THERMOPHYSICAL, INTERFACIAL AND DECOMPOSITION ANALYSES OF POLYHYDROXYALKANOATES INTRODUCED AGAINST ORGANIC AND INORGANIC SURFACES

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The development of a “cradle-to-cradle” mindset with both material performance during utilization and end of life disposal is a critical need for both ecological and economic considerations. The main limitation to the use of the biopolymers is their mechanical properties. Reinforcements are therefore a good alternative but disposal concerns then arise. Thus the objective of this dissertation is to investigate a biopolymer nanocomposite where the filler is a synthetically prepared layer double hydroxide (inorganic interface); and a biopolymer paper (organic interface) based coating or laminate. The underlying issues driving performance are the packing density of the biopolymer and the interaction with the reinforcement. Since the polyhydroxyalkanoates or PHAs (the biopolymers used for the manufacture of the nanocomposites and coatings) are semicrystalline materials, the glass transition was investigated using dynamic mechanical analysis (DMA) and dielectric spectroscopy (DES), whereas the melt crystallization, cold crystallization and melting points were investigated using differential scanning calorimetry (DSC). Fourier transform infrared (FTIR) spectroscopy was used to estimate crystallinity in the coated material given the low thermal mass of the PHA in the PHA coating. The significant enhancement of the crystallization rate in the PHA nanocomposite was probed using DSC and polarized optical microscopy (POM) and
analyzed using Avrami and Lauritzen-Hoffman models. Both composites showed a significant improvement in the mechanical performance obtained by DMA, tensile and impact testing. The degradation and decomposition of the two composites were investigated in low microbial activity soil for the cellulose paper (to slow down the degradation rate that occurs in compost) and in compost. An in-house system according to the American Society for Testing and Materials ASTM D-98 (2003) was engineered. Soil decomposition showed that PHA coating into and onto the cellulose paper can be considered to be a useful method for the assessment of the degradability of the biopolymer. PHA nanocomposite showed enhanced compostability.
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CHAPTER 1

SCOPE OF THE DISSERTATION

Biopolymers are employed in a variety of applications such as therapeutic aids, medicines, coatings, food products, packaging and agricultural materials. Although a range of applications have been developed for biopolymers, their industrial application is unfortunately limited to the design of low cost sacrificial materials [1]. The main reason for the restrictive development of biopolymers is their weak properties (mechanical, thermal stability, slow crystallization rate and gas barrier properties) and high sensitivity to environmental variables such as temperature and humidity [1,2]. There is a critical need to evaluate and improve the properties of the biopolymers to make them fully competitive with common non-degradable thermoplastics [3]. Therefore the main question is how to retain the properties when the biopolymers are used while having them decompose at the end of their utilization. Efforts to improve the properties of biopolymers have included dispersion of nanoparticles into the host matrix to form a biopolymer nanocomposite or manufacture of a biopolymer coating by introducing an organic substrate against the host matrix. The general premise is that biopolymerer nanocomposite and coating show the remarkable advantage of displaying biocompatibility, biodegradability and, in some cases, functional properties provided by either the renewable component, the inorganic moiety or the substrate.
1.1 Objectives

The knowledge and improvement of the properties of biopolymer nanocomposites and coatings are a critical need to guaranty high performance during their lifetime. This implies gaining better understanding of the structure-properties relationship. In general, interfaces in biopolymer nanocomposites and coatings play an important role in controlling the properties. It is thus important to study the impact of interface region in biopolymers nanocomposites and coatings on the effective properties in order to optimize these properties [1].

Environmental degradation can involve enzymatic pathways and microorganisms such as bacteria and fungi, or chemical pathways such as hydrolysis. It is important that biopolymers have an adequate life span for applications - their biodegradability makes them ideal for use in resorbable medical products such as sutures, in short-term packaging applications for fast foods and fresh groceries, and for sanitary uses. The degradation of biodegradable polymers has been investigated for many years [4,5]. Several microorganisms have been detected to degrade biopolymers [6,7]. Although their biodegradation have been reported, it is however very useful during the development of biodegradable polymers to access extensive knowledge for their degradability, particularly when they are introduced against inorganic (synthetic clay) and organic (cellulose paper substrate) surfaces. It is very important to consider microbial degradation of these biopolymers in order to understand what is necessary for biodegradation and the mechanism involved. This requires understanding of the interactions between biopolymers and microorganisms and the biochemical changes involved. Therefore, the
The investigation of the improvement of the degradability of these biopolymers is of great importance.

The objective of this dissertation is to explore the properties of two biopolyesters poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] by themselves and when introduced against inorganic (synthetic clay) and organic (cellulose paper substrate) surfaces through an understanding of thermophysical, interfacial and decomposition analyses.

Synthetic clay which is layered double hydroxide or LDH was dispersed in P(3HB-co-3HV) to form biopolymer nanocomposite. A biopolymer coating was manufactured by introducing P(3HB-co-4HB) against cellulose Kraft paper. P(3HB-co-3HV) and P(3HB-co-4HB) are members of polyhydroxyalkanoates or PHAs family.

The thermophysical properties of P(3HB-co-3HV) and P(3HB-co-4HB) in the composites were investigated. The interfacial area between P(3HB-co-3HV) and LDH on one hand, between P(3HB-co-4HB) and cellulose Kraft paper on other hand was investigated. The degradability of both biodegradable polymers by themselves and when introduced against LDH and cellulose Kraft paper was evaluated.

The dissertation is organized as follows. Chapter 2 provides an overview of PHAs, PHAs-based nanocomposites and PHAs-based paper coatings, the interfacial area and the biodegradation of PHAs. We then first lay down the properties of the P(3HB-co-3HV) nanocomposites. Chapter 3 describes the effect of the addition of LDH on the structure, mechanical and thermal properties of P(3HB-co-3HV). The annihilation of the
cold crystallization peak and development of a crystallization peak in the P(3HB-co-3HV) prompted us to examine the crystallization kinetics. These are probed in chapter 4 by means of Avrami and Lauritzen-Hoffmann models. The free volume and packing effects as well as frequency dependent relaxation is examined in chapter 5 using dynamic mechanical analysis (DMA) and dielectric spectroscopy (DES). We then examine the compostability potential of P(3HB-co-3HV) nanocomposite in chapter 6. The design of a composting system and the evaluation of the biodegradability of P(3HB-co-3HV) by itself and when introduced against LDH in a thermophilic composting environment are both covered. Having established the structure-property relationships in P(3HB-co-3HV) reinforced through nanocomposites, we then examine the results in the organic P(3HB-co-4HB) coating or laminate. The first challenge with using cellulose paper with its inherent low thermal stability and porous absorptive surface is to develop a reliable process methodology. Thus, in chapter 7, the thermophysical and mechanical performance of the biodegradable coating made of P(3HB-co-4HB) and cellulose Kraft paper are discussed using both solvent and non-solvent processes. The coating as well as the biopolymer and the cellulose paper were then examined for decomposition mechanisms. First, in order to probe the gradual decomposition and ascertain the correlation between mechanical and structural breakdown in the coating, we utilize a low microbial activity soil medium. Thus, the evaluation of the degradability of P(3HB-co-4HB) by itself and when coated into and on to cellulose Kraft paper using soil medium operating at room temperature, having low microbial activity and low relative humidity; was studied in chapter 8. In order to bridge these results with those of the inorganic
nanocomposite system, we examined the compostability in chapter 9. Here a comparative study of 2 weeks degradation in soil and compost of P(3HB-\textit{co}-4HB) is analyzed. The overarching conclusions that provide the big picture of how organic and inorganic reinforcements influence PHAs [P(3HB-\textit{co}-3HV) and P(3HB-\textit{co}-4HB)] performance are provided in chapter 10.
1.2 References


CHAPTER 2
BACKGROUND

In this chapter, four main sections are reviewed. The first section which provides the extensive overview of polyhydroxyalkanoates is followed by the second section in which polyhydroxyalkanoates-based nanocomposites and coatings are discussed. The interfacial region which drives the structure and the properties of polyhydroxyalkanoates-based nanocomposites and coatings is discussed in the third section. The mechanism by which molecular structure of polyhydroxyalkanoates breaks down through degradation is discussed in the last section.

2.1 Polyhydroxyalkanoates or PHAs

PHAs are a family of linear polyesters, synthesized by a wide variety of bacteria through the fermentation of sugars, lipids, alkanes, alkenes and alkanoic acids. They are found as discrete cytoplasmic inclusions in bacterial cells. Once extracted from the cells, PHAs exhibit thermoplastic and elastomeric properties [1]. The chemical structure of PHAs is shown in Figure 2.1. They are recyclable, are natural materials and can be easily degraded to carbon dioxide and water. They are excellent replacements for petroleum-based plastics in terms of processability, physical characteristics and biodegradability. In addition, they are biocompatible and have several medical applications [1-3]. Their molecular mass is in the range of 50,000-1,000,000 Daltons and varies with the PHAs.
producer. All the monomeric units of PHAs are enantiomerically pure and in the R-configuration [4].

\[
\begin{align*}
\text{R} & \quad \text{O} & \quad \text{CH} & \quad \left( \text{CH}_2 \right)_{n} & \quad \text{C} & \quad \left( \text{CH}_2 \right)_{m}
\end{align*}
\]

Figure 2.1 General structure of PHAs: R=alkyl groups C1-C13; n=1-4; m=100-30,000.

2.1.1 History

In 1923, Maurice Lemoigne, a French microbiologist at the Institute Pasteur, demonstrated that aerobic spore-forming bacillus formed quantities of 3-hydroxybutyric acid in anaerobic suspensions [1]. He proceeded to investigate further and was successful in estimating the quantity of 3-hydroxybutyric acid formed. Finally, in 1927, he was able to extract a substance from bacillus using chloroform and proved that the material was a polymer of 3-hydroxybutyric acid known as poly(3-hydroxybutyrate) or P(3HB) [1,5]. However, it was not until the early 1960s that the production of P(3HB) was explored on a commercial scale. Baptist and Weber at W.R. Grace & Co. (Columbia, MD) were the pioneers in developing P(3HB). Their work earned them several patents to produce and isolate P(3HB). They began using this biopolymer to fabricate products such as sutures and prosthetic devices. However, their efforts had to be terminated as the fermentation yields were low and the biopolymer was tainted with bacterial residues.

In 1970s, the petroleum crisis provided a boost in the quest for alternative plastics [1]. Imperial Chemical Industries ICI (UK) was able to formulate conditions for the bacteria
*Alcaligenes latus* to produce P(3HB) up to 70% of its dry cell weight. However, P(3HB) material was brittle, with poor mechanical properties and high production costs. Therefore, after the oil crisis subsided, the development of P(3HB) as the future material came to a temporary standstill [1,6]. In the meantime, ICI produced a novel biopolymer named BIOPOL™ – a copolymer of 3-hydroxybutyrate (3HB) and 3-hydroxyvalerate (3HV). BIOPOL™ had improved properties, such as lower crystallinity and more elasticity than P(3HB) [1,7]. ICI split in June 1993 and the Zeneca BioProducts branch of ICI started dealing with BIOPOL™. Zeneca then sold their BIOPOL™ technology to Monsanto (St Louis, MO) in April 1996. Metabolix Inc. (Cambridge, MA) obtained the license from Monsanto in 1998 [1,8]. In 1998, following collaboration between Metabolix Inc. and Children’s Hospital, Boston, a new spin off company named Tepha Inc. (Lexington, MA) was initiated. Tepha leads the technology in developing medical devices from biologically derived biodegradable polymers including bioengineered heart valves, surgical sutures, meshes and orthopedic fixtures [1].

2.1.2 Biosynthesis of PHAs

A wide variety of bacteria both gram-negative and gram-positive such as *Pseudomonas, Bacillus, Ralstonia, Aeromonas, Rhodobacter* and certain archaea, especially members of the halobactericeae, like *Haloferax sulfurifontis*, synthesize polyhydroxyalkanoates. Also, marine prokaryotes, both bacteria and archaea, produce PHAs that have remarkable commercial application. PHAs accumulate intracellularly in these microorganisms to levels as high as 90% of dry cell weight under conditions of
nutrient stress [1,4, 9]. They function as carbon and energy storage materials; and are present in the cells as insoluble granules in the cytoplasm [1,10]. The above facts show the widespread occurrence of PHAs-producing microorganisms in the environment. A representative image showing the accumulation of PHAs in the cells is displayed in Figure 2.2.

![Accumulation of PHA granules in Rhodobacter shaeroides](http://www.unil.ch/webdav/site/dbmv/shared/Poiriers/Fig1.jpg)

Figure 2.2  A representative image showing the accumulation of PHAs in the cells (image obtained from: http://www.unil.ch/webdav/site/dbmv/shared/Poiriers/Fig1.jpg).

Polyhydroxyalkanoates are divided into two groups known as short-chain-length (SCL) PHAs or PHA\textsubscript{SCL} and medium-chain-length (MCL) PHAs or PHA\textsubscript{MCL} based on the number of constituent carbon atoms in their monomer units. PHA\textsubscript{SCL} consist of monomers with 3-5 carbon atoms while PHA\textsubscript{MCL} contain 6-14 carbon atoms [1, 4, 10].
PHA\textsubscript{SCL} are stiff and brittle with high degree of crystallinity whereas PHA\textsubscript{MCL} are flexible, have low crystallinity, tensile strength and melting point [1, 11, 12].

The supply of the substrate monomer and the polymerization of this monomer are the two main steps involved in the biosynthesis of PHAs. The PHA synthesized by a microbe is dependent on the carbon source used. Carbon sources have been classified as “related” and “unrelated” sources. Carbon from “related” source gives rise to monomers that are structurally identical to that particular carbon source whereas carbon from “unrelated” source generates monomers that are completely different from the given carbon source. The cause of this difference can be explained from the metabolic pathways operating in the microorganism [1]. There are three well-known PHA biosynthetic pathways as reported in Figure 2.3 [1,13].

Pathway I used by \textit{Cupriavidus necator} (previously known as \textit{Wauterisia eutropha} or \textit{Ralstonia eutrophus}) is the best known among the PHA biosynthetic pathways. The classical poly(3-hydroxybutyrate) or P(3HB) pathway consist of reactions catalyzed by three distinct enzymes encoded by \textit{phaA}, \textit{phaB} and \textit{phaC}. In this pathway, 3-hydroxybutyrate or 3-HB monomers are generated by the condensation of two acetyl-CoA molecules, from the tricarboxylic acid (TCA) cycle to form acetoacetyl-CoA by the enzyme $\beta$-ketothiolase (encoded by \textit{phaA}) [1,14,15]. Then acetoacetyl-CoA reductase (encoded by \textit{phaB}) acts on acetoacetyl-CoA to form 3-hydroxybutyryl-CoA. Finally, the PHA synthase enzyme (encoded by \textit{phaC}) catalyses the polymerization via esterification of 3-hydroxybutyryl-CoA into poly(3-hydroxybutyrate) or P(3HB).
Pathways involved in fatty acid metabolism generate different hydroxyalkanoate monomers utilized in PHA biosynthesis [1,16]. The fatty acid β-oxidation pathway (Pathway II) generates substrates that can be polymerized by the PHA synthases of Pseudomonads belonging to the ribosomal RNA homology group I such as Pseudomonas aeruginosa. These microbes can synthesise PHA<sub>MCL</sub> from various alkanes, alkenes and alkanoates. The monomer composition is related to the carbon source used. In Aeromonas caviae, the β-oxidation intermediate, trans-2-enoyl-CoA is converted to (R)-hydroxyacyl-CoA by a (R)-specific enoyl-CoA hydratase [1,17,18].

Huijberts et al. showed that PHA synthases (such as that of Pseudomonas putida) that catalyze PHA synthesis from fatty acids are also responsible for PHA synthesis from glucose [19]. The intermediates for this channel of synthesis were obtained from the fatty acid de novo biosynthetic pathway (Pathway III). Pathway III is of significant interest because it helps generate monomers for PHA synthesis from structurally unrelated and simple, inexpensive carbon sources such as glucose, sucrose and fructose. The (R)-3-hydroxyacyl intermediates from the fatty acid biosynthetic pathway are converted from their acyl carrier protein (ACP) form to the CoA form by the enzyme acyl-ACP-CoA transacylase (encoded by phaG). This enzyme is the key link between fatty acid synthesis and PHA biosynthesis [1,20].
Figure 2.3 PHA biosynthetic pathways. Reproduced from S. Philip et al. [1].
2.1.3 Production of PHAs by Genetically Engineered Plants

As the production of PHAs in bacteria is expensive, its production in plants may be an attractive alternative. Plants are ideal candidates for synthesizing PHAs because they have been shown to be efficient producers of biomass compared to bacteria [21]. For example, *Ralstonia eutropha* and potato tuber accumulate up to 85% dry weight of their commodities, P(3HB) and starch, respectively. One hectare of potatoes can produce about 20,000 kg of starch at about USD 0.2/kg compared to bacterially-produced P(3HB) at USD 15/kg [21,22]. In addition to being highly productive, genetically engineered plants have demonstrated the ability to produce foreign proteins that are biologically active such as antibodies [21,23]. Plants are also the supplier of carbon sources through photosynthesis, so it is much more efficient to use plants as a direct carbon source for PHA production by eliminating the carbon input cost. In fact, the cost of producing PHAs in plants may even become comparable to petroleum-based plastics because of no cost of feedstock and fermentation settings. It was estimated that PHAs could potentially be produced at a cost of USD 0.2-0.5/kg if plants could produce to a level of 20-40% dry weight [21, 24, 25]. Another advantage to using plants is the presence in plant cells of acetyl-CoA, the precursor to the PHAs biosynthetic pathway. In general, the production of P(3HB) from plant’s acetyl-CoA required genetic engineering of *phbA*, *phbB* and *phbC* genes of *Ralstonia eutropha*, whereas, the *bktB*, *phbB*, *phbC* genes of *Ralstonia eutropha* and *ilvA* gene of *Escherichia coli* were required for poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or P(3HB-co-3HV) production from plant’s acetyl-CoA and propionyl-CoA. In order to locate the expression of these genes in plant cells, at least two
important considerations has to be given to the most appropriate subcellular compartment, the presence of acetyl-CoA and the available space for storage of P(3HB) and P(3HB-co-3HV). In plant cells, acetyl-CoA is present in the cytoplasm, plastid, mitochondria and peroxisome. Thus, the synthesis of PHAs could be achieved in any of these compartments. However, considering the high storage space available, the cytoplasm and plastids seem to be the most appropriate subcellular compartments. Cytoplasm is normally occupied by oils bodies and plastids which act as storage tissues with a large storage capacity to store a high amount of starch and lipids.

2.2 PHAs-based Nanocomposites and Based Paper Coatings

2.2.1 PHAs-based Nanocomposites

Biopolymer nanocomposites can be considered as a new generation of hybrid nanostructured materials in the frontier of materials science, life science and nanotechnology [26]. “Bionanocomposites” has become a common term assigned to those nanocomposites involving biopolymers in combination with inorganic fillers (nanoparticles) showing at least one dimension on the nanometer scale. They are very promising materials since they show improved properties with preservation of the material biodegradability and biocompatibility. They are mainly intended to biomedical applications, and different short-term applications (e.g. packaging, agriculture or hygiene devices). They therefore represent a strong and an emerging solution for improved and eco-friendly materials.
Several reasons are known to explain the resurgence of interest in biopolymer nanocomposites. The first reason is that nanoscale particles often have properties that are different from the bulk properties of the same material [27]. These features of nanoparticles provide an opportunity for creating biopolymer nanocomposites with unique properties. The second reason is that nanoscale particles are small defects. Micrometer-scale particles are similar in size to the critical crack size causing early failure [27-29] while nanoparticles are an order of magnitude smaller. This can prevent early failure, leading to bionanocomposites with enhanced ductility and toughness [27, 30, 31]. The third reason is that the large surface area of the nanoparticles causes the bionanocomposites to have a larger volume of interfacial matrix material with properties different from those of the bulk biopolymer [27].

As stated early in the chapter 1, some drawbacks of PHAs, such as brittleness, poor thermal stability, and slow crystallization rate restrict their developments and uses. Therefore, bionanocomposites appear as a possible answer to overcome these problems and to improve different properties (e.g., crystallization). A variety of nanoparticles of precise size and shape have been used as nanofillers in PHAs matrix. They can be classified as layered (e.g. clay), spherical (e.g. silica) and acicular nanoparticles (e.g. carbon nanotubes). Their specific geometrical dimensions, and thus aspect ratios, partly affect the final materials properties. Layered silicate clays offer high surface area, more than 700 m²/g, i.e., huge interface with the biopolymer matrix, which governs the final materials properties [32]. Beside their economical, environmental friendly characteristics, layered silicate clays are well known for their exceptional barrier properties and
reasonably well understood intercalation and exfoliation chemistry [33,34]. Table 2.1 presents the characteristics of some commercial clay.

Table 2.1 Commercial organically modified montmorillonite (OMMT) and their characteristics. Reproduced from Bordes et al.[32].

<table>
<thead>
<tr>
<th>Commercial clays Supplier/trade name/designation</th>
<th>Clay type</th>
<th>Organomodifier’s type</th>
<th>Modifier concentration (meq/100 g)</th>
<th>ΔW&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>d-spacing (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Southern Clay Products (USA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cloisite®Na</td>
<td>CNa</td>
<td>MMT</td>
<td>-</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td>Cloisite®15A</td>
<td>C15A</td>
<td>MMT</td>
<td>N'(Me)&lt;sub&gt;2&lt;/sub&gt;(tallow)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>125</td>
<td>43</td>
</tr>
<tr>
<td>Cloisite®20A</td>
<td>C20A</td>
<td>MMT</td>
<td>N'(Me)&lt;sub&gt;2&lt;/sub&gt;(tallow)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>95</td>
<td>38</td>
</tr>
<tr>
<td>Cloisite®25A</td>
<td>C25A</td>
<td>MMT</td>
<td>N'(Me)&lt;sub&gt;2&lt;/sub&gt;C&lt;sub&gt;14&lt;/sub&gt;(tallow)</td>
<td>95</td>
<td>34</td>
</tr>
<tr>
<td>Cloisite®93A</td>
<td>C93A</td>
<td>MMT</td>
<td>NH'(Me)(tallow)&lt;sub&gt;2&lt;/sub&gt;</td>
<td>90</td>
<td>37.5</td>
</tr>
<tr>
<td>Cloisite®30B</td>
<td>C30B</td>
<td>MMT</td>
<td>N'(Me)(EtOH)&lt;sub&gt;2&lt;/sub&gt;(tallow)</td>
<td>90</td>
<td>30</td>
</tr>
<tr>
<td>Süd-Chemie (Germany)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nanofil®804</td>
<td>N804</td>
<td>MMT</td>
<td>N'(Me)(EtOH)&lt;sub&gt;2&lt;/sub&gt;(tallow)</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Laviosa Chimica Mineraria (Italy)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dellite®LVF</td>
<td>LVF</td>
<td>MMT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dellite®43B</td>
<td>D43B</td>
<td>MMT</td>
<td>N'(Me)(CH&lt;sub&gt;2&lt;/sub&gt;-φ)(tallow)</td>
<td>105</td>
<td>4-6</td>
</tr>
<tr>
<td>CBC Co. (Japan)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somasif</td>
<td>MEE</td>
<td>SFM</td>
<td>N'(Me)(EtOH)&lt;sub&gt;2&lt;/sub&gt;(coco alkyl)</td>
<td></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>MAE</td>
<td>SFM</td>
<td>N'(Me)&lt;sub&gt;2&lt;/sub&gt;(tallow)&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>120</td>
</tr>
</tbody>
</table>

Tallow: ~65% C18; ~30% C16; ~5% C14.

<sup>a</sup> %Weight loss on ignition.

Biopolymer-layered material nanocomposites can, in principle, be formed using three methods [35]. The solvent intercalation method consists in swelling the layered materials in a biopolymer solvent to promote the macromolecules diffusion in the interlayer spacing. In the in-situ intercalation method, the layered materials are swollen in the monomer or monomer solution before polymerization. Finally, the melt intercalation method is based on biopolymer processing in the molten state such as extrusion.

The three methods of preparation lead to biopolymer-layered material nanocomposites with three different types of structure. A microcomposite is obtained
when the layers are still stacked and the biopolymer chains are not intercalated due to poor polymer-layered material affinity. Thus, the nanocomposite presents phase separation. An intercalated nanocomposite is obtained when the biopolymer chains are partially intercalated between the layers. In the intercalated structure, the layers are still stacked but the interlayer spacing has increased. Finally, an exfoliated nanocomposite can be obtained and is characterized by individual and well-dispersed clay platelets into the polymer matrix. In the exfoliated structure, the layered structure does not exist anymore. Figure 2.4 illustrates the different structures of layered material in biopolymers. These different structures can be characterized by transmission electron microscopy (TEM) and wide angle X ray diffraction (WAXD).

For the case called “immiscible nanocomposite” in Figure 2.4, the nanoplatelets exist in aggregates more or less as they were in the pure layered material powder, i.e., no separation of nanoplatelets. Thus, the WAXD scan of the biopolymer nanocomposite is expected to look essentially the same as that obtained for the pure layered material powder; there is no shifting of the X-ray d-spacing. Generally, such scans are made over a low range of angles, 2θ, such that any peaks from a crystalline biopolymer matrix are not seen since they occur at higher angles. For completely exfoliated layered material, no WAXD peak is expected for the nanocomposite since there is no regular spacing of the nanoplatelets and the distances between nanoplatelets would, in any case, be larger than what WAXD scattering can detect [35].

Often WAXD scans of biopolymer nanocomposites show a peak reminiscent of the layered material peak but shifted to lower 2θ or larger d-spacing. The fact that there is
a peak indicates that the nanoplatelets are not exfoliated. The peak shift indicates that the gallery has expanded (larger d-spacing), and it is usually assumed that biopolymer chains have entered or have been intercalated in the gallery. Placing biopolymer chains in such a confined space would involve a significant entropy penalty that presumably must be driven by an energetic attraction between the biopolymer and the layered material [35,36,37].

In general TEM micrographs exhibit dark lines embedded in white domain. The dark lines (also called nanoplatelets) are the sections of the layers and the white domain represents the biopolymer matrix. The thickness, the length and the distance between the nanoplatelets can be determined.

![Illustration of different states of dispersion of layered materials in biopolymer matrix.](image)

Figure 2.4 Illustration of different states of dispersion of layered materials in biopolymer matrix. Reproduced from Paul et al. [35].

The studied PHA/clay nanocomposites are summarized in Table 2.2 and described here after. Maiti et al. [32,38] prepared P(3HB)-based nanocomposites by melt extrusion. P(3HB) was then reinforced using organo modified fluoromicas (MEE and MAE) or montmorillonite (OMMT) containing 2 wt% and up to 4 wt% of clay, respectively. MEE and MAE fluoromicas (see Table 2.1) as well as OMMT modified with octadecylammonium [MMT-NH3+(C18)] were selected. WAXD and TEM revealed
well-ordered intercalated nanocomposites with decreasing d-spacing with the increase of clay content. Dynamic mechanical analyses (DMA) revealed a better reinforcing effect of fluoromica compared to montmorillonite. The storage modulus $E'$ increased with clay content reaching an increment of $+35\%$ with 3.6 wt% of MMT-C$_{18}$, $+33\%$ and $+40\%$ with 2 wt% of MAE and MEE, respectively. These authors explained this behavior by an enhanced degradation in presence of OMMT, due to the presence of Al Lewis acid sites in the inorganic layers which catalyze the ester linkages hydrolysis. This phenomenon does not occur in the case of fluoromica since they are based on magnesium. Nevertheless, recently, Hablot et al. [32,39] reported that P(3HB) enhanced degradation can also be caused by decomposition products of clay organomodifiers which have a catalyzing effect on the thermal or thermomechanical degradation. Eventually, the biodegradation studies also highlighted the difference between montmorillonite and fluoromicas since the initial degradation rate of P(3HB) with [MMT-NH$_3^+$(C$_{18}$)] was higher than with fluoromicas [38]. In this case, the degradation rates have been considerably reduced and even suppressed with MEE.

A similar study on P(3HB)-based nanocomposites was carried out by Lim et al. [32,40]. Nevertheless, they used solvent intercalation route to obtain P(3HB)/Cloisite 25A (C25A) with 3, 6 and 9 wt% of clay content. WAXD data led to the conclusion of intercalated structures, the interlayer distance reaching 35 Å, but no dependence on clay content was observed. These results were confirmed by Fourier transform infra red (FTIR) spectroscopy analyses showing that two distinct different phases coexisted. These structural observations were completed by the thermal stability investigation. Indeed,
thermogravimetric analysis (TGA) results indicated an increase of the onset temperature of weight loss and a decrease of the degradation rate with 3 wt% of C25A. This was attributed to the nanoscale OMMT layers dispersion decreasing the diffusion of volatile decomposition products. At higher clay contents (> 6 wt%), although the onset of thermal degradation did not increase because of the organomodifier’s thermal sensitivity, the bionanocomposites degradation rates decreased due to restricted thermal motion of the polymer chains in the OMMT interlayer.

Researchers were also interested in the development of P(3HB-co-3HV)-based nanocomposites since the P(3HB-co-3HV) presents better properties than P(3HB) and better processability. Choi et al. [32,41] described the microstructure as well as the thermal and mechanical properties of P(3HB-co-3HV)/Cloisite30B (C30B) nanocomposites with low clay content. These materials were prepared by melt intercalation using a Brabender mixer. WAXD and TEM clearly confirmed that intercalated nanostructures were obtained. Such structures were formed due to the strong hydrogen bond interactions between P(3HB-co-3HV) and the hydroxyl groups of the C30B organomodifier. They demonstrated that the nanodispersed organoclay acted as a nucleating agent, increasing the temperature and rate of P(3HB-co-3HV) crystallization. Moreover, differential scanning calorimetry (DSC) thermograms revealed that the crystallite size was reduced in the presence of nanodispersed layers since the P(3HB-co-3HV) melting temperature are shifted to lower temperatures. Nanocomposites thermal stabilities were also studied. TGA results revealed that the temperature corresponding to 3% of weight loss increased with C30B content (+10 ºC with 3 wt% of filler). They
explained these trends by the nanodispersion of the silicate layers into the matrix and thus concluded that the well-dispersed and layered structure accounts for an efficient barrier to the permeation of oxygen and combustion gas. Eventually, the mechanical properties showed that clays can also act as an effective reinforcing agent since the Young’s modulus significantly increases from 480 to more than 790 MPa due to strong hydrogen bonding between P(3HB-co-3HV) and C30B.

Wang *et al.* [32,42] and Chen and co-workers [32,43,44] have investigated the structure and the properties of P(3HB-co-3HV)/OMMT nanocomposites. They synthesized nanocomposites made of P(3HB-co-3HV) having 3 and 6.6 mol% of 3-HV units and organomodified MMT obtained via cationic exchange in an aqueous solution with hexadecyl-trimethylammonium bromide [MMT-N+(Me)3(C16)]. Nanocomposites were prepared by the solution intercalation method by first adding 1, 3, 5 or 10 wt% OMMT to a chloroform solution of P(3HB-co-3HV) and then exposing the resulting dispersions to an ultra-sonication treatment. These conditions led to intercalated structures shown by WAXD, but clay aggregation occurred when increasing the clay content to 10 wt%. A detailed study of the P(3HB-co-3HV)/OMMT crystallization behavior was achieved. It was shown that OMMT acted as a nucleating agent in the P(3HB-co-3HV) matrix, which increased the nucleation and the overall crystallization rate, leading to more perfect P(3HB-co-3HV) crystals [43]. With increasing amount of OMMT, the predominant crystallization mechanism of P(3HB-co-3HV) was shifted from the growth of crystals to the formation of crystalline nuclei. The nucleation effect of the organophilic clay decreased with the increase of the clay content. Wang *et al.* [42] postulated that the
nanoscaled OMMT layers affect the crystallization in two opposite ways. On one hand, a small part of OMMT can increase the crystalline nuclei thus causing a more rapid crystallization rate. On the other hand, owing to the interaction of OMMT layers with P(3HB-co-3HV) chains, most of the OMMT layers restrict the motion of the P(3HB-co-3HV) chains. Therefore, the crystallization rate increased whereas the relative degree of crystallinity decreased with increasing amount of clay in the P(3HB-co-3HV)/OMMT nanocomposites. Furthermore, the P(3HB-co-3HV) processing behavior could be improved with OMMT based nanocomposites since the processing temperature range enlarged by lowering melting temperature with the increasing clay content. The tensile properties of the corresponding materials were improved by incorporation of 3 wt% of clay [44]. Above this clay content, aggregation of clay occurred and tensile strength and strain at break decrease. DMA, through the study of the modulus and the glass transition temperature $T_g$ revealed that the interface was maximized because of the nanometer size OMMT which restricts segmental motion near the organic-inorganic interface. Thus, it confirmed that intercalated nanocomposites were formed. Eventually, the biodegradability of these nanocomposites systems in soil suspension decreased with increasing OMMT. This was related to the interactions between P(3HB-co-3HV) and OMMT, but also to water permeability, the degree of crystallinity, and the anti-microbial property of OMMT.

Bruzaud and Bourmaud [45] studied P(3HB-co-3HV)/MLS nanocomposites prepared by the solution intercalation method. Their WAXD results showed that intercalated and/or exfoliated nanocomposites were obtained. The mechanical behavior showed significant
improvement in terms of modulus, tensile stress and hardness with increasing clay loading. All the nanocomposites had greater thermal stability than pure P(3HB-co-3HV).

Table 2.2 Structure of the studied PHA/clay bionanocomposites. Reproduced from P. Bordes et al. [32] and completed.

<table>
<thead>
<tr>
<th>PHA</th>
<th>Process</th>
<th>System</th>
<th>Structure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
<td>Solvent intercalation</td>
<td>MMT-N'(Me)<em>{2}(C</em>{16}),(tallow)/chloroform</td>
<td>Intercalated</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>Melt intercalation</td>
<td>MMT-N'(Me)(EtOH)_{2}(tallow)/MA-g-P(3HB)</td>
<td>Intercalated</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SFM-N'(Me)(EtOH)_{2}(coco alkyl)</td>
<td>Intercalated and well delaminated</td>
<td>[47]</td>
</tr>
<tr>
<td>P(3HV-co-3HB)</td>
<td>Solvent intercalation</td>
<td>MMT-N'(Me)<em>{3}(C</em>{16})/chloroform</td>
<td>Intercalated</td>
<td>[42-44]</td>
</tr>
<tr>
<td></td>
<td>Melt intercalation</td>
<td>MMT-N'(Me)<em>{2}(tallow)</em>{2}/chloroform</td>
<td>Intercalated and/or delaminated</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MMT-N'(Me)(EtOH)_{2}(tallow)</td>
<td>Intercalated, nanodispersed</td>
<td>[41]</td>
</tr>
</tbody>
</table>

Eventually, Misra et al. developed a novel solvent-free method to prepare P(3HB) functionalized by maleic anhydride [MA-g-P(3HB)] [32, 46]. The functionalization was successfully achieved by free radical grafting of maleic anhydride using a peroxide initiator by reactive extrusion processing. Then, they have mixed MA-g-P(3HB) with C30B to make the organomodifier hydroxyl functions react with the MA [47]. Although, the d-spacing was comparable to P(3HB)/C30B prepared by melt blending, the decrease in intensity of WAXD signals and TEM images showed that more delaminated platelets were obtained.
Other interesting inorganic layered materials are layered double hydroxides, characterized by similar shape and structure to natural clay that have more recently appeared in the field of biopolymer nanocomposites [34].

Layered double hydroxides or LDHs are the class of anionic clays whose structure is based on brucite [Mg(OH)₂]-like layers in which some of the divalent cations have been substituted by trivalent ions to form positively charged sheets. LDHs can be represented by the general formula \( [M^{2+}_{1-x}M^{3+}_x(OH)_2]^+A^{n-}\times/n\cdot m\cdot H_2O \), where \( M^{2+} \) and \( M^{3+} \) are divalent and trivalent metal cations, such as Zn²⁺, Al³⁺, respectively, and \( A \) is an interlayer anion, such as NO₃⁻, CO₃²⁻, SO₄²⁻ [33,34,48]. LDH structure is represented in Figure 2.5 and consists of stacks of positively charged mixed metal hydroxide layers with hydrated anions between the sheets to maintain overall charge neutrality. They are available both as naturally occurring and synthetic minerals resembling natural hydrotalcite, which has the formula \( \text{Mg}_6\text{Al}_2(\text{OH})_{16}\text{CO}_3.4\text{H}_2\text{O} \), and for this reason they also known as hydrotalcite-like materials.

The most common method for LDH synthesis is coprecipitation of \( M^2 \) and \( M^3 \) salts (chloride, nitrate, sulfate, carbonate, etc.) from homogenous solution [34,49-51]. LDHs grow as hexagonal crystals, where each cation is octahedrally surrounded by hydroxide groups, the octahedral sharing edges forming two dimensional sheets [34,52]. Therefore, LDH sheets are composed of one polyhedral-made layer, often corrugated, and for this reason they are considered more flexible than other bidimensional frameworks such as 2:1 layered silicate [34]. Most LDHs are binary systems, i.e. with two kinds of metal cations within the hydroxide layers, but ternary LDHs have also been
synthesized. LDH might be tailored to produce functional materials with properties required for specific applications, e.g. as precursors for magnets, heat retention additive in horticultural plastic films, flame retardants, HCl absorbing agents for poly(vinyl chloride) (PVC) and other halogenated polymers, adsorbing agents for dyes, pigment dispersion stabilizers for coloring polymers, in biology and medicine for the storage, delivery and controlled release of pharmaceuticals and other biomolecules, as sensing materials for determination of water level, toxic additives, as biosensors for electrode, as catalysts for polymerization or decomposition of noxious gases, etc [33,51,53].

Figure 2.5  Schematic representation of LDH. Reproduced from C. Del Hoyo [51].

Up to now there is only one literature article on PHA/LDH nanocomposites. PHA/LDH nanocomposites were investigated by Hsu et al [54]. They prepared P(3HB)/layered double hydroxides (LDHs) nanocomposites by mixing P(3HB) and
poly(ethylene glycol) phosphonates (PEOPAs)-modified Mg-Al LDH (PMLDH) in chloroform solution. Both WAXD results and TEM micrographs of P(3HB)/PMLDH nanocomposites indicate that the PMLDHs are randomly dispersed and exfoliated into the P(3HB) matrix. In their study, the effect of PMLDH on the isothermal crystallization behavior of P(3HB) was investigated using DSC and polarized optical microscopy (POM). Isothermal crystallization results using Avrami model of P(3HB)/PMLDH nanocomposites show that the addition of 2 wt% PMLDH into P(3HB) induced more heterogeneous nucleation in the crystallization significantly increasing the crystallization rate and reducing their activation energy. More PMLDH into the P(3HB) probably causes more steric hindrance of the diffusion of P(3HB), reducing the transportation ability of biopolymer chains during crystallization, thus increasing the activation energy. The analysis of kinetic data using a modified Lauritzen-Hoffman equation indicates that P(3HB) has the highest values of product of the lateral and folding surface free energy. These results suggest that the incorporation of PMLDH into P(3HB) probably induces heterogeneous nucleation of P(3HB) crystallization and then decreases the surface energy barrier for P(3HB) crystallization. Mechanical properties results of the nanocomposites measured by DMA show significant improvements in the storage modulus when compared with that of neat P(3HB).

In sum, most of the articles reported the preparation of PHA-based nanocomposite by solvent and melt intercalation method, and whatever the elaboration route, full exfoliation state was neither obtained nor clearly demonstrated.
2.2.2 PHAs-based Paper Coatings

Commercially available PHA products include BIOPOL™ from Metabolix Inc. (Cambridge, MA), NODAX™ from Procter and Gamble (Cincinnati, OH). PHAs possess excellent film-forming and coating properties and are water resistant due to their high hydrophobicity [55,56]. P(3HB)-coated paperboard has been used for packaging of ready meals, while P(3HB-co-3HV)-coated board has been used for dry products, dairy products and beverages [55,57]. The studied PHA-based paper coatings are described here after. Lim et al. [58] prepared solution based P(3HB) coatings obtained by P(3HB) penetration into and adhesion onto cellulose paper. The coating biodegradation efficiency index of P(3HB) coated paper was investigated through solution enzymatic degradation. The results indicated that P(3HB) coated paper exhibited the lowest degradability by cellulases (enzymes that degrade cellulose paper) with respect to pure cellulose paper. Kuusipalo [59,60] investigated coatings obtained by P(3HB-co-3HV) extrusion coated onto a series of paper and paperboard substrates. The physical properties of the coatings showed that the adhesion between the biopolymer and the substrate was poor when corona and flame pretreatments were used. On the other hand, the adhesion was sufficient when the substrate was primed with an acrylic-based primer. The results of the heat sealability of P(3HB-co-3HV) extrusion coatings show that the sealing temperature increased with increased substrate basis weight. The water vapor barrier of the coatings was shown to be lowered by incorporation of wax or tall oil rosin. Krook et al. [55,61] investigated the creasability of P(3HB-co-3HV) compression molded onto paperboard. Their results showed that upon application of creasing and bending stresses, P(3HB-co-
3HV) coating showed no evidence of cracks, and the delamination was observed to decrease with increased molding temperature due to improved paperboard-coating adhesion.

2.3 Interfacial Area in PHAs-based Nanocomposites and Based Paper Coatings

The incorporation of nanoscale inclusions in PHA can cause improvement in mechanical, electrical, thermal and other physical properties in this latter. In addition to the inclusions themselves, the interface, a special region of biopolymer chains in the vicinity of the nanofillers, also can play an important role in the improvement of the biopolymer nanocomposite properties [62]. The existence of nanofiller surfaces in the biopolymer can alter the mobility of the biopolymer chains surrounding them. Such perturbations in the biopolymer molecular mobility can extend several radii of gyration and create regions of biopolymer, the interface, with properties and response are different from that of the host bulk biopolymer [62]. Due to the large surface to volume ratio of the nanofillers, the amount of interface biopolymer generated in nanocomposites can be considerable.

The intimate interaction between a biopolymeric resin and a paper substrate can also cause an enhancement in mechanical and other physical properties of biopolymer-based paper coating. The interface which can be characterized as a region with the formation of the adhesive bonds between the cellulose paper substrate and the biopolymeric material can play an important role in the enhancement of the properties of
the biopolymer-based paper coating. In biopolymer-based paper coating, the interface can be mainly considered for its contribution to the load transfer.

The structure and the properties of the interfacial region are not only different from the bulk materials but are also critical to controlling properties of the overall biopolymer-based nanocomposites and paper coating. Therefore it is relevant to explore the structure and properties of the interfacial biopolymer. The interfacial biopolymer can exhibit change in crystallinity, mobility, chain conformation, chain entanglement density, molecular weight and charge distribution [27].

The nanofiller surface can alter the degree of crystallinity, the phases present, the lamellar size and organization, and even the spherulite structure of the biopolymer [27]. DSC and POM can be used to probe the above changes. The influence of the above changes is important particularly in materials where the crystalline phase and spherulite structure impact the mechanical and the tribological behavior which can be investigated through DMA, tensile testing and tribological study.

The significant change of the viscoelastic properties is one example that cannot be well explained without considering the contribution of the interface biopolymer [62]. Recent experimental studies on the nanocomposites show that the transition zones of storage/loss modulus and loss tangent curves may be broadened (if the interface creates only local changes in the biopolymer relaxation time) or shifted (if the interface causes global changes in the biopolymer relaxation time) in the time/temperature domain [62-65]. Since the properties of the nanofillers are not time/temperature dependant within the experimental measurement scale, those enhancements cannot be simply attributed to the
existence of the nanofillers. DMA through time temperature superposition (TTS) investigation can contribute in accessing the above change.

In amorphous domain of biopolymer matrix, it is qualitatively understood that an attractive interface will decrease the mobility of the biopolymer chains and a repulsive interface will increase the mobility [27,66]. One way for probing this change in the mobility of the biopolymer chains in the interfacial region is to measure the glass transition temperature using DSC, DMA and dielectric spectroscopy (DES). Using these methods can show that a glass transition temperature of a biopolymer can be raised or lowered (if the interface causes global changes in the biopolymer relaxation time) with the addition of the nanoparticles with attractive and repulsive interaction with the matrix, respectively.

Tensile test, impact test, DMA and DSC are analytical tools that can also be used to investigate the interfacial region in biopolymer-based paper coating.

2.4 Biodegradation of PHAs

Possibly one of the unique advantages that PHAs hold over other biodegradable polymers is their ability to degrade under both aerobic and anaerobic conditions. They can also be degraded by thermal means or by enzymatic hydrolysis. In a biological system, PHAs can be degraded using microbial depolymerases as well as by non-enzymatic and enzymatic hydrolysis in animal tissues [1,67].

In general, the biodegradability of a polymer is governed mainly by its physical and chemical properties [1]. It has been found that low molecular weight PHAs are more
susceptible to biodegradation. The melting temperature is another important factor to be considered when studying biodegradation. As the melting point increases, the biodegradability decreases. With increasing melting temperature, the enzymatic degradability decreases. Tokiwa and Suzuki [1,68] found that lipases cannot hydrolyze the optically active P(3HB). This could be due to the high melting temperature of the latter (178°C). Mochizuki and Hirami [1,69] explained that biodegradation of solid polymers is influenced by chemical structure (especially functional groups and hydrophilicity–hydrophobicity balance) and highly ordered structures (mainly crystallinity, orientation and morphological properties). Nishida and coworkers [1,70] reaffirmed that crystallinity plays a very important role in biodegradability. They also identified that highly ordered structures, i.e. highly crystalline materials have lower biodegradability. In addition, the microbial population in a given environment, the temperature and the relative humidity also contribute to biodegradability in the environment [1,71].

2.4.1 Biodegradation in the Environment

P(3HB) has been taken as a prototype in the biodegradation studies of PHAs. Micro-organisms from the families *Pseudonocardiaeae, Micromonosporaceae, Thermomonosporaceae, Streptosporangiaceae* and *Streptomycetaceae* predominantly degrade P(3HB) in the environment [1,71]. In addition, most PHA-producing bacteria are able to degrade the biopolymer intracellularly.
During intracellular degradation, the PHA depolymerases in the cell breaks down P(3HB) to give 3-hydroxybutyric acid. A dehydrogenase acts on the latter and oxidizes it to acetylace and a $\beta$-ketothiolase acts on acetylace to break it down to acetyl-CoA. The $\beta$-ketothiolase enzyme plays an important role in both the biosynthetic and the biodegradation pathways. Under aerobic conditions, the acetyl-CoA enters the citric acid cycle and is oxidized to CO$_2$ [1,72]. Very little is known about the intracellular depolymerases since they are always found to be intimately connected to the P(3HB) granules and the overall process is very complex [1, 71, 73].

Extracellular depolymerases degrade PHAs present in the environment [1,71]. The bacteria, algae and fungi present in the environment attack the biopolymers on the surface [1,74]. These microbes secrete extracellular enzymes that hydrolyze the water-insoluble PHAs and the resultant water-soluble products are then absorbed through their cell walls and utilized [1,75]. The PHA depolymerases enzymes act on the biopolymer mainly by hydrophobic interactions. Degradation by these depolymerases initially produces oligomers. Some microbes produce an additional dimer hydrolase, which further breaks down the oligomers into the corresponding monomer [76]. The behavior of these extracellular depolymerases is quite well understood [73]. The rate of biodegradation of PHAs depend on environmental conditions like temperature, moisture, pH, nutrient supply and those related to the PHA materials themselves, such as, monomer composition, crystallinity, additives and surface area [1,77].

Environmental degradation tests can be conducted in fresh and marine water, sludge, soil and compost following suitable standards. There are a range of international
standards and test methods developed specifically to access the biodegradability, products safety and also for compost derived products. These are the American Society for Testing and Materials (ASTM), European Standardization Committee (CEN), International Standards Organization (ISO), Institute for Standards Research (ISR) and German Institute for Standardization (DIN).

2.4.2 Biodegradation in Living Systems

The mechanism of biodegradability of PHAs in vivo is of huge significance because the rate of degradation of the material should equal the regenerative rate of tissue, in order to be used effectively as scaffolds in tissue engineering applications [1]. Literature on this aspect is limited and gives contradictory views. Studies by Miller and Williams suggest that P(3HB) and P(3HB-co-3HV) monofilaments implanted in animals do not lose their mass and maintain their physical and mechanical properties over a period of 6–12 months [1,78]. However, Duvernoy et al., have reported that P(3HB) films lose their mass by 30–80% within a year [1,79].

In vitro studies carried out usually investigate the stability of the biopolymers in model systems that mimic in vivo conditions, using varying values of pH, temperature and medium salinity [1]. The biopolymers used in these tests are of different compositions, molecular mass and crystallinity. The degradation is monitored by tracking the reduction in molecular mass and degree of crystallinity. Based on studies carried out, it is clear that PHA degradation is multiphasic. In the first few weeks, amorphous regions are eroded by random scission. Over a few weeks, biopolymer chains are disrupted and
the crystallinity of the biopolymer increases. Monomers, dimers and tetramers are formed due to which the molecular mass decreases. Later on, erosion processes are initiated leading to reduction in biopolymer mass. This can occur over 2–3 years and is dependent on environmental circumstances and the physicochemical properties of the PHA [1,80].

In vivo, PHAs are also degraded by the enzymes present in blood and animal tissues. Atkins and Peacock [1,81] studied the PHA-depolymerising activity of calf serum, pancreatic and synthetic gastric juice on P(3HB-co-3HV) microspheres with caprolactone. Weight loss was observed in the following order: bovine serum > pancreatic juice > synthetic gastric juice.

2.4.3 Techniques to Evaluate the Biodegradation of PHAs

Several standard test methods and a number of other techniques are available to access the biodegradability of polymeric material through the measurements of the chemical, physical, mechanical properties and percent degradation [82]. Figure 2.6 summarizes the different analytical techniques to access the biopolymer degradation.
Figure 2.6 Different analytical techniques to access the polymer degradation.
2.5 References


CHAPTER 3

POLY [(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE)] / LAYERED DOUBLE HYDROXIDE NANOCOMPOSITES *

3.1 Introduction

Poly[(3-hydroxybutyrate-co-3-hydroxyvalerate)] P(3HB-co-3HV) has been recognized as a potential environmentally-friendly substitute for traditional plastics such as polyethylene. P(3HB-co-3HV) is a member of the polyhydroxyalkanoates (PHAs) family. It is a fully biodegradable plastic material produced by microorganisms such as Alcaligenes eutrophus and Pseudomonas oleovorans [1]. Poly(3-hydroxybutyrate) or P(3HB) and P(3HB-co-3HV) are the main representatives of the PHAs family. P(3HB) is a highly crystalline (more than 60%) and brittle polyester with a melting point around 180°C, a sub-ambient glass transition temperature of 4-7°C and mechanical properties comparable to those of isotactic polypropylene [2]. Thermoplastic P(3HB) is a naturally occurring biodegradable material, which presents some drawbacks such as melt instability, degradation at elevated temperature near its melting point, brittleness and low strength. These factors limit the widespread applicability of P(3HB) [3]. Therefore, a

* This entire chapter is reproduced from Koffi L. Dagnon, Hua H. Chen, Lucia H. Innocentini-Mei and Nandika A. D’Souza, Poly[(3-hydroxybutyrate-co-3-hydroxyvalerate)] / Layered double hydroxide nanocomposites*, Polymer International 58: 133-141 (2009), with permission from Wiley Interscience.
P(3HB-co-3HV) co-polyester consisting of randomly arranged (R)-3-hydroxybutyrate (3HB) and (R)-3-hydroxyvalerate (3HV) units was developed [1]. P(3HB-co-3HV) has a lower melting point and higher flexibility than the P(3HB) homopolymer. The lower melting point of P(3HB-co-3HV) improves the melt stability and increases the processing window of the biopolymer. P(3HB-co-3HV) is characterized by improved chemical and physical properties for a wide range of applications [1-6] and the amount of 3HV content in P(3HB-co-3HV) co-polyester strongly influences its properties such as crystallinity, melting point and crystallization rate [7]. P(3HB-co-3HV) is potentially useful in biomedical and pharmaceutical applications such as tissue engineering and drug delivery systems [6,8-10]. It is also an attractive material for environmental waste management [1,11,12], and can substitute for conventional polymers when recovery for recycling or incineration is difficult or not cost effective [13]. Although mechanical properties have been found to meet those of many engineering plastics, a slow crystallization rate has limited the performance of P(3HB-co-3HV) [4,5]. Enhancing the crystallization rate via the use of nucleating agents has therefore received some attention [14].

The development of systems based on nanostructured hybrid organic-inorganic materials is of increasing interest. In this context, much attention has been focused on systems in which layered materials (montmorillonite in particular and more recently layered double hydroxides) are dispersed at a nanometric level in a polymeric matrix. More frequently investigated systems are those based on clay or layered silicates, due to the availability of the clay starting material and reasonably well-understood intercalation and exfoliation chemistry [15-20]. Wang et al.[4] and Chen et al.[5] investigated P(3HB-
co-3HV) and organically modified montmorillonite layered silicate (MLS) nanocomposites. The addition of the organophilic clay caused an increase in the overall crystallization rate of P(3HB-co-3HV), but did not influence the mechanism of nucleation or the growth of the P(3HB-co-3HV) crystals [4]. Experimental results showed that the melting temperature and the enthalpy of melting of the nanocomposites decreased. The crystallinity of the neat P(3HB-co-3HV) reduced and the spherulite size decreased with an increasing amount of MLS in the nanocomposites [4]. The mechanical properties in terms of tensile strength and modulus of the organoclay-based P(3HB-co-3HV) were enhanced. Indeed, with the incorporation of 3 wt% MLS, the tensile strength of the nanocomposite increased by 32% and the modulus by 2.8% over those of the original P(3HB-co-3HV) [5]. Choi et al.[21] incorporated MLS into P(3HB-co-3HV) by melt extrusion. Their research showed that the nanodispersed organoclay acted as a nucleating agent, increasing the temperature and rate of crystallization. The thermal stability and tensile properties of their nanocomposites were enhanced. Bruzaud and Bourmaud [22] studied P(3HB-co-3HV)/MLS nanocomposites prepared by the solution intercalation method. Their wide angle x-ray scattering (WAXS) results showed that intercalated and/or exfoliated nanocomposites are obtained. The mechanical behavior showed significant improvement in modulus, tensile stress and hardness with increasing the clay loading. All the nanocomposites had greater thermal stability than the pure P(3HB-co-3HV). Other additives have also been studied as nucleating agents for P(3HB-co-3HV). Lai et al.[13] investigated P(3HB-co-3HV) and multi-walled carbon nanotubes (MWNTs) composites formed by solution processing. Their calorimetric measurements
on the MWNT-based nanocomposites indicated that crystallization rate and crystallization temperature increased by the addition of MWNTs. The thermal stability of the MWNT-based nanocomposites was enhanced. A similar enhancement in crystallization rate of P(3HB-co-3HV) was reported by Kai et al.[14]. They investigated the nucleating activity of talc and boron nitride on P(3HB-co-3HV). Their non-isothermal crystallization results showed a shift to higher melt crystallization temperatures (T_{mc}) and lower cold crystallization temperature (T_{cc}) of the composites with respect to that of the neat P(3HB-co-3HV). The melt crystallization peak became sharper on addition of the nucleating agents. From their results, they inferred a faster crystallization process in the nanocomposites with respect to the pure P(3HB-co-3HV). This conclusion was also supported by their isothermal studies. Buzarovska et al.[23] investigated the kinetics of non-isothermal crystallization and melting behavior of P(3HB-co-3HV) in model, bulk and compatibilized P(3HB-co-3HV)/Kenaf fiber composites using DSC. Their result indicated that the Kenaf fibers, as well as their content, do not affect significantly the crystallization kinetics of the biopolymer P(3HB-co-3HV) matrix.

To overcome some of the limits, such as purity, posed by natural clays, synthetic clays such as layered double hydroxides (LDHs) have been increasingly investigated as additives in polymers. The composition of LDHs may be generally represented by the ideal formula \([M^{2+}_{1-x}M^{3+}_x(OH)_2]^{x+}A^{x/-}_{n/m}H_2O\), where \(M^{2+}\) and \(M^{3+}\) are divalent and trivalent metal cations, such as \(Zn^{2+}\), \(Al^{3+}\), respectively, \(A\) is an anion, such as \(NO_3^{-}\), \(CO_3^{2-}\), \(SO_4^{2-}\)[24, 25]. LDHs are important layered crystals due to their wide range of applications such as catalysts, flame retardants, stabilizers, medical applications, etc [24].
In general, modified LDHs can be prepared using simple procedures, at high level of purity. They are cheap and eco-compatible, and can be organically modified with a variety of organic anions. This latter characteristic will make the layered compounds compatible with a large variety of polymers leading to new hybrid biodegradable polymeric materials [16].

P(3HB-co-3HV) is characterized by a slow crystallization rate which is a limiting factor for the biopolymer processing. Because of this slow crystallization rate, a film made from P(3HB-co-3HV) with a higher contents of 3-HV units will stick to itself even after cooling; a substantial fraction of P(3HB-co-3HV) chains remains amorphous and tacky for a long period of time [26]. The addition of layered double hydroxide organically modified with stearate anions on the biopolymer is anticipated to enhance crystallization. With this in mind we probe the dispersion, crystallization, mechanical properties and thermal stability of these nanocomposites

3.2 Experimental

3.2.1 Materials

The modified anionic clay used was Zn-Al nitrate LDH (Zn-Al NO₃ LDH) organically modified with stearate anions. Zn-Al NO₃ LDH was synthesized by the following procedure. Zn (NO₃)₂·6H₂O (2.499 g, 8.40 mmol, 98%, Sigma-Aldrich) and Al (NO₃)₃·9H₂O (1.047 g, 2.79 mmol, 98.0 - 102.0 %, Aesar Aesar) were dissolved in 50 ml deionized water. To this solution, 0.88 ml of 50% NaOH solution (16.80 mmol, reagent grade, Alfa Aesar) was added under nitrogen gas with stirring. The mixture was aged
overnight with stirring at a nominal external oil bath temperature of 95-110°C for 24 h. The mixture was cooled and the precipitate was then centrifuged, and washed twice with deionized water to afford the Zn-Al NO₃ LDH precipitate.

P(3HB-co-3HV) (Mw=250,000-400,000 Daltons) with 18 mol% 3-HV content was supplied by the School of Chemical Engineering (State University of Campinas, Brazil) and dried in oven for 48 hours at 40°C. Chloroform (>99.8% purity, EMD), sodium hydroxide (50% NaOH solution; Sigma-Aldrich) and stearic acid (95%; Sigma-Aldrich) were used as received.

3.2.2 Intercalation of Stearate Anions in Zn-Al NO₃ LDH

An ion-exchange reaction was performed on the Zn-Al NO₃ LDH precipitate by adding a solution of stearic acid prepared by dispersing 0.794 g stearic acid (2.79 mmol, 95% Aldrich) in 50 ml deionized water neutralized with 0.10 ml of 50% NaOH solution. The mixture was stirred for 1 h, and then the resulting precipitate was centrifuged, washed several times with deionized water. The as-collected precipitate was dried in a vacuum over molecular sieves for several days. Elemental analysis of the LDH-stearate shows that, compared to the theoretical value, the stearate is slightly overexchanged (Table 3.1). The Zn-Al NO₃ LDH organically modified with stearic acid is designated in this work as LDH-SA.
<table>
<thead>
<tr>
<th>LDH Sample</th>
<th>Zn (%)</th>
<th>Al (%)</th>
<th>C (%)</th>
<th>H (%)</th>
<th>Zn/Al</th>
<th>C/Al</th>
<th>Residue (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-prepared Zn Al-stearate</td>
<td>20.2</td>
<td>4.19</td>
<td>42.4</td>
<td>8.43</td>
<td>1.99</td>
<td>22.7</td>
<td>28.0</td>
</tr>
<tr>
<td>$\text{Zn}_2\text{Al(OH)}<em>6\text{(stearate)}</em>{1.1.2}\text{H}_2\text{O}$</td>
<td>22.6</td>
<td>4.66</td>
<td>41.0</td>
<td>8.37</td>
<td>2.00</td>
<td>19.8</td>
<td>38.1</td>
</tr>
</tbody>
</table>

### 3.2.3 Preparation of the Nanocomposites Films

Nanocomposites were fabricated by the solution casting technique. Initially, LDH-SA powder was dispersed in chloroform and stirred for 10 min. Then $\text{P(3HB-co-3HV)}$ was added and the mixture was heated at $60^\circ\text{C}$ under vigorous stirring for 4 h. The resulting dispersion was allowed to age for 24 h. Nanocomposite films were prepared by spin casting (using a custom-built spin caster without any substrate). The samples were then dried under vacuum at $40^\circ\text{C}$ for 4 days to remove residual solvent. The LDH-SA content of the produced films was 1, 3, 5, and 7 wt%. Samples are designated as $\text{P(3HB-co-3HV)/SAn}$, where n is the amount of LDH-SA present in the nanocomposite. Two batches of each sample are prepared to establish reproducibility and measurements were conducted on each of them.

Spin cast films of the neat polymer as well as of its nanocomposites were used for wide angle X-ray diffraction (WAXD), transmission electron microscopy (TEM), dynamic mechanical thermal analysis (DMTA), tensile testing, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) and polarized optical microscopy (POM).
3.2.4 WAXD Experiments

WAXD was conducted on a Rigaku model D/Max–Ultima III (Cu-Kα wavelength of 1.542 Å generated at 44 mA and 40 kV). Diffractogram of the neat P(3HB-co-3HV) and the nanocomposite samples were recorded at room temperature in an angular range of 1.5° to 50° with a step size of 0.03 and a scanning rate of 0.02°s⁻¹. All experiments were repeated twice and duplicate X-ray analyses were performed.

3.2.5 TEM Experiments

TEM was used to complement WAXD measurements on nanoplatelet dispersion. Bright-field TEM images were recorded on a Philips EM 420 transmission electron microscope at an accelerating voltage of 120 kV. Prior to the analysis, samples of P(3HB-co-3HV)/LDH-SA nanocomposites were sandwiched and glued between two sheets of epoxy resin. The sandwiched samples were then cut into slices of a nominal thickness of 90 nm with a diamond knife on RMC MT 6000 ultra microtome at room temperature. The sections were transferred from water at room temperature onto a 200-mesh copper grid.

3.2.6 DMTA Experiments

The viscoelastic properties of P(3HB-co-3HV) and the nanocomposites were measured using a Rheometric Solids Analyzer 3 (RSA III) DMTA instrument operating in the tensile mode. For the viscoelastic measurements, films measuring 25x5x0.12mm³ were scanned at a heating rate of 2°C/min, a frequency of 1 Hz and a strain amplitude of 0.25% (determined from a separate strain amplitude sweep at 1 Hz to establish the linear
viscoelastic region), between -140°C to 100°C. All DMTA measurements were done in triplicate to obtain average viscoelastic values.

3.2.7 Tensile Testing

Tensile properties were measured using films of 30x5x0.12 mm$^3$ with Rheometric Solids Analyzer 3 (RSA III) DMTA instrument operating in the tensile mode. Measurements were performed following the ASTM D 3039-92 standard with a strain rate of 0.04 min$^{-1}$. An average of 3 tests was chosen as the tensile test value.

3.2.8 TGA Experiments

Thermal degradation studies were conducted using a Perkin-Elmer TGA 7 analyzer, under a nitrogen atmosphere. Around 10 mg of the sample was weighed in a standard ceramic pan. The measurements were performed from 25 to 800°C at 20°C min$^{-1}$. All TGA measurements were done in duplicate.

3.2.9 DSC Experiments

Thermal analysis of the P(3HB-co-3HV) and the nanocomposites was performed on a Perkin-Elmer DSC 6 calorimeter, under a nitrogen atmosphere, calibrated with an indium standard. Around 5 mg of the sample was weighed out in a standard aluminium pan. Heating and cooling rates were maintained at 10°C min$^{-1}$ during DSC runs. Specimens were held at 10°C for 2 min, heated to 185°C, maintained at this temperature
for 5 min. The specimens were cooled down to 10°C, reheated to 185°C and cooled down to 10°C to obtain the second run. All DSC measurements were done in duplicate.

3.2.2 POM Experiments

A Nikon polarized optical microscope equipped with an Instec STC200 hot stage was used to investigate the superstructure of the nanocomposites. Thin films of pure P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites (about 100 μm thick) were sandwiched between two thin glass slides and heated with the help of the hot-stage to 200°C at a rate of 10°C min⁻¹. The samples were then held at 200°C for 5 min. They were quenched to 87°C (Tc) at a rate of 20°C min⁻¹ and crystallized isothermally.

3.3 Results and Discussion

3.3.1 Dispersion of the LDH-SA in P(3HB-co-3HV)

Figure 3.1 shows the WAXD patterns in the range of 2θ=1.2-35° of the pristine P(3HB-co-3HV), LDH-SA and the nanocomposites. LDH-SA sample has a reflection at 2θ = 2.76°, corresponding to the basal spacing d_(003) of 3.2 nm (Table 3.2). The second, third and the fourth reflections of LDH-SA correspond to the higher harmonics of the interlayer distance. The peaks are sharp, indicating an ordered arrangement of the organic anions intercalated in the nanofiller interlayer galleries. In all hybrids an increase in basal spacing of the LDH-SA is observed from 3.2 nm in the neat LDH-SA to 5.3, 4.9, 4.8 and 5 nm, corresponding to P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. This indicates some degree of
intercalation of P(3HB-co-3HV) chains within the LDH-SA interlayer galleries but no compositional effect on the degree of intercalation. The existence of sharp Bragg peaks after solution casting indicates that the dispersed LDH-SA still retains an ordered structure. This proves that the LDH-SA crystals remain essentially integral within the P(3HB-co-3HV) matrix and, as a consequence, exfoliation of LDH-SA does not take place.

Figure 3.1  WAXD patterns of P(3HB-co-3HV), LDH-SA and P(3HB-co-3HV)/SA nanocomposites: (a) P(3HB-co-3HV), (b) P(3HB-co-3HV)/SA1, (c) P(3HB-co-3HV)/SA3, (d) P(3HB-co-3HV)/SA5, (e) P(3HB-co-3HV)/SA7 and (f) LDH-SA.
Table 3.2  WAXD characteristic peaks of LDH-SA

<table>
<thead>
<tr>
<th>LDH-SA</th>
<th>(003)</th>
<th>(006)</th>
<th>(009)</th>
<th>(0012)</th>
<th>(0015)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflection Angle, 2θ (deg.)</td>
<td>2.76</td>
<td>5.40</td>
<td>8.65</td>
<td>11.68</td>
<td>12.59</td>
</tr>
<tr>
<td>Interlayer spacing (nm)</td>
<td>3.20</td>
<td>1.63</td>
<td>1.02</td>
<td>0.76</td>
<td>0.62</td>
</tr>
<tr>
<td>Full width at half maximum</td>
<td>0.59</td>
<td>0.49</td>
<td>0.41</td>
<td>0.39</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The effect of the LDH-SA on the biopolymer P(3HB-co-3HV) was also examined. The WAXD diffractogram of neat P(3HB-co-3HV) shows that this polyester is a semicrystalline material. Its characteristic reflections have been reported in the literature [6,7]. These characteristic peaks of P(3HB-co-3HV) are indicated in Figure 3.1 and correspond to a rhombic cell. P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7 show P(3HB-co-3HV) reflections at the same values as the neat biopolymer, indicating that, in the nanocomposites, P(3HB-co-3HV) crystallizes in the typical crystalline form. Its unit cell is not changed after being incorporated with LDH-SA. However, the addition of LDH-SA results in sharper reflections for (020), (021) and (101) P(3HB-co-3HV) reflections compared to those of pure P(3HB-co-3HV). The crystallite size $d_{hkl}$ (nm) for the direction normal to hkl plane was determined by Scherrer equation: $d_{hkl}$ (nm) = $0.9\lambda/\beta\cos\Theta$, where $\lambda$ is the wavelength of the X-ray radiation, $\Theta$ is the Bragg’s angle, and $\beta$ is the full width at half maximum (FWHM). Table 3.3 shows the effect of LDH-SA on the crystalline lamella size for (020) and (021) reflections. The lamella size for (020) and (021) directions increased with
increasing LDH-SA indicating that the crystalline lamella size increased in the presence of LDH-SA.

Table 3.3  Effect of LDH-SA content on the crystallite size for (020) and (021) reflections.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\beta_{(020)}$ (degree)</th>
<th>$d_{(020)}$ (nm)</th>
<th>$\beta_{(021)}$ (degree)</th>
<th>$d_{(021)}$ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>0.404</td>
<td>19.6</td>
<td>0.649</td>
<td>12.2</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>0.400</td>
<td>19.9</td>
<td>0.630</td>
<td>12.6</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>0.381</td>
<td>20.8</td>
<td>0.619</td>
<td>12.9</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>0.368</td>
<td>21.6</td>
<td>0.604</td>
<td>13.3</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>0.359</td>
<td>22.1</td>
<td>0.510</td>
<td>15.7</td>
</tr>
</tbody>
</table>

TEM micrographs of the P(3HB-co-3HV)/SAn nanocomposites are shown in Figure 3.2 and the intercalated structure of LDH-SA component can be observed. Poor long range dispersion is indicated by significant LDH–SA aggregation. There is limited dispersion of LDH-SA in the P(3HB-co-3HV) matrix as the LDH-SA concentration increases. Micrographs in Figure 3.3 are obtained at higher magnification. The dark entities are the cross section of stacked intercalated and/or exfoliated LDH-SA layers whereas the bright area represents the biopolymer P(3HB-co-3HV) matrix. For P(3HB-co-3HV)/SA1, the layers are mostly parallel and their average length varied from 150 to 200 nm. On the other hand, the micrographs of P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7 samples showed a mixture of intercalated and exfoliated structure. All nanocomposites samples show an average line thickness of 1.30 - 1.60 nm and interplate distance of 4.8-6.1 nm, which falls well within the range predicted.
by the WAXD. Considering the d spacing, one can infer that the plates themselves are well dispersed but on a larger spatial scale, they show signs of aggregation.

Figure 3.2  TEM micrographs of P(3HB-co-3HV)/SAn nanocomposites showing dispersion of LDH-SA in P(3HB-co-3HV) matrix (scale bar: 100 nm).
3.3.2 Thermomechanical Properties

DMTA for the pure P(3HB-co-3HV) and P(3HB-co-3HV)/Sa nanocomposites was carried out to see the effect of the LDH-SA on the thermo-mechanical properties in the linear viscoelastic region. Figure 3.4 and 3.5 illustrate the dynamic storage modulus (E’) and the loss tangent (tan δ), respectively, as a function of temperature for all the
compositions. Table 3.4 summarizes the glass transition temperatures (temperature obtained at \( \tan \delta_{\text{max}} \)), \( \beta \)-relaxation temperatures (\( T_{\beta} \)) and dynamic tensile modulus at fixed temperatures (-125 and 25°C). \( E' \) represents the ratio of the in-phase stress to the applied strain. It represents the stiffness of a viscoelastic material and is proportional to the energy stored per cycle of deformation. The loss factor or \( \tan \delta \) is the ratio of loss modulus (\( E'' \)) to the storage modulus (\( E' \)). It is a measure of the energy lost, expressed in terms of the recoverable energy, and represents mechanical damping or internal friction in a viscoelastic material. As can be observed from the maxima of the \( \tan \delta \) (Figure 3.5 and Table 3.4), the \( \beta \)-relaxation (\( T_{\beta} \)) of the matrix polymer (around -101°C), which is conventionally associated with local crankshaft motion of the (CH\(_2\))\(_n\) segment, is unaffected by the addition of the LDH-SA. \( T_g \) of the hybrids is also unaffected by the addition of LDH-SA. The P(3HB-co-3HV)-based nanocomposites have higher storage moduli than pure P(3HB-co-3HV) as shown by Figure 3.4. The higher \( E' \) values of P(3HB-co-3HV)/SAn nanocomposites reflect the reinforcement potential of LDH-SA in the biopolymer matrix. The increase in rubbery modulus can be ascribed to the reinforcing effect of the nanofiller. Although \( E' \) generally increased with increasing LDH-SA content, P(3HB-co-3HV)/SA7 showed \( E' \) less than that of P(3HB-co-3HV)/SA3 and P(3HB-co-3HV)/SA5. The decrease in the \( E' \) of P(3HB-co-3HV)/SA7 can be attributed to increased aggregate formation leading to a decrease in the mechanical properties.
Figure 3.4  Dynamic thermo-mechanical properties in term of E’ of P(3HB-co-3HV) and P(3HB-co-3HV)/SA\textsubscript{n} nanocomposites.

Figure 3.5  Dynamic thermo-mechanical properties in term of Tan \( \delta \) of P(3HB-co-3HV) and P(3HB-co-3HV)/SA\textsubscript{n} nanocomposites.
Table 3.4  Dynamic thermomechanical properties of P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (°C)</th>
<th>$T_β$ (°C)</th>
<th>$E'(GPa)$ 125 °C</th>
<th>$E'(GPa)$ 25 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>13</td>
<td>-101.8</td>
<td>6.61 (±0.05)</td>
<td>2.45 (±0.05)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>12</td>
<td>-102.5</td>
<td>6.92 (±0.07)</td>
<td>2.40 (±0.03)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>10</td>
<td>-102.4</td>
<td>7.59 (±0.10)</td>
<td>3.21 (±0.10)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>10</td>
<td>-102.6</td>
<td>8.13 (±0.10)</td>
<td>3.40 (±0.10)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>10</td>
<td>-102.0</td>
<td>7.24 (±0.09)</td>
<td>2.87 (±0.07)</td>
</tr>
</tbody>
</table>

3.3.3 Tensile Properties

The influence of LDH-SA content on the mechanical properties of the nanocomposites is shown in the Figure 3.6 and Table 3.5. With the incorporation of 1, 3, 5 and 7 wt% LDH-SA, the Young’s modulus ($E$), increased to 1.23, 1.33, 1.42, and 1.24 GPa, which is about 10, 19, 27 and 11% higher than that of the pure P(3HB-co-3HV) (1.12 GPa). This enhancement in modulus reflects reinforcement by LDH-SA in P(3HB-co-3HV) (complementary to DMTA measurements) and the effectiveness of stress transfer across the LDH-SA interface. This can be attributed to the intercalated dispersion of LDH-SA nanoplatelets, resulting in a greater P(3HB-co-3HV) and LDH-SA interfacial area. The trends reflect those of the DMTA storage modulus where increased P(3HB-co-3HV) results in enhancement of stiffness, but aggregation for the 7 wt% LDH-SA composite results in limited enhancement. The tensile strength increased for 1 and 3 wt% LDH-SA and decreased for 5 and 7 wt% LDH-SA. This can be explained by the fact that, at lower LDH-SA content, the biopolymer matrix was restrained, resulting in lower strain at comparatively higher stresses. At nanofiller contents above 3 wt%, the formation of
aggregates results in decreased mechanical properties due to decreased flow stress. Enhanced mechanical properties in the nanocomposites at nanometric scales can be attributed to a high degree of dispersion of the nanoplatelets within the biopolymer matrix leading to a strong interaction between the biopolymer and the layered double hydroxide. Lastly, the hybrids showed a decrease in the strain at break with increasing LDH-SA content indicating an alteration in plastic deformation of the matrix with the incorporation of the nanofiller.

Figure 3.6  Stress-strain curves of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites.

Table 3.5  Mechanical properties of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites.
### 3.3.4 Thermal Stability

The thermal degradation of pristine P(3HB-co-3HV) and its hybrids with LDH-SA loadings are presented in Figure 3.7. P(3HB-co-3HV) thermally degrades drastically above 332 °C due to chain scission reactions leading to a reduction of molecular weight and formation of volatile acid products such as crotonic acid [2]. The shape of the curve for the nanocomposites is very similar to that of the parent P(3HB-co-3HV), which showed a single weight loss zone, although the temperature of the decomposition step is lower. The maximum weight loss temperature ($T_p$) decreased from 332 °C for pure P(3HB-co-3HV) to 324, 319, 313 and 309 for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. Figure 3.7 also describes the relation of the LDH-SA content with the onset decomposition temperature ($T_{onset}$) of the hybrids. $T_{onset}$ is considered to be the temperature that the material loses 2% of its weight. The thermal stability of the biopolymer is reduced with increasing LDH-SA content. It decreased from 298°C for the neat P(3HB-co-3HV) to 286, 281, 275 and 270°C for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. This behavior is due to the fact that

<table>
<thead>
<tr>
<th>Sample</th>
<th>Modulus (GPa)</th>
<th>Tensile strength (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>1.12 (±0.09)</td>
<td>25.10 (±0.30)</td>
<td>4.03 (±0.50)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>1.23 (±0.05)</td>
<td>28.20 (±0.15)</td>
<td>3.50 (±0.40)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>1.33 (±0.08)</td>
<td>28.50 (±0.55)</td>
<td>3.25 (±0.18)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>1.42 (±0.09)</td>
<td>24.20 (±0.25)</td>
<td>2.50 (±0.20)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>1.24 (±0.10)</td>
<td>24.40 (±0.45)</td>
<td>2.70 (±0.25)</td>
</tr>
</tbody>
</table>
LDH-SA degradation is accompanied by water loss which would accelerate P(3HB-co-3HV) decomposition.

Figure 3.7 TGA curves for P(3HB-co-3HV) with various compositions of LDH-SA.

3.3.5 Crystallization Effects of LDH-SA on P(3HB-co-3HV)

Figure 3.8 show the DSC results of the neat P(3HB-co-3HV) and nanocomposites. The melting temperature ($T_m$), melting enthalpy ($\Delta H_m$), cold
crystallization temperature (T_{cc}), cold crystallization enthalpy (\Delta H_{cc}), melt recrystallization temperature (T_{mc}), melt recrystallization enthalpy (\Delta H_{mc}) and the degree of crystallinity (X_c) were obtained from the thermograms. The results of the first heating and cooling curves and the second heating and cooling curves are summarized in Table 3.6 and 3.7, respectively. It was found that P(3HB-co-3HV) as received pellets, P(3HB-co-3HV) spun films and P(3HB-co-3HV)/SAn nanocomposite samples showed bimodal endothermic melting peaks (158 and 172°C) for the first heating (Figure 3.8A). These peaks correspond to the T_m of the crystalline phases, suggesting the presence of two crystalline phases in the samples and the fraction of the two \Delta H_m reflect the relative amount of the crystalline phases in the P(3HB-co-3HV)/SAn nanocomposites. No change in both melting points was observed with increasing LDH-SA content, whereas total \Delta H_m increased from 53.4 Jg^{-1} for the parent P(3HB-co-3HV) to 55.0, 55.4, 57.4.4 and 58.4 Jg^{-1} for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. An evaluation of the two melting points in terms of peak height showed that the low-temperature melting peak (158°C) was unchanged whereas the high-temperature melting peak (172°C) increased slightly with increasing LDH-SA content. It was inferred that the low-temperature melting peak was probably related to homogenous nucleation of P(3HB-co-3HV), which started spontaneously by P(3HB-co-3HV) chain aggregation below the melting point. The high-temperature melting peak was probably related to heterogeneous nucleation of P(3HB-co-3HV) [4]. Other authors have also reported the bimodal endothermic peaks in the P(3HB-co-3HV) and its nanocomposites [4,13]. In the DSC of P(3HB-co-3HV)/MLS nanocomposites, Wang et al.[4] reported a
change in the bimodal endothermic peaks of P(3HB-co-3HV)/MLS nanocomposites characterized by an increase in the high-temperature endothermic peak and suppression of the low-temperature endothermic peak when MLS increased. On the other hand, Lai et al. [13] in their DSC study of P(3HB-co-3HV)/MWNTs nanocomposites showed that for pure P(3HB-co-3HV) the upper melting peak was dominant with increasing heating rate whereas for the P(3HB-co-3HV)/MWNTs nanocomposites the lower melting peak was more pronounced. In our LDH-SA nanocomposites, we do not detect a change in the nanocomposite peaks compared to the P(3HB-co-3HV) peaks.

$T_{mc}$ values during dynamic cooling were analyzed as a function of the concentration of LDH-SA for the first and second cooling scans. The cooling thermograms of the samples showed that the parent P(3HB-co-3HV) did not show any $T_{mc}$ exotherm peak for both first and second scans. The P(3HB-co-3HV)/SA$n$ nanocomposites however, did (Figure 3.8B and 3.8C). P(3HB-co-3HV) melt recrystallization became more pronounced with the addition of LDH-SA. Low LDH-SA concentrations gave a broad melt recrystallization peak with $T_{mc}$ at around 48°C. For the P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, a substantial transition in melt recrystallization was evident. $\Delta H_{mc}$ and $T_{mc}$ of the nanocomposites for the first and second cooling showed that P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7 exhibited narrower and more symmetrical melt recrystallization curves with respect to the rest of the nanocomposites and the neat P(3HB-co-3HV). These characteristics suggest that the melt recrystallization of P(3HB-co-3HV) becomes faster, and that the former samples
comprised more stable crystals, which implied better crystal perfection for concentrations above 5 wt%.

The effect of thermal history of the LDH-SA was probed further by examining the second heating thermograms. These thermograms, after first cooling, showed significantly different behavior compared to that observed in the first heating. As shown in Figure 3.8D, a cold crystallization peak (T_{cc}) appeared at low-temperature. For pure P(3HB-co-3HV), P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3 and P(3HB-co-3HV)/SA5, T_{cc} peaked at 42, 46, 45 and 36°C, respectively. With respect to T_{cc} of neat P(3HB-co-3HV), a slight increase for P(3HB-co-3HV)/SA1 and P(3HB-co-3HV)/SA3 occurs. A decrease in T_{cc} for P(3HB-co-3HV)/SA5 was followed by complete elimination of the cold crystallization peak in P(3HB-co-3HV)/SA7. T_{mc} and T_{cc}, respectively, during the dynamic cooling are the indirect measures of the crystallization rate. Usually, lower T_{cc} indicates faster crystallization, whereas lower T_{mc} indicates slower crystallization. In the context of peak area, P(3HB-co-3HV) showed a noticeable large and narrow cold crystallization peak which became smaller with increasing LDH-SA content. However, P(3HB-co-3HV)/SA7 did not show any obvious cold crystallization peak. This is caused by the fact that 7 wt% nanofiller greatly accelerated the crystallization of P(3HB-co-3HV), so most of the biopolymer recrystallized during the cooling process. The low-temperature melting point of P(3HB-co-3HV) is suppressed and only the T_{m} at 170°C was preserved for pure P(3HB-co-3HV). The peak doublet evident in the first heat was annihilated by the thermal history and merged into a single peak. A shift to a lower temperature for the high-temperature melting peak with increasing amount of LDH-SA
was observed. It shifted from 170°C for the pure P(3HB-co-3HV) to 166, 164, 163 and 162°C for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. ΔH_m increased from 67.4 Jg⁻¹ for pure P(3HB-co-3HV) to 68.1, 73.3, 74.4 and 76.0 Jg⁻¹ for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. All values were higher than those of the first heating. This behavior indicated that the total crystallinity of P(3HB-co-3HV) was influenced by LDH-SA content. The degree of crystallinity for the second heating is calculated according to the relation X_c = ΔH_f/ΔH_f°, where ΔH_f is the enthalpy of fusion and ΔH_f° is taken as 109 J/g [23]. It can be noted that X_c increased for all the compositions with increasing the nanofiller content in comparison to P(3HB-co-3HV). The combination of increased enthalpy of melting and increased temperatures of melt recrystallization established a clear crystallization-promoting effect of LDH-SA in P(3HB-co-3HV). We note that the value of the melting point decreased for all samples from the first to the second heating. The peak doublet was also eliminated and a single peak with lower melting point observed. The decrease in melting point indicates an increase in lamella thickness which supports the Scherrer-based analysis of the WAXD results of the P(3HB-co-3HV) reflections. The increase in the degree of crystallinity coupled to the decrease in melting point could imply that while the lamella thickness increased, the crystal density also increased.
Figure 3.8 DSC traces for P(3HB-co-3HV) and P(3HB-co-3HV)/SA\textsubscript{n} nanocomposites: (A) first heating; (B) first cooling; (C) second cooling; (D) second heating - [a, P(3HB-co-3HV); b, P(3HB-co-3HV)/SA\textsubscript{1}; c, P(3HB-co-3HV)/SA\textsubscript{3}; d, P(3HB-co-3HV)/SA\textsubscript{5}; e, P(3HB-co-3HV)/SA\textsubscript{7}].
Table 3.6 Thermal properties of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites: first heating and cooling

<table>
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<th>1st Cooling</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T m (°C)</td>
<td>ΔH m (Jg⁻¹)</td>
<td>T mc (°C)</td>
<td>ΔH mc (Jg⁻¹)</td>
</tr>
<tr>
<td></td>
<td>Peak 1</td>
<td>Peak 2</td>
<td>Peak 1</td>
<td>Peak 2</td>
</tr>
<tr>
<td>P(3HB-co-3HV)</td>
<td>158.1</td>
<td>172.2</td>
<td>12.3</td>
<td>41.1</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>158.4</td>
<td>172.7</td>
<td>12.6</td>
<td>42.4</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>158.3</td>
<td>172.5</td>
<td>12.8</td>
<td>42.6</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>157.6</td>
<td>171.8</td>
<td>13.1</td>
<td>44.3</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>158.1</td>
<td>172.1</td>
<td>13.5</td>
<td>44.9</td>
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Table 3.7 Thermal properties of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites: second heating and cooling

<table>
<thead>
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<th>Sample</th>
<th>2nd Heating</th>
<th></th>
<th>2nd Cooling</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T cc (°C)</td>
<td>T m (°C)</td>
<td>ΔH cc (Jg⁻¹)</td>
<td>ΔH m (Jg⁻¹)</td>
</tr>
<tr>
<td>P(3HB-co-3HV)</td>
<td>41.9</td>
<td>170.0</td>
<td>-38.2</td>
<td>67.4</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>45.8</td>
<td>166.5</td>
<td>-34.3</td>
<td>68.1</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>45.5</td>
<td>164.4</td>
<td>-32.2</td>
<td>73.3</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>36.3</td>
<td>163.0</td>
<td>-6.5</td>
<td>74.4</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>-</td>
<td>161.8</td>
<td>-76.0</td>
<td>69.8</td>
</tr>
</tbody>
</table>

3.3.6 Morphology of P(3HB-co-3HV) Spherulites and Effect of LDH-SA on Crystallization

Figure 3.9 shows the POM photomicrographs of spherulites of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites crystallized isothermally at 87°C. Ring-banded spherulites were observed. P(3HB-co-3HV) revealed integrated spherulitic structure with clear spherical surface. For P(3HB-co-3HV)/SAn nanocomposites, the
shapes of the spherulites were interrupted and an increase in the size of the spherulites can be seen. LDH-SA affected the crystallization and size of P(3HB-co-3HV) spherulites.

Figure 3.9 POM images of P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites isothermally crystallized at $T_c=87^\circ$C (scale bar = 100 µm).
For all samples, interspherulitic boundaries can be seen. In addition, the structure of the P(3HB-*co*-3HV) spherulites is characterized by the nucleation site (small ring located in the center) and the presence of a homogenous amorphous phase (lighter domains which consist of uncrystallized P(3HB-*co*-3HV) chains) situated mainly in interlamellar regions of crystalline phase (darker domains which consist of lamellar chain folded crystallite) [27].

3.4 Conclusion

The incorporation of LDH-SA improved the mechanical properties of P(3HB-*co*-3HV). In particular, the elastic modulus and the tensile strength increased with increasing the loading of LDH-SA. The thermomechanical properties are modified by the presence of LDH-SA. Dynamic elastic modulus (E’) of the nanocomposites increased while the β-transition and the glass transition temperature (T_g) did not change. WAXD indicated an intercalated dispersion but TEM indicated significant agglomeration in the nanocomposites. From the DSC results, the nanodispersed LDH-SA acted as a nucleating agent and a pronounced recrystallization temperature, absent in pure P(3HB-*co*-3HV), was induced in all nanocomposites. A cold crystallization transition was induced by the thermal history of the first heating in the second heating scans. However, the induced cold crystallization peak decreased with increasing LDH concentration and was completed eliminated at higher loadings of the P(3HB-*co*-3HV)/SA7. Polarized optical microscopy indicated that large-sized P(3HB-*co*-3HV) spherulites were formed. TGA
results showed that the nanofiller destabilized the biopolymer matrix leading to decreased thermal stability of the nanocomposites with increasing LDH-SA content. The decreased thermal stability could be due to the release of water from LDH-SA which hydrolyses the ester bonds of P(3HB-co-3HV). Compared to previous work on P(3HB-co-3HV)/MLS nanocomposites [5], where the addition of MLS resulted in ca 3% increase in modulus and decreased enthalpy of melting, the LDH-SA was an effective crystallization promoting additive with >10% improvement in modulus for all compositions investigated.
3.5 References


CHAPTER 4

SYNTHETIC SURFACE ACTIVE CLAYS FOR ENHANCED POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE CRYSTALLIZATION *

4.1 Introduction

Biodegradable polymers or biopolymers have been extensively investigated from ecological and biomedical perspectives. As a consequence, many researchers and manufacturers are motivated to move towards materials that can be produced from renewable resources and would not remain in the environment after disposal [1]. Among these biomaterials, poly(hydroxyalkanoic acid)s (PHAs) family, poly-lactic acid (PLA), thermoplastic starch (TPS), and their copolymers or blends with other biodegradable polymers are attracting much interest. Since biopolymers originate from renewable resources, they represent a potential substitute to petroleum based synthetic polymers. However, the majority of these biopolymers is expensive and exhibit inferior mechanical properties. When processed under conventional methods such as injection molding and extrusion, their slow crystallization rate leads to part tackiness and low dimensional stability. There is thus a critical need to improve the crystallization of the biopolymers to make them fully competitive with conventional thermoplastics. One approach is to combine the biopolymers with nano-sized fillers [1].

Polymer nanocomposites can be defined as polymers containing fillers with one dimension smaller than 100 nm. They constitute an interesting route to combine specific properties from the polymer and nanoparticles in one final product by means of blending processes such as in situ polymerization, intercalation of the polymer from a solution, direct intercalation of the molten polymer and sol-gel technology. Many experimental studies have shown that by incorporating nanoscale inclusions in a polymer matrix, polymer nanocomposites can result in significant improvement in mechanical, thermal, electrical, and other physical properties in comparison to the parent materials [2].

PHAs are polyesters of hydroxyalkanoic acids (HAs) synthesized by bacteria as intracellular-carbon and energy storage compounds and accumulated as granules in the cytoplasm of cells [3]. As biodegradable and biocompatible materials, they have attracted much attention in diverse applications [4]. A frequently utilized member of PHAs family is poly(3-hydroxybutyrate) P(3HB), a polymerized 3-hydroxybutyrate (3-HB) discovered in 1927 by Lemoigne. This biomaterial has distinct properties including biodegradability, biocompatibility, and piezoelectric characteristics [5]. It also has the particular advantage that it is thermoplastic and therefore, in principle, can be processed using existing processing equipment. However, there are also many drawbacks to the use of P(3HB), mainly its tendency to be a rather brittle and stiff material compared to common chemosynthesized plastics as well as a narrow processing window, limiting manufacturability [3,6]. These drawbacks prevent its practical application, and various copolyesters of P(3HB) have been developed to surmount these shortcomings. Frequently utilized copolyesters of P(3HB) include poly(3-hydroxybutyrate-co-3-hydroxyvalerate).
or P(3HB-co-3HV) which is 3-hydroxyvalerate (3-HV) units polymerized with 3HB units and poly(3-hydroxybutyrate-co-4-hydroxybutyrate) or P(3HB-co-4HB), which is a random copolymer of 4-hydroxybutyrate (4-HB) units with 3-HB units. With respect to the homopolyester P(3HB), these copolyesters are characterized by more desirable processing properties [3,7]. Molar fractions of 3-HV and 4-HB strongly affect the physical properties of P(3HB) copolyesters [3,8].

The copolyester P(3HB-co-3HV) presented in Figure 4.1 was first developed by Imperial Chemical Industries (ICI). ICI produced copolyester of 3-HB and 3-HV via a bacterial fermentation process with high purity in which alcaligenes eutrophus was grown in a culture media containing propionic acid and glucose as carbon sources [9] and marketed it as Biopol® in the composition range of 0 to 30 mol% HV units. P(3HB-co-3HV) is a semi crystalline material which is both biodegradable and biocompatible. These properties make it attractive as a biomedical and environmentally friendly material. On the other hand, the application of P(3HB-co-3HV) is hindered by some disadvantages such as the development of interlamellar secondary crystallization on storage, slow crystallization rate, high brittleness and high production costs [10]. Its brittleness is due to the inherent defects in the big spherulites whereas its low nucleation density is due to its high purity [11]. Therefore, the expansion of commercial applications for P(3HB-co-3HV) will necessitate the improvement of the crystallization and processing behavior, diminution of overall cost, improvement of the brittleness and enhancement of the mechanical properties of the biopolymer [12].
As mentioned earlier, P(3HB-co-3HV) is characterized by a slow crystallization rate, and this property becomes serious with increasing 3-HV comonomer content. The low crystallization rate has negative impacts on the processing of the biopolymer. In injection molding the part is soft and may not be properly ejected unless a long cooling time is allowed. In extrusion, it is tacky and tends to stick to itself. In addition, a large fraction of P(3HB-co-3HV) remains amorphous and slowly undergoes crystallization during storage [13]. Increased temperature and rate of crystallization of P(3HB-co-3HV) can be obtained by the addition of a small amount of nucleating agent. Chen et al. [14] and Ma et al.[11] studied the effect of nucleating agents such as silicate layers and fumed silica on the crystallization behavior of P(3HB-co-3HV). A number of additives including boron nitride, thymine and melamine; talc and boron nitride [15] have been also proposed to overcome the crystallization shortcomings of P(3HB-co-3HV). We have in the chapter 3 reported on the mechanical property enhancements and the crystallization behavior improvement in P(3HB-co-3HV) using an anion exchanged layer double hydroxide (LDH-SA). Based on dynamic heating and cooling, we determined that while P(3HB-co-3HV) had no recrystallization peak when cooled from the melt, the introduction of LDH-
SA resulted in an increasingly prominent recrystallization exothermic peak. Layered double hydroxides (LDHs) are synthetic clays [16] with a high surface activity of 1 reactive site per nm$^2$ and have been increasingly investigated as additives in polymers [16-20]. The composition of LDHs may be generally represented by the ideal formula $[\text{M}^{2+}_{1-x}\text{M}^{3+}_x\text{(OH)}_2]^{x^+}\text{A}^{n-}_{x/n}\text{mH}_2\text{O}$, where $\text{M}^{2+}$ and $\text{M}^{3+}$ are divalent and trivalent metal cations, such as $\text{Zn}^{2+}$, $\text{Al}^{3+}$, respectively, $\text{A}$ is an anion, such as $\text{NO}_3^-$, $\text{CO}_3^{2-}$, $\text{SO}_4^{2-}$. In general, modified LDHs can be prepared with simple procedures, at high level of purity. They are cheap and eco-compatible, and can be organically modified with a variety of organic anions.

In this study, P(3HB-co-3HV) and Zn-Al nitrate LDH organically modified with stearic acid nanocomposites have been prepared by a solution route. The isothermal and non-isothermal crystallization kinetics of P(3HB-co-3HV) and its nanocomposites were performed using a differential scanning calorimetry (DSC). Polarized optical microscopy (POM) was also used to complement the isothermal crystallization of P(3HB-co-3HV) and its nanocomposites. The Avrami parameters (Avrami exponent and crystallization rate constant), activation energy and the Lauritzen-Hoffman parameters (nucleation constant and folded surface free energy as well as the work of chain folding of isothermal crystallization) were derived and discussed.
4.2 Experimental Section

4.2.1 Materials

P(3HB-co-3HV) (Mw=250,000-400,000 Daltons) with 18 mol% 3-HV content was used as polymeric material. Its description is given in the chapter 3 section 3.2.1. The Zn-Al nitrate layered double hydroxide, organically modified with stearic acid or LDH-SA was used as nanofiller. Its synthesis in our laboratory has been described in the chapter 3 section 3.2. Chloroform (>99.8% purity, EMD Chemicals, Gibbstown, NJ) was used as received.

4.2.2 Preparation of the Nanocomposites Films

Nanocomposites were fabricated by the solution casting technique described in Chapter 3 section 3.2.3. The LDH-SA content of the fabricated films was 1, 3, 5, and 7 wt%. As in Chapter 3, samples in this work are designated as P(3HB-co-3HV)/SAn, where n is the amount of the LDH-SA used in the preparation of the nanocomposite.

4.2.3 Characterization

A Perkin-Elmer DSC-6 (Waltham, MA) differential scanning calorimeter (DSC) was used to characterize the thermal transitions and to monitor the rate of heat flow from the P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites samples during non-isothermal and isothermal crystallization from the melt. The instrument was calibrated with an indium standard. The measurement was conducted under a nitrogen atmosphere. The sample weight was kept in the 5-6 mg range.
For non-isothermal crystallization, samples were heated from 25°C to 190°C at 50°C min\(^{-1}\) and held at that temperature for 5 min to erase all prior thermal history. Then, they were cooled to 25 °C at a rate of 10°C min\(^{-1}\).

The procedure for isothermal crystallization kinetic experiment was as follows: all samples were first heated from 25 to 190°C at 50°C min\(^{-1}\) and held at that temperature for 5 min to erase all prior thermal history. They were quenched to the desired crystallization temperature, \(T_c\), at a rate of 50°C min\(^{-1}\). For all the samples, \(T_c\)'s were selected to be mostly in the vicinity of the peak of non-isothermal melt recrystallization temperature. The crystallization temperatures \(T_c\)'s investigated in this study are 63, 66, 69 and 72°C since they corresponded to the temperature range over which an isothermal exothermic peak was obtained. The exothermic crystallization peak was recorded as a function of time at \(T_c\). Subsequently, the samples were reheated to 190°C at a rate of 10°C min\(^{-1}\) to examine the melting behavior. The heat generated during the development of the crystalline phase was recorded up to a vanishing thermal effect and analyzed according to the usual procedure to obtain the relative degree of crystallinity, \(X_{rel}\) [14].

\[
X_{rel} = \frac{X_c(t)}{X_c(\infty)} = \frac{\int_0^t (dH / dt) dt}{\int_0^\infty (dH / dt) dt} \quad (1)
\]

Where the first integral is the heat of crystallization generated after time \(t\) and the second integral is the total heat of crystallization for \(t = \infty\). All DSC measurements were done and analyzed in duplicate.
A Nikon polarized optical microscope (POM) equipped with an Instec STC200 hot-stage was used to investigate the super-structure of the nanocomposites. Thin films of pure P(3HB-co-3HV) and its nanocomposites (about 100 µm thick) were sandwiched between two thin glass slides and heated to 190°C at a rate of 50°C min$^{-1}$. The samples were then held at 190°C for 5 min. Subsequently, they were quenched to the appropriate crystallization temperature ($T_c$) at a rate of 50°C min$^{-1}$. The samples were then held at $T_c$ ($35°C \leq T_c \leq 125°C$, 3°C increment) and the growth of spherulites as a function of time was monitored every minute for 5 min. With the aid of an interfaced video-camera, real-time spherulites growth measurements were performed. In order to minimize the thermal degradation effects, a new sample was used for each crystallization measurement.

4.3 Results and Discussion

4.3.1 Non-isothermal Crystallization by DSC

The effect of LDH-SA on the non-isothermal crystallization behavior of P(3HB-co-3HV) from the melt is shown in Figure 4.2. We observe that the neat biopolymer did not show any melt recrystallization peak ($T_{mc}$) upon cooling. However, with the addition of the nanofiller, P(3HB-co-3HV) exhibited $T_{mc}$ peaks between 35 and 75°C which varied with LDH-SA concentration. Indeed, broad melt recrystallization peaks can be seen for low LDH-SA content (1 and 3 wt%) whereas high LDH-SA content (5 and 7 wt%) exhibited narrower and more symmetrical one. Also, in the nanocomposites, $T_{mc}$ shifted to higher temperature with increasing the nanofiller content. The presence of the melt recrystallization peak in the P(3HB-co-3HV) nanocomposites coupled with its
shifting towards higher temperature with increasing LDH-SA content could have indicated some degree of faster crystallization in the P(3HB-co-3HV)/SAn nanocomposites. Since the nanocomposites showed melt recrystallization peaks between 35 and 75°C, the crystallization temperature $T_{cs}$ (63, 66, 69 and 72 °C) used in the DSC isothermal crystallization kinetics study were selected to be mostly in the vicinity of the peak of non-isothermal melt recrystallization temperature.

Figure 4.2  DSC non-isothermal crystallization thermograms of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites at a cooling rate of 10°C min$^{-1}$ showing the crystallization promoting effect of LDH-SA – A: P(3HB-co-3HV), B: P(3HB-co-3HV)/SA1, C: P(3HB-co-3HV)/SA3, D: P(3HB-co-3HV)/SA5, E: P(3HB-co-3HV)/SA7.
4.3.2 Isothermal Crystallization Kinetics

Polymer crystallization is a topic of continuous interest and it has been studied widely for several decades. It is a complex phenomenon in materials and pharmaceutical processing that strongly affects microscopic structure and properties of polymer products [21]. Crystallization of polymers, which involves two consecutive phenomena—nucleation and growth, is governed by both thermodynamics and kinetics considerations. The presence of a solid surface in contact with a polymer melt usually results in heterogeneous nucleation. Therefore, investigating or understanding the thermodynamics and kinetics of polymer crystallization is an important scientific challenge. Because P(3HB) and P(3HV) are miscible polymers, the crystallization behavior of the random copolymer, P(3HB-co-3HV), is similar to that of the P(3HB) homopolymer, with some defects consisting of 3HV units [14].

4.3.2.1 Equilibrium Melting Point $T^0_m$ of P(3HB-co-3HV) and Nanocomposites by DSC

To evaluate $T^0_m$, the effect of the cooling thermal history on subsequent melting endotherms was determined. The melt-recrystallized P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites samples were heated directly from $T_c$s after isothermal crystallization was performed. The thermograms of pure P(3HB-co-3HV) and P(3HB-co-3HV) nanocomposites samples displayed bimodal melting endotherms ($T_{m1}$ and $T_{m2}$). For each sample, we observe that the higher melting temperature peak, $T_{m2}$, is independent of the $T_c$s whereas the lower temperature melting peak, $T_{m1}$, is dependent of the $T_c$s. It was
postulated that $T_{m2}$ is due to the melting of crystals that are recrystallized during heating of the samples in the DSC pans. On the other hand, $T_{m1}$ is related to the melting of crystals formed during isothermal crystallization from the melt [13]. For our study, $T_{m1}$ and $T_{m2}$ data plotted as a function of $T_c$ are shown in Figure 4.3 for all samples. For each sample $T_{m1}$ increased gradually with increasing $T_c$s whereas $T_{m2}$ is almost unchanged. The two-peak behavior can be caused either by a phase-separated structure or as a result of the melt/recrystallization process. The lower of the two endothermic peaks is usually regarded as unstable and not a perfect crystal, whereas the higher is regarded as a stable and perfect one [11]. Our results show that the neat P(3HB-co-3HV) exhibited the highest $T_{m1}$ and $T_{m2}$ with respect to the P(3HB-co-3HV)/SAn nanocomposites. The trend in the melting temperatures is inversely related to the extent of crystal perfection. As can be seen for the nanocomposites, the higher the concentration of LDH-SA in the biopolymer matrix, the lower the melting points $T_{m1}$ and $T_{m2}$. Our results are inconsistent to that of P(3HB-co-3HV) nanocomposites with montmorillonite layered silicates investigated by Chen et al. [14].

The relation between $T_{m1}$ and $T_c$ can be described using the Hoffman and Weeks equation [22]:

$$T_m = T_m^o \left[ 1 - \frac{1}{\gamma} \right] + \frac{T_c}{\gamma} \quad (2)$$
Where $T_{m}^{o}$ is the equilibrium melting temperature of crystals with infinite lamellar thickness and $\gamma$ is the ratio of the final to initial thickness. The approximate value for $T_{m}^{o}$ can be obtained by extrapolating $T_{m1}$ versus $T_{c}$ curve to the point where $T_{m1} = T_{c}$. For all the samples investigated in this work, plots of $T_{m1}$ against $T_{c}$ exhibited linear relationships. The extrapolated equilibrium melting temperatures $T_{m}^{o}$ for P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites are listed in Table 4.1. For neat P(3HB-co-3HV), $T_{m}^{o}$ value is 187.7 °C. This result is in good agreement with other studies of P(3HB-co-3HV) having different concentrations of 3HV [14, 23, 24]. The value of $T_{m}^{o}$ decreased with increasing LDH-SA content. $T_{m}^{o}$ decreased from 187.7 °C to 183.6, 180.4, 177.2 and 171.3 °C corresponding to 1, 3, 5 and 7 wt% LDH-SA in P(3HB-co-3HV), respectively. A similar result was reported by Hsu et al.[18] in their nanocomposites of poly(3-hydroxybutyrate)/layered double hydroxides nanocomposites. This result suggested that the crystalline structure of P(3HB-co-3HV)/SA nanocomposites was less perfect than that of the pure P(3HB-co-3HV). This behavior could result from the presence of more heterogeneous nucleation induced by the nanofiller and more restriction of the biopolymer chains to pack between the nanofiller, leading to less perfect crystal that caused the $T_{m}^{o}$ shifted to lower temperatures [18]. This depression of the equilibrium melting point can allow processing of P(3HB-co-3HV) at lower melting temperature avoiding the degradation of the biopolymer. Thus, this characteristic enlarges the processing windows of P(3HB-co-3HV).
Figure 4.3 The plot of $T_m$ as a function of $T_c$ for neat P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites showing a decrease in the equilibrium melting point ($T_m^0$) with increasing LDH-SA content.
4.3.2.2 Analysis Based on Avrami Equation by DSC

The isothermal crystallization kinetics of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites can be determined by Avrami equation [25]:

\[ 1 - X_{rel} = \exp(-Kt^n) \] (3)

Here, \( n \) is the Avrami exponent, which is determined by the mode of crystal nucleation and the crystal growth geometry under the experimental conditions and the parameter \( K \) is an isothermal crystallization rate constant associated with both the nucleation and the growth processes. \( X_{rel} \) is the relative degree of crystallinity defined in Equation 1. Taking a ‘double logarithm’ of Equation 3 gives the following:

\[ \log[-\ln(1-X_{rel})] = \log K + n \log t \] (4)

Since the basic Avrami equation (Equation 3) is based on many assumptions such as linear crystal growth, primary nucleation, constant volume, and so forth it is usually applicable at low conversions as long as impingement is not serious [13]. As a result, in this study the \( n \) and \( K \) were calculated from the early linear segment of the Avrami plot. The plot of \( \log[-\ln(1-X_{rel})] \) against \( \log t \) gives a straight line, whose slope is \( n \) and its intercept on the ordinate is \( \log K \).

When \( X_{rel} = 0.5 \) in Equation 3, the half crystallization time, \( t_{1/2} \), which is the time taken for the completion of 50% of ‘total volume’ crystallization, is given as [15]:

\[ t_{1/2} = \left( \ln \frac{2}{K} \right)^{1/n} \] (5)

The plots of relative degree of crystallinity \( X_{rel} \) as a function of crystallization time are shown in Figure 4.4. The slopes of the curve at each point are a measure of the rate of
crystallization. It can be observed that the rate of crystallization keeps constant for $X_{\text{rel}}$ between 0.2-0.7 because those segments of the curves are almost straight. The deviation from the linearity starts at $X_{\text{rel}} = 0.7$, indicating that other factors such as secondary nucleation, exert influences at high conversions.

Figure 4.4  The plot of relative degree of crystallinity $X_{\text{rel}}$ as a function of time at various $T_c$ for P(3HB-co-3HV) and P(3HB-co-3HV)/SA$n$ nanocomposites.
The isothermal Avrami plots of P(3HB-co-3HV) and its nanocomposites are shown in Figure 4.5 and the parameters n, K and t_{1/2} measured in our study are given in Table 4.1. We note that for pure P(3HB-co-3HV), P(3HB-co-3HV)/SA1 and P(3HB-co-3HV)/SA3 samples, the graphs at higher T_c show an initial linear segment during the early stage of crystallization and a tendency to level off due to the existence of secondary crystallization at a later stage [15]. This secondary crystallization is the result of slower crystallization, crystal perfection, and/or spherulites impingement in the later stage of the crystallization process. On the other hand, the upper segment of the graphs of P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7 show almost a straight line. This indicates that at higher concentration of the nucleating agent, the secondary crystallization in the P(3HB-co-3HV) is reduced at higher T_c,s. Also, we observed that for a given sample, all of the fitted plots of the isothermal kinetics observed at various T_c,s are almost parallel with each other.

The Avrami exponent n varies in a narrow range, from 2.34 to 2.69 for neat P(3HB-co-3HV), 2.59 to 2.83 for P(3HB-co-3HV)/SA1, 2.45 to 2.68 for P(3HB-co-3HV)/SA3, 2.67 to 2.87 for P(3HB-co-3HV)/SA5, and from 2.66 to 2.91 for P(3HB-co-3HV)/SA7. This illustrates that the crystal growth may not occur in three dimensions at an equal rate, and hence a low n value may be obtained. According to the Avrami equation, in the ideal state of nucleated crystallization for three-dimensional crystallization growth, the n value should be exactly 3 [15]. However, in the actual process of crystal growth, the practical circumstances cannot satisfy the ideal state that the Avrami equation is supposed to have; hence causing the deviation of the n value from
3. The non-integral n value was reported by other authors [11,15,18] and may be considered because of the presence of mixed nucleation and growth mechanisms. Hence, these results indicate an athermal nucleation process followed by mixed three-dimensional and two-dimensional crystalline growth for P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites, suggesting that the addition of the nanofiller did not change the crystallization mechanism of the biopolymer [18].

For each sample, the value of the crystallization rate constant K increases with decreasing $T_c$, while the half crystallization time $t_{1/2}$ value increases with increasing $T_c$. These results indicated that with the increase of $T_c$, the crystallization rate decreases. The K values of P(3HB-co-3HV)/SAn nanocomposites are much higher than those of the neat P(3HB-co-3HV) at the same crystallization temperature, 63, 66, 69 and 72°C, whereas the $t_{1/2}$ values are much lower. This indicates that the crystallization rate of the P(3HB-co-3HV) in the LDH-SA based nanocomposites is much faster than that of neat P(3HB-co-3HV) at the same crystallization temperature. The addition of the LDH-SA into P(3HB-co-3HV) increases the rate of formation of crystalline phase. Also the values of K increase while those of $t_{1/2}$ decrease with increasing LDH-SA concentration. These results indicate that the incorporation of the LDH-SA enhances the crystallization rate of P(3HB-co-3HV) and the large amount of the nanofiller is more effective than the small one. For example the $t_{1/2}$ value of the neat P(3HB-co-3HV) at 63°C is 1.7, 2.3, 4.3 and 5.9 times as large as that of P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. Thus, the crystallization rate of
P(3HB-co-3HV) in the nanocomposites increases rapidly with LDH-SA concentration at the same temperature.

Figure 4.5 The plots of log [-ln (1-X_w)] as a function of log (t) at various T_c for P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites.
4.3.2.3 Isothermal Crystallization Activation Energy ($\Delta E_A$) by DSC

The crystallization process for the biopolymer and its nanocomposites is assumed to be thermally activated. Therefore the crystallization rate constant parameter $K$ can be approximately described by the following Arrhenius equation to obtain the activation energy [18]:

$$\frac{1}{n(\ln K)} = \ln K_0 - \frac{\Delta E_A}{R T_c}$$  \hspace{1cm} (6)

Where $K_0$, $\Delta E_A$, $R$, $n$ and $T_c$ are the pre-exponential factor, activation energy (Jmol$^{-1}$), universal gas constant ($8.314$ Jmol$^{-1}$K$^{-1}$), Avrami exponent and the absolute crystallization temperature (Kelvin), respectively.

![Figure 4.6 Arrhenius plots of 1/n (lnK) versus 1/RTc for P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites.](image)

Figure 4.6 Arrhenius plots of $1/n (\ln K)$ versus $1/RT_c$ for P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites.

Plotting $1/n(\ln K)$ vs. $(RT_c)^{-1}$ (Figure 4.6), $\Delta E_A$ can be obtained and its value for each sample is shown in Table 4.1. $\Delta E_A$ is the total activation energy which represents both the energy required to transport molecular segments across the phase boundary to the
crystallization surface and the nucleation activation energy, which is the free energy of formation of critical size nuclei at $T_c$. The absolute magnitude of $\Delta E_A$ increases with the incorporation of LDH-SA in the P(3HB-co-3HV) matrix. The addition of LDH-SA into P(3HB-co-3HV) results both in the increased heterogeneous nucleation (higher recrystallization temperatures with increased LDH-SA concentration as shown and discussed in the non-isothermal crystallization section) but more LDH-SA induces more steric hindrance, thus reducing the transport of polymer chains during crystallization.

4.3.2.4 Spherulitic Morphology and Growth Rate Analysis by POM

The rate of isothermal crystallization from the melt of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites was also followed by a hot-stage optical microscopy. The biopolymer and its nanocomposites crystallized with spherulitic morphology. At the crystallization temperatures investigated ($35^\circ C \leq T_c \leq 125^\circ C$), space-filling spherulites were observed to growth at a constant rate until their final impingement. Only large spherulites were observed in the P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites on crystallization. During the spherulites growth, the non-crystallizable LDH-SA nanoparticles remain trapped in the interlamellar space, because their diffusion rate is much slower than the spherulites growth rate. It has been reported that the bacterial origin of P(3HB) and P(3HB-co-3HV) copolymers determines their exceptional purity. It is precisely this purity that facilitates the growth of very large spherulites [26, 27]. Figure 4.7 showed typical spherulitic superstructures of neat P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites isothermally crystallized at $72^\circ C$. 

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Figure 4.7  Polarized optical micrograph of P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites isothermally crystallized at 72°C (scale bar: 100 µm).

All the spherulites exhibit the characteristic of Maltese cross section pattern. Another attractive aspect of the P(3HB-co-3HV) spherulites is the appearance of defined
“banding” extinction patterns. The extinction pattern exhibit double-ringed bands indicating a bi-axial optical character, expected for a polymer crystallizing with an orthorhombic structure [26].

The spherulitic radial growth rate (G) of the neat P(3HB-co-3HV) and its nanocomposites as a function of crystallization temperature (T_c) is reported in Figure 4.8.

![Figure 4.8](image_url)

**Figure 4.8** Spherulitic radial growth rate (G) as a function of crystallization temperature (T_c) for P(3HB-co-3HV) and P(3HB-co-3HV)/SA_n nanocomposites.

A bell-shaped curve is obtained for each sample, indicating the dependence of G on T_c. For pure P(3HB-co-3HV), it can be seen that G has a maximum value at about 72°C. This value shifts to higher values with increasing the content of the nanofiller in the biopolymer matrix (74°C for P(3HB-co-3HV)/SA1, 77°C for P(3HB-co-3HV)/SA3, 79°C for P(3HB-co-3HV)/SA5 and 82°C for P(3HB-co-3HV)/SA7), indicating the
crystallization promoting effect of the nanofiller. It is well known that the crystallization window of a crystalline polymer lies between the glass transition temperature $T_g$ and melting temperature $T_m$. The shift in $T_c^{\text{max}}$ can be explained by the changes in $T_g$ and $T_m$ of the nanocomposites.

The regime theory of polymer crystallization may be used to analyze the growth rate and obtain values for surface energies within crystals [28]. The radial growth rate $G$ of spherulites may be described qualitatively by Turnbull and Fisher equation [29,30]:

$$G = G_o \exp \left[ -\frac{\Delta E^*}{kT_c} \right] \exp \left[ -\frac{\Delta F^*}{kT_c} \right]$$

(7)

$G_o$ is a pre-exponential factor, $k$ is Boltzman constant and $T_c$ is the crystallization temperature. The first term, $\exp \left[ -\frac{\Delta E^*}{kT_c} \right]$ describes the surface nucleation process in which $\Delta E^*$ is the free energy of formation of a surface nucleus with critical size. The second term, $\exp \left[ -\frac{\Delta F^*}{kT_c} \right]$ is a molecular diffusion term in which $\Delta F^*$ is the free energy for transporting molecular segments from the super-cooled phase to the crystalline phase. The two terms are in opposition in the sense that as the crystallization temperature is decreased, the first term decreases and the second increases, explaining the presence of a maximum in the behavior of the growth rate $G$. The transporting term should be dominant for the crystallization rate when $T_c$ approaches the glass transition temperature $T_g$, and the nucleation term should be dominant when $T_c$ approaches the melting temperature $T_m$. As shown in the chapter 3 section 3.3.2 and 3.3.5, the biopolymer exhibited $T_g$ at 13 °C and $T_m$ at 170°C. In this work, $T_c$ ranges from 35 to 125 °C, where both the nucleation and transport processes control the crystallization rate.
On the basis of Turnbull and Fischer equation and to make the later equation compatible for the polymers crystallization in a large degree of supercooling, Lauritzen and Hoffman obtained the famous Lauritzen–Hoffman equation. This equation is thought to govern the linear spherulitic growth rate $G$ versus crystallization temperature profiles. The linear growth rate $G$ of chain-folded polymer crystals is given as [31]:

$$G = G_0 \exp \left[ -\frac{U^*}{R(T_c - T_\infty)} \right] \exp \left[ -\frac{K_g}{fT_c \Delta T} \right]$$  \hspace{1cm} (8)

Where $G_0$ is a growth rate constant that is essentially temperature independent, $U^*$ is the activation energy for the transport of crystallizable segments to the crystal front through the subcooled melt, $R$ is the gas constant equals to 8.314 JK$^{-1}$mol$^{-1}$, $T_\infty$ is the temperature below which segmental motions cease, $\Delta T = T_m^0 - T_c$ the degree of supercooling where $T_m^0$ is the equilibrium melting point and $T_c$ is the crystallization temperature. The correction factor $f$ for the temperature dependence of the equilibrium enthalpy of fusion is equal to $2T_c/(T_m^0 + T_c)$. All the temperature must be expressed in Kelvin. $K_g$ the nucleation parameter representing the free energy required to form a nucleus of critical size, can be expressed as:

$$K_g = \left[ \frac{\beta b_o \sigma \sigma_e T_m^0}{k \Delta H_m^0} \right]$$  \hspace{1cm} (9)

Where $b_o$ is the molecular layer thickness, $\sigma$ and $\sigma_e$ are the lateral and fold surface free energy of the growing crystals, respectively, $k$ the Boltzmann constant equals to $1.38 \times 10^{-23}$ JK$^{-1}$, and $\Delta H_m^0$ the heat of fusion of perfect crystal. The parameter $\beta$ is related to the characteristic of the crystallization growth and may have the value of 2 or 4. Hoffman proposes that there are three distinct regimes (I, II and III) of growth of polymer crystals,
depending on the relative rates of formation of new secondary nuclei on the growth front and the rate at which the nuclei once formed spread along the growth front. At low supercoolings the rate of spreading is so large compared to the rate of nucleation that a nucleus once formed spreads right across the growth front: regime I. At higher supercoolings, several nuclei form and spread across the front together, the separation between them decreasing as supercooling increases: regime II. At a sufficiently high supercooling the separation is of the order of the molecular width, when no more spreading takes place: Regime III. The three regimes may be distinguished by the value of the constant $\beta$ in Equation 9. $\beta$ changes from 4 in regime I to 2 in regime II and back to 4 in regime III. This latest theory due to Hoffman differs from all earlier theories in that it introduces the third regime (regime III) at high supercoolings.

Equation 8 can be converted as:

$$\ln G + \frac{U^*}{R(T_c - T_\infty)} = \ln G_0 - \left[ \frac{K_g}{fT_c\Delta T} \right]$$

Equation 10

To analyze the growth kinetics of P(3HB-co-3HV) spherulites in the framework of Lauritzen-Hoffman theory, the first member of Equation 10 can be plotted against $1/fT_c\Delta T$. Thus, $K_g$ and $G_0$ can be obtained. The data represented in the growth rate curves for P(3HB-co-3HV) were used in an attempt to fit Equation 10. In order to model this equation, thermodynamic values of $T_m^0$, $U^*$ and $T_\infty$ need to be estimated. $T_m^0$ are obtained from $T_m$ against $T_c$ plots and are summarized in Table 4.1. Organ and Barham [28] found that $U^*= 10.25 \text{ kJmol}^{-1}$ showed excellent fits for P(3HB) and P(3HB-co-3HV). $T_\infty (288K)$ is taken from chapter 3 section 3.3.2. Akhtar et al.[32] showed that $T_\infty$
values of about 293-323 K gave good fits for P(3HB-co-3HV) biopolymer. For our estimation, $T_\infty$ taken as $T_g - 30K$ (258K) showed excellent fits. For neat P(3HB-co-3HV), the best-fit plot derived from the thermodynamic values is plotted according to Equation 10 and presented in Figure 4.9. The most notable feature on the plot in Figure 4.9 for P(3HB-co-3HV) is that all the growth rate data (covering a crystallization temperature range from 35 to 125°C) fall on a single line. This meant that no regime transition was shown from 35 to 125°C. Similar result was obtained by Organ et al.[28] and Pen et al.[29]. They identified the transition from regime II to regime III around 130-140 °C. Therefore, our data for the crystallization temperature range studied (35-125°C) for P(3HB-co-3HV) can be attributed to regime III. The slope $K_g$ and the intercept $G_0$ values derived for P(3HB-co-3HV) from the fitted growth rate data are given in Table 4.2. The slope is $4.03 \pm 0.37 \times 10^5$ K$^2$. Peng et al.[29] investigated the isothermal crystallization behavior by POM between 75 and 115 °C of P(3HB-co-3HV) containing 8 mol% of HV. Their nucleation parameter $K_g$ obtained from a regime III was found to be $3.14\pm0.07 \times 10^5$ K$^2$, which is smaller than ours.

To confirm to which regime, the growth rate data in selected crystallization temperature range belong to, we applied the Lauritzen Z test [33]:

$$Z = 10^3 \left( \frac{L}{2a_0} \right)^2 \exp \left[ - \frac{X}{T_c \Delta T} \right]$$ (11)

Where L is the effective lamellar thickness and $a_0$ is the molecular layer width in the crystal. According to this test, if the substitution of $X = K_g$ into the test results in $Z \leq 0.01$, regime I or III kinetics are followed. On the other hand if $X = 2K_g$ into the test
yields $Z \geq 1.0$, regime II kinetics are followed. As pointed out by Lauritzen and Hoffman, it is more convenient to use a known value of $K_g$ and the inequalities for $Z$ to obtain the values of $L$ in regime I, II or III and to estimate if such values of $L$ are realistic.

Kunioka et al. [8] studied the crystalline structure of P(3HB-co-3HV). They found that, when the 3-HV content in P(3HB-co-3HV) is lower than 37 mol%, only the parameter $a$ of the unit cell changes with increasing 3-HV content in P(3HB-co-3HV). For the 3-HV content up to 37 mol%, the crystal lattice of P(3HB-co-3HV) is the same as that of P(3HB). Therefore, the unit cells of P(3HB-co-3HV) (18 mol% 3-HV content) investigated in our study can be considered orthorhombic (space group P2$_1$2$_1$2$_1$) with $a = 5.8$ Å, $b = 13.2$ Å and $c$ (fiber axis) = 5.96 Å [8]. The values of the molecular layer width $a_0$ and thickness $b_0$ (in Equation 9) can be calculated and are equal to 6.6 Å and 5.8 Å, respectively [29].

Assuming $Z \leq 1.0$ and substituting $X = 2K_g$ into the test, $L \leq 3.5 \times 10^4$ Å, which is clearly impossible. Assuming $Z \leq 0.01$ and substituting $X = K_g$ into the test, $L \leq 12.5$ Å, and this calculation is reasonable for P(3HB-co-3HV). This calculation confirmed that the crystallization regime of the present data at $35 \ ^\circ C \leq T_c \leq 125^\circ C$ is attributed to be regime III, and the parameter $\beta = 4$ can be adopted.

In view of the analysis of P(3HB-co-3HV) spherulite growth in the presence of LDH-SA, additional terms must be considered to account for the presence of the nanofiller, [14, 34] and consequently Equation 8 must be adapted. For polymer-diluent system, Equation 8 was modified by Boon and Azcue [35] to describe the growth rate of spherulites of a crystallizable polymer in a single-phase melt containing a diluent:
\[ G = \varphi_2 G_0 \exp \left( \frac{-U^*}{R(T_c - T_\infty)} \right) \exp \left( -\frac{K_g}{fT_c\Delta T} \right) + \left( \frac{2\sigma T_m^0 \ln \varphi_2}{f b_0 \Delta H_m^0 \Delta T} \right) \] (12)

Where \( \varphi_2 \) is the volume fraction of crystallizable polymer. The pre-exponential factor \( G_0 \) is multiplied by \( \varphi_2 \) to account for the dilution due to the nanofiller because the rate of nucleation is proportional to the concentration of the polymer crystallizable units. The additional term in the second exponential is an entropic contribution to the free energy of activation for nucleus formation, which accounts for the probability of selecting the required number of crystallizable polymer sequences from the mixture at the given \( \varphi_2 \) concentration.

It is suitable to rearrange Equation 12 in the form:

\[ \ln G + \frac{U^*}{R(T_c - T_\infty)} - \ln \varphi_2 (1 + \frac{2\sigma T_m^0}{f b_0 \Delta H_m^0 \Delta T}) = \ln G_0 - \left[ \frac{K_g}{fT_c\Delta T} \right] \] (13)

The left-hand side (hereafter named as Kappa) of Equation 13 predicts a linear dependence on \( 1/fT_c\Delta T \). The slope and the intercept of the line yield \( K_g \) the nucleation constant and \( G_0 \) the growth rate constant, respectively for P(3HB-co-3HV)/SAn nanocomposite samples.

In order to model the Equation 13 and to obtain the best fit, the literature value of the activation energy \( U^* = 10.25 \text{ kJmol}^{-1} \) was used, whereas \( T_m^0 \) were taken from Table 4.1 and \( T_\infty \) was kept at 258 K. The heat of fusion \( \Delta H_m^0 \) of 100\% crystalline P(3HB-co-3HV) was not measured directly, but was taken from literature and is equal to \( 1.65 \times 10^8 \text{ Jm}^{-3} \) [29]. For each P(3HB-co-3HV)/SAn nanocomposite sample, kappa was plotted against \( 1/fT_c\Delta T \) and reported in Figure 4.9. For each nanocomposite sample, the growth
rate data fall on the single line as in the case for neat P(3HB-co-3HV). The derived $K_g$ and $G_0$ values for each nanocomposite sample were listed in Table 4.2. In our work, we observe that $K_g$ and $G_0$ decrease with increasing the content of the nanofiller. This result meant that the presence of LDH-SA in P(3HB-co-3HV) matrix led to a decrease in the energy barrier for secondary nucleation.

![Figure 4.9](image)

Figure 4.9  Plot of Kappa versus $1/(fT_c \Delta T)$ for P(3HB-co-3HV) and P(3HB-co-3HV)/SAn nanocomposites: Kappa = $\ln G + \left[ U^*/R(T_c-T_\infty) \right] - \ln \varphi_2 \left[ 1 + (2\sigma T_m/fb_0 \Delta H_m \Delta T) \right]$. 
In the same way as for neat P(3HB-co-3HV), we conducted Lauritzen Z test on
the P(3HB-co-3HV)/SAn nanocomposite samples and concluded that their crystallization
belonged to the regime III too.

4.3.2.5 Surface Free Energies and Work of Chain Folding by POM

The lateral surface energy $\sigma$ of the developing crystals can be calculated by
using the relation suggested by Lauritzen and Hoffman [36] based on Thomas-Staveley
relation [37].

$$\sigma = \alpha_{LM} \Delta H_m^0 \left( \frac{a_0 b_0}{2} \right)^{1/2}$$  \hspace{1cm} (14)

Where $\alpha_{LM}$ is an empirically determined constant and is equal to 0.25 for high-melting
polyesters, $a_0 b_0$ is the cross sectional area of the P(3HB-co-3HV) chain [36]. The value of
$\sigma$ calculated from Equation 14 is $2.6 \times 10^{-2}$ Jm$^{-2}$ (26 erg.cm$^{-2}$).

The derived $K_g$ (Equation 9) can be used to calculate the fold surface energy $\sigma_e$ for
P(3HB-co-3HV) and its nanocomposites. Table 4.2 summarized $\sigma_e$ and the product $\sigma \sigma_e$
for P(3HB-co-3HV) and its nanocomposites. The value of $\sigma_e$ for the neat biopolymer is
$32.91 \pm 0.03$ erg.cm$^{-2}$. We observe that the value of $\sigma_e$ decreased with increasing the
content of the nanofiller. Generally speaking, the smaller the fold surface free energy $\sigma_e$,
the faster the crystallization rate of polymer crystal. In the P(3HB-co-3HV)/SAn
nanocomposites, the fold surface free energy $\sigma_e$ decreased with increasing the content of
LDH-SA, indicating that the incorporation of the nanofiller into P(3HB-co-3HV) may
induce the heterogeneous nucleation of P(3HB-co-3HV) crystallization and then decrease
the surface energy barrier for P(3HB-co-3HV) crystallization. The result showed that the conclusion based on Lauritzen-Hoffman theory is consistent with the analysis by Avrami equation.

Finally, the work of chain folding, $Q$, which is most closely correlated with molecular structure, can be calculated from the following relation [29]:

$$Q = 2a_0b_0\sigma_e$$ \hspace{1cm} (15)

The values of $Q$ are given in Table 4.2. $Q$ obtained for the neat P(3HB-co-3HV) is $1.26 \times 10^{13}$ erg per molecular chain fold. We observed that the value of $Q$ decreased with increasing the content of LDH-SA. This decrease could have meant that P(3HB-co-3HV) in the nanocomposites will take less energy than the neat P(3HB-co-3HV) to yield fold-chain crystals, indicating the crystallization promoting effect of LDH-SA.

In sum, we investigated in details the effect of LDH-SA on P(3HB-co-3HV) crystallization in view of the Avrami, Arrhenius and Lauritzen-Hoffman models. We concluded that LDH increased P(3HB-co-3HV) crystallization and a large amount of the nanofiller is effective than a small amount. LDH-SA enhanced the arrangement of P(3HB-co-3HV) molecular chains and caused an increase of crystallizing the biopolymer chains.
Table 4.1 Effect of LDH-SA on the crystallization parameters of P(3HB-co-3HV).

<table>
<thead>
<tr>
<th>Sample</th>
<th>T_c (°C)</th>
<th>n ± 0.10</th>
<th>K± 0.001</th>
<th>t_{1/2} ± 0.002</th>
<th>ΔE_A</th>
<th>R^2(a)</th>
<th>T''_m (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>63</td>
<td>2.34</td>
<td>0.041</td>
<td>3.348</td>
<td>-60.16 ± 2</td>
<td>0.98</td>
<td>187.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>2.39</td>
<td>0.028</td>
<td>3.829</td>
<td>-72.23 ± 3</td>
<td>0.99</td>
<td>183.6 ± 0.4</td>
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<tr>
<td></td>
<td>69</td>
<td>2.62</td>
<td>0.012</td>
<td>4.703</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2.69</td>
<td>0.005</td>
<td>6.255</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>63</td>
<td>2.83</td>
<td>0.111</td>
<td>1.956</td>
<td>-81.41 ± 5</td>
<td>0.99</td>
<td>180.4 ± 0.3</td>
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<tr>
<td></td>
<td>66</td>
<td>2.79</td>
<td>0.061</td>
<td>2.435</td>
<td>-99.48 ± 4</td>
<td>0.99</td>
<td>177.2 ± 0.4</td>
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<tr>
<td></td>
<td>69</td>
<td>2.67</td>
<td>0.035</td>
<td>2.632</td>
<td></td>
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<tr>
<td></td>
<td>72</td>
<td>2.59</td>
<td>0.022</td>
<td>3.250</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>63</td>
<td>2.68</td>
<td>0.276</td>
<td>1.472</td>
<td>-111.10 ± 5</td>
<td>0.99</td>
<td>171.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>2.68</td>
<td>0.107</td>
<td>2.207</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>2.56</td>
<td>0.076</td>
<td>2.558</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>72</td>
<td>2.45</td>
<td>0.035</td>
<td>3.195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>63</td>
<td>2.59</td>
<td>1.391</td>
<td>0.782</td>
<td>-111.10 ± 5</td>
<td>0.99</td>
<td>171.3 ± 0.5</td>
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<td>66</td>
<td>2.67</td>
<td>0.638</td>
<td>1.032</td>
<td>-99.48 ± 4</td>
<td>0.99</td>
<td>177.2 ± 0.4</td>
</tr>
<tr>
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<td>69</td>
<td>2.81</td>
<td>0.239</td>
<td>1.461</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2.87</td>
<td>0.090</td>
<td>2.037</td>
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<td></td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>63</td>
<td>2.79</td>
<td>3.430</td>
<td>0.564</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66</td>
<td>2.91</td>
<td>1.370</td>
<td>0.791</td>
<td>-111.10 ± 5</td>
<td>0.99</td>
<td>171.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>2.89</td>
<td>0.482</td>
<td>1.134</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>2.66</td>
<td>0.207</td>
<td>1.575</td>
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</tbody>
</table>

(a) Regressional analysis for ΔE_A determination
Table 4.2  Values of $T_c$ at $G_{max}$, $K_g$, and $G_0$, $\sigma_e$ and $Q$ for P (3HB-co-3HV) and P (3HB-co-3HV) /SA$n$ nanocomposites.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_c$ at $G_{max}$ ($^\circ$C)</th>
<th>$K_g \times 10^5$ (K$^2$)</th>
<th>$R^2$ (a)</th>
<th>$G_0$ (cm$^{-1}$)</th>
<th>$\sigma_e$ (erg cm$^{-2}$)</th>
<th>$\sigma_\sigma_e$ (erg$^2$ cm$^{-4}$)</th>
<th>$Q$ (erg$\times 10^{13}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>72.0</td>
<td>4.03 ± 0.37</td>
<td>0.99</td>
<td>36879</td>
<td>32.91</td>
<td>855.66</td>
<td>1.26</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA1</td>
<td>74.0</td>
<td>3.39 ± 0.25</td>
<td>0.99</td>
<td>22026</td>
<td>27.90</td>
<td>725.40</td>
<td>1.07</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA3</td>
<td>77.0</td>
<td>2.76 ± 0.15</td>
<td>0.97</td>
<td>13359</td>
<td>22.87</td>
<td>594.62</td>
<td>0.88</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA5</td>
<td>79.0</td>
<td>2.15 ± 0.08</td>
<td>0.99</td>
<td>9996</td>
<td>17.94</td>
<td>466.44</td>
<td>0.69</td>
</tr>
<tr>
<td>P(3HB-co-3HV)/SA7</td>
<td>82.0</td>
<td>1.38 ± 0.03</td>
<td>0.98</td>
<td>4914</td>
<td>11.67</td>
<td>303.42</td>
<td>0.45</td>
</tr>
</tbody>
</table>

(a) Regressional analysis for $K_g$ determination

4.4 Conclusion

Crystallization kinetics of P(3HB-co-3HV) and its nanocomposites have been investigated. Non-isothermal crystallization from the melt showed the parent P(3HB-co-3HV) lacked a melt recrystallization peak $T_{mc}$. With the addition of the LDH-SA, $T_{mc}$ appeared which shifted to higher crystallization temperature with increasing the content of the nanofiller. The above fact indicated a crystallization promoting effect of LDH-SA in P(3HB-co-3HV). To explore in detail the crystallization behavior of P(3HB-co-3HV), the Avrami model and Lauritzen-Hoffman theory were employed. Isothermal crystallization results from DSC measurements showed that the addition of the nanofiller induced more heterogeneous nucleation in the crystallization significantly increasing the crystallization rate and the absolute magnitude of the activation energy $\Delta E_A$. Hoffman-
Weeks plots were employed to estimate the equilibrium melting points $T^0_m$ of P(3HB-co-3HV) and its nanocomposites. In neat P(3HB-co-3HV), $T^0_m$ was $187.7 \pm 0.5 \, ^\circ C$. Increasing the content of LDH-SA in the P(3HB-co-3HV) matrix resulted in lowering the equilibrium melting point after isothermal crystallization. The value of Avrami exponent $n=2.34-2.91$ illustrated that crystal growth may not occur in three dimensions at an equal rate and the addition of LDH-SA did not change the mechanism of nucleation and growth of P(3HB-co-3HV). The experimental data of spherulitic growth rate were analyzed according to the polymer-diluent theory based on Lauritzen-Hoffman model. The results indicated regime III crystallization for P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites. The nucleation constant $K_g$, the folded surface free energy $\sigma_c$ and the work of chain folding $Q$ of P(3HB-co-3HV) crystals are $4.03 \pm 0.37 \, K^2$, $32.91 \pm 0.04 \, \text{erg/cm}^2$ and $1.26 \times 10^{13} \, \text{erg}$, respectively. These values decreased with increasing LDH-SA content. These results suggested that the incorporation of the LDH-SA into P(3HB-co-3HV) induced heterogeneous nucleation of the biopolymer crystallization and decreased the surface energy barrier for P(3HB-co-3HV) crystallization.
4.5 References


CHAPTER 5

VISCO-ELASTIC PROPERTIES OF POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) / LAYERED DOUBLE HYDROXIDE NANOCOMPOSITES STUDIED BY DYNAMIC MECHANICAL ANALYSIS AND DIELECTRIC SPECTROSCOPY

5.1 Introduction

The addition of the nanoparticles in polymeric material is one way for changing the viscoelastic properties of this latter. The viscoelastic properties can be altered while the desired characteristics of the polymer matrix, such as chemical inertness, thermal stability, mechanical, electrical properties, or self-healing properties can be maintained or improved. In addition to the inclusions themselves, the interface, a special region of polymer chains in the vicinity of the nanoparticles, also can play an important role in the improvement of the polymer nanocomposite properties [1]. The existence of nanoparticles surfaces in the polymer can alter the mobility of the polymer chains surrounding them. Such perturbations in the polymer molecular mobility can extend several radii of gyration and create regions of polymer, the interface, with properties and response different from that of the host bulk polymer [1]. Due to the large surface to volume ratio of the nanoparticles, the amount of interface polymer generated in nanocomposites can be considerable.
The significant change of the viscoelastic properties is one example that cannot be well explained without considering the contribution of the interface polymer [1]. Recent experimental studies on the polymer nanocomposites show that the transition zones of storage/loss modulus and loss tangent curves may be broadened (if the interface creates only local changes in the polymer relaxation time) or shifted (if the interface causes global changes in the polymer relaxation time) in the time/temperature domain [1-4]. Since the properties of the nanoparticles are not time/temperature dependant within the experimental measurement scale, those enhancements cannot be simply attributed to the existence of the nanoparticles. Dynamic mechanical analysis (DMA) through time temperature superposition (TTS) investigation can contribute in accessing the above change.

In amorphous domain of polymer matrix, it is qualitatively understood that an attractive interface will decrease the mobility of the polymer chains and a repulsive interface will increase the mobility [5,6]. One way for probing this change in the mobility of the polymer chains in the interfacial region is to measure the glass transition temperature using DMA, dielectric spectroscopy (DES) and differential scanning calorimetry (DSC). Using these methods can show that a glass transition temperature of a polymer nanocomposite can be raised or lowered (if the interface causes global changes in the polymer relaxation time) with the addition of the nanoparticles with attractive and repulsive interaction with the polymer matrix, respectively.

DMA and DES have proven to be very useful tools to study the structure and the dynamic of polymeric systems [7]. DMA and DES measure changes in the properties of a
material as a response to the application on it of a time dependant mechanical force and electric field, respectively. An advantage of DES over DMA is the possibility of using a wider frequency range. In addition, dielectric measurements are extremely sensitive to small changes in material properties. This enables detection of transitions which would not be possible through DMA. In particular, DES complements DMA for characterization of the internal motions in polymers.

In an effort to fully understand the effect of the addition of layered double hydroxide on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or P(3HB-co-3HV), a comprehensive study of the viscoelastic properties of the biopolymer was carried out. DMA through time-temperature superposition (TTS) was used to determine the effect of the addition of the nanoparticles on the storage modulus and loss tangent $T_g$ of the biopolymer. DES was used to complement the result on $T_g$.

5.2 Experimental

5.2.1 Materials

P(3HB-co-3HV) (Mw=250,000-400,000 Daltons) with 18 mol% 3-HV content was used as polymeric material. Its description is given in the chapter 3 section 3.2.1. The Zn-Al nitrate layered double hydroxide, organically modified with stearic acid or LDH-SA was used as nanofiller. Its synthesis in our laboratory has been described in the chapter 3 section 3.2. Nanocomposites of P(3HB-co-3HV) and LDH-SA were fabricated by the solution casting technique described in the chapter 3 section 3.2.3. The LDH-SA content of the fabricated films was 1, 3, 5, and 7 wt%. As in the chapter 3 and 4, samples
in this work are designated as P(3HB-\textit{co}-3HV)/SAn, where \(n\) is the amount of the LDH-SA used in the preparation of the nanocomposite.

5.2.2 Dynamic Mechanical Analysis (DMA)

The dynamic properties of P(3HB-\textit{co}-3HV) and P(3HB-\textit{co}-3HV)/SAn nanocomposites were measured by means of Rheometric Scientific Analyzer 3 (RSA3, Newcastle, DE) using rectangular specimen (length 25 mm, width 5 mm, and thickness 0.12 mm) in a tensile mode. Data acquisition was done using RSI Orchestrator software. Frequency sweep was carried out in the temperature range of -30 to 50°C and data were taken at every 5°C. A ramp and soak program was used to control the temperature profile. At each temperature, the specimen was soaked for 5 min before testing. Storage modulus, \(E'\) and loss modulus, \(E''\) were measured in the frequency range of 16-0.16 Hz. Strain amplitude (0.25%) and an initial static force (200 g) were used in all the measurements.

5.2.3 Dielectric Spectroscopy (DES) Measurements

DES measurements of P(3HB-\textit{co}-3HV) and its nanocomposites were performed by the means of Advanced Rheometric Expansion System (ARES, TA Instruments, Newcastle, DE) interfaced with an Agilent E4980 A Precision LCR meter (20 Hz-2 MHz). Circular parallel plate geometry having a diameter of 25 mm was used. Dielectric loss of P(3HB-\textit{co}-3HV) and the nanocomposites (diameter 25 mm and thickness 0.5 mm)
were measured in compression mode at a frequency of 500 Hz and at temperature of -150 to 100 °C.

5.3 Results and Discussion

5.3.1 DMA: Time-Temperature Superposition (TTS)

Since the behavior of a polymer at high frequency is related to the behavior at very low temperature, and vice versa, the frequency sweep tests at over a range of temperature can be used to understand the behavior at extremes temperatures outside the experimental range, using time-temperature-superposition (TTS) method.

TTS has long been used to obtain temperature-independent master curves for polymer systems by shifting the values of storage/loss modulus (E’) towards the frequency axis. One reference temperature $T_{\text{ref}}$ should be chosen (here $T_{\text{ref}} = 10^\circ\text{C}$) and the viscoelastic variables of interest (e.g., storage modulus) at other temperatures are shifted to the corresponding values at $T_{\text{ref}}$. A horizontal shift factor, $a_T$, which is a function of temperature enables to obtain the master curves. For neat biopolymer and its nanocomposites $a_T$ versus temperature curves are shown in Figure 5.1.

For thermorheologically simple materials, at temperatures less than the glass transition $T_g$, the $a_T$ curve follows an Arrhenius relationship as indicated by Equation 1.

$$\ln a_T = \frac{E_A}{R} \left(\frac{1}{T} - \frac{1}{T_g}\right) \quad (1)$$
One can relate the shift factor, $a_T$, to the glass transition temperature, $T_g$, and to the activation energy $E_A$, which is related to the steepness of the energy barrier required to change the quasi-equilibrium state of the system from one configuration to another [8]. At temperature greater than $T_g$, the substantial effects of free volume make $a_T$ curve to follow the Williams-Landel-Ferry (WLF) relationship as indicated by Equation 2.

\[
\log a_T = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \quad (2)
\]

One can relate the shift factor, $a_T$, to $T_g$ of a polymer and two constant, $C_1$ and $C_2$, which have been found to be characteristic of the polymer molecular structure (Equation 2).

Figure 5.2 shows log($E'$) versus log(frequency) time-temperature master curves of P(3HB-co-3HV) and its nanocomposites. Reinforcement of the biopolymer with the addition of the LDH-SA can be seen over the frequency range studied. The storage modulus of the nanocomposites increases with increasing the content of the nanofiller in the biopolymer matrix. This increase is due to the restricted movement of the biopolymer chains and rigidity of nanocomposite samples. However the extent of reinforcement depends on the nanofiller concentration. 7 wt% nanofiller exhibit low reinforcement with comparison to 5 and 3 wt%. These results are consistent with those of single frequency DMA obtained in the chapter 3 section 3.3.2.
Figure 5.1 Horizontal shift factor $a_T$ versus temperature for P(3HB-co-3HV) and P(3HB-co-3HV)/SA nanocomposites showing Arrhenius and WLF regions from $T_{ref}$.

Figure 5.2 Dynamic mechanical time–temperature master curve at a reference temperature ($T_g = 10^{\circ}C$) of the storage modulus of P(3HB-co-3HV) and P(3HB-co-3HV) / SA nanocomposites.
Figure 5.3 shows log$(E'' \rangle$ versus log(frequency) time-temperature master curves of P(3HB-co-3HV) and its nanocomposites. The depression of the maximum $E''$ peak can be seen with increasing the amount of the nanofiller. This result could indicate a modification in the relaxation time of the biopolymer as a result of positive interaction between this latter and the LDH-SA.

Table 5.1 summarizes the activation energy $(E_A)$ parameter according to Arrhenius Equation and the WLF constants $C_1$, $C_2$ derived from Williams-Landel-Ferry (WLF) Equation. We observe that $E_A$, $C_1$ and $C_2$ increase with increasing the content of the nanofiller. $E_A$ increases due to strong interaction between the biopolymer and the
nanofiller. The WLF constants \( C_1 \) (19.2-45.0) and \( C_2 \) (76.5-196.3) are different from their universal values which are 17.4 and 51.6 K for \( C_1 \) and \( C_2 \), respectively. An increase in \( C_1 \) and \( C_2 \) values after addition of the nanofiller indicates a decrease in \( f_g \) fractional free volume at \( T_g \) and \( \alpha_f \) volumetric expansion of free volume as shown by Equation 3 and 4, respectively. The decrease in \( f_g \) indicates better biopolymer-nanofiller interaction.

\[
f_g = \frac{1}{2.303C_1} \quad (3)
\]

\[
\alpha_f = \frac{f_g}{C_2} \quad (4)
\]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arrhenius</th>
<th>WLF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( E_A ) (kJmol(^{-1}))</td>
<td>( R^2 )</td>
</tr>
<tr>
<td>P(3HB-co-3HV)</td>
<td>338.02</td>
<td>0.9946</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA1</td>
<td>347.13</td>
<td>0.9966</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA3</td>
<td>355.33</td>
<td>0.9964</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA5</td>
<td>373.03</td>
<td>0.9893</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA7</td>
<td>380.18</td>
<td>0.9941</td>
</tr>
</tbody>
</table>

\( R^2 \): Regressional analysis

5.3.2 DES: Dielectric Loss (\( \varepsilon'' \))

\( \varepsilon'' \) data of P(3HB-co-3HV) and its nanocomposites were used to access the interaction between the biopolymer and the inorganic filler. Figure 5.4 shows \( \varepsilon'' \) versus temperature curves for all the samples. Temperature dependence of \( \varepsilon'' \) can be seen. Neat biopolymer exhibits two relaxations: one at about -60°C denoted as \( \beta \) and second-
some 15°C, as α (Figure 5.4 and Table 5.2). We observe that the addition of the nanofiller did not change β relaxation whereas it did change α. The α relaxation which is considered as glass transition temperature shifted to higher temperature with the addition of the inorganic filler, indicating global changes in the relaxation time of the biopolymer. It shifted from 15°C for neat P(3HB-co-3HV) to 22, 23, 25 and 28 for P(3HB-co-3HV)/SA1, P(3HB-co-3HV)/SA3, P(3HB-co-3HV)/SA5 and P(3HB-co-3HV)/SA7, respectively. We explain this behavior as attractive interactions between the biopolymer and the nanofiller at interfaces. Moreover, the depression of the maximum ε’’ peak can be seen with increasing the amount of the nanofiller. This result could indicate a modification in the relaxation time of the biopolymer as a result of positive interaction between this latter and the LDH-SA. This result is consistent with the loss modulus E’’ time-temperature master curves results. The shift to higher glass transition temperature shows that DES measurements are extremely sensitive to small changes in material properties in comparison to DMA.

Table 5.2 Effect of LDH-SA on the glass transition temperature T₉ of P(3HB-co-3HV).

<table>
<thead>
<tr>
<th>Sample</th>
<th>T₉ ( °C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-3HV)</td>
<td>15</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA1</td>
<td>22</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA3</td>
<td>23</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA5</td>
<td>25</td>
</tr>
<tr>
<td>P(3HB-co-3HV) / SA7</td>
<td>28</td>
</tr>
</tbody>
</table>
Figure 5.4  Temperature dependence of dielectric loss $\varepsilon''$ of P(3HB-co-3HV) and P(3HB-co-3HV) / SAn nanocomposites showing the effect of the addition of LDH-SA

5.4 Conclusion

The effect of the addition of LDH-SA on the viscoelastic properties of P(3HB-co-3HV) was investigated using DMA and DES. The storage modulus results show that LDH-SA acted as a reinforcement of the biopolymer matrix over the frequency range studied. The loss modulus results show peak depression, indicating modification in the relaxation time of the biopolymer as a result of positive interaction between this latter and the nanofiller. We observe that $E_A$, $C_1$ and $C_2$ increase with increasing the content of LDH-SA. $E_A$ increases due to strong interaction between the biopolymer and the nanofiller. Increase in $C_1$ and $C_2$ values after addition of the nanofiller indicates a
decrease in $f_g$ fractional free volume at $T_g$. The decrease in $f_g$ indicates better biopolymer-nanofiller interaction. The dielectric loss results show a shift and a depression of the glass transition of the biopolymer with increasing LDH-SA content, indicating global changes in the relaxation time of the biopolymer as a result of positive interaction between this latter and the LDH-SA. DES results complement those of DMA for characterization of the internal motions in P(3HV-co-3HV) and its nanocomposites.
5.5 References


CHAPTER 6

EFFECT OF A SYNTHETIC NANOCLAY ON THE AEROBIC BIODEGRADABILITY BEHAVIOR OF POLY (3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) IN A LABORATORY AUTOMATED MULTI-UNIT COMPOSTING SYSTEM

6.1 Introduction

Environmental problems caused by non-degradable plastics have led to the development and use of biodegradable plastics. Biodegradable plastics are of growing importance as one of the alternatives to traditional resistant petroleum-based plastics. Tokiwa and Calabia [1] summarized the advantages of biodegradable plastics hereafter. They can be composted with organic wastes and return to enrich soil. In comparison to traditional synthetic plastics, their utilization will not only diminish injuries to wildlife but will also lessen the labor cost for the removal of plastic wastes in the environment because they degrade naturally. In addition, the decomposition of biodegradable plastics will help increase the longevity and stability of landfills by reducing the volume of garbage. Lastly, these bioplastics could be recycled to useful monomers and oligomers by enzymatic and microbial treatment.

Biodegradable plastics must be designed to meet both durable and biodegradable approaches i.e. the designed materials must be resistant during their utilization and must have biodegradable properties at the end of their valuable life [2]. There is a vital need to
study the biodegradability of biodegradable plastics in natural environment in order to expand their use. Therefore, exploring and understanding the mechanism that involve in the biodegradation of polymeric biodegradable materials will contribute in developing future materials. For the determination of the biodegradability of polymeric materials, composting has been recognized as one of the most valuable method because it is the most environmentally friendly, and thus recommended, method for treating organic solid wastes [3,4].

Standard composting test methods such as American Standardization for Testing and Materials (ASTM); and International Organization for Standardization (ISO) are used to evaluate the biodegradation processes of biodegradable polymeric materials [5, 6]. These test methods measure the bio-oxidation of organic materials to carbon dioxide, water, mineral salts and biomass. The quantity of carbon dioxide evolved under the designated composting conditions of moisture, pH and temperature can be determined. The carbon dioxide produced during the composting is related to the total carbon content of the material.

ASTM D-5338-98 (2003) recommends that oxygen O₂ is supplied evenly to all the composting bioreactors [7]. The carbon dioxide CO₂ from the outlet gas of the composting bioreactors can be measured using a CO₂-trapping apparatus and titration equipment. However, these devices can be replaced by a gas flow meter plus a gas chromatograph, or other apparatus equipped with suitable detector and column.

We have developed a laboratory automated multi-unit composting system following the requirements of ASTM D-5338-98 (2003). To measure the concentration of
CO₂ in the outlet gas of each bioreactor, a nondispersive infrared (NDIR) gas analyzer was integrated into the system. A detailed description of the system is described in this work. For the evaluation of the biodegradability using this system, the results showing the effect of a synthetic clay-layered double hydroxide on the biodegradability of a biopolyester, poly(3-hydroxybutyrate-co-3-hydroxyvalerate), were presented and compared to those of cellulose used as a reference material.

6.2 Experimental
6.2.1 Materials

Microcrystalline cellulose powder (particle size of 20 µm) was obtained from Sigma-Aldrich (St Louis, MO). Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or P(3HB-co-3HV), a biopolymer, and Zn-Al stearate Layered double hydroxide or LDH-SA, a synthetic clay, were used. Both materials were described in the chapter 3 sections 3.2.1 and 3.2.2, respectively. Films of pure P(3HB-co-3HV) and nanocomposite made of 95 wt% P(3HB-co-3HV) and 5 wt% LDH-SA were ground into fine pieces. The preparation of these films was described in the chapter 3 section 3.2.3. The physical, thermal and mechanical properties of the biopolymer and its nanocomposite samples were discussed in the chapter 3 sections 3.3.1 to 3.3.5. The carbon contents determined by CHN elemental analysis (Elemental Analysis Inc., Lexington, KY) of the biopolymer, nanocomposite and the microcrystalline cellulose were 56.19, 55.74 and 42.10 %, respectively. Neat P(3HB-co-3HV) and nanocomposite made of 95 wt% P(3HB-co-3HV) and 5 wt% LDH-SA samples are designated as P(3HB-co-3HV) and P(3HB-co-3HV)/SA5, respectively.
6.2.2 Description of the Automated Multi-unit Composting System (AMUCS)

AMUCS is designed following the American Society for Testing and Materials ASTM D-5338-98 (2003) standard [7]. This standard allows for the minimum of twelve composting bioreactors. A representative block diagram and a picture of AMUCS are shown in Figure 6.1 and 6.2, respectively. AMUCS can be divided into three subsystems which are the water bath system (WBS), the gas distribution system (GDS), and the hardware control and data acquisition system (HCDAQS).

![Figure 6.1 Representative diagram of the automated multi-unit composting system (AMUCS).](image_url)

Figure 6.1 Representative diagram of the automated multi-unit composting system (AMUCS).
6.2.2.1 Water Bath System (WBS)

WBS is designed to meet the incubation requirements according to ASTM D-5338-98 (2003) standard which requires the composting bioreactors to be held inside a temperature range of 58°C (±2 ºC) for the entire duration of the composting experiment to allow the thermophilic microbes to reproduce. It is designed using a 30 gallon acrylic fish tank (FT), a 30 gallon water heater (WH), a transfer pump (TP) and three resistance temperature detectors (RTDs).

The fish tank is used as a water bath to hold twelve composting bioreactors which are 500 mL Erlenmeyer flasks. It was wrapped in foam duct board and placed inside of a stainless steel box. This helps to maintain the water temperature more efficiently during the experiment and provides safety and structural integrity.

A water heater is used as a heating source due to its large heating capacity and relatively low cost compared to water temperature controllers on the market. Most water
heaters are advertised to have a maximum temperature of approximately 65.5°C, which exceeds the temperature requirements of the ASTM 5338-98(2003) standard. Resistance temperature detectors or RTDs are placed in the water bath to measure the average temperature and are monitored with the hardware control and data acquisition system (HCDAQS).

The transfer pump is also monitored by HCDAQS and is used to pump water from the water heater into the fish tank as follows: during composting experiments HCDAQS triggers the transfer pump to circulate water through the fish tank and the water heater to maintain the required composting incubation temperature.

6.2.2.2 Gas distribution system (GDS)

The gas distribution system is a very important part of AMUCS. It is needed in order to keep the microorganisms inside of the compost medium alive and to measure the biodegradation by the carbon dioxide CO₂ metabolized from the degradation of the materials. As mentioned early in the introduction, ASTM D-5338-98 (2003) recommends that oxygen O₂ is supplied evenly to all the composting bioreactors. The CO₂ from the outlet gas of each composting bioreactor can be measured using a CO₂-trapping apparatus and titration equipment. However, these devices can be replaced by a gas flow meter plus a gas chromatograph, or other apparatus equipped with suitable detector and column. AMUCS uses a nondispersive infrared (NDIR) gas analyzer to measure the concentration of CO₂ in the outlet gas of each composting bioreactor.
GDS is designed to supply compressed air to the twelve composting bioreactors at a defined rate via a flow divider shown in Figure 6.3. The flow divider consists of a nylon manifold that splits the compressed air into twelve inlet channels (one for each composting bioreactor). A dial flow controller is placed on each inlet channel to control and verify that each composting bioreactor is receiving the same volumetric flow rate of compressed air.

![Figure 6.3](image) A representative picture of the nylon manifold (left) and flow controller (right).

Each inlet channel is connected to a composting bioreactor through a stainless steel tube that passes through a rubber stopper sealing the bioreactor to the bottom of the bioreactor. This to ensure that the compost is thoroughly aerated from the bottom as displayed in Figure 6.4. The inlet compressed air passes through the flow controller to the bottom of each bioreactor by the means of fully enclosed, air-tight inlet channel, stainless steel tube and bioreactor.
An outlet, an air-cooled Graham condenser, is placed on the top of each composting bioreactor as indicated in Figure 6.5. In addition to being an outlet, the condenser is used to remove the water vapor from the outlet gas released from each bioreactor. It causes the water vapor to condense and drain back into the composting bioreactor. Each condenser is connected to a gas multiplexer to form an outlet channel. Figure 6.6 shows the picture of the gas multiplexer. Indeed, in order to sample all twelve outlet channels with a single CO₂ gas analyzer, a multiport valve or gas multiplexer is designed so that each of the twelve outlet channels (of each composting bioreactor) can be sampled by the CO₂ gas analyzer individually.

To construct the gas multiplexer, twelve three-way solenoid valves, twelve check valves and a nylon manifold are used to reduce the twelve outlet channels down to one sample channel. The sample channel is then connected to a mass flow meter and a NDIR gas analyzer as shown in the Figure 6.6. The outlet gas released by each bioreactor
passes through each condenser into the gas multiplexer by the means of fully enclosed, air-tight condenser, outlet channel and gas multiplexer.

Figure 6.5  Composting bioreactor showing the air-cooled Graham condenser.

Figure 6.6  Gas multiplexer showing 3-way solenoid valves, check valves and nylon manifolds.

After the outlet gas (released by each composting bioreactor) is dehydrated by the condenser, its flow rate is measured using a Sierra-820 mass flow meter (Sierra Instruments, Inc.: CA, USA) and the CO₂ concentration within the outlet gas is sampled by the Li-COR 820 CO₂ NDIR gas analyzer (Li-COR Biosciences, Inc.: NE, USA). Figure 6.7 shows a picture of the mass flow meter and the CO₂ gas analyzer.
The three-way solenoid valves are sequenced using LabVIEW 8.6 software to pass the outlet gas of only one of the composting bioreactors to the mass flow meter and CO$_2$ NDIR gas analyzer at a time. After a solenoid valve is activated to be measured, the outlet gas flows through a check valve. This latter then leads the outlet gas to the mass flow meter and the CO$_2$ NDIR gas analyzer for measurements. Check valves allow the outlet gas to flow only in one direction and are placed between the solenoid valve and the nylon manifold (Figure 6.6). Non-activated solenoid valves exhaust their outlet channel’s contents. The activation of the solenoid valves and the measurement of the outlet gas are operated using a cyclic procedure and Table 6.1 illustrates the first four steps in the valve cycling.
Table 6.1 Representative first four steps in the valve sequence

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6.2.2.3 Hardware Control and Data Acquisition System (HCDACQS)

The computer program used in this work was LabVIEW 8.6 of the National Instrument CO. The hardware control and data acquisition system (HCDAQS) is an important component of the composting system. HCDAQS is powered by National Instruments (NI) LabVIEW 8.6 software and compact data acquisition (cDAQ) hardware to create a user interface. It is in charge of acquiring and logging data from the RTDs, flow meter and gas analyzer as well as controlling the water bath temperature, transfer pump and cycling the solenoid valves. A valve and pump control interface (VPCI) was
also used in order to interface the NI hardware with the solenoid valves and transfer pump.

6.2.3 GDS and CO₂ Gas Analyzer Validation

The gas distribution system was checked for gas leaks by use of a soap solution on each connection point. This is achieved by supplying all composting bioreactors with compressed air at a rate of 0.2 standard liters per minute (slpm) and dispensing a small amount of soap solution on each of the approximately 100 connection points on the system.

The Li-COR 820 gas analyzer was also validated. This was performed by flowing research grade oxygen O₂ (<0.1 ppm CO₂) through the gas analyzer for 10 min. The reading of the CO₂ measured on the gas analyzer was consistent with that of the research grade O₂. Once the reading stabilized, it was tared in the Li-COR software to set the zero point. A second validation point was also measured to confirm the accuracy of the CO₂ measurements. This was performed by passing compressed air through the gas analyzer. According to a 2008 study of the National Oceanic and Atmospheric Administration (NOAA), CO₂ measured in atmospheric air consist of approximately 385 ppm or 0.0385% [8]. The compressed air was allowed to flow through the gas analyzer for 10 min and measured an average of 402 ppm CO₂ or 0.0402% CO₂. Comparing this to the NOAA estimation resulted in 4.4% difference. This atmospheric CO₂ measurement was also compared with a study performed by Jayasekara et al. [4]. They determined the CO₂ concentration to be 400 ppm or 0.04% which resulted in 0.005% difference. From the
above statement, it was concluded that the accuracy of the CO₂ gas analyzer was validated in our case.

6.2.4 Outlet Gas Flow Rate Reading and Carbon Dioxide Measurements

When an outlet channel is activated, its content first flows through the mass flow meter which measures the volumetric flow rate in standard liters per minute (slpm). The mass flow meter has two functions. First, it verifies and confirms that each composting bioreactor is supplied the same flow rate of compressed air. Second, for a composted material the flow rate is used to calculate the weight of carbon (CO₂-C) lost in grams and is discussed in the section 6.2.5.

After exiting the mass flow meter, the outlet channel content passes into the measurement chamber of the Li-COR 820 CO₂ gas analyzer where its CO₂ concentration is measured. This gas analyzer was chosen for the following reasons. It is inexpensive and does not require a carrier gas or external pump to pull the outlet channel gas into the sampling chamber to be measured as in the case of gas chromatographs. In addition, it does not require that a reference gas be added to the outlet channel content to increase the accuracy and precision of the CO₂ concentration measurement. Finally, Li-COR 820 reads the real-time CO₂ concentration as the gas is flowing through the measurement chamber. The CO₂ measurement range within this gas analyzer is 0-2000 ppm.

After an outlet channel is activated to be measured, its content flows first through the mass flow meter and the CO₂ gas analyzer for 1 min to flush both devices. Then, the reading of the volumetric flow rate of the channel content and the measurement of the
CO₂ within this channel content were recorded after the stabilization of the content flow for another 1 min.

For Li-COR 820 CO₂ gas analyzer, the mechanism of measuring the CO₂ is explained here after [9]. This gas analyzer is a single beam, dual wavelength NDIR sensor. The CO₂ measurement is a function of the absorption of IR energy as it travels through the optical path (shown in Figure 6.8). The IR source emits radiation into the optical path, where CO₂ absorbs photons of a certain wavelength. The CO₂ sampling channel uses an optical filter centered at 4.26 µm, corresponding to the absorption band for CO₂, while the reference channel uses a nonabsorbing band at 3.95 µm. A pyroelectric detector measures the absorption of the infrared beam passing through the optical path. The ratio of the sample and reference signals indicates the amount of light absorption by CO₂, and thus, the gas concentration. A heating element placed underneath the pyroelectric terminal regulates the detector temperature precisely at 50ºC so that the detector can determine the thermal gradient noise absorbed from the 4.24 µm band.

Figure 6.8 Optical path of the Li-COR NDIR CO₂ gas analyzer. Reproduced from Li-COR Biosciences-Li-820 CO2 Gas Analyzer Instruction Manual 2002 (Lincoln, NE).
The CO₂ concentration in the outlet channel content is obtained in part per million (ppm) using Equation 1. C is the CO₂ concentration in ppm, \( f_c \) is a 6th ordered CO₂ calibration polynomial, \( \alpha_c \) is the CO₂ absorptance, \( g_c(\alpha_c, P) \) is the pressure correction, \( T \) is the temperature (°C) of the sample gas and \( T_o \) is the temperature of the pyroelectric detector which is 50°C [9].

\[
C = 10 f_c(\alpha_c, g_c(\alpha_c, P))\left(\frac{T + 273}{T_o + 273}\right) \quad (1)
\]

6.2.5 Calculations

Degradation of materials is measured by calculating the amount of carbon or (CO₂-C) released. After \( C \) (Equation 1) is measured by the CO₂ gas analyzer, it is acquired and logged along with the outlet channel content flow rate obtained using National Instruments data acquisition hardware and LabVIEW 8.6. The mass flow meter and CO₂ gas analyzer data were obtained in slpm, and in ppm, respectively. These two units are used to calculate the total CO₂ (in g) metabolized by the materials using variants of the ideal gas laws and the methodology described here after.

First, the hourly flow rate or \( F_{\text{Hourly}} \) (l/hr) of the outlet channel content for each bioreactor is calculated. This is achieved by multiplying the flow rate \( F \) (l/min) obtained using the mass flow meter by (60 min/1 hr) to convert the flow rate to l/hr as shown by Equation 2.

\[
F_{\text{Hourly}} = F\left(\frac{l}{\text{min}}\right) \times \left(\frac{60 \text{ min}}{1 \text{ hr}}\right) \quad (2)
\]
The next step is to correct the CO\textsubscript{2} concentration or CO\textsubscript{2(meas)} of the outlet channel content for each bioreactor. The corrected CO\textsubscript{2} measurement denoted as CO\textsubscript{2(corr)} is achieved by subtracting the CO\textsubscript{2} present in the compressed air or CO\textsubscript{2(CA)} from the CO\textsubscript{2(meas)} as shown by Equation 3.

\[
CO_{2(corr)} (ppm) = CO_{2(meas)} - CO_{2(CA)} \quad (3)
\]

CO\textsubscript{2(corr)} is then used to determine the weight of CO\textsubscript{2} per sampled liter of gas or CO\textsubscript{2 (g/l)} by taking the product of the CO\textsubscript{2 (corr)} and the ratio of the molecular weight of CO\textsubscript{2} or MW\textsubscript{CO2} (44 g/mol) to the molar volume of a perfect gas or MV\textsubscript{PG} (22.414 L/mol at room temperature T\textsubscript{0}=273.15 K and pressure P\textsubscript{0}=1 atm). This obtained product is corrected by the ratio of the experimental temperature T\textsubscript{Exp} to T\textsubscript{0} as shown by Equation 4.

\[
CO_{2(g/l)} = CO_{2(corr)} \left[ \frac{MW_{CO2}}{MV_{PG}} \times \frac{T_0}{T_{Exp}} \right] \quad (4)
\]

The previous equation determined the CO\textsubscript{2 (g/l)}. However, biodegradation of materials is determined only by the carbon weight or (CO\textsubscript{2-C}) (g/l) metabolized during the composting process. To negate the weight of the oxygen atoms, CO\textsubscript{2 (g/l)} is multiplied by the ratio of carbon weight (12 g mol\textsuperscript{-1}) to the molecular weight of CO\textsubscript{2} (44 g mol\textsuperscript{-1}) as shown by Equation 5.

\[
(CO_2 - C) \left( \frac{g}{l} \right) = CO_{2(g/l)} \times \frac{12}{44} \quad (5)
\]

After calculating (CO\textsubscript{2-C}) (g/l), the gross (CO\textsubscript{2-C}) (g) metabolized from the degradation of material is determined by taking the product of (CO\textsubscript{2-C}) (g/l), the hourly flow rate, and
the time elapsed in hours since the last measurement as shown in Equation 6. For our experiment, CO₂ measurements were recorded every 8 h. We then recorded 3 measurements per day.

\[(CO_2 - C)_{\text{Gross}} (g) = (CO_2 - C) \left( \frac{g}{l} \right) \times F_{\text{Hourly}} \times \Delta T_{\text{Hour}} \quad (6)\]

As mentioned earlier, only (CO₂-C) weight obtained during the degradation of the material samples is needed to calculate the percent biodegradation or mineralization. So, the (CO₂-C) weight metabolized by the compost medium needs to be negated from the (CO₂-C)Gross. This is achieved by subtracting the average (CO₂-C) value of the compost medium of the blank bioreactors (containing only compost) from the (CO₂-C)Gross measurement of the material sample obtained using Equation 6 to obtain the average net (CO₂-C) or (CO₂-C)net as shown by Equation 7

\[(CO_2 - C)_{\text{net}} (g) = (CO_2 - C)_{\text{Gross}} - (CO_2 - C)_{\text{Compost}} \quad (7)\]

(CO₂-C)net is then added together to determine the cumulative weight (CO₂-C) released or (CO₂-C)Cum over the entire duration n of the test as shown by Equation 8.

\[(CO_2 - C)_{\text{Cum}} (g) = \sum_{n=0}^{\infty} (CO_2 - C)_{\text{net}} \quad (8)\]

Once the (CO₂-C)Cum is calculated, the percent biodegradation or % biodegradation can be determined. To calculate the % biodegradation, the carbon content or %C of the material samples must be used. This can be obtained by conducting CHN elemental analysis. The total carbon content weight (C_Tot) can be obtained by taking the product of the %C and the original weight or m (in grams) of the material sample used for the
composting experiment. The % biodegradation is obtained by dividing $(CO_2-C)_{Cum}$ by $C_{Tot}$ and multiplying the result by 100% as shown in Equation 9

$$\% Bio\ deg\ radiation = \left( \frac{(CO_2-C)_{Cum}}{C_{Tot}} \right) \times 100\% \quad (9)$$

6.2.6 Compost Medium and its Characterization

A compost medium was purchased from Collin County Municipal Waste Facility (TX, USA) and is made of food waste and yard trimming including grasses, leaves, and sticks. The compost was ground and sieved on a screen of 10 mm to prepare homogenous sample.

The moisture content of the compost was determined following ASTM D 2974 Test Methods. Compost sample of 25 g was dried in an oven at 105ºC until constant weight was obtained. The measurements were conducted in triplicate and the moisture content result obtained was 56.49% (±1.5).

The procedure for the determination of total solids (TS) and volatile solids (VS) is described hereafter. First, compost sample of 25 g was dried in an oven at 105ºC overnight to obtain the % TS. The dried sample was then heated in a furnace at 550ºC for 1 h to obtain the % VS. The measurements were conducted in triplicate and the %TS and %VS were 43.51 (±1.5) and 20.60 (±1.5), respectively.

The pH of the compost was determined with an Oakton Acorn® pH 6 Meter. The measurements were done in triplicate on 5 g compost samples in 25 mL distilled water.
after homogenization for 5 min at room temperature. The pH result obtained was 8.81 (±0.3) which is above 7.

Carbon (16.13%) and nitrogen (0.71%) contents of the compost medium were determined by CHN elemental analysis (Elemental Analysis Inc., Lexington, KY). The ratio of C/N is 22.72 which is within the recommended range of 10-40.

6.2.7 Composting Procedure

The biodegradability test by composting using AMUCS was conducted on the basis of ASTM D 5338-98 (2003) standard [7]. This test method determines the degree and rate of aerobic biodegradation of plastic materials on exposure to a controlled-composting environment under laboratory conditions.

200 g of homogenized compost was weighed into each glass bioreactor vessel and mixed with up 2 g of the biopolymer and its nanocomposite samples, and 4 g of microcrystalline cellulose. Microcrystalline cellulose was used as a positive reference material. Three blank bioreactors were included in the biodegradation testing system. Each of them contained only 200 g of the compost medium without testing material. After mixing, all the bioreactors were weighted and incubated under optimal oxygen, temperature and moisture conditions for a test period of 45 days. The compressed air flows were regulated to the amount of 0.2 standard liters per minute (slpm) throughout the experiment to ensure enough oxygen for the biodegradation process. The temperature and moisture content were kept at 56.3°C and 56.5%, respectively. The biodegradation of
the testing materials, microcrystalline cellulose and the compost medium was done in triplicate.

The water content in the bioreactors was controlled every 4 days to adjust the moisture level to 56.5%. This is accomplished by first stopping the experiment and weighing each bioreactor to record its weight loss. Then distilled water of approximately 20 cm$^3$ corresponding to the amount of weight loss was added to each bioreactor contents to restore the initial weight. The contents were then mixed homogenously using a spatula.

6.3 Results and Discussion

6.3.1 Quality of the Compost Medium and Validation of the Composting Test Conditions

An important criterion concerning the quality of the compost medium and the validation of the composting test conditions is the biodegradation of the positive reference of cellulose. ISO 14855 stipulates that the degree of biodegradation of reference material is more than 70% after 45 days. Figure 6.9(A) and (B) show the net cumulative carbon dioxide (CO$_2$-C) productions and the percentage mineralization of the microcrystalline cellulose, respectively. The result of this experiment showed that the degree of biodegradation of the positive reference material (microcrystalline cellulose) was 72.05% in the compost medium after 45 days at 56.3°C.
6.3.2 Biodegradation Behavior of P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 Nanocomposite

A convenient way to compare the biodegradation behavior of polymeric materials and their nanocomposites is to determine the carbon dioxide metabolized during the composting test. The net cumulative carbon dioxide (CO$_2$-C) productions and the percentage mineralization of P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 nanocomposite are shown in Figure 6.10 (A) and (B), respectively. Mineralization of P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 samples proceeded slowly at first (with a lag period of 4 days) but then increased slowly from about day 5 to day 15. After day 15, the % mineralization was 20.32 and 17.43 for P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 nanocomposite, respectively. After day 15, the net CO$_2$-C production increased rapidly for another 20 days. However, the increase is higher for P(3HB-co-3HV)/SA5 sample than for the neat P(3HB-co-3HV). After day 35, the % mineralization was 60.86 and 70.65 for P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 nanocomposite, respectively. After day 35, the net (CO$_2$-
C) is produced at a much slower rate before reaching a plateau in the case of P(3HB-co-3HV)/SA5 sample at about 45 days. For neat P(3HB-co-3HV), no plateau is reached. After day 35, the % mineralization was 65.31 and 73.65 for P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 nanocomposite, respectively.

The above analysis suggested that the overall biodegradability (i.e. rate, degree and ease of degradation) of the biopolymer P(3HB-co-3HV) was significantly affected by the addition of the synthetic clay LDH-SA. Indeed, under the same controlled composting test conditions, the incorporation of 5 wt% LDH-SA into P(3HB-co-3HV) matrix yielded nanocomposite with significantly improved biodegradability.

![Graph showing net cumulative (CO2-C) productions and percentage biodegradation](image)

**Figure 6.10** Net cumulative (CO2-C) productions (A) and percentage biodegradation (B) of P(3HB-co-3HV) and P(3HB-co-3HV)/SA5 nanocomposite.

### 6.4 Conclusion

The system described in this work uses twelve inlet channels to supply compressed air to 12 composting bioreactors of 500 ml capacity. Twelve outlet channels
containing the outlet gas were converted into a single channel by the means a gas multiplexer. The single channel is connected to a mass flow meter which measures the volumetric flow rate of the outlet gas. After exiting the mass flow meter, a non-dispersive infrared gas analyzer samples the CO₂ concentration within the outlet gas.

The system is controlled by a hardware control and data acquisition system (HCDAQS). HCDAQS is powered by National Instruments (NI) LabVIEW 8.6 software and compact data acquisition (cDAQ) hardware to create a user interface, acquire and log data, and control other hardware components of the system.

The system developed is simple and contains no sophisticated devices or attachments in comparison with other systems used for measurement of biodegradation [7]. Therefore, its initial cost is low compared to other systems [7].

For the biodegradation study according to ASTM D 5338-98 (2003), the system is validated with the use of cellulose as a reference material. Under controlled composting conditions, the mineralization of microcrystalline cellulose yielded 72.05% which is slightly higher than the 70% mineralization requirement.

The system is used to investigate the effect of the addition of synthetic clay on the biodegradability behavior of P(3HB-co-3HV) biopolymer. The mineralization result suggested that the overall biodegradability (i.e. rate, degree and ease of degradation) of the biopolymer P(3HB-co-3HV) was significantly affected by the addition of the synthetic clay LDH-SA. Indeed, under the same controlled composting test conditions, the incorporation of 5 wt% LDH-SA into P(3HB-co-3HV) matrix yielded nanocomposite with significantly improved biodegradability with respect to the neat P(3HB-co-3HV).
6.5 References


CHAPTER 7

POLY(3-HYDROXYBUTYRATE-CO-4-HYDROXYVALERATE) COATING ON KRAFT PAPER *

7.1 Introduction

Polymeric coatings are useful for a wide range of industries including food, aerospace, automotive, biomedical, and electronics. Coatings are used for protective, decorative, and functional purposes on many kinds of surfaces [1]. Coating of polymeric materials can be conducted on a variety of substrates using a number of different techniques such as melt-extrusion, dispersion coating and solution methods. These techniques give vast opportunities to form coated, multilayer and laminated structures. Most coatings are the result of the intimate interaction between two different materials – an organic/inorganic substrate and a polymeric resin that form adhesive bonds with each other. The quality and durability of the coating is directly related to the level of adhesion [2]. The military consumes significant quantities of paper and fiberboard. By utilizing a biocompostable polymer and paper, a potential biocompostable packaging can be realized.

* This entire chapter is reproduced from Koffi. L. Dagnon, Christopher Thellen, Jo Ann Ratto and Nandika A. D’Souza, “Poly (3-hydroxybutyrate-co-4-hydroxybutyrate) Coatings on Kraft paper”, Journal of Biobased Materials and Bioenergy, in press.
Kraft paper is a universal material made essentially from wood pulp that is produced by a modified sulfate pulping process (Kraft Process). It comprises a matrix of long cellulose fibers held together by hydrogen bonding between hydroxyl groups. Cellulose is an environmentally friendly and renewable biomaterial [3]. It is a linear polysaccharide of D-glucose units (C₆H₁₀O₅) linked by β-1,4-glycosidic bonds. Kraft paper is widely used in packaging applications for both food and non-food materials due to its high mechanical resistance to tearing and to tensile forces. These properties are due to the long fibers used in the manufacture of Kraft paper and its basis weight of 30-150 g/m² [4]. In addition, its environmentally friendly nature has helped the material to resist substitution by petroleum-based plastics. However, due to its hygroscopic properties and poor barrier to water and water vapor, Kraft paper is not suitable for long-term storage of dehydrated products, whose shelf life would be reduced [4,5].

Environmental concerns, such as the limitation of greenhouse gases, the accumulation of plastic wastes over last few decades and the presumably higher cost of petrochemicals in the future, have made renewable materials more interesting and attractive as a substitute for synthetic packaging materials such as ethylene-vinyl alcohol copolymers, poly (vinylidene chloride), polyesters and polyamides. Therefore biodegradable green plastics are being looked upon as possible substitutes to petroleum-based plastics. Polyhydroxyalkanoates or PHAs have occupied a special position among biodegradable green polyesters. PHAs are a family of natural, biodegradable polyesters which are intracellularly synthesized as a carbon and an energy reserve materials by various microorganisms such as alcaligenes eutrophus, and pseudomonas oleovorans [6].
PHAs have been recognized as a potential environmentally-friendly substitute for fossil based plastics such as polyethylene, polystyrene, and other consumer plastics etc. They are fully biodegradable, thermoplastic polyesters and their applications can be found in the food industry (as packaging and antioxidant materials), agriculture (as coating material for seeds, fertilizers and pesticides), medicine and pharmacology. Their physico-chemical properties, diversity and the feasibility of producing PHAs-based composites with various materials make them the materials of 21st century. Poly (3-hydroxybutyrate) [P(3HB)], poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] are representatives of the PHAs family.

In an effort to produce more environmentally friendly materials, PHAs have been investigated as paper coating materials. PHAs possess excellent film-forming and coating properties and are water resistant due to their high hydrophobicity [7, 8]. For example, P(3HB)-coated paperboard has been used for packaging of ready meals, while [P(3HB-co-3BV)] coated board has been used for dry products, dairy products and beverages [7, 9]. The US Patent and Trademark Office awarded to Robert Whitehouse and co-inventors (Metabolix: Cambridge, MA, USA) the US Patent 7,094,840 covering low molecular weight PHAs for use as hot melt adhesives, waxes and protective coatings [10]. Coating compositions include a PHA oligomer and also stabilizers, plasticizers, dyers, emulsifiers, thickening agents, antioxidants, preservatives, cross-linking agents, other biologically degradable polymers, and anti-fungal agents. The coatings are used in coating cheese and other food products [10]. Lim et al.[11] prepared solution based
P(3HB) coatings obtained by P(3HB) penetration into and adhesion onto cellulose paper. The coating biodegradation efficiency index of P(3HB) coated paper was investigated through solution enzymatic degradation. The results indicated that P(3HB) coated paper exhibited the lowest degradability by cellulases (enzymes that degrade cellulose paper) with respect to pure cellulose paper. Kuusipalo [12, 13] investigated coatings obtained by P(3HB-co-3HV) extrusion coated onto a series of paper and paperboard substrates. The physical properties of the coatings showed that the adhesion between the biopolymer and the substrate was poor when corona and flame pretreatments were used. On the other hand, the adhesion was sufficient when the substrate was primed with an acrylic-based primer [12]. The results of the heat sealability of P(3HB-co-3HV) extrusion coatings show that the sealing temperature increased with increased substrate basis weight. The water vapor barrier of the coatings was shown to be lowered by incorporation of wax or tall oil rosin [13]. Krook et al. [14] investigated the creasability of P(3HB-co-3HV) compression molded onto paperboard. Their results showed that upon application of creasing and bending stresses, P(3HB-co-3HV) coating showed no evidence of cracks, and the delamination was observed to decrease with increased molding temperature due to improved paperboard-coating adhesion.

The main objective of this work was to investigate the structure and performance of Kraft paper coated with poly(3-hydroxybutyrate-co-4-hydroxybutyrate) or P(3HB-co-4HB). Solvent and non-solvent (melt) methods are employed to manufacture the coating. Solvent based approaches included a dip coating method and the deposition of a solution between the nips of a two roll coating system (roll coating method). The non-solvent
based approach was the application of the compression molded P(3HB-co-4HB) films to the Kraft paper. The coating structure and quality were examined using environmental scanning microscopy (ESEM), Fourier transform infrared spectroscopy (FTIR) and wide angle X ray diffraction (WAXD) while the mechanical properties were examined using dynamic mechanical analysis (DMA) and impact testing.

7.2 Experimental

7.2.1 Materials

Brown Kraft paper was supplied by US Army Natick Soldier Research Development and Engineering Center (Natick, MA). The Kraft papers used for the coating had weights of 60 and 75 pounds (lbs.) and a thickness of 0.22 and 0.20 mm (±0.01), respectively. Poly (3-hydroxybutyrate-co-4-hydroxybutyrate) or P(3HB-co-4HB) (Lot # MBX CS06082205; $M_w = 437,084 \text{ g} \text{mol}^{-1}; M_n = 189,902 \text{ g} \text{mol}^{-1}; \text{PDI} = 2.3$) was supplied by Metabolix (Cambridge, MA). P(3HB-co-4HB) pellets were dried in an oven for 48 hours at 40°C before use. The biopolymer chemical structure is illustrated in Figure 7.1 and its thermal properties obtained by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) are summarized in Table 7.1. The biopolymer displayed two endotherm peaks $T_{m1}$ at 148 °C and $T_{m2}$ at 162 °C, indicating the presence of two crystalline phases in the sample and the two $\Delta H_m$ values reflect the relative amount of the crystalline phases. $T_{m1}$ is related to the melting of crystals formed during non isothermal crystallization whereas $T_{m2}$ is due to the melting of crystals that are recrystallized during the heating of the biopolymer sample in the DSC pan [15]. A melt
crystallization peak $T_{mc}$ is obtained at 105°C. The biopolymer displayed a maximum decomposition temperature $T_p$ at 299 °C obtained by TGA. Analytical grade dichloromethane (>99.8% purity, EMD Chemicals: Gibbstown, NJ) was used as received as the biopolymer solvent.

![Chemical structure of P(3HB-co-4HB)](image)

Figure 7.1 Chemical structure of P(3HB-co-4HB).

<table>
<thead>
<tr>
<th>Sample</th>
<th>$a T_{m1}$ ($^\circ$C)</th>
<th>$a T_{m2}$ ($^\circ$C)</th>
<th>$b \Delta H_{m1}$ (J/g)</th>
<th>$b \Delta H_{m2}$ (J/g)</th>
<th>$c T_{mc}$ ($^\circ$C)</th>
<th>$d \Delta H_{mc}$ (J/g)</th>
<th>$e T_p$ ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-4HB)</td>
<td>148</td>
<td>162</td>
<td>12.7</td>
<td>37.7</td>
<td>105</td>
<td>47.5</td>
<td>299</td>
</tr>
</tbody>
</table>

$^a$ Melting ($T_m$) and $^b$ enthalpy of melting ($\Delta H_m$) obtained from second heating differential scanning calorimetry (DSC) at 10°C/min.
$^c$ Melt crystallization ($T_{mc}$) and $^d$ enthalpy of melt crystallization ($\Delta H_{mc}$) obtained from the cooling differential scanning calorimetry at 10 °C/min.
$^e$ Temperature at which the weight loss rate is maximum ($T_p$): obtained from thermogravimetric analysis (TGA) at 20°C/min.

### 7.2.2 Solution Coatings

10 grams of (P3HB-co-4HB) were stirred in 200 ml of dichloromethane. The solution was heated to 50°C overnight to obtain complete dissolution of the biopolymer. Roll and dip coating techniques were used for solution coating. For the roll coating, the
solution of (P3HB-co-4HB) was poured uniformly onto the substrate which is then rolled between two cylindrical bars. Both sides of the substrate were coated. Controlled thickness can be obtained by adjusting the gap between the two cylindrical bars. In the case of dip coating, a solution bath of size 30×20 cm² was used. The paper substrates were immersed with the aid of a weighted screen (3kg) to prevent them from floating and withdrawn from the solution bath after 10 min. In both cases, the liquid film formation of the biopolymer on the paper is obtained by evaporation of the solvent. The coated papers were dried in ambient conditions for 48 h.

7.2.3 Coatings by Compression Molding

Melt coated papers were obtained by compressing molding 15 grams of the biopolymer pellets between two sheets of Teflon using a Carver press. First, the Carver press was pre-heated to 180°C, and a total pressure of 6 MPa was applied at 0.5 MPa per min to produce the biopolymer film. Then, the melt processed biopolymer film was applied on the paper (50 cm x 50 cm) at 180°C for 1 minute with a total pressure of 2 MPa. Only one side of the Kraft paper substrate was coated. However, we observed that diffusion of the biopolymer occurred on the opposite side. Compression molded biopolymer film with thickness of 0.12 (±0.02) mm was also made and used as control sample for different characterizations. All the samples were sectioned for characterization. Sample nomenclature employed in describing the results is the Kraft paper weight followed by manufacturing process.
7.2.4 Characterization of Coatings

7.2.4.1 Thickness and Coating Weight

The thickness of the biopolymer, paper substrate and the coated samples was measured by a micrometer screw gauge. The coating weight of the samples was measured using an analytical balance. In both cases, measurements were made before and after impregnation, these data being compared to determine the biopolymer coated film thickness and coating weight.

7.2.4.2 Environmental Scanning Electron Microscopy: ESEM

The surface and the cross section structures of the uncoated, coated Kraft paper samples were observed using a FEI Quanta 2000 Environmental Scanning Electron Microscope (Hillsboro, OR). Cross section samples were immersed in liquid nitrogen before fracture. All samples were coated with gold before imaging and were examined using an accelerating voltage of 12.5 to 20 kV. Thickness measurements were also conducted on the ESEM images using Image-J (Java-based online image processing program developed by National Institutes of Health, Bethesda, Maryland). Reported coating thicknesses correspond to total thickness of the coatings on both sides of the paper.

7.2.4.3 Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

Attenuated Total Reflection-Fourier transform-infrared (ATR FT-IR) measurements of neat Kraft paper, neat biopolymer and the coated samples were
performed using a Nicolet Nexus 6700 FT-IR spectrometer (Thermo Fisher Scientific Inc, Waltham, MA) having a resolution of 4 cm\(^{-1}\), a scan range of 4000 to 400 cm\(^{-1}\). A total of 64 scans per sample were performed. The Ge (Germanium) crystal has been used for ATR FT-IR measurements.

7.2.4.4 Wide Angle X-ray Diffraction: WAXD

The existence of crystallinity in the neat P(3HB-co-4HB), Kraft papers and the coated samples was observed using a WAXD (Rigaku model D/Max -2000 / PC series: The Woodlands, TX). An angular range of 10° to 50° with a Cu-K\(\alpha\) wavelength of 1.542 Å generated at 44 mA and 40 kV, and a scanning rate of 0.02°/s were used.

7.2.4.5 Dynamic Mechanical Analysis: DMA

The thermomechanical properties of the pure P(3HB-co-4HB), uncoated and coated Kraft paper samples were measured by means of a dynamic mechanical analyzer RSA III (TA Instruments, New Castle, DE) operating in the tension mode. For the DMA measurements, the pure biopolymer film, uncoated and coated Kraft papers were cut into 30 x 10 mm\(^2\) dimensions. A single frequency temperature scan was used for the measurement. The specimens were heated at 2°C/min from -50°C to 50°C under an oscillatory strain of 0.3% and a force of 250 grams (previously determined from a separate strain amplitude sweep of the biopolymer at 1 Hz to establish the linear viscoelastic region) at a frequency of 1 Hz. All DMA measurements were done in triplicate.
7.2.4.6 Impact Testing

Instrumented impact testing was conducted on an Instron instrumented tester 9250 (Norwood, MA) connected to a piezotup unit capable of measuring up to 500 pounds of load. An impact weight of 8.5 kg was used at a height of 0.4 m. The velocity of the impact is 2.80 m/s and the tests were measured under ambient conditions. Pieces (10 cm x 10 cm) of uncoated and coated Kraft papers were used for the instrumented impact testing.

7.3 Results and Discussion

7.3.1 Coating Cross-sections and Surfaces Analyzed by ESEM

The surfaces of the representative uncoated and coated paper (60 lbs) are shown in Figure 7.2(A) to 7.2(D). The surface of the Kraft paper [Figure 7.2(A)] shows a heterogeneous porous and fibrous material. The micrographs of the coated samples in Figures 7.2(B) to 7.2(D) indicated a biopolymer layer was formed on the surface of the Kraft paper. The porous fibrous structure was covered and filled with P(3HB-co-4HB). Solution based coatings exhibited a higher degree of roughness than the melt coated sample [Figures 7.2(B) and 7.2(C) compared to 7.2(D)]
Figure 7.2  Representative ESEM micrographs of the surface of: (A) - Kraft paper 60lbs showing porous and fibrous structure; (B) - 60 Dip coating; (C) - 60 Roll coating and (D) - 60 Melt coating (scale bar: 50 µm).

Figures 7.3 to 7.6 show the cross sections of the cryofractured uncoated and coated 60 lbs paper samples (representative data). The uncoated paper (Figure 7.3) exhibited a heterogeneous fibrous structure. For both the solvent and melt processed samples, after the impregnation of the Kraft paper with P(3HB-co-4HB), the coating was in close contact with the base paper and filled out the irregularities of the substrate cross-section surface as shown in Figures 7.4(A), 7.5(A) and 7.6(A). The cross-sectional morphology of the coated paper samples showed a layer-by-layer structure. Figures 7.4(B), 7.5(B) and 7.6(B) showed the penetration of the biopolymer into the cellulose structure of paper for all coatings. This suggested that the biopolymer fused with fibers,
causing an increased compaction in the coated papers. Dip [Figure 7.4(B)] and melt coated paper [Figure 7.6(B)] exhibited lower void presence compared to the roll coated paper [Figure 7.5(B)]. This indicates that during the application process, more impregnation of the paper was obtained through the dip and melt coating process. Dip coating by virtue of its enhanced time in the bath compared to the roll coating enables higher solution transport. The low melt viscosity ascribed to the PHA family can explain the enhanced penetration of the paper in the melt coated sample. Both enhanced penetration and decreased void fraction as well as improved coating uniformity was obtained for the melt and dip coated samples. However, the examination of the cross-section of 60 Roll sample in Figure 7.5(A) showed that this lacked a defined boundary as obtained for 60 Dip and 60 Melt samples. This observation could have been due to the fact that 60 Roll sample was compressed between the nips of a two roll coating system. As a result of this, there was no clearly defined boundary between the coating and the base paper. Furthermore, the coating film for 60 Roll sample seemed to be very thin and discontinuous; and the fibers shape could be distinguished throughout the biopolymer layer. This result could have indicated that the interface region between the thin coating and the paper surface contained irregularities such as voids (the biopolymer bonding to the paper substrate appeared to be low) as a result of few contact points between the biopolymer and the paper substrate. Thus, this type of composite could have been characterized as having limited sites of physical contact between the biopolymer and the paper substrate.
Figure 7.3  Representative ESEM micrograph of a cross-section of Kraft paper 60lbs showing fibrous structure (scale bar: 100 µm).

Figure 7.4  Representative ESEM micrographs of a cross-section of 60 Dip: (A) - A, Kraft paper and biopolymer; B, biopolymer layer (scale bar 100 µm) / (B)-A region showing the penetration of the biopolymer into Kraft paper (scale bar 40 µm).
7.3.2 Thickness and Coating Weight of the Samples

The influences of the impregnation with the biopolymer on the thickness and the weight of the uncoated Kraft paper sample were presented in Table 7.2.
The average thickness values for all the coatings samples are shown in Table 7.2. The coating thickness for the solution based approaches was determined by first conducting the dip coating method with a range of concentrations to achieve a uniform coating. For roll coating, the gap of the rolls was then set to manufacture a coated paper that matched the thickness of the dip coated sample. The thickness of the melt coated sample was set to correspond to the solution based coating approach. However, due to the variability involved in making a film and applying pressure over a small gap in the compression molding on one hand; and compressing the coated paper between the nips of a two roll coating system on the other hand, variation in both techniques persisted. The melt processed samples have the highest coating thickness, while the roll coated samples the lowest. The average thickness values of the coating samples obtained through the cross-sectional ESEM micrographs analysis using Image-J are almost close to those measured with a micrometer screw gauge as indicated in Table 7.2.

The average coating weight values determined by comparing 5 coated and uncoated weight of the same area of each sample are also reported in Table 7.2. Melt coated samples showed the highest value whereas roll coated one the lowest. This result is consistent with the variation in thickness of the different samples.
Table 7.2  Thickness and coating weight measurements of Kraft paper 60 and 75lbs and their coating samples.

<table>
<thead>
<tr>
<th></th>
<th>60 Melt</th>
<th>60 Dip</th>
<th>60 Roll</th>
<th>75 Melt</th>
<th>75 Dip</th>
<th>75 Roll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (µm)</td>
<td>45</td>
<td>35</td>
<td>20</td>
<td>55</td>
<td>45</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>(±5)</td>
<td>(±2)</td>
<td>(±2)</td>
<td>(±5)</td>
<td>(±2)</td>
<td>(±2)</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>33</td>
<td>18</td>
<td>53</td>
<td>44</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>(±4)</td>
<td>(±2)</td>
<td>(±3)</td>
<td>(±2)</td>
<td>(±2)</td>
<td>(±3)</td>
</tr>
<tr>
<td>Coating weight (g/m²)</td>
<td>35.50</td>
<td>22.07</td>
<td>10.75</td>
<td>37.43</td>
<td>25.85</td>
<td>12.85</td>
</tr>
<tr>
<td></td>
<td>(±0.56)</td>
<td>(±0.85)</td>
<td>(±0.48)</td>
<td>(±0.67)</td>
<td>(±0.55)</td>
<td>(±0.42)</td>
</tr>
</tbody>
</table>

* Average coating thickness values determined by comparing the thickness of coated and uncoated sample.

* Average coating thickness values determined by ESEM.

* Average coating weight values determined by comparing 5 coated and uncoated weight of the same area of each sample.

7.3.3 Analysis of the Coating by ATR FT-IR and WAXD

Since the coated samples were not infrared transparent, an attenuated total reflectance (ATR) method was employed to evaluate the coatings by FTIR. Good contact between the sample and the crystal is essential to record a good qualitative and quantitative ATR spectrum. The representative spectra of the pure components (biopolymer and paper 75lbs) and their coatings are shown in Figure 7.7. Luo et al.[16] and Xu et al. [17] reported on the characteristic bands of P(3HB-co-4HB) and other polyhydroxyalkanoates (PHAs), respectively. The bands at 980, 1227, 1279 and 1722 cm⁻¹, respectively, are characteristic of the crystalline phase in the biopolymer while that at 1181 cm⁻¹ is characteristic of the amorphous phase. The band at 1722 cm⁻¹ represents the stretching vibration of carbonyl groups (C=O) in the crystalline phase. The band at
1279 cm$^{-1}$ corresponds to the symmetric CH$_2$ stretching. The band at 1227 cm$^{-1}$ was proposed as the conformational band of the helical chains. The bands at 1181 and 1133 cm$^{-1}$ are characteristic of the asymmetric and the symmetric stretching vibration of the C–O–C group, respectively.

The characteristic bands of Kraft paper were reported elsewhere [18]. In the spectrum of Kraft paper, a broad band at 3350 cm$^{-1}$ is attributed to O-H stretching vibrations. The band at 2875 cm$^{-1}$ represents the aliphatic C–H stretching vibration. The band observed at 1050 cm$^{-1}$ is due to skeletal vibration involving C-O stretching.

We conducted the ATR FT-IR measurements on variable points of the sample surface and observed, by comparing the coated Kraft papers and P(3HB-co-4HB) samples spectra, that the biopolymer crystalline phase reflection peaks are predominant on the coatings. This could imply a certain degree of the biopolymer bonding to the paper substrate as shown in the ESEM analysis. As proposed by Xu et al.[17], a crystallinity index (CI) defined as the ratio of the intensity of a band at 1453 cm$^{-1}$ which is insensitive to the crystallinity and composition to that of the band at 1181 cm$^{-1}$, was used to quantitatively measure the crystallinity of the biopolymer since the coatings were obtained through solution and melt processed techniques. The CI is not an absolute measure of the degree of crystallinity but is useful as a comparison criterion. The results for the P(3HB-co-4HB) and coatings on 60 and 75 lbs paper are shown in Table 7.3. It can be observed that CI does not change for melt processed coating while it decreases for solution processed coating (both roll and dip coated samples), reflecting the decreased crystallinity of the biopolymer during solution coating.
Figure 7.7  ATR FT-IR spectrum of P(3HB-co-4HB), Kraft paper 75lbs and their coatings.

Table 7.3  The crystallinity index (CI) defined as the ratio of the intensities of 1453 cm\(^{-1}\) peak and that of 1181cm\(^{-1}\) peak in the FTIR spectra of P(3HB-co-4HB) and its coating samples.

<table>
<thead>
<tr>
<th></th>
<th>P(3HB-co-4HB)</th>
<th>60Melt</th>
<th>60Dip</th>
<th>60Roll</th>
<th>75Melt</th>
<th>75Dip</th>
<th>75Roll</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>0.34</td>
<td>0.34</td>
<td>0.26</td>
<td>0.22</td>
<td>0.34</td>
<td>0.26</td>
<td>0.22</td>
</tr>
</tbody>
</table>

The representative X-ray diffractogram of the pure components (biopolymer and paper 75lbs) and their coatings are shown in Figure 7.8. The X-ray diffractogram of the pure biopolymer showed that the copolyester is a semicrystalline material. Its characteristic reflections have been reported in the literature [19,20,21]. These reflections indicated in correspond to a rhombic cell. Since Kraft paper comprises a matrix of long cellulose fibers held together by hydrogen bonding between hydroxyl groups, its
crystalline structure might be derived from these fibers. The crystalline structure of cellulose has been well studied [3,22]. The diffractogram of the Kraft paper shows a well-defined principal peak at $2\theta = 22.5^\circ$ and a secondary peak at $2\theta = 15.5^\circ$. This diffractogram is characteristic of cellulose I [22]. The diffractogram of all the coatings samples showed different characteristics in terms of peak reflections and intensity. We observed that the crystalline phase of the biopolymer was present in the melt processed coating samples (60 and 75 Melt). This behavior could be due to the thick biopolymer layer of this composite. However, a small decrease in the intensity can be observed in the reflection peaks of the coatings with respect to that of pure P(3HB-co-4HB). An overview of the diffractogram of the solution processed coating samples (60 Dip, 60 Roll, 75 Dip and 75 Roll) indicates few reflection peaks with low intensity of the biopolymer, reflection peaks which could have been masked by the broad amorphous reflection peaks of the paper substrate. These results are consistent with the crystallinity index one. Roll coated samples (60 and 75 Roll) exhibited the poorest characteristic in terms of biopolymer reflection peaks, characteristic which can be due to the thin thickness of the biopolymer layer and both solvent coated papers samples were inferior to the melt processed papers samples.
7.3.4 Effect of Coatings on the Viscoelastic and Mechanical Properties

Figures 7.9 and 7.10 are representative data and illustrate the trends of the dynamic storage modulus ($E'$) and the loss tangent ($\tan \delta$), respectively, as a function of temperature for all the samples for pure components (Kraft paper 60lbs and biopolymer) and their coating. $E'$ represents the stiffness of a viscoelastic material and is proportional to the energy stored during a loading cycle. $\tan \delta$ is the ratio of loss modulus ($E''$) to storage modulus ($E'$). It is a measure of the energy lost, expressed in terms of recoverable energy, and represents mechanical damping or internal friction in a viscoelastic region. Table 7.4 summarizes the glass transition temperature ($T_g$: temperature at $\tan \delta_{\text{max}}$) and the dynamic tensile modulus of the samples at various temperatures (−40, 0, 25 and 40°C).
The glass transition of the pure biopolymer was 2 °C. As can be observed from the maxima of the tan δ (Figures 7.10, Table 7.4), the glass transition temperature shifted to significantly higher temperatures for the coated samples. For 60 lbs paper coated samples, T_g shifted from 2°C for the neat biopolymer to 5.2, 9.4 and 11.20 °C for 60 Melt, 60 Dip and 60 Roll, respectively. Likewise, for 75 lbs paper coated samples, T_g shifted from 2°C for the neat biopolymer to 4.5, 6.90 and 10.80 °C for 75 Melt, 75 Dip and 75 Roll, respectively. The shift to high temperature could have indicated favorable attractive interaction at the interface between the biopolymer and the paper substrate. Furthermore, the decrease in the T_g peak intensity for the coated samples could have indicated a modification in the relaxation time of the biopolymer as a result of positive interaction between this latter and the paper substrate. Since the glass transition process is related to the molecular motion, the T_g is considered to be affected by molecular packing, chain rigidity and linearity. For both paper weights, the glass transition temperature was significantly higher in the solvent based coating than in the melt based coating. Between the dip and roll coated samples, the roll coated samples showed higher glass transition temperatures than the dip coated samples. We noted that the higher glass transition feature of the roll coated samples compared to their lower crystallinity index obtained by the ATR FT-IR could have indicated a higher amorphous character.

In sub-ambient conditions, the dynamic storage modulus E’ of the coatings is higher than that of the biopolymer and the uncoated Kraft papers (Figure 7.9, Table 7.4). Over the temperature range, E’ of uncoated Kraft papers showed insignificant change. As the temperature increased, E’ of coated samples and pure P(3HB-co-4HB) decreased.
However the decrease is higher for the neat biopolymer than for the coated samples. Table 7.4 shows that for both coated paper weights, $E'$ was significantly higher in the melt based coating than in the solvent based coating. Also, with respect to the uncoated paper weight (60lbs and 75lbs) an increase in $E'$ of the coated paper weights is noticeable in sub-ambient. For example at -40°C $E'$ for 60lbs paper coated samples increased to 25.3, 22.5 and 21% for 60 Melt, 60 Dip and 60 Roll sample, respectively. Similarly, $E'$ for 75lbs paper coated samples increased to 55.6, 29.5 and 15.8% for 75 Melt, 75 Dip and 75 Roll sample, respectively. The increase in $E'$ reflected the coating structure results obtained by ESEM, FTIR and WAXD for each coated sample. The increase in the $T_g$s and the dynamic modulus of the coated samples in comparison with the P(3HB-co-4HB) can be due to good adhesion between the biopolymer and the uncoated Kraft papers. We utilized a micromechanical model for predicting the coating properties from its pure components to that obtained experimentally.

$$E_{\text{coating}} \times A_{\text{coating}} = E_{\text{paper}} \times A_{\text{paper}} + E_{\text{biopolymer}} \times A_{\text{biopolymer}}$$

Where, $E$ is the modulus and $A$ the area.

Using this, we found that the experimentally obtained values of $E'$ are significantly greater than the predicted composite values (Table 7.5). This indicated that the coating interaction with the paper significantly enhances its own mechanical contributions to the coated paper. Furthermore, we noticed that the reinforcement potential is more effective in melt processed coating samples than solution processed coating samples, with Roll coating samples displaying less reinforcement.
Figure 7.9  Characteristics of E’ of P(3HB-co-4HB), Paper 60lbs and their coatings.

Figure 7.10  Characteristics of Tan δ of P(3HB-co-4HB) and 60lbs coatings.
Table 7.4 Dynamic mechanical summary for P(3HB-co-4HB), Kraft paper 60lb, 75lb and the coatings.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E'(GPa) -40 °C</th>
<th>E'(GPa) 0 °C</th>
<th>E'(GPa) 25°C</th>
<th>E'(GPa) 40 °C</th>
<th>Tg(°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB-co-4HB)</td>
<td>5.40 ±(0.15)</td>
<td>3.20 ±(0.13)</td>
<td>1.39 ±(0.15)</td>
<td>1.20 ±(0.16)</td>
<td>2.00 ±(0.2)</td>
</tr>
<tr>
<td>60 lbs</td>
<td>4.75 ±(0.20)</td>
<td>4.32 ±(0.20)</td>
<td>4.19 ±(0.18)</td>
<td>4.09 ±(0.20)</td>
<td>-</td>
</tr>
<tr>
<td>60 Melt</td>
<td>5.95 ±(0.35)</td>
<td>5.18 ±(0.33)</td>
<td>4.51 ±(0.30)</td>
<td>4.33 ±(0.29)</td>
<td>5.20 ±(0.3)</td>
</tr>
<tr>
<td>60 Dip</td>
<td>5.75 ±(0.18)</td>
<td>4.89 ±(0.15)</td>
<td>3.92 ±(0.19)</td>
<td>3.67 ±(0.15)</td>
<td>9.40 ±(0.3)</td>
</tr>
<tr>
<td>60 Roll</td>
<td>5.75 ±(0.23)</td>
<td>4.22 ±(0.21)</td>
<td>2.89 ±(0.18)</td>
<td>2.60 ±(0.20)</td>
<td>11.20 ±(0.2)</td>
</tr>
<tr>
<td>75 lbs</td>
<td>4.95 ±(0.20)</td>
<td>4.55 ±(0.19)</td>
<td>4.48 ±(0.20)</td>
<td>4.43 ±(0.20)</td>
<td>-</td>
</tr>
<tr>
<td>75 Melt</td>
<td>7.70 ±(0.17)</td>
<td>5.90 ±(0.16)</td>
<td>4.55 ±(0.15)</td>
<td>4.42 ±(0.16)</td>
<td>4.50 ±(0.5)</td>
</tr>
<tr>
<td>75 Dip</td>
<td>6.41 ±(0.22)</td>
<td>5.44 ±(0.20)</td>
<td>4.37 ±(0.19)</td>
<td>4.40 ±(0.20)</td>
<td>6.90 ±(0.3)</td>
</tr>
<tr>
<td>75 Roll</td>
<td>5.73 ±(0.15)</td>
<td>5.06 ±(0.14)</td>
<td>4.19 ±(0.17)</td>
<td>3.91 ±(0.15)</td>
<td>10.80 ±(0.4)</td>
</tr>
</tbody>
</table>

Table 7.5 Dynamic mechanical advantage of composite over the pure components.

<table>
<thead>
<tr>
<th>Sample</th>
<th>-40 °C</th>
<th>0 °C</th>
<th>25°C</th>
<th>40 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 Melt</td>
<td>32.41%</td>
<td>25.45%</td>
<td>21.41%</td>
<td>20.30%</td>
</tr>
<tr>
<td>60 Dip</td>
<td>23.82%</td>
<td>17.37%</td>
<td>03.80%</td>
<td>02.75%</td>
</tr>
<tr>
<td>60 Roll</td>
<td>19.70%</td>
<td>09.6%</td>
<td>03.10%</td>
<td>01.40%</td>
</tr>
<tr>
<td>75 Melt</td>
<td>48.77%</td>
<td>37.85%</td>
<td>13.15%</td>
<td>09.80%</td>
</tr>
<tr>
<td>75 Dip</td>
<td>27.52%</td>
<td>25.90%</td>
<td>10.72%</td>
<td>08.84%</td>
</tr>
<tr>
<td>75 Roll</td>
<td>16.90%</td>
<td>15.31%</td>
<td>05.94%</td>
<td>01.93%</td>
</tr>
</tbody>
</table>

The damage resistance and damage tolerance under impact loading are of the most importance of composite materials characteristics because they are often susceptible to impact. Velocity impact can introduce severe internal damages to composite structures and significantly reduce their load-carrying capacity [23]. Velocity impact damages may
consist of cracks, delaminations, and fiber breakage in a zone surrounding the impact point. When a foreign object impacts a composite material, the impact energy is absorbed by the composite and damages such as delaminations, fiber breakage, and cracks occurred in the composite structure. The mechanical properties of the specimens were evaluated and Figures 7.11 and 7.12 illustrate the representative data of load as a function of deflection and time, respectively for Kraft paper 75lbs and their coating. Four tests were run on each specimen. The average maximum load, total energy for Kraft papers and the coatings were recorded in the Table 7.6. From Figure 7.11, it is clear that the 75 Melt samples showed more elastic features than other specimens since 75 Melt samples responded more quickly upon impact loading. After the impact, it was observed that the melt processed samples showed large crack lines whereas the solution processed one exhibited medium crack lines with respect to the pure Kraft paper samples as showed by the representative data in Figures 7.13 to 7.16. These different characteristics of the coatings can be related to the difference in the coating texture which plays an important role in coating performance.

Under high impact, the coated papers indicated better resistance than uncoated paper. However, melt applied biopolymer samples showed enhanced adhesion and the delamination occurred in multiple stages resulting in enhanced fracture toughness. Table 7.6 indicated that both 60 and 75 lbs papers showed similar trends in impact performance. The total energy was higher for the melt processed samples compared to the solvent processed samples. Improvements of around 45% were observed for the melt processed samples while solvent based samples showed around 30% improvement in energy.
absorption capability. The maximum load followed the same trend and improvements of around 85% were observed for the melt processed samples while solvent based samples showed around 75%.

Figure 7.11  Load versus deflection plots of Kraft paper 75lbs and the coatings.

Figure 7.12  Load versus time plots of Kraft paper 75lbs and the coatings.
Figure 7.13  Fractured surface of 75lbs paper after impact test.

Figure 7.14  Fractured surface of 75 Roll sample after impact test.
Figure 7.15 Fractured surface of 75 Dip sample after impact test.

Figure 7.16 Fractured surface of 75 Melt sample after impact test.
7.4 Conclusion

This study has shown that P(3HB-co-4HB) coated paper can be obtained through solution and melt processed technique. ESEM results indicated the melt coated samples showed both better surfaces and penetration of the polymer into the paper. FT-IR results showed that the reflection peaks were from the biopolymer, indicating retention of the chemical structure of the pure P(3HB-co-4HB) in the coatings. WAXD results indicated that the coatings samples showed the melt processed coated samples showing most of the crystalline structure of the biopolymer. DMA results showed that the melt processed sample exhibited better storage modulus in subambient conditions and the improvement was higher for the higher weight Kraft paper. At room temperature and above, the coatings showed the values of the storage modulus close to those of Kraft paper but higher than those of the biopolymer. Impact results conducted at room temperature showed that the melt based samples had a higher total energy absorption capability indicating superior adhesion to the solution processed samples. Of the two solvent coated papers, the dip coating offered superior performance over that of the roll coated. We attribute that to the additional soaking and contact time that dip coating has over the roll coating. We further observed that nip pressure minor variances could possibly affect uniformity of the coating application.
7.5 References


CHAPTER 8

PHYSICAL AND THERMAL ANALYSIS OF POLY(3-HYDROXYBUTYRATE-CO-4-HYDROXYBUTYRATE) COATED PAPER DEGRADATION IN SOIL

8.1 Introduction

Paper remains an optimal material for a highly biodegradable packaging choice. It is a critical component of packaging applications for shipping and handling of food and non-food products. However its hygroscopic nature limits its potential when shelf life concerns are taken into account. To ensure the barrier properties and to retain dimensional stability, paper and paperboard intended for packaging materials are often coated with common commercial polymers such as poly-vinylidene chloride, poly(ethylene-co-vinyl alcohol) and oriented polyethylene terephthalate [1]. Polyolefin-derived materials represent the major materials for everyday life. Their products are easily produced, convenient, inexpensive, long lasting and widely used in many fields of applications including, food packaging and agriculture.

Food packaging represents a high volume commodity using paperboard based products for shipping and handling purposes. When discarded, food packaging products

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can become the most evident source of litter generated by human beings. This has caused increasing environmental concerns. The Army, Air Force and Marine Corps consume approximately 46.6 million operational rations each year, generating 14,117 tons of packaging waste [2]. Shipping containers fabricated from fiberboard are necessary to transport and store food and other military items for all warfighters, including sailors on Navy vessels. However, there are numerous disadvantages in the process used to produce fiberboard: the process is costly, uses cellulose and hazardous chemicals, depletes natural resources in our environment and creates hazardous waste. In recent years, due to environmental concerns, the development of biodegradable packaging materials from renewable natural resources has received widespread support in many countries as well as in many national or international organizations [3]. The objectives in the development of biodegradable packaging materials can be summarized as a combination of two issues: use renewable raw materials (crops instead of crude oil) and facilitate integrated waste management approaches to reduce the amount of waste sent to landfills [3].

Biodegradable packaging made of paper or paperboard and biopolymers would have the dual advantages of avoiding the cost of removal and disposal, and mitigating environmental concerns. Also, synergistic advantages in terms of mechanical, water and gas barrier properties can be obtained from the composites of paper or paperboard and biopolymer. Thus, in an effort to produce more environmentally friendly packaging materials, renewable and biodegradable biopolymers have been investigated as paper, paperboard and fiberboard coating materials by a number of researchers [1,4-14]. Among them, polyhydroxyalkanoates or PHAs have been investigated as paper coating materials.
PHAs are a diverse group of biopolymesters, synthesized intracellularly by a wide range of microorganisms as carbon and energy reserves [15]. Their physicochemical properties are similar to those of traditional thermoplastics such as polypropylene and polyethylene. They have attracted attention for industrial, medical and agricultural applications [16,17] because of their biodegradability and biocompatibility [15,18]. Poly(3-hydroxybutyrate) or P(3HB) is the common member of PHAs family, a highly crystalline and brittle material. These physico-chemical properties limit P(3HB) utilization. Copolymerization of 3-hydroxybutyrate or 3-HB with units of 4-hydroxybutyrate or 4-HB, and 3-hydroxyvalerate or 3-HV to yield P(3HB-co-4HB) and P(3HB-co-3HV), respectively, modifies the physico-chemical properties of P(3HB) making this latter more flexible, less crystalline for practical applications like coatings [19]. PHAs possess excellent film-forming and coating properties and are water resistant due to their high hydrophobicity [5]. For example, P(3HB)-coated paperboard has been used for packaging of ready to eat meals, while P(3HB-co-3HV) coated board has been used for dry products, dairy products and beverages [4,6]. The US Patent and Trademark Office awarded to Robert Whitehouse and co-inventors (Metabolix: Cambridge, MA, USA) the US Patent 7,094,840 covering low molecular weight PHAs for use as hot melt adhesives, waxes and protective coatings [6]. Coating compositions include a PHA oligomer and also stabilizers, plasticizers, dyers, emulsifiers, thickening agents, antioxidants, preservatives, cross-linking agents, other biologically degradable polymers, and anti-fungal agents. The coatings are used in coating cheese and other food products [7]. The paper substrate, Kraft paper, is a universal material made essentially from wood pulp produced by a
modified sulfate pulping process (Kraft process). It comprises a matrix of long cellulose fibers held together by hydrogen bonding between hydroxyl groups. Cellulose is an environmentally friendly and renewable biomaterial [20]. It is a linear polysaccharide of D-glucose units (C₆H₁₀O₅) linked by β-1,4-glycosidic bonds. Kraft paper is widely used in packaging applications for both food and non-food materials due to its high mechanical resistance to tearing and to tensile forces. These properties are due to the long fibers used in the manufacture of Kraft paper and its basis weight of 30-150 g/m² [21]. Kraft paper is usually brown in color but can be bleached to produce white paper. It is used for paper grocery bags, multiwall sacks, fiberboard, envelopes and other packaging due to its high mechanical resistance to tearing and to tensile forces [21,22]. In addition, its environmentally friendly nature has helped the material resist substitution by petroleum-based plastics. Kraft paper is the main raw material for corrugated packaging industries. Corrugated fiberboard, the most widely used distribution container material, represents more than 80% of the volume of all paper-based packaging materials [12]. However, due to its hygroscopic properties and poor barrier to water and water vapor, Kraft paper is not suitable for long-term storage of dehydrated products because it reduces their shelf life [21, 23].

The investigation of the preparation and degradation, both based on physical weight loss and biodegradation of biopolymer based paper coatings has received some interest. Lim et al. [8] prepared solution based P(3HB) coating obtained by P(3HB) penetration into and adhesion onto cellulose paper. The coating efficiency index of P(3HB) coated paper was investigated through solution enzymatic degradation. They
showed that the biodegradability of P(3HB) coated paper was greater than that of P(3HB) powder. They concluded that the penetration of P(3HB) into the cellulose paper by coating improved the biopolymer degradability. The physical and thermal consequences of the weight loss have been monitored for a P(3HB-co-3HV) based copolymer by Luo and Netravali [24]. FTIR, DSC and tensile measurements of the biopolymer in a composting environment were investigated. The surface pitting of the sample over time in the composting medium led to lower yield stress values since crack initiation was promoted at the porous surface. The effect of a biodegradable additive such as abaca fiber in a P(3HB-co-3HV) has been investigated by Teramoto et al. [25]. They showed increased weight loss in P(3HB-co-3HV) as a consequence of adding abaca fiber. Thermophysical effects were not examined.

The objective of this study is to investigate the physical and thermal effects that the weight loss generates in P(3HB-co-4HB), cellulose paper and their coatings. While composting environments provide the optimum conditions for high degradation rates, we utilize a soil mixture at 30°C which slows the degradation rate in order to monitor the mechanism and retain sample integrity over the measurement period.

8.2 Experimental

8.2.1 Materials

Brown Kraft paper was supplied by the U.S. Army Natick Soldier Research Development and Engineering Center (NSRDEC). It had a weight of 75 lbs and a thickness of 0.22 mm. P(3HB-co-4HB) (Lot # MBX CS06082205 ; $M_w = 437,084$ gmol$^{-1}$
P(3HB-co-4HB) pellets were dried in an oven for 48 hours at 40°C. The chemical structure of P(3HB-co-4HB) is shown in Figure 8.1. Dichloromethane (>99.8% purity, EMD Chemicals: Gibbstown, NJ) was used as received.

8.2.2 Preparation of Coated Kraft Paper Samples

10 g of P(3HB-co-4HB) pellets were dissolved in 200 ml of dichloromethane. The solution was heated at 50°C and magnetically stirred for 10 min. After that, the solution was kept overnight at 40°C under agitation for complete dissolution of the biopolymer. For the coating process, a solution bath of 30 × 20 cm² was used. The substrate was immersed with the aid of a weighted screen (3 kg) to prevent it from floating and was withdrawn from the bath after ten min. A coating was obtained by evaporation of the solvent at ambient condition. The average thickness of the coated sample is 0.35 ± 0.01 mm, and the coating weight is 41.2 ± 2.8 g/m². This was determined by comparing 5 coated and uncoated weight of the same area of sample. Control P(3HB-co-4HB) films of 0.20 (± 0.02) mm thickness were also prepared at 190°C for 5 min by compression molding between teflon sheets using a Carver hot press (Wabash, IN).
8.2.3 Aging in Soil

Uncoated and coated Kraft paper and biopolymer samples were cut into $5 \times 5$ cm$^2$ pieces, weighed, and used for the weight loss investigation. Three replicates of each sample were subjected to a soil burial test for 1, 2, 5 and 8 week periods. The soil medium used was constructed of 1 part topsoil, 1 part sand, and 1 part manure that contained 20% water by weight. The soil chambers were kept in an incubator at a constant temperature of 30°C. These conditions decrease the microbial activity or the weight loss of the biopolymer and enable monitoring the mechanism of degradation. The samples removed from the soil medium were brushed lightly to remove any soil particulates, thoroughly rinsed using distilled water, dried in a vacuum oven at 60°C temperature for 24 hours, and weighed. The dried samples were used for subsequent analyses.

8.2.4 Surface Morphology

A Nikon Digital Camera D40X was used to observe the surface of the uncoated, coated Kraft paper and biopolymer samples before and after aging in soil. Enhanced resolution was obtained through additional use of a FEI Quanta 2000 Environmental Scanning Electron Microscope (Hillsboro, OR). All samples were coated with gold before imaging and were examined using an accelerating voltage of 12.5 kV.
8.2.5 Dynamic Mechanical Thermal Analysis (DMTA)

The viscoelastic properties of the uncoated Kraft paper, coated Kraft paper and biopolymer samples before and after degradation in soil were measured using a dynamic mechanical analyzer RSA III (TA Instruments, New Castle, DE) operating in the tensile mode. For the viscoelastic measurements, samples were scanned at a heating rate of 5°C/min, a frequency of 1 Hz and a strain amplitude of 0.3% (determined from a separate strain amplitude sweep of the biopolymer at 1 Hz to establish the linear viscoelastic region) between -140°C to 100°C. The dimensions for the different samples are of 25 × 5 × 0.22 mm³ for the Kraft paper, 25 × 5 × 0.20 mm³ for the biopolymer and 25 × 5 × 0.35 mm³ for the coating. All DMA measurements were done in triplicate. Frequency sweep was carried out on pure and 8 weeks aged biopolymer samples in the temperature range of -50 to 60°C and data were taken at every 5°C. A ramp and soak program was used to control the temperature profile. At each temperature, the specimen was soaked for 5 min before testing. Storage modulus, E' and loss modulus, E'' were measured in the frequency range of 24-0.16 Hz. Strain amplitude (0.3%) and an initial static force (350 g) were used in all the measurements. All frequency sweep measurements were done in duplicate.

8.2.6 Differential scanning calorimetry (DSC)

Thermal analysis of the coated Kraft paper and biopolymer samples before and after degradation in soil was performed on a DSC6 PerkinElmer apparatus (Waltham, MA) under a nitrogen atmosphere. The system was calibrated with an indium standard.
Approximately 5 mg of each sample was used for DSC measurement. In order to assess the aging process on the thermal transitions of the samples, one heating scan was performed from 25°C to 190 °C at 10°C/min. For the biopolymer samples, a second heating scan was performed after a dynamic cooling from the melt to investigate the change in the melting point and the crystallinity. Measurements were done in duplicate.

8.3 Result and discussion

8.3.1 Weight loss during aging in soil

Figure 8.2 shows the results of weight loss for uncoated and coated Kraft paper and for the biopolymer in soil. The graph displays weight loss (normalized to sample thickness) as a function of exposure time in soil and provides indications of the degradation potential of the samples in the soil medium. The rate of weight loss during soil burial, as a percentage of initial weight, varies from sample to sample. From Figure 8.2, one observes that the rate of weight loss is smaller in the beginning, but increases with time. The severe weight loss of the uncoated paper after 8 weeks resulted in torn or disintegrated material which was difficult to weigh. The coated paper underwent a lower weight loss rate than the uncoated paper. This shows that the biopolymer is protecting the paper from water migration and microorganisms. After a 5 week period of exposure in soil, the rate of weight loss was 75% for the uncoated paper and 31% for the coated paper. On the other hand, P(3HB-co-4HB) samples started to show weight loss only after a 2 weeks period and exhibited a very low rate of weight loss of 5% over the entire 8 weeks period of aging, reflecting the lower activity of the soil conditions.
Figure 8.2 Weight loss for uncoated, coated Kraft paper and the biopolymer samples buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks.

Luo and Netravali [24] showed through weight loss measurements of pure P(3HB-co-3HV) that the microbial activity in a composting environment changed with time. It was smaller initially, increased with time, and finally slowed down. Many types of microorganisms are known to degrade cellulose (Kraft paper) and the biopolymer P(3HB-co-4HB). Endocellulases and exocellulases [14], and PHA depolymerases [18] are secreted by several types of bacteria and fungi. Many authors have reported on the degradation of polymer. Philip et al. [16] explained that the biodegradability of a polymer is governed primarily by its physical and chemical properties. It has been found that low molecular weight PHAs are more susceptible to biodegradation. Also, the melting point is another important factor to be considered when investigating biodegradation. As the melting point increases, the biodegradability decreases. With increasing melting point, the enzymatic degradability decreases. Tokiwa et al. [26]
investigated the hydrolysis of polyesters by lipases and found that these enzymes cannot hydrolyze the optical active P(3HB) due to the high melting temperature (around 178°C) of the latter. On the other hand, Mochizuki et al.[27] clarified that the biodegradation of solid polymer such as fibers and films is influenced not only by chemical structure of the polymers, especially the presence of functional groups and hydrophilicity-hydrophobicity balance, but also by the highly ordered structures such as crystallinity, orientation and other morphological properties. Volova et al.[28] reaffirmed that the crystallinity plays a very important role in biodegradability. They confirmed the existing opinion that highly crystalline material [e.g., P(3HB)] has lower degradability with less availability to degrading microorganisms and enzymes. In addition, the microbial population and activity, as well as temperature and moisture content, also contribute to biodegradability in the environment [27].

Thus, in our study, the high rate of weight loss of the uncoated paper samples could be due to the availability of the endo- and exocellulases, temperature and moisture content in the soil medium. The low rate of weight loss displayed by the biopolymer samples could be due to the lack of PHA depolymerases. Since the soil conditions were the same for all samples, the results indicated that low temperature and moisture content contributed to the low rate of weight loss for all samples. However, the increased weight loss for the biopolymer coated samples reflects the synergistic gains in using biopolymer coatings together with paper, since enhanced degradation rates are obtained in the biopolymer due to increased microbial activity from the paper.
8.3.2 Effect of Aging on the Surface Topography

Photographs of the uncoated Kraft paper, the coated Kraft paper and the biopolymer samples before and after soil burial are presented in Figures 8.3(A, B, C, D, E), Figures 8.4(A, B, C, D, E) and Figure 8.5(A, B, C, D, E), respectively. As seen in the figures, aging occurs randomly at the sample surface, making it rough by forming cavities of microbial colonization and water penetration on it (visual observation). This change, insignificant at the beginning, expanded on the surface of the samples as aging increased. For the coated paper sample, the protective role of the biopolymer is evident. The uncoated Kraft paper tore into small pieces after 8 weeks while the coated papers remained almost intact. For the biopolymer samples, the observed change suggested that after 8 weeks, they may be more resistant to degradation. The above observations are supported by the rate of weight loss data. We note that we have conducted our experiments at 30°C which is below the recommended 50°C (and above) for PHAs via composting [29] but since paper shows a high degradation rate at 50°C (and above) [30] we utilized a lower temperature to retain physical integrity of the samples.
Figure 8.3  Photographs of uncoated Kraft paper buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks.

Figure 8.4  Photographs of coated Kraft paper buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks.
Figure 8.5  Photographs of P(3HB-co-4HB) films buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks.

ESEM micrographs of uncoated Kraft paper, coated Kraft paper and biopolymer samples are shown in the Figure 8.6(A, B, C, D, E), Figure 8.7(A, B, C, D, E) and Figure 8.8(A, B, C, D, E), respectively. Figure 8.6(A) displays the micrograph of uncoated Kraft paper before soil burial, displaying a fibrous and porous structure. Impregnation with the biopolymer modified the surface of the material, making it more homogenous, as shown in Figure 8.7(A). Rhim et al.[12] also showed through SEM images that polylactic acid (PLA) coating on paperboard produced a more homogenous and smooth coating surface than its uncoated counterpart. Furthermore, they revealed that the coating surface of the substrate became smoother with increasing coating weight.

The micrographs of the uncoated paper in Figure 8.6(B, C, D, and E) show that, by 5 weeks, microorganisms have extensively colonized the surface and the interior.
Fibrillar breakage and defibrillation are evident on the sample surface and interior with increased exposure time in soil. As a result, the initial porous and fibrous structure of the paper was completely destroyed after 8 weeks of aging.

![Micrographs of uncoated Kraft paper buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks (scale bar: 50 µm).](image)

Figure 8.6 ESEM micrographs of uncoated Kraft paper buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks (scale bar: 50 µm).

Microorganism growth and colonization were also widespread on the surface of the P(3HB-co-4HB) coated paper at 5 weeks [Figures 8.7(B), 8.7(C), 8.7(D), 8.7(E)]. However, compared with the uncoated paper, the microorganism penetration to the interior of the coated paper was limited. This is due to the protective role of the biopolymer, which may resist degradation more than the uncoated paper. After 8 weeks
exposure in soil, the degradation reaches the porous and fibrous structure of the paper, as shown in the Figure 8.7(E). The low rate of weight loss of the coated samples with respect to that of uncoated samples supports this determination.

In Figure 8.8(A), it can be seen that the biopolymer sample shows uniform structure throughout the surface before burial. After exposing the biopolymer samples to the soil medium, they became rougher than the unaged sample. Microbial colonization and surface erosion were evident as the degradation time increased. ESEM micrograph of samples exposed for 5 weeks [Figure 8.8(D)] shows significant cracks and fissures on the
surface. With increasing aging time, cracks and fissures increased in depth, with the appearance of multiple holes on the surface as shown by 8 week biopolymer samples [(Figure 8.8(E)]. In terms of change in the surface topography of P(3HB-co-4HB) samples after 8 weeks in soil medium, one can conclude, that during the initial degradation period, a few microorganism colonies that existed were spread far apart. They resulted in the surface erosion shown on the biopolymer surface after 2 weeks [Figure 8.8(C)]. As the microorganism colonies increased, cracks and fissures emerged as displayed on the surface of the biopolymer after 5 weeks of aging. As the colonies spread on the samples, deeper cracks and fissures appeared with the formation of holes as shown in the 8 weeks samples. However, after 8 weeks, the surface degradation was not even, which could result in somewhat smoother surface. This observation suggests that after 8 weeks of aging, P(3HB-co-4HB) samples may be more defiant to microbial degradation in the low activity soil environment. The low weight loss obtained after 8 weeks of exposure in soil medium supported the above observation.
Figure 8.8. ESEM micrographs of P(3HB-co-4HB) films buried in soil for prescribed periods: 0, 1, 2, 5 and 8 weeks (scale bar: 50 µm).

We conclude from the photographs and ESEM micrographs analyses that the biopolymer, Kraft paper and their coating samples used in this study were readily colonized and partially degraded by soil microorganisms. The surface erosion and the low weight loss rate of the biopolymer could indicate an enzymatic degradation. It seemed like Kraft paper could have helped in degrading the biopolymer faster. Thus, these samples show clear signs of degradation and provided evidence for their degradability in laboratory conditions. The results also confirmed the suitability of the
applications of P(3HB-co-4HB), Kraft paper and their coating involving degradation in soil under natural conditions.

8.3.3 Effect of Aging on the Thermomechanical Properties

Besides the analysis of morphological changes, the aging process was also studied by means of the characterization of the thermomechanical behavior of the uncoated Kraft paper, coated Kraft paper and biopolymer samples before and after soil burial. Figures 8.9 to 8.12 show the relaxation spectrum in terms of storage modulus (E’) and loss tangent (tan δ) versus temperature at the frequency of 1 Hz for the biopolymer, uncoated Kraft paper and coated Kraft paper; non-aged and aged uncoated Kraft paper; non-aged and aged coated Kraft paper; non-aged and aged biopolymer samples, respectively. Table 8.1 and 8.2 summarize the dynamic mechanical properties of P(3HB-co-4HB), Kraft paper and their composite coating at fixed temperatures (-140, -50 and 25°C) before and after aging (up to 2 weeks, paper integrity limits the analysis for longer times). E’ represents the stiffness of a viscoelastic material and is proportional to the energy stored during a loading cycle. On the other hand, tan δ is defined as the ratio of loss modulus E’’ to storage modulus (E’’/E’). It is a measure of the energy lost, expressed in terms of recoverable energy, and represents mechanical damping or internal friction in a viscoelastic system. The transitions were measured from the temperatures corresponding to the maxima in tan δ plot.
Figure 8.9 Mechanical spectra in terms of $E'$ and $\tan \delta$ of uncoated Kraft paper (A), coated Kraft paper (B) and the biopolymer (C) showing the mechanical advantage of the composite over the pure components.

The data in Figure 8.9 show that the biopolymer P(3HB-co-4HB) exhibited two transitions at around -104°C and +2°C, corresponding to $\beta$ and $\alpha$ relaxation processes, respectively. The $\beta$ relation ($T_\beta$) of the biopolymer is conventionally associated with local crankshaft motion of the $(\text{CH}_2)_n$ segment [31] whereas the $\alpha$ relaxation, known as glass transition ($T_g$), constitutes the most important mechanical property for all polymers.

Cellulose is the most abundant chemical species found in paper. As a viscoelastic polymer, cellulose exhibits time and temperature dependent mechanical properties which are the result of molecular level relaxation processes. Pankonin and Habeger [32] investigated the relaxation processes in cellulose. They reported two secondary relaxations below room temperature at low frequencies (~ 1Hz): $\gamma$ at -93°C and $\beta$ at -23°C. They also reported on the effect of moisture in these relaxation processes. In dry cellulose, the $\gamma$ relaxation is large, and the $\beta$ is absent. However, as moisture is added, the
β relaxation appears and strengthens relative to the γ relaxation. The presence of moisture shifts both relaxations to lower temperatures, suggesting water acts as a plasticizer for both processes. They attributed the γ relaxation to a hindered conformation change (rotation) of the methylol groups in the disordered regions of cellulose and the β relaxation to the rotation of methylol-water complexes, also in the disordered areas. Thus, the tan δ plot of our Kraft paper exhibited a broad peak with two maxima at -95 and -8°C (Figure 8.9), which could be attributed to γ and β relaxations, respectively.

In the subambient region, the E’ of the biopolymer is slightly higher than that of the uncoated Kraft paper (Figure 8.9). The brittle structure of the biopolymer could have been responsible for its high storage modulus in subambient as compared to Kraft paper, which has a fibrous and porous structure. Over the temperature range of our study, the magnitude of E’ of Kraft paper was similar to that reported by other authors [33]. Around the glass transition temperature Tg, E’ of the biopolymer reduced more markedly. Above Tg, the E’ of the uncoated paper is higher than that of the biopolymer with increasing temperature.

The impregnation of the Kraft paper with the biopolymer promoted significant changes in its thermomechanical properties. A significant enhancement of E’ in the subambient temperature range is evident. This can be related to the synergistic advantages gained in terms of mechanical properties by combining the properties of both biomaterials in a single, strong biodegradable composite. Above room temperature, the coating did not provide a significant enhancement in the E’ values as compared to the uncoated paper.
Table 8.1 also showed the reinforcement imparted to the coating by the biopolymer and Kraft paper. At lower temperature, the reinforcement imparted by the biopolymer was higher whereas at room temperature and above Kraft paper’s reinforcement was considerably predominant. After the impregnation of the Kraft paper with the biopolymer, the coating showed two relaxation transition temperatures attributable to the biopolymer. However, we observed that in the composite coating, $T_\beta$ was unaffected whereas $T_g$ was affected. $T_g$ increased from +2°C for the biopolymer to +12°C for the composite coating. The increase may be related to the restriction of the biopolymer chain mobility and, this, elevated the glass transition $T_g$. This could indicate favorable attractive interaction at the interface between the biopolymer and the paper substrate. We also note that the $T_g$ of the coating sample was less-resolved compared to that of the neat biopolymer sample. The decrease in the $T_g$ peak intensity for the coated sample could indicate a modification in the relaxation time of the biopolymer as a result of positive interaction between this latter and the paper substrate. Since the glass transition process is related to the molecular motion, the $T_g$ is considered to be affected by molecular packing, chain rigidity and linearity. We utilized a micromechanical model for predicting the coating properties from its pure components to that obtained experimentally.

\[ E_{coating} \times A_{coating} = E_{paper} \times A_{paper} + E_{biopolymer} \times A_{biopolymer} \quad (1) \]

Where, $E$ is the modulus and $A$ the area.

Using this, we found that the experimentally obtained values of $E'$ before aging are significantly greater than the predicted composite values (Table 8.1). This shows the mechanical advantage of the composite over the pure components and indicates that the
coating interaction with the paper significantly enhances its own mechanical contributions to the coated paper. After 2 weeks aging in soil, the coating retains its mechanical advantage over the pure components in subambient (Table 8.2).

The thermomechanical spectra of the uncoated paper (Figure 8.10) show that, by 5 weeks, the weight loss was so severe that no further experiment could be conducted on the samples. It can be seen that the weight loss affects $E'$ significantly. $E'$, which represents the stiffness of a viscoelastic material, decreased with exposure time. The decrease in the fiber cohesion due to breakage and defibrillation explains the loss of the stiffness. No change in $\gamma$ and $\beta$ relaxations with exposure time can be seen. The $E'$ result is consistent with the morphological study obtained by photographic review and ESEM.

![Figure 8.10](image)

Figure 8.10  Mechanical spectra in terms of $E'$ and tan $\delta$ of uncoated Kraft paper buried in soil for prescribed periods 0, 1 and 2 weeks.

A decrease in the stiffness was also observed for the P(3HB-co-4HB) coated paper with exposure time (Figure 8.11). However, compared with the uncoated paper,
thermomechanical behavior at 5 weeks can be extracted up to 40°C. Above 40°C, the test samples break so that the experiment has to be stopped. The 8 week samples were impossible to test, since breakage occurred as soon as the experiment began. The surface embrittled and the brittle coating can be primarily attributed to the biopolymer, indicating the protective role of this component. The coating can also be attributed as the cause of the lower weight loss in the coated material compared to the uncoated paper. These observations were supported by the results of morphological study obtained by photographic analysis and ESEM.

![Mechanical spectra](image)

Figure 8.11 Mechanical spectra in terms of E’ and tan δ for coated Kraft paper buried in soil for prescribed periods 0, 1, 2 and 5 weeks.

For the biopolymer samples (Figure 8.12), the surface and/or internal alteration after 8 weeks exposure in soil could have affected the stiffness of this latter resulting in the decrease of E’. No change in either relaxation process could be seen with aging.
Figure 8.12  Thermomechanical spectra of P(3HB-co-4HB) films in soil after different periods of time: 0, 1, 2, 5 and 8 weeks.

Table 8.1  Dynamic mechanical properties of P(3HB-co-4HB), Kraft paper and their coating.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_g$ (°C)</th>
<th>E'(GPa)</th>
<th>Mechanical advantage of composite over the pure components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-140  °C</td>
<td>-50  °C</td>
<td>25  °C</td>
</tr>
<tr>
<td>P(3HB-co-4HB)</td>
<td>2 ±(0.2)</td>
<td>7.3 ±(0.5)</td>
<td>5.7 ±(0.4)</td>
</tr>
<tr>
<td>Kraft paper</td>
<td>-</td>
<td>6.8 ±(0.3)</td>
<td>4.9 ±(0.3)</td>
</tr>
<tr>
<td>Coating</td>
<td>12 ±(0.3)</td>
<td>8.5 ±(0.8)</td>
<td>6.8 ±(0.7)</td>
</tr>
</tbody>
</table>
Table 8.2 Dynamic mechanical properties of P(3HB-co-4HB), Kraft paper and their coating with aging time – i :1 week; ii : 2 weeks.

<table>
<thead>
<tr>
<th>Sample</th>
<th>E’(GPa)</th>
<th>Mechanical advantage of composite over the pure components</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-140 °C</td>
<td>-50 °C</td>
</tr>
<tr>
<td>P(3HB-co-4HB) i</td>
<td>6.6 ±(0.4)</td>
<td>4.8 ±(0.4)</td>
</tr>
<tr>
<td>Kraft paper i</td>
<td>5.5 ±(0.3)</td>
<td>4.1 ±(0.1)</td>
</tr>
<tr>
<td>Coating i</td>
<td>7.5 ±(0.3)</td>
<td>5.2 ±(0.2)</td>
</tr>
<tr>
<td>P(3HB-co-4HB) ii</td>
<td>6.4 ±(0.1)</td>
<td>4.1 ±(0.3)</td>
</tr>
<tr>
<td>Kraft paper ii</td>
<td>4.9 ±(0.2)</td>
<td>3.7 ±(0.3)</td>
</tr>
<tr>
<td>Coating ii</td>
<td>6.8 ±(0.4)</td>
<td>4.5 ±(0.3)</td>
</tr>
</tbody>
</table>

Since the behavior of a polymer at high frequency is related to the behavior at very low temperature, and vice versa, the frequency sweep tests at over a wide range of temperature (-50 to 60°C) can be used to understand the behavior at extremes temperatures outside the experimental range, using time-temperature-superposition (TTS) method. We have applied TTS to the neat and 8 weeks soil aged biopolymer samples.

TTS has long been used to obtain temperature-independent master curves for polymer systems by shifting the values of storage/loss modulus (E’) towards the frequency axis. One reference temperature T_{ref} should be chosen (here T_{ref} =5°C) and the viscoelastic variables of interest (e.g., storage modulus) at other temperatures are shifted to the corresponding values at that reference temperature. A horizontal shift factor, a_{T},

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which is a function of temperature enables to obtain the master curves. For unaged and 8 weeks aged biopolymer, $a_T$ versus temperature curves are shown in Figure 8.13.

![Figure 8.13](image)

**Figure 8.13** Horizontal shift factor $a_T$ versus temperature for the unaged and 8 weeks soil aged P(3HB-co-4HB) samples showing Arrhenius and WLF regions from $T_{ref}$. 

For thermorheologically simple materials, at temperatures less than the glass transition $T_g$, the $a_T$ curve follows an Arrhenius relationship as indicated by Equation 2.

$$\ln a_T = \frac{E_A}{R} \left( \frac{1}{T} - \frac{1}{T_g} \right) \quad (2)$$

One can relate the shift factor, $a_T$, to the glass transition temperature, $T_g$ and to the activation energy $E_A$ which is related to the steepness of the energy barrier required to change the quasi-equilibrium state of the system from one configuration to another.
At temperature greater than $T_g$, the substantial effects of free volume make $\alpha_T$ curve to follow the Williams-Landel-Ferry (WLF) relationship as indicated by Equation 3.

$$\log \alpha_T = \frac{-C_1(T-T_g)}{C_2 + (T-T_g)} \quad (3)$$

One can relate the shift factor, $\alpha_T$, to $T_g$ of a polymer and two constant, $C_1$ and $C_2$, which have been found to be characteristic of the polymer molecular structure.

Figure 8.14 shows $\log(E')$ versus $\log$(frequency) time-temperature master curves of non aged and 8 weeks aged P(3HB-co-4HB) samples. At higher frequency or lower temperature and at lower frequency or high temperature, the storage modulus of the aged sample shows a decrease. Physical aging due to cracks and fissures in the aged sample can explained this behavior.

![Figure 8.14](image)

**Figure 8.14** Dynamic mechanical time–temperature master curve at a reference temperature ($T_g = 5^\circ C$) of the storage modulus for the unaged and 8 weeks soil aged P(3HB-co-4HB) films.
Figure 8.15 shows log($E''$) versus log(frequency) master curves of non aged and aged P(3HB-co-4HB) samples. Neat P(3HB-co-4HB) sample shows a maximum $E''$ peak which is related to the glass transition. In the aged sample this peak shift to lower frequency or high temperature. This result could indicate a modification in the relaxation time of the biopolymer as a result of physical aging in the soil medium.

Table 8.3 summarizes the activation energy ($E_A$) parameter according to Arrhenius Equation and the constants $C_1$, $C_2$ derived from Williams-Landel and Ferry (WLF) Equation. We observe that $E_A$, $C_1$ and $C_2$ decrease in the aged sample with respect to the unaged one. The decrease in $E_A$ indicates the reduction in the steepness of the energy barrier required to change the quasi-equilibrium state of the system from one configuration to another. The WLF constants $C_1$ (15.80-20.50) and $C_2$ (57.48-68.55) are different from their universal values which are 17.4 and 51.6 K for $C_1$ and $C_2$, respectively. A decrease in $C_1$ and $C_2$ after 8 weeks aging in soil indicates an increase in $f_g$ fractional free volume at $T_g$ and $\alpha_f$ volumetric expansion of free volume as shown by Equation 4 and 5, respectively.

$$f_g = \frac{1}{2.303C_1} \quad (4)$$

$$\alpha_f = \frac{f_g}{C_2} \quad (5)$$
Figure 8.15 Dynamic mechanical time–temperature master curve at a reference temperature ($T_g = 5^\circ C$) of the loss tangent for the unaged and 8 weeks soil aged P(3HB-co-4HB) films.

Table 8.3 Arrhenius and WLF parameters for the neat and 8 weeks soil aged P(3HB-co-4HB) films.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arrhenius</th>
<th>WLF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E_A \text{ (kJmol}^{-1}\text{)}$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>P(3HB-co-4HB) 0 week</td>
<td>296.09</td>
<td>0.9979</td>
</tr>
<tr>
<td>P(3HB-co-4HB) 8 weeks</td>
<td>248.08</td>
<td>0.9909</td>
</tr>
</tbody>
</table>

$R^2$: Regressional analysis

8.3.4 Effect of Aging on the Biopolymer Thermal Properties

Figure 8.16(A), Figure 8.16(B) and Figure 8.16(C) showed the first heating, first cooling and second heating thermograms of the non-degraded and partially degraded biopolymer samples, respectively. Figure 8.17 displayed the first heating thermograms of the non degraded and partially degraded coating samples.
The biopolymer sample before aging in soil [Figure 8.16(A)] showed a bimodal endothermic peak: a low melting temperature at 148°C and a high melting temperature at 162°C. These values are consistent with those reported elsewhere [34]. These peaks might correspond to the melting temperatures of the crystalline phases. This suggested two crystalline phases probably presented in the biopolymer. The thermograms of the partially aged samples are different from those of the non-aged sample. One can notice the presence of a third endothermic peak before the double endothermic peaks. In fact, for the samples of 1 and 2 weeks, a small peak appeared between 70 and 86°C whereas for those of 5 and 8 weeks, a large peak showed between 110 and 135°C. This observation could indicate a certain degree of physical aging of the biopolymer due to water penetration and microbial colonization during exposure in the soil medium. This physical aging, insignificant initially, increased with exposure time in the soil medium.

Cong et al.[35] reported three endothermic peaks in the P(3HB-co-4HB) biopolymer, having 9, 11, 17 and 20 mol% 4-HB unit. They assigned the lowest temperature melting peak to the crystalline phase of the 4-HB rich units, the middle one to that of 3-HB rich units and the highest one to a recrystallization process in which the lattice of the copolymer had enough time to be rearranged at a given DSC heating rate, that was because the intensity of this peak relative to that of the peak at a lower temperature decreased with the heating rate. In our study, the appearance of the lowest endothermic peak could be related to phase separation phenomena in the biopolymer as a result of weight loss. In the as-received sample, the melting peak corresponding to the 4-HB unit was incorporated into the 3-HB crystallites. However, the results show that aging leads to
phase separation in the copolymer, leading to the secondary phase separation. In addition, this phase separation increased with aging time.

Figure 8.16 DSC thermograms of P(3HB-co-4HB) films buried in soil for prescribe periods: 0, 1, 2, 5 and 8 weeks showing the aging effect on the biopolymer - (A): first heating; (B): cooling; (C): second heating.

Figure 8.16(B) showed the melt recrystallization thermograms of P(3HB-co-4HB) samples before and after aging. Melt recrystallization temperature ($T_{mc}$) and enthalpy ($\Delta H_{mc}$) were observed between 102.7 and 105.0°C, and 47.5 and 37.6 J/g, for control and
partially aged samples, respectively (Table 8.4). A slight increase in the $T_{mc}$ and a marked decrease in the $\Delta H_{mc}$ with aging time can be observed. The slight increase in the $T_{mc}$ could be related to a slight increase in the crystallization rate whereas the marked decreased in the $\Delta H_{mc}$ to a change in the overall crystallinity.

The thermograms of the second heating [Figure 8.16(C)] supported the observations of the non-isothermal crystallization. In Table 8.4, the low and high melting temperature ($T_m$) were observed between 147.9 and 149.4°C, 162.1 and 162.8 °C, for control and partially aged samples, respectively. Likewise, the low and high melting enthalpy ($\Delta H_m$) were seen between 12.7 and 11.8 J/g, 37.7 and 28.5 J/g, for control and partially aged samples, respectively. A slight increase in the $T_m$ and a noticeable decrease in the $\Delta H_m$ with aging time can be seen. The decrease in the $\Delta H_m$ is more evident for the high temperature melting peak than for the low temperature melting peak. The slight increase in the $T_m$ values could be attributed to the continued crystallization of the samples in the soil medium. The marked decrease in the $\Delta H_m$ values could be related to a change in the crystallinity of the P(3HB-co-4HB) samples after aging in soil. Luo et al.[24] studied the change in the thermal properties of P(3HB-co-3HV) during degradation in a composting medium. Their results indicated that both melting temperature and enthalpy of the degraded polyester films increased with degradation time. They concluded that the degradation of the biopolymer is enzymatic rather than hydrolytic and occurs from surface and the degraded material leaches out. Their result in terms of the enthalpy of melting is in opposite with ours.
Table 8.4  DSC thermal properties of P(3HB-co-4HB) films in soil after different periods of time: 0, 1, 2, 5 and 8 weeks.

<table>
<thead>
<tr>
<th>Sample</th>
<th>2nd Heating $T_m$ (°C)</th>
<th>2nd Heating $\Delta H_m$ (J/g)</th>
<th>2nd Heating $T_{mc}$ (°C)</th>
<th>2nd Heating $\Delta H_{mc}$ (J/g)</th>
<th>Cooling $T_m$ (°C)</th>
<th>Cooling $\Delta H_m$ (J/g)</th>
<th>Cooling $T_{mc}$ (°C)</th>
<th>Cooling $\Delta H_{mc}$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before burial</td>
<td>147.9</td>
<td>162.1</td>
<td>12.7</td>
<td>37.7</td>
<td>102.7</td>
<td>47.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>148.0</td>
<td>162.0</td>
<td>12.7</td>
<td>37.4</td>
<td>102.8</td>
<td>47.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>148.3</td>
<td>162.1</td>
<td>12.7</td>
<td>35.7</td>
<td>103.3</td>
<td>45.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 weeks</td>
<td>149.2</td>
<td>162.6</td>
<td>12.8</td>
<td>34.8</td>
<td>104.5</td>
<td>44.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>149.4</td>
<td>162.8</td>
<td>11.8</td>
<td>28.5</td>
<td>105.0</td>
<td>37.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For the non-aged biopolymer coating sample (Figure 8.17), the transition shown on the thermogram corresponds to that of the biopolymer after solution coating. We observed that the double melting point on the non-aged biopolymer sample was merged into a single melting point at around 158°C in the non-aged biopolymer coated sample. The thermograms of the partially degraded samples are different from that of the non-degraded sample. Multiple overlapped endothermic peaks can be seen on the thermograms with increasing exposure time, indicating a change in the total crystallinity of the biopolymer. The above remark could indicate a certain degree of physical aging of the samples due to water penetration and microbial colonization during the exposure in soil medium. Finally, we observed a similarity between the thermograms of 5 and 8 week of aged biopolymer and aged coated samples in terms of endothermic peaks.
Figure 8.17  DSC first heating thermograms of coated Kraft paper buried in soil for prescribed periods 0, 1, 2, 5 and 8 weeks showing the aging effect on the coated sample.

8.4  Conclusion

The results obtained in this work showed that significant reduction in the rate of weight loss and improvement in the thermomechanical properties of Kraft paper might well be achieved through biopolymer P(3HB-co-4HB) coating. Two factors, including the filling of the Kraft paper surface and internal pores by the biopolymer, reduce significantly the rate of weight loss of the coated paper, and the combination of synergistic thermomechanical properties of both materials improves the stiffness of the biodegradable coating during soil burial. P(3HB-co-4HB) sample exhibited a low weight loss rate after 8 weeks period of aging in soil medium. The surface topography of the partially aged samples presents cracks, fissures and holes, indicating surface aging in the soil medium. DSC results show a change in the crystallinity of the partially degraded
biopolymer samples and the thermomechanical properties indicate a decrease in the stiffness of the partially degraded biopolymer samples with respect to the control samples.

In this study, the biopolymer coating of Kraft paper offers an interesting alternative for the improvement of thermomechanical properties of the Kraft paper during exposure in soil medium. Also, coating of the biopolymer onto the cellulose Kraft paper can be considered to be a useful method for the assessment of the degradability of biodegradable polymer. Furthermore, this type of coating would have the advantages to be biodegradable and to have synergistic mechanical properties. Thus, it can be used as linerboard for the fabrication of corrugated fiberboard boxes for both food and non-food packaging where both good mechanical properties and environmental concerns are requirements.
8.5 References


9.1 Introduction

Biopolymers can be degraded in soil and compost by the action of a wide range of microorganisms such as gram-positive and gram-negative bacteria, streptomycetes and fungi. The rate of degradation depends mainly on the temperature, relative humidity and the number of characteristic microbial populations in soil and composting medium. In addition, physical and chemical properties of the biopolymers also affect the rate of degradation [1]. Chowdhury in 1963 reported on various microorganisms that excrete extracellular depolymerases to degrade polyhydroxyalkanoates or PHAs into water-soluble oligomers and monomers, and that utilize the resulting products as nutrients [2]. Since then, a number of aerobic and anaerobic PHA depolymerases have been isolated from various ecosystems [3]. A PHA depolymerase is composed of two functions of catalytic and substrate binding domains. First, this enzyme binds to the biopolymer substrate then subsequently catalyzes a hydrolytic cleavage [4]. Hydrophobic properties of the binding domains are strongly dependent on the environment where PHA depolymerases live [2].

The present work studies the change in surface topography and thermal properties of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) or [P(3HB-co-4HB)] in soil and in
compost medium. Various characterization techniques such as photographic analysis, environmental scanning electron microscopy (ESEM), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) were used.

9.2 Experimental

9.2.1 Materials

P(3HB-co-4HB) was used. This material was described in the experimental section of chapter 7. P(3HB-co-4HB) films were prepared by compression molding. The procedure was described in the chapter 7 section 7.2.3. The thickness of compression molded P(3HB-co-4HB) film is 0.12 (±0.02) mm.

9.2.2 Characterization

9.2.2.1 Aging in Soil and Compost Medium

The biopolymer films were buried in soil and compost medium for 2 weeks. The procedure for composting and soil burial was described in the chapter 6 section 6.2.4 and chapter 8 section 8.2.3, respectively. Samples geometry of 5 × 5 cm² was used.

9.2.2.2 Analytical Procedures

To analyze the change in the surface topography of the biopolymer, photographic and environmental scanning electron microscopy (ESEM) were used. For both techniques, the procedure was described in the experimental section of chapter 8.
To investigate the change in the thermal properties and stability of the biopolymer, differential scanning calorimeter (DSC) and thermogravimetric analysis (TGA) were used. The procedure for DSC measurements was reported in the experimental section of Chapter 8. The procedure for TGA measurements is described hereafter. Thermal decomposition was observed in terms of weight loss as a function of temperature by using a TA Instruments Q50 TGA (New Castle, DE). The samples were evenly and loosely distributed in an open sample pan with an initial sample amount of 8–10 mg. The temperature change was controlled from 25°C to 600°C at 20°C/min in nitrogen atmosphere. The thermogravimetric (TG) and the derivative thermogravimetric (DTG) curves obtained were analyzed by using Universal Analysis 2000 software from TA Instruments.

9.3 Results and Discussion

9.3.1 Effect of Aging on the Biopolymer Surface Topography

The microbial degradation of the biopolymer films in soil at room temperature and in compost at thermophilic conditions was observed by photographic analysis and ESEM. Photographs of the unaged control film, aged film in soil and in compost for 2 weeks are presented in Figure 9.1(A), 9.1(B) and 9.1(C), respectively. Before degradation, neat biopolymer sample exhibits some uniformity throughout its surface. After 2 weeks in soil and composting medium, a change can be seen on the samples surface. Indeed, marks can be observed. The change is pronounced for composted samples than for the soil one. The surface of the composted samples shows multiple
holes. Furthermore, these samples tore into pieces. For the soil aged samples, the lack of change suggested that after 2 weeks in soil medium, they may be more resistant to degradation. Temperature, relative humidity and microbial populations can explain this difference in the topographical pattern of the biopolymer films in soil and compost medium.

Figure 9.1 Photographs of P(3HB-co-4HB) films buried in soil and compost for 2 weeks.

(A) : Before burial

(B) : 2 weeks in soil

(C) : 2 weeks in compost

Figure 9.1 Photographs of P(3HB-co-4HB) films buried in soil and compost for 2 weeks.

The surface topography was further investigated using ESEM. ESEM micrographs of the unaged control film, aged film in soil and in compost for 2 weeks are presented in Figure 9.2 (A), 9.2(B) and 9.2(C), respectively. High magnification micrographs were displayed in Figure 9.3(A)-(C). The surface of the neat biopolymer
was uneven, which resulted from its preparation using compression molding technique [Figure 9.2(A) and 9.3(A)].

Figure 9.2  ESEM micrographs of P(3HB-co-4HB) films buried in soil and compost for 2 weeks (scale bar: 100 µm).

(A) : Before burial  (B) : 2 weeks in soil

(C) : 2 weeks in compost

After exposure in the soil and composting medium, the surface of the samples becomes rougher. The roughness is more pronounced for the composted samples [Figure 9.2(C) and 9.3 (C)] than the soil one [Figure 9.2(B) and 9.3 (B)]. Indeed, ESEM micrographs of the samples exposed in compost medium for 2 weeks show significant
number of pits on the surface. This result suggests that the composting conditions strongly favor the rate of degradation of the biopolymer with respect to the soil burial conditions. This observation is in good agreement with the photographic analysis.

Figure 9.3  ESEM micrographs of P(3HB-co-4HB) films buried in soil and compost for 2 weeks (scale bar: 50 µm).

A) : Before burial  B) : 2 weeks in soil  C) : 2 weeks in compost
9.3.2 Effect of Aging on the Biopolymer Thermal Properties and Stability

Figure 9.4(A), 9.4(B) and 9.4(C) showed the first heating, first cooling and second heating thermograms of the control and partially degraded biopolymer samples in soil and in compost medium, respectively.

Figure 9.4  First heating (A), first cooling (B) and second heating (C) DSC thermograms: (a) neat P(3HB-co-4HB), (b) P(3HB-co-4HB) aged in soil for 2 weeks and (c) P(3HB-co-4HB) aged in compost for 2 weeks.
The biopolymer sample before exposure in soil and compost medium [thermogram (a) in Figure 9.4(A)] showed a bimodal endothermic peak: a low melting temperature at 148°C and a high melting temperature at 162°C. These values are consistent with those reported elsewhere [5]. These peaks might correspond to the melting temperatures of the crystalline phases. This suggested two crystalline phases probably presented in the biopolymer.

The first heating thermograms of the partially degraded samples in soil [thermogram (b)] and in compost [thermogram (c)] medium are different from those of the non aged sample. One can notice the presence of a third endothermic peak (marked by an arrow) before the double endothermic peaks. In fact, for the samples of 2 weeks, a small peak appeared between 70 and 85°C. This observation could indicate a certain degree of physical aging of the biopolymer in soil and compost medium. Cong et al.[6] reported three endothermic peaks in the P(3HB-co-4HB) biopolymer, having 9, 11, 17 and 20 mol% 4-HB unit. They assigned the lowest temperature melting peak to the crystalline phase of the 4-HB rich units, the middle one to that of 3-HB rich units and the highest one to a recrystallization process in which the lattice of the copolymer had enough time to be rearranged at a given DSC heating rate, that was because the intensity of this peak relative to that of the peak at a lower temperature decreased with the heating rate. In our study, the appearance of the lowest endothermic peak could be related to phase separation phenomena in the biopolymer as a result of partial degradation.

The melt recrystallization thermograms of P(3HB-co-4HB) samples before and after partial degradation are shown in Figure 9.4(B). Melt recrystallization temperature
(T_{mc}) and enthalpy (\Delta H_{mc}) were reported in Table 9.1. Neat biopolymer exhibits T_{mc} at 102.7 °C and \Delta H_{mc} of 47.5 J/g. There was almost no change in T_{mc} of the biopolymer after 2 weeks in soil medium. However, the composted sample showed a broad melt recrystallization peak which shifted to lower temperature [marked by an arrow in Figure 9.4(B), thermogram (c)]. T_{mc} shifted from 102.7 to 41.9 °C, indicating a decrease in the crystallization rate of the composted sample. A change in \Delta H_{mc} is also observed. This change is significant in the composted sample with respect to the soil sample. Indeed, \Delta H_{mc} decreases from 47.5 J/g in the neat biopolymer to 45.3 and 21.8 J/g for soil and composted sample, respectively.

The thermograms of the second heating are shown in Figure 9.4(C). Cold crystallization temperature (T_{cc}) and enthalpy (\Delta H_{cc}); melting temperature (T_{m}) and enthalpy (\Delta H_{m}) are summarized in Table 9.1. The shapes of the thermograms of neat biopolymer sample [thermogram (a)] and the partially degraded sample in soil medium [thermogram (b)] are similar. However, for the partially composted sample, the thermogram showed significantly different behavior compared to that observed during the first heating. As shown in Figure 9.4(C), a T_{cc} peak appeared at low temperature at 42°C (marked by an arrow). This order-disorder transition can be explained by physical aging of the biopolymer in the composting medium. Table 9.1 showed no change in the high melting temperature for all the samples. The low melting point is also unchanged for the neat biopolymer sample and partially soil degraded sample. However, the partially composted sample showed a decrease in the low melting temperature. It decreased from 147.9 to 140°C. Furthermore, the low temperature melting peak showed an important
depression and is almost suppressed [marked by an arrow in Figure 9.4(C)]. Also, the total melting enthalpy ($\Delta H_m$) was affected with the composted samples showing the significant change. It decreased from 50.4 J/g for the biopolymer to 47.8 and 42.8 J/g for the partially soil aged and composted sample, respectively.

Based on the above observations, we concluded that the thermal properties results confirmed the change in the total crystallinity of the composted sample over the soil aged sample.

Table 9.1 DSC thermal properties of P(3HB-co-4HB) films after 2 weeks in soil and compost.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$T_{cc}$ (°C)</th>
<th>$\Delta H_{cc}$ (J/g)</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_m$ (J/g)</th>
<th>$T_{mc}$ (°C)</th>
<th>$\Delta H_{mc}$ (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before burial</td>
<td>-</td>
<td>-</td>
<td>147.9</td>
<td>162.1</td>
<td>12.7</td>
<td>37.7</td>
</tr>
<tr>
<td>2 weeks in soil</td>
<td>-</td>
<td>-</td>
<td>148.3</td>
<td>162.1</td>
<td>12.1</td>
<td>35.7</td>
</tr>
<tr>
<td>2 weeks in compost</td>
<td>42.0</td>
<td>-19.5</td>
<td>140.0</td>
<td>162.4</td>
<td>2.7</td>
<td>40.1</td>
</tr>
</tbody>
</table>

The thermal decomposition of the neat biopolymer, partially soil and compost degraded samples is shown in Figure 9.5. As exhibited by the TG curve, the biopolymer thermally degrades drastically above 350°C due to chain scission reactions leading to a reduction of molecular weight and formation of volatile acid products. The shapes of the curves for the partially degraded samples are very similar to that of the neat biopolymer, which shows a single weight loss zone with a maximum weight loss temperature ($T_p$) of 305°C. $T_p$ did not change in the partially soil degraded sample whereas it did in the partially composted sample. $T_p$ decreased from 305°C from the neat biopolymer to 304.5
and 296°C for the partially soil degraded and composted sample, respectively. The thermal stability results supported the thermal properties one.

Figure 9.5   TG (A) and DTG (B) curves: (a) neat P(3HB-co-4HB), (b) P(3HB-co-4HB) aged in soil for 2 weeks and (c) P(3HB-co-4HB) aged in compost for 2 weeks.

9.4 Conclusion

Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) samples were subjected to soil and composting medium for 2 weeks, and the degradation in their physical and thermal properties were characterized using photographic analysis, ESEM, DSC and TGA. The surface topography of the partially composted samples presents more cracks, fissures and holes over the soil aged samples, indicating more microbial activity in the compost medium than in the soil medium. DSC results show a significant change in the crystallinity of the partially composted biopolymer over the soil aged samples. TGA results indicate a decrease in the thermal stability of the composted samples over the soil
aged samples. Thermal properties and stability of the different samples corroborate well with the topographical pattern observed by photographic analysis and ESEM.
9.5 References


The thermophysical properties, interfacial interaction and decomposition of PHAs reinforced with organic (cellulose paper) and inorganic (layered double hydroxides functionalized with stearate) constituents have been investigated. Individual chapters offer the conclusions to be derived within their scope. The inferences we determine across the preceding chapters are outlined below.

10.1 Processing Effects

The processing effects of the inorganic reinforcements in the nanocomposites are reflected in the degree of dispersion of the nanofillers as discussed in chapter 3. For P(3HB-co-3HV) reinforced with layer double hydroxides functionalized with stearate (LDH-SA), WAXD coupled with TEM indicated an intercalated dispersion but presence of significant agglomeration in the nanocomposites was evident with increased LDH-SA loading.

In contrast, when a porous cellulose paper is used as reinforcement for P(3HB-co-4HB), care had to be taken to resolve an appropriate processing methodology as discussed in chapter 7. Solvent and non-solvent (melt) methods were employed to manufacture the coatings. The coatings performance was compared and correlated to the coating structure. ESEM results indicated that the melt coated samples showed both
better surfaces and penetration of the biopolymer into the paper. FT-IR results showed that the reflection peaks were from the biopolymer, indicating retention of the chemical structure of the pure P(3HB-co-4HB) in all the coatings samples. WAXD results indicated that among the coatings samples, only the melt processed coated samples showed most of the crystalline structure of the biopolymer.

10.2 Mechanical Properties

Both organic and inorganic reinforcements improved the mechanical performance of the PHA. In chapter 3, the incorporation of LDH-SA improved the tensile properties of P(3HB-co-3HV). Also, the thermomechanical properties obtained by DMA (at 1 Hz) are modified by the presence of the nanofiller. Indeed, dynamic elastic modulus (E’) of the nanocomposites increased while the primary (glass transition temperature T_g) and secondary (β transition) relaxations did not change.

In the P(3HB-co-4HB) coated samples, DMA (at 1 Hz) results indicated that the melt processed sample exhibited better storage modulus in subambient conditions as discussed in chapter 7. At room temperature and above, the coatings showed the values of the storage modulus close to those of cellulose paper but higher than those of the biopolymer. Also, impact results conducted at room temperature showed that the melt based samples had a higher total energy absorption capability indicating superior adhesion to the solution processed samples.
10.3 Crystallization Effects

The nature of the interface and its geometry of interaction had a significant influence on crystallization. This is probed in chapter 4. For the P(3HB-co-3HV) nanocomposites, it was enhanced nucleation. Non-isothermal crystallization results from DSC showed that the nanodispersed LDH-SA acted as a nucleating agent increasing the crystallization rate of the biopolymer. To explore in detail the crystallization behavior of P(3HB-co-3HV) with the addition of LDH-SA, the Avrami model and Lauritzen-Hoffman theory were employed. Isothermal crystallization from DSC measurements (Avrami model) showed that the addition of the nanofiller induced more heterogeneous nucleation in the crystallization significantly increasing the Avrami crystallization rate constant $K$ and the absolute magnitude of the activation energy $\Delta E_A$. Hoffman-Weeks plots were employed to estimate the equilibrium melting points $T_m^0$ of P(3HB-co-3HV) and its nanocomposites. Increasing the content of LDH-SA in the P(3HB-co-3HV) matrix resulted in lowering the $T_m^0$ after isothermal crystallization. The value of Avrami exponent $n$ illustrated that crystal growth may not occur in three dimensions at an equal rate and the addition of LDH-SA did not change the mechanism of nucleation and growth of P(3HB-co-3HV). The experimental data of spherulitic growth rate obtained by POM were analyzed according to the polymer-diluent theory based on Lauritzen-Hoffman model. The results indicated regime III crystallization for P(3HB-co-3HV) and its nanocomposites. The nucleation constant $K_0$, the folded surface free energy $\sigma_e$ and the work of chain folding $Q$ of P(3HB-co-3HV) crystals decreased with increasing LDH-SA content. These results suggested that the incorporation of the LDH-SA into P(3HB-co-3HV)
3HV) induced heterogeneous nucleation of the biopolymer crystallization and decreased the surface energy barrier for P(3HB-co-3HV) crystallization.

In contrast, the as prepared cellulose paper-P(3HB-co-4HB) coating obtained by solvent method showed a decrease in both melting point and enthalpy of melting when compared to the pure P(3HB-co-4HB), indicating change in total crystallinity of the biopolymer (chapter 8). However the cellulose paper surface was not effective in nucleating crystallites.

10.4 Interfacial Interaction

Against inorganic surfaces the nanocomposite architecture offers a high surface area where two ranges of relaxation are observed. By comparing the low frequency response (single frequency: 1 Hz and multiple frequency: 0.16 to 16 Hz) via dynamic mechanical analysis (DMA) to the high frequency (500 Hz) dielectric spectroscopy (DES), a distinction in the glass transition is determined as discussed in chapter 3 and 5. Against the clay interface and between the P(3HB-co-3HV) spherulites, a relative high level of chain folding or packed morphology would be expected to occur given the rigid interface. Nucleation originating from the clay interface would be expected to render an additional constraint to the P(3HB-co-3HV) chains. We therefore infer from our results in chapter 3 that the relatively unchanged glass transitions from the low frequency at 1 Hz (high relaxation time) response, indicates that away from the nucleating or trapped surfaces the amorphous mobility is not affected to a significant extent. In chapter 5, multiple frequency (0.16-16 Hz) DMA through time-temperature superposition (TTS)
was used to determine the effect of the addition of the nanoparticles on the storage modulus and loss modulus of the biopolymer. Arrhenius and Williams-Landel-Ferry (WLF) relationships were applied to TTS data. We observe that the Arrhenius activation energy ($E_A$), as well as the WLF constants ($C_1$ and $C_2$) increase with increasing the content of LDH-SA. $E_A$ increases due to strong interaction between the biopolymer and the nanofiller. Increase in $C_1$ and $C_2$ values after addition of the nanofiller indicates a decrease in fractional free volume $f_g$ of the biopolymer. The decrease in $f_g$ indicates better biopolymer-nanofiller interaction. The dielectric results at 500 Hz showed a shift and a depression of the dielectric loss peak of the biopolymer with increasing LDH-SA content, indicating global changes in the relaxation time of the biopolymer as a result of positive interaction between this latter and the LDH-SA. DES results complement those of DMA for characterization of the internal motions in P(3HV-co-3HV) and its nanocomposites.

For cellulose paper-P(3HB-co-4HB) coating samples obtained by solvent and melt method, the positive hydrophilic interactions between the biopolymer and the cellulose caused an increase in the glass transition, indicating attractive interactions between both components as discussed in chapter 7.

10.5 The path to Decomposition and Decomposition Mechanism

Both inorganic and organic surfaces provided an ability to develop better compostable bioplastics. In chapter 6, we have engineered a laboratory automated multi-unit composting system following the requirements of ASTM D-5338-98 (2003) to study the biodegradability of materials by measuring the CO$_2$ metabolized during material
biodegradation by the means of a nondispersive infrared (NDIR) gas analyzer. This system is used to investigate the effect of the addition of LDH-SA on the biodegradability behavior of P(3HB-co-3HV). The mineralization results suggested that the overall biodegradability (i.e. rate, degree and ease of degradation) of the biopolymer was significantly affected by the addition of the nanofiller. In fact, under the same controlled composting test conditions of 56.3°C and 56.5 % moisture content, the incorporation of 5 wt% LDH-SA into P(3HB-co-3HV) matrix yielded nanocomposite with significantly improved biodegradability with respect to the neat P(3HB-co-3HV). The increased evolution of CO₂ during the degradation of the nanocomposite in compost indicated the benefits of the nanofiller in improving the compostability of the biopolymer.

In chapter 8, we have investigated the physical and thermal effects that the weight loss generates in P(3HB-co-4HB), cellulose paper and their coating sample obtained by solvent method. While composting environments provide the optimum conditions for high degradation rates, we utilized a soil mixture at 30°C and 20% moisture content which slows the degradation rate in order to monitor the mechanism and retain sample integrity over the measurement period. The results obtained in this work showed that significant reduction in the rate of weight loss and improvement in the thermomechanical properties of the cellulose paper might well be achieved through biopolymer P(3HB-co-4HB) coating. Two factors, including the filling of the cellulose paper surface and internal pores by the biopolymer, reduce significantly the rate of weight loss of the coated paper, and the combination of synergistic thermomechanical properties of both materials improves the stiffness of the biodegradable coating during soil burial. P(3HB-co-4HB)
sample exhibited a low weight loss rate after 8 weeks period of aging in soil medium. The surface topography of the partially aged samples presents cracks, fissures and holes, indicating surface aging in the soil medium. DSC results show a change in the crystallinity of the partially degraded biopolymer samples and the thermomechanical properties indicate a decrease in the stiffness of the partially degraded biopolymer samples with respect to the control samples. In this study, the biopolymer coating of cellulose paper offers an interesting alternative for the improvement of the thermomechanical properties of the cellulose paper during exposure in soil medium. Also, coating of the biopolymer into and onto the cellulose paper can be considered to be a useful method for the assessment of the degradability of biodegradable polymer. Furthermore, this type of coating would have the advantages to be biodegradable and to have synergistic mechanical properties. Thus, it can be used as linerboard for the fabrication of corrugated fiberboard boxes for both food and non-food packaging where both good mechanical properties and environmental concerns are requirements.

In chapter 9, a comparative study showing the effect of the conditions of soil and compost medium on the degradability of P(3HB-co-4HB) samples was investigated. P(3HB-co-4HB) samples were subjected to soil (at 30°C and 20% moisture content) and composting medium (at 56.3°C and 56.5% moisture content) for 2 weeks, and the degradation in their physical and thermal properties were characterized using photographic analysis, ESEM, DSC and TGA. The surface topography of the partially composted samples presents more cracks, fissures and holes over the soil aged samples, indicating more microbial activity in the compost medium than in the soil medium. DSC
results show a significant change in the crystallinity of the partially composted biopolymer over the soil aged samples. TGA results indicate a decrease in the thermal stability of the composted samples over the soil aged samples. Thermal properties and stability of the different samples corroborate well with the topographical pattern observed by photographic analysis and ESEM.