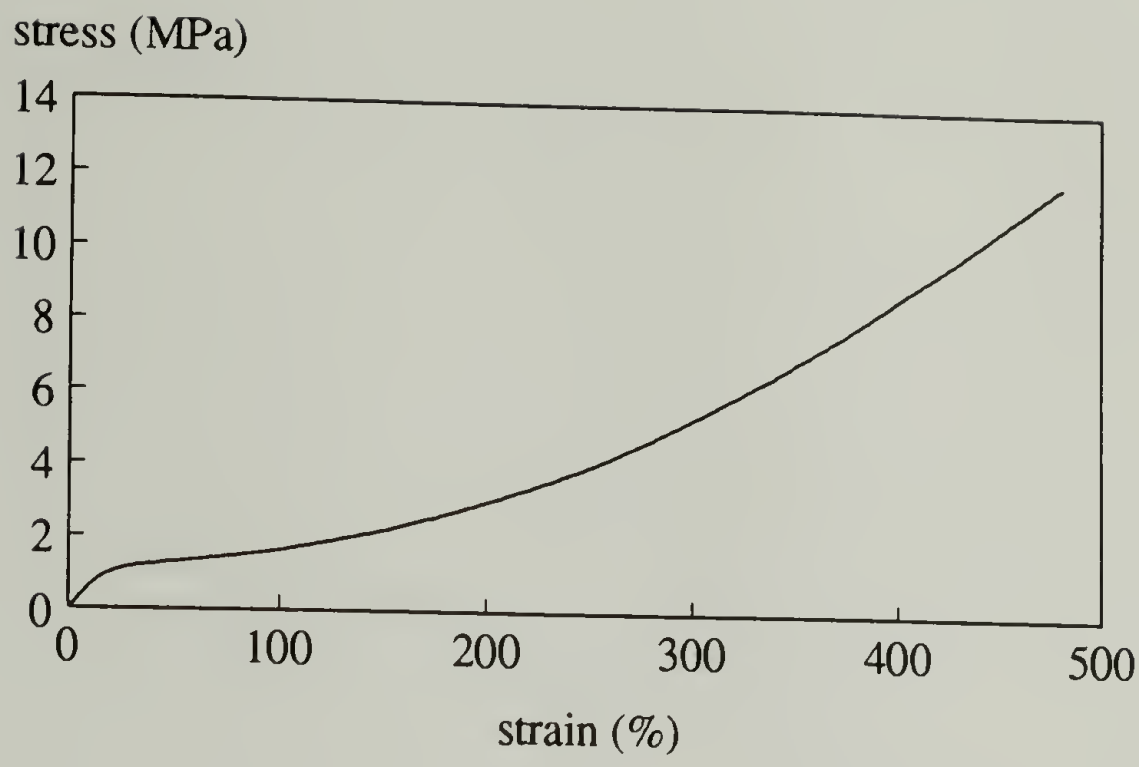

Figure 4.13 Typical stress-strain curve for PHO crystallized for two years.

Table 4.2 Summary of mechanical properties of PHO crystallized at different temperatures and for different lengths of time.

T_c	-20 °C		5 °C		20 °C		
	1 yr.	2 yrs.	1 yr.	2 yrs.	2 wks.[a]	1 yr.	2 yrs.
E [MPa]	2.9	7.0	5.8	12.1	8	8.6	17.7
Tensile Strength [MPa]	7.2	12.8	8.4	15.4	9	11.7	nd[b]
Ultimate Elongation [%]	470	500	350	435	380	310	nd[b]
100% Modulus [MPa]	1.0	1.6	1.7	2.3	2	2.8	nd[b]
300% Modulus [MPa]	3.3	5.3	6.3	8.4	7	11.1	nd[b]

[a] 2 min^{-1} strain rate. All other values for 1 min^{-1} strain rate.

[b] nd - no data

4.3.5 Tensile Set

Tensile set was substantial for all films, but a slightly lower tensile set occurred in the material crystallized at -20 °C as shown in Figure 4.14. As a comparison, tensile set for PHO crystallized at room temperature for only 2 weeks is included. No difference was noted in tensile set between PHO crystallized for 2 weeks or extended times.

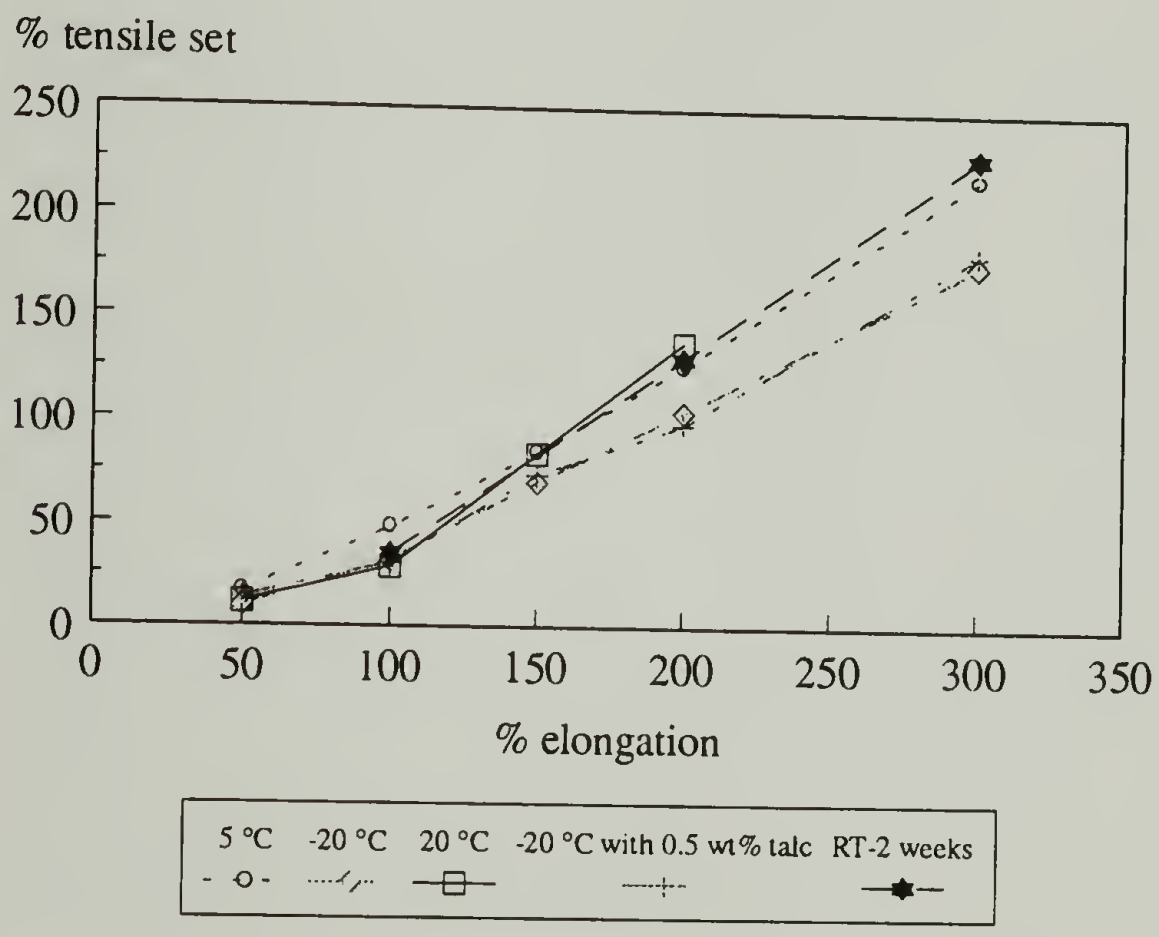

Figure 4.14 The tensile set evaluation results for PHO crystallized from the melt for 28 weeks at the various temperatures indicated. As a comparison, the tensile set results for PHO crystallized from the melt at room temperature for 2 weeks are also included in the figure.

To investigate the possible reasons for the high tensile set in PHO, thermal analysis was conducted on the strips used in the tensile set measurements from the film which had

been crystallized at 20 °C. Samples for the DSC measurements were taken from the stretched region of the strips. Table 4.3 lists the DSC parameters obtained for the samples tested. The heat of fusion, initially decreased 10% after 50% elongation, but upon further elongation, it increased to the initial value prior to elongation. The melting temperature also exhibited a decrease after 50% elongation with no further decrease observed on samples elongated to higher extents. No change in the glass transition temperature was observed.

Table 4.3 Thermal analysis results for PHO film crystallized from the melt at 20 °C and stretched to various elongations. From DSC thermograms at a heating rate of 20 °C/min.

% elongation	ΔH_m (J/g)	T_m (°C)	T_g (°C)
0	27	62	-32
50	24	59	-32
100	25	58	-33
150	27	59	-32
200	27	58/45	-32

Some unusual changes were observed in the shape in the melting endotherm as seen in Figure 4.15. A shoulder can be seen forming after the 100% and 150% elongations, but after 200% elongation a new distinct melting peak emerged at a lower melting point, 45 °C. This new peak may indicate that a different crystal structure was formed with stretching. Another possibility is that the deformation induced chain degradation. A bimodal distribution in the molecular weight could explain the double melting peak.

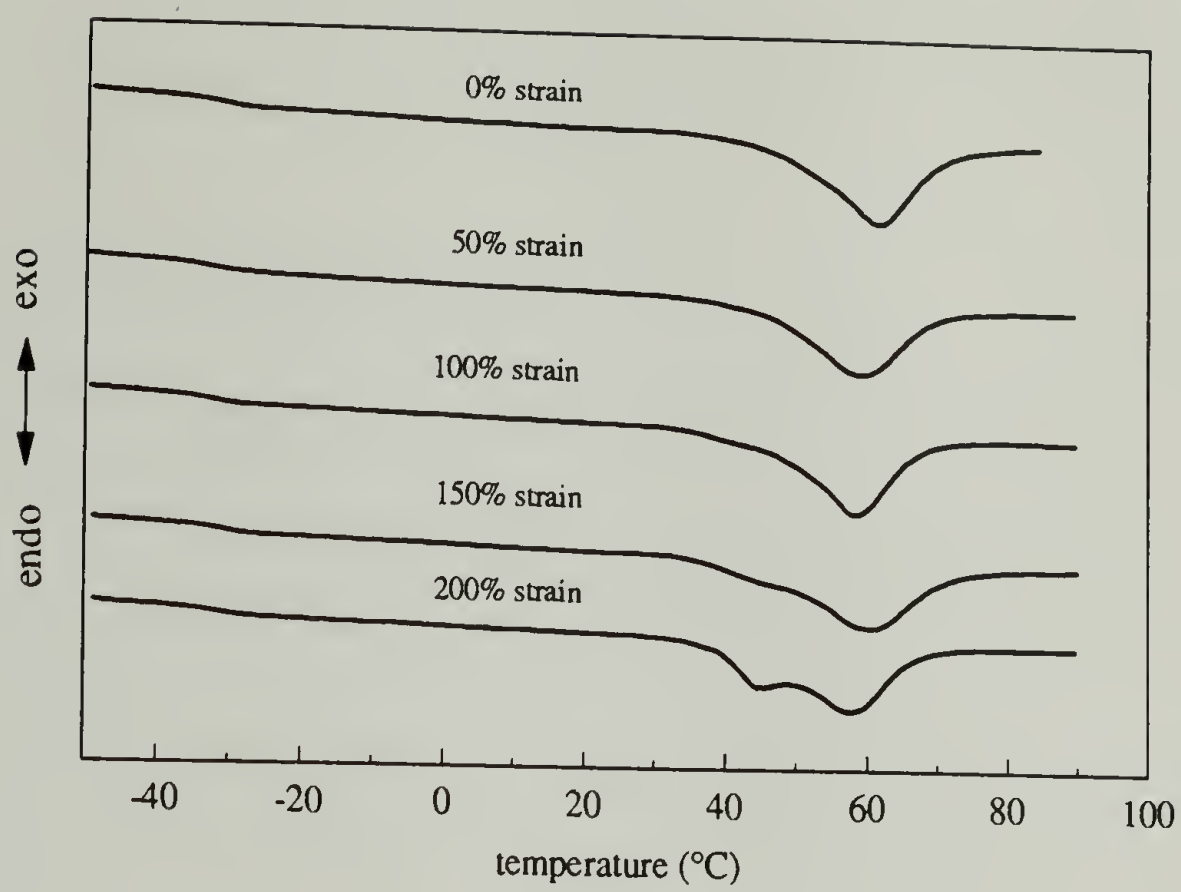

Figure 4.15 DSC thermograms obtained from PHO films stretched to different elongations. Prior to stretching, the samples were crystallized at 20 °C from the melt for 28 weeks.

4.3.6 Gel Permeation Chromatography

Because of the unusual double peak melting endotherm observed after stretching PHO 200% as shown in Figure 4.15, GPC analysis was conducted on the stretched samples to determine if any change to the molecular weight distribution had occurred because of the deformation. The results are shown in Figure 4.16 which shows no change in molecular weight distribution was observed. However PHO stretched 200% did shift to a slightly higher molecular weight. This shift corresponds to approximately a 10% change in molecular weight which is within the error of this technique. The double melting peak

observed for PHO stretched 200% does not appear to be the result of a deformation induced change in the molecular weight distribution of the polymer.

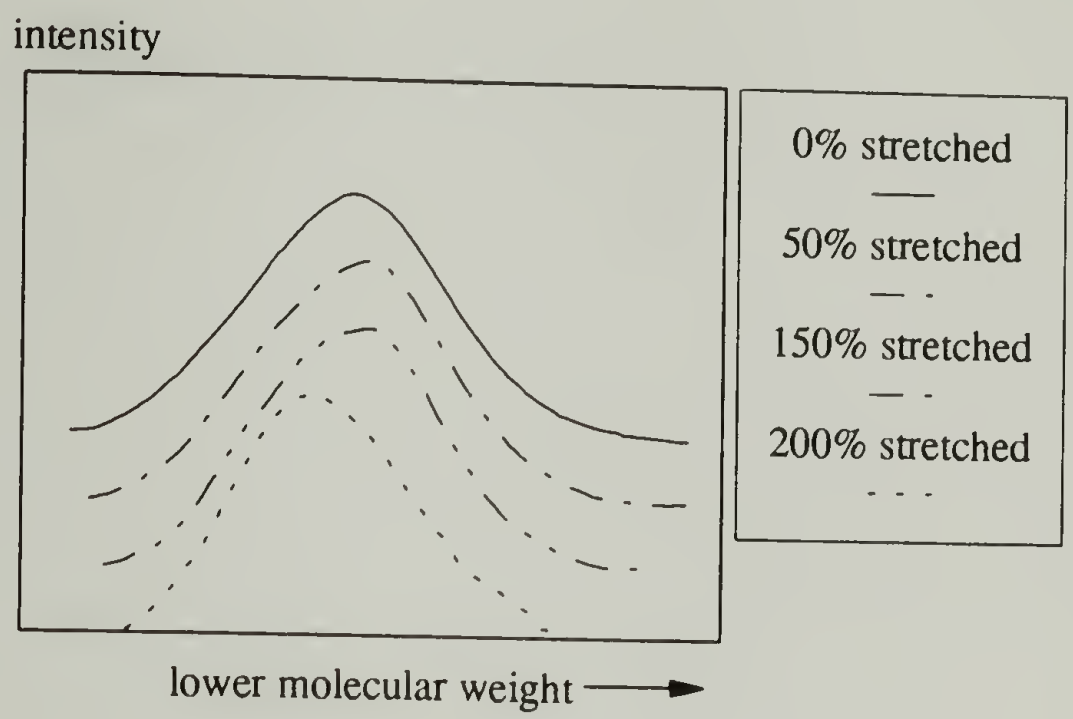

Figure 4.16 GPC chromatograms of PHO before and after stretching.

4.4 Conclusions

PHO crystallization, based on the heat of fusion as the indicator of extent of crystallinity, occurred fastest at 0 °C to 5 °C. The equilibrium melting point of PHO was determined to be approximately 68 °C. Long term crystallization is believed to be a combination of crystallization and annealing because the T_g of PHO is below room temperature. PHO crystallizes very slowly requiring approximately 7 weeks at 20 °C and 16 weeks at 5 °C to attain stable levels of crystallinity with an unchanging melting endotherm peak shape. The ΔH_m continues to increase over time at room temperature. Eventually PHO may behave like a thermoplastic if a high enough level of crystallinity is

reached. Property results after two years at room temperature indicate the material still behaves elastomerically.

The thermal history greatly affected the mechanical properties of the material crystallized for 24 weeks with Young's Modulus varying from 9 MPa to 2.5 MPa, tensile strength at break from 6 MPa to 10 MPa, and ultimate elongation from 300% to 450% depending on the crystallization temperature. Tensile set was extensive for PHO crystallized at all three of the temperatures evaluated, approximately 35% after 100% elongation. Elongation of PHO films crystallized for extended times showed unusual changes in the melting endotherm peak shape, and a new distinct, lower melting point peak emerged at 45 °C after 200% elongation. This new peak does not appear to be the result of a change in the molecular weight distribution. Perhaps the deformation induced a different crystal structure. Wide angle X-ray diffraction studies would help resolve this unusual finding.

4.5 References

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CHAPTER 5

CHEMICAL CROSSLINKING

5.1 Background

The motivation behind conducting chemical crosslinking experiments on PHO and PHOU is to improve the elastic response of these materials. The crystalline regions which act as the physical crosslinks in PHO changed upon deformation and resulted in substantial tensile set as discussed in Sections 3.3.3 and 4.3.5. The goal of chemical crosslinking is to eliminate all crystallinity and rely solely upon chemical crosslinks for the polymer chain junction points.

Two common chemistries used for rubber crosslinking are peroxide crosslinking and sulfur vulcanization.[1-3] A comparison of these different crosslinking chemistries is made in Table 5.1. Multifunctional coagents were used with peroxide crosslinking to promote crosslinking versus chain scission reactions.[3]

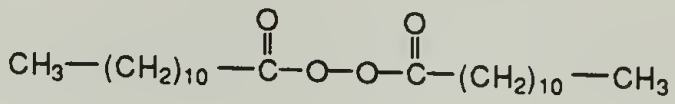
Four different peroxides were included in the experiments, two low temperature peroxides [4], lauroyl and benzoyl, and two vinyl specific peroxides [5], dicumyl and 2,5-dimethyl-2,5-di(t-butyl)hexane (or Varox®). Two different multifunctional crosslinking agents [2] were employed, a difunctional agent, ethylene glycol dimethacrylate, and a trifunctional agent, triallyl cyanurate. Several multifunctional crosslinker to peroxide concentrations were tested. The chemical structure of the peroxides and multifunctional coagents used are shown in Figure 5.1.

Table 5.1 Comparison between peroxide and sulfur chemistries used to crosslink PHO and PHOU.

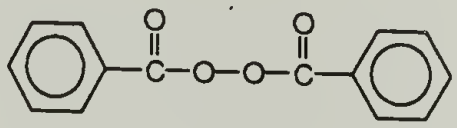
PEROXIDE	SULFUR
• crosslinks both saturated and unsaturated polymers	• crosslinks only unsaturated polymers
• reaction site less predictable except with vinyl specific peroxides	• reacts only with unsaturated moiety
• controlled cure temperature through choice of peroxide	• generally higher cure temperatures required
• expensive chemicals	• less expensive chemicals
• free radical chemistry can cause chain scission reactions [6]	• chain scission reduced
• transparent films	• opaque tan films

PEROXIDES

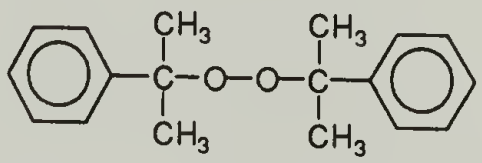
lauroyl
(80 ° C, 8 hours)

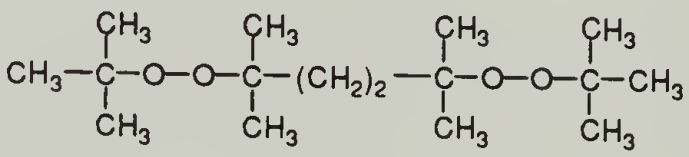


benzoyl
(80 °C, 8 hours)



dicumyl
(160 °C, 1 hour)

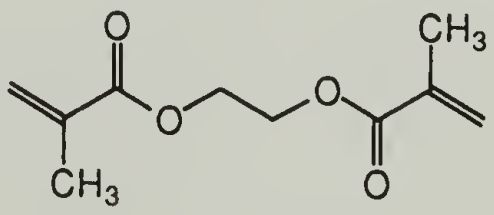




2,5 dimethyl-2,5-di(t-butyl peroxy) hexane (Varox®)
(160 °C, 5 hours)

COAGENTS (MULTIFUNCTIONAL CROSSLINKERS)

<u>difunctional</u> - ethylene glycol dimethacrylate



<u>trifunctional</u> - triallyl cyanurate

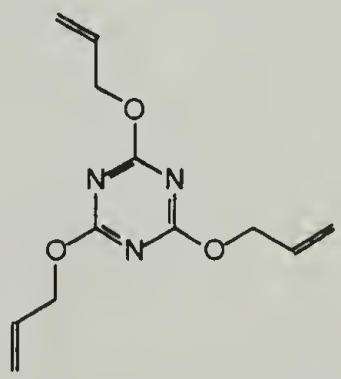


Figure 5.1 Chemical structures of the different peroxides and multifunctional coagents used in crosslinking experiments. Cure temperatures and times used for each peroxide are also included. [4-7]

A comparative evaluation of the different peroxides and the effect of olefin groups was accomplished by using a peroxide efficiency model first developed for radiation crosslinked polymers but extended successfully to chemical crosslinking.[8] Equation 5.1 was used:

$$S + S^{0.5} = p_o/q_o + (2 * E * M_n * [i])^{-1} \qquad (5.1)$$

where

S = soluble fraction of partially crosslinked polymer.

p_o/q_o = ratio of probability of degradation to crosslink formation per monomer per unit initiator.

E = number of crosslinks per decomposed peroxide molecule.

[i] = peroxide concentration in moles per gram of polymer.

Figure 5.2 shows that the inverse of the slope provides the efficiency and the y-intercept indicates the tendency of degradation to crosslink formation. A y-value of 2 indicates that no gel formation has occurred and corresponds to the minimum peroxide concentration needed to instigate gel formation.

Although elemental sulfur alone was first used to vulcanized natural rubber, accelerated sulfur cures are predominately used today.[1-3] Elemental sulfur which has a tendency to form non-productive cyclic sulfur linkages, has been replaced by sulfur donating vulcanizing agents which require accelerators and accelerator activators to optimize the cure cycle and properties. Trial and error appears to be the most widely used method of optimizing the amount and combination of these chemicals to produce the required properties.

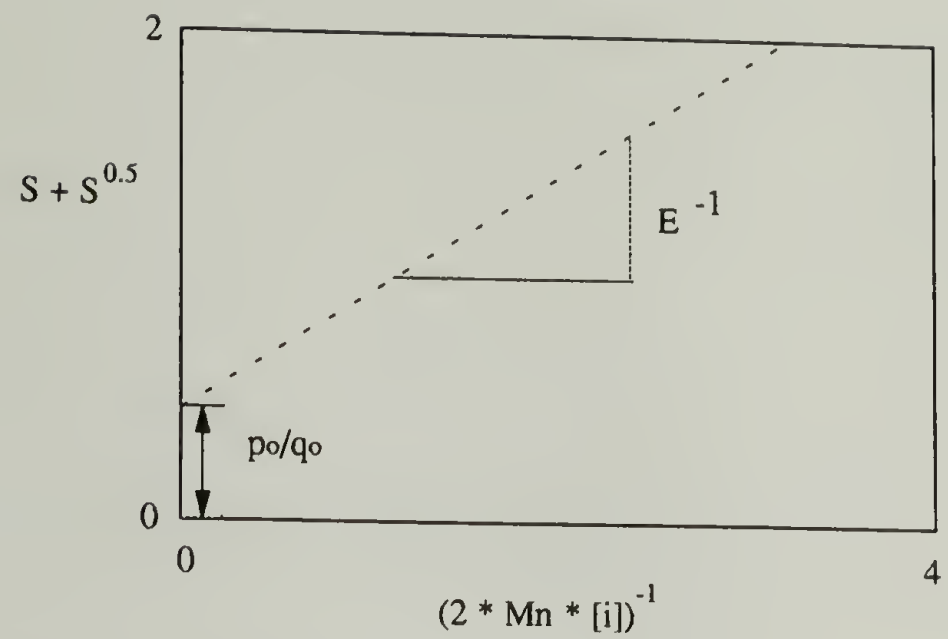


Figure 5.2 Graphical use of the peroxide efficiency equation to determine the peroxide efficiency (inverse of slope) and ratio of probability of causing chain degradation to forming crosslinks (y-intercept).

Two different vulcanizing agents, elemental sulfur and dipentamethylene thiuram tetra/hexa sulfide (DPTT), four different accelerators, zinc dibutyl dithiocarbamate di-n-butylamine combination (ZBUDX), zinc dibutyl dithiocarbamate (BZ), 2-benzothiazole sulfenamide (BBTS), and 2,2'-dibenzothiazyl disulfide (MBTS), and one combination accelerator activator, zinc oxide / stearic acid were included in this study. [9] Figure 5.3 shows the chemical structures and required cure temperatures for the different compounds.

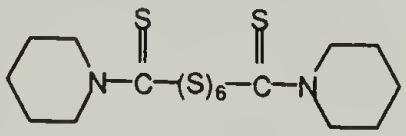
The need for an olefin containing polymer is essential for sulfur crosslinking and is believed to improve the efficiency of peroxide crosslinking. [6] Fortunately, *Pseudomonas oleovorans* can produce an olefin containing polymer when fed an appropriate carbon source. For this study, a mixture of carbon sources was used to control the amount of olefin in the polymer designated PHOU (refer to Sections 2.2.1 and 2.3.2). Five different compositions of PHOU were included: (95/5), (93/7), (91/9), (80/20), and (74/26).

Two other crosslinking approaches will also be discussed. The use of a tetrafunctional sulfur compound requiring a radical initiator was included which has

VULCANIZING AGENTS

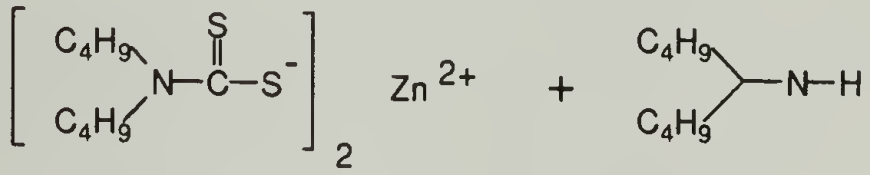
sulfur S

DPTT - dipentamethylene thiuram tetra/hexa sulfide

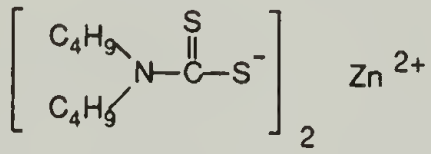


ACCELERATORS

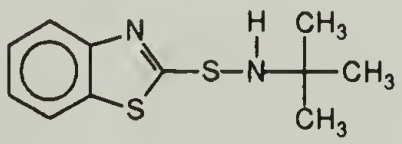
110 °C ZBUDX - zinc N,N-di-n-butyl dithiocarbamate, di-n-butylamine



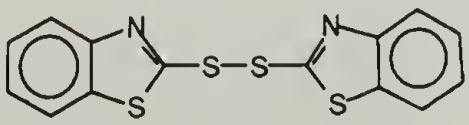
140 °C BZ - zinc dibutyl dithiocarbamate



160 °C BBTS - 2-benzothiazolesulfenamide



160 °C MBTS - 2,2'-dibenzothiazyl disulfide (thiazole)



ACCELERATOR ACTIVATORS

zinc oxide ZnO

stearic acid

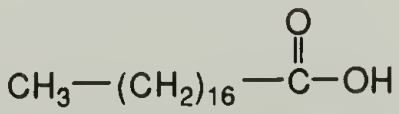


Figure 5.3 Chemical structures of the various sulfur vulcanization chemicals grouped as vulcanization agents, accelerators, or accelerator activators. Required cure temperatures indicated for the different accelerators.

been successfully employed as a crosslinking agent for silicone containing low levels of unsaturation. [10] The advantage of this reaction is the low temperature required for initiator activation, 88 °C, and the tetrafunctional crosslinking agent. Epoxidation of the olefin group followed by crosslinking was also attempted. This approach attempted to duplicate results of a previous researcher in our group. [11] Figures 5.4 and 5.5 show the chemical compounds involved with these different approaches.

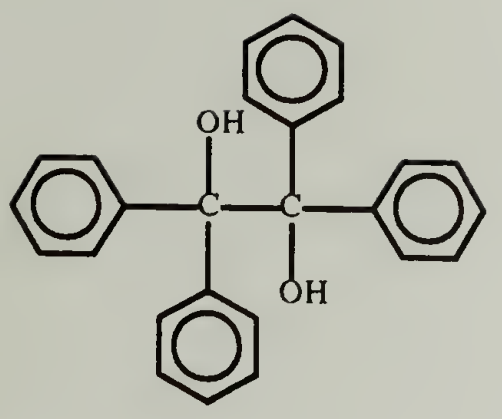
5.2 Experimental

5.2.1 Peroxide Crosslinking Sample Preparation

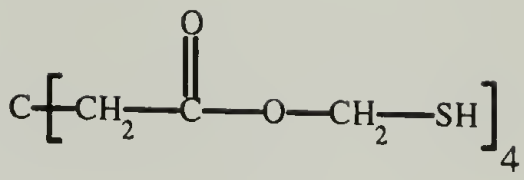
PHO, PHOU(95/5), PHOU(93/7), PHOU(80/20), or PHOU(74/26), peroxide, and multifunctional coagent (if used) were all dissolved in chloroform and then pipeted into a glass casting dish. The solvent was allowed to evaporate over approximately one week to insure network formation was done in the absence of solvent. The polymer films, typically 0.5 mm thick were heated in a vacuum oven under either a vacuum or nitrogen atmosphere. The cure temperature and time depended on the peroxide, see Figure 5.1. Typically a series of 4 to 8 different peroxide concentrations were used for each polymer. Several coagent to peroxide concentration ratios were tested usually at one peroxide concentration where gel formation was previously confirmed. Table 5.2 summarizes the various samples tested.

5.2.2 Sulfur Vulcanization Sample Preparation

Approximately 0.5 g (or 4 g for samples which underwent full mechanical property evaluation) of PHOU(91/9), PHOU(93/7), PHOU(80/20), or PHOU(74/26) was softened by heating the polymer in a watchglass then the polymer was allowed to cool.

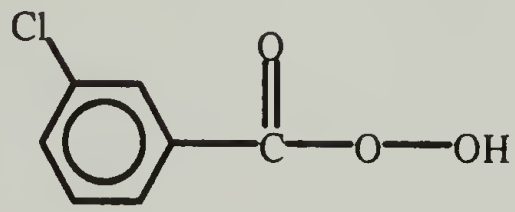


benzopinacole



pentaerythritol tetrakis (3-mercaptopropionate)

Figure 5.4 Chemical structures for the tetrafunctional crosslinking approach.



meta-chloroperoxybenzoic acid

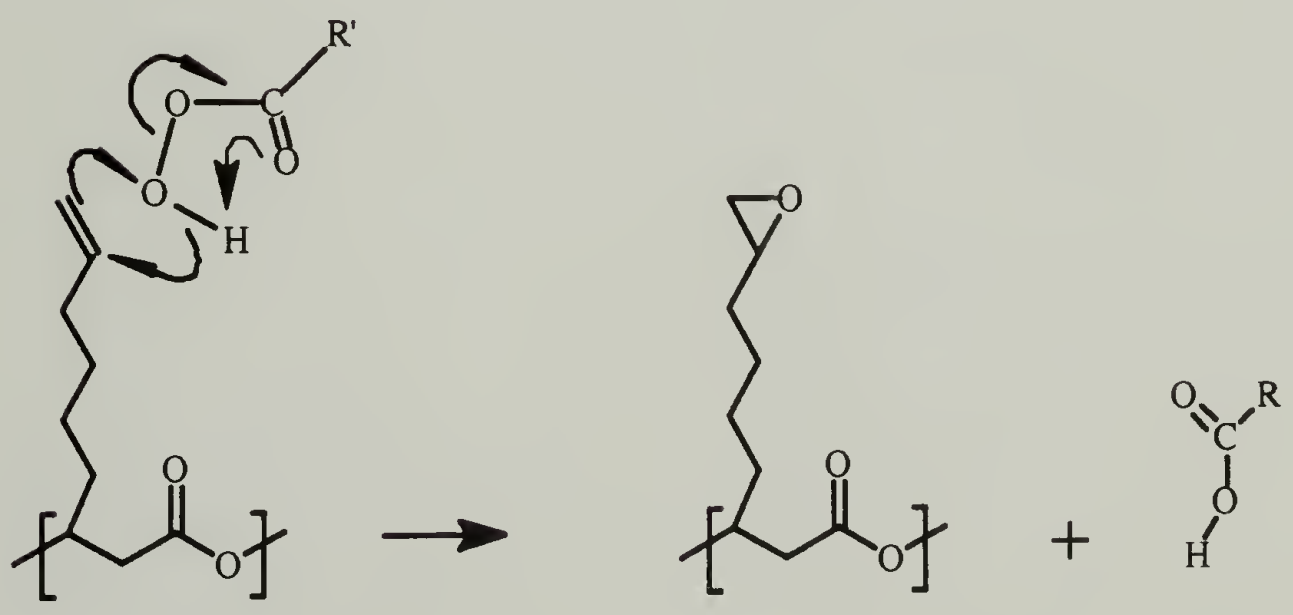


Figure 5.5 Chemical structure and proposed mechanism for the epoxidation of the olefin group.

Table 5.2 Summary of peroxide crosslinked samples tested. Weight % peroxide or weight % coagent/peroxide listed.

		SAMPLE							
PEROXIDE	**POLYMER**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Lauroyl	PHO	0.05	0.1	0.5	1.0	1.5	2.0	3.0	4.0
1.5 wt %* & di**	PHO	15	25	50					
1.5 wt % & tri	PHO	15	25	50					
	PHOU(95/5)	0.05	0.1	0.5	1.0	1.5	2.0	3.0	4.0
	PHOU(93/7)	0.5							
0.5 wt % & di	PHOU(93/7)	15	25	50					
0.5 wt % & tri	PHOU(93/7)	15	25	50					
Benzoyl	PHO	0.05	0.1	0.5	1.0	1.5	2.0	3.0	4.0
1.0 wt % & di	PHO	25							
1.0 wt % & tri	PHO	25							
	PHOU(95/5)	0.05	0.1	0.5	1.0	1.5	2.0	3.0	4.0
	PHOU(93/7)	0.1	1.0	3.0					
Dicumyl	PHOU(93/7)	0.05	0.1	0.25	0.5	1.0	1.5		
0.5 wt % & di	PHOU(93/7)	25							
0.5 wt % & tri	PHOU(93/7)	25							
	PHOU(80/20)	0.05	0.1	0.25	0.4				
	PHOU(74/26)	0.05	0.1	0.25	0.4				
Varox®	PHOU(93/7)	0.05	0.1	0.5	1.0	2.0	3.0		
0.5 wt % & di	PHOU(93/7)	25							
0.5 wt % & tri	PHOU(93/7)	25							

* wt % refers to peroxide level

* * di = ethylene glycol dimethacrylate, tri = triallyl cyanurate.

All the sulfur vulcanization ingredients were kneaded into the softened polymer which was then formed into a ball in preparation for curing.

For the 0.5 g samples, the vulcanization curing process was monitored with a Rheometrics RMS or RDS II Rheometer using a parallel plate geometry, 2 to 5 rad/sec frequency, and cure temperature appropriate for the specific chemistry (see Figure 5.4). The sample was loaded onto the preheated bottom plate. The plates were closed which typically resulted in a 0.5 to 1 mm gap. The storage modulus was monitored as a function of time. Table 5.3 summarizes the various samples tested.

For tensile property and tensile set testing, 4 g samples of PHOU(93/7), PHOU(80/20), and PHOU(74/26) were placed in a 140 mm diameter mold, 0.25 mm thick and compression molded and cured using a preheated Carver Press set at 140 °C. The vulcanization time was determined from the rheometry experiments. The cured films were dusted with talc for easier handling.

5.2.3 Tetrafunctional Sulfur Reagent Sample Preparation

PHOU(93/7) and the tetrafunctional sulfur compound, pentaerythritoltetrakis(3-mercaptopropionate), were both dissolved in chloroform. The initiator was added and the solution stirred approximately 15 minutes then pipeted into a glass casting dish and placed in a vacuum oven.. The vacuum was oscillated with a nitrogen flush to quickly remove the solvent. Heating was started after all solvent had evaporated to insure crosslinking was conducted in the absence of solvent. The film was heated at 95 °C for 48 hours in a nitrogen atmosphere. Several different polymer olefin to tetrafunctional sulfur compound sulfur ratios were tested including 1:1, 1:2, 1:3 and 1:4, while the initiator concentration was kept constant.

Table 5.3 Summary of sulfur vulcanization samples tested. Values given are phr, parts per hundred rubber, where polymer used is 100 phr.

CHEMISTRY	INGREDIENTS	SAMPLE				
		1	**2**	**3-6**	**7-9**	**10-12**
DPTT & BZ	PHOU	(91/9)	(95/5)	(91/9)	(80/20)	(74/26)
	DPTT	1	2	2.5, 3.5, 4.5, or 5.5	3.5, 4.5, or 5.5	3.5, 4.5, or 5.5
	BZ	0.5	0.5	0.5	0.5	0.5
	zinc oxide	3	3	5	5	5
	stearic acid	1	1	3	3	3
S & ZBUDX	PHOU	(91/9)	(93/7)	(93/7)		
	S	2	3	3		
	ZBUDX	1.5	1.5	3		
	zinc oxide	5	5	5		
	stearic acid	2	2	2		
DPTT & ZBUDX	PHOU	(93/7)	(93/7)	(93/7)		
	DPTT	3	3	2.5		
	ZBUDX	2	1	1		
	zinc oxide	3	3	3		
	stearic acid	1	1	1		
DPTT & BBTS	PHOU	(93/7)	(93/7)	(93/7)		
	DPTT	3	2.5	3		
	BBTS	1.5	1.5	1.5		
	zinc oxide	5	5	5		
	stearic acid	2	2	2		
S & BBTS	PHOU	(93/7)				
	S	2				
	BBTS	1.5				
	zinc oxide	5				
	stearic acid	2				
DPTT & MBTS	PHOU	(93/7)	(93/7)			
	DPTT	3	3			
	MBTS	1.5	1.5			
	zinc oxide	5	5			
	stearic acid	2	2			
S& MBTS	PHOU	(93/7)				
	S	2				
	MBTS	1.5				
	zinc oxide	5				
	stearic acid	2				

5.2.4 Epoxidation Reaction Preparation

The chemical compound and solvent were first purified based on procedures described by the researcher who previously conducted this reaction and a standard laboratory purification guide.[11,12] Metachloroperoxybenzoic acid, (MCPBA), was purified by first washing the powder in a pH 7.4 buffer (KH_2PO_4/NaOH), collecting the powder using a Büchner funnel and flask, then drying the powder under vacuum for 2 days at room temperature. The chloroform was washed with water to remove the ethanol preservative, dried with potassium carbonate (K_2CO_3), refluxed with phosphorus pentaoxide (P_2O_5) overnight, then distilled under nitrogen. The purified chloroform was stored in the dark.

PHOU(93/7), the purified MCPBA, and the dried and purified chloroform were dissolved together at room temperature under a nitrogen atmosphere for approximately 6 hours. A 1:1 stoichiometric amount of MCPBA to olefinic groups was used in the reaction. The reaction was quenched and the acid by-product washed out using a 10% aqueous solution of sodium sulfate (Na_2SO_4). The organic layer was collected and dried over magnesium sulfate ($MgSO_4$). The organic layer was then pipeted into a glass casting dish. Crosslinking was expected to occur upon evaporation of the solvent due to the excess acid catalyzing the hydrolysis of an epoxide group which would then react with another epoxide group. An ether linkage would be formed in this reaction scheme. Another possible mechanism could be the acid catalyzed hydrolysis of the epoxide followed by reaction with a remaining olefin group forming a carbon carbon bond with a pendant hydroxyl group. This scheme could occur if only partial epoxidation of the olefin groups had occurred. A small sample of the organic layer was tested prior to evaporation in a ^1H NMR to determine what percentage of the olefin groups had reacted with the MCPBA.

5.2.5 Sol-Gel Analysis

Sol-gel analysis [13] was conducted on all films and was the primary means of determining if crosslinking had occurred. A sample of the cured film (typically 0.1 g to 0.5 g) was weighed then placed in a round bottom flask containing approximately 3 ml to 10 ml of chloroform. The film was allowed to sit in the solvent for 2 hours at room temperature and was agitated periodically. If a gel was present after 2 hours, it was retrieved by filtering off the sol and catching the gel on filter paper using a Büchner filter flask under vacuum. The gel was placed in a glass dish and dried overnight in a vacuum oven at room temperature. The gel was re-weighed and the % sol calculated from equation 5.2:

$$\% \text{ sol} = \frac{\text{sol}}{(\text{sol} + \text{gel})} * 100 \qquad (5.2)$$

5.2.6 Gel Permeation Chromatography

All molecular weight data was obtained using a Rabbit Model solvent delivery system with a Waters Model R401 differential refractometer detector and three Polymer Labs PL 5 μ gel columns with mean pore diameters of 10^5Å, 10^4Å, and 10^3Å. A 2 ml/min tetrahydrofuran flow rate was used with a toluene flow marker. The molecular weight was based on polystyrene standards.

5.2.7 Thermal Analysis

Differential scanning calorimetry was conducted using a TA Instruments DSC Model 2910. Samples were tested from -85 to 100 °C at a 20 °C/min heating rate. The glass transition temperature reported is at the inflection point. The melting temperature reported is the peak temperature.

Thermogravimetric analysis was conducted using a TA Instruments TGA Model 2950. TGA was conducted in both an air and nitrogen atmosphere from 30 °C to 500 °C at a 20 °C/min heating rate. Degradation temperatures are reported as the onset temperature of the weight loss.

5.2.8 Tensile Set

All testing was conducted using an Instron Universal Testing Instrument, Model TTBM. Strip samples approximately 2 to 5 mm wide by 2 to 4 cm were used. Testing was based on the procedures described in ASTM D412 [14]. The samples were extended at a 1 or 2 min^{-1} strain rate and held for 10 minutes at the intended elongation then released at the same strain rate. The samples were then allowed to recover for 10 minutes prior to final measurement of the gage length.

5.2.9 Tensile Testing

All tensile testing was conducted using an Instron Universal Testing Instrument, Model TTBM with an Interface SM-10 load cell. Ring samples per ASTM D 412 [15], Type 2, were punched from the films using a steel rule die and Carver Hot Press Model C at room temperature. Special grips were made to hold the ring specimens. The ring was supported at each end by a pin, approximately 5 mm in diameter. Each pin was engaged

at both ends in the inner track of a ball bearing to insure the stress and strain remained equal on both sides of the ring during testing. The pins are removable for loading the sample. The initial gage length was based on half the average circumference of the ring die. A strain rate of 1 min^{-1} was used for all tensile tests.

5.2.10 Impulse Viscoelasticity

Impulse viscoelasticity, a dynamic mechanical technique, affords direct measurement of the equilibrium shear modulus, G_{eq}.[15] From G_{eq}, the molecular weight between crosslinks, M_c, can be calculated using equation 5.3 (see Section 3.3.1 for variable definitions).

$$M_c = \frac{\rho RT}{G_{eq}} \qquad (5.3)$$

A double lap shear sample geometry was used. After a baseline was established, a small known step strain was placed on the sample for a duration of 10 seconds then the strain was removed. The material was allowed to relax for 60 to 120 seconds before another pulse was applied. The relaxation times were chosen to insure the material had ample time to relax back to the established baseline. The stress and strain were integrated over the entire pulse and relaxation time and the equilibrium modulus, G_{eq}, was calculated from the ratio of the stress/strain integrals. Figure 5.6 illustrates the use of this technique.

Several samples while still mounted in the apparatus, were heated above the melting temperature of PHO and allowed to cool then retested. This procedure would assess if crystallites had been present because the equilibrium shear modulus would decrease significantly when evaluated after the heat treatment.

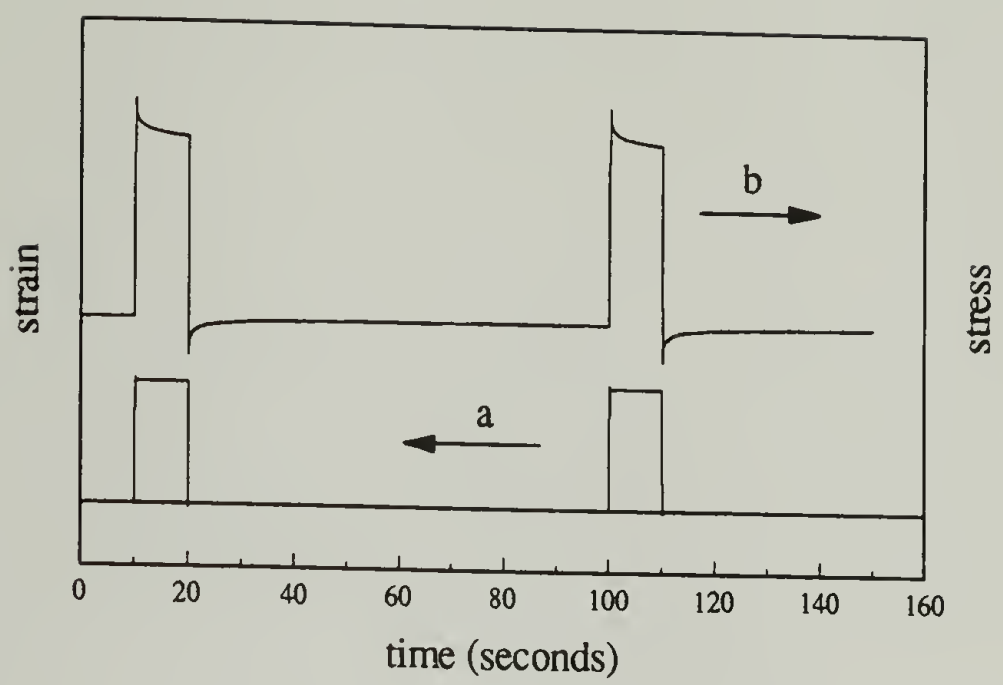


Figure 5.6 Typical shear pulse-strain deformation for a material exhibiting a viscoelastic stress response. a) forced strain function and b) stress response of material.

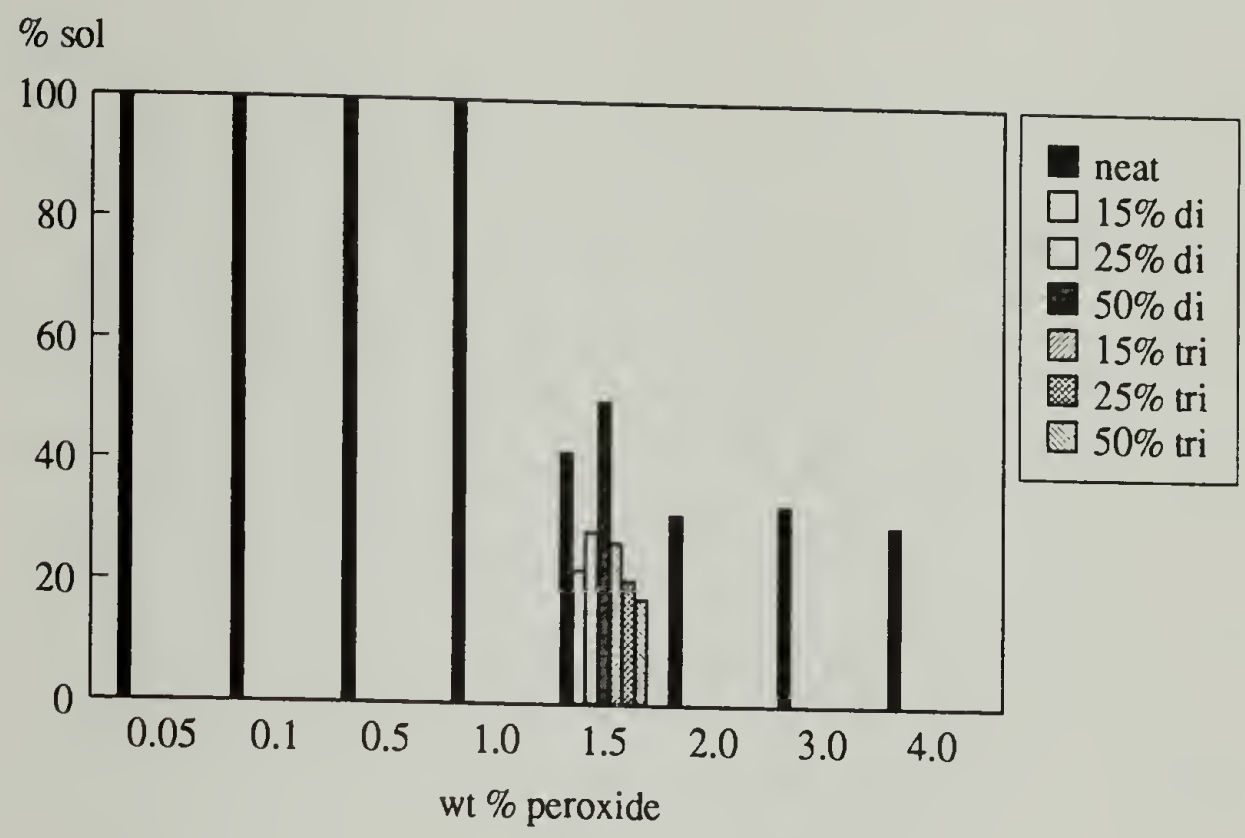
5.3 Peroxide Crosslinking Results and Discussion

5.3.1 Sol-Gel Analysis

The sol-gel analysis results are shown for several peroxide cures. Figure 5.7 depicts the results for PHO crosslinked with either lauroyl or benzoyl peroxides both with and without coagents. The results for PHOU(95/5) crosslinked with either lauroyl or benzoyl peroxide are shown in Figure 5.8. Figure 5.9 shows the results for PHOU(93/7) crosslinked with lauroyl, Varox® or dicumyl peroxide including the effect of coagents.

A crosslinked material did result in all cases as long as enough peroxide was used. The % sol at first decreased as the amount of peroxide increased. This trend was followed by a leveling off of % sol even though the peroxide concentration was increased further. The final % sol was not zero indicating there was always some polymer not incorporated

a) lauroyl peroxide



b) benzoyl peroxide

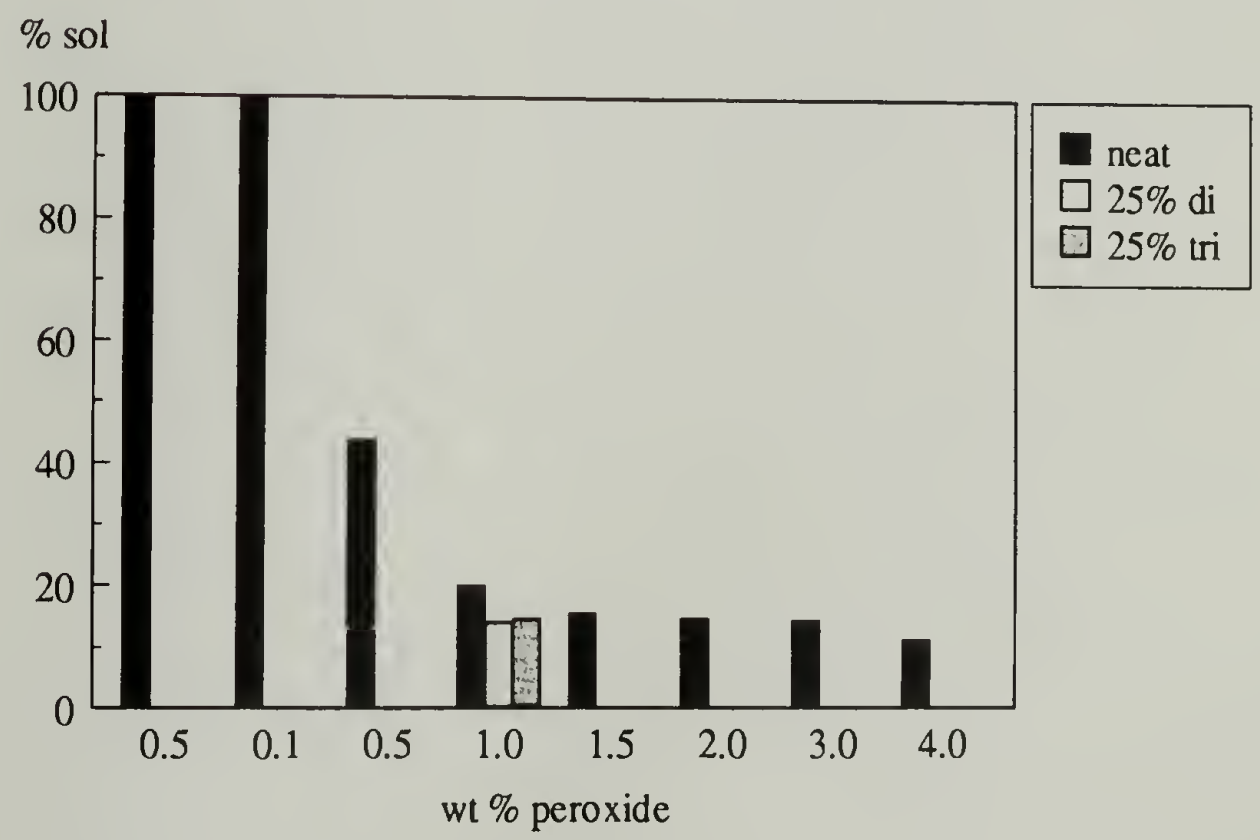


Figure 5.7 Sol-gel analysis results of PHO crosslinked with either a) lauroyl or b) benzoyl peroxides both with and without coagents. Di = difunctional coagent, tri = trifunctional coagent. Percentages indicate the coagent to peroxide weight ratio.

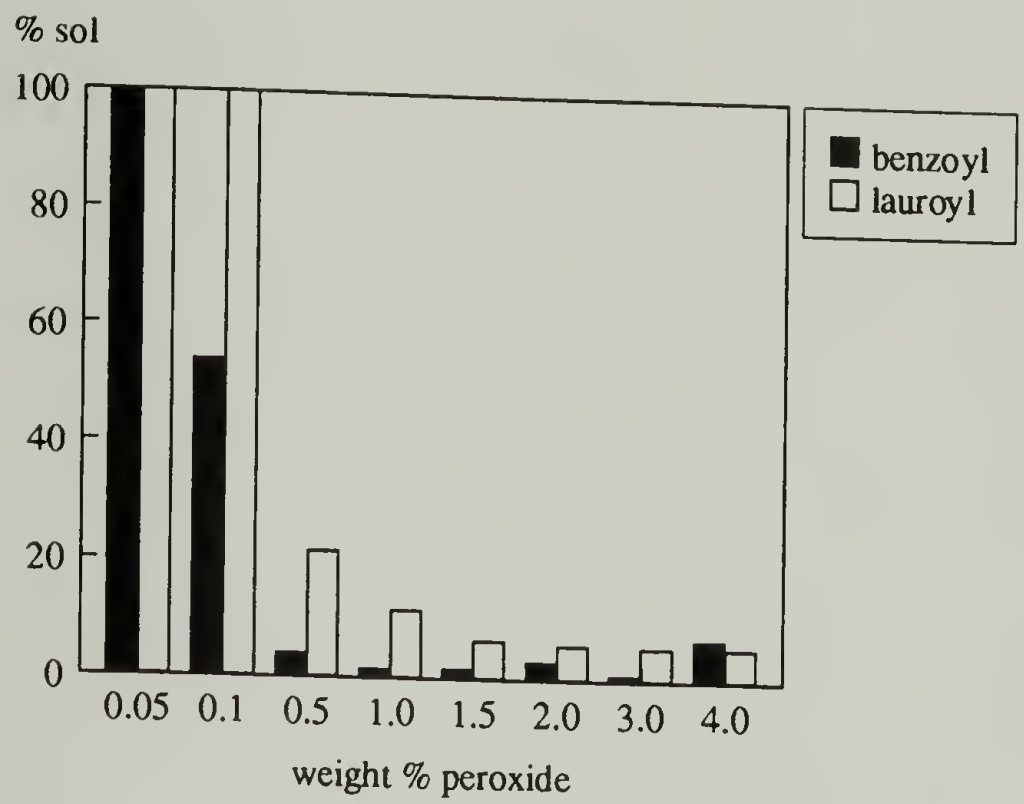


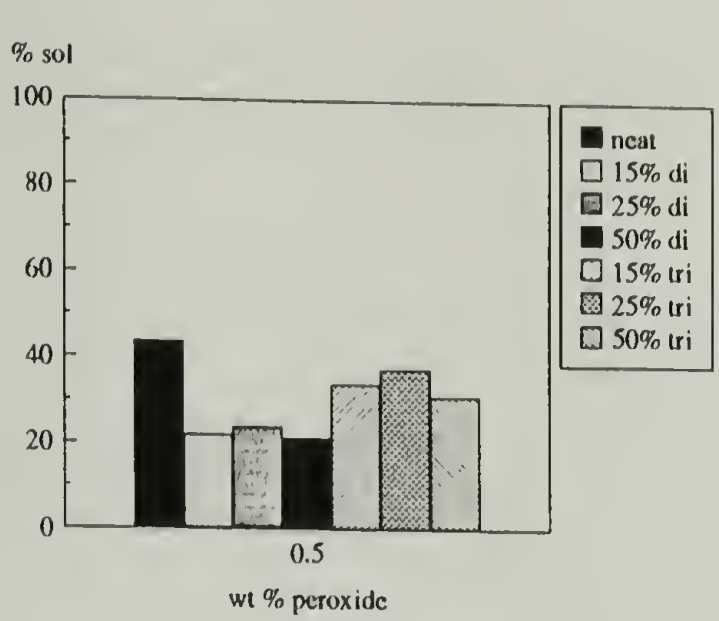
Figure 5.8 Sol-gel analysis results of PHOU(95/5) crosslinked with lauroyl or benzoyl peroxide.

into the gel no matter how much peroxide was used. Perhaps a loss of chain entropy prevented total gel formation or perhaps this sol was the result of chain scission reactions. A molecular weight analysis using gel permeation chromatography (see Section 5.3.3) was conducted on the sol fraction to better address these findings.

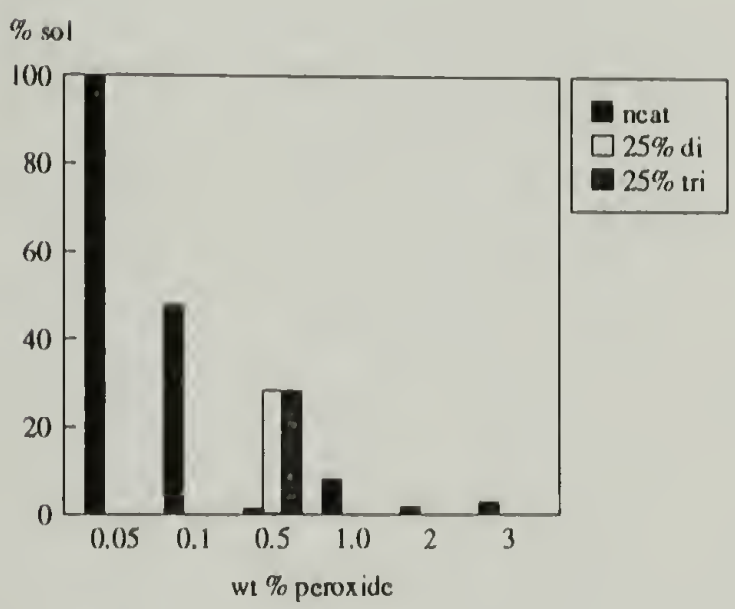
The effect of the coagent on the extent of gel formation was complex and depended on the amount and type of coagent and which peroxides were used.

For the non-vinyl specific peroxides, lauroyl and benzoyl peroxide, the addition of coagents generally improved the crosslinking reaction in that less extractables were recovered. This was the finding independent of whether saturated or unsaturated polymers were used. Refer to Figures 5.7a and b and Figure 5.9a. In contrast, the vinyl specific peroxides and coagents resulted in a higher amount % sol, sometimes a dramatic increase as was the case for Varox® peroxide, see Figure 5.9b. Perhaps the vinyl specific

a) lauroyl peroxide



b) Varox® peroxide



c) dicumyl peroxide

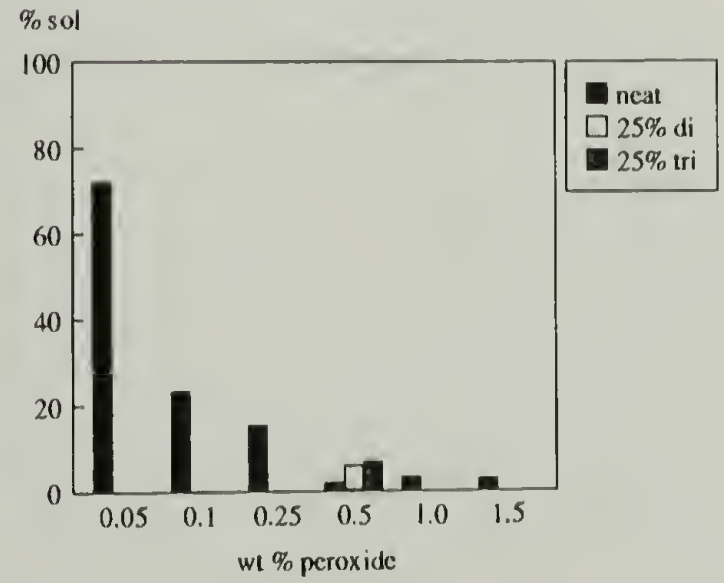


Figure 5.9 Sol-gel analysis results of PHOU(93/7) crosslinked with either a) lauroyl or b)Varox® or c) dicumyl peroxide both with and without coagents.

peroxides were capable of homopolymerizing the coagents. This undesired side reaction would consume the coagents and the resulting polymer could add to the % sol.

An interesting trend was noted for PHO crosslinked with lauroyl peroxide and the coagents. The more difunctional coagent added the more sol fraction was detected, while the more trifunctional added the less sol fraction was detected. This was not observed for the 7% olefin containing polymer. Compare Figures 5.7a and 5.9a. For the olefin containing polymer, it appeared that the coagents improved the crosslinking reaction (lower % sol) independent of the coagent concentration (same % sol independent of coagent concentration).

Several PHOUs with varying olefin content were crosslinked with dicumyl peroxide to study the effect of olefin content on gel formation. The results are shown in Figure 5.10. Very brittle gels had formed which were difficult to retrieve for final weighing. This problem explains some higher than expected sols.

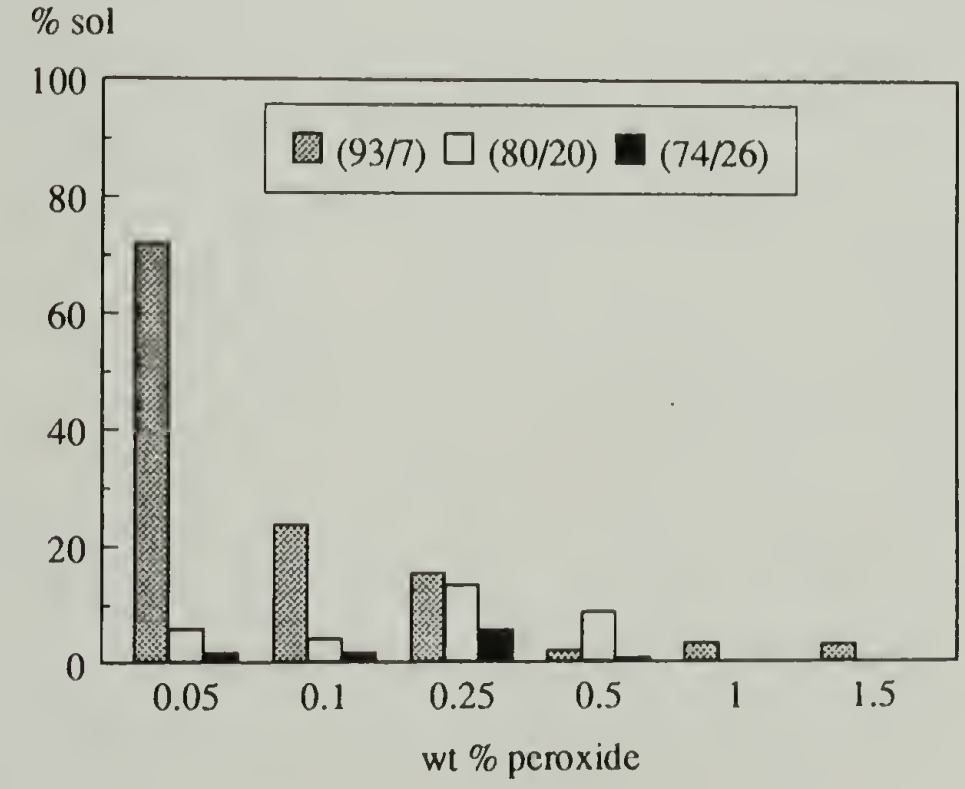


Figure 5.10 Sol gel analysis of several PHOUs with varying olefin content after being crosslinked with dicumyl peroxide.

5.3.2 Efficiency Determination

The peroxide efficiency model allows comparison of peroxides using two criteria. The first criteria is the amount of peroxide required to crosslink the polymer which implies the efficiency of the thermal degradation of the peroxide to produce viable free radicals that are not susceptible to non-productive side reactions. The second criteria is whether the free radicals cause more chain scission than crosslinking.

The comparison between PHO crosslinked with lauroyl and benzoyl peroxide is shown in Figure 5.11. Lauroyl peroxide was slightly more efficient than benzoyl peroxide as seen by the smaller slope, but lauroyl peroxide had a very high y-intercept which indicated that degradation was much more likely to occur than crosslinking.

The use of a polymer containing just 5% olefin groups altered the picture dramatically. Lauroyl peroxide was now much less likely to cause degradation but was now slightly less efficient than benzoyl peroxide as seen in Figure 5.12. The effect of the olefin group is readily seen in Figure 5.13 which depicts PHO, PHOU(95/5), and PHOU(93/7) crosslinked with benzoyl peroxide. As the number of olefin groups was increased, the efficiency of peroxide crosslinking improved and the tendency for degradation to occur decreased. This efficiency analysis was attempted with PHOU(93/7), (80/20), and (74/26) all crosslinked with dicumyl peroxide but the results were nonsensical. Very low levels of sol were extracted from the four peroxide concentrations tested. The analysis appears to require a larger range of % sol for meaningful analysis.

The results of PHOU(93/7) crosslinked with lauroyl, benzoyl, dicumyl, & Varox® peroxides is shown in Figure 5.14. This comparison showed dicumyl peroxide was the most efficient peroxide while Varox® showed the least tendency towards degradation. These results are more readily seen in Table 5.4 (page 135) which summarizes all the findings for peroxide crosslinking.

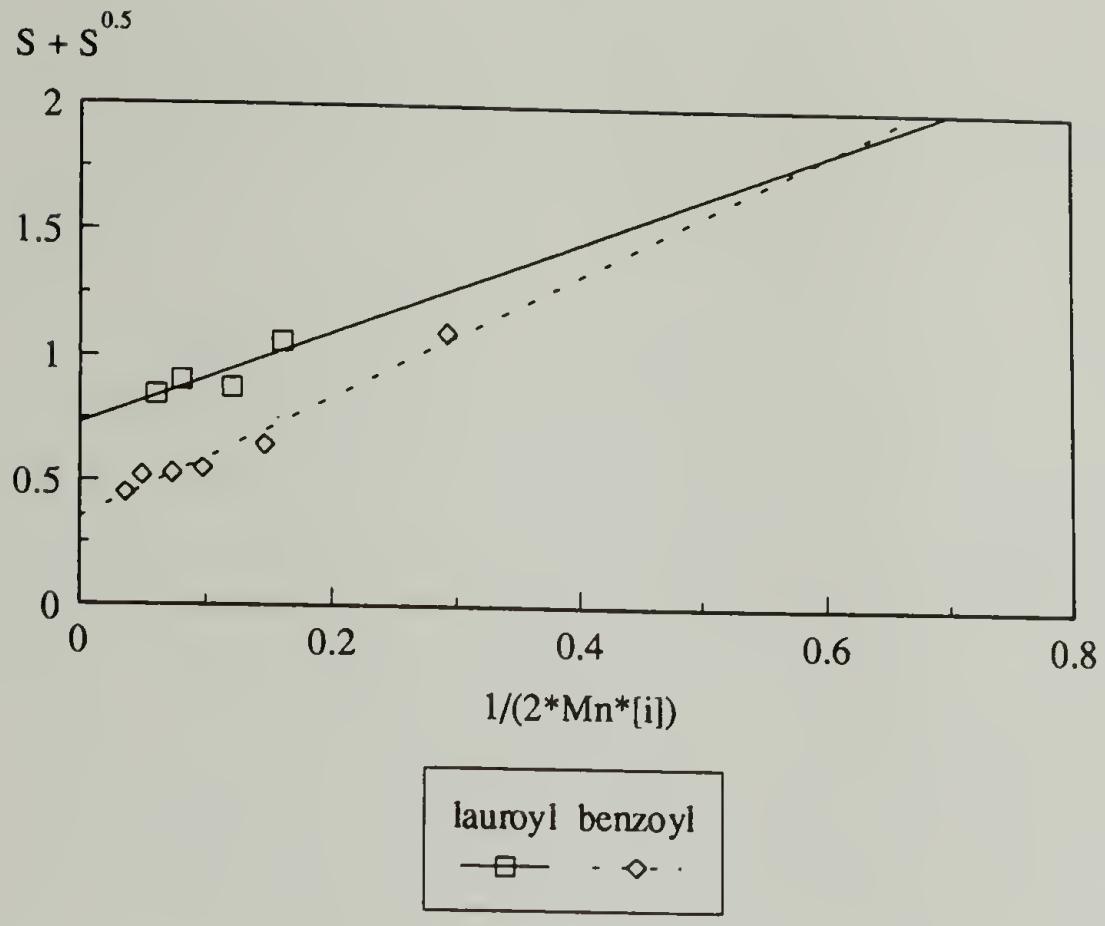


Figure 5.11 Efficiency comparison between PHO crosslinked with lauroyl or benzoyl peroxide.

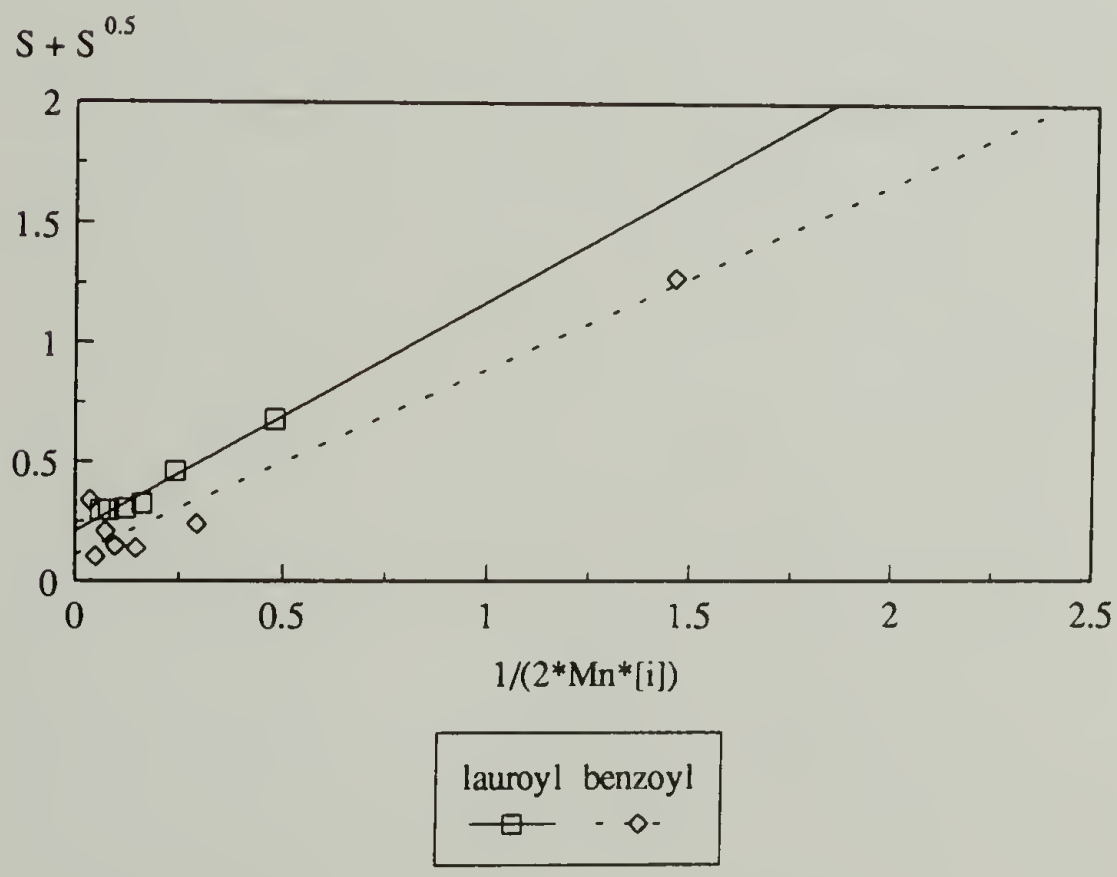


Figure 5.12 Efficiency comparison between PHOU(95/5) crosslinked with lauroyl or benzoyl peroxide.

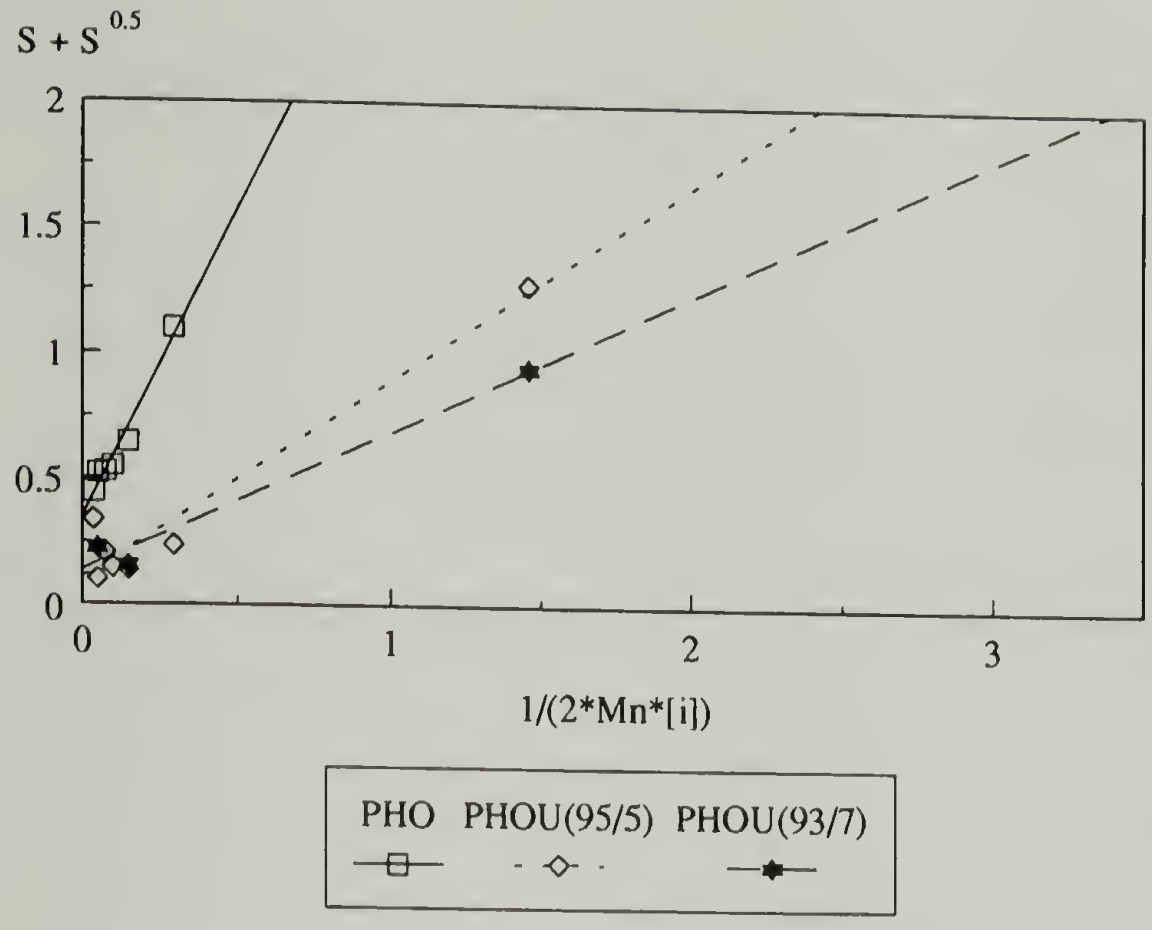


Figure 5.13 Efficiency comparison between PHO, PHOU(95/5), and PHOU(93/7) crosslinked with benzoyl peroxide.

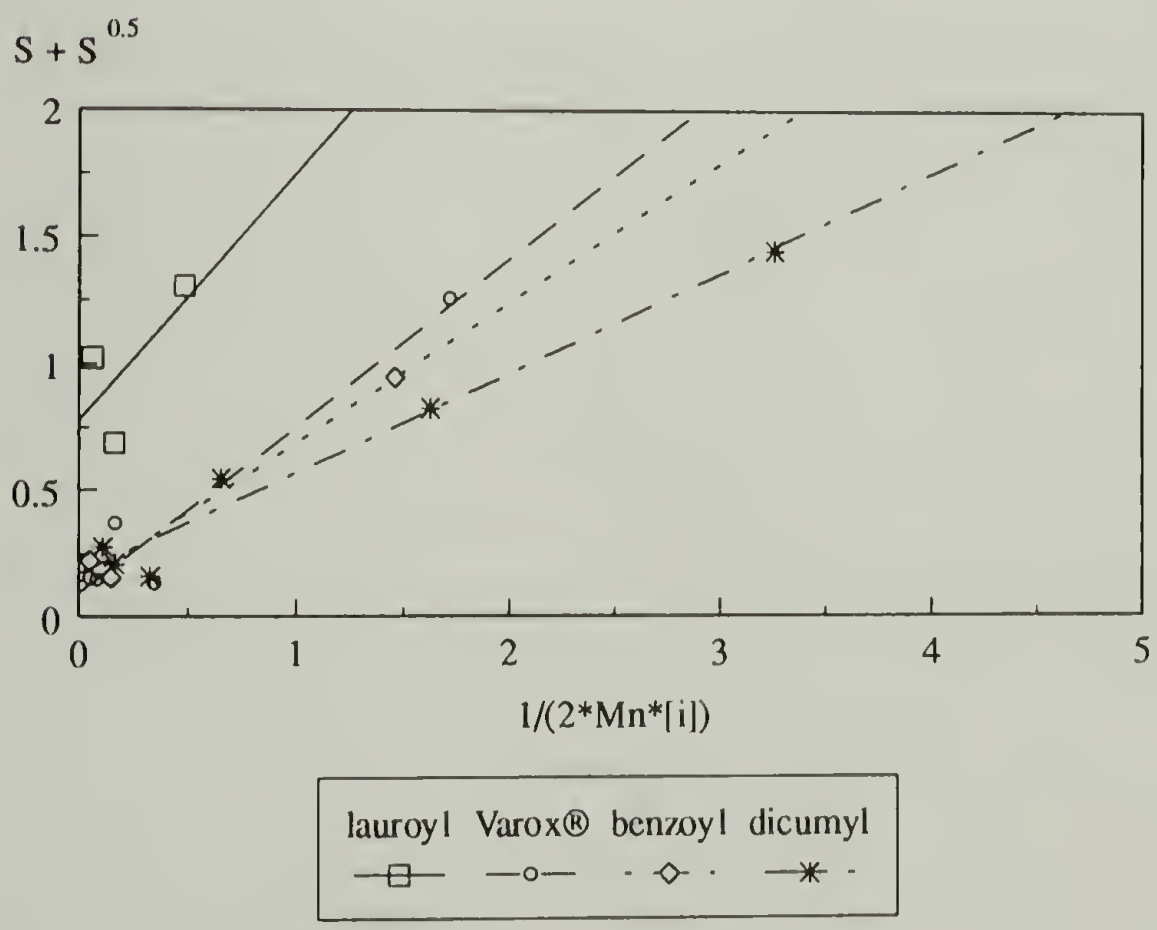


Figure 5.14 Efficiency comparison between PHOU(93/7) crosslinked w/ lauroyl, benzoyl, dicumyl, and Varox® peroxides.

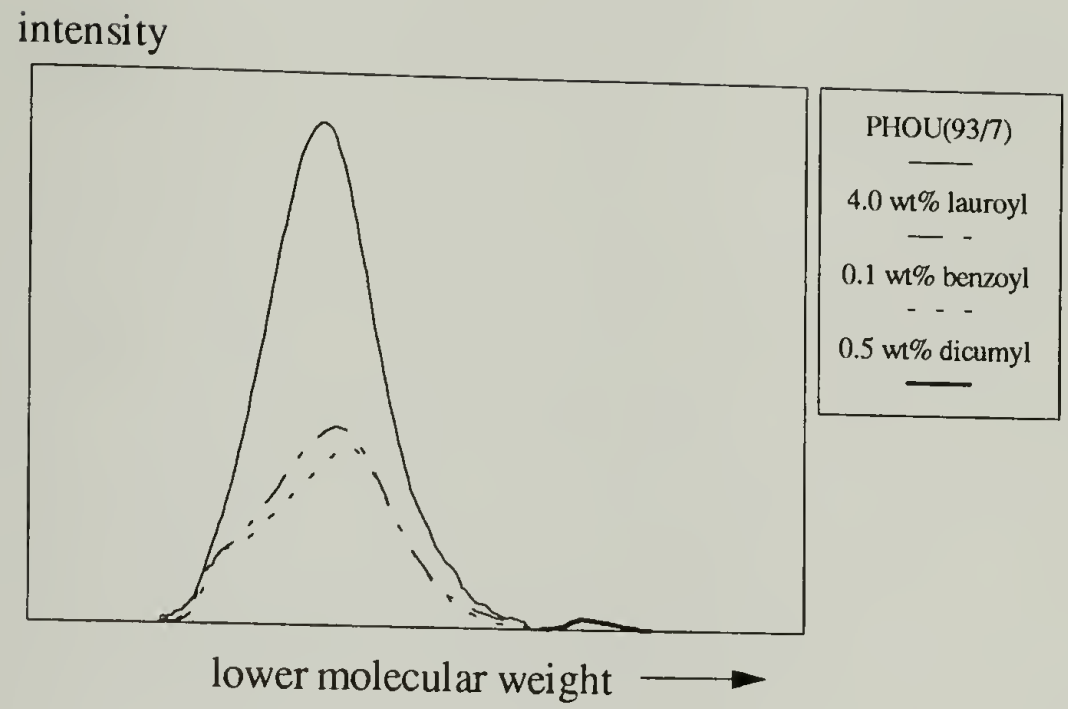
5.3.3 Gel Permeation Chromatography

Several sol fractions extracted from the crosslinked polymers were evaluated for molecular weight distribution. Figure 5.15 shows the results of the sol fraction for PHOU after undergoing peroxide crosslinking reactions with three different peroxides. The sol fraction molecular weight distribution for lauroyl and benzoyl peroxide crosslinked PHOU(93/7) revealed a Mw and Mn that was higher and broader than the original polymer with a definite high molecular weight shoulder observed. These results imply that chain extension had occurred but from the high % sol, not enough reactions had occurred to completely gel the material. There was not a significant difference observed in the molecular weight distribution between the sol from lauroyl and benzoyl peroxides. The efficiency model indicated that excessive chain degradation occurred with lauroyl peroxide but this prediction was not reflected in the GPC data.

The very low molecular weight sol obtained from the dicumyl crosslinked polymer, lower than any fraction found in the original polymer, suggested that some chain degradation had occurred.

5.3.4 Thermal Analysis

One goal of chemical crosslinking was to totally eliminate crystallinity and rely solely on a chemically crosslinked network. The presence of crystallinity was implied by the formation of a translucent hazy film versus a transparent film. The haziness was due to crystallites scattering light. In addition, it was observed that if after cure the films were still extremely sticky and gummy but firmed up overnight, crystallites were most likely present.



	Mw	Mn	PDI
PHOU(93/7)	165,000	62,000	2.6
lauroyl sol (39.4%)	327,000	88,000	3.7
benzoyl sol (35.2%)	1,386,000	369,000	3.8
dicumyl sol (2%)	650	490	1.3

Figure 5.15 Gel permeation chromatographs and data of PHOU(93/7) before crosslinking and the sol fraction of the polymer after undergoing peroxide crosslinking.

Thermal analysis was conducted on many of the cured films to determine if peroxide crosslinking was successful in eliminating crystallinity. If enough chemical crosslinking is present, crystallinity should be eliminated because of a reduction in chain entropy. An increase in the glass transition temperature could also result from chemical crosslinking.

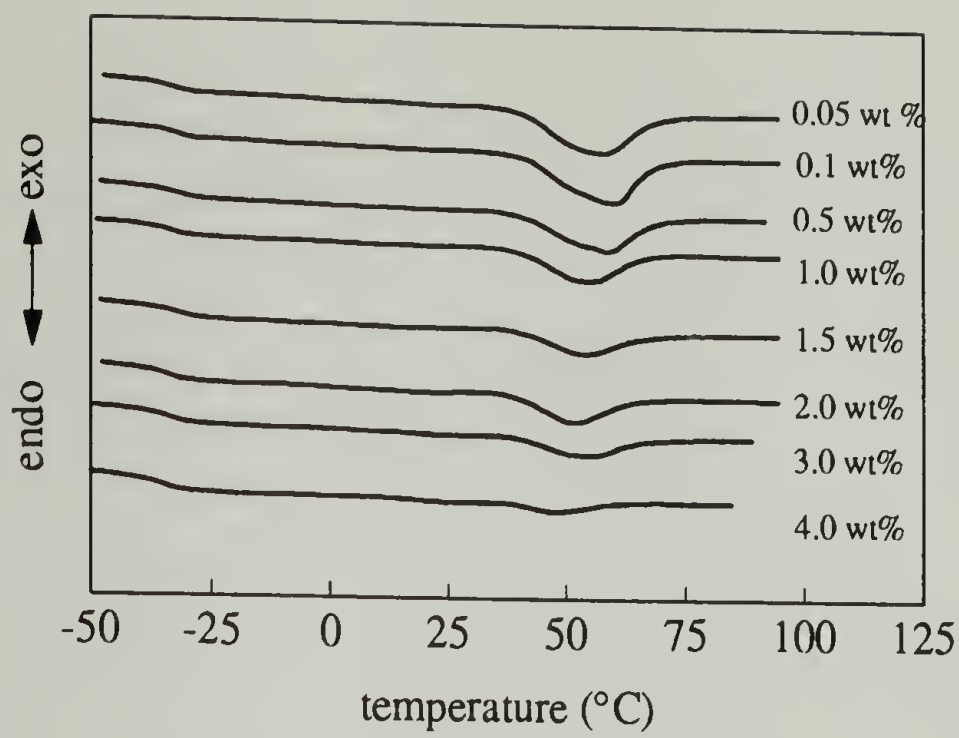
Figure 5.16 shows the DSC results of PHO crosslinked with either lauroyl or benzoyl peroxide. As the wt % of peroxide was increased, the amount of crystallinity, represented by the area under the melting peak, was reduced. For benzoyl peroxide, by 1.0 wt %, crystallinity was eliminated. For lauroyl peroxide, not even 4 wt % peroxide was sufficient to eliminate all crystallinity.

For PHOU(95/5), both lauroyl and benzoyl peroxide totally eliminated crystallinity by 1.0 wt % peroxide as shown in Figure 5.17. It is interesting that the addition of 5% olefin groups would so drastically improve the efficiency of the crosslinking reaction and eliminate crystallinity at a much lower peroxide concentration. The disappearance of crystallinity at lower peroxide levels concurs with the efficiency model results which indicated that less peroxide was needed to crosslink olefin containing polymers.

Several samples of PHOU(93/7) crosslinked with Varox® peroxide were tested for the presence of crystallinity while mounted in the impulse viscoelasticity test apparatus as described in Section 5.2.10. The results indicated that all crystallinity was eliminated at and above the 0.5 wt % peroxide concentration.

Thermogravimetric analysis of both non-crosslinked and peroxide crosslinked polymers revealed no change in the decomposition temperature as a result of the crosslinking. The onset of decomposition occurred at approximately 290 °C for all samples tested which included PHO and PHOU(95/5) both non-crosslinked and crosslinked with lauroyl or benzoyl peroxide, and PHOU(93/7) non-crosslinked. The shape of all the decomposition curves were also similar.

a) lauroyl peroxide



b) benzoyl peroxide

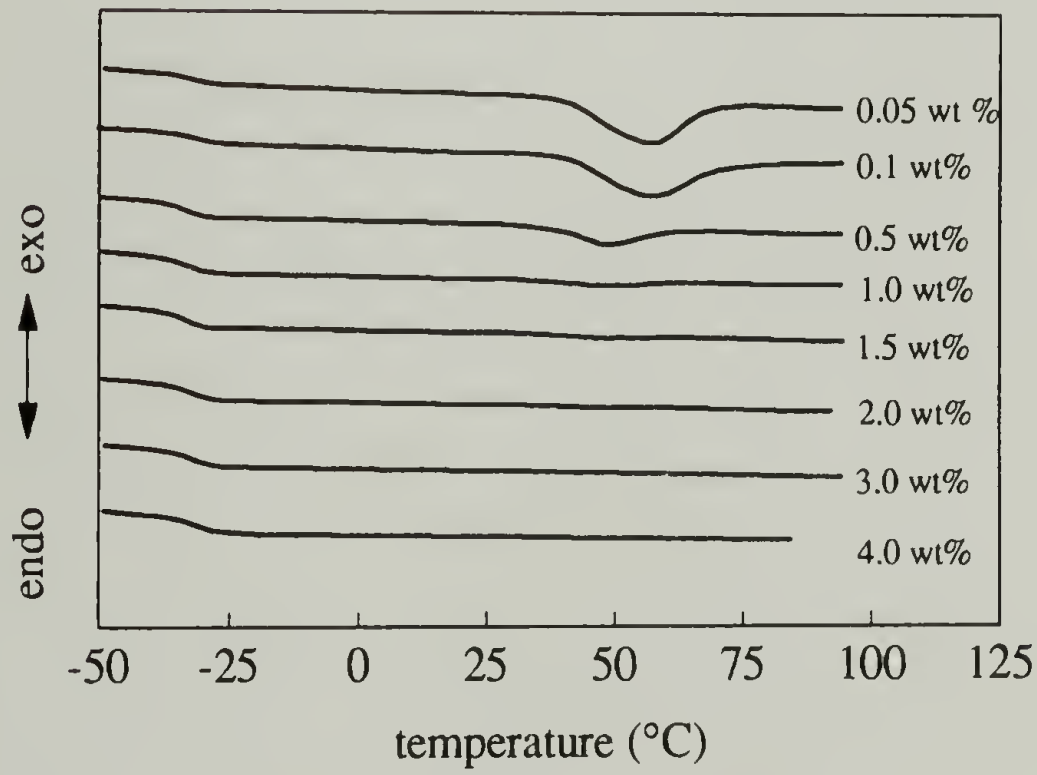
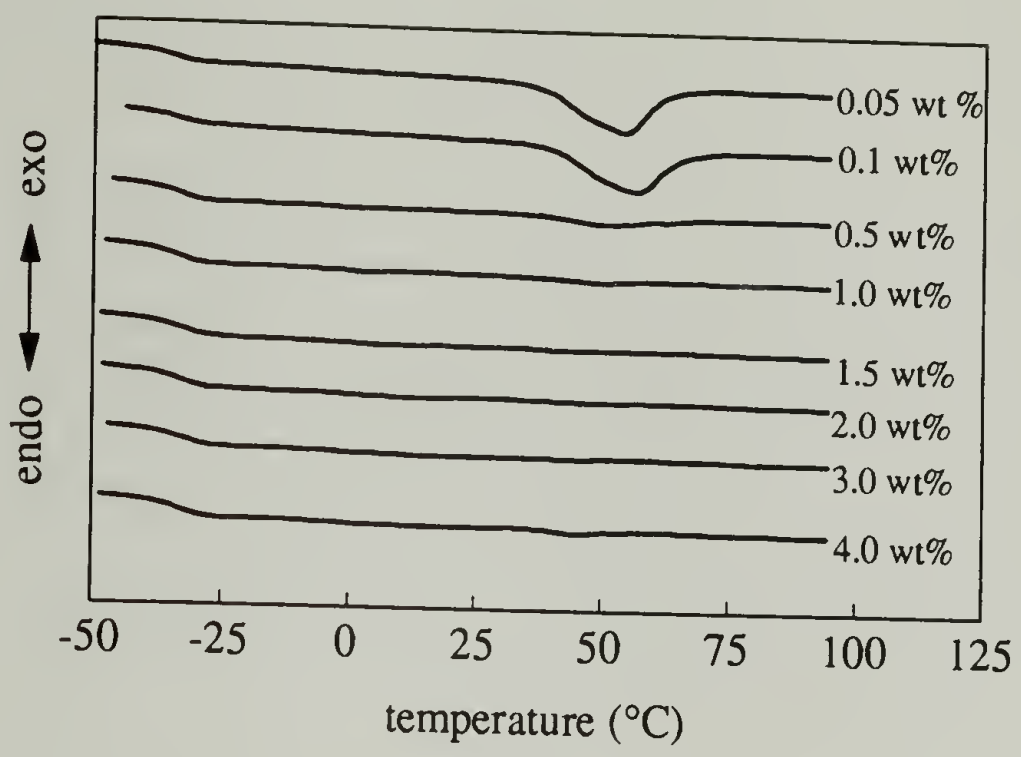


Figure 5.16 DSC results for PHO crosslinked with either a) lauroyl or b) benzoyl peroxide.

a) lauroyl peroxide



b) benzoyl peroxide

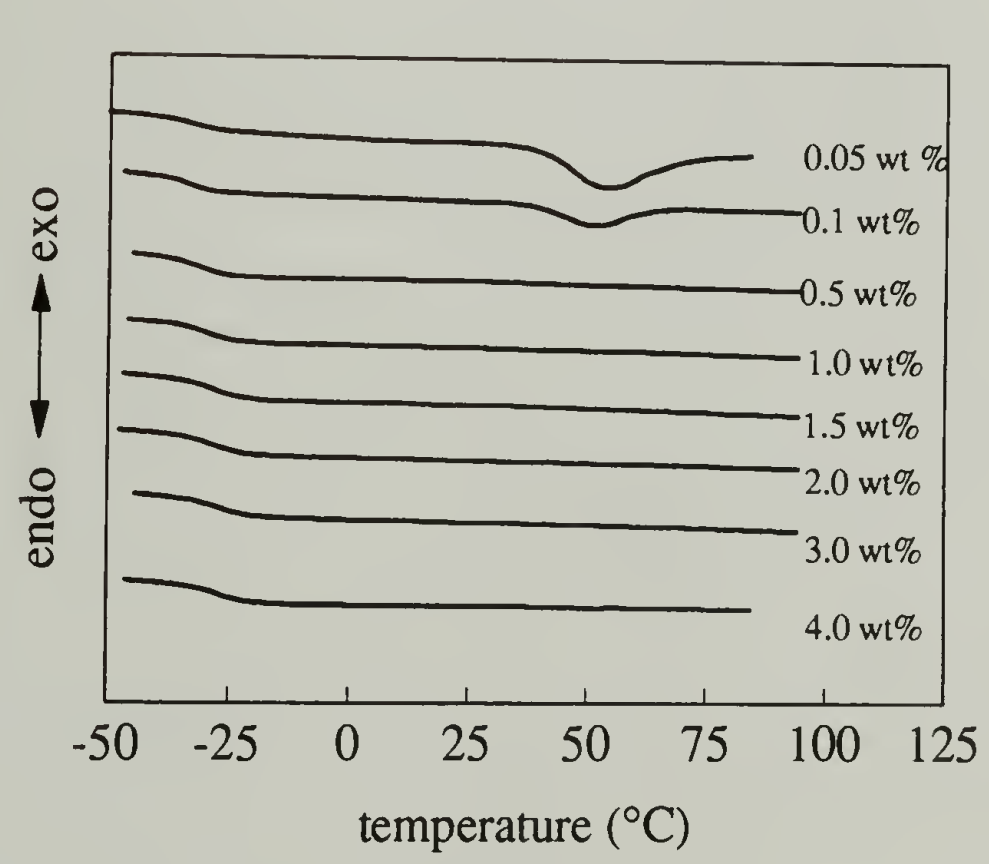


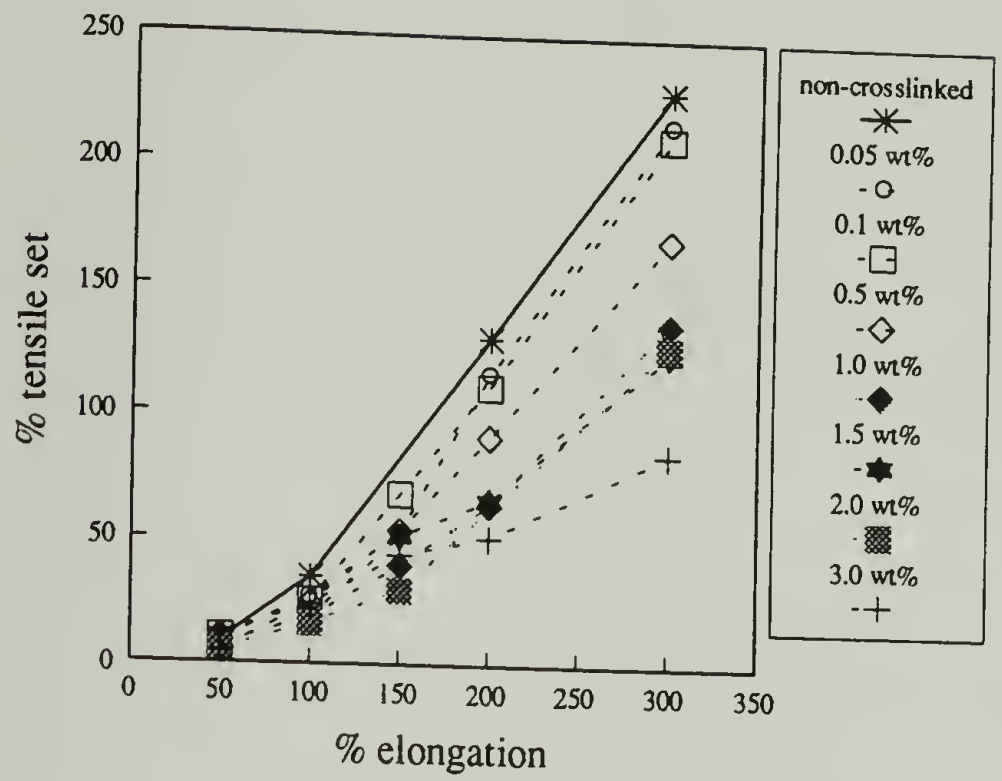
Figure 5.17 DSC results for PHOU(95/5) crosslinked with either a) lauroyl or b) benzoyl peroxide.

5.3.5 Tensile Set

Another goal of chemical crosslinking was improvement in the elastic response of the material. Peroxide crosslinking did improve the elastic response of the polymer as shown in Figures 5.18 and 5.19. For PHO and PHOU(95/5), as the amount of peroxide was increased, the tensile set decreased. With PHO cured with benzoyl peroxide and PHOU(95/5) cured with either peroxide, most of the samples could not be evaluated. The material integrity had been compromised by the crosslinking which resulted in a material with little or no tensile strength or tear resistance, best described as 'cheesy'. The samples could not be removed from the glass casting dishes intact so material property evaluations were impossible. Why the addition of 5% olefin groups to the polymer would so drastically change the crosslinking reaction and produce such a cheesy material is not understood. The peroxide efficiency model had indicated that the addition of olefin groups would reduce the probability of degradation. Perhaps the gain of chemical crosslinks did not offset the loss of crystallinity.

To explore the concept that the loss of crystallinity was not overcome by the amount of chemically crosslinking, higher olefin content polymers, PHOU(80/20) and PHOU(74/26) were biosynthesized. If more olefin groups provided more crosslinks perhaps the loss of material integrity associated with the loss of crystallinity would be surmounted. These polymers were crosslinked with several different concentrations of dicumyl peroxide. These materials still displayed very poor mechanical integrity.

a) lauroyl peroxide



b) benzoyl peroxide

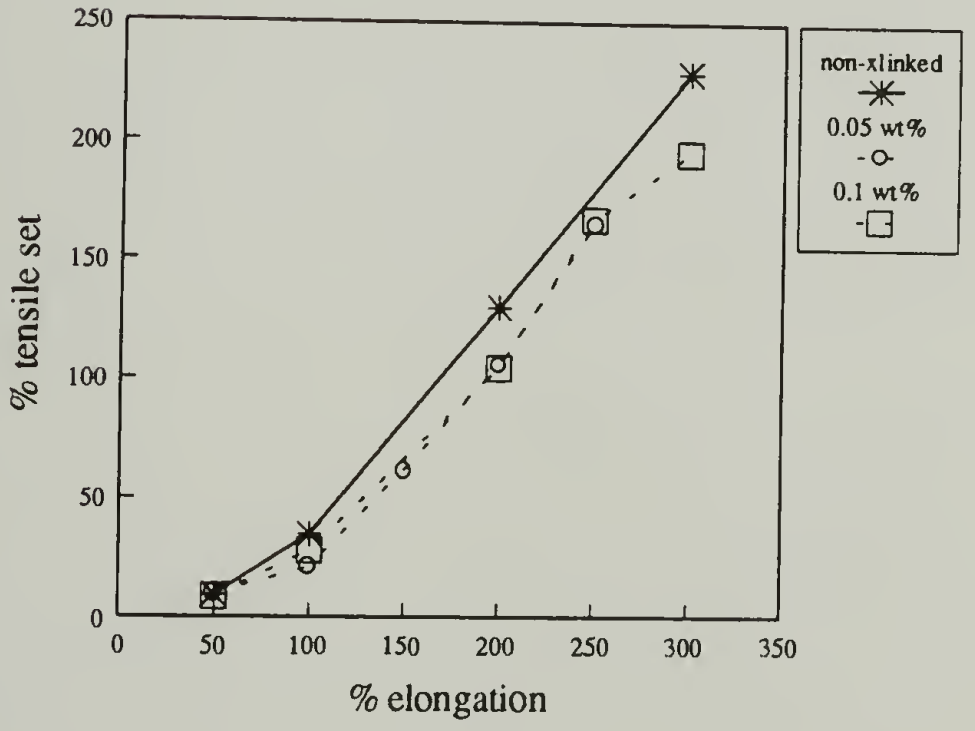
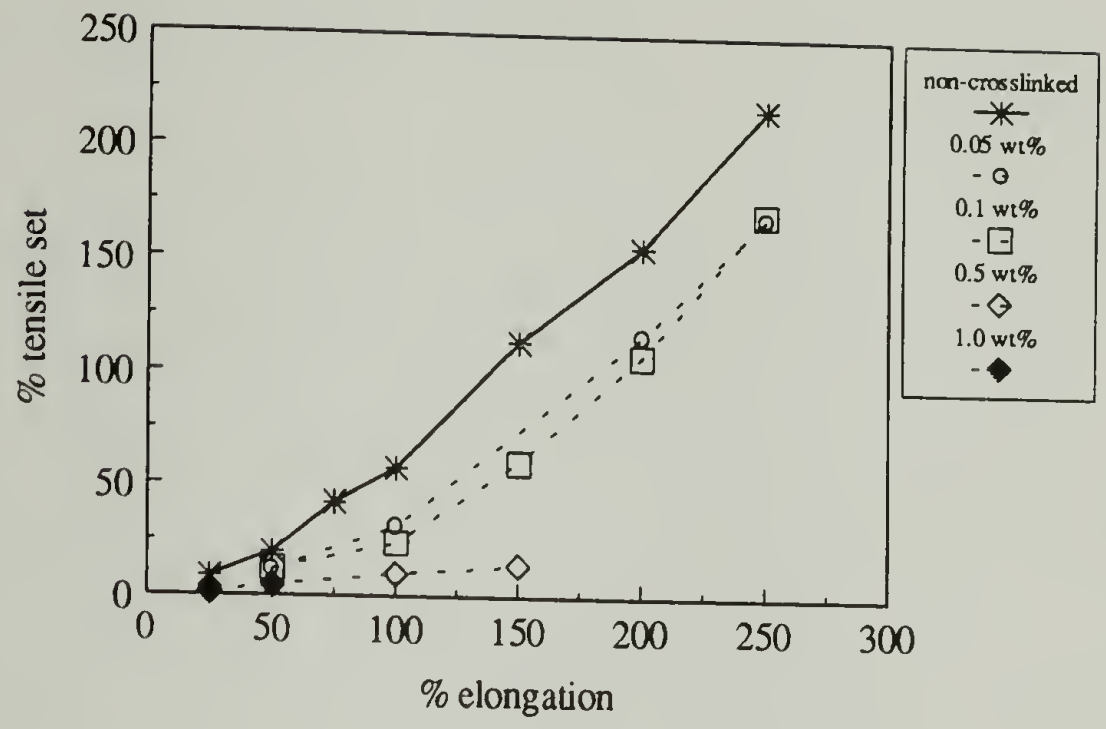


Figure 5.18 Tensile set of PHO crosslinked with either a) lauroyl or b) benzoyl peroxide. Non-crosslinked PHO results are also included for comparison.

a) lauroyl peroxide



b) benzoyl peroxide

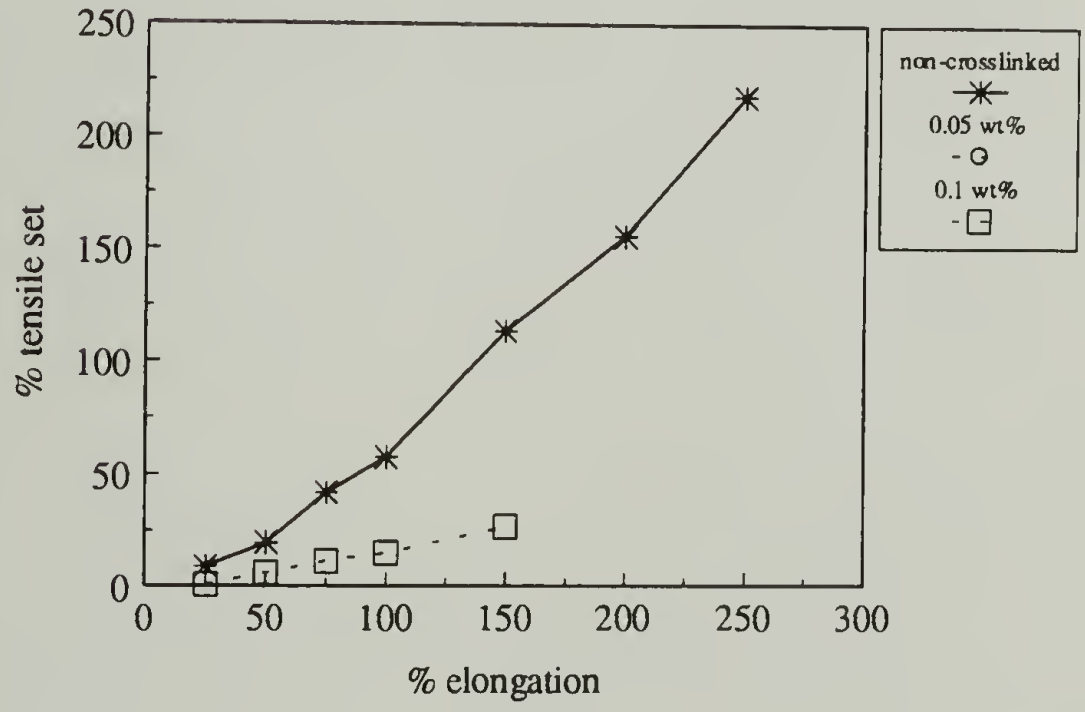


Figure 5.19 Tensile set of PHOU(95/5) crosslinked with either a) lauroyl or b) benzoyl peroxide. Non-crosslinking PHOU(95/5) results are also included for comparison.

5.3.6 Tensile Properties

Figure 5.20 reveals the modulus as a function of wt % peroxide for PHO, PHOU(95/5), and PHOU(93/7) crosslinked with various peroxides. Again, results could only be obtained on a limited number of samples because of the lack of material integrity. The results obtained did show a drastic decrease in the modulus as the amount of peroxide was increased. Only PHO crosslinked with lauroyl peroxide could be tested at most peroxide levels. As mentioned in Section 5.3.3 this combination of polymer and peroxide never totally eliminated all crystallinity even at high peroxide concentrations. These results support the argument that material integrity is compromised by the loss of crystallinity and not offset by the gain of chemical crosslinks.

5.3.7 Network Structure

The network structure as revealed by the molecular weight between crosslinks, M_c, was determined for PHOU(93/7) crosslinked with Varox® peroxide using the impulse viscoelasticity technique. The M_c was estimated at 3000 g/mol for the polymer crosslinked with 0.5 wt % to 2.0 wt % peroxide.

5.3.8 Summary

The various peroxides were all successful in producing crosslinked materials. The efficiency or amount of peroxide required to reach a maximum amount of gel varied. The effect of olefin groups dramatically increased the peroxide efficiency. The goal of eliminating the crystalline regions which acted as the physical crosslinks was achievable for most peroxides if enough peroxide was used. The elastic response was improved but in

general the material integrity was very poor. A summary of the results for peroxide crosslinking is given in Table 5.4.

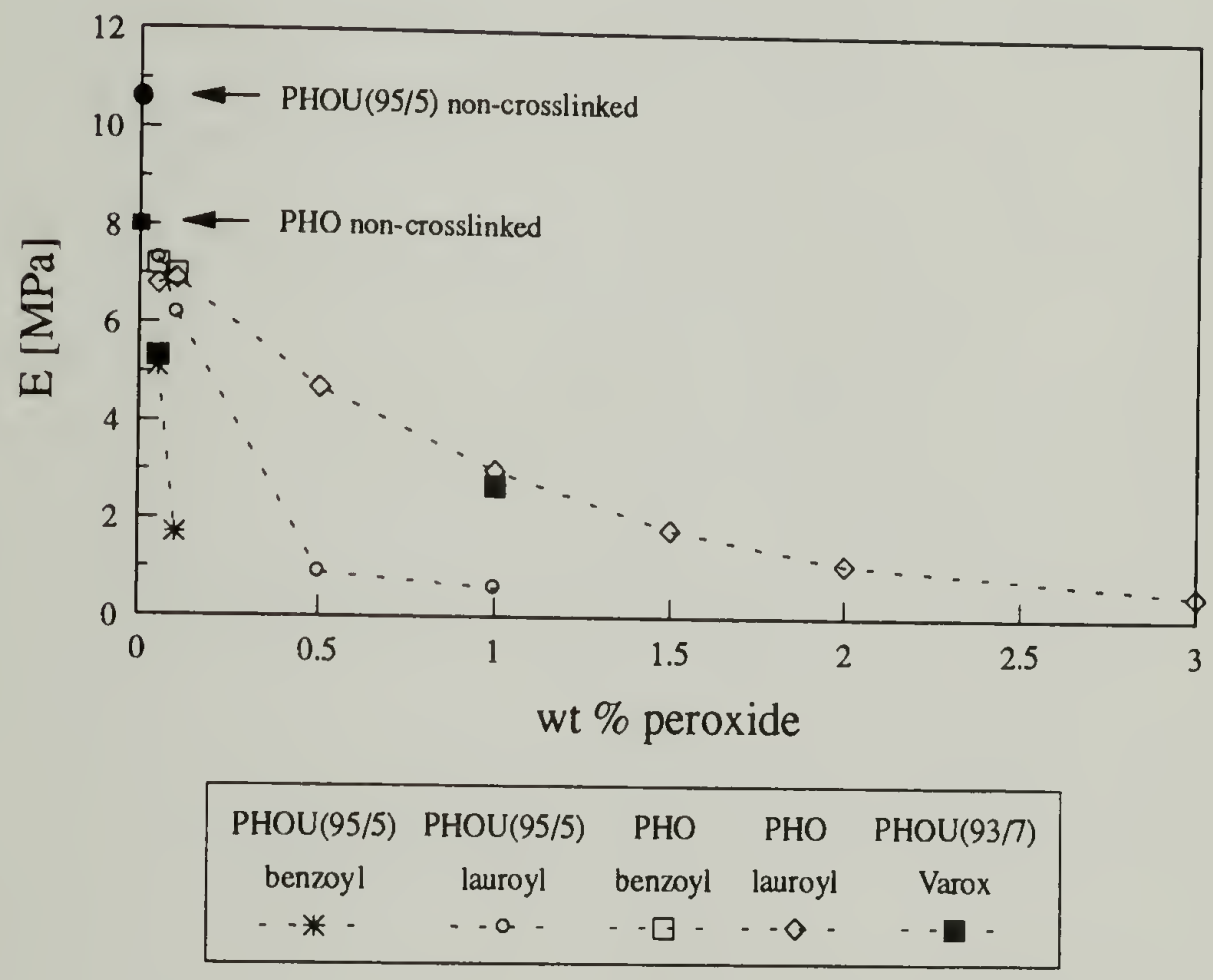


Figure 5.20 Tensile modulus as a function of peroxide concentration. Sample geometry: strips.

Table 5.4 Summary of peroxide crosslinked sample results including model efficiency parameters, molecular weight between crosslinks, M_c, and crystallinity.

Description	Peroxide Efficiency	p_o/q_o	M_c (g/mol)	crystallization
PHO				
lauroyl	0.55	0.73	—	YES
benzoyl	0.41	0.35	—	YES to NO
PHOU(95/5)				
lauroyl	1.04	0.21	—	YES
benzoyl	1.28	0.11	—	YES TO NO
PHOU(93/7)				
lauroyl	1.03	0.78	—	YES
benzoyl	1.82	0.14	—	YES TO NO
Varox®	1.52	0.10	2400-4200	YES TO NO
dicumyl	2.54	0.17	—	YES TO NO

5.4 Sulfur Vulcanization Results and Discussion

5.4.1 Vulcanization

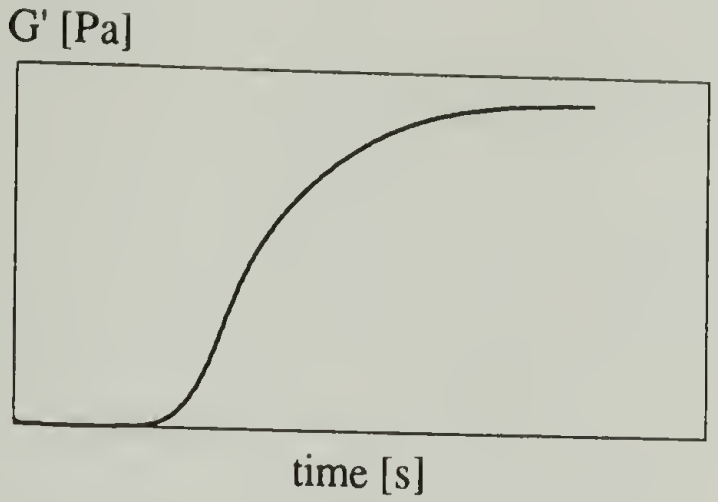
The vulcanization process is typically monitored using a modified rheometer called a cure meter which measures the torque as a function of time during isothermal curing. [16] For this study, the storage modulus which is proportional to the torque, was monitored over time using a rheometer. Cure curves can take on three different shapes: plateau, reversion, or marching depending on how the torque (or storage modulus in this study) varies with time.[16] Figure 5.21 shows examples of these different types of cure curves. It is important to know which type of cure a specific chemistry will produce to insure the cure is optimized.

All samples were an opaque cream color after compounding. After cure, most samples had darkened to a tan or dark tan color and remained opaque. This tan color is similar to a sulfur vulcanized rubber band in appearance. One sample cured with the sulfur and BBTS chemistry turned into a dark brown liquid shortly after heating began and was discarded.

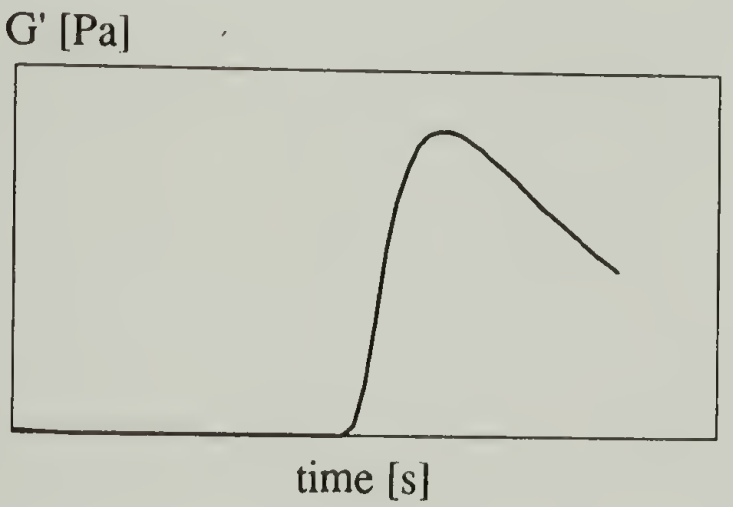
Figure 5.22 shows a series of cure curves typically observed for the thiuram (DPTT) dithiocarbamate (BZ) sulfur chemistry. As the olefin content was increased, the final dynamic modulus attained at the plateau also increased. The higher modulus reflects the higher crosslink density achieved with more reactive groups available for crosslinking.

PHOU(80/20) cured at several vulcanizing agent concentrations is shown in Figure 5.23. The trend of an increasing plateau dynamic modulus with increasing vulcanization agent was observed. The increase was substantial from 3.5 to 4.5 phr vulcanizing agent but insignificant when the concentration was raised to 5.5 phr. These results imply that the vulcanization agent did not limit the curing process after 4.5 phr vulcanizing agent was used. Similar results were obtained for PHOU(74/26).

a) plateau cure curve



b) reversion cure curve



c) marching cure curve

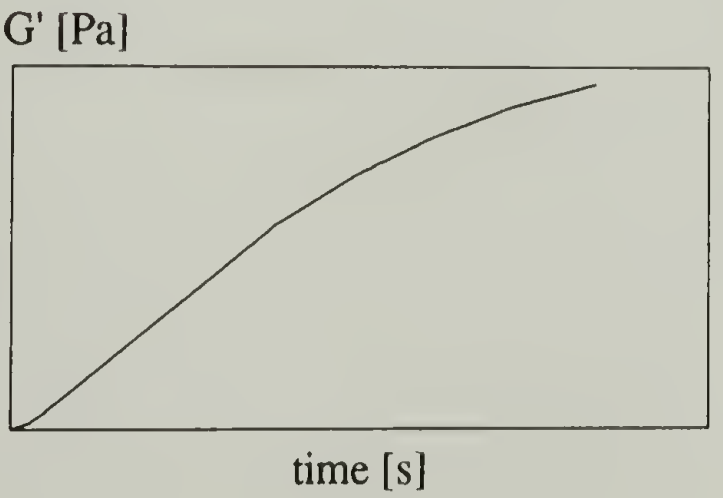


Figure 5.21 Examples of different cure curves encountered with sulfur vulcanization.

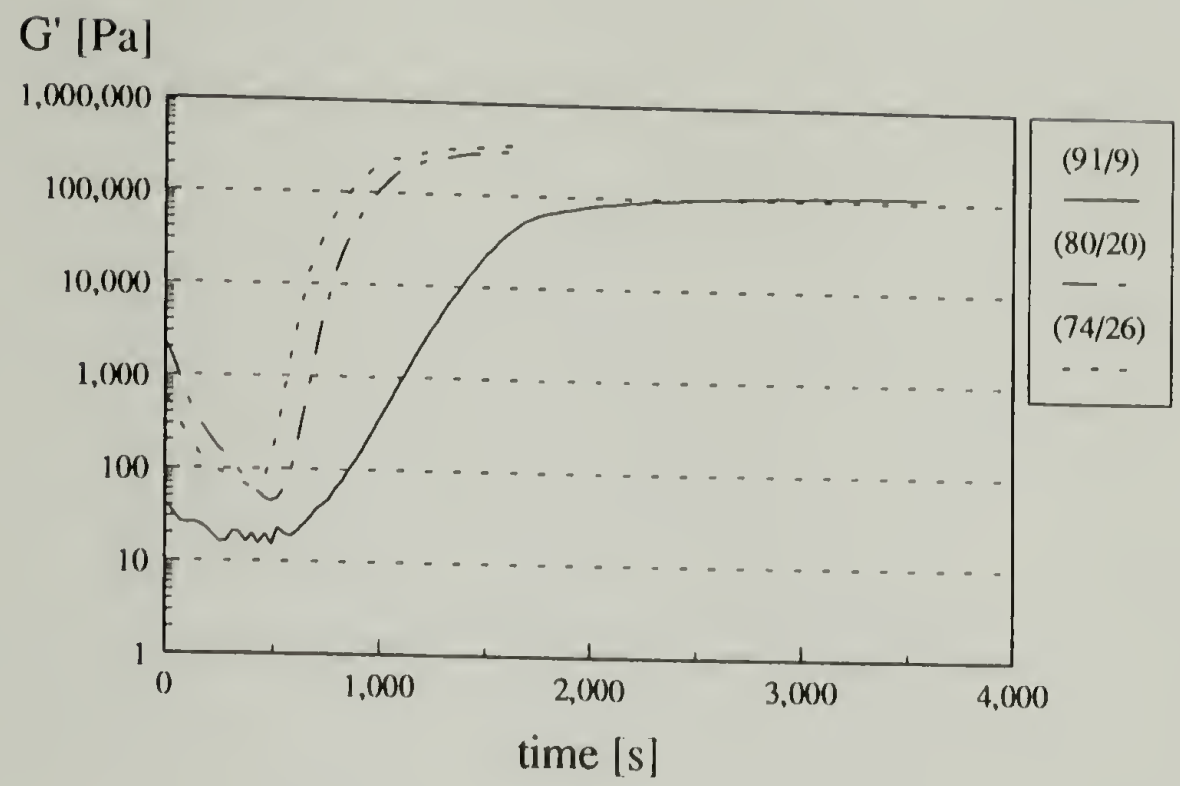


Figure 5.22 Effect of olefin content on cure of several PHOUs using 4.5 phr DPTT and 0.5 phr BZ. Cure temperature 140 °C.

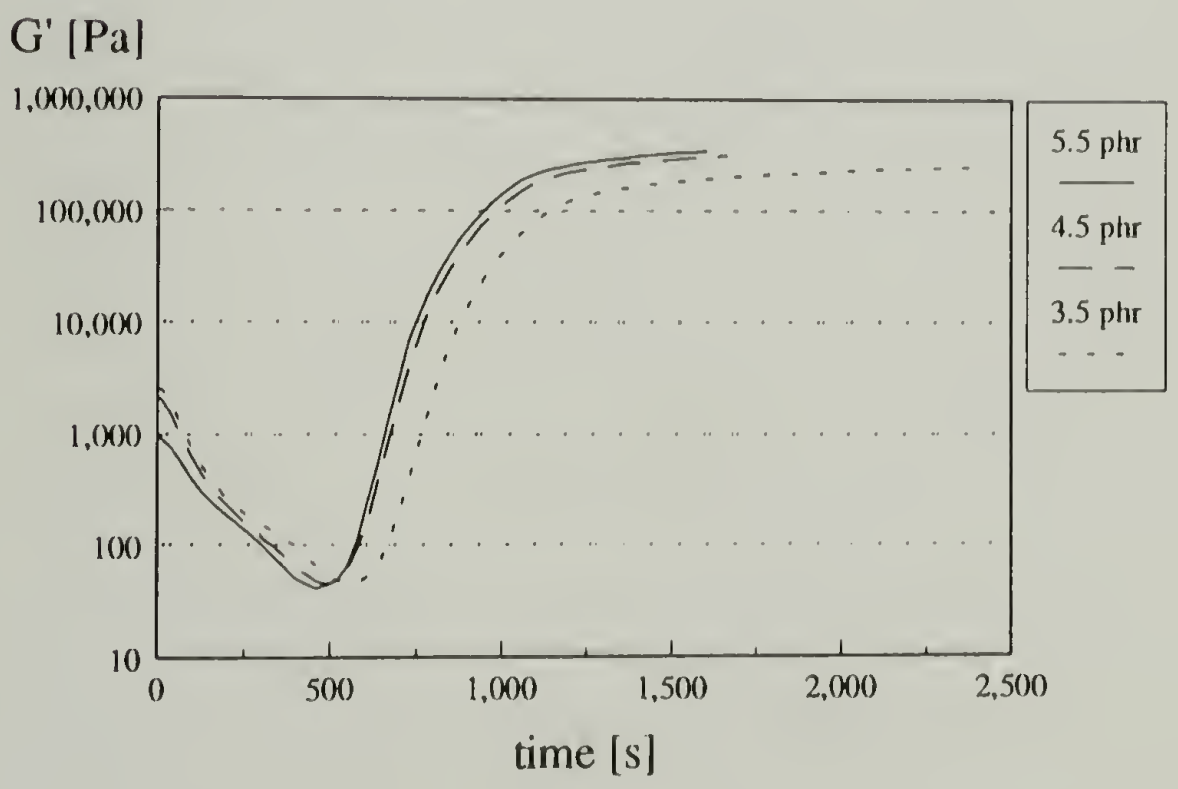


Figure 5.23 Effect of vulcanizing agent concentration on cure of PHOU(80/20) using DPTT and 0.5 phr BZ. Cure temperature 140 °C.

The feel of the material after cure also changed with olefin content. The low olefin content PHOU(93/7) remained sticky and was soft after curing whereas PHOU(80/20) and PHOU(74/26) were dry to the touch and harder.

5.4.2 Sol-Gel Analysis

A sol-gel analysis was conducted on the many of the vulcanized samples. Figure 5.24 compares the results of the different sulfur chemistries. All sulfur cures produced a gel. Generally a trend of decreasing sol with increasing sulfur vulcanization agent was observed.

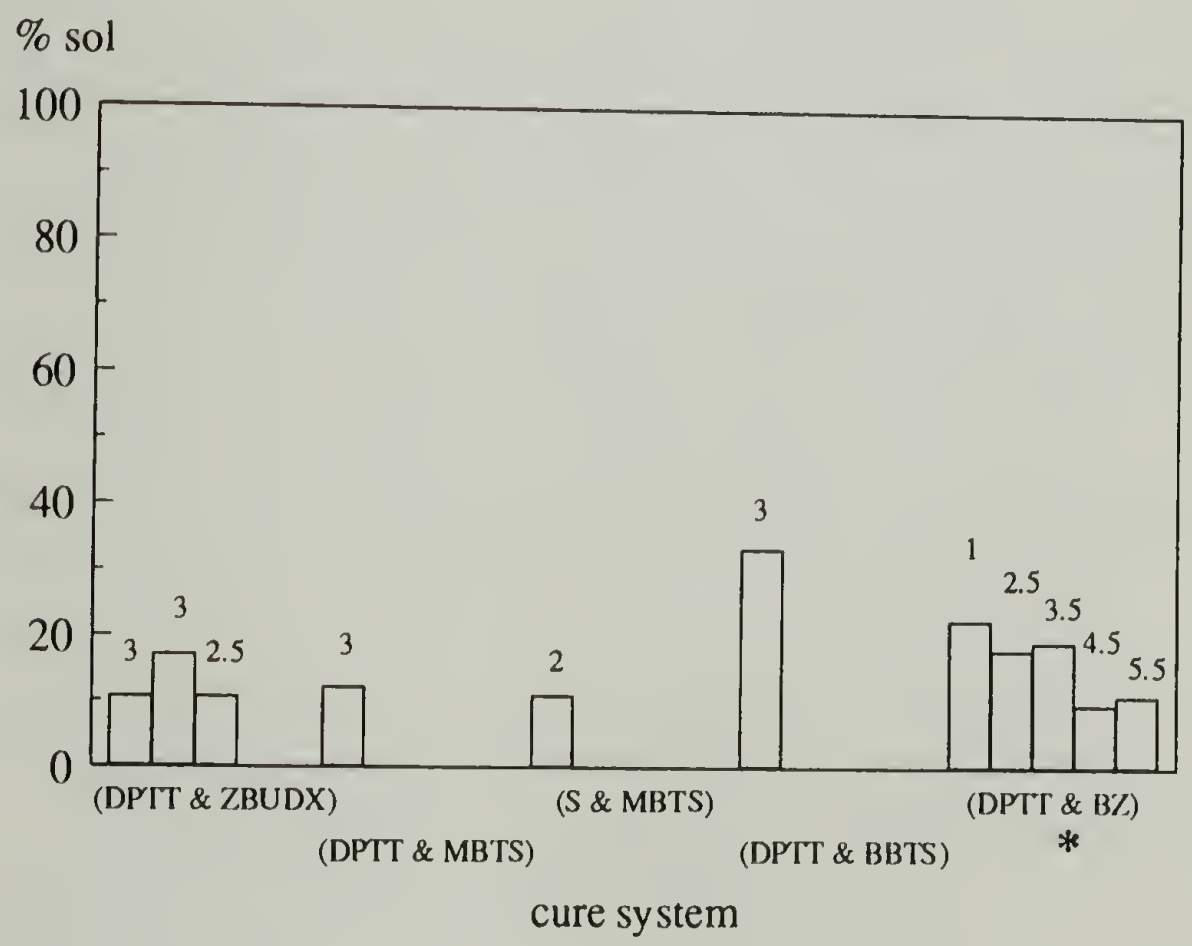


Figure 5.24 Sol-gel analysis results for PHOU(93/7) sulfur vulcanized with several different sulfur chemistries. The numbers above the bars indicated the amount of vulcanizing agent in phr. The * indicates PHOU(91/9) was used for these samples.

One chemistry which showed promising results was evaluated further with polymers containing different levels of olefins. The sol-gel results of these samples are given in Figure 5.25. The trends observed in the % sol results reflect the results obtained from the cure curves. Increasing the olefin content produced a material which was more thoroughly crosslinked as indicated by a decreasing % sol in each group of three bars. The use of more vulcanizing agent also decreased the amount of extractables but with less dramatic an influence than the olefin content.

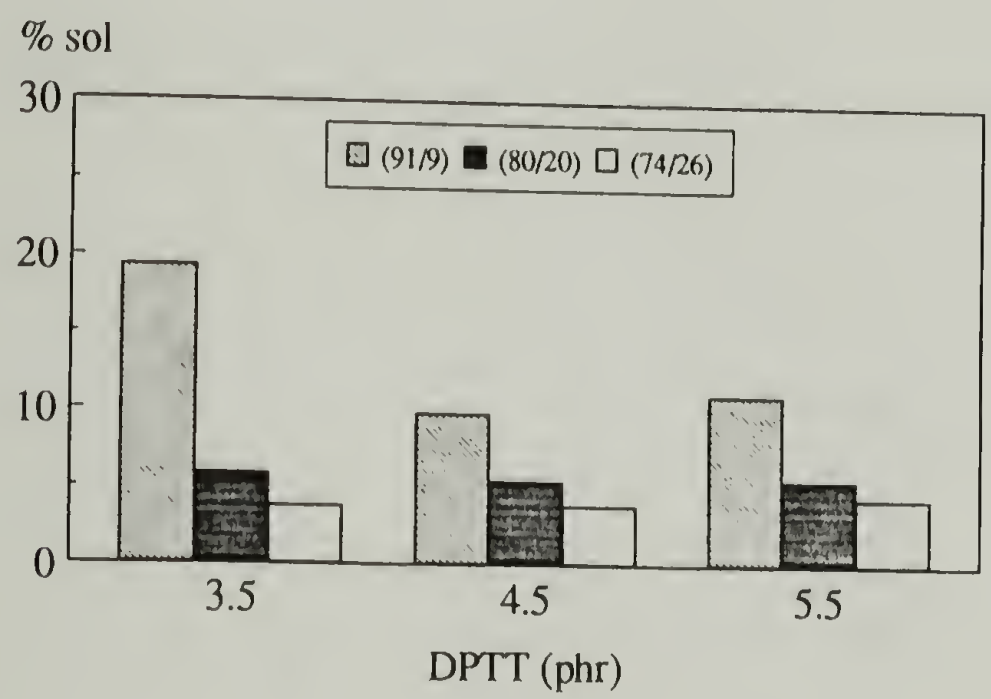
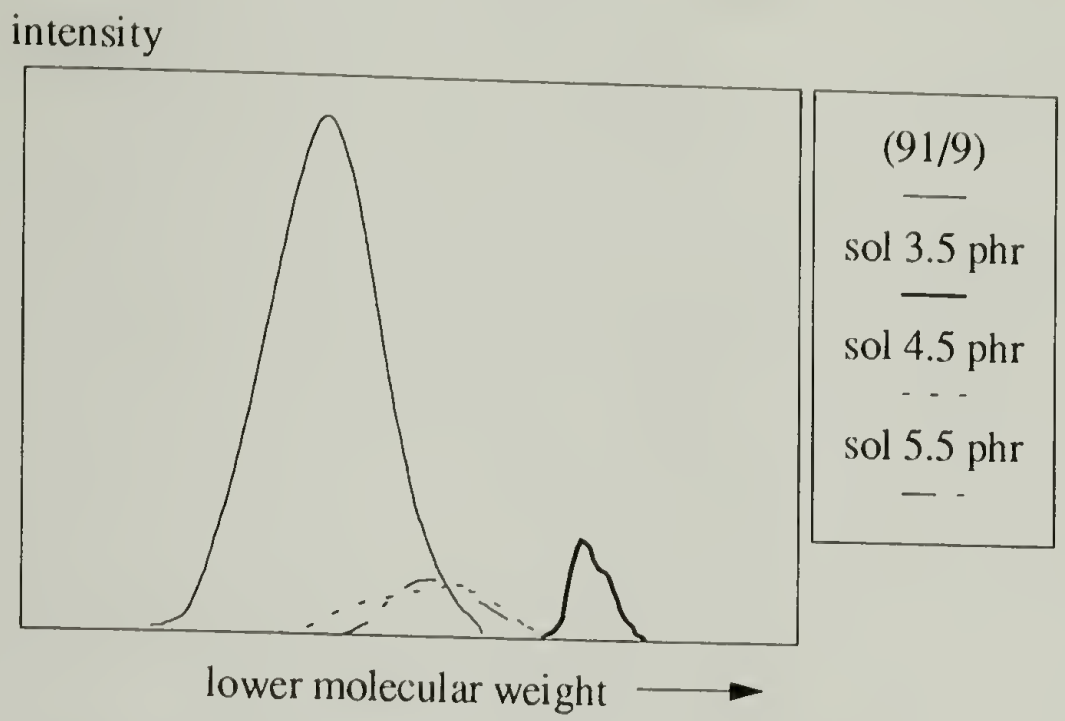


Figure 5.25 Sol-gel analysis showing the effect of polymer olefin content and concentration of vulcanizing agent on % sol.

5.4.3 Gel Permeation Chromatography

The sol fractions extracted from several sulfur vulcanized samples were evaluated for molecular weight distribution. Figure 5.26 shows the results of the sol fractions for PHOU(91/9) after undergoing sulfur vulcanization with three different vulcanizing agent concentrations. The samples were vulcanized in the rheometer from 25 to 60 minutes.



	Mw	Mn	PDI
PHOU(91/9)	148,000	64,000	2.3
3.5 phr sol (19.3 %)	600	490	1.2
4.5 phr sol (9.7%)	27,000	12,000	2.3
5.5 phr sol (11.0%)	18,000	12,000	1.6

Figure 5.26 Gel permeation chromatographs and data for PHOU(91/9) before vulcanization and the sol fraction of the polymer after undergoing sulfur vulcanization with various levels of DPTT and 0.5 phr BZ.

The molecular weight distribution changed shape and shifted to a lower molecular weight fraction for all the sols analyzed. The sol fraction from the sample vulcanized with 3.5 phr DPTT exhibited the lowest molecular weight being totally beyond the molecular weight range found in the original polymer. The sols from the 4.5 and 5.5 phr DPTT samples exhibited a slight shift to a lower molecular weight than the original polymer and also exhibited a pronounced broadening of the distribution. The shifts suggests that some chain scission reactions had occurred during cure. It is unknown whether the low molecular weight fractions were generated from the vulcanization process of simply because of heating to 140 °C for approximately 25 minutes.

5.4.4 Thermal Analysis

The presence of crystallinity could be implied if the material after cure was still sticky and gummy but hardened up overnight. To corroborate the observations, DSC testing was conducted approximately one week after vulcanization giving ample time for the polymer to crystallize. The goal of eliminating all crystallinity was achieved with several chemistries. Generally a trend of decreasing crystallinity and increasing glass transition temperature was observed as the amount of vulcanization agent was increased as shown in Figure 5.27. These results indicated that a more highly crosslinked network was produced when more vulcanization agent was used. The polymer chains were constrained in such a way that crystallization was prevented or disrupted and the glass transition was shifted to a higher temperature due to the extra junction points. These results indicate that other chemistries which did not eliminate all crystallinity may still be viable candidates if higher vulcanization agent concentrations were employed.

Interestingly, for 3.5 phr DPTT, the glass transition for a 7% olefin containing vulcanized polymer appeared higher than for either the 20% or 26% olefin containing polymers. The difference was approximately 2 °C.

Thermogravimetric analysis showed that the thermal decomposition of sulfur vulcanized PHOU occurred approximately 20 °C below that of as extracted PHOU yet retained the same decomposition curve shape. The onset of thermal decomposition was independent of the amount and type of vulcanization agent used. Perhaps the added compounds promoted the mechanism of decomposition in the bacterial polyesters.

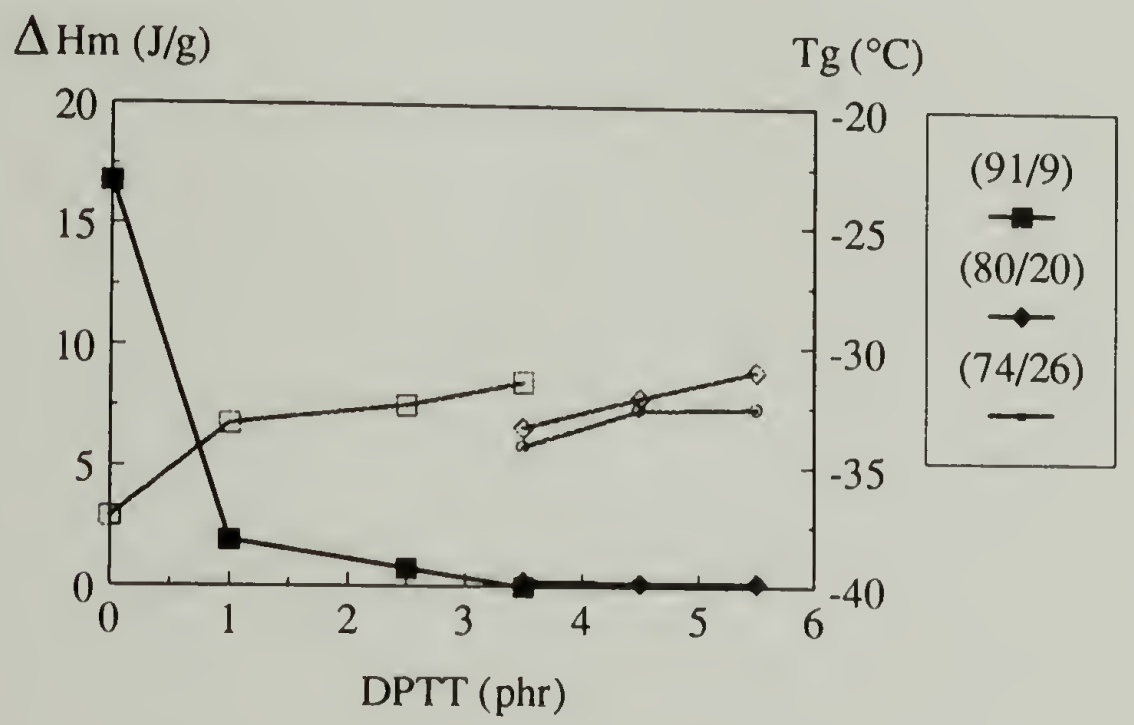


Figure 5.27 DSC results for several PHOUs vulcanized with DPTT and BZ. Open symbols indicate T_g. Closed symbols indicate ΔH_m.

5.4.5 Tensile Set

Tensile set was virtually eliminated in the sulfur vulcanized PHOUs as shown in Figure 5.28. Even after 200% elongation, PHOU(93/7) showed less than 5% tensile set. Non-crosslinked PHOU(93/7) would typically exhibit a tensile set of approximately 150%

after 200% elongation. Unfortunately, the tensile strength was so compromised in the higher olefin content PHOUs that less than 100% elongation was possible. But even at these low strains, the tensile set was reduced fivefold. Typical values for non-modified PHOU would be a tensile set of approximately 20% after 50% elongation.

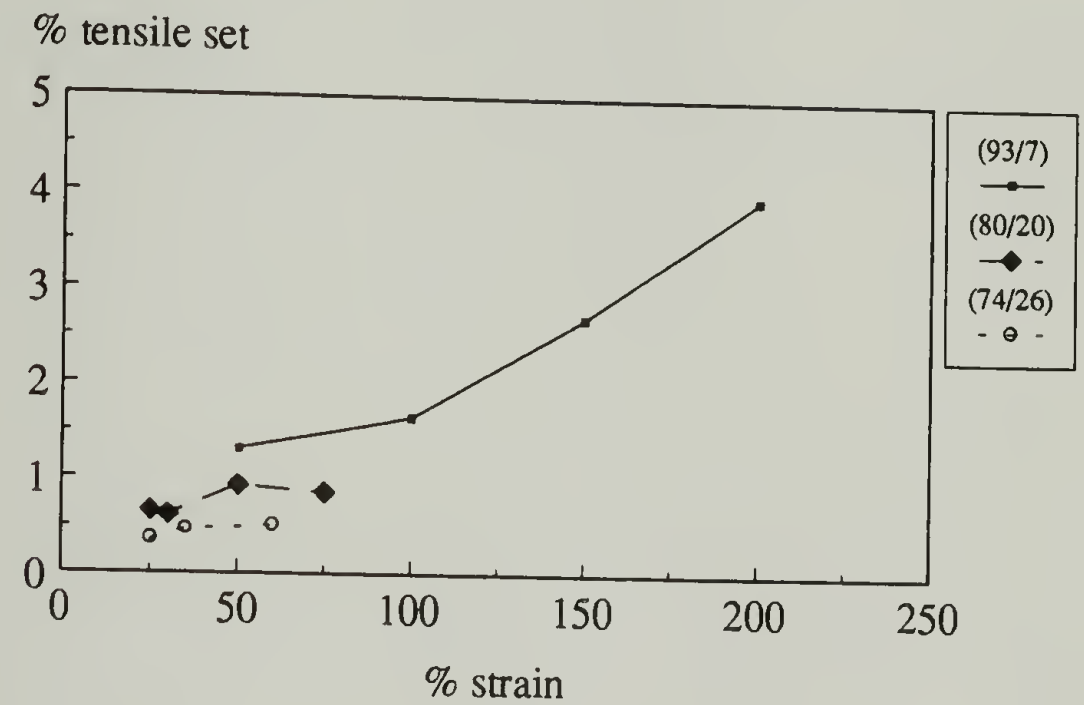


Figure 5.28 Tensile set of several PHOUs sulfur vulcanized with 4.5 phr DPTT.

5.4.6 Tensile Properties

The mechanical properties of several vulcanized polymers are summarized in Table 5.5 and compared to a non-crosslinked PHOU. The modulus, tensile strength, and elongation were all substantially below the values for non-modified polymer. The PHOU with only a 7% olefin content still retained a relatively high elongation.

Table 5.5 Tensile properties of PHOU(91/9) non-crosslinked and several PHOUs sulfur vulcanized with 4.5 phr DPTT. Strain rate 1 min^{-1}. Standard deviation approximately 20%.

Polymer	E [MPa]	Tensile Strength [MPa]	Ultimate Elongation [%]
PHOU(91/9) non-crosslinked[a]	8	10	370
PHOU(93/7)	1.2	0.8	250
PHOU(80/20)	1.8	0.8	90
PHOU(74/26)	1.2	0.8	80

[a] Samples tested at a 2 min^{-1} strain rate.

The tensile modulus was determined at several strain rates for three different PHOUs. The results are summarized in Table 5.6. The modulus increased slightly when the olefin content was increased from 7% to 20%. The modulus did not increase further when the olefin content was increased to 26%. The effect of strain rate was negligible even when varied between 1 min^{-1} and 25 min^{-1}. In addition, the modulus did not change after repeated stretching of the same sample at any of the strain rates.

Table 5.6 Sulfur vulcanized PHOU tensile modulus values at several strain rates. Standard deviation approximately 20%.

strain rate [min^{-1}]	PHOU(93/7)	PHOU(80/20)	PHOU(74/26)
25	0.7	1.5	1.1
10	0.7	1.4	1.2
5	0.7	1.3	1.2
2.5	0.8	1.7	1.3
1	1.2	1.8	1.2

5.4.7 Network Structure

Impulse viscoelasticity experiments were conducted on several sulfur vulcanized samples. Figure 5.29 shows the equilibrium shear modulus values obtained. Increasing the vulcanizing agent concentration increased the shear equilibrium modulus. A dramatic increase in the modulus was noted when the olefin content of the polymer was increased from 7% to 20%. An increase to 26% olefin content did not alter the modulus value significantly above the 20% value. An increase in modulus corresponds to a decrease in the molecular weight between crosslinks. In other words, the more vulcanization agent used or the higher olefin content polymer, the higher the crosslink density.

The molecular weight between crosslinks, M_c, was calculated from equation 5.3 using the equilibrium shear modulus values. Sulfur vulcanized PHOU(93/7) exhibited an M_c that was one order of magnitude larger, approximately 20,000, than those for peroxide cured PHOU(93/7) which had an M_c of approximately 3000. The olefin content dramatically affected the M_c with the value decreasing to approximately 6, 000 when the olefin content was increased to 26%.

An interesting calculation revealed that if all the olefin groups in PHOU(80/20) resulted in a crosslink point, the resulting M_c should be approximately 750. This indicates that only 10% to 15% of all olefin groups reacted during sulfur vulcanization. Perhaps all the olefin groups are somehow prevented from participating in the reaction. This idea would be supported by the concept of reduced chain entropy occurring after partial crosslinking.

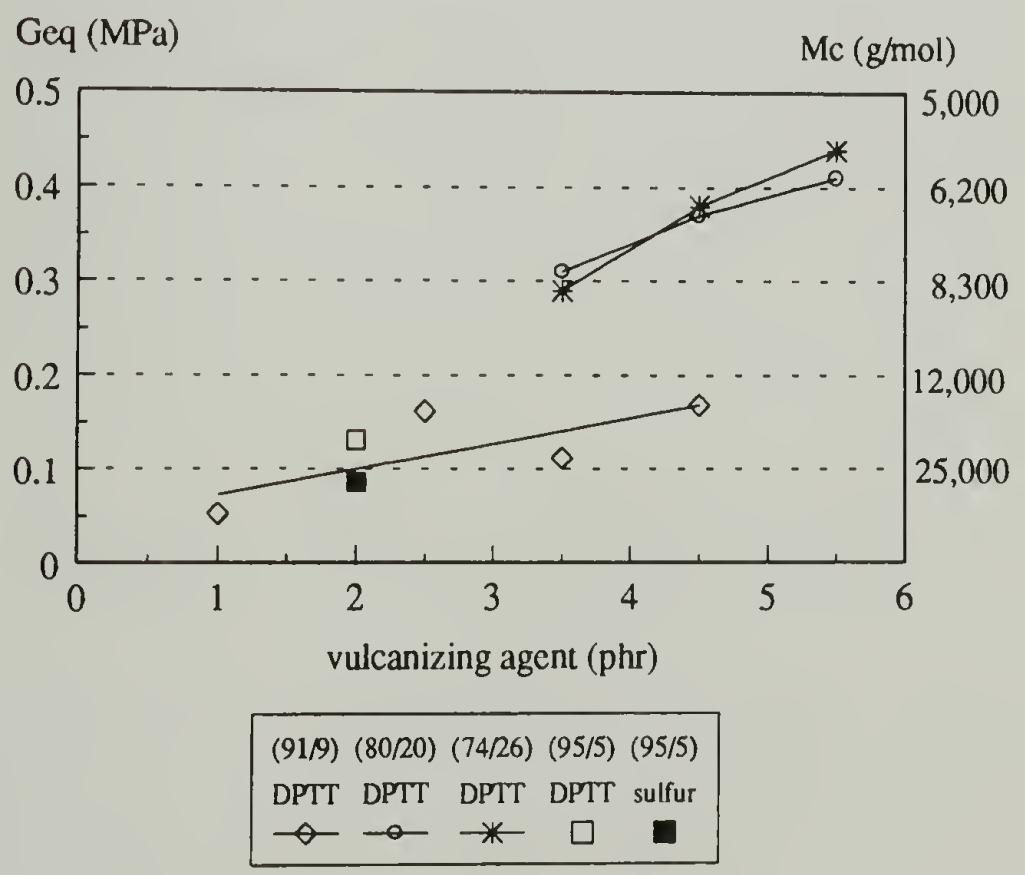


Figure 5.29 Shear equilibrium modulus, G_{eq}, and molecular weight between crosslinks, M_c, as a function of the vulcanizing agent concentration for several systems.

5.4.8 Summary

Several sulfur vulcanization chemistries were successful in producing crosslinked PHOUs. The higher the olefin content, the less extractables, the lower the level of crystallinity detected (if at all), and the lower the molecular weight between crosslinks. The materials exhibited less than 5% tensile set even after 200% elongation. The tensile modulus and tensile strength of the material were reduced dramatically compared to non-modified PHOU. Table 5.7 summarizes the findings for sulfur vulcanized PHOUs.

Table 5.7 Summary of sulfur vulcanization results including % sol, crystallinity, and molecular weight between crosslinks, M_c.

CURE CHEMISTRY	% SOL	CRYSTALS	M_C (g/mol)
DPTT & BZ	10 - 30	YES TO NO	6K - 34K
DPTT & ZBUDX	10 - 20	YES TO NO	20K - 28 K
S & ZBUDX	—	YES	—
DPTT & BBTS	33	YES	—
S & BBTS	100	YES	—
DPTT & MBTS	12	YES	18K
S& MBTS	10	VERY LOW	25K

5.5 Tetrafunctional Sulfur Crosslinker

Several stoichiometries including 1:1, 1:2, 1:3, and 1:4 polymer olefin to sulfur compound sulfurs were tested. The sol-gel analysis results are shown in Figure 5.30. A gel was produced using these compounds. All the cured materials were transparent. The 1:1 stoichiometry film was slightly sticky after cure but firmed up overnight indicating that some crystallinity may be present. The other stoichiometries produced firmer films. However, the mechanical integrity of all the films was compromised; the material exhibited a 'cheesy' character similar to peroxide cured PHOU. The similarity to peroxide cured films may be due to the use of a free radical initiator. The expected chemistry which relies on heat to produce a free radical initiator which attacks the sulfur compound may not be the only reaction occurring. The polymer may also be involved directly as in peroxide crosslinking especially with the use of a substantial 0.1 wt % initiator concentration.

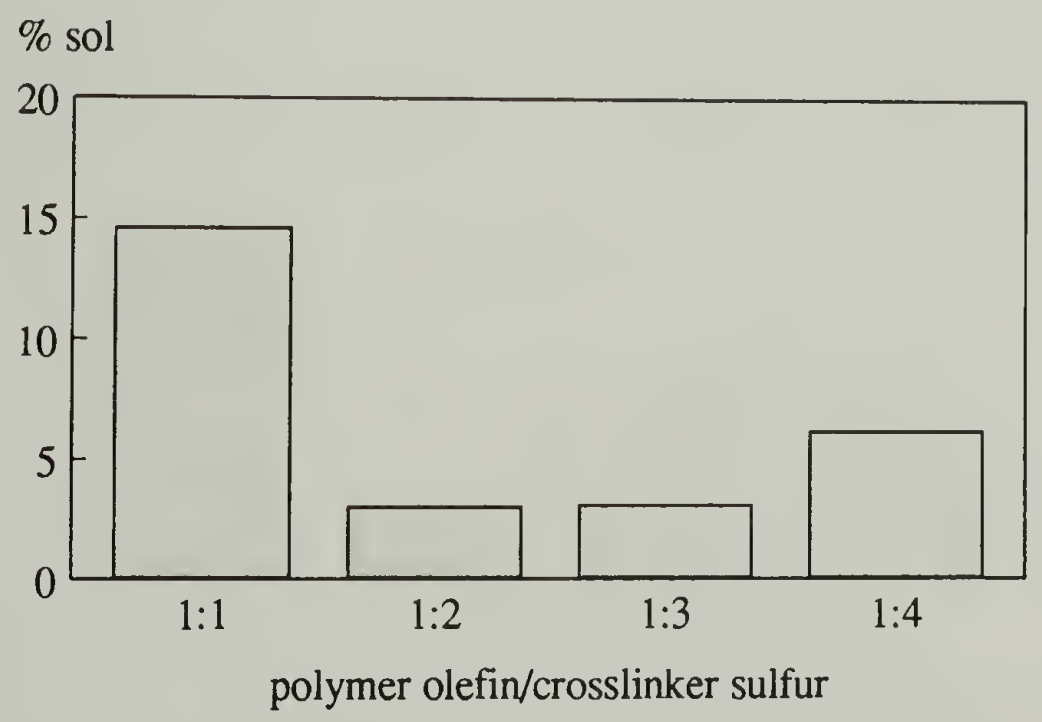


Figure 5.30 Sol-gel analysis results for PHOU(93/7) crosslinked with the tetrafunctional sulfur compound.

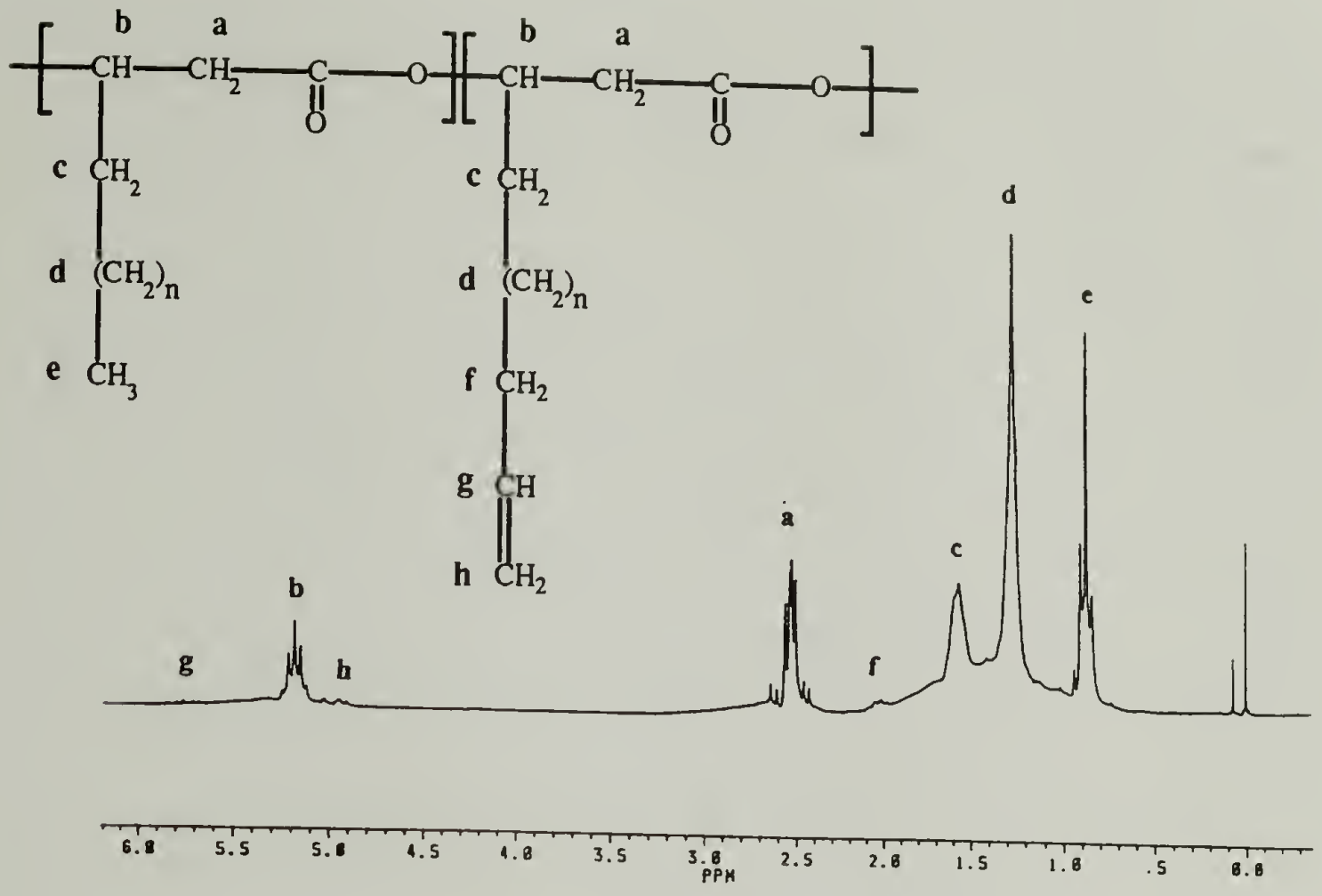
Several peroxides at the 0.1 wt % level did produce gels. The sulfur reagent did influence the amount of gel produced implying that the initiator was not the only reagent involved in promoting crosslinking.

5.6 Epoxidation Results

The ^1H NMR results shown in Figure 5.31 indicated that the reaction of the polymer with metachloroperoxybenzoic acid was successful in eliminating the olefinic proton signals. However, the presence of epoxy proton signals (g, h_1, and h_2) expected at 2.47, 2.74, and 2.90 ppm were not present. The film formed after solvent evaporation was still liquid-like after two weeks and appeared to contain a large amount of a white compound which had crystallized and was intimately mixed with the polymer. No crosslinking had occurred. This approach to crosslinking was abandoned.

5.7 Conclusions

Peroxide crosslinking was successful in producing a gel. The crystallinity was reduced or eliminated and the tensile set was reduced in the samples tested. However, the loss of tensile strength was a general feature of peroxide crosslinked PHO and especially PHOU both with and without coagents. It is believed that the gain of chemical crosslinks was not substantial enough to overcome the loss of crystallinity in providing mechanical integrity to the material even when high olefin content PHOUs were used. It is possible that chain scission was a factor, a common problem with free radical chemistry, although the peroxide efficiency model did not indicate this was the case. Perhaps the "vinyl specific" peroxides for silicones are not vinyl specific for polyesters and random crosslinking (and chain scission) had occurred.



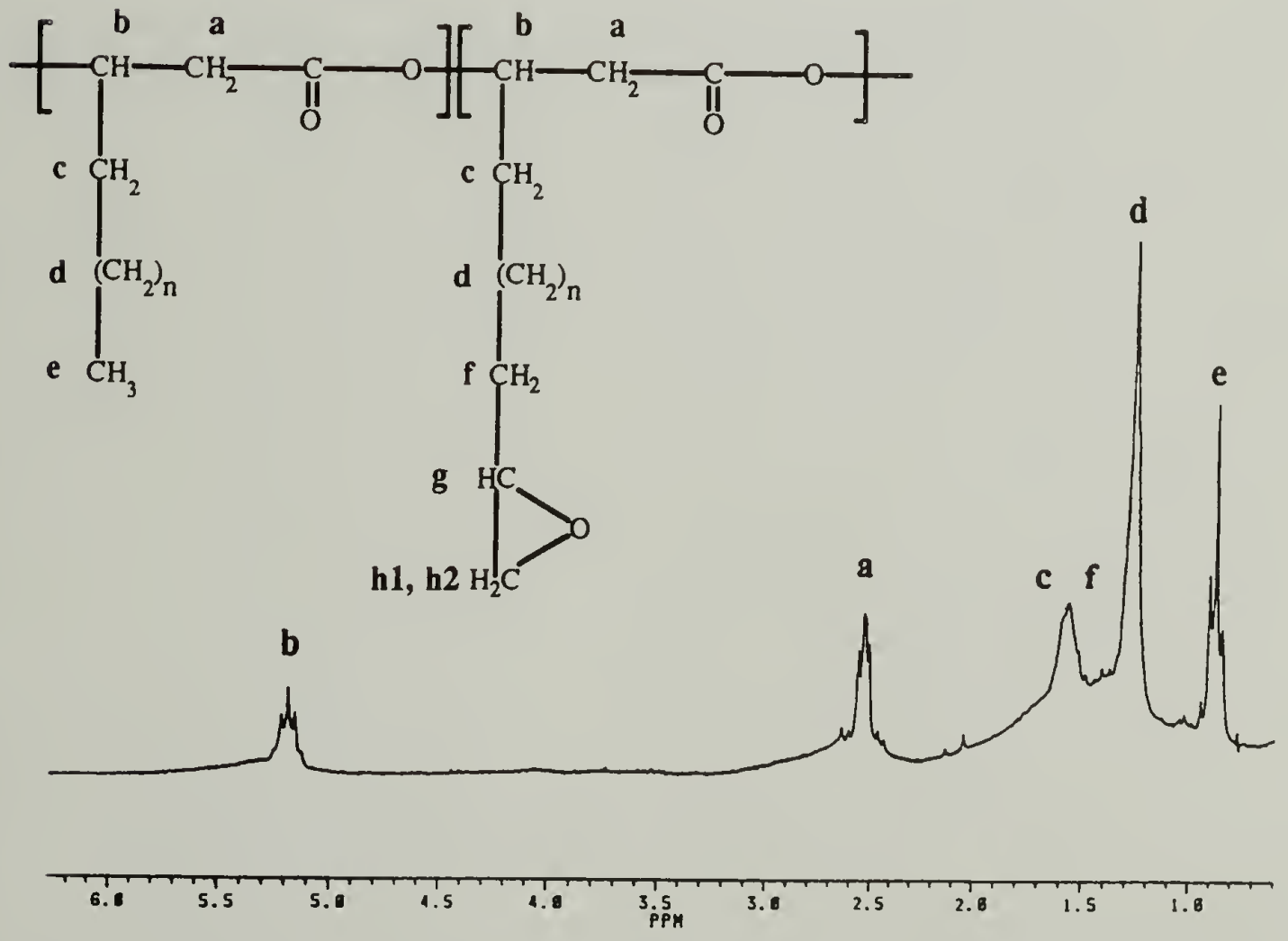


Figure 5.31 ^{1}H NMR spectra from PHOU(93/7) both before and after reaction with *meta*-chloroperoxybenzoic acid.

Another explanation may be the effect of chain ends which would be more pronounced in a random crosslinking reaction. Generally synthetic rubbers which are to be peroxide crosslinked must have a high molecular weight, on the order of 200,000 to 500,000 g/mol. The high molecular weight is required to overcome the network defects introduced by chain ends.[17] The bacterial polyesters used have substantially lower molecular weights, approximately 80,000 g/mol. At this time the bacteria cannot be coaxed to alter the molecular weight of the polymers produced. However, one end of each polymer chain terminates with a hydroxyl group. The reaction of the polymer with a diisocyanate would double the molecular weight. Perhaps this higher molecular weight polymer would exhibit superior mechanical properties after peroxide crosslinking.

Sulfur crosslinking was also successful in producing a gel, reducing or eliminating crystallinity, and almost eliminating tensile set. The tensile properties of the vulcanized materials were dramatically reduced and a soft elastomer was produced. Sulfur crosslinking requires the use of many chemicals which brings up the concern of environmental fate when (and if) the crosslinked material biodegrades.

The use of a tetrafunctional sulfur agent did produce a crosslinked material. However, the material properties were similar to the peroxide cured materials, low tensile strength and low tear resistance. A reaction conducted by a previous researcher which involved epoxidation followed by crosslinking could not be duplicated. The procedure was unsuccessful in creating a gel.

The long term crystallization study discussed in Section 4.3.3 indicated that crystallization is very slow and annealing can also occur over the long term. Will a peroxide crosslinked or sulfur vulcanized film devoid of crystallinity stay crystallite free? PHOU(91/9) which had been sulfur vulcanized with DPTT and BZ was re-evaluated for crystallinity using the impulse technique approximately 11 months after vulcanization. The equilibrium modulus dropped significantly after the material was heated above the melting

point and allowed to cool to room temperature implying that crystallization had occurred over the 11 months. Higher olefin content PHOUs which produced a tighter network upon vulcanization need to be investigated after long times to determine if the higher crosslink density would prevent crystallization from ever occurring.

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CHAPTER 6

BIODEGRADATION

6.1 Background

Biodegradation is a term used often but not often defined. This confusion has led to a mistrust of materials reputed to be biodegradable. At the First International Scientific Consensus of Degradable Materials, environmentally degradable was broken down into three forms of degradation: photodegradable, chemically degradable, and biodegradable.[1] Biodegradable was further defined as disintegratation of plastic material where the primary mechanism is through the action of microorganisms.

A recent article [2] tries to explain the complexities of defining biodegradability and charges that the real concern is environmental fate. The article discusses the ASTM standards that are being established which would outline laboratory test protocols for determining the biodegradability of polymers. A more meaningful term, mineralization, is applied to materials which under the influence of aerobic microorgansims are totally converted to carbon dioxide, water, and salts (plus methane for anaerobic minerialization).

Work to date on evaluating the biodegradability of bacterial polymers has relied heavily on two main methods: development of enzymatic assays and placing the polymers directly in different environments. Biodegradation has been established by monitoring weight loss, deterioration in mechanical properties, microbial colonization, formation of a clear zone [3], and changes in pH [4].

Most work has focused on determining the biodegradability on the more readily available PHB and PHB/HV polymers. Several bacteria have been identified that excrete PHB depolymerase which can be concentrated for the development of laboratory assays.[5,6]

PHB and PHB/HV have been placed in a wide range of environments have been found to biodegrade in anaerobic sewage, well watered soil, sea and lake water sediments, aerobic sewage, leaf compost, the rumen of cattle, sea and lake water, in vivo (subcutaneously or intramuscularly) and in vitro with negligible biodegradation in moist air. [7, 8] Good reviews on the biodegradation studies on PHB and PHB/HV have been written. [9, 10] One reviewer comments on the expected changes in biodegradation rates with poly-β-hydroyalkanoates such as PHO. The more hydrophobic nature of the polymer due to the long alkyl pendant groups would most likely extend the mineralization time.

The number of studies conducted on PHO to date is rather limited mainly because of the scarcity of material. An early study on the biodegradability of PHO showed that PHO was not degraded by the depolymerase known to degrade PHB.[11] A recent study has identified 26 bacteria from various soils, lake water, and activated sludge that used PHO as their sole carbon source. [12] One microorganism from the study was identified as *P. fluorescens* and was found to excrete a PHO depolymerase into the culture media. The extracellular PHO depolymerase was isolated and characterized. Interestingly, this enzyme did not degrade PHB/HV.

Studies on the mechanism of PHB or PHB/HV biodegradation can shed light on the general requirements for biodegradation of PHAs. The chiral backbone of the PHA is believed to be essential for enzymatic attachment and attack because enzymes are also chiral. However one study has showed that biodegradation did occur on a 77% [R] atactic and 50% [R] syndiotactic PHB. [4] The study mainly dealt with initial biodegradation rates and did not quantitatively determine if all the non-natural [S] stereoisomer was completely mineralized.

The effect of crystallinity on the rate of biodegradation has been the subject of several papers. [4, 13, 14] The results indicated that the amorphous regions were more quickly degraded by the microorganisms. Also large surface area increased the rate of degradation.[15] PHO and long pendant group PHAs generally have a much lower degree

of crystallinity, typically 35% compared to either PHB at 85% or PHB/HV which can be reduced to 60% crystallinity. PHO may exhibit a faster degradation rate because of the low level of crystallinity once environments which degrade the polymer are established.

A joint study with the Microbiology Department has been initiated to investigate the biodegradation of PHO. Establishing the environment(s) and conditions for PHO biodegradation is an essential part of the material investigation.

Both field (leaf compost and sewage) and laboratory (enrichment and culture) studies have been conducted to determine the conditions required for the biodegradation of PHO. PHO crosslinked with lauroyl peroxide was also included in the leaf compost study.

An enrichment culture is an aqueous growth media (Minimal Basal Medium [MBM] and vitamins) containing a film sample of PHO that is inoculated with a sample from an ecosystem that contains a variety of microorganisms. Subsequent transference of a sample of this media to fresh media and PHO film eventually eliminates all carbon sources except for the PHO sample. The new culture will flourish only if organisms capable of using PHO as a carbon source are present. Both anaerobic and aerobic enrichment cultures were studied. Several aqueous cultures (MBM with vitamins) inoculated with fungal isolates were also tested. All studies were conducted by two colleagues within our reseach group and one colleague in the Microbiology Department at the University of Massachusetts, Amherst.

6.2 Experimental

6.2.1 Leaf Compost

Drs. David Gilmore and Anton George conducted a field study for biodegradation using a Muncipal Leaf Compost Facility located in Springfield, Massachusetts. Leaf

mounds or windrows, approximatley 30 m long by 3 m wide by 2 m high are formed from collected leaf waste in autumn. The windrows are aerated by turning the piles approximately every 2 weeks with machinery. By May of the following year, the leaves have turned into soil and the experiments were ended. The temperature of the windrows was measured and recorded monthly. Approximately 0.5 mm thick film samples of PHO and PHO crosslinked with roughly 5 weight % lauroyl peroxide were attached to wooden stakes along with PHB and PHB/HV samples and placed in the leaf windrows. The stakes were removed during aeration of the windrows. Samples were removed monthly for evaluation. Control samples were incubated in sterile leaves at 55 °C. Weight loss was the primary means of determining biodegradation. Microbial colonization was used as another sign of biodegradation.

6.2.2 Sewage Sludge

Dr. David Gilmore conducted a field study for biodegradation using activated sewage sludge located in a nearby municipal facility. Film samples approximately 0.5 mm thick of PHO were attached to wooden stakes which were hung using nylon rope into the first aeration area of the sewage treatment plant. PHB and PHB/HV samples were also included in the study. Control samples were incubated in sterile sewage at 10 to 20 °C. Weight loss was the means of determining biodegradation.

6.2.3 Enrichment Cultures

Both anaerobic and aerobic enrichment cultures were tested. All aqueous media consisted of 50 ml minimal basal media, 0.25 ml vitamins, 50 mg PHO, and inoculm. For the anaerobic cultures, the head space consisted of 80% nitrogen and 20% carbon dioxide.

anaerobic inocula:

- muck from Hawley Bog, MA
- muck from Hardwick Bog, MA
- muck from a Winogradsky column, standard lab anaerobic inoculum

The cultures were incubated at 30 °C in the dark. After 2 weeks, samples from the Winogradsky column were transferred into 4 fresh MBM, vitamin, and PHO containing flasks and grown under the same conditions.

aerobic incocula:

- garden compost
- forest soil

The cultures were incubated at 37 °C on a slow shaker. After one month, samples from each flask were transferred to two new flasks with fresh MBM, vitamins, and PHO and grown under the same conditions. After one more month, samples were transferred again to new MBM, vitamin, and PHO containing flasks. The cultures were incubated at 30 °C on a slow shaker.

6.2.4 Fungal Cultures

To insure no other organisms were present except for the inoculum, the following steps were taken.

1) flasks and MBM autoclaved
2) added pre-weighed PHO
3) added vitamins
4) inoculated
5) incubated on slow shaker at 37 °C

The PHO was either presterilized with a 70% ethanol soak for 15 minutes then dried in a sterile petri dish or after addition of the PHO, the entire flask contents were heat treated at 55 °C for 30 minutes.

Fungal inoculi, types of penicillum, were designated T3A, M2A, and G1. These fungi had been shown to degrade PHB and PHB/HV. Two flasks of each were inoculated. After one month, the protein content and PHO weights were determined. Controls were included in this study and consisted of uninoculated PHO, MBM, and vitamins.

Protein content was evaluated as a means to determine if the fungi were flourishing while polymer weight loss was used to monitor biodegradation. Two different methods were used to separate the PHO from the liquid media for evaluation. For one method, the liquid was poured off and measured. The PHO was sonicated twice for 2 minutes each time to break-up and remove any organisms then dried and re-weighed. The other method consisted of combining equal volumes of media and chloroform in a separatory funnel. When the polymer had dissolved, the organic layer was drained into a pre-weighed aluminum weigh pan, the solvent allowed to evaporate, and the polymer weight determined. The MBM/protein layer was collected in a test tube and a protein assay conducted using a colorimetric technique.

6.3 Results and Discussion

6.3.1 Leaf Compost

The leaf windrows averaged approximately 45 °C to 55 °C. The PHO samples appeared to have melted and soaked into the wooden stakes used to hold the samples. No determination of weight loss could be made. The peroxide crosslinked PHO became sticky in the leaf compost. Leaf material became imbedded in the samples and could not

be removed with washing. Weight loss could not be determined for the PHO samples. By comparison, PHB/HV had nearly disintegrated after 6 months.

6.3.2 Sewage Sludge

PHO was submerged in the activated sewage sludge for several months. No weight loss was observed. The material properties did not appear to alter during the course of the experiment. By comparison, a 65% weight loss was reported for PHB/HV after 150 days of exposure.

6.3.3 Enrichment Cultures

The anaerobic enrichment cultures showed little or no growth on PHO.

After 6 weeks, two of the four aerobic enrichment cultures showed some growth, the liquid was cloudy. Samples were transferred to two new flasks and are presently incubating at 30 °C on a slow shaker.

6.3.4 Fungal Cultures

After one week, growth was observed in all flasks. A gray-greenish and/or silver fuzz was noted on the PHO samples. Also growth clumps were floating in the liquid.

The results of the PHO weight and protein content analysis are recored in Table 6.1.

Table 6.1 Summary of biodegradation study on PHO using fungal cultures.

Fungual Culture	Separation Method	Original Weight of PHO (mg)	Change in Weight (mg)	PHO % Weight Loss	Protein (g/flask)
T3A	sonication	53.0	0.7	1.3	1180
T3A	$CHCl_3$				430
M2A	sonication	52.1	3.4	6.5	1430
M2A	$CHCl_3$				----
G1	sonication	49.8	3.7	7.4	840
G1	$CHCl_3$				750
control 1	$CHCl_3$			3.5	0
control 2	$CHCl_3$			1.8	0

6.4 Conclusions

With the leaf windrows averaging 55 °C, it is not surprising that the PHO melted or that the peroxide crosslinked PHO became sticky. Relying on weight loss determinations for confirmation of PHO biodegradation does not appear feasible using leaf composting facilities.

Activated sewage sludge does not appear to have the required microorganisms to degrade PHO. However, another explanation could be that the time required for biodegradation of PHO may be longer than the length of the experiment. Microorganisms require an aqueous environment to flourish. The biodegradation of PHO would be expected to be slower than for PHB or PHB/HV because PHO has a more hydrophobic

nature due to the longer alkyl side chains. [9] The use of activated sewage sludge may require a very long time to establish whether PHO is biodegradable in that environment.

The enrichment culture work is continuing. Growth appears to be occurring in the aerobic systems under evaluation.

The fungal cultures appear the most promising. The results did show weight loss for PHO in the range of 1.3% to 7.4%. However, the controls also showed weight loss of 1.8% to 3.5% presumably due to the separation technique. The control weight loss values are very close to the weight losses reported for PHO so firm conclusions cannot be drawn at this point. The use of the chloroform separation technique will not be possible with crosslinked PHO or PHOU because the material will not dissolve. A separation technique designed for both crosslinked and non-crosslinked material should be developed.

The use of fungi that were shown to degrade PHB and PHB/HV may be dubious. The bacteria *P. fluorescens* which could degrade PHO could not degrade PHB/HV. [12] Investigating other fungi or obtaining other organisms known to degrade PHO appears more appropriate. One species, *P. fluorescens*, has already been identified in the literature. Another bacteria, *Xanthomonas malta*, has been identified as excreting a PHO depolymerase and has been obtained for evaluation. [16]

Determining environments which degrade PHO requires further investigation. Establishing the conditions and the degradation characteristics of PHO is essential before the effect of chemical crosslinking can be assessed. Because sulfur vulcanization and crosslinking with vinyl specific peroxides presumably limits the sites of crosslinking to the terminal pendant olefin group, the chiral backbone would remain unperturbed. Biodegradation, if it indeed relies on the chiral backbone, may be least effected by these types of crosslinking chemistries. Interestingly, a preliminary evaluation of the enzymatic degradation of peroxide crosslinked PHB/HV was conducted. The results indicated that

peroxide crosslinking had not affected the biodegradability of the material. [17] Further investigations are required before any conclusions can be drawn.

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CHAPTER 7

CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

Waste management of polymers has sparked interest in biodegradable polymers. Bacterial polyesters appear to be truly biodegradable or minerializable by the action of microorganisms in several environments. But if these materials were made part of the consumer market, where would these materials biodegrade? The main disposal system used today, the landfill, has been shown to be lacking in sufficient microbial activity to enable significant degradation of disposed items.[1] Archeological type digs of old landfills have found food that is still recognizable and newspapers that are still intact and readable.[1] The landfill system appears to preserve the waste for all time.

This new understanding about landfills and the fact that space is running out in the depositories has started generating ideas on ways to handle waste. These main ideas include:

- source reduction
- recycling
- composting
- incineration
- depolymerization

Each method has advangates and disadvantages and is beyond the scope of this work to explore the options thoroughly.

With the bacterial polyesters in mind, the idea of bioreactors may be viable. This idea is a combination of composting, depolymerization, and recycling. The disposed

biomaterials could be used as a feed stock for bacteria which would break down the waste and generate new polymers.

Making consumer products out of the bacterial polymers has begun with a shampoo bottle being marketed in Europe. Biosynthesizing, extracting and purifying the polymers from bacteria is still an expensive process. A combination of understanding the metabolic pathway of the bacteria and development of more sophisticated biotechnology tools will improve the situation. Ultimately, the use of genetic engineering will undoubtably be a key to decreasing the cost of these biopolymers where the amount of polymer accumulated, the prevention of internal degradation or utilization of the accumulated polymer, and easier extraction and purification techniques could be envisoned.

With all this in mind, the first step is still the basic exploration into the types of polyesters bacteria can produce or be coaxed into producing. An understanding of the structure property relationship of the new polymers is needed to determine if the material properties would fill a need. The goal of this dissertation was to understand why PHAs from *Pseudomonas oleovorans* exhibited elastic material properties and quantitatively measure the material properties. In trying to improve the elastic response, chemical modification of several PHAs was accomplished. These samples raised the question as to whether biodegradation will still occur after chemical modification. The answer is unknown at this time but crosslinked samples are now available for testing.

7.1.1 Thermoplastic Elastomeric PHO

PHO is a thermoplastic elastomer with a random copolymer chemical structure which is unique for a thermoplastic elastomer. The natural source of the polymer, bacteria, endows the chemical structure with an absolute [R] stereochemistry at every chiral carbon resulting in a 100% isotactic polymer. The tacticity allows crystallization to

take place but because the metabolism of the bacteria produces a copolymer even when grown on one carbon source, the level of crystallinity is low. The low level of crystallinity together with a glass transition temperature well below room temperature results in elastic behavior at room temperature with the crystalline regions acting as the physical crosslinks. The network structure is similar to a segmented polyurethanne where the molecular weight between crosslinks is approximately 4000 g/mol and each chain is involved with approximately 10 to 20 crosslink sites.

The elastic response of PHO decreased as the amount of strain increased due to deformation induced changes in the crystalline regions and overall crystallinity of the material. However, the material properties of PHO compared well with examples of all six classes of commercial thermoplastic elastomers.

The crystallization rate of PHO is very slow requiring at least 10 hours even at the fastest crystallization temperature before the material forms a film with mechanical integrity. Attempts to increase the rate of crystallization through the incorporation of possible nucleating agents was unsuccessful.

The crystallization temperature influences the mechanical properties significantly. Regulation of the material properties can be achieved through the use of specific crystallization temperatures. It is unknown if the material will remain elastic since crystallization and annealing are continuing at room temperature. However, the material still exhibited an elastomeric shaped stress strain curve after crystallization and annealing for over 2 years.

7.1.2 Crosslinked PHO and PHOU

Pseudomonas oleovorans was capable of producing a polymer with a predictable olefin content by controlling the mix of different carbon sources during biosynthesis. These new polymers enabled testing of several crosslinking chemistries. Peroxide

crosslinking and sulfur vulcanization were studied extensively. The elastic response of the bacterially polyesters were improved by both types of chemical crosslinking. Peroxide crosslinking in general produced a 'cheesy' material which exhibited little tensile strength or tear resistance. Sulfur crosslinking produced a material with excellent elastic response, less than 5% tensile set was measured after 200% elongation. However, the tensile strength and tear resistance were much lower than in the unmodified polymer. The effect the crosslinks may have on the biodegradability of the material is unknown at this time. Establishing environments where PHO degrades must first be accomplished.

7.2 Future Work

Many questions have been raised by this investigation into the strucutre property relationships of PHO and PHOU. Several studies have been proposed below to answer some of these questions and to futher extend the knowledge about these polymers.

- PHO when stretched after crystallizing for many months exhibited an unusual melting endotherm. Another lower melting temperature peak developed as the strain was increased. Determining why this new peak formed would be interesting. Perhaps a new crystal structure was induced by the deformation.

- A continued investigation into long term annealing effects on PHO seem appropriate. Perhaps crystallinity will develop to such an extent that the material will no longer behave as an elastomer.

- Developing a correlation between degree of crystallinity (through WAXS studies and heat of fusion (DSC studies) would be informative and allow the application of the Avrami model of crystallization kinetics.

- Determination of the average crystallite size through SAXS studies would enable a better determination of the network structure. In addition, changes to the crystallite size after deformation could be directly measured.

- The peroxide crosslinked materials showed poor mechanical integrity. The use of fillers such as fumed silica, may be a way to improve the tensile strength and tear resistance.

- The poor mechanical properties of PHO and PHOU after the random crosslinking reaction of peroxide may be due to the low molecular weight of the starting material. Doubling the molecular weight could be achieved with a diisocynanate since one end of each chain terminates in a hydroxyl group.

- Several other rubber crosslinking chemistries could be investigated including phenolic resins, quinone derivatives, maleimides, and metal oxides (ZnO & MgO).

- Environments that biodegrade PHO and PHOU need to be established along with the degradation characteristics (how long, environmental fate). The influence that chemical crosslinking has on the biodegradation of PHO and PHOU then needs to be established. Procedures are being developed to accurately determine biodegradation through a carbon balance methodology using lab composting equipment. These test procedures and equipment are all outlined in the new ASTM standard on biodegradation. PHO film samples have been prepared for inclusion in a study.

- If chemical crosslinking does not prevent biodegradation, experimenting with other PHAs which do not crystallize would seem pertinent. Relying on crosslinking to disrupt crystallinity would no longer be a concern.

- A biodegradable thermoplastic elastomer with an improved elastic response would result from a triblock of different PHAs. A hard block from a highly crystalline, higher melting temperature PHA such as PHB coupled with a soft block of a PHA which does not crystallize and has a very low glass transition temperature.

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