

BLENDING OF POLY(LACTIC ACID) AND
POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) – THE EFFECT OF MALEATED PLA AS
A REACTIVE COMPATIBILIZER AND BIODEGRADATION STUDY

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ABSTRACT

BLENDING OF POLY(LACTIC ACID) AND POLY(3-HYDROXYBUTYRATE-CO-3-HYDROXYVALERATE) – THE EFFECT OF MALEATED PLA AS A REACTIVE COMPATIBILIZER AND BIODEGRADATION STUDY

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Poly(lactic acid), PLA was blended with poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, based on a crossed mixture-factorial experimental design with three levels of factorial variable of the type of pre-produced maleated PLA, *PLAgMA-type*, used as the blend compatibilizer, and three components mixture variable which were the contents of *PLA*, *PHBV*, and *PLAgMA*, included in the polymer blends. The mixture model was based on the constrained level of the weight fraction of each mixture component as follows: $0.2 \leq PHBV \leq 0.7$, $0.2 \leq PLA \leq 0.7$, and $0.05 \leq PLAGMA \leq 0.15$. The design of experiment yielded 16 runs of compatibilized blends, with 2 runs of non-compatibilized blend and 2 runs of neat polymers, PLA and PHBV, for comparison. The model of relationship between variables was derived based on the multiplication of a linear relationship of one factorial variable with a quadratic Scheffe model of the mixture ingredients.

Multiple formulas of the blend compatibilizer, maleated PLA (PLA-*g*-MA), were pre-produced by a reactive melt blending method to functionalize maleic anhydride, MA, on the PLA backbone in a twin-screw co-rotating extruder. Dicumyl peroxide, DCP, was used as a free radical initiator in the reactive blending. The formulas were designed using response surface experimental design to determine the effect of the contents of MA and DCP on the amount of grafted MA, *MA-grafting yield*, and the molecular weight properties, M_n , M_w , IV , and *dispersity* of PLA-*g*-MA. The model regression indicated a significant effect of DCP with increasing DCP tending to reduce the *MA-grafting yield*, M_n , M_w , and IV , and increase the *dispersity*. The optimum point that maximized the desirability of these responses simultaneously was with the content of DCP = 0.1 wt. % and MA = 3.94 wt. % (PLA basis).

Blending of PLA and PHBV clearly increased the crystalline fraction of the blends compared to neat PLA, which affects the barrier properties of the materials. Inclusion of PHBV at 25 wt. % in the non-compatibilized blend and at 45 – 60 wt. % in compatibilized blends resulted in more than 60% reduction of water and O₂ permeability compared to PLA. The compatibilized PLA/PHBV blend with PLA weight

fraction of 0.45 achieved 300% increase in the tensile strength compared to the neat PHBV; this level of improvement was equivalent to the non-compatible blend containing PLA 75 wt. %. This was attributed to enhanced interfacial adhesion that was evidently supported by increased miscibility between the blend components in compatibilized blends which was exhibited through the shifting of T_g of PLA and the decrease of k constants based on the Gordon-Taylor equation of the compatibilized blends. The factorial-mixture model regression suggested the validity of the mixture variable of *PLA*, and *PHBV* in both tensile and barrier properties; the *PLAgMA* had a significant effect only on the tensile performance of the polymer blends. The overlapped contour plots as well as the desirability functions could be used to optimize the mixture of the PLA/PHBV blend components that provide desirable tensile and barrier properties.

A biodegradation study was conducted on neat PLA, PHBV, non-compatible blend of 75:25 PLA/PHBV, and compatibilized blend of 65:15:20 PLA/PLA-*g*-MA/PHBV. PLA/PLA-*g*-MA/PHBV was the fastest to reach 100% mineralization, followed by PLA and PLA/PHBV samples, according to the CO₂ evolution and % mineralization, whereas PHBV reached only 81% mineralization at the end of the test of 180 days. The facilitation of anhydride present in PLA-*g*-MA on the hydrolysis of PLA was a major cause of the fast biodegradation of PLA/PLA-*g*-MA/PHBV. A sharp increase in enthalpy of fusion, ΔH_f , as well as a rapid reduction of the molecular weight of PLA/PLA-*g*-MA/PHBV compared to PLA and PLA/PHBV support the occurrence of an elevated rate of hydrolysis. The PHBV sample showed the biodegradation was barely affected by abiotic hydrolytic degradation as the thermal properties did not show any shifting of the melting transition and the ΔH_f remained stable until 30 days of the test; the main mechanism was the enzymatic microbial degradation causing an erosion at the surface rather than affecting the bulk properties such as the molecular weight. The scanning electron micrographs also revealed the biodegradation of PHBV that initially occurred was from the surface and later showed the degradation of the crystalline structure. The PLA crystals formed during the biodegradation of PLA/PHBV and PLA/PLA-*g*-MA/PHBV samples could be seen from SEM photos.

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1 INTRODUCTION

1.1 *Motivation and objective*

Growing concerns regarding the use of conventional petroleum-based polymers (e.g., depletion of limited non-renewable resources, large carbon footprint relating to their production, and their durability that allows them to last for several hundred years in the environment) emphasize the interest in alternative materials that are more environmentally friendly. Especially in the packaging area where the problem is easily visible due to its presence in almost every product and service, and also its short service life, using sustainable materials is prioritized. Among the list of polymers with renewable and biobased sources and biodegradability, polyhydroxyalkanoates and poly(lactic acid) draw significant attention.

Polyhydroxyalkanoates (PHAs) are a family of polymers which are well-known as biobased polymers harvested from microbial cultures. PHAs have promising biodegradability. They are biodegraded in different environments including soil, compost, and marine sediment, in both aerobic and anaerobic conditions [1]. A wide range of bacteria that can degrade these polymers have been isolated and identified. This expands the biodegradation capability of this group of polymers. With diverse thermal and mechanical properties and biocompatibility, members of the PHA polymer family are widely used in medical applications. In a packaging application, PepsiCo's Frito-Lay recently launched a compostable snack bag in September 2021 following the company's 2018 award-winning compostable flexible packaging innovation in collaboration with Danimer Scientific, where the polymer resins being used for this innovation were blends of Danimer's PHA and mineral fiber [2–4].

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) is a copolymer, one of the well-known members of the PHA family. It is a result of copolymerization of poly(3-hydroxybutyrate) or P(3HB) with poly(3-hydroxyvalerate) or P(3HV). The copolymer has improved mechanical and processing properties compared to the homopolymer of P(3HB). With mechanical properties comparable to a petroleum-based commodity polymer like polypropylene (PP), PHBV has the potential to be used in industrial, agriculture, and packaging applications. Together with its environmental benefits, PHBV is one of the most attractive alternatives to petroleum-based packaging polymers. However, because of a much higher price due to the high production cost, its application in the field is quite limited [5]. To make PHBV become cheaper enough to be able to be used more in packaging applications is inspiring, especially in terms of an

expected environmental gain. Besides the upstream attempt to reduce the production cost by boosting the polymer productivity, using cheaper raw material, and utilizing industrial wastes as substrates, to modify and composite PHBV with inexpensive materials can optionally expand the use of the copolymer in the packaging area. Blending of PHBV with other biodegradable materials such as poly(lactic acid) (PLA) could be an engaging solution.

Poly(lactic acid) (PLA) holds the same position as PHBV as a biobased and biodegradable polymer. It is more widespread in packaging applications due to its competitive cost, and mechanical, and processing properties that are comparable to other commodity polymers [5,6]. PLA has a relatively high glass transition temperature ranging from 35 to 60 °C that makes it brittle at room temperature and limits its service temperature range. Modification of PLA by copolymerization, blending, and plasticization, was done to improve flexibility and toughness. Nevertheless, the biodegradability of PLA is relatively inferior compared to PHBV. Biodegradation of PLA is possible under industrial composting conditions starting with a hydrolysis process; then it is degraded as a result of the action of microorganisms occurring at an elevated temperature (58 °C) [6]. The blend of PLA and PHBV could not only bring down the cost of using PHBV but also alter the characteristics of the polymers in many thought-provoking aspects.

The blending of PHBV and PLA has been of interest and been widely studied. The combination of these two polymers can preserve their attractive characteristics of biobased, biodegradability, and biocompatibility, at the same time tweaking the flaws of each polymer. The early research regarding this, using physical or non-compatibilized blending, resulted in composite materials that were immiscible blends where the expected properties were hard to achieve due to the phase separation. The use of compatibilizers as well as reactive blending then later were an option that contributed enhanced miscibility and could emphasize the advantages of each polymer in the blended material.

The overall goal of this research is to investigate the polymer blend of PHBV and PLA using maleated poly(lactic acid) (PLA-*g*-MA) as a reactive compatibilizer by considering the effect of the compatibilizer on the polymer blend performance as a packaging material (i.e. mechanical properties and barrier properties) and its compostability.

1.2 Specific objectives

Objective 1: Production and characterization of PLA-*g*-MA, and evaluation of MA and peroxide initiator content factors on the MA grafting yield and molecular weight properties of PLA-*g*-MA.

Objective 2: Melt blending and characterization of PHBV/PLA compatibilized and non-compatibilized blends.

Objective 3: Determination and optimization of the effect of the PLA-*g*-MA compatibilizer properties on the polymer blend properties including miscibility, mechanical properties, and barrier properties

Objective 4: Investigation of the biodegradation behavior of the compatibilized PHBV/PLA blend compared to neat PHBV and PLA and non-compatibilized blends

1.3 Research plan

Phase 1: Grafting of maleic anhydride (MA) on PLA

In the experimental design, response surface methodology (RSM) was used to evaluate the effect of MA content and dicumyl peroxide (DCP) content on the final properties of PLA-*g*-MA. Spectroscopic analysis using FTIR was conducted to verify the grafting of MA on PLA. MA grafting yield and molecular weight were determined. The obtained data was used to evaluate the significance of the factors and optimize the PLA-*g*-MA production.

Phase 2: PHBV/PLA blending

The factors of the blend composition content (*i.e.*, PHBV, PLA, and PLA-*g*-MA) were used to plan the experiment of PHBV/PLA blending using mixture experimental design. The significance of the content factors was determined using RSM regarding the miscibility, mechanical properties, and barrier properties of the produced blends.

Phase 3: Biodegradation Study of PHBV/PLA Blends

The biodegradation of the compatibilized blend compared to neat PHBV, PLA and the product of the non-compatibilized blending process were planned to study using a direct measurement respirometric (DMR) system under controlled composting conditions. The evolved CO₂, mineralization, molecular weight reduction, as well as the surface morphology were evaluated during the biodegradation study.

The organization of this study is as follows; chapter 2 is the review of the literature relating to the scope of the study, chapter 3 presents the experiment and results of the grafting of MA on PLA, chapter 4 presents the study of the melt blending of PLA and PHBV, and chapter 5 is the biodegradation study of PLA/PHBV blends. Lastly, chapter 6 provides the summary of this study and the recommendations for future work.

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2 LITERATURE REVIEW

2.1 Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) - PHBV

2.1.1 PHBV chemistry and production

Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or PHBV is a copolymer that belongs to the family of polyhydroxyalkanoates (PHAs). This group of polymers is well-known as biobased plastics that are harvested from microbial cultures. PHAs are biosynthesized and stored as intracellular carbon and used for energy storage by numerous types of bacteria under growth conditions characterized by an excess amount of carbon with a limitation of essential growth nutrients such as nitrogen and phosphate [1]. More than 80 hydroxyalkanoates (HAs) have been found; different combinations of HA constituents result in many types of polyesters with various mechanical properties ranging from hard crystalline polymers to elastic rubbers, depending on the incorporated monomer units [2]. PHAs have the general structural formula shown in Figure 2-1 where poly(3-hydroxybutyrate) or P(3HB) or PHB is the most common. However, PHB has a weakness of a very narrow processing temperature range since its melting temperature is very close to the decomposition temperature. Copolymerization of PHB with poly(3-hydroxyvalerate) results in a copolymer, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) or P(3HB-co-3HV) or PHBV with improved mechanical and processing properties.

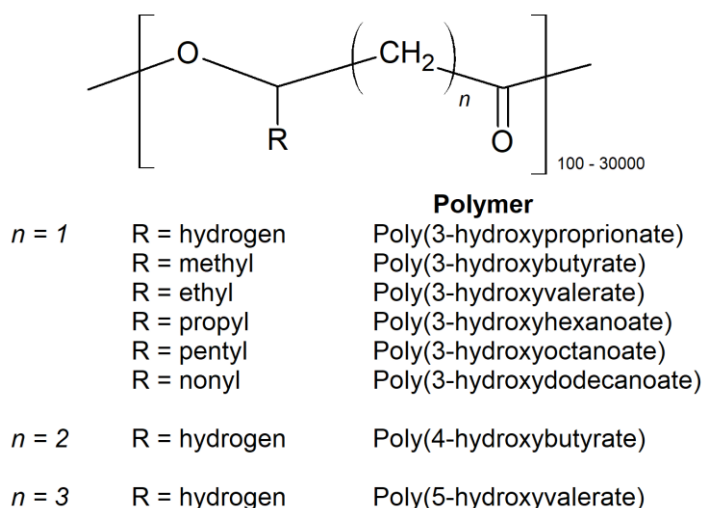


Figure 2-1 General Structure of Polyhydroxyalkanoates adapted from Lee (1995)

PHBV is among four major members of PHAs including 1) PHB, 2) PHBV, 3) poly(3-hydroxybutyrate-co-3-hydroxyhexanoate [P(3HB-co-HHx)] and 4) poly(3-hydroxybutyrate-co-4-

hydroxybutyrate) [P(3HB-co-4HB)] that are produced on a commercial scale [3]. Even though numerous types of bacteria were found to be able to synthesize PHAs, the economic factors including expensive carbon sources, growth rate, polymer synthesis rate, the extent of polymer accumulation, and the cost of polymer recovery, result in only limited kinds of bacteria having been selected to produce PHAs on an industrial scale [2]. Specifically, only *Ralstonia eutropha* which is also known as *Cupriavidus necator* (previously named *Hydrogenomonas eutrophus* and *Alcaligenes eutropha*) is currently being used to produce PHBV using glucose and propionate as carbon sources [3].

PHAs have a significant flaw in their high price due to the high production cost. The need for expensive pure carbon sources to produce an economic yield is a significant factor. In response to that problem, the potential to produce PHAs using cheap carbon sources and the production process optimization have been widely studied. Specific to the production of PHBV, crude glycerol (byproduct from biodiesel production), waste rapeseed oil, rapeseed meal, and palm oil mill effluent could be used as carbon sources by fermentation using *Cupriavidus necator* [4–7]. The optimization of PHBV productivity by varying the carbon source content was studied by Aramvash et al. [8], and Berezina [9], where the combination of fructose + propanol + beef extract, and levulinic acid + sodium propionate were investigated, respectively. The study of Shantini, Yahya, & Amirul [10] reported the effect of feeding frequency and dissolved oxygen level control on PHBV productivity using oleic acid and 1-pentanol as carbon sources. A fermentation system with external cell recycle was proposed by Schmidt et al. [11] to efficiently utilize raw materials used to prepare the medium for the bacterial fermentation. Moreover, the co-culture of *Ralstonia eutropha* with *Bacillus subtilis* and *Bacillus amyloliquefaciens* could produce PHBV directly from sucrose as the *Bacillus* hydrolyzed the sugar into glucose and fructose for the fermentation by *R. eutropha* to produce the copolymer [12].

The ability to produce PHBV using the engineered recombinant strains of other bacteria was also studied, in addition to the wild-type strains of *Cupriavidus necator*. The recombinant strains of *Rhodospirillum rubrum* S1 and *Escherichia coli* harboring *R. eutropha* PHAs biosynthesis genes can increase PHBV productivity with their fast growth rate and their ability to accumulate a large amount of the copolymer in their cells [2,13]. In the study of Yee et al. [14], recombinant *E. coli* JM109 harboring the plasmid containing PHA biosynthesis genes of *Comamonas* sp. EB172 could produce PHBV from both

glucose and mixed organic acids. This could broaden the selection of the fermentation substrates beyond sugar to oil industry byproducts that contain a large amount of fatty acid. The modified genetic strains of *R. eutropha* H16 studied by Zhang, Liu, Weng, Ding, & Liu [15] can produce a high content of PHBV solely from glucose without the addition of propionate, one of the significant costs of the copolymer.

Various bacterial strains other than *Cupriavidus necator* were also reported to be able to produce PHBV using alternative carbon sources. *Haloferax mediterranei* can generate PHBV from glycerol, cheese whey hydrolysate containing glucose and galactose, and olive mill wastewater [16–18]. Aqueous extracts of powders of dried orange peels, banana peels, and bagasse were also used to feed *Halomonas campisalis* during the fermentation [19]. Obruca et al. [20] also found that the levulinic acid present in the hydrolysate of spent coffee grounds could increase the amount of 3HV synthesized by *Burkholderia cepacian*. Another group of bacteria, *Bacillus megaterium* and *Bacillus* spp., used: 1) glycerol together with urea, and 2) multiple sugars including monosaccharides (glucose and fructose), disaccharides (sucrose), pentoses (xylose and arabinose), organic acids (acetic acid, propionic acid and octanoic acid) and the acid pre-treated liquor of sugarcane trash, respectively, as substrates [21,22]. Methane and methanol with the presence of valerate or *n*-pentanol or valeric acid were the carbon sources used by the methylotrophs, *Methylocystis* sp. WRRC1 and *Methylobacterium* sp. GW2, in PHBV production [23–25].

In addition to the conditions where the organic substrates were required to produce PHBV, several species of cyanobacteria can photoautotrophically grow and accumulate PHB resulting from photosynthesis using carbon dioxide (CO₂) and water. Based on the recent study of Taepucharoen, Tarawat, Puangcharoen, Incharoensakdi, & Monshupanee [26], the selected strain of *Oscillatoria okeni* TISTR 8549 can produce the copolymer PHBV. Even though this method had less productivity compared to the traditional bacterial system, it consumed less energy and did not require any organic carbon source, which is considered the primary factor for the copolymer's high production cost. The researchers also pointed out the advantage of the system that instead of releasing CO₂ as the traditional method does, it consumes CO₂ from the environment.

Additionally, *Cupriavidus necator* has an autotrophic ability to grow and accumulate PHA using CO₂ as the sole carbon source and hydrogen (H₂) as an energy source. In the system where the CO₂ and

valeric acid are supplied, the bacteria can produce the copolymer, PHBV, where CO₂ is the precursor for the 3-hydroxybutyrate (3-HB) and valeric acid is the precursor for 3-hydroxyvalerate (3-HV). The recent work of Ghysels, Mozumder, De Wever, Volcke, & Garcia-Gonzalez [27] summarized the state of the art of PHBV production using this mechanism and reported the modeling of the system parameters including continuous sparging of CO₂ with a pulse and pH-stat feeding of valeric acid to produce PHBV with predefined content of 3-HV.

In summary, the current status of the research based on PHBV production is leading to more economical ways to produce PHBV to allow more opportunity for the copolymer production at a commercial scale. The possibility of utilizing cheap substrates, using alternative bacterial strains and production pathways, also designing and modeling of the production parameters, are the crucial areas of research effort.

2.1.2 PHBV properties and applications

As previously mentioned, PHBV is the copolymer of 3-HB and 3-HV; the basic structure of PHBV is as shown in *Figure 2-2*. With the number of carbon atoms between 3-5 within a monomer, both 3-HB and 3-HV are both considered as short chain length PHAs (scl-PHAs) where the medium chain length PHAs (mcl-PHAs) contain 6-14 carbon atoms. The scl-PHAs have high crystallinity, generally ranging between 55 to 80% [3]. The homopolymer of 3-HB or PHB has a high melting temperature (T_m) of about 180 °C where PHBV with less stereoregularity and crystallinity has lower T_m between 137-170 °C. PHBV also has a lower glass transition temperature (T_g) down to -6 °C, compared to PHB (at around 4 °C) [3]. However, the thermomechanical properties of PHBV vary with the content of 3HV in the copolymer, which is dependent on the type of bacteria producing the copolymer and the fermentation conditions. Table 2-1 shows the thermal and mechanical properties of PHBV with different percentages of HV content compared to the homopolymer, PHB, poly(lactic acid) (PLA), and polypropylene (PP).

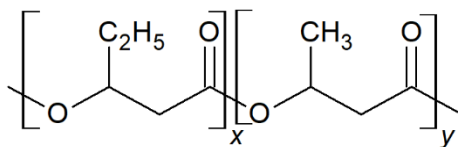


Figure 2-2 Basic Structure of PHBV where x is the number of 3-HV monomers and y is the number of 3-HB monomers.

Different 3HV content affects the thermal properties because it relates to the crystallization behavior of the copolymer. PHBV crystallizes in either P(3HB) or P(3HV) crystal lattices where the monomer of one type partially includes the crystal lattice of the other [28]. The crystallization of the copolymer experiences the phenomena called *isomorphism* where at a specific content of 3HV, there is a transformation of the crystallization from the P(3HB) lattice to the P(3HV) lattice [28]. The transformation limits were reported to be within the range of 30 to 41 mol% of the 3HV composition [28]. At a low percentage of 3HV, below the transformation limit, the crystallization occurs in the P(3HB) lattice and vice versa. This influences the change of the crystallinity degree (X_c) of PHBV with varied content of 3HV. Generally, X_c of the copolymer decreases when the fraction of different monomer becomes larger. The presence of 3HV has more effect on the crystallization in the P(3HB) lattice than the presence of 3HB in the P(3HV) lattice. Increasing 3HV content at the lower range below the transformation limit causes a faster decrease of the crystallinity degree (X_c) than its increase above the transformation limit. This results in a decrease and increase of the T_m which is directly related to the X_c in the same way.

Table 2-1 Comparison of thermal and mechanical properties of PHBV, PHB, PP, and PLA

		T_m (°C)	T_g (°C)	Young's modulus (GPa)	Elongation at break (%)	Tensile strength (MPa)
PHBV^a	Tianan 20% HV	171	-3.1	8.37	1.26	15.2
	Tianan 5% HV	171	-2.67	13	1.39	22.1
	Aldrich 12%HV	156	-7.39	8.67	4.49	20.9
	Aldrich 5% HV	166	-0.9	-	-	-
PHB^b		179	4	3.5	40	5
PP^c	non-oriented	160-175	-10	1.140 – 1.550	38 ^b	31-42
PLA^d		130 – 180	50-80	8.6	3-30	0.88

^a data reported by Modi, Koelling, & Vodovotz [29]

^b data reported by Możejko-Ciesielska and Kiewisz [3]

^c data reported by Selke, Culter, & Hernandez [30]

^d data reported by Castro-Aguirre, Iniguez-Franco, Samsudin, Fang, & Auras [31]

With comparable properties to conventional polymers such as PP, and their useful properties of biodegradability and compostability, PHAs have gained a lot of interest for use as an alternative to petroleum-based polymers. However, with their high price, previously, the demand in bulk product manufacture including packaging and agricultural applications was not very successful. Nevertheless, PHAs are promising materials in the biomedical field due to their biocompatibility, non-toxicity,

biodegradability, and thermoplasticity. They are fabricated to be used in several biomedical applications ranging from the cardiovascular system to wound management, and also bone and tissue reconstruction [3,32]. On the other hand, the use of PHAs in the drug delivery system is inferior compared to other polymers commonly used in the field due to their limited encapsulation efficiency [32]. PHB and PHBV are among the PHA family that have been studied most in the area. PHBV with improved processability, flexibility, and strength is more attractive than PHB as a drug carrier, specifically in the long-term release platform due to its slow degradation [32]. Plenty of studies based on the application of PHB and PHBV in biomedical applications were well summarized elsewhere [28,33–37].

Regarding the use as packaging materials, PHAs were first commercially recognized back in the 1980s when Imperial Chemical Industries (ICI/Zeneca) released Biopol®, resins of PHBV, and launched a trial of Wella™ shampoo bottles made from Biopol® as the “world’s first totally biodegradable product” in 1992 [38]. However, with uneconomical turnover due to high production cost, the resin production was transferred to Monsanto in 1996 and later to Metabolix (now Yield10 Bioscience, since 2016) in 2001 [38,39]. Even though there has been no official launch of commercial packaging made from PHAs since then, a number of companies are listed as suppliers of PHAs for packaging applications, including Biomer, Goodfellow, Procter & Gamble Chemicals (P&G), Tianan Biologic Material, PHB Industrial S.A. and so forth [3,38,40]. In 2018, the global production of PHAs accounted for 1.4 % of the total 2.11 million tons of bioplastic production, which includes both bio-based/non-biodegradable polymers and biodegradable polymers [41]. The production capacity of PHAs is expected to quadruple in the next five years [41]. Packaging is a major application of bioplastics. The potential and performance of PHAs as packaging materials are being actively researched with PHB and PHBV as the leading members of interest.

2.1.3 PHBV blends and composites

Biodegradable polymers, natural starches, natural fibers, agricultural and industrial byproducts, as well as nanomaterials were selected by researchers to be mixed with PHBV in their studies as listed in Table 2-2. These studies anticipated economic, environmental, as well as material performance benefits. By incorporating other cheaper materials, the cost of the resulting PHBV based materials can be reduced; thus, the use of this polymer could expand. At the same time, materials that are considered as wastes,

such as natural fiber from agriculture byproducts, could find additional pathways of utilization and value-added. Mixing PHBV with nature-based materials was also expected as a possible way to maintain the blend components' environmentally friendly characteristics such as renewable sourced and biodegradability. Blending and compositing of PHBV were also aimed to reinforce and improve its performance flaws, e.g., brittleness, low melt strength, and narrow processing temperature range. It is crucial to evaluate the properties of the output materials whether they are equivalent or comparable to the main polymer matrix. This essentially suggests the target application of the developed materials where they could either be used to substitute for the original material or be utilized in other areas that fit their set of properties.

Table 2-2 Processing techniques used in PHBV based polymer blends and composites

Blend/Composite	Fabrication Technique	References
Natural PHBV/synthetic atactic PHB	Solution blending in chloroform	[42]
Cornstarch-PHBV	Extrusion + injection molding	[43]
Bamboo fiber-PHBV	Extrusion + injection molding	[44]
Wood fiber-PHBV	Extrusion + injection molding	[44]
Agro-residue (corn straw, soy stalk, and wheat straw) – PHBV	Extrusion + injection molding	[45]
Distiller's dried grains with solubles (DDGS) – PHBV	Extrusion + injection molding	[46]
Carbon fiber – PHBV	Extrusion + injection molding	[47]
switchgrass fiber – PHBV/PBAT	Extrusion + injection molding	[48]
PHBV/PBAT, PHBV/PBSebT ^a	Extrusion + compression molding, extrusion blow molding (mono-layered and bi-layered films)	[49]
Nanokeratin fiber – PHBV	Extrusion + compression molding, electrospinning	[50]

^a PBSebT – an experimental film blowing grade biodegradable and compostable bioplastics with the commercial name of Mater-Bi, a proprietary formulation of Novamont

Materials that are mixed in with PHBV can alter the mechanical properties of the output material in different ways. Natural fibers (i.e. bamboo fiber, wood fiber, agro-residue fiber, switchgrass fiber)-PHBV and carbon fiber (CF)-PHBV composites were found to have increased tensile modulus and flexural modulus compared to neat PHBV [44,45,47,48]. The improvement in modulus properties was attributed to the high modulus of the fibers themselves and uniform dispersion of these fibers in the PHBV matrix, leading to even fiber-matrix stress transfer. On the other hand, tensile strength, impact strength, and % elongation at break of these bio-fiber composites were lower due to lack of sufficient interfacial adhesion between fibers and the polymer matrix [44–46]. Weak interfacial adhesion could also be observed in SEM micrographs of the composite fractured surfaces where voids resulting from fiber pullouts were visible [44]. At the same time, an agglomerate formation of the filler could act as a stress raiser that reduces the energy required to break the specimens [45]. On the contrary, CF-PHBV composites had increased tensile strength and fractural strength with increased CF loading [47]. The fractured surface from the tensile test of CF composites showed debonding between the CF and the matrix, but not all fibers were completely loose. This probably indicated stronger interfacial adhesion of CF-PHBV matrix than that of natural fibers.

To improve the interfacial adhesion, Zarrinbakhsh et al. [46] included polymeric methylene diphenyl diisocyanate (PMDI) as a compatibilizer into the composite system of distiller's dried grains with solubles (DDGS)¹ and PHBV/PBS polymer blend. The compatibilized composites showed enhanced tensile and flexural strength compared to uncompatibilized ones. The stronger interfacial adhesion of the compatibilized composites could also be inferred from a smooth fracture surface morphology from the impact test specimens, as well as their lower impact energy. The researchers explained that with the filler particles firmly attached to the matrix with covalent bonds initiated by the compatibilizer, the fracture tends to occur by breaking directly through the filler particles rather than propagating around the particles creating pullouts. With less surface area of fracture, lower energy was required to break the compatibilized composites. PMDI was also used to compatibilize the composites of switchgrass and

¹ Distiller's dried grains with solubles (DDGS) is defined by Zarrinbakhsh et al. [46] as "a coproduct of the corn ethanol industry in which corn is converted to approximately equal weight of ethanol, DDGS, and carbon dioxide."

PHBV/PBAT polymer blend [48]. The compatibilization helped in improving the tensile strength, tensile modulus, and impact strength, which was essentially a result of the enhanced interfacial adhesion.

Mechanical properties of the PHBV-based polymer blends were varied from those of neat PHBV depending on the properties of the polymer that was blended in. For instance, in the study of Scandola et al. [42] where synthetic atactic PHB (a-PHB) was blended with natural PHBV, while the elastic modulus of the blends was decreased with increasing a-PHB as a result of crystallinity decrease, the elongation at break increased by 30 fold compared to the neat PHBV. In the other case, to reduce the brittleness of PHBV, it was blended with 30 wt.% of poly(butylene succinate) (PBS) [46]. In this study, while the modulus of PHBV was reported to be more than five times higher than that of PBS, the elongation at break of PHBV was almost fifty-fold less than PBS. Consequently, tensile modulus and flexural modulus of the blend were found to decrease from that of neat PHBV and fall around the middle range of the two blend components. The elongation at break of this blend was almost three times higher than that of neat PHBV.

Thermo-mechanical properties, i.e., storage modulus (E'), loss modulus (E''), and $\tan \delta$ peaks give information about how rigid the material is and the matrix molecular mobility at different temperatures. This explains the change in mechanical properties of PHBV based blends and composites compared to the polymer matrix. An increase in storage modulus (E') was observed in bamboo fiber-PHBV, agro-residue fiber-PHBV, DDGS-PHBV/PBS, CF-PHBV, and switchgrass-PHBV/PBAT composites [44–48]. Lower $\tan \delta$ or damping peaks of the composites compared to those of the polymer matrix revealed that the presence of the fillers appeared to limit the mobility of the polymer chains [44,46,48]. The reduction of cold crystallization temperatures of bamboo fiber-PHBV composites was observed in the curve of storage modulus as a function of temperature [44]. It was assumed that the fineness and uniform distribution of the bamboo fibers could allow a nucleating effect of bamboo fibers in the PHBV matrix [44]. As $\tan \delta$, the ratio of E'' to E' , depicts the energy dissipated as heat during the dynamic testing, widening of $\tan \delta$ peaks indicating an increase of heterogeneity was observed in the bamboo fiber-PHBV composites [44]. This was also supported by the fractured surface morphology SEM micrograph. Comparing to bamboo fiber-PP composites, better compatibility of PHBV matrix to bamboo fiber was emphasized where a shift of dynamic glass transition temperature occurred only in PHBV based

composites [44]. This finding agreed with the drastic drop in tensile strength and elongation at break of the PP based composites.

Heat deflection temperature (HDT), the maximum temperature that a polymeric material can be used as a rigid material, of natural fiber and agro-residue reinforced PHBV based composites were found to increase compared to the base polymer matrix [44–46,48]. The increase in HDT of CF-PHBV composites was significantly higher than that of the natural fiber composites [47]. This agreed with increasing tensile modulus of these composites.

Fourier transform infrared spectroscopy (FTIR) is another technique used to characterize polymer blends and composites. In the study of bamboo fiber-PHBV-based composites, the increase of crystallinity with addition of bamboo fiber was confirmed in the FTIR results with rising *crystallinity index (CI)*, which is calculated as a ratio of intensity of the band sensitive to crystallization to the band insensitive to crystallization [44]. The absorption peaks at 1228 cm^{-1} and 1176 cm^{-1} were identified as the bands sensitive and insensitive to crystallinity, respectively. The *CI* of PHBV increased around 12 % with the addition of 30 wt.% bamboo fiber, indicating a nucleating agent effect of bamboo fiber. This increased crystallinity supported the mechanical property results that the composite had higher tensile modulus with bamboo fiber addition. FTIR is also a powerful tool used to verify the interaction between the secondary phase or the filler and the polymer matrix induced by the compatibilizer. In the study of switchgrass-PHBV/PBAT composites where PMDI was added as a compatibilizer, a comparison of FTIR spectra of PMDI, switchgrass, PHBV/PBAT blend, non-compatibilized composite, and compatibilized composite indicated the compatibilization effect caused by PMDI [48]. Disappearance of the peaks specifically belonging to PMDI in the spectra of the compatibilized composite revealed that PMDI was consumed during the compatibilization reaction. At the same time, new peaks associated with amide groups, which are a result of the reaction between the isocyanate groups of PMDI and hydroxyl groups of switchgrass or carbonyl groups of the polyester components of the matrix, PHBV or PBAT, were present in the spectra of the compatibilized composite. The bonds occurring between the composite components improved the interfacial adhesion and as a result the strength of the material was increased.

Referring to Table 2-2, the fabrication techniques that were mostly used are extrusion compounding followed by injection molding or compression molding to produce test specimens for

characterization. Extrusion blow molding was used in a study where PHBV was blended or co-extruded with other polymers with higher melt elasticity, shear viscosity, and toughness, i.e., PBAT and PBSebT (an experimental film blowing grade polymeric compound of Novamont) [49]. By blending with these polymers, the film blowing ability of the polymer blends was improved compared to neat PHBV. Mechanical properties (Young's modulus and strain at break) of the PHBV/PBAT and PHBV/PBSebT blown films from different molder settings (blow up ratio (BUR): 2.3 – 2.5, take up ratio (TUR): 4.3 – 5.8, and nine combinations of cooling fan speed and air ring aperture where each setting has three levels of min, med, and max) were found to be comparable and ruled by the PHBV matrix which accounted for 70 wt.% of the blends. A small mechanical anisotropy of the blown film was found only in the PHBV/PBAT blend from high BUR and TUR settings. However, it was found in this study that the step prior to blow molding, melt blending, caused thermal degradation of PHBV that was evidenced with significantly higher *melt flow Index (MFI)* of the polymer blends compared to the blend components, resulting in a drastic viscosity drop due to chain scission of PHBV. Consequently, co-extrusion was adopted as an option to avoid the thermal degradation. Coextrusion film blowing was claimed to be easier than the equivalent film blowing of the polymer blend. Coextruded bilayer PHBV/PBAT film was found to have half less thickness variation in the machine direction compared to the film blown from the blend. The bilayer films, nonetheless, had poor adhesion between layers and delamination often occurred both during specimen cutting and tensile testing. Regarding food and non-food packaging applications, the coextruded bilayer films tended to have advantages over the films blown from the polymer blend with better transmittance and extended range of water vapor permeability. The bilayer film also had elevated tear resistance when PBAT was exposed to the tear. A higher strain of welded joints was observed in the bilayer films (between 8-14 % of PHBV/PBAT blend welded joint and 358-539% of the bilayer film welded joint when PBAT was the welded material).

Incorporating of nanomaterials in PHBV was studied by Fabra et al. [50], where nanokeratin extracted from poultry feathers was combined with PHBV. Two techniques; 1) direct melt compounding, and 2) melt compounding of PHBV with pre-electrospun masterbatch of nanokeratin-PHBV (further referred as the preincorporation method), were adopted to produce the biobased-nanocomposites. Pre-electrospinning of nanokeratin in PHBV as a masterbatch prior to melt compounding with PHBV helped in

improving the dispersion of the nano-additive in the polymer matrix. The effect of uniform distribution of nanokeratin could be observed from the mechanical properties of the composites. While the composites obtained from the blending method tended to be stiffer with addition of nanokeratin, stretchability of the sample from the preincorporation method was still equivalent to neat PHBV. Addition of nanokeratin improved the barrier properties of the resulting materials. Water vapor permeability (WVP) and oxygen permeability (O_2P) of the nanocomposites obtained from both techniques were found to decrease compared to the neat polymer matrix, with the preincorporation method showing a significant improvement. Also, in this study, a multilayer system of nanokeratin-PHBV was investigated as a method to improve the hydrophobicity of the nanokeratin film. Nanokeratin film was solution casted and then coated with an electrospun PHBV fiber mat and annealed under heat. The PHBV coated-nanokeratin film showed improved barrier properties compared to the single layer nanokeratin film. WVP of the multilayer film decreased up to 60%. O_2P at high humidity condition (80% RH), which was unable to be measured in single layer nanokeratin film due to a partial disintegration of the film due to moisture exposure, was measurable in the multilayer film. The decrease of water sorption was reported as a main factor in the improvement.

In summary, the combination of PHBV with various materials ranging from other polymers, biomass, and nanomaterials results in alteration of mechanical properties, thermal properties, as well as barrier properties. These characteristics of the blends and composites are generally ruled by multiple factors including miscibility or interaction between the polymer matrix and the dispersion phase or the filler, how uniform the filler is distributed in the matrix, size of the filler, the properties belonging specifically to each composite or blend component, and its content in the mixed material. Characterization and measurement techniques were adopted to examine, validate, and explain the phenomena that resulted from the combination of PHBV and other materials. The extended properties beyond the original range of the neat PHBV expand the potential use and application of the polymer. At the same time, when utilizing renewable material as a reinforcement phase, the obtained composite is fully biobased.

2.2 Poly(lactic acid) – PLA

2.2.1 PLA chemistry and production

Poly(lactic acid) or PLA is the currently most widespread biobased, biodegradable aliphatic polymer used in various applications. The polymer's global production capacity is among the top of the biodegradable bioplastics together with the starch blends [41]. With its versatile properties, PLA is used in many industrial applications including medical, fibers and textiles, packaging and serviceware, agriculture, and environmental remediation, where the major target market segment of this polymer is rigid packaging following by flexible packaging and textiles [31,41]. High molecular weight (M_w) PLA ($M_w > 100$ kDa) used for these purposes is generally produced by polycondensation and ring opening polymerization using lactic acid as the starting building block. While lactic acid can be produced by both bacterial fermentation of carbohydrate sources or by chemical synthesis, the major PLA producers, NatureWorks LLC. and Corbion®, prefer using the former method due to the advantages regarding production capacity and costs [31]. This means PLA in the market is mostly biobased with natural raw materials including glucose and maltose from corn or potatoes fed to microorganisms for lactic acid fermentation [31]. Three pathways to produce high M_w PLA on an industrial scale include: 1) direct condensation polymerization; 2) direct polymerization in an azeotropic solution; and 3) polymerization through lactide formation. While method 1) uses chain coupling agents to generate high M_w PLA from low M_w polymer resulting from the condensation process of lactic acid, method 2) obtains high M_w PLA by a direct condensation polymerization for an extended period of time with continuous removal of condensation water from the system to prevent a reverse reaction by the azeotropic distillation. The solvent is kept refluxing through the system throughout the period while the obtained high M_w polymer is kept in the reaction vessel by 3-Å molecular sieves. At the end the polymer can be collected as is or be purified by dissolving and precipitation. Unlike the former two methods, method 3) depolymerizes low M_w prepolymer PLA into lactides following by the ring opening of lactides then polymerizing to produce high M_w polymer. Since lactic acid has two stereoisomers of L-lactic acid or (S)-lactic acid and D-lactic acid or (R)-lactic acid, when forming into lactide, three diastereomeric structures are obtained: L-lactide or (S-S)-lactide, D-lactide or (R-R)-lactide, and meso-lactide or (S-R)-lactide. With different boiling points of each lactide structure and different levels of stereochemical purity, the properties including molecular weight and crystallinity of PLA

derived from lactide monomers can be varied based on different ratios of these lactides [31]. The synthesis of PLA and the effect of the amount of different lactide are well described in Hartmann [51] and Castro-Aguirre et al. [31], respectively.

2.2.2 PLA properties and applications

Based on the thermal, rheological, and crystallinity behaviors of PLA, it can be processed through generic melt processing methods including extrusion, injection molding, injection stretch blow molding, casting, thermoforming, foaming, and fiber spinning. The mechanical, thermal, and barrier properties of PLA are comparable to other commercial polymers such as polypropylene (PP), poly(ethylene terephthalate) (PET), and polystyrene (PS). PLA made its first landing in medical applications with its biocompatibility properties and the high production cost at the beginning. With continuous decrease of the polymer's price together with increasing environmental consciousness of consumers, PLA demand took a lead in the biodegradable bioplastic market with a growing target segment in packaging applications. The application of PLA in fibers and textiles is also well recognized, such as water absorbents, substitution of petroleum-based polymers in textile automotive parts, as well as in apparel [31].

2.2.3 PLA blends and composites

One common rationale behind the studies of PLA-based polymer blends and composites is to tailor its properties. Even though PLA possesses many attractive strengths including; 1) biobased, biodegradable, and biocompatible nature, 2) competitive cost compared to traditional petroleum-based commodity polymers, and 3) beneficial mechanical and physical properties of high modulus, high strength, and good clarity; the weaknesses that limit its use are undeniable. While the processability of PLA is suppressed by its low melt strength and slow crystallization, the application of this biobased polymer is limited by its brittleness, low toughness, and low glass transition temperature. Polymer blending and composites are known to be an easy way to alter and improve these properties.

PLA-based polymer blending has been extensively studied. More than 900 published articles relating to PLA-based blends since 1996 – 2018 were recorded in the recent review of Nofar et al. [52]. Among the categories that the reviewers classified all these studies based on the blend components other than PLA, the group of binary blend systems containing PLA and non-biobased, biodegradable polymers had the largest number of studies recorded. It might be inferred that whether or not the final

blends retain the biobased and biodegradable characteristics could be one of the factors that researchers who did studies about PLA blending considered when choosing material(s) to be blended with PLA. In this review [52], miscibility, morphology, thermal and mechanical properties and target applications of the blending, as well as techniques that were used to improve the performance of the blends of PLA with a certain material were well discussed. The original review article is recommended for interested readers to obtain the latest updates of this topic.

2.3 Polymer blends

Polymer blend is defined by Utracki, Mukhopadhyay, & Gupta [53] as “a mixture of at least two macromolecular substances, polymers or copolymers, in which the ingredient content is above 2 wt.%”. Polymer blending is a cheaper and less time-consuming option beyond inventing of new polymeric materials that require the development of new monomers and new polymerization routes [54]. Polymer blending can produce new polymeric materials with combining outstanding properties of more than one existing polymer [54]. The performance of a polymer blend depends on many factors including the intrinsic properties of the blend components, miscibility, compatibilization, surface and interfacial properties, morphology, rheology, and processing.

Morphology is one of the most important characteristics that control the performance of a polymer blend. The concept regarding the morphology of polymer blends essentially explained by Kamal et al. [55] is summarized in this paragraph. Morphology depends on the blend concentration and the thermodynamic and rheological properties of the blend components. The system of mixing between a small quantity of one polymer in another polymer is a blend composed a matrix (the major component) and the dispersed phase (the minor component). From low to high concentration, the morphology of the dispersed phase could change from discontinuous nearly spherical drops to interconnected drops, then rods, fibers, and sheets. At a certain concentration where the dispersed and matrix phases are no longer differentiated, the morphology of the system becomes co-continuous, and this concentration is marked as *the phase inversion volume fraction*, Φ_I . Phase co-continuity is a crucial aspect of blend morphology as it contributes to synergism of properties such as advantageous combination of high modulus and high impact strength in commercial blends. In immiscible polymer blends, the change in morphology relates to the percolation threshold volume fraction, Φ_{perc} , at which when the volume fraction of a dispersed phase

exceeds this value, the phase co-continuity suddenly increases. At equilibrium and within the region of low volume fraction of the dispersed phase, $\Phi < \Phi_{perc}$, droplets are expected, while at $\Phi > \Phi_{perc}$ a co-continuous morphology, e.g., fibers or lamellae, is usually observed. Determination of the blend composition that co-continuity can be formed and the phase inversion volume fraction is related to the volume fraction and viscosity ratio of the polymer blend components; more detail can be found in the chapter of Kamal et al. [55].

Generally, most polymers form immiscible blends where the miscibility of the polymer blend can be determined by the free energy of mixing [54]:

$$\Delta G_m = \Delta H_m - T \cdot \Delta S_m \quad \text{Eq. 2-1}$$

where the gain in entropy, ΔS_m is negligible when two high molecular weight polymers are blended and ΔH_m is the heat of mixing. A polymer blend is miscible when it is homogeneous down to the molecular level, with the negative value of ΔG_m : $\Delta G_m \approx \Delta H_m \leq 0$ [53]. ΔH_m can be negative only when the mixing is exothermic, which could happen when the blend components form specific interactions ranging from strong ionic to weak and nonbonding interactions, such as hydrogen bonding, ion-dipole, dipole-dipole, and donor-acceptor interactions [54]. In general, when polymers are mixed, only Van der Waals interactions form, which explains why completely miscible blends of polymers are quite rare [54]. Measurement of the glass transition temperature (T_g) is the most widely used tool to detect the miscibility. The *completely miscible blend* has a significant characteristic of one T_g located in between the T_g values of the blend components relating to the blend composition. Another type of polymer blend based on the miscibility is a *partially miscible blend* where a small part of the blend components dissolve in each other forming a fine phase morphology [54]. In this type, T_g values belonging to each blend component are still exhibited but they tend to shift toward each other. The last type is a *fully immiscible blend*, where a coarse phase morphology forms in the blending, the adhesion between the blend phase is poor, and the T_g of the blend components are likely to be constant with the blend composition.

According to Hess et al. [56], “homogeneity at fairly fine level is necessary for optimum performance, but some degree of microheterogeneity is usually desirable to preserve the individual properties of respective polymer components”. With the fact that nearly all commercial polymer blends are immiscible, the miscibility is usually assessed mainly to design better compatibilizers and

compatibilization strategies rather than to develop single-phase commercial blends [57]. Compatibilization is defined as “a process of modification of the interfacial properties in immiscible polymer blend, resulting in reduction of the interfacial tension coefficient and stabilization of the desired morphology, thus leading to the creation of a polymer alloy” [53]. Performance of a polymer alloy depends on the blend components, their concentration, and morphology. Stable and predictable morphology of polymer blends are contributed by the two main components of the polymer alloying process: an appropriate dispersing method, i.e. mechanical mixing, solution or latex blending) and compatibilization [53].

According to Utracki et al. [53], the appropriate dispersing method should be able to reduce the interfacial tension causing the blend to form a fine dispersion, maintain the stability of the morphology against thermal or shear effects during the processing steps, and contribute interfacial adhesion in the solid state. Regarding the compatibilization of immiscible polymer blends, numerous reviews summarizing the strategies have been published. The list of these studies is worth to review in the chapter by Brown [58]. Examples of these methods include: 1) addition of a small amount of co-solvent; 2) addition of a copolymer containing parts that are miscible with the blend components (e.g., premade graft or block copolymers); 3) reactive compounding resulting in modification of at least one polymer component that induces local miscibility regions, etc.

Reactive compatibilization is when compatibilizing agents or copolymer species or interaction inducing miscibility regions between the blend components are generated *in situ* during the melt processing [59]. Investigation of these strategies has become more active over time both in industrial and academic research due to economic and performance advantages. Only a small amount of reaction initiators or compatibilizers are required to add into the process.

2.3.1 Maleated PLA compatibilizer

Polymer blend compatibilization by addition of reactive polymer is one of the most familiar methods of polymer blend reactive compatibilization. Inert polymer that is miscible in one blend component can be functionalized with a reactive group. When added into the blending process, these reactive functionalized polymer chains can increase the interaction with the other blend component, and at the same time have a good adhesion to a component they are miscible with.

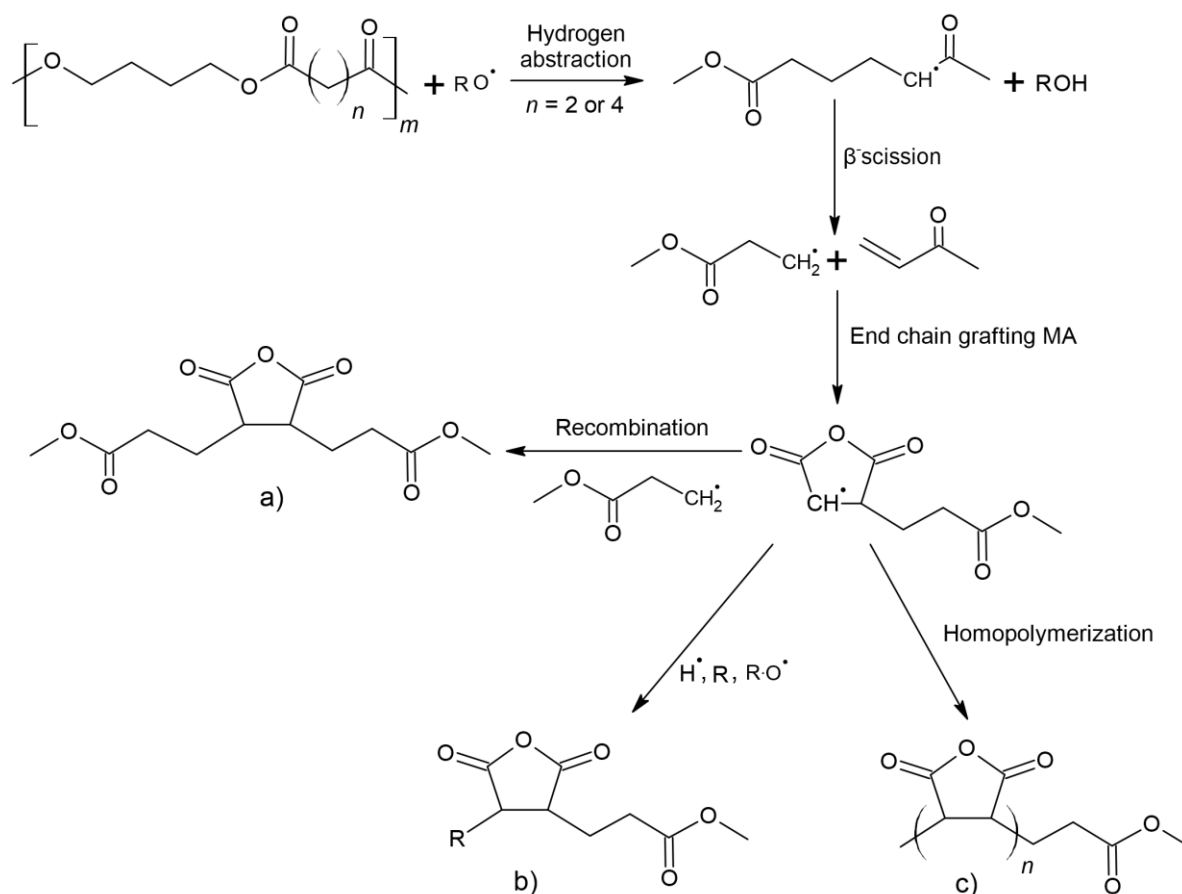
Anhydride is one of the versatile reactive groups that are chemically suitable with many functional groups of polymers such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH₂) [59]. Maleic anhydride (MA) is widely used since it can be grafted on many polymers at normal melt processing temperature without homopolymerization [59]. MA was grafted on a variety of polyolefins and polyesters. A study of Mani et al. [60] investigated grafting of MA on polyesters, i.e. polybutylene succinate (PBS), polybutylene succinate adipate copolyesters (PBSA), and poly(lactic acid) (PLA). The grafting efficiency was found to be influenced by different kinds of free radical initiators and their concentrations, processing temperature, and also the amount of free radical sites on the polymer backbone [60].

Several studies aimed at compatibilization of PLA based blends used maleated PLA (PLA-*g*-MA) as a reactive compatibilizer. Performance of the blend could be impacted by qualities of the compatibilizer. In a study by Detyothin et al. which focused on reactive compatibilization of the blend between PLA and thermoplastic cassava starch (TPCS), they found that the blends' tensile properties were affected by the amount of grafted MA on PLA-*g*-MA compatibilizer and their number average molecular weights, M_n [61]. These characteristics of PLA-*g*-MA were reported to be influenced by the contents of initiator and MA used in the functionalization. Increase of 2,5-bis(tert-butylperoxy)-2,5-dimethyl hexane (L101) which was used as a radical initiator resulted in an increase of grafting yield with a decrease of M_n of PLA-*g*-MA polymer chains [62]. The composition of 4.5 wt.% of MA and 0.45-0.65 wt.% L101 with the screw speed of 20 rpm were also reported as an optimal condition for grafting MA on PLA [62]. Other radical initiators such as 3,6,9-Triethyl-3,6,9-trimethyl-1,4,7-triperoxonane (T301) [63], benzoyl peroxide [64], and dicumyl peroxide (DCP) [65] were also used in the reactive maleation of PLA.

The performance of PLA/TPCS blends using PLA-*g*-MA with different initiators of L101 and DCP were evaluated in the study of Bher et al.[66], who found that the interfacial adhesion resulting from both cases was improved, however, the morphology of PLA-*g*-MA initiated with DCP showed a finer domain of TPCS and better interfacial adhesion on the PLA matrix. The elongation at break of all blends was improved; tougher samples were obtained when the PLA-*g*-MA initiated with DCP was used [66].

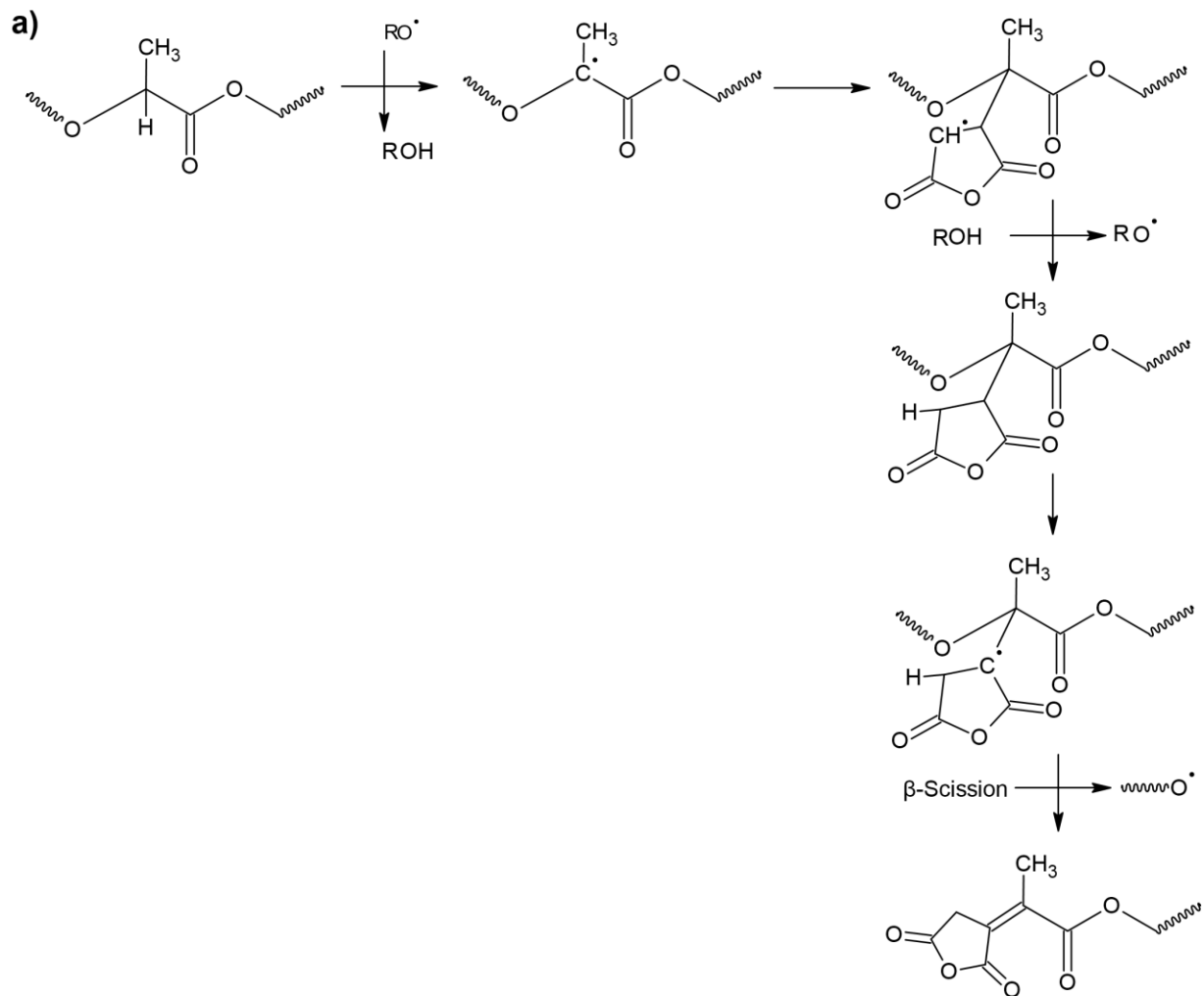
2.3.2 Maleation

Grafting of polymer with suitable functional groups can be done by a number of methods including melt grafting, solid-state grafting, solution grafting, suspension grafting in aqueous or organic solvents, and redox system grafting [60]. The reactive extrusion method which is the most widespread melt state process to produce graft polymer was used in the studies to graft maleic anhydride (MA) onto polyesters [60,67]. Maleation mechanisms occurring in the grafting process were proposed by Mani et al. [60] and Carlson et al. [67] in Scheme 2-1 and Scheme 2-2, respectively. Scheme 2-1 was proposed based on the maleation of polybutylene succinate (PBS) and polybutylene succinate adipate copolyesters (PBSA). The results from the study of Mani et al. [60] explained that the reaction starts with the homolytical scission of the peroxide initiator, followed by the hydrogen abstraction of the α -carbon of the ester carbonyl group forming a polyester macroradical which later goes through the β -scission and results in simultaneous formation of a radical chain end and vinylidene chain end. A single MA molecule is grafted onto the radical end. After that termination reactions (Scheme 2-1b), chain transfer processes (Scheme 2-1a), oligomerization of MA (Scheme 2-1c), and other possible reactions could occur. However, according to their result, homopolymerization of MA was not observed.



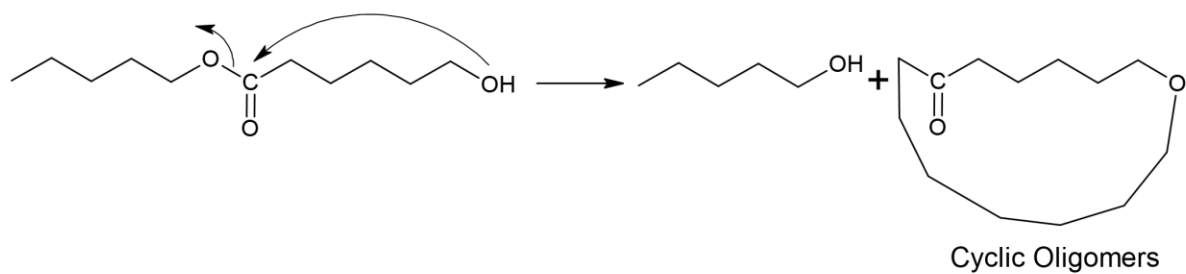
Scheme 2-1 Maleation mechanisms of polybutylene succinate (PBS) and polybutylene succinate adipate copolyesters (PBSA) showing β -scission occurs after the formation of a polyester macroradical due to the hydrogen abstraction of the α -carbon of the ester carbonyl group (adapted from Mani et al. [60])

Carlson et al. [67] proposed the maleation reaction of PLA (Scheme 2-2a). The formation of macroradical occurs through the hydrogen abstraction at the α -carbon relative to the carbonyl group induced by the peroxide radical similarly explained by Mani et al. [60]. After that the grafting of MA on PLA radical happens along with the combining of the polymer radical with other radicals, i.e. peroxide, other polymer radicals, or hydrogen. Different from what is described by Mani et al. [60], β -scission was proposed to occur after MA grafting through the back-biting or thermohydrolysis (Scheme 2-2b). Even though the oligomerization of MA was discussed to be able to be limited in the melt maleation process since the processing temperature is higher than the typical ceiling temperature for the formation of poly(MA), Carlson et al. assumed, without experimental supporting evidence, that there is still a possibility of MA homopolymerization.

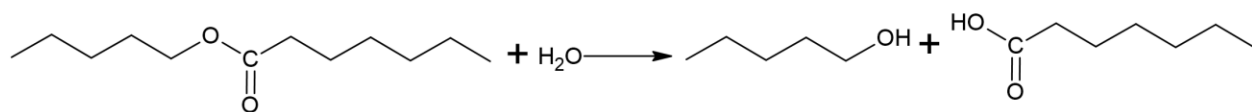


b)

Back-biting



Thermohydrolysis



Scheme 2-2 Maleation reaction of PLA proposing β -scission to occur after MA grafting through the back-biting or thermohydrolysis (adapted from Carlson et al. [67])

2.4 Blending between PHBV and PLA

With aims to preserve the biobased, biodegradable, and biocompatible characteristics of PHBV and PLA, and at the same time to improve the properties of the materials, the blending of these polymers has been widely studied. The early studies in this topic were done by *solvent casting* where the polymers were dissolved in the solvents, mainly chloroform, and then cast into film samples for characterization. The *melt mixing* method using compounders and extruders was later often used. Calorimetric analysis reflecting miscibility and crystallization behavior, heat stability, morphology, and mechanical properties were usually investigated in these studies. The blending in the studies during the early stage was done by a non-compatibilized method. Variation between studies includes characteristics of PLA and PHBV such as molecular weight and percentage of hydroxyvalerate (HV) in PHBV, and the fabricating methods. Later studies proceeded adding a third polymer, nanofillers, and natural fibers to reinforce and compatibilize the polymer blends. Reactive compatibilization by peroxides and functionalized polymers were also investigated. Regarding the blending composition, the blending of this pair of polymers was studied throughout all the ratios of both polymers from 0 to 100% resulting in the investigation of composition-dependent phenomena of all properties of the obtained blends.

2.4.1 Miscibility

The extent of miscibility is one of the important factors governing the performance of the polymer blend. According to the studies of the polymer blending between PHBV and PLA that concerned the blend miscibility, three main concepts were applied to evaluate this aspect of the blends including: 1) the theory of Flory and Huggins for miscibility: Eq. 2-2 and Eq. 2-3, 2) the k parameter of the Gordon-Taylor equation for calculating the T_g of the blend: Eq. 2-4, and 3) the Fuoss-Kirkwood equation: Eq. 2-5 [68,69].

The following equation is applied from Flory and Huggins theory for determining the miscibility of the blend:

$$\frac{\Delta G}{R \cdot T \cdot V} = \left(\frac{\Phi_1}{V_{1m}} \cdot \ln \Phi_1 + \frac{\Phi_2}{V_{2m}} \cdot \ln \Phi_2 + \frac{\Phi_1 \cdot \Phi_2}{R \cdot T} (\delta_1 - \delta_2)^2 \right) ; \quad \text{Eq. 2-2}$$

$$(\delta_1 - \delta_2)^2 = \frac{R \cdot T}{V_m} \cdot \chi_{12} \quad \text{Eq. 2-3}$$

where ΔG is the free enthalpy of mixing, R the gas constant, V the volume of the system, Φ_1 and Φ_2 are, respectively, the volume fractions, V_{1m} and V_{2m} are, respectively, the molar volumes, δ_1 and δ_2 are, respectively the solubility parameters, and χ_{12} is the Flory-Huggins Interaction parameter [69].

Jost and Kopitzky [69] investigated the miscibility of the blends between PLA and PHBV by reviewing the previous studies of these blends based on thermodynamic aspects as well as conducting their own experiments. The theory of Flory-Huggins for miscibility was adopted in their study to examine the miscibility of the blending systems they reviewed as well as the ones they produced. Regarding the Flory-Huggins theory, the extent of miscibility was indicated by the critical solubility parameter difference, $(\delta_1 - \delta_2)_{cr}$ of PHBV and PLA used in the blending system. $(\delta_1 - \delta_2)_{cr}$ was calculated by taking into consideration the molecular weight and the density of the blend components together with the temperature. The blend system that contributed the true solubility parameter difference above $(\delta_1 - \delta_2)_{cr}$ was marked as immiscible.

The Gordon-Taylor equation (G-T model) for calculating the T_g of the blend is shown below.

$$T_{g,mix} \approx \frac{w_1 \cdot T_{g1} + k \cdot w_2 \cdot T_{g2}}{w_1 + k \cdot w_2} \quad \text{Eq. 2-4}$$

$T_{g,mix}$ and T_{gi} are the glass transition temperature of the blend and of the components, w_i is the weight fraction of component i and k is an adjustable fitting parameter. Fitting of the T_g data from the blend system that results in k value closer to 1 indicates better miscibility between the blend components [68,70].

The following is a form of the Fuoss-Kirkwood equation used in the work of Iannace et al. [68]:

$$E''(w, T) = 2E''_{max} \frac{(w\tau)^m}{1 + (w\tau)^{2m}} \quad \text{Eq. 2-5}$$

where τ is the characteristic relaxation time of the material at the temperature T which is assumed to follow an Arrhenius-type equation:

$$\tau(T) = \tau_0 \exp \left[\frac{E_a}{R} \left(\frac{1}{T} - \frac{1}{T_0} \right) \right] \quad \text{Eq. 2-6}$$

where τ_0 is the relaxation time at temperature T_0 , E_a is the activation energy of the relaxation phenomenon, R is the universal gas constant, and m is a parameter that considers the distribution of all

relaxation times and can assume values between 0 and 1. A decrease of E_a and the parameter m of the blend compared to a neat polymer indicates the molecular level interactions that occur between the blend components.

2.4.2 Non-reactive compatibilized blending

According to the previous studies, PHBV/PLA blends are generally immiscible. Non-compatibilized blending by solvent casting [68,71] and melt blending [70,72–76] resulted in separated-phase morphology and separated calorimetric features: glass transition temperature (T_g), melting temperature (T_m), and crystallization temperature (T_c), belonging to each polymer. The calorimetric analysis results of Iannace et al. [68] and Ferreira et al. [71], where PHBV and PLA were blended by solvent casting without compatibilization, showed that the melting temperature (T_m) tended to be constant with the variation of the blend composition. T_m values were 145 °C and 160 °C for PHBV and 175 °C for PLLA [71]. The specific heat of fusion of PLLA decreased with increasing PHBV content. By adding PHBV into the matrix, the dispersion of the PLLA amorphous phase in the PHBV phase resulted in not all PLLA able to crystallize, which caused decreasing of the crystallinity of PLLA.

The glass transition temperature (T_g) of a polymer blend indicates the miscibility of the component polymers of the blend. A single composition-dependent T_g located between the T_g values of the pure blend components is evidence of miscibility of the component polymers in the amorphous phase [70]. According to Iannace et al. [68] and Ferreira et al. [71], two distinct T_g values were characterized from the blends they studied; the first belonged to the PHBV phase at about -1 to -4 °C and the second at about 50 to 60 °C of the PLA phase. The presence of these two values suggested the separated phases after melting. However, the T_g of the PHBV phase slightly increased with increasing PLA content while the T_g of PLA gradually decreased with increasing PHBV content [73]. The findings involving the T_g were analyzed using the following relation derived from the G-T model:

$$w_1(T_{g,1} - T_{g,mix}) + kw_2(T_{g,2} - T_{g,mix}) = 0 \quad \text{Eq. 2-7}$$

The best fit of the experimental $T_{g,i}$ and W_i that gives k equals or closes to 1 represents the behavior of the blend with the experimental $T_{g,mix}$ that is weighted by the two T_g values of the blend components [68]. The analysis of Iannace et al. [68] by plotting $T_{g,PLA}$ versus w_{PLA} gave $k = 88.22$ while

the study of Richards et al. [72] obtained $k = 0.18$ by plotting $T_{g,mix}$ by w_{PHBV} . The value of k that is away from 1 indicates poor miscibility.

Separated crystallization temperatures (T_c), 58 °C for PHBV and 95 °C for PLLA were observed from the blends [71]. Based on the result from the wide-angle X-ray diffractograms, no peak shifts when comparing between different blend compositions suggested that the distances of the crystalline structure were not affected and there was no co-crystallization between materials [68].

These findings of separate T_m and T_g belonging to each blend component and no variation of T_m and T_g with different blend compositions confirmed the conclusion that the blends between PHBV and PLLA were immiscible.

Nanda et al. [70] investigated the miscibility of non-compatibilized blends of PLA and PHBV using Gibb's free energy:

$$\Delta G_m = \Delta H_m - T(\Delta S_m^c + \Delta S_m^e) \quad \text{Eq. 2-8}$$

where ΔG_m is Gibb's free energy, ΔH_m is the heat of mixing, T is the temperature, ΔS_m^c is the combinatorial entropy of mixing and ΔS_m^e is the excess entropy of mixing. For the ideal mixed solution, the ΔG_m is always negative. Their obtained ΔG_m values for the blend of PHBV/PLA 70:30, 60:40, and 50:50 ranged from 0.562 to 0.669 indicating slight miscibility of the polymers in the blends [70].

The presence of PLA affected the molecular mobility of the PHBV chains; the partial dispersion of low molecular weight PLA or the interphase of low molecular weight fractions of both polymers widened the T_g range of the polymer blends instead of initiating a significant shifting [68]. This also reduced the crystallinity of the PLA phase which resulted in a slight lowering of its T_m [68].

Regarding mechanical properties, tensile strength, tensile modulus, and elongation at break of non-compatibilized PHBV/PLA blends from various published studies are shown in Figure 2-3 to Figure 2-5. Tensile strength and tensile modulus of the blends were in between the values of the neat polymers used as the blend components. Most studies found that the properties of the blends were improved compared to the pure polymers [28,68,70,71,74,76–79]. With addition of PLA, the polymer blends became stronger and more rigid compared to the neat PHBV. The elongation at break did not show a clear trend of change with changing contents of the blend components. However, some studies agreed

that the value compared to neat PLA tended to increase with inclusion of PHBV up to a certain content, about 20 – 30 wt.%, then started to decrease with more PHBV [68,70,74,79].

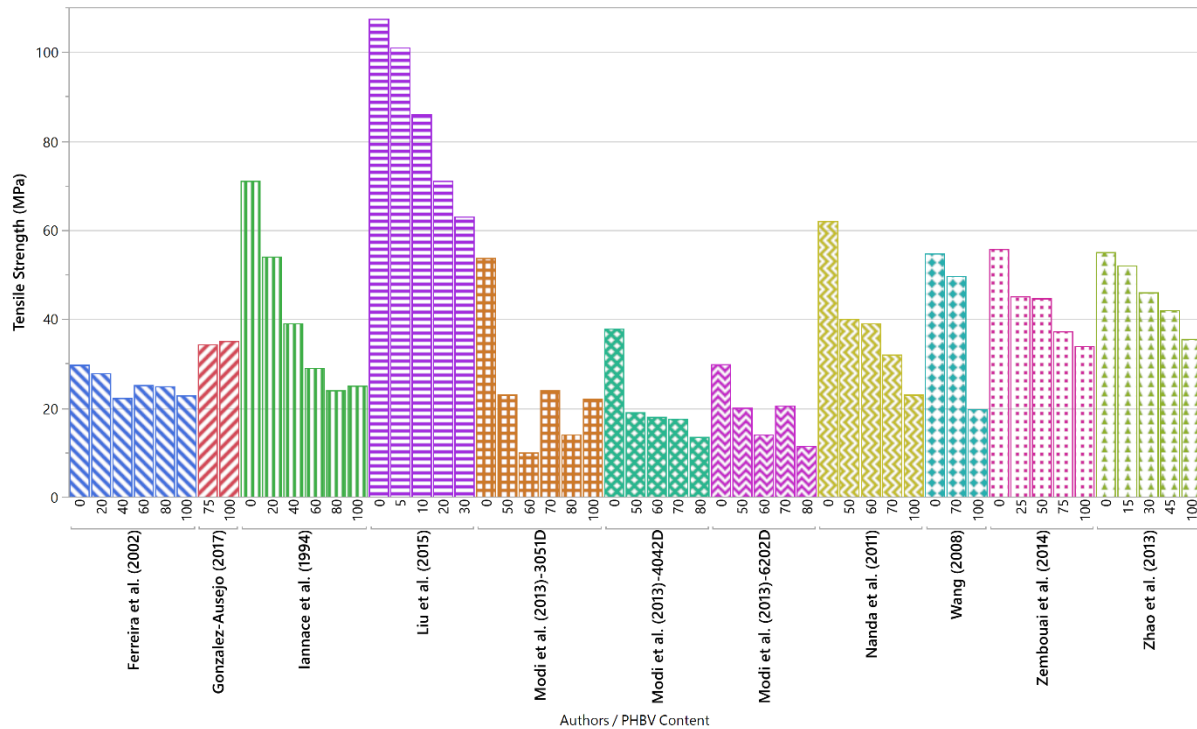


Figure 2-3 Tensile Strength of non-compatibilized PHBV/PLA blends

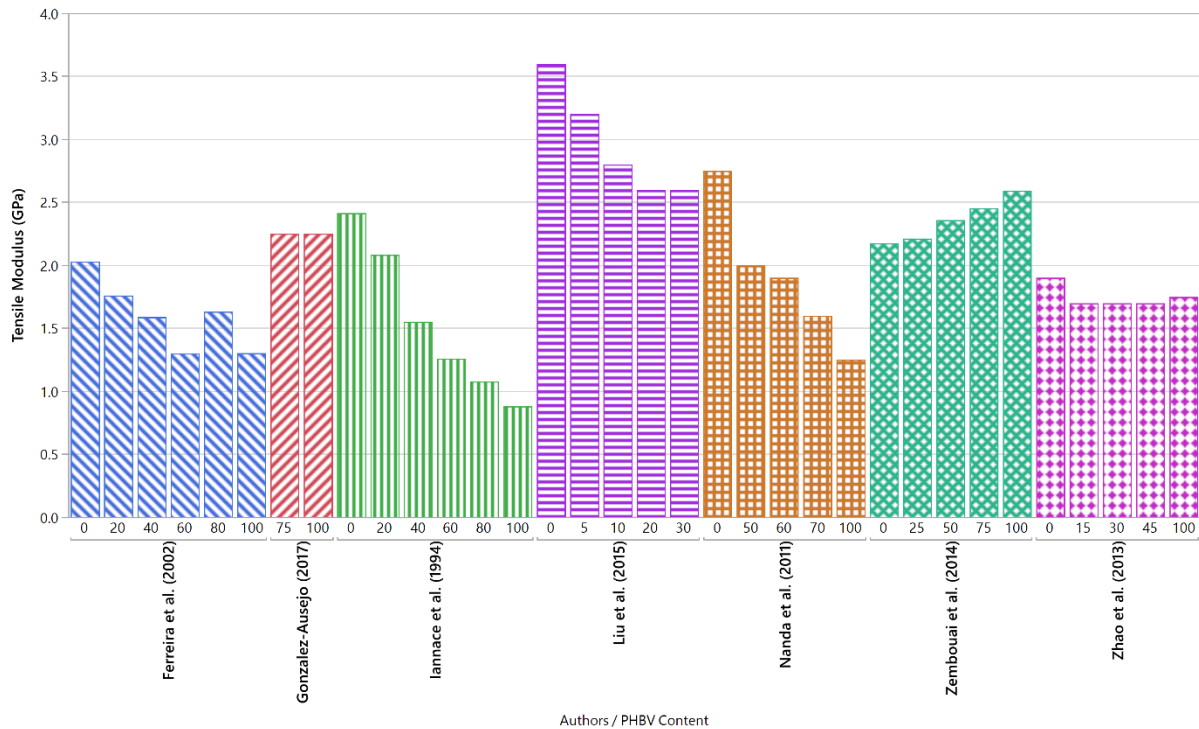


Figure 2-4 Tensile modulus of non-compatible PHBV/PLA blends

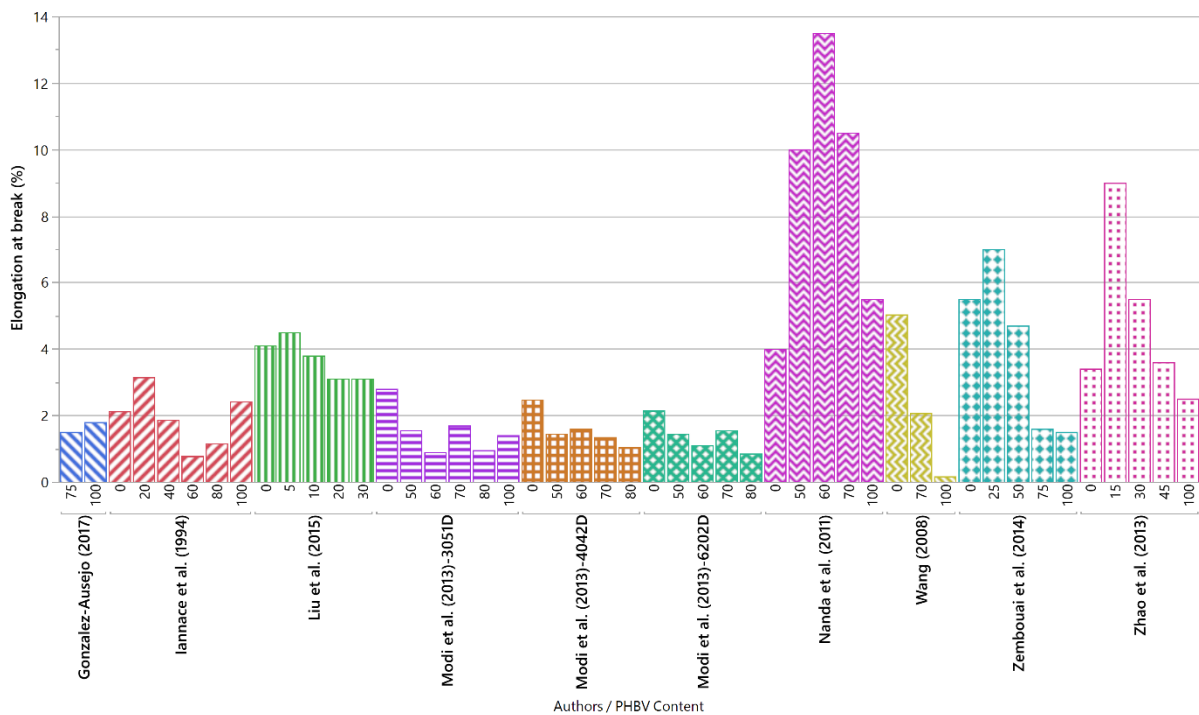


Figure 2-5 Elongation at break of non-compatible PHBV/PLA blends

PHBV/PLA blends were also compatibilized by filler addition using chitin nanowhiskers, titanium dioxide (TiO₂), and organo-modified montmorillonite [80–82]. Additionally, the polymers were ternary blended with poly(butylene succinate) [83], and were studied using a multilayer co-extrusion process [84].

2.4.3 Reactive blending

Another pathway to compatibilize polymer blends that was adopted for the blending of PHBV and PLA is *reactive blending* or *reactive compatibilization*. This method involves the process of compatibilization when compatibilizing agents due to the functionalization are generated *in situ* or chemico-physical interactions or interfacial reactions are induced during the blending process [59,85]. A few studies on the blend of PHBV and PLA were found to use this method to improve the miscibility of the blend. Compatibilizers and additives that were used in these studies are listed in Table 2-3. PHBV-grafted-maleic anhydride (PHBV-g-MAH) and diisocyanates were used as reactive compatibilizers in PHBV/PLA blending [77,79]. In the studies of Zembouai et al., PHBV-g-MAH obtained by grafting maleic anhydride (MAH) on the PHBV backbone through the thermal decomposition of dicumyl peroxide (DCP) was used as a compatibilizer in the blend of PHBV, PLA, and Cloisite 30B [79]. The blended material was reported to have better interfacial adhesion and miscibility [79].

Gonzalez-Ausejo et al. used hexamethylene diisocyanate (HMDI), poly(hexamethylene diisocyanate (polyHMDI) and 1,4-phenylene diisocyanate (PDI) as compatibilizing agents in PHBV/PLA blends [77]. The presence of isocyanates improved overall mechanical properties of the blends, caused reduction of PHBV crystallinity and increased the final complex viscosity at low frequencies compared to the uncompatibilized blends [77]. The thermoforming of these blends was also investigated. It was found that when an appropriate amount of diisocyanates was added, there is a remarkable improvement in the thermoforming capacity and the thermoforming window was widened [86]. The barrier performance was slightly decreased with the presence of PLA as the authors predicted [86]. However, the biodegradability in composting condition was not negatively affected compared to neat PHBV [86].

Table 2-3 List of reactive compatibilizers and additives used in PHBV/PLA blends

PHBV	PLA	Compatibilizers/additives	References
Tianan: ENMAT Y1000P	NatureWorks 7001D	PHBV-g-MA (DCP as a free radical initiator)	[79]
Tianan: ENMAT Y1000P	NatureWorks 2003D	• HMDI: hexamethylene diisocyanate • PolyHMDI: poly(hexamethylene) diisocyanate • PDI: 1,4-phenylene diisocyanate	[77]
Tianan: ENMAT Y1000P	NatureWorks 2002D	Surface-active compatibilizers • PEG-PE: Poly-(ethylene-block-polyethylene glycol) • PEGDO: Polyethylene glycol 400 dioleate • PMMA: Polymethyl methacrylate Peroxides • DCP: Dicumyl peroxide • VB: Divinyl benzene • HAD: Hexanediol diacrylate • DEGDM: Diethylene glycol dimethacrylate • PEGDO: Polyethylene glycol 400 dioleate Transesterification catalysts • Fe(acac)₃: Iron(III) acetyl acetate • Zr(acac)₄: Zirconium(IV) acetyl acetate • Zn(acac)₂: Zinc(II) acetyl acetate • Cu(acac)₂: Copper(II) acetyl acetate • TiBu₄: Titanium butylate • Zn acetate: Zinc(II) acetate monohydrate	[69]

Jost and Kopitzky [69] investigated the influence of multiple compatibilization strategies (listed in Table 2-3), which were aimed to enhance the miscibility between PLA and PHBV, on the mechanical and barrier properties of the blends. In this study, the ratio of PLA:PHBV at 75:25 was selected to be processed based on the results from their earlier study on the miscibility of this pair of polymers using Flory and Huggins theory, and the Gordon-Taylor equation. This ratio contributed a combination of better miscibility and cost performance [69]. Most of the compatibilization strategies tended to contribute softer materials with decreasing Young's modulus and tensile strength. The blends resulting from transesterification processes showed significant improvement in the stretchability with an increase of the elongation at break ranging from 177% to 1088% across the different strategies compared to neat PLA. Tensile properties of reactive compatibilized PHBV/PLA blends from published articles are shown in Figure 2-6.

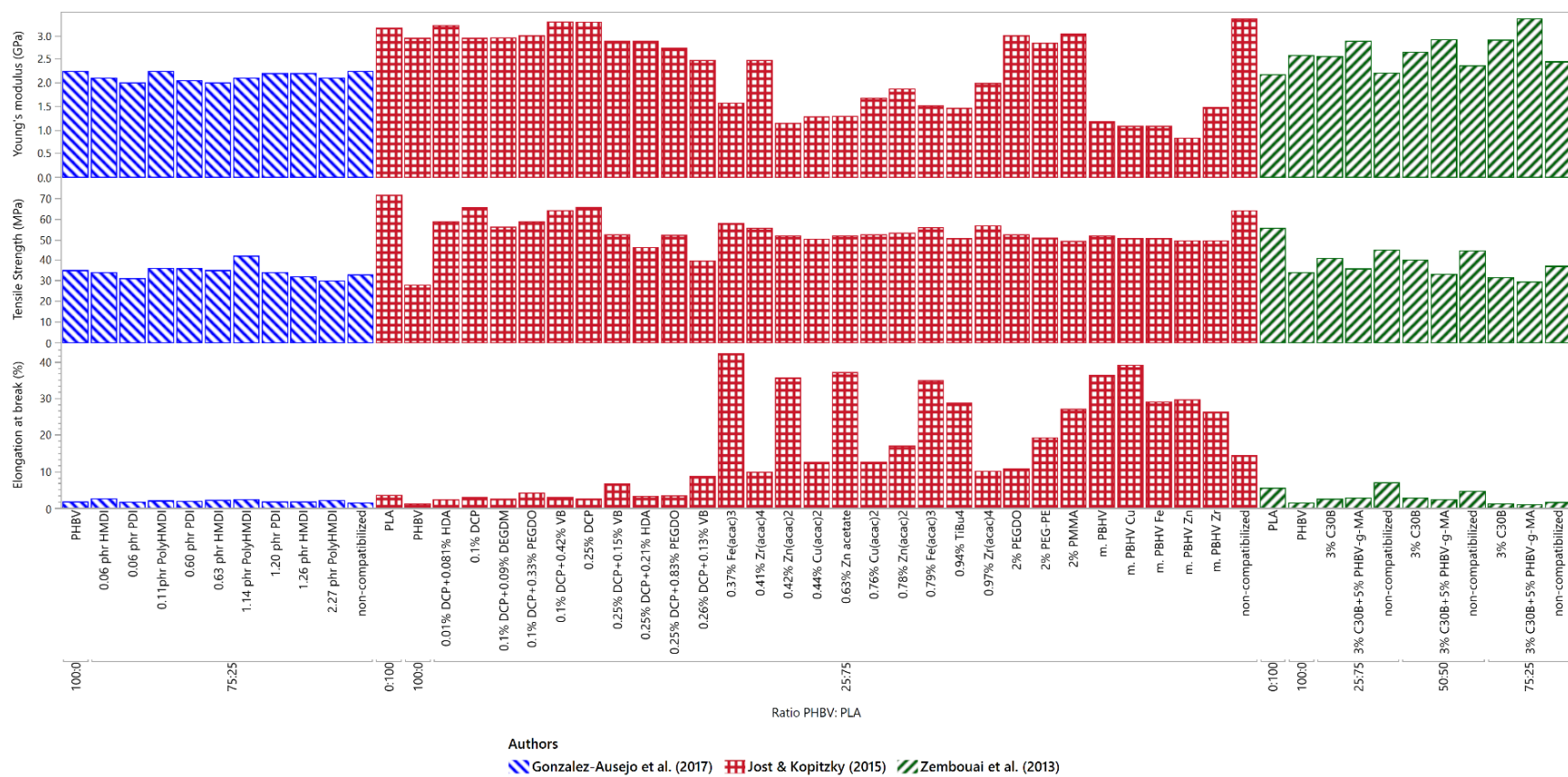


Figure 2-6 Mechanical Properties of PHBV/PLA reactive compatibilized blends

Regarding the barrier properties, PHBV, with its semi-crystalline nature and higher crystallinity, features better oxygen and water barrier than PLA. Blending of PHBV and PLA efficiently promoted the barrier properties of PLA even with a low content of PHBV. An inclusion of 25 wt.% of PHBV could decrease the oxygen and water permeability by 35% and 23%, respectively [75]. The oxygen and water vapor permeability coefficients of PLA decreased with increasing ratio of PHBV in the blended material. Zembouai et al. [75] and Gonzalez-Ausejo et al. [86] were in agreement that with a 75 wt.% ratio of PHBV, the oxygen permeability decreased by more than 80% compared to the value of the neat PLA. The enhanced barrier properties were explained as the result of the crystallinity change and the formation of phase interfaces in the two-phase morphology microstructure of the blends [75,86]. The blending in of PHBV causes the inclusion of impermeable crystallites that reduces the amount of amorphous phase in PLA that the gas molecules can permeate [75]. At the same time, the gas transport paths could get more tortuous with the presence of more crystals [75].

Addition of different isocyanates as a method to reactively compatibilize the blend of PHBV and PLA was reported by Gonzalez-Ausejo et al. [86] to have a relatively small impact on the barrier properties compared to the uncompatibilized blend. Jost and Kopitzky [69] observed the effect of different compatibilization methods on the barrier properties of PHBV/PLA blends by comparing the experimental results obtained from the produced materials to the values calculated based on the system of two-layer structure of PLA/PHBV. The values based on the two-layer structure were preferred as a reference rather than the ones based on the additivity rule due to the immiscibility of the blends. The equations below were used for the permeability calculation where Q is the permeability, P is the permeation coefficient, P_i and l_i are the permeation coefficient and the thickness of individual layers, respectively.

$$Q = \frac{P}{l}; \quad \text{Eq. 2-9}$$

$$\frac{1}{P_{multilayer}} = \frac{1}{l_{total}} \sum_{i=1}^{i=n} \frac{l_i}{P_i} \quad \text{Eq. 2-10}$$

The barrier properties including water vapor transmission rate, oxygen permeability, nitrogen permeability, and carbon dioxide permeability were found to be varied by groups of compatibilization methods, i.e., 1) addition of surface active compatibilizers, 2) addition of peroxides, 3) transesterification

of PLA and PHBV using catalysts, and 4) addition of peroxide-modified PHBV together with the transesterification catalysts. Among the methods used to compatibilize 25:75 PHBV/PLA blends, Jost and Kopitzky [69] found the transesterification methods gave the most promising barrier performance with WVTR, and gases permeability comparable to the uncompatibilized blend and less variation between different catalysts used. This also reflected that the transesterification did not significantly change the morphology [69]. The material obtained from most of peroxide addition methods had higher permeability than the uncompatibilized blend except when DCP + PEGDO was used. The values obtained from this group of methods were found to vary the most. The presence of insoluble gel-particles in the morphology was hypothesized as a cause of higher permeability since they tend to increase the diffusion pathway in the materials [69].

2.5 Response surface methodology

Response surface methodology (RSM) is an approach combining mathematical and statistical techniques to model a functional relationship between a response and multiple influencing variables. RSM is useful for developing, improving, and optimizing processes. It is well applied in the design, development, and formulation of new products, also the improvement of existing product and process design. In general, the relationship approximating *Response* can be written in the following low-degree polynomial form

$$y = f(x_1, x_2, \dots, x_k) + \varepsilon \quad \text{Eq. 2-11}$$

where y is the response value in a continuous scale, f is the unknown function of response, $x_1, x_2, \dots, x_k$ are the independent variables, k is the number of the independent variables, and ε is the error term. The models that are commonly used in RSM include the first-degree model:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \varepsilon \quad \text{Eq. 2-12}$$

and the second-order model:

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{1 \leq i \leq j}^k \beta_{ij} x_i x_j + \varepsilon \quad \text{Eq. 2-13}$$

where β_0 is the constant term, β_i depicts the coefficient of the linear parameter, and x_i represents the variables [87]. In the second order model, β_{ij} represents the coefficients of the interaction parameters [87]. The first-degree model is used when the responses do not present any curvature such as in the case where a very narrow range of the variable level is selected.

Conducting an optimization study using RSM requires three main stages including: 1) preliminary work to determine the independent parameters and their levels; 2) selection of experimental design, prediction and verification of the model equation; and 3) to obtain the response surface plot and contour plot of response as a function of the independent parameters and determination of optimum point [88].

Response is usually affected by many parameters at different magnitudes. In the first stage, due to the limitations of the experiment such as limited cost and availability of material, important parameters having major effects on the response should be selected. This selection may be done by a screening experiment such as factorial design. Meanwhile, the level of the selected parameter that affects the response within the range of interest should be defined based on the experimenter's knowledge and experience. The success of the optimization process directly relates to these selected levels [88].

In the second stage, the common experimental designs for fitting first-degree models called first-order designs include 2^k factorial design, Plackett–Burman Design, and the Simplex Design [87]. For fitting second-degree models, the second-order designs either the 3^k factorial design, Central Composite Design (CCD), or the Box–Behnken Design can be used. The main differences between these designs are the number of points of the experimental runs; the selection mostly depends on the range of interest and also the limitations regarding the experiment. After obtaining the experimental data, the prediction of the model equation is processed using the method of least squares (MLS) [88]. The model adequacy can be verified by several techniques such as residual analysis, scaling residuals, prediction error sum of squares (PRESS) residuals, and testing of lack of fit [88].

In the last stage, two plots visualizing the predicted model equation are obtained: 1) the *response surface plot* – a three-dimensional plot showing the relationship between the response and the independent variables; and 2) the *contour plot* – a two-dimensional display of the surface plot [88]. The shape of a response surface is demonstrated on the contour plot with lines of constant response drawn in the plane of the independent variables. The maximum or minimum response can be seen on the contour

plot as ellipses or circles which are called a stationary point. In the case that the contour plot displays a hyperbolic or parabolic system, the stationary point is called a saddle point. At the stationary point of a second order equation, the first derivative of the function equals to zero. The values of the independent variables corresponding to the optimum response (either maximum or minimum) can be solved for.

2.5.1 Mixture Design

A mixture experiment is a type of response surface experiment where the factors are the ingredients or components of a mixture, and the response varies as a function of the proportion of each component [89]. The analysis of mixture experiments is complicated due to the constraint that the components have to sum to one [90]. Suppose there is a mixture system of q components, let x_i represents the proportion of the i th component in the mixture, then

$$x_i \geq 0, \quad i = 1, 2, \dots, q \quad \text{Eq. 2-14}$$

and

$$\sum_{i=1}^q x_i = x_1 + x_2 + \dots + x_q = 1.0 \quad \text{Eq. 2-15}$$

The constraint of the equation Eq. 2-15 makes the values of x_i to be constrained proportions. By altering the proportion of one component in the system, at least one other component is changed proportionally. The experimental region or factor space is defined by the values of x_i in a regular $(q - 1)$ -dimension simplex. For $q = 2, 3$, and 4 , the factor spaces are a straight line, an equilateral triangle, and a tetrahedron, respectively. Figure 2-7 demonstrates the factor spaces for $q = 2$ and $q = 3$.

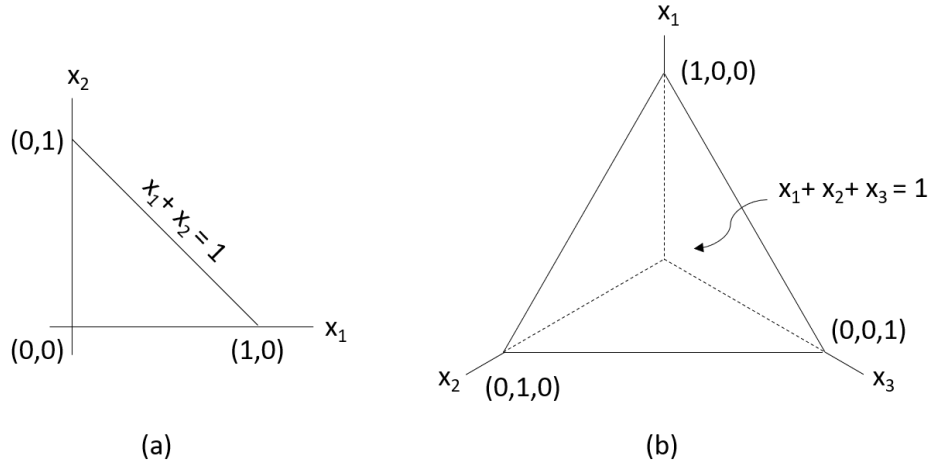


Figure 2-7 Simplex factor spaces for (a) $q = 2$ and (b) $q = 3$ (adapted from [91])

The aims of performing a mixture problem are to empirically predict the response at any combination of the mixture system, and to measure the influence of a single or a mixture of ingredients on the response [91]. In order to achieve these goals, the mixture surface needs to be modelled with some form of mathematical equation [91]. The common standard mixture designs for model fitting include *Simplex-Lattice* designs, and *Simplex-Centroid* designs [90].

In *Simplex-Lattice* designs, points in a $\{q, m\}$ design for q components are defined by the assumed proportions of each component takes the $m + 1$ equally spaced from 0 to 1, $x_i = 0, \frac{1}{m}, \frac{2}{m}, \dots, 1$ for $i = 1, 2, \dots, q$ and all possible combinations of the proportions are used. For example, in the mixture system of $q = 3$ components with $m = 2$ then

$$x_i = 0, \frac{1}{2}, 1 \text{ for } i = 1, 2, 3$$

All points of the possible combinations include

$$(x_1, x_2, x_3) = (1, 0, 0), (0, 1, 0), (0, 0, 1), \left(\frac{1}{2}, \frac{1}{2}, 0\right), \left(\frac{1}{2}, 0, \frac{1}{2}\right), \left(0, \frac{1}{2}, \frac{1}{2}\right)$$

The regression function to fit the data from the mixture experiment is different for the traditional polynomial fit due to the restriction that $x_1 + x_2 + \dots + x_q = 1.0$. The function is often referred to as the canonical polynomial. The form is derived using the general form of the regression function that fits to data collected at the points of a $\{q, m\}$ simplex-lattice design and substituting into this function the dependence relationship among the x_i terms [90].

The general canonical forms of the mixture models are as follows:

Linear:

$$E(y) = \sum_{i=1}^q \beta_i x_i \quad \text{Eq. 2-16}$$

Quadratic:

$$E(y) = \sum_{i=1}^q \beta_i x_i + \sum_{i<j=2}^q \sum_{j=2}^q \beta_{ij} x_i x_j \quad \text{Eq. 2-17}$$

Full Cubic:

$$E(y) = \sum_{i=1}^q \beta_i x_i + \sum_{i<j=2}^q \sum_{j=2}^q \beta_{ij} x_i x_j + \sum_{i<j=2}^q \sum_{j=2}^q \delta_{ij} x_i x_j (x_i - x_j) + \sum_{i<j=2}^q \sum_{j=2}^q \sum_{k=3}^q \beta_{ijk} x_i x_j x_k \quad \text{Eq. 2-18}$$

Special Cubic:

$$E(y) = \sum_{i=1}^q \beta_i x_i + \sum_{i<j=2}^q \sum_{j=2}^q \beta_{ij} x_i x_j + \sum_{i<j=2}^q \sum_{j=2}^q \sum_{k=3}^q \beta_{ijk} x_i x_j x_k \quad \text{Eq. 2-19}$$

In *simplex-centroid* designs, the experimental points are the same as in *simplex-lattice* designs, but the centroid points are included. A q -component simplex-centroid design has $2^q - 1$ design points.

The polynomial model used to fit the data is as follows:

$$E(y) = \sum_{i=1}^q \beta_i x_i + \sum_{i<j=2}^q \sum_{j=2}^q \beta_{ij} x_i x_j + \sum_{i<j=2}^q \sum_{j=2}^q \sum_{k=3}^q \beta_{ijk} x_i x_j x_k + \cdots + \beta_{12\dots q} x_1 x_2 \dots x_q \quad \text{Eq. 2-20}$$

For example, in a simplex-centroid design with $q=3$ components, the model can be written as:

$$E(y) = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 \quad \text{Eq. 2-21}$$

Since *simplex-lattice* designs, and *simplex-centroid* designs are boundary-point designs, in which all the design points are on the boundary of the simplex, in the case that the interior is the point of interest, the *augmented simplex-centroid design*, where the axial runs are included in with the centroid, is recommended. Cornell [92] defined the axial design as “the axis of component i is the imaginary line extending from the base point $x_i = 0$, $x_j = 1/(q - 1)$ for all $j \neq i$, to the vertex where $x_i = 1$, $x_j = 0$ for all $j \neq i$.”

Other design techniques including *constrained mixture design* and *pseudo-simplex design* are introduced to solve the problem of the standard designs that limits researchers from exploring the entire simplex region due to the constraint of upper or lower bounds of the components. Various types of mixture design analysis, and model building were well discussed by Cornell [92].

2.6 Biodegradation studies of PHBV and PLA

Biodegradation study is an important part of biodegradable polymer material development and modification to verify the biodegradability of the material. Biodegradation of polymers occurs through mainly three stages of biodeterioration, biofragmentation, and assimilation, together with the involvement of abiotic factors [93]. To what extent these phenomena can happen is strongly dependent on the intrinsic properties of a certain polymer material under the biodegradation conditions. These properties include: 1) first order structures (chemical composition, molecular weight and molecular weight distribution), 2) higher order structures (glass transition temperature, melting temperature, modulus of elasticity, degree of crystallinity, crystal size and structure), and 3) surface properties (hydrophobicity, roughness, specific surface) [94,95]. The sensitivity of the material toward the environmental factors, i.e., mechanical forces, light, heat, chemicals, and moisture, that cause the abiotic degradation, and the specificity of enzymes and microorganisms that cause the biodegradation of a certain material, are governed by the aforementioned properties. By observing and measuring products and evidence occurring at each stage of biodegradation, researchers could explain the mechanism that happens. Methods including visual observations, weight loss measurements, changes in molecular structure, mechanical properties and molecular weight, and CO₂ evolution/O₂ consumption are used to measure the erosion and degradation of the polymers during the biodegradation experiments. The information obtained from these measurements not only indicates how much the material degrades over time but also reveals the mechanism specific to a certain material.

Biodegradation of PHBV, PLA, and modified materials based on them were widely studied not only to validate their biodegradability but also to observe and explain the mechanism of degradation happening with the materials. Biodegradation of these materials was evaluated in various conditions including soil burial tests, controlled composting conditions, and simulated fresh-water and marine

environments. These experimental conditions imitated the end-of-life of the materials either in the landfill, composting system, or even in the natural environment. In the controlled condition, the observation of biodegradation could be based on either a specific microorganism culture, or mixed microbial strains naturally present in different sources including soil, sewage sludge, food waste, etc. While the use of a selected microbial population generally aims to verify an ability of the microbe of interest in degrading a certain material, the use of mixed cultures directly obtained from the source measures the biodegradability of the material in a specific environment and conditions.

The biodegradation studies of PHBV, PLA, and the modified materials based on these two polymers suggested different mechanisms of the biodegradation belonging to each of them. The biodegradation of PHBV mainly relies on the surface erosion caused by the microbial enzymatic hydrolysis rather than the abiotic hydrolysis which is a major phenomenon initiating the PLA biodegradation process. Change in molecular weight properties was an important indicator of this difference. The decrease in number average molecular weight (M_n) as a function of degradation time reflects the bulk change of a polymer as a result of the abiotic hydrolytic chain scissions. Due to the steric hindrance, the enzymes produced by the microorganisms were not supposed to diffuse into the bulk of the material; the enzymatic reaction that occurs mainly at the surface could not cause a noticeable decrease in M_n [95]. Salomez et al. [95] found the M_n and dispersity reduction were weakly pronounced in their PHBV composting experiment throughout the incubation time. The results based on the PLA biodegradation study of Castro-Aguirre et al. [96] clearly indicated the M_n reduction of PLA in the early stage of degradation. The contribution of abiotic hydrolysis in the initial stage of PLA degradation was also confirmed in their study as the M_n reduction occurred with no significant difference either in uninoculated vermiculite media, where no microorganisms were presented, or in the compost and inoculated vermiculite.

Mineralization curves of PHBV and PLA also revealed different kinetic degradation behavior of these polymers. While the mineralization curve of PLA clearly contained three phases of lag, biodegradation, and plateau phases [96], the lag phase was hardly seen on the curve of PHBV [95,97,98]. The curve of PHBV tends to look similar to the ones of natural polymers like cellulose and starch in which the biodegradation starts almost immediately and a very short lag phase is assumed to be

due to the adaptation of the microorganisms [96]. The extended lag phase of PLA generally is attributed to the abiotic hydrolysis causing the high molecular weight polymer chains to fragment until the molecular weight is low enough, about 2 – 3 kDa, that the oligomers and monomers become water soluble and can be assimilated by the microbes [96]. The length of the lag phase of PLA depends on factors such as its crystallinity, T_g , and initial molecular weight [96].

The change in crystallinity during the degradation provides information regarding the process of degradation in amorphous and crystalline regions. PHBV and PLA are both semi-crystalline polymers where the presence of the crystal structures could affect how fast the polymers are biodegraded. The crystallinity (X_c) obtained from DSC analysis of the PHBV samples pulled out from the controlled composting tests at different incubating times demonstrated slight changes of crystallinity with increasing degradation time [95,99]. This suggested an equivalent rate of degradation between crystalline and amorphous regions, even though the SEM observation of Salomez et al. [95] on the PHBV films showed a very rough surface revealing spherulite crystals after the primary surface erosion which could lead to the belief that amorphous regions could preferably be degraded before the crystalline ones. The slower degradation of the crystalline region seemed to be compensated by more area being exposed to the degrading enzymes that allowed faster crystalline region degradation to occur [95]. Oppositely, in the degradation of PLA in compost, the crystallinity increased with degradation time [100]. This was explained as a result of the hydrolysis that provide extra mobility due to the chain cleavage allowing lower molecular weight polymer chains to crystallize as well as the remaining crystalline phase to reorganize [95,100].

SEM photomicrographs of the polymer samples at different degradation times revealed important information regarding the erosion patterns as well as the possible microorganisms taking a role in the degradation. In the recent study of Salomez et al. [95] where the degradation mechanisms of PHBV and PBSA were comparatively observed, the SEM results indicated that while PHBV tended to become porous with an eroded shape, PBSA fractured in the form of sharp edges. The presence of filament footprint also appeared only on the surface of PHBV which suggested the colonization of fungi to be an important process in the PHBV biodegradation. The progression of erosion of PHBV under composting was described by Luo and Netravali [101] that from the smooth surface at the beginning, a few deep pits started to form far apart, probably as the result of a few microorganism colonies at the initial stage. As the

colonies multiplied, the number of pits increased and spread to other areas. After the colonies covered the entire specimen, the surface erosion became even, resulting in a smoother surface. However, Nishida and Tokiwa [102] observed a different progression pattern starting from small pits; then the size increased with the proceeding degradation. Different degradation mediums were hypothesized to be factors in the pattern variation as Luo and Netravali [101] used composting medium, while Nishida and Tokiwa [102] used an isolated bacterium, strain SC-17, in their study.

FTIR spectroscopy was used in some PHBV and PLA biodegradation studies to observe the chemical changes occurring during the degradation. Similar characteristic peaks appeared on the FTIR spectra obtained from PHBV samples at different degradation times. This emphasized the idea that the degradation of the polymer was mainly caused by the erosion by microorganisms starting from the surface then spreading gradually to the interior without any change of the chemical structures [97,98]. Luo and Netravali [101], suggested that no detectable changes of the spectra at different times was the result of the leaching out of the biodegradation byproducts (i.e., oligomers formed by the chain scission) into the compost medium or these products could be washed out during their sample cleaning using deionized water. The measurement of the relative surface crystallinity (SCI) was presented in the study of Luo and Netravali [101] to explain the differential degradation of PHBV in crystalline and amorphous regions. The FTIR-ATR from the study was used to calculate SCI by normalizing the intensity of the band at 1182 cm^{-1} , which decreases with the degree of crystallinity, to that of the band at 1378 cm^{-1} , which is the most insensitive to the degree of crystallinity. It was found that throughout the composting time (0 – 40 days), the SCI remained unchanged, and the researchers concluded that the rate of degradation in the crystalline and amorphous phase was not different [101]. However, due to some conflicts with the result of Koyama and Doi [103] that had proposed the predominant hydrolysis of PHB depolymerase of the PHB chains in the amorphous phase, Luo and Netravali proposed that differential degradation could occur within the period of 10 days before they pulled the sample for the first characterization [101].

Besides the polymer's characteristic peaks, the FTIR spectra of PLA samples under composting gave evidence regarding the formation of biofilm on the surface of the samples. Sedničková et al. [104] who studied the degradation of PLA, PLA plasticized with triacetin, and the blend of PLA/PHB plasticized with triacetin, reported the presence of a broad band in the region of valence bands related to hydroxyls,

–OH, between 3100 and 3500 cm^{-1} with the maximum at 3340 cm^{-1} in PLA samples after 16 days of composting. The authors referred to the work of Abrusci et al. where the band had been assigned to a layer of protein formed on the polymer surface. In the same study [104], a possible amide / bond –NH–CO could be observed at 1650 cm^{-1} with the decay of bonds –CH– and –C=O in the spectra of the PLA plasticized with triacetin samples after 8 days of composting at the same time the broad band between 3100 and 3500 cm^{-1} appeared. The amide / peak and the broad band became increasingly intense with the composting time. Since the amide / bond does not belong to the degradation products, it was proposed to support the presence of the protein biofilm on the surface of the sample during the biodegradation process [104]. The broad band appeared in PLA/PHB blend samples sooner than in PLA samples, after 2 days of composting [104].

Biodegradation of the blends containing PLA and the polymers in the polyhydroxyalkanoate family as the result of physical blending or plasticized blending were studied and reported. In a study on the disintegration of poly(lactic acid)-poly(hydroxybutyrate) (PLA-PHB) blends (75:25 wt.% ratio) using different plasticizers including poly(ethylene glycol), acetyl-tri-n-butyl citrate, and D-limonene, it was reported that PHB acted as a nucleating agent for the blends and slowed down the disintegration of PLA [105].

The biodegradation of the blends between poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB,4HB)] and PLA under real soil environment was studied by Weng et al. [106]. Their results including the surface morphology recorded by a digital camera, SEM microscopy, FTIR spectra, and the element analysis, indicated a decreasing rate of degradation with increasing ratio of PLA in the blends. The soil burial depth, i.e., 20 and 40 cm, affected the degradation rate of these polymer blends. While P(3HB,4HB) samples and 75% P(3HB,4HB) blend samples demonstrated a slower degradation rate at 40 cm of soil compared to that at 20 cm, the blends containing 50% and 75% PLA as well as the neat PLA showed faster degradation in the deeper soil. The anaerobic condition at 40 cm depth of soil was indicated to be more favorable for the biodegradation of PLA. Different mechanisms of degradation of PLA in aerobic and anaerobic conditions were indicated by their FTIR results. The shift of the absorption peak of C=O stretching vibration from 1749 cm^{-1} to 1758 cm^{-1} indicated that lower molecular weight polymer with carboxyl groups was produced in the aerobic condition (20 cm soil). However, no shifting of

this peak was observed in the specimens from 40 cm soil, so the authors concluded that in the anaerobic condition, high molecular weight PLA was rather degraded into lower molecular weight ones, which made the C=O stretching vibration unchanged.

Sikorska et al. [107] studied the degradation of the blend of PLA and 15% poly[(R,S)-3-hydroxybutyrate] (a-PHB) in the industrial composting and compost extract (sterilized and non-sterilized) to investigate the mechanism and observe the effect of biotic and abiotic factors on the degradation of the material. Abiotic hydrolysis was indicated to be a major process occurring during the first weeks of incubation since the decreasing of molecular weight could be observed during this time without any visual sign of surface erosion [107]. Due to the fact that PLA was the matrix of the blend, in other words having a much higher ratio in the blend compared to a-PHB, this result could be expected. The hydrolytic degradation of the polymer in the compost pile was noticeably slower than that in the compost extract, indicating the significant role of water in the process [107]. The lack of change in the pH as well as the non-detection of the degradation products based on the mass spectroscopy results of the non-sterilized compost extract during the incubation suggested the role of the microorganisms on either direct microbial depolymerization or assimilation of the water-soluble product from abiotic hydrolysis of PLA [107].

Selective biodegradation of PLA and PHB components in the blends of PLA/poly(butylene adipate-co-terephthalate) (PBAT) and PHB/PBAT was reported by Tabasi and Ajji [108]. The SEM micrographs of these blend samples showed a porous three-dimensional network after 15 days of composting which later analysis using ATR-FTIR showed were rich in PBAT content [108]. This result together with the rate of CO₂ evolution during the composting supported the idea that the inclusion of PBAT retarded the biodegradation process of the blends compared to the pure polymers [108].

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3 PRODUCTION AND CHARACTERIZATION OF PLA-*g*-MA

3.1 Introduction

In polymer blending, in addition to the intrinsic properties of the blend components that dictate the performance of the blended material, the miscibility or compatibility of the blend also has an important role. In general, most polymers form immiscible polymer blends, including biobased polymers like poly(lactic acid) (PLA), that are often blended with other materials in order to improve some of its drawbacks such as low heat deflection temperature and fragility. In PLA based blending, compatibilization essentially increases surface adhesion between the blend components that result in desirable properties of the PLA blends.

Addition of reactive polymers is one of the widely used methods for polymer blend compatibilizing. A reactive group that is functionalized on a polymer that is miscible in one polymer blend component can increase the tendency of interaction with another blend component. Anhydride, as one of the versatile reactive groups that are chemically suitable with many functional groups of polymers such as hydroxyl (-OH), carboxyl (-COOH), and amine (-NH₂) [1], has been utilized in several studies to be grafted on PLA and used as a compatibilizer for blending. In this study, we aimed to produce maleic anhydride (MA) grafted PLA (PLA-*g*-MA) to be used in the blending between PLA and poly(hydroxybutyrate)-co-(hydroxyvalerate) or PHBV. It has been reported that the quality of PLA-*g*-MA, including the amount of grafted MA and the molecular weight of PLA-*g*-MA, affected the morphology and the mechanical properties of its composite [2]. These characteristics of PLA-*g*-MA are influenced by the content of initiator and MA used in the functionalization [2]. Therefore, we planned this study to investigate the use of dicumyl peroxide as a free radical initiator in the functionalization of PLA-*g*-MA and the effect of its content as well as the content of MA on the grafting yield and the molecular weight properties of the produced PLA-*g*-MA. DCP was chosen due to the report that it contributed to fine dispersion of the thermoplastic cassava starch (TPCS) phase in a PLA/TPCS composite [3].

Response surface methodology (RSM) was adopted in this study to design the experiment and evaluate the effect of the factor variables, i.e., the content of MA and DCP, on the grafting yield and molecular weight properties that were evaluated as responses.

3.2 Experimental

3.2.1 Response surface experimental design

Response surface experimental design was performed using JMP 14.1.0 software (SAS Institute Inc., Cary, NC). The central composite design (CCD) was selected with the following boundaries of the factors: 1) x_1 = MA, 2 – 7 wt % based on PLA weight, and 2) x_2 = DCP, 0.1 – 0.9 wt % based on PLA weight. Ten runs of experiments were generated with the pattern and actual level of the variables listed in Table 3-1.

Table 3-1 Pattern and actual level of MA and DCP in PLA-g-MA reactive functionalization

Run no.	Pattern	MA (wt %)	DCP (wt %)	Run ID
1	+-	7	0.1	R1-M7D1
2	--	2	0.1	R2-M2D1
3	0a	4.5	0.1	R3-M45D1
4	A0	7	0.5	R4-M7D5
5	00	4.5	0.5	R5-M45D5
6	++	7	0.9	R6-M7D9
7	a0	2	0.5	R7-M2D5
8	0A	4.5	0.9	R8-M45D9
9	-+	2	0.9	R9-M2D9
10	00	4.5	0.5	R10-M45D5

Note: - Low value, + High value,
0 Midrange (center value), a Low axial value,
A High axial value

3.2.2 Materials

PLA (Ingeo™ Biopolymer 2003D) was obtained from NatureWorks LLC. (Minnetonka, MN). Maleic anhydride (MA), and dicumyl peroxide (DCP) were purchased from MilliporeSigma (St. Louis, MO, USA) and used as received.

3.2.3 Reactive extrusion of PLA-g-MA

Ten runs of PLA-g-MA reactive extrusion were prepared using a twin-screw co-rotating extruder. Before processing, PLA was dried at 80 °C for 24 h to remove excess moisture. PLA then was manually tumble-mixed with DCP and MA. The mixture was fed into a Century ZSK-30 twin-screw co-rotating extruder (Traverse City, MI) using a mechanical feeder set at a rate of 70 g min⁻¹. Ten temperature zones of the barrel were set using the following temperature profile: 140/150/160/160/160/170/170/170/170/160

°C. The screw speed was set at 120 rpm. Unreacted MA was removed during the extrusion process by the vacuum suction on the vent port of the extruder. The extrudate was cooled in a water bath, then pelletized in a BT 25 pelletizer (Scheer Bay Co., Bay City, MI). The pellets were dried at 60 °C for 24 h to evaporate moisture from the cooling process and then stored in a freezer (~ -18 °C) before further processing. One additional run of the control sample was produced with the same process setting using only neat PLA without addition of DCP and MA (Run ID: nPLA).

3.2.4 PLA-g-MA characterization

3.2.4.1 ATR-FTIR spectroscopy

Chemical functional groups of the PLA-g-MA were measured using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR). PLA-g-MA pellets were fabricated into a thin sheet using an M CARVER compression molder (Carver Laboratory Press, Menomonee Falls, WI) before characterization using ATR-FTIR. Approximately five grams of dried resin was placed between poly(tetrafluoroethylene) (PTFE) sheets, preheated at 160 °C for 10 min, pressed at 680 kg for 5 min, then removed from the compression molder. The sheet was wrapped with aluminum foil and cooled down in a freezer at about -15 °C for 15 min. The samples were kept in a desiccator at 25 °C for at least 72 h; then the FTIR-ATR spectrum was measured using an IR-Prestige 21 (Shimadzu, Columbia, MD) over the wavenumber range of 4000-400 cm⁻¹ at a resolution of 4 cm⁻¹ for 40 consecutive scans.

3.2.4.2 MA-grafting yield

MA-grafting yield of PLA-g-MA was determined by a titration method described by Detyothin [4]. First, PLA-g-MA samples were dried at 130 °C for 24 h in vacuum to evaporate unreacted MA. Then 1.51 ± 0.01 g of the sample was dissolved in 150 mL of chloroform and 1.5 mL of 1M hydrochloric acid was added. The solution was vigorously stirred for 45 min followed by the precipitation of the grafted polymer using 700 mL of methanol. The precipitant was then dried at 80 °C for 15 h. To determine the amount of MA grafted, the solution of chloroform and methanol (3/2 v/v) was used to dissolve dry precipitant (1.20 ± 0.05 mg). 8 to 10 drops of phenolphthalein in ethanol (1% w/v) were added. 0.03 N standardized potassium hydroxide (KOH) in methanol was used to titrate the solution. Color and pH were observed to determine the end point. Neat PLA was used as a control. The following equations were used to calculate the grafting yield.

$$\text{Acid number (mgKOH} \cdot \text{g}^{-1}) = \frac{V_{\text{KOH}} \times N_{\text{KOH}} \times 56.1}{\text{Weight of the sample in g}} \quad \text{Eq. 3-1}$$

$$\text{Grafted MA (\%)} = \frac{\text{Acid number} \times 98}{2 \times 561} \quad \text{Eq. 3-2}$$

where V_{KOH} and N_{KOH} were volume (mL) and normality of the standardized KOH, respectively.

3.2.4.3 Molecular weight

The molecular weight properties of neat PLA and PLA-g-MA were determined using Waters gel permeation chromatography (GPC). The samples were prepared by dissolving 20 ± 5 mg of neat PLA or PLA-g-MA resins in 10 mL of HPLC grade tetrahydrofuran (THF) for 24 h at ambient temperature. The solution was then filtered using a 0.45 μm PTFE filter. 100 μL of the filtrate was injected to a gel permeation chromatograph (GPC) unit from Waters (Milford MA, USA) equipped with an isocratic pump (Waters 1515), and an autosampler (Waters 717); a refractive index detector (Waters 2414) was used with THF running with a flow rate of 1 mL/min. A series of HR Styragel® HR4, HR3, HR2 (300 mm x 7.8 mm (I.D)) columns with a controlled temperature of 35 °C were used. The molecular weight distribution (MWD) curve for each sample was determined by analyzing the raw data obtained using Breeze™ 2 Software (Waters, Milford MA, USA) with the Mark-Houwink constants (K and α values) of PLA in THF at 35 °C obtained from the values of the PS standard of 0.000164 mL g⁻¹ and 0.704, respectively.

3.2.4.4 Data analysis

The *MA-grafting yield* and *molecular weight* response data from ten runs were analyzed using JMP software to get the optimum concentration of MA and DCP that resulted in high grafting yield while maintaining the molecular weight at a high level. The software processes the data by fitting the responses to a second order response surface model using the least squared error method and analysis of variance (ANOVA) is performed to determine the significance of the parameters. The software solves for the optimization using the desirability function as described in [5] and explained in detail in Section 3.3.5. The regression model is as follows:

$$E(y) = b_0 + \sum_i^q b_i x_i + \sum_{1 \leq i \leq j}^q b_{ij} x_i x_j \quad \text{Eq. 3-3}$$

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad \text{Eq. 3-4}$$

Where $E(y) =$	Estimate response of interest
$x_1 =$	MA content (wt% on PLA basis)
$x_2 =$	DCP content (wt% on PLA basis)
$b_0 =$	Intercept
$b_1, b_2 =$	Linear main effects
$b_{11}, b_{22} =$	Quadratic main effects
$b_{12} =$	Interaction effects

JMP software was used to analyze and visualize the fitted models.

The significant differences ($P < 0.05$) between samples were also analyzed and determined using one-way analysis of variance and Tukey's honestly significant difference (HSD) tests using JMP software.

3.3 Results and discussion

3.3.1 FTIR Spectrum

PLA-g-MA samples were measured by ATR-FTIR spectroscopy compared to the neat PLA sample (nPLA) to verify the presence of MA grafted on PLA. The FTIR spectrums of all PLA-g-MA samples including the one of nPLA are presented in Figure 3-1. The peak that was clearly seen appearing on the spectrum of all PLA-g-MA samples was a strong peak at 696 cm^{-1} corresponding the C=C bending of disubstituted (cis) alkene in DCP that was used as a peroxide initiator in the reactive functionalization process [6,7]. The characteristic bands representing three possible structures of MA grafted on PLA were found in PLA-g-MA samples. First, the bands at the wavenumbers of $1840 - 1842 \text{ cm}^{-1}$ and $1760 - 1780 \text{ cm}^{-1}$ belonged to the ranges that typically ascribe the symmetric and the asymmetric C=O stretching of conjugated 5-membered ring carboxylic anhydrides [8]. These bands corresponded to the presence of the cyclic anhydride by oligomeric end-grafting [4]. Second, the weak band at 1827 cm^{-1} is in the typical range of symmetric C=O stretching of open chain saturated aliphatic anhydrides; in our case this could indicate the presence of maleic acid end-grafting [4,8]. Third, the weak band at $1860 - 1870 \text{ cm}^{-1}$ and the band at $1788 - 1790 \text{ cm}^{-1}$ of symmetric and asymmetric C=O

stretching, which are generally attributed to conjugated 5-membered anhydrides, were found. These bands represented the chemical structure of saturated cyclic anhydride rings which were the result of succinic anhydride end grafting [4,8]. These absorption bands could be seen most clearly on the spectrums of sample R1-M7D1, R2-M2D1, and R3-M45D1. This probably indicates the most grafting of MA on the PLA backbone among all PLA-*g*-MA samples. However, the bands in the wavenumber range below 1780 cm^{-1} were quite hard to recognize since the added amount of MA was small, the intensity of the bands was weak, and they could overlap with the stretching of C=O of the PLA.

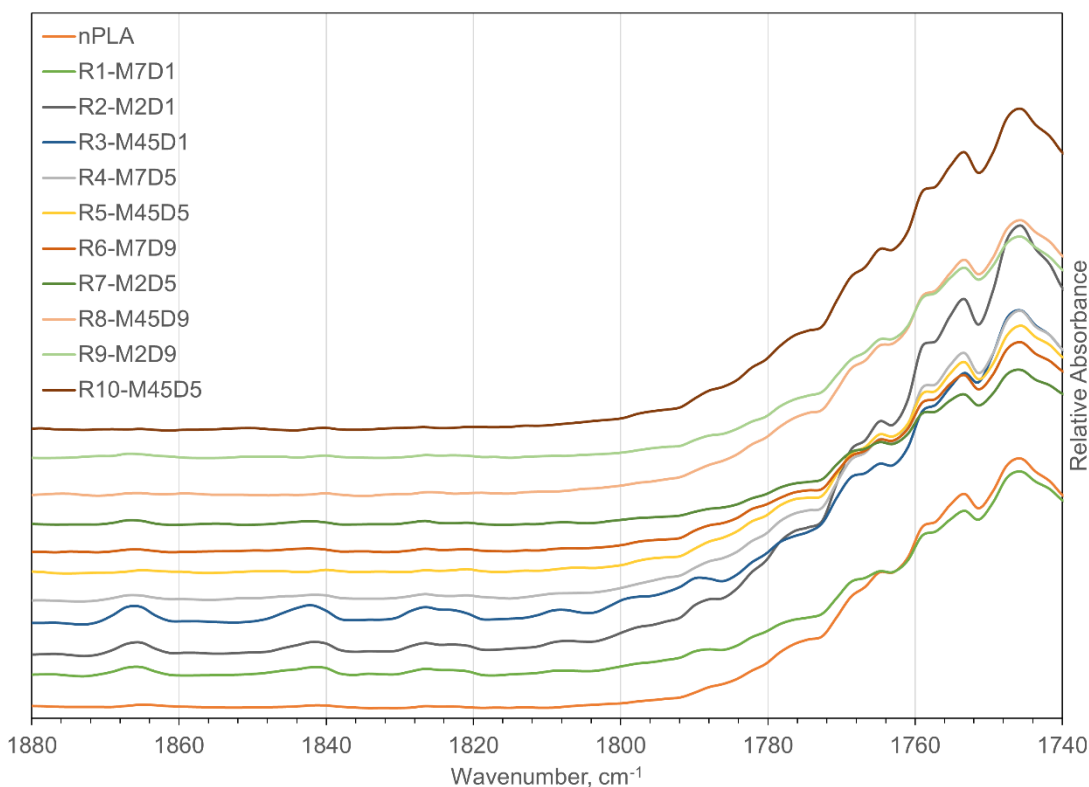


Figure 3-1 FTIR spectrums of PLA-*g*-MA samples in the range of wavenumber that the characteristic bands of grafted MA presented.

3.3.2 MA-grafting yield and molecular weight properties

The average MA-grafting yield, M_n , M_w , dispersity, and IV of each sample run are listed in Table 3-1. In overview, the content of DCP tended to have an inverse relationship with the molecular weight. At the same level of MA content, with increasing amount of DCP, M_n , M_w , and IV of the PLA-*g*-MA samples

decreased. The *dispersity* increased indicating broader distribution of molecular sizes. The content of MA and DCP did not show any certain pattern of impact on the MA-grafting yield.

Table 3-2 MA-grafting yield and molecular weight properties of PLA-g-MA samples

Run no.	Pattern	Content, wt. % (PLA basis)		MA-grafting yield, wt. % Mean $\pm$ SD	M_n , kDa Mean $\pm$ SD	M_w , kDa Mean $\pm$ SD	Dispersity ($\bar{D}$) Mean $\pm$ SD	IV Mean $\pm$ SD
		MA	DCP					
11	PLA	0	0	n/a	87.1 $\pm$ 13.5 ab	175.9 $\pm$ 25.5 bc	2.0 $\pm$ 0.0 de	0.8 $\pm$ 0.1 ab
2	--	2	0.1	0.053 $\pm$ 0.005 abc	92.3 $\pm$ 2.3 a	194.1 $\pm$ 10.6 ab	2.1 $\pm$ 0.1 bcde	0.8 $\pm$ 0.0 ab
7	a0	2	0.5	0.052 $\pm$ 0.006 abc	61.1 $\pm$ 6.1 cd	137.1 $\pm$ 4.4 de	2.2 $\pm$ 0.2 abcd	0.7 $\pm$ 0.0 cde
9	++	2	0.9	0.047 $\pm$ 0.003 abc	45.0 $\pm$ 5.4 e	109.1 $\pm$ 9.4 e	2.4 $\pm$ 0.1 a	0.6 $\pm$ 0.0 e
3	0a	4.5	0.1	0.058 $\pm$ 0.005 a	100.4 $\pm$ 2.2 a	205.0 $\pm$ 4.5 abc	2.0 $\pm$ 0.0 cde	0.8 $\pm$ 0.1 a
5	00	4.5	0.5	0.055 $\pm$ 0.006 ab	74.6 $\pm$ 2.9 bc	160.1 $\pm$ 3.0 cd	2.2 $\pm$ 0.1 bcde	0.7 $\pm$ 0.0 bc
10	00	4.5	0.5	0.040 $\pm$ 0.003 c	51.7 $\pm$ 3.1 de	127.6 $\pm$ 8.2 e	2.5 $\pm$ 0.1 a	0.6 $\pm$ 0.0 de
8	0A	4.5	0.9	0.044 $\pm$ 0.005 bc	49.5 $\pm$ 3.2 de	114.3 $\pm$ 4.9 e	2.3 $\pm$ 0.1 abc	0.6 $\pm$ 0.0 de
1	+-	7	0.1	0.047 $\pm$ 0.003 abc	94.4 $\pm$ 1.8 a	183.0 $\pm$ 2.6 a	1.9 $\pm$ 0.0 e	0.8 $\pm$ 0.0 ab
4	A0	7	0.5	0.055 $\pm$ 0.007 ab	64.6 $\pm$ 5.2 cd	137.6 $\pm$ 6.3 de	2.1 $\pm$ 0.2 bcde	0.7 $\pm$ 0.0 cd
6	++	7	0.9	0.044 $\pm$ 0.005 bc	54.8 $\pm$ 2.3 de	128.3 $\pm$ 6.1 e	2.3 $\pm$ 0.0 ab	0.6 $\pm$ 0.0 cde

3.3.3 RSM model of MA-grafting yield

RSM regression results of the model Eq. 3-4 of MA-grafting yield are presented in column a) of Table 3-3. The regression indicates that the model was not appropriate to predict the *MA-grafting yield* response with the R^2 very low at 0.22, and the F-test of ANOVA suggested the regression was not statistically significant ($\alpha=0.1$). Reconsidering the data, it could be seen from Table 3-2 that the *MA-grafting yield* of Run no.10 is significantly lower than that of Run no.5, even though they are both the center points of the central composite design we used for the experiment and were made with the same contents of MA (4.5 wt. %) and DCP (0.5 wt. %). According to Rheem and Oh [9], the existence of an outlier at the center point could adversely influence the statistical modeling and must be either corrected or eliminated to improve the quality of response surface analysis. The regression results with elimination of the data of Run no.10 are in column b) of Table 3-3. The p-value of ANOVA that decreases from 0.2242 in column a) to 0.0164 in column b) suggested statistical significance of the model at $\alpha = 0.05$. DCP shows a significant effect on the *MA-grafting yield* response with a negative relationship. Increasing of DCP tended to result in decreasing grafting yield.

Table 3-3 Parameter estimates and significant regression models for MA-grafting yield using original data and with the data of Run no.10 eliminated.

Term	a) MA-grafting yield (Using original data)	b) MA-grafting yield (Run no.10 eliminated)
Parameter Estimates		
Intercept	0.05729***	0.06277***
MA	-0.000447	-0.000447
DCP	-0.009915**	-0.009915***
(MA-4.5)*(MA-4.5)	7.208e-5	-0.000454
(MA-4.5)*(DCP-0.5)	0.0008525	0.0008525
(DCP-0.5)*(DCP-0.5)	-0.01129	-0.03185
Summary of fit		
R ²	0.24	0.46
ANOVA, Prob>F	0.2242	0.0164**
Lack of fit, Prob>F	0.0778	0.1176

Note: ***, and ** indicate statistical significance at type I error (α) of 0.01, and 0.05, respectively.

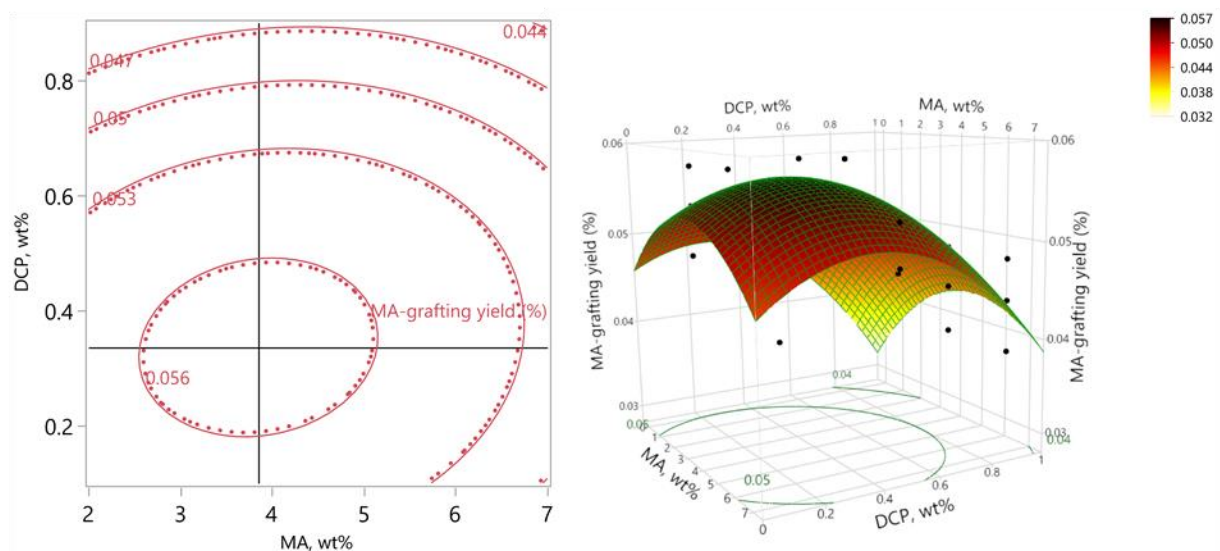


Figure 3-2 Contour plot and surface plot of MA-grafting yield based on the effect of MA and DCP contents. The actual data points are included on the surface plot.

3.3.4 RSM model of molecular weight

Table 3-4 Parameter estimates and significant regression models for the molecular weight properties of PLA-g-MA

Term	M_n	M_w	Dispersity	IV
Parameter Estimates				
Intercept	88157.28***	189087.81***	2.170***	0.7654***
MA	1026.62	573.76	-0.02498	0.004827
DCP	-57496.39***	-95962.08***	0.4205***	-0.2257***
(MA-4.5)*(MA-4.5)	-324.47	-996.88	-0.005241	-0.001791
(MA-4.5)*(DCP-0.5)	1929.92	7560.1667**	0.01753	0.0159515
(DCP-0.5)*(DCP-0.5)	62862.95***	100563.54***	-0.3165	0.2632***
Summary of fit				
R ²	0.90	0.91	0.60	0.87
ANOVA, Prob>F	<.0001***	<.0001***	0.0003***	<.0001***
Lack of fit, Prob>F	0.6835	0.3036	0.4953	0.292

Note: ***, and ** indicate statistical significance at type I error (α) of 0.01, and 0.05, respectively.

The analyses of RSM regression of M_n , M_w , *dispersity*, and *IV* (Table 3-4) suggest the model is significant with non-significant lack of fit for all responses. While the R² of 0.90, 0.91, and 0.87 indicate good fit of the experimental data to the model prediction for M_n , M_w , and *IV*, respectively; the *dispersity* shows a lower R² of 0.60.

DCP had a highly significant effect on all responses ($P < 0.01$); its quadratic effect was also significant in M_n , M_w , and *IV*. With increasing DCP, M_n , M_w , and *IV* all decreased. That caused *dispersity* to increase as the molecular size distribution of the PLA-g-MA became broader due to the increasing amount of low molecular weight chains. This could be seen clearly from the contour and surface plots of M_n (Figure 3-3a), as MA was insignificant in the model. While the M_n remained stable regardless of changing MA content, it showed a drastic decrease with increased DCP content. A similar impact of DCP on M_w and *IV* could be anticipated as M_n , M_w , and *IV* properties are related. The contour and surface plots of *dispersity* (Figure 3-3b) show its increase due to the influence of DCP content. However, the increase of *dispersity* was retarded at a higher amount of MA; this could be because of an interaction between MA and DCP ($P < 0.05$) on M_w .

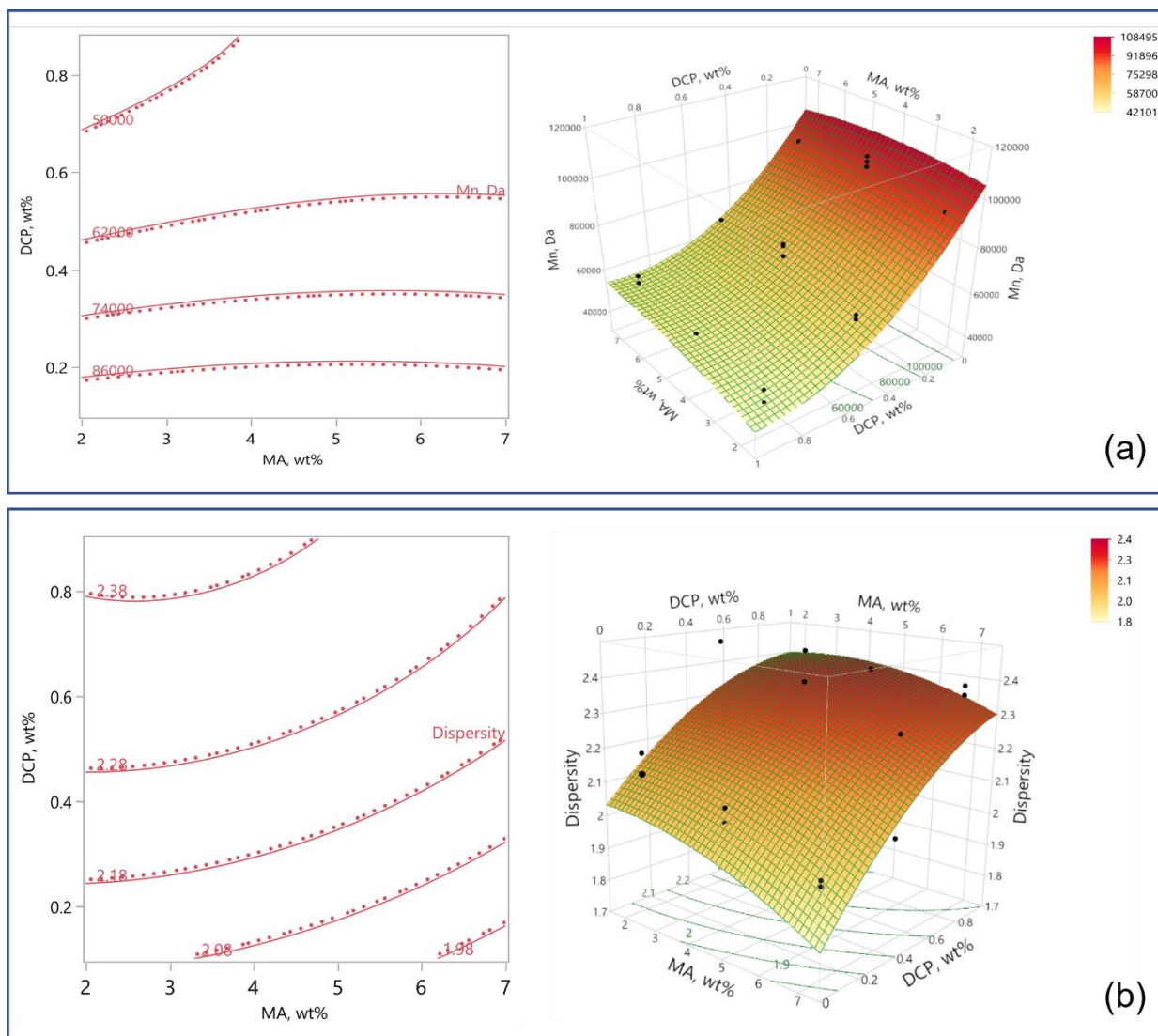


Figure 3-3 Contour plot and surface plot of (a) Mn and (b) dispersity of PLA-g-MA based on the effect of MA and DCP contents. The actual data points are included on the surface plot.

3.3.5 Optimization of grafting yield and molecular weight using desirability functions

Multiple responses can be simultaneously optimized using an overall desirability function which is the geometric mean of the desirability functions for the individual responses. In JMP software, the individual desirability function for maximizing or minimizing a response is a three-part smooth piecewise smooth function that consists of interpolating cubics between the user-defining low, middle, and high points, and exponentials in the tails. The overall desirability, D is expressed by the following equation:

$$D = d_1^{1/k} \times d_2^{1/k} \times \dots \times d_k^{1/k} \quad \text{Eq. 3-5}$$

where $d_1, d_2, \dots, d_k$ are the individual desirability functions of k responses.

When a certain response is prioritized over other responses, *Importance* values can be assigned for each response by the user. The *Importance* values are scaled by the software to sum to 1 and integrated into the overall desirability function. This overall desirability is defined as a weighted geometric mean of the individual desirability functions:

$$D = d_1^{w_1} \times d_2^{w_2} \times \dots \times d_k^{w_k} \quad \text{Eq. 3-6}$$

where $w_1, w_2, \dots, w_k$ are the scaled *importance* values of k responses.

In this study, *MA-grafting yield* and *molecular weight* responses were optimized using desirability profiling in the JMP software. The parameter settings for the desirability profiling analysis are listed in Table 3-5 and we analyzed the overall desirability in two scenarios with different *importance* values of the *MA-grafting yield* and *Mn* responses. The results of scenario 1 and 2 analysis with the DCP and MA contents that maximize the desirability are shown in Figure 3-4; the crossed red dotted line shows the point of maximum desirability. The overall desirability for each scenario is listed at the bottom left corner of the chart in Figure 3-4a, and Figure 3-4b for scenario 1 and 2, respectively. In scenario 1, when the *Importance* values of the two responses were set to be equal, the desirability was maximum with the content of DCP = 0.1 wt. % and MA = 3.94 wt. % (PLA basis); the resulting *Mn* was maximized while *MA-grafting yield* is lower than its possible maximum. This might indicate that the desirability was more relevant to *Mn* than *MA-grafting yield*. We proposed scenario 2 to simulate the situation in which the *MA-grafting yield* is prioritized; the maximum desirability moved toward the maximum *MA-grafting yield* with

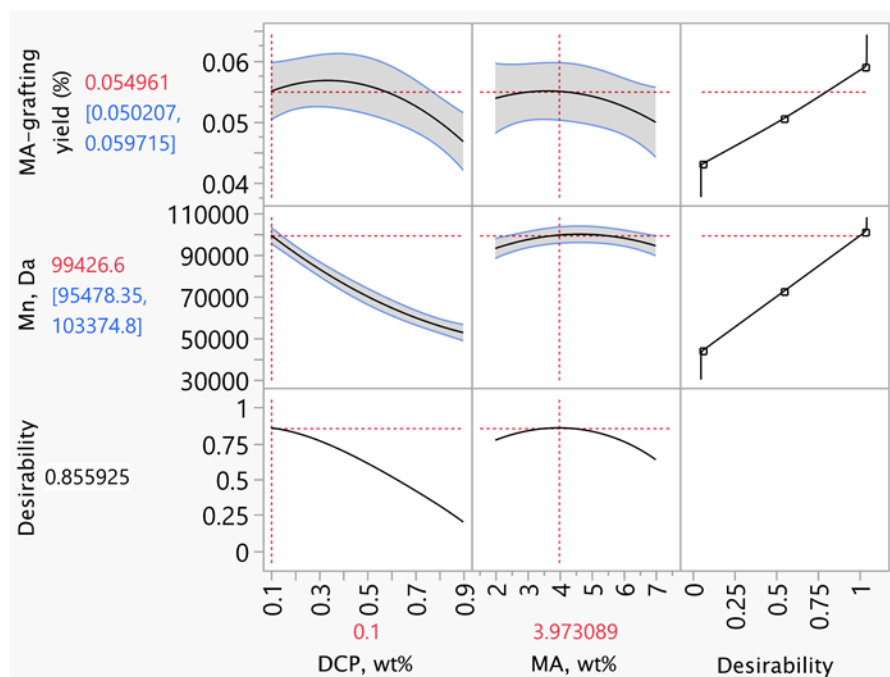
increasing content of DCP and a slight decrease of MA; *Mn* decreased from 99 kDa in scenario 1 to 90 kDa. These results suggested a competing criterion when we try to optimize the two responses together so that in order to maximize one response, we need to sacrifice another. Therefore, it is important to determine the priority among the responses in order to assign an appropriate importance value for each response. In general, knowledge and experience of the researcher are a key in this justification, where in our experiment, the potential application of the resultant material is an important factor to consider. In our case, the *MA-grafting yield* and molecular weight properties of PLA-*g*-MA, which will be used as a compatibilizer in a polymer blend, could affect performance of the final product differently; for example, a slight increase of *MA-grafting yield* might significantly improve the tensile strength of a polymer blend while the decreased *Mn* does not cause an equivalent effect. In this case, we might assign an *importance* value of *MA-grafting yield* to be 1 and the value of *Mn* to be 0 in order to maximize the *MA-grafting yield* and the *Mn* decreased to ~ 80 kDa with DCP = 0.34 wt. % and MA = 3.85 wt. % PLA basis (the result is not shown here).

Table 3-5 Parameter settings for desirability profiling analysis of MA-grafting yield and Mn responses

	<i>MA-grafting yield</i> , %	<i>Mn</i> , Da	Desirability
<u>Control points</u>			
High	0.0590	101000	0.99
Middle	0.0505	72500	0.50
Low	0.0430	44000	0.01
<u>Importance values</u>			
Scenario 1	1	1	n/a
Scenario 2	0.75	0.25	n/a

Optimizing of multiple responses can also be done by examining overlaid contour plots of responses of interest. Figure 3-5 demonstrates overlaid contour plots of *MA-grafting yield* (red lines) and *Mn* (blue lines) responses. Dots next to the line show the direction with higher value of response and the light red shaded area shows the *MA-grafting yield* of above 0.565 %. A certain overlaid area shows a boundary of a combination of DCP and MA content factors contributing certain levels of the responses. For example, if a desired level of *Mn* is above 89.5 kDa and one would like to maintain the *MA-grafting yield* to be above 0.056 %, the content of DCP must be not higher than 0.2 wt. % PLA basis and MA can be between ~3.2 and 4.5 wt. %. However, this method might not be suitable if there are more than two factors or independent variables.

(a) Scenario 1



(b) Scenario 2

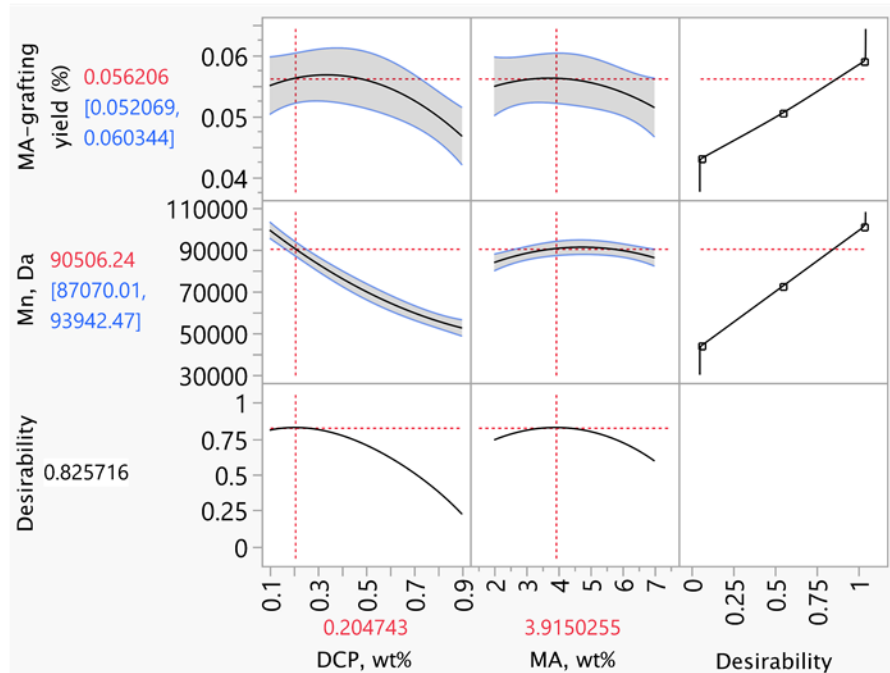


Figure 3-4 DCP and MA contents from desirability profiling analysis of Scenario 1 and 2

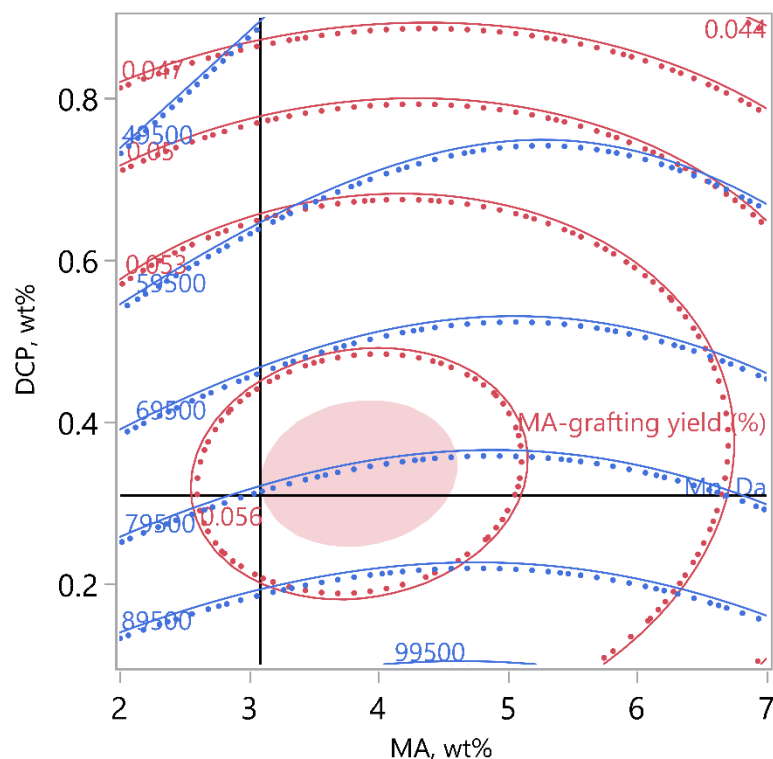


Figure 3-5 overlaid contour plots of MA grafting yield and M_n responses

3.4 Conclusions

With the aim to produce PLA-g-MA to be used as a compatibilizer in PLA based polymer blending as well as to investigate the effect of MA and DCP content on the characteristics of the PLA-g-MA including the grafting yield of MA on the PLA backbone and the molecular weight properties, the central composite design (CCD) was selected to design the experiment to produce PLA-g-MA with 10 runs for the response surface methodology analysis. FTIR spectrums of all produced PLA-g-MA showed characteristic absorption bands indicating the grafting of MA on PLA. The regression of *MA-grafting yield* using the data of all 10 runs indicated an insignificance of the model due to an effect of the presence of an outlier at one center point of the designed experiment (Run no.10). The model regression with the data of run no.10 eliminated resulted in significance of the model with increased R^2 . The content of DCP has a significant effect on *MA-grafting yield* response with a negative relationship. Increasing DCP tended to decrease the *MA-grafting yield*. The regression of M_n , M_w , *dispersity*, and *IV*, were statistically significant

with DCP having a highly significant effect on all responses. Increasing of DCP resulted in reduced M_n , M_w , and IV ; at the same time, the *dispersity* increased with an increasing amount of short polymer chains. Two scenarios of desirability analysis were demonstrated in optimizing *MA-grafting yield* and the molecular weight, M_n of PLA-g-MA based on the effect of MA and DCP content. The first scenario that assigned equal importance to both responses suggested the optimum point with the content of DCP = 0.1 wt. % and MA = 3.94 wt. % (PLA basis). The M_n response was maximized in this scenario. In the second scenario assigned higher importance to *MA-grafting yield* (*importance* value = 0.75) than to M_n (*importance* value = 0.25); the optimum moved forward the maximum of *MA-grafting yield* while the M_n decreased by 9 kDa compared to the first scenario. This emphasized the importance of considering the priority of each response in multiple response optimization.

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4 MELT BLENDING OF PHBV/PLA

4.1 Introduction

Environmental concerns regarding the use of petroleum-based polymeric materials increasingly draw the interest of consumer and product manufacturers to alternative renewable biobased, biodegradable, and compostable polymers. Poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs) are among the group of bioplastic-biodegradable materials with top global capacity [1]. PHAs are a family of biobased microbially synthesized polymers with a reputation of superior biodegradable properties. Poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) are the most popular members of the family according to the world production capacity. PHBV has comparable properties to utility polymers such as polypropylene (PP). However, due to its high production cost, the use of PHBV in general applications such as agriculture or packaging has not been very successful. To make possible using this polymer in more general applications beyond its current area of biomedical is environmentally reasonable. Polymer blending is a good solution to produce a cheaper material based on PHBV. PLA is an ideal candidate to be blended with PHBV. Due to their common characteristics of being biobased and compostable, the obtained blended materials can retain this strength. The thermal, mechanical and barrier properties of the blends can be expected to provide an interesting resultant material that might be able to extend applications beyond those of the original blend components.

PHBV/PLA blending has been widely studied, and the results from early studies indicate immiscibility of the polymer blends without compatibilization. As stable and reproducible morphology is necessary for tailoring the properties of polymer blends, some of the later studies focused on methods to compatibilize the blend system in the hope of improving the overall properties. Addition of a third polymer, nanofillers, and natural fibers to reinforce and compatibilize the polymer blends as well as reactive compatibilization by peroxides and functionalized polymers have been investigated. In this study, we were interested in maleated PLA (PLA-*g*-MA) as a reactive compatibilizer due to its miscibility with PLA and the tendency of anhydride groups to react with the hydroxyls of PHBV. Compatibility between the blend components can be expected. The main objective of this study is to investigate the properties of PHBV/PLA blends which were compatibilized by the addition of PLA-*g*-MA. Chemical and thermal properties were assessed for better understanding their connection on the mechanical and barrier

properties of the polymer blends. The influence of the content of the blend components and characteristics of PLA-*g*-MA, i.e. molecular weight, and the amount of grafted maleic anhydride, on the properties of the blends were investigated through models based on the mixture experimental design where the factorial variable of different types of PLA-*g*-MA was included.

4.2 Experimental

4.2.1 Mixture experimental design

A crossed factorial-mixture design was adopted for the experimental design of this study. *PLAgMA*-type was assigned as a factorial variable to be crossed with three mixture variables, *PHBV*, *PLA*, *PLA-g-MA*, the weight fractions of PHBV/*PLA* polymer blend components including two neat polymers, PHBV and *PLA*, and a compatibilizer, maleated *PLA* or *PLA-g-MA*. Three pre-produced formulas of *PLA-g-MA* were used in this blending experiment and were assigned as three levels of *PLAgMAType* factorial factor: A, B, and C. Characteristics of the *PLA-g-MA* are listed in Table 4-1. The mixture model was based on the constrained level of each mixture variable as follows: $0.2 \leq PHBV \leq 0.7$, $0.2 \leq PLA \leq 0.7$, and $0.05 \leq PLA-g-MA \leq 0.15$. Because the concentration of PHBV of about 20 – 35% in *PLA* has been reported to contribute the most improved properties [2], evaluation at higher concentrations could be helpful to understand the influence of the compatibilizer in the blend system.

Table 4-1 Contents, MA-grafting yield, and molecular weight of *PLA-g-MA* type A, B, and C

PLAgMA-Type	MA Grafting contents (wt % <i>PLA</i> basis)		MA-grafting yield, wt%	M_n , kDa	M_w , kDa
	MA	DCP			
A	7	0.5	0.055 ± 0.007 a	64.6 ± 5.2 ab	137.6 ± 6.3 b
B	4.5	0.5	0.052 ± 0.011 b	74.6 ± 2.9 a	160.1 ± 3.0 a
C	2	0.5	0.052 ± 0.006 b	61.1 ± 6.1 b	137.1 ± 4.4 b

Note: Lowercase letters show the comparison between runs ($P < 0.05$). Levels not connected by same letter are significantly different.

The set of design points was generated using JMP 14.1.0 software (SAS Institute Inc., Cary, NC) using the simplex centroid design where a q -component simplex centroid design has $2^q - 1$ design points. In this study, with three components, a mixture design of 7 runs was generated. With 3 levels of factors, the total number of runs could be anticipated to be $7 \times 3 = 21$ runs. However, the software fractionated the

model to minimize the number of experimental runs by removing 1 run from the *PLAgMA-Type B* and 2 runs from *PLAgMA-Type C*. In the actual experiment, the centroids of *PLAgMA-Type A* and *B* were also removed as the equal content blends were not within the interests of the authors. However, two non-compatible PHBV/PLA blends of 75:25 and 25:75 were included for observation. The actual performed experimental runs are listed in Table 4-2 and illustrated in Figure 4-1.

Table 4-2 Performed experimental runs with actual levels of the blend component mixture

Mixture			PLA-g-MA Type		
			A	B	C
PHBV	PLA	PLA-g-MA	Blend ID		
0.7	0.2	0.1	BR1	BR18	
0.2	0.75	0.05	BR4	BR12	BR3
0.65	0.2	0.15	BR6	BR15	BR5
0.75	0.2	0.05	BR8	BR16	BR14
0.2	0.65	0.15	BR13	BR17	BR9
0.2	0.7	0.1	BR10		
0.45	0.45	0.1			BR11
1	0	0	nPHBV (neat PHBV sample)		
0	1	0	nPLA (neat PLA sample)		
0.75	0.25	0	BNR1 (non-compatible blend)		
0.25	0.75	0	BNR2 (non-compatible blend)		

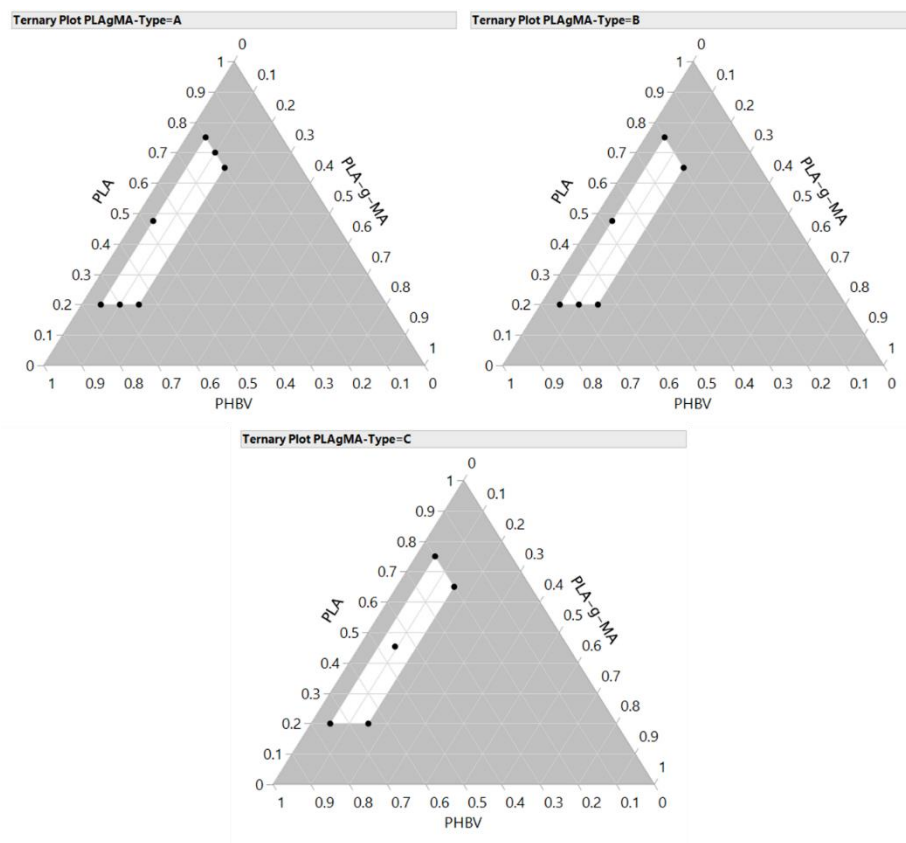


Figure 4-1 Ternary plots of the design points separated by PLAGMA-Type factorial variables generated from JMP software

4.2.2 PLA-g-MA preparation

Three types of compatibilizer PLA-g-MA were pre-produced and characterized, with some characteristics shown in Table 4-1. PLA-g-MA was prepared by reactive functionalization of MA (MilliporeSigma, St. Louis, MO, USA) on PLA (Ingeo™ Biopolymer 2003D, NatureWorks LLC., Minnetonka, MN, USA) using dicumyl peroxide (DCP) (MilliporeSigma, St. Louis, MO, USA) as an initiator. PLA was dried at 80°C for 24 h prior to processing to remove excess moisture. All components, PLA, MA, and DCP, were manually mixed and then fed into a Century ZSK-30 twin-screw co-rotating extruder (Traverse City, MI, USA) using a mechanical feeder set at a rate of 70 g min⁻¹. The temperature profile of the barrel was set at: 140/150/160/160/160/170/170/170/170/160 °C with the screw speed of 120 rpm. Unreacted MA was removed during the extrusion process by the vacuum suction on the vent port of the extruder. The extrudate was cooled in a water bath, then pelletized in a BT 25 pelletizer

(Scheer Bay Co., Bay City, MI, USA). The pellets were dried at 60 °C for 24 h to evaporate the moisture from the cooling process and then stored in a freezer (~ -18 °C) for further processing.

4.2.3 Melt Blending of PHBV and PLA

Compatibilizers described in section 4.2.2 were used in compounding PHBV and PLA.

PLA (Ingeo™ Biopolymer 2003D) was obtained from NatureWorks LLC. (Minnetonka, MN). The polymer, according to the data sheet, had the following main properties: density = 1.24 g cm⁻¹, MFI = 6 g/10 min (210 °C, 2.16 kg) and melting temperature, T_m = 210 °C.

PHBV (TianAn ENMAT™ Y1000P resin) was from TianAn Biologic Materials Co., Ltd. (Beilun Port, Ningbo City, PRC). According to the manufacturer and the study of Jost and Kopitzky [2] which used the same grade of PHBV, the polymer, which was in pellet form, had a density of 1.25 g cm⁻¹, T_m between 170-176 °C, and the content of valeric acid, %HV was approx. 3%.

The viscosity-averaged molecular weight, M_η of PLA and PHBV was calculated according to Mark-Houwink equation [3] using the intrinsic viscosity, $[\eta]$ obtained from the measurement following ASTM D2857 – 16 [4] using chloroform, CHCl₃ as a solvent. Mark-Houwink equation is as follows:

$$[\eta] = K \cdot M_\eta^a \quad \text{Eq. 4-1}$$

where K and a are constants which depend on the nature of the polymer and solvent as well as on temperature. For PLA in CHCl₃: $K = 2.21 \times 10^{-4}$ dL/g, $a = 0.77$ [5]. For PHBV in CHCl₃: $K = 7.70 \times 10^{-5}$ dL/g, $a = 0.82$ [6]. M_η of PLA and PHBV by own measurement were 9.77×10^4 and 4.02×10^4 , respectively.

Before processing PLA, PHBV and PLA-g-MA pellets were dried for 24 h at 65 °C. Then the materials were prepared according to the weight ratios listed in Table 4-2. The compounding was also performed in the Century ZSK-30 twin-screw co-rotating extruder with ten-temperature zone profile of 95/140/160/170/170/170/170/165/165/160 °C. The feeder rate and screw speed were set at 160 and 100 rpm, respectively. The extrudate was water-cooled, then pelletized in a BT 25 pelletizer. The pellets were dried for 24 h to evaporate the moisture, then stored in a freezer at -18 °C for further use.

4.2.4 PHBV/PLA Blend Characterization

For the characterization, the PHBV/PLA blends obtained from each run were fabricated into thin sheet samples using an M CARVER compression molder (Carver Laboratory Press, Menomonee Falls,

WI, USA). Approximately five grams of dried resin was placed between poly(tetrafluoroethylene) (PTFE) sheets, then put in between a pair of metal plates and loaded into the compression molder with temperature set at 180 °C. The preheat time and compression time and pressure were set at two different conditions: 1) the PHBV-rich blend was preheated for 3 min, compressed at 1814 kg for 1 min; 2) the PLA-rich blend was preheated for 4.5 min, and compressed at 4500 kg for 2.5 min. At the end of the compression time, cooling water was opened to cool down the heated press to about 70 °C; the set of PTFE sheets and metal plates holding a sample sheet was unloaded from the machine and left for ambient temperature air cooling for 15 min. The sample was then stored in the freezer at -18 °C for further characterization.

4.2.4.1 ATR-FTIR spectroscopy

Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) was used to observe and track the change of chemical functional units in the blends. FTIR spectra of neat polymer samples and blend samples were recorded using an IR-Prestige 21 Shimadzu FTIR spectrophotometer (Shimadzu, Columbia, MD) over the wavenumber range of 4000-400 cm^{-1} at a resolution of 4 cm^{-1} for 40 consecutive scans.

4.2.4.2 Thermal properties

A differential scanning calorimeter DSC Q100 (TA Instruments, New Castle, DE) was used to measure the thermal properties, i.e. glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c), and crystallinity, of the obtained blends. 5-10 mg of sample was accurately weighed and placed in a standard non-hermetic DSC pan to be measured with an empty pan as a reference. The sample was heated at a rate of 10 °C min^{-1} to 200 °C for the first heating scan, then cooled down to -20 °C, and reheated to 200 °C for the second heating scan. The machine operated with a refrigerated cooling system with a nitrogen flow of 70 mL min^{-1} . T_g , T_c , T_m , and enthalpies of melting and cold crystallization were analyzed from the heating curves using TA universal analysis software. Overlapped endothermic melting peaks of the blend samples were deconvoluted using Fityk 1.3.1 software with Pearson 7Area peak shape [7]. The degree of crystallinity (X_c) of the blend components, PLA and PHBV, in the samples was calculated using the following equation:

$$X_c = \left(\frac{\Delta H_f}{m\Delta H_{f0}} \right) \times 100 \quad \text{Eq. 4-2}$$

where ΔH_f is the observed enthalpy of fusion (J g^{-1}), ΔH_{f0} is enthalpy of fusion for the 100% crystalline substance, and m is the weight fraction of the polymer. ΔH_f normally equals to $\Delta H_m - \Delta H_{cc}$ where ΔH_m is the enthalpy of melting and ΔH_{cc} is the enthalpy of cold crystallization of the polymer acquired for the DSC thermogram. The ΔH_{f0} of PLA and PHBV used in this calculation are 93.7 and 109 J g^{-1} , respectively [8]. In the compatibilized blend samples where the compatibilizer, PLA-*g*-MA, was added, the fraction of PLA in the compatibilizer was included in the weight fraction of PLA in the calculation of X_c of PLA in the blend sample. The weight fraction of PLA in the compatibilized blend could be calculated using the following equation:

$$m_{PLA} = W_{PLA} + \left(W_{PLAgMA} \times \frac{100}{100 + (C_{MA} + C_{DCP})} \right) \quad \text{Eq. 4-3}$$

where m_{PLA} is the weight fraction of PLA for the calculation of X_c , W_{PLA} and W_{PLAgMA} are the weight fractions of PLA and PLA-*g*-MA from the mixture design that can be looked up from Table 4-2, C_{MA} and C_{DCP} are the contents of MA and DCP used in the grafting of PLA-*g*-MA which were specific to the type of PLA-*g*-MA used for each blend sample. The values of C_{MA} and C_{DCP} can be looked up from Table 4-1.

TGA and DTG thermograms of the samples were obtained using a TGA Q50 (TA Instruments, New Castle, DE). The specimens were cut and weighed approximately 5-10 mg and tested with a heating rate of 10 °C from the ambient temperature to 600 °C under a nitrogen atmosphere. Onset, maximum, and end decomposition temperature, as well as the non-volatile residuals were obtained from TA universal analysis software.

4.2.4.3 Mechanical Properties

The mechanical properties including tensile strength, tensile modulus, and elongation at break were obtained from tensile testing using an Instron 5565 Universal Testing Machine, following ASTM D882-18. Samples were conditioned at 23 ± 2 °C and 50 ± 10 % relative humidity at least 40 h before testing, then cut into strips of 10 mm width and the thickness accurately measured. The length of the strip specimens was at least 100 mm since the initial grip separation was set to be 50 mm. The rate of grip separation or crosshead speed was set to be 50 mm min⁻¹. The tensile modulus obtained from the test

was also reported, however, with a remark that the gage length used in the test was shorter than the recommendation of the standard at 250 mm.

4.2.4.4 Barrier Properties

Water vapor transmission rate (WVTR), and oxygen transmission rate (OTR) of the polymer blends were measured using MOCON PERMATRAN W 3/31 WVTR analyzer and MOCON OX-TRAN Model 2/21 OTR analyzer (MOCON, Inc., Brooklyn Park, MN), respectively. The test specimens were cut into the size that could securely cover the opening of the aluminum foil mask where circular openings of two sizes, 3.14 and 0.495 cm², were used in the measurement of WVTR and OTR, respectively. The WVTR of the sample was measured at 23 °C and 100% relative humidity, and OTR was measured at 50% relative humidity at the same temperature using N₂ as a carrier gas. The permeability of water and oxygen was calculated using the following equations:

$$P'_{H_2O} = WVTR \times \frac{1}{\Delta P_{H_2O}} \times l \quad \text{Eq. 4-4}$$

$$P'_{O_2} = OTR \times \frac{1}{\Delta P_{O_2}} \times l \quad \text{Eq. 4-5}$$

where P'_{H_2O} is water permeability, ΔP_{H_2O} is the difference of the partial pressure of water vapor across the film sample, P'_{O_2} is oxygen permeability, ΔP_{O_2} is the difference in the partial pressure of oxygen across the sample, and l is the thickness of the test specimen.

4.2.4.5 Data analysis

Significant differences ($P < 0.05$) between measurement results of the samples were analyzed and determined using one-way analysis of variance and Tukey's honestly significant difference (HSD) tests with JMP software.

The *tensile properties* and *barrier properties* response data of all blends were analyzed using JMP software by fitting to a second order factorial-mixture model using the least squared error method, and analysis of variance (ANOVA) was performed to determine the significance of the parameters. The model analyzed was a multiplication of the *PLAgMA-Type* factorial model and the mixture model to accommodate their interaction. To generate this combination model, we adopted the method described

by Snee et al. [9] starting by using x_i for a mixture variable, z_i for a factorial variable, b_i for a mixture coefficient, and a_i for a factorial variable coefficient, $f(x)$ and $g(x)$ are used for a model with only mixture ingredients, and for a model with only the factorial variables, respectively. $f(x)$ are fitted by a quadratic Scheffe model that includes linear terms and all interaction terms between components:

$$E(y) = \sum_{i=1}^q b_i x_i + \sum_{1 \leq i < j}^q b_{ij} x_i x_j \quad \text{Eq. 4-6}$$

$g(x)$ with one factorial variable could be fitted by the following linear relationship:

$$E(y) = a_0 + \sum_{i=1}^r a_i z_i \quad \text{Eq. 4-7}$$

Adapting to our experiment, the mixture variables were assigned as follows: *PBHV* as x_1 , *PLA* as x_2 , *PLA-g-MA* as x_3 , and the factor variable *PLAgMA-Type* was assigned as z_1 . Then $f(x)$ and $g(x)$ could be written as:

$$f(x) = b_1 x_1 + b_2 x_2 + b_3 x_3 + b_{12} x_1 x_2 + b_{13} x_1 x_3 + b_{23} x_2 x_3 \quad \text{Eq. 4-8}$$

$$g(z) = a_0 + a_1 z_1 \quad \text{Eq. 4-9}$$

The combination of the mixture and factorial model including the presence of their interaction is demonstrated by:

$$\begin{aligned} c(x, z) &= f(x) \times g(z) \\ c(x, z) &= b_1 x_1 \cdot a_0 + b_1 x_1 \cdot a_1 z_1 + b_2 x_2 \cdot a_0 + b_2 x_2 \cdot a_1 z_1 + b_3 x_3 \cdot a_0 + b_3 x_3 \cdot a_1 z_1 \\ &\quad + b_{12} x_1 x_2 \cdot a_0 + b_{12} x_1 x_2 \cdot a_1 z_1 + b_{13} x_1 x_3 \cdot a_0 + b_{13} x_1 x_3 \cdot a_1 z_1 + b_{23} x_2 x_3 \\ &\quad \cdot a_0 + b_{23} x_2 x_3 \cdot a_1 z_1 \end{aligned} \quad \text{Eq. 4-10}$$

4.3 Results and discussion

4.3.1 ATR-FTIR spectroscopy

Figure 4-2 shows the FTIR spectra of the neat PHBV and PLA samples, nPHBV and nPLA. Since PHBV and PLA are both polyesters, they produce some similar characteristic absorption bands, however, with some minor location and intensity differences. The band at 1454 cm^{-1} that represents the C-H bending of the methyl group is almost identical in both polymers. The band corresponding to the C-H bending of alkane at 1377 cm^{-1} of PHBV appears to be stronger than the one at 1360 cm^{-1} of PLA due to the longer alkane of the valerate of PHBV. A strong absorption band at 1182 cm^{-1} of C-O-C stretching in PLA relates to its amorphous phase [10]; the one of PHBV at 1184 cm^{-1} appears less intense. Strong bands attributed to the C-O stretching of secondary alcohols are found at 1128 and 1126 cm^{-1} in PHBV

and PLA, respectively. The literature reports, specifically to PHBV, the absorption bands at 1282 and 1053 cm^{-1} are attributed to the C-O-C stretching of the crystalline parts [10]. Our PHBV sample shows doublets at 1271 and 1258 cm^{-1} and one at 1042 cm^{-1} that could be inferred to represent the same stretching as well as a highly crystallized structure of PHBV. The PLA sample also has a similar mild peak at 1256 cm^{-1} . The absorption band of carbonyl stretches, C=O, of PHBV and PLA were located at 1713 cm^{-1} and 1746 cm^{-1} , respectively. The crystal structure results in the CH_3 group in one helical structure and the C=O group in the other helical being in close proximity, as indicated by the presence of the band above 2993 cm^{-1} implying the formation of C-H $\cdots$ O hydrogen bonds between the groups [10].

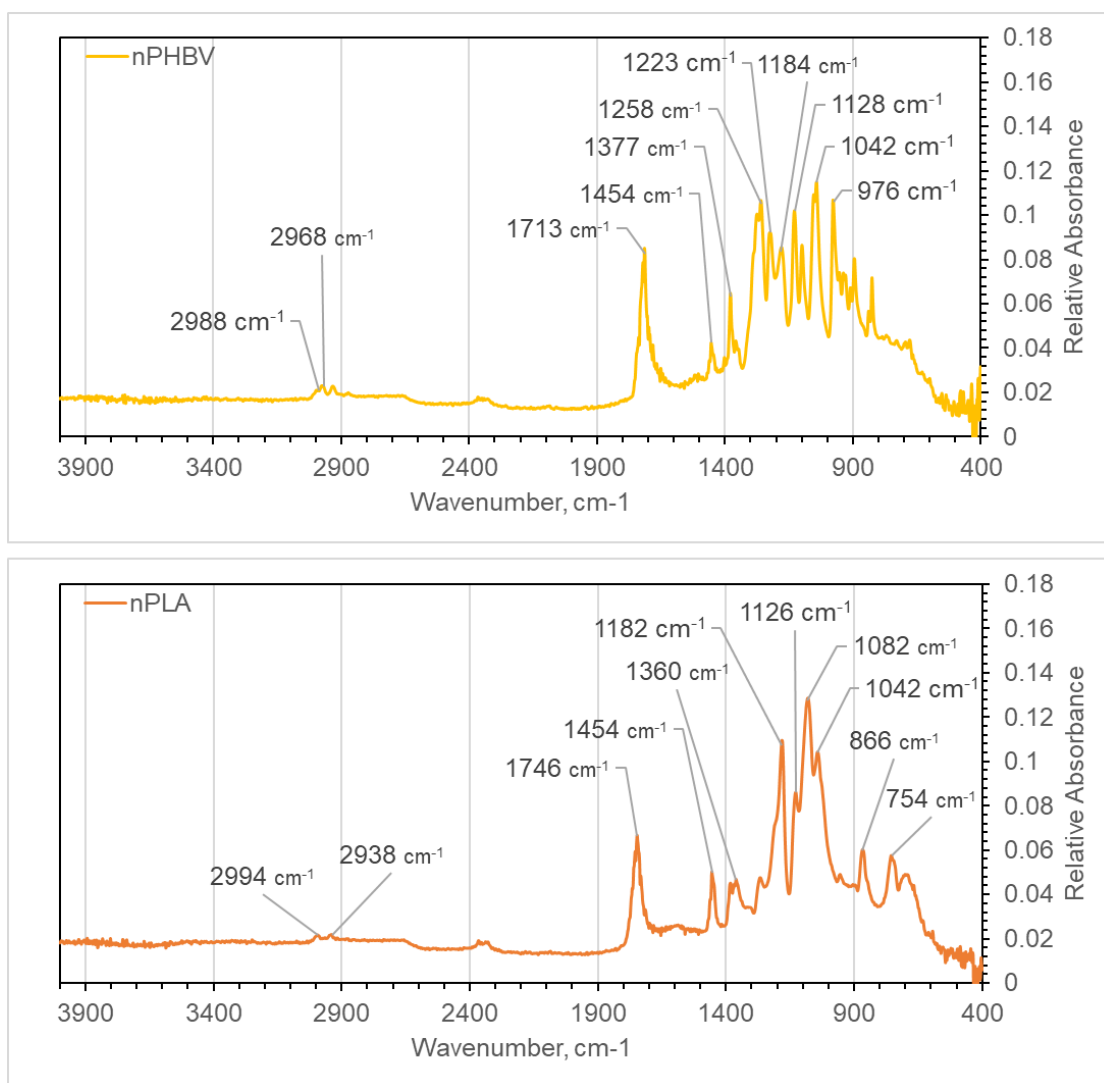


Figure 4-2 FTIR spectra of nPHBV and nPLA samples

To observe the impact of blending, the spectra of the blend samples are demonstrated in Figure 4-3. The spectra are grouped as PHBV-rich blends (weight fraction of PHBV > 0.5) and PLA-rich blends (weight fraction of PLA > 0.5) to facilitate the comparison as the pattern of differences can be seen between the groups. The spectra of PHBV-rich blends show all characteristic absorption bands originally appearing in the neat PHBV without any visible change even though a certain amount of PLA was blended in. The bands associated with the presence of PLA are hardly seen. This can be observed in Figure 4-3a by comparing the spectra of nPLA and nPHBV to all spectra above them. No significant difference between the non-compatibilized blend, BNR1, and the compatibilized ones, BR samples, are found, either. In contrast, the spectra of PLA-rich blends clearly show characteristic absorption bands of both base polymers. Grey vertical strips highlight the difference at the same wavenumber range in the spectra of PHBV-rich blends and PLA-rich blends. Strips number 1 to 4 show the absorption bands that represent the presence of PLA in the blends which, as in the discussion earlier, are not visible on the spectra of the PHBV-rich blends. These absorption bands of PLA show up together with the bands of PHBV even though not more than 25% of PHBV was in these blends.

Strip number 5 shows the decrease in intensity of the absorption band at 976 cm^{-1} in the compatibilized blends, BR samples. According to the report of Zembouai et al. [10], this band and the presence of the band at 895 cm^{-1} were assigned to the C-C backbone stretching with CH_3 stretching vibration and the C-O-C vibration band of the crystalline phase. Strip number 6 exhibits the band at 854 cm^{-1} of PLA on the spectra of both BNR2 and all BR sample that relates to its amorphous phase [10].

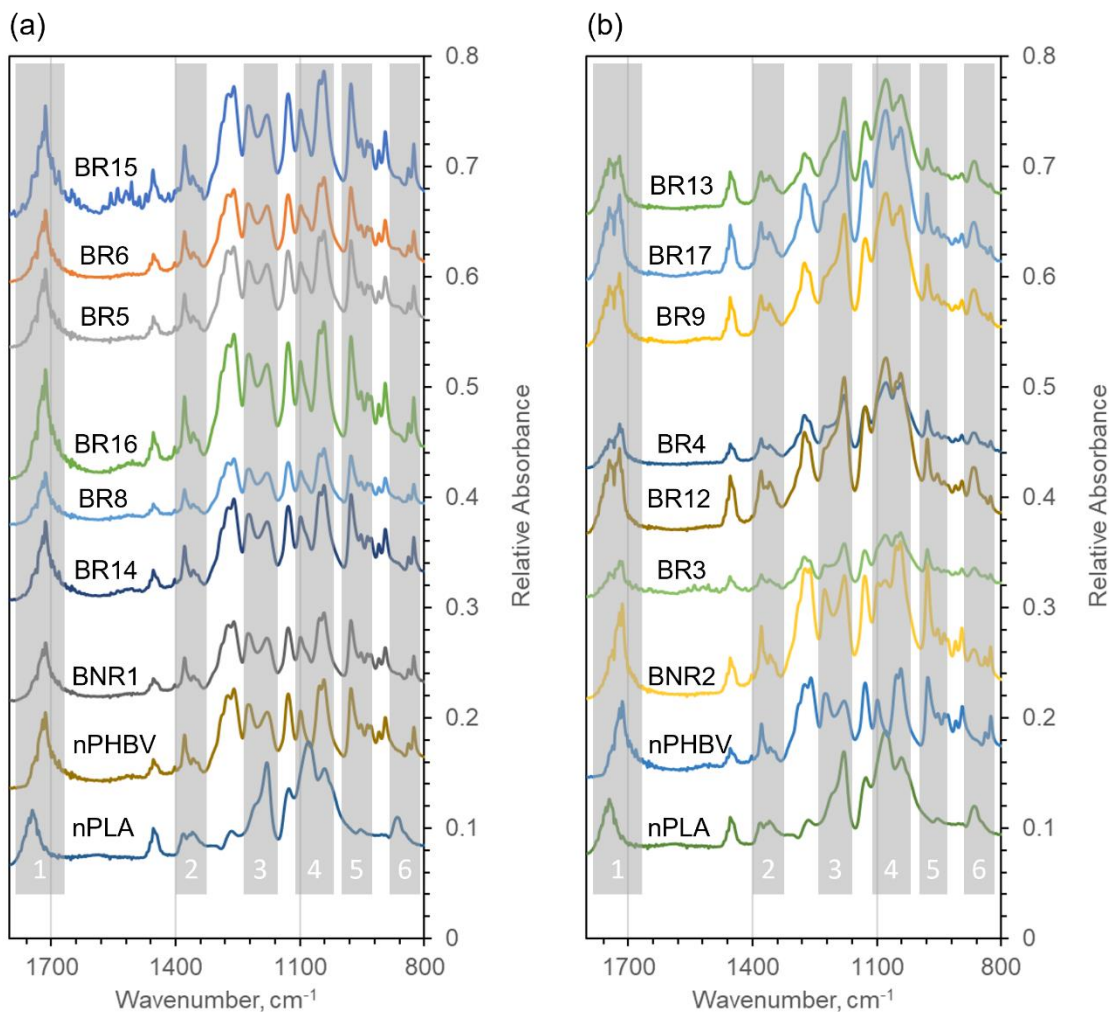


Figure 4-3 FTIR spectra of a) PHBV-rich blends, and b) PLA-rich blends. Each graph includes the spectra of nPLA, nPHBV, and non-compatible blends, BNR1 and BNR2. The spectra are shifted along the vertical axis to ease the comparison.

4.3.2 Thermal properties

The TGA and DTG thermograms of the base polymers and non-compatible blends are displayed in Figure 4-4. Both neat polymers, nPHBV and nPLA, show a single step decomposition (Figure 4-4a). nPHBV shows a steep decomposition with setting at 254.03 ± 3.49 °C and ending at 282.15 ± 0.98 °C, with about 100% weight loss occurring within less than a 30 °C range. nPLA exhibits a slower decomposition process starting at the onset temperature of 345.03 ± 4.57 °C and ending at 388.62 ± 2.06 °C. The maximum decomposition of nPHBV and nPLA occurs at 271.48 ± 0.63 and 369.55 ± 2.85 °C, respectively. The thermal stability of PHBV/PLA non-compatible blends, BNR1 (75/25) and BNR2

(25/75) was influenced by the base polymers. Both blends demonstrate two-step decomposition with higher onset temperatures compared to nPHBV (Figure 4-4b). Shifting of the decomposition to the higher range of temperature indicates an increase in the thermal stability when PLA was included in the blends. While the first weight loss on the thermograms of BNR1 and BNR2 represents the decomposition of the PHBV portion of the blend, the second weight loss is of PLA.

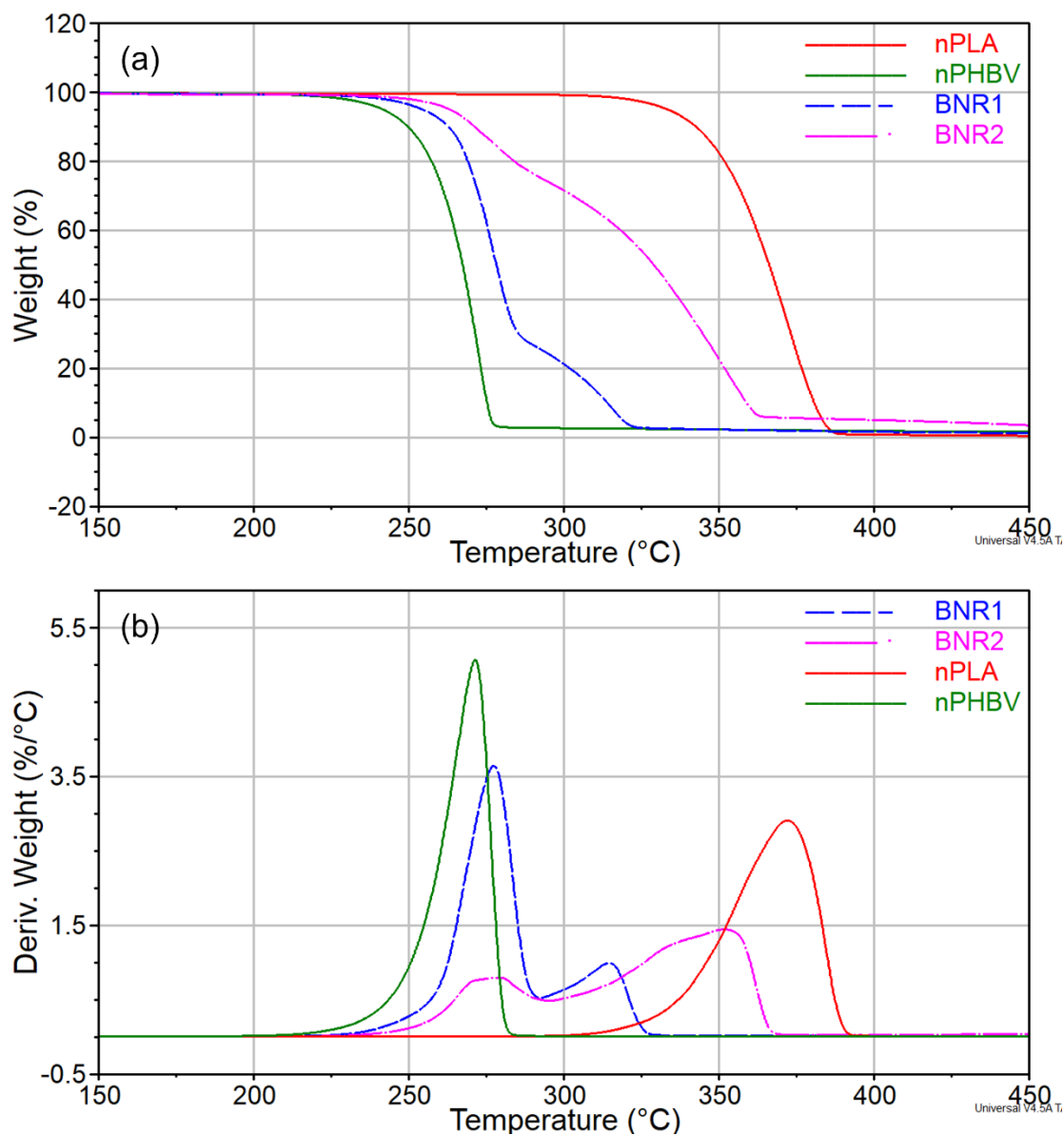


Figure 4-4 (a) TGA, and (b) DTG thermograms of nPHBV, nPLA, BNR1, and BNR2

Figure 4-5 to Figure 4-7 show the TGA and DTG thermograms of compatibilized blends with PLA-*g*-MA type A, B, and C with the ones of nPHBV and nPLA included in each graph for comparison. Shifting of the decomposition to a higher temperature range due to the increased content of PLA in the blends could be seen, like what happened in non-compatibilized blends as mentioned above. To observe the effect of the compatibilizer on the thermal stability of the compatibilized blends, plots of the increase of temperature of decomposition at 5%, 50% and 80% (T_5 , T_{50} , and T_{80}) as a function of PLA content with different PLA-*g*-MA types added are demonstrated in Figure 4-8. Overall, the increase of T_5 , T_{50} , and T_{80} of the compatibilized blends are considerably lower than the increase occurring in non-compatibilized blends. Addition of PLA-*g*-MA in the blending process could induce random chain scission of PLA, and increase active main chain groups and impurities that enhance the thermal degradation [11]. The blends with PLA-*g*-MA type A had the lowest increase of T_5 , T_{50} , and T_{80} compared to the blends with PLA-*g*-MA types B and C. This probably relates to the highest concentration of maleic anhydride in the PLA-*g*-MA type A that could initiate reactions on the backbone of PLA and PHBV more than other types of PLA-*g*-MA, causing the resulting blends to be more vulnerable to thermal decomposition.

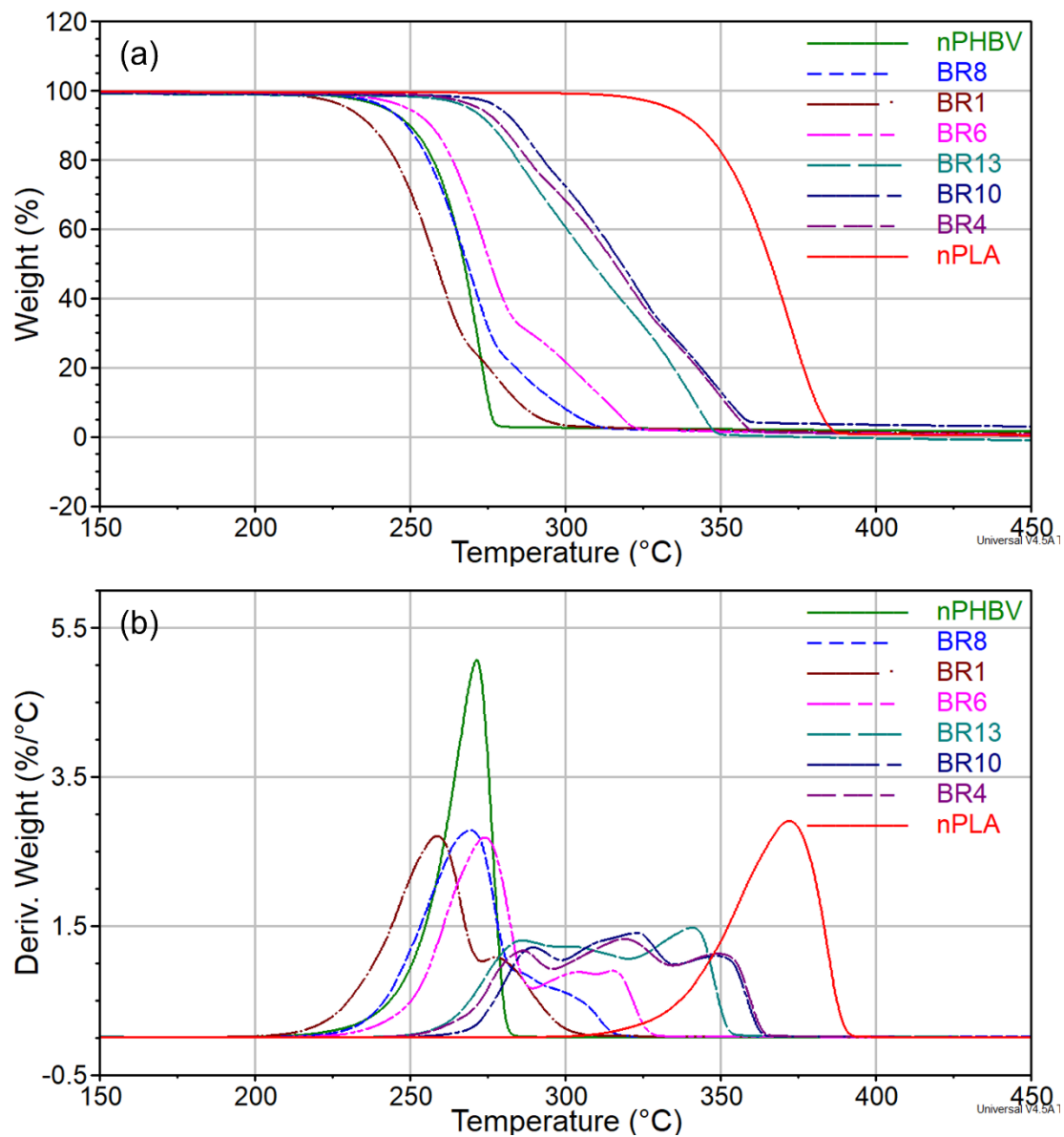


Figure 4-5 (a) TGA, and (b) DTG thermograms of compatibilized blends with PLA-g-MA type A

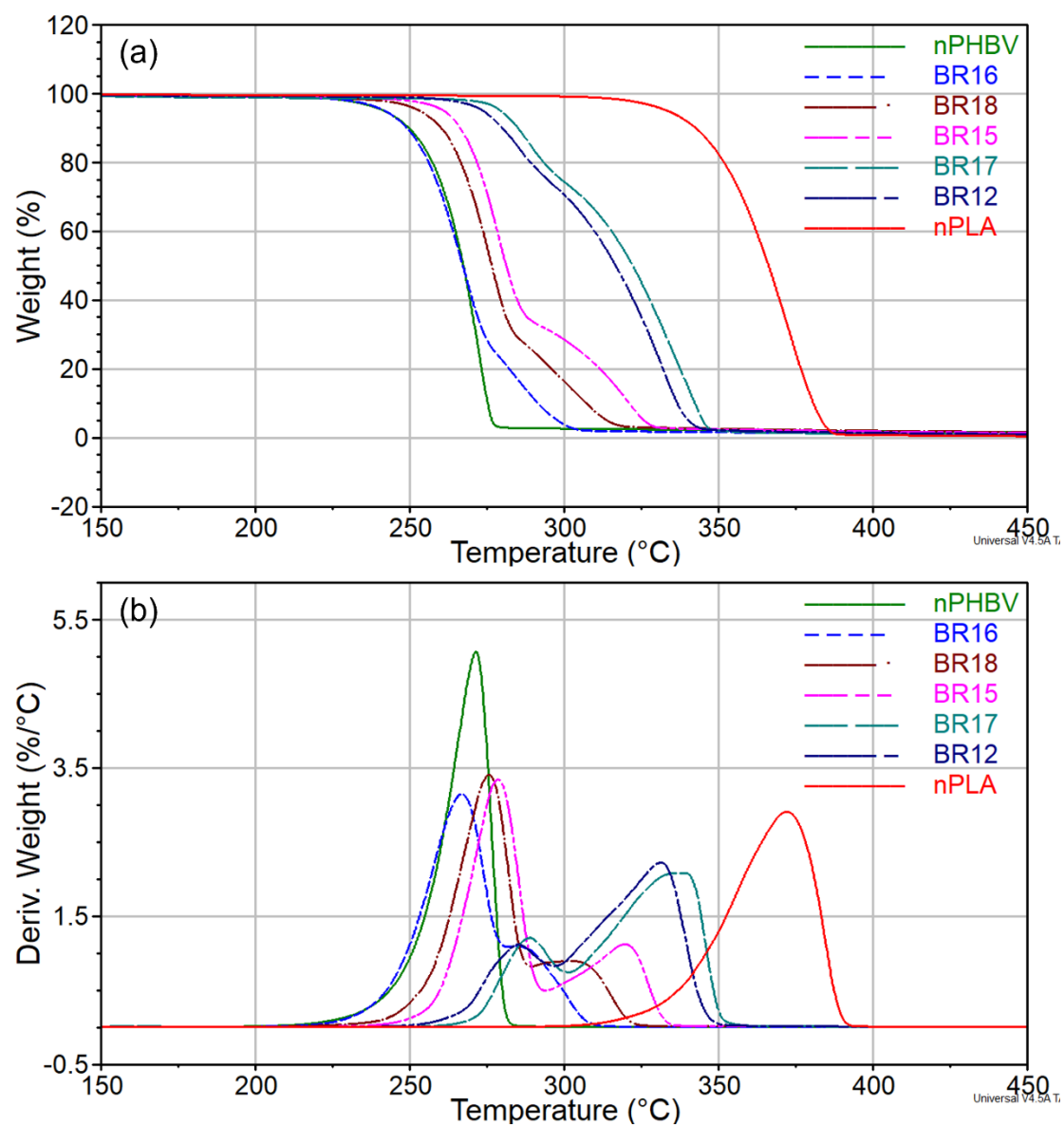


Figure 4-6 (a) TGA, and (b) DTG thermograms of compatibilized blends with PLA-g-MA type B

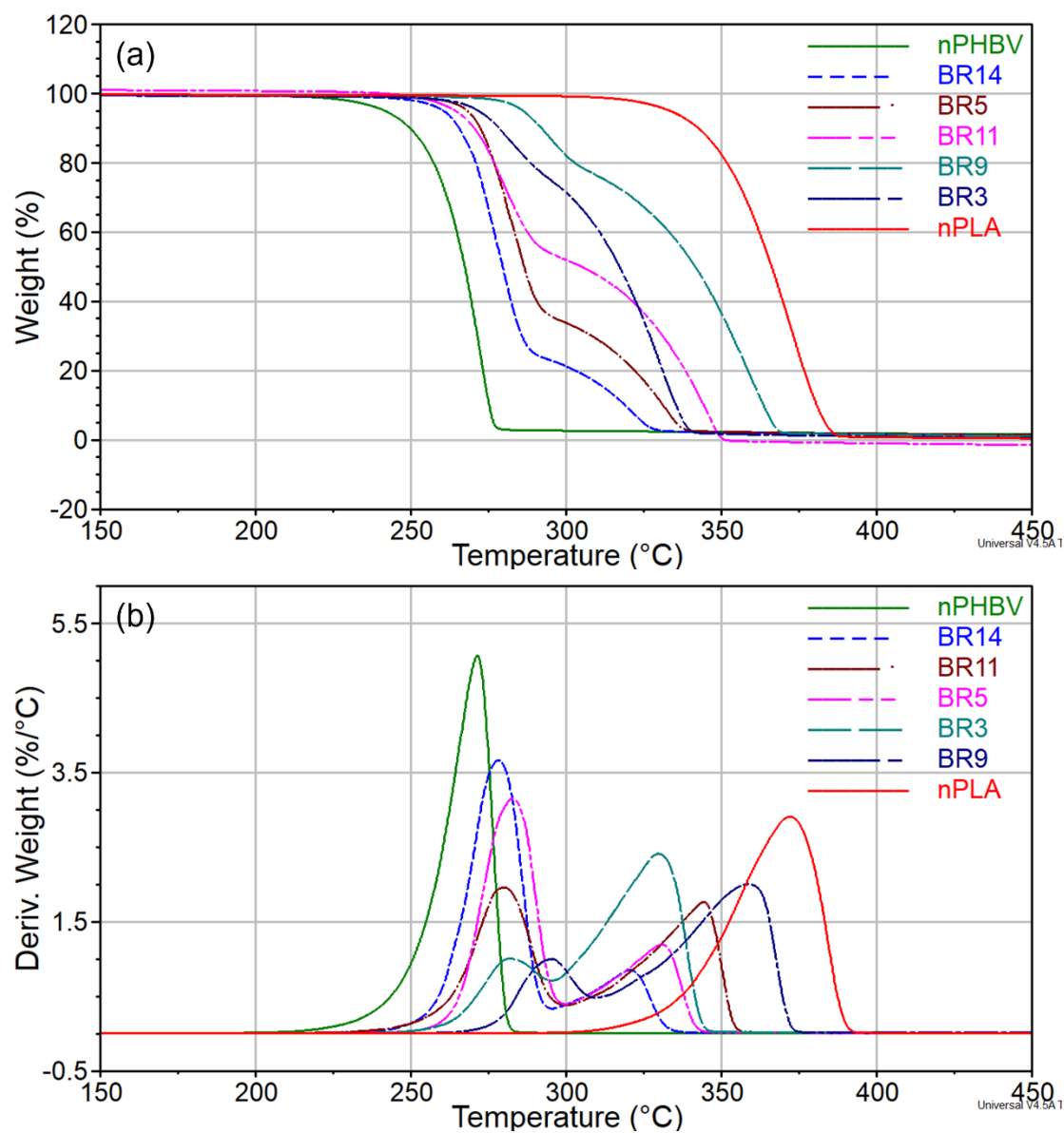


Figure 4-7 (a) TGA, and (b) DTG thermograms of compatibilized blends with PLA-g-MA type C

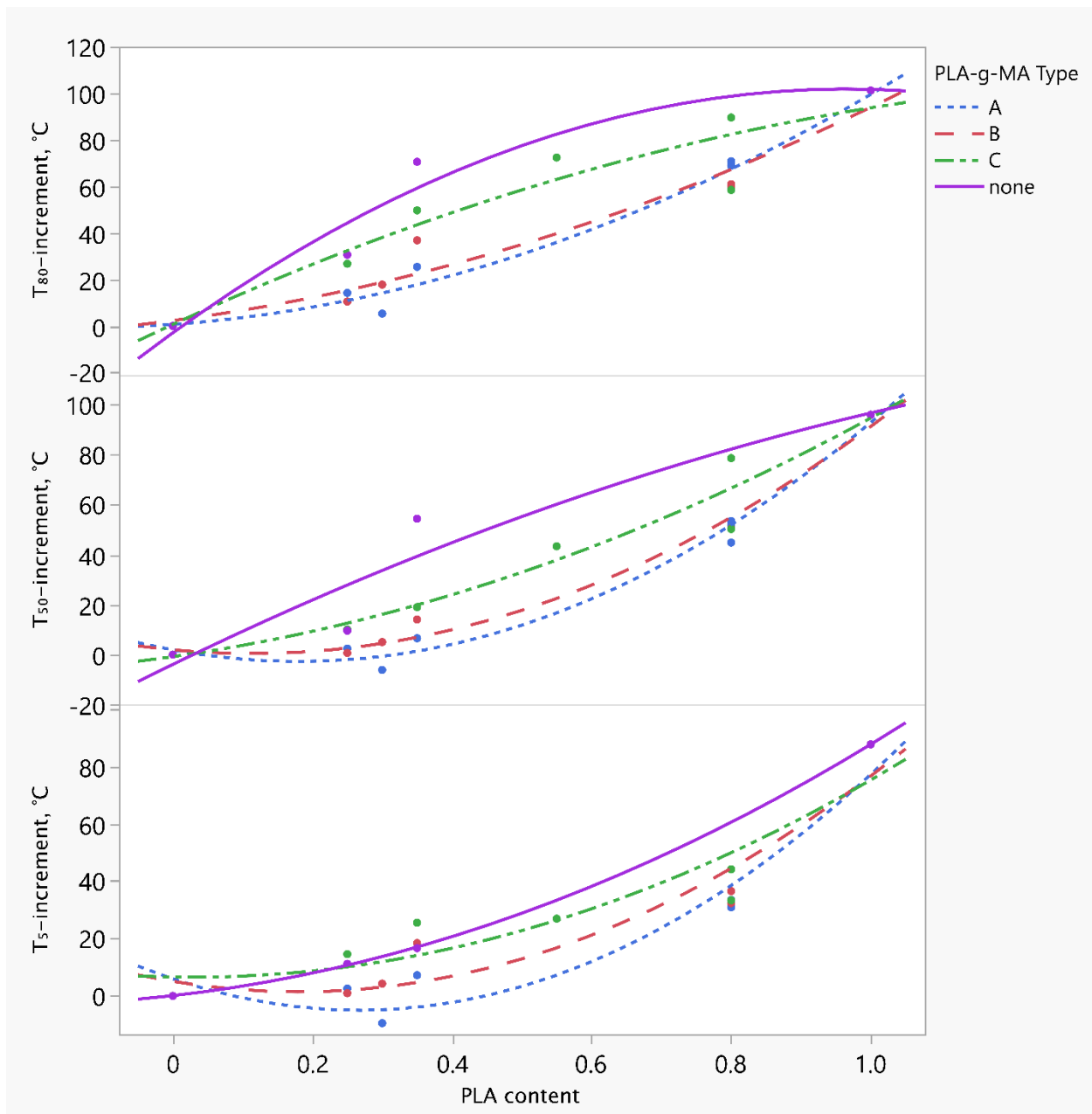
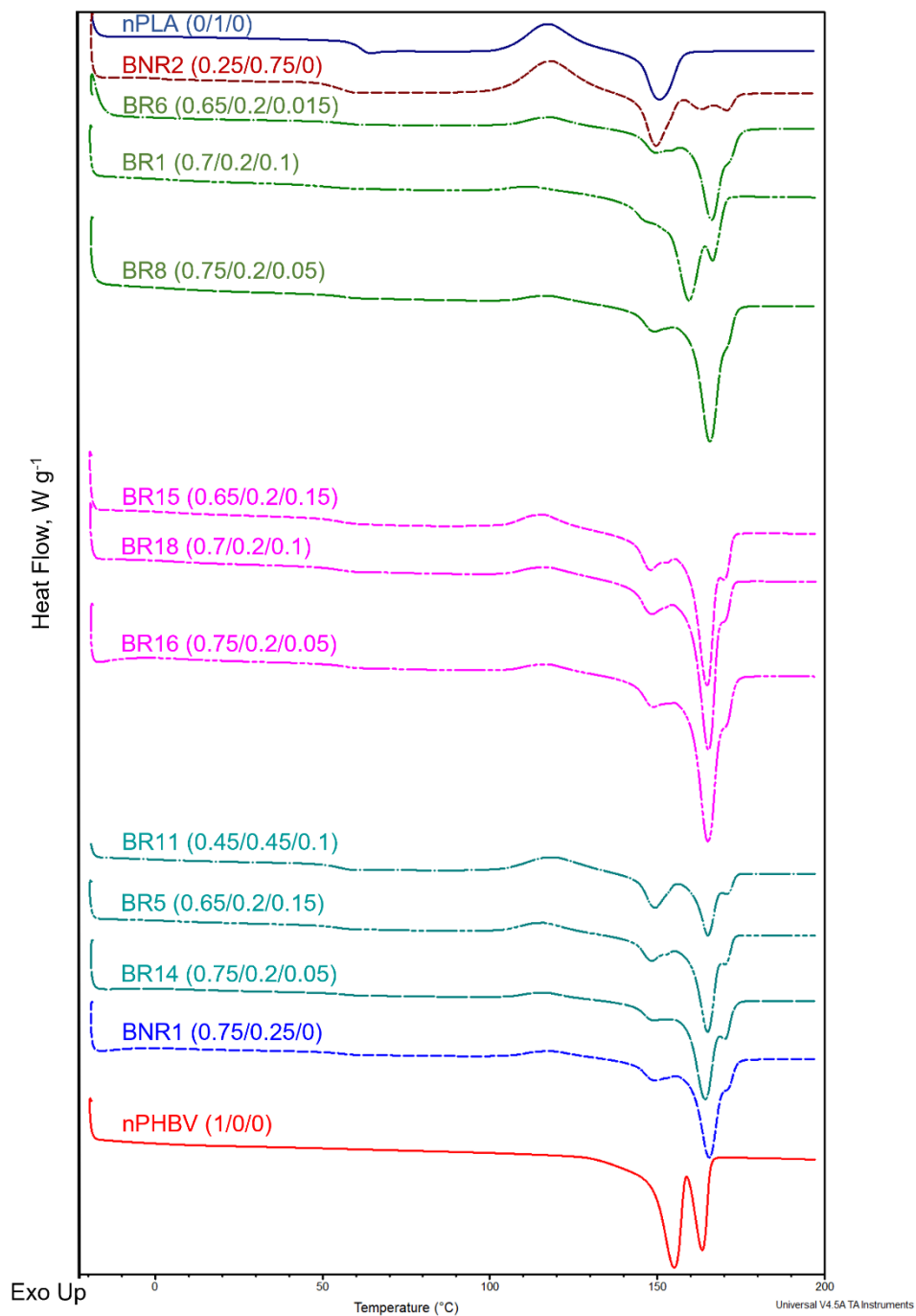


Figure 4-8 T_5 , T_{50} , and T_{80} increment as a function of PLA content overlayed by PLA-g-MA types. The lines represent the quadratic polynomial regression $y = \beta_0 + \beta_1x + \beta_{11}x^2$ where y is the T_5 , T_{50} , and T_{80} increment and x is the PLA content.

DSC analysis of PHBV, PLA, and their blended materials gave the information regarding the glass transition temperature (T_g), crystallization point (T_c), melting point (T_m), enthalpy of crystallization (ΔH_c), enthalpy of fusion (ΔH_f), and degree of crystallinity (X_c). The detailed data are presented in Table 4-10 and Table 4-11 in the APPENDIX. The DSC curves of all samples are illustrated in Figure 4-9 for

PHBV-rich blends and Figure 4-10 for PLA-rich blends. The thermograms of the neat polymers and non-compatible blends, BNR1 and BNR2, are included in both figures to facilitate the comparison. While the neat PLA sample shows the melting peak at 150 ± 0.09 °C, a bimodal melting peak can be observed in nPHBV where the melting points are at 155.24 ± 0.17 °C and 163.64 ± 0.12 °C. According to Verhoogt et al. [12], two melting transitions of PHBV might be due to crystal thickening and/or recrystallization that occurred during the heating in the DSC. Sato et al. [13] reported splitting of melting temperature into two peaks in PHBV with low %HV (6 and 16 mol%), where the rearrangement of the crystal structure can occur easier than when there is a higher %HV due to the steric hindrance of side chains of the HV unit. The melting peak at lower temperature shows primary melting of the crystal structure while the one at higher temperature shows rearrangement of the crystal [13]. DSC curves of the blend samples show recognizable melting peaks around the peaks of the neat polymers; therefore, in further discussion, the melting peak at the lower temperature was referred to as the melting peak of the PLA phase of the blended material (T_{m-PLA}) and the melting peaks of the PHBV phase are at the higher temperature and represented by T_{m-PHBV} . Increased T_{m-PHBV} compared to the ones of nPHBV sample was observed in all blends. The inclusion of PLA could hinder the melting of PHBV which was also reflected in increased thermal stability of the blended material. In PHBV-rich blends, the second melting peak of PHBV, $T_{m-PHBV-2}$, became a shoulder rather than a sharp peak as on the curve of nPHBV. This could be because the rearrangement of the crystals, which causes the appearance of the second melting peak, is slower in the blends than in pure polymer, probably due to the reaction of the compatibilizer that reduces chain mobility.

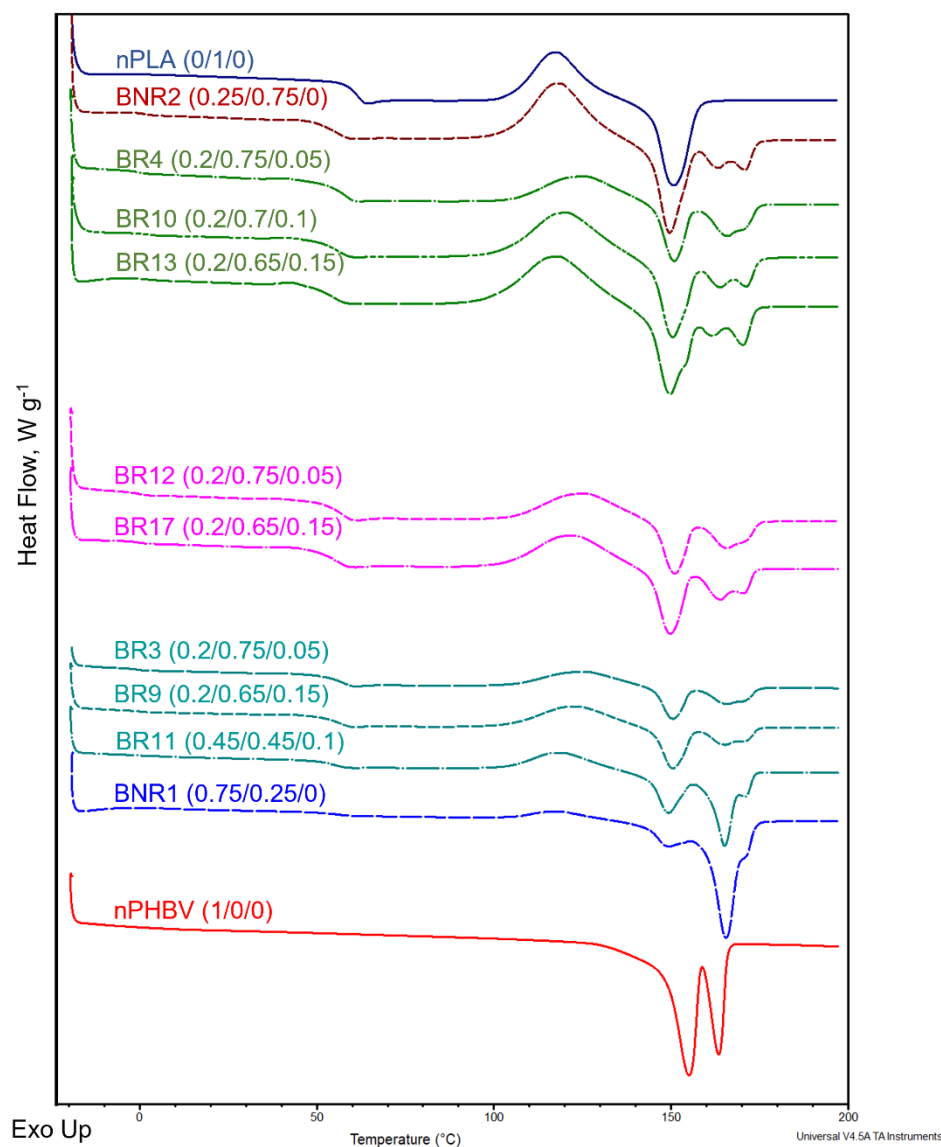
The cold crystallization temperature of PLA, T_c , of PHBV-rich samples did not show significant differences between the blends with different content ratios. However, in PLA-rich blends, most of the compatibilized blends demonstrated increased T_c compared to the non-compatible blend and the neat PLA. This agrees with the work of Arrieta et al. [14] that reported an increase in PLA cold crystallization temperature from 118.1 °C to 130 °C in the PLA/PHB blend with 25 wt. % of PHB. The study attributed this effect to the transesterification reaction between PHB and PLA during the blending.



Note:

- The thermogram label: Blend ID (wt. fraction of PHBV/PLA/PLA-g-MA).
- Thermograms of the blends with PLAGMA-Type A are in green.
- Thermograms of the blends with PLAGMA-Type B are in pink.
- Thermograms of the blends with PLAGMA-Type C are in teal.

Figure 4-9 DSC thermograms of PHBV-Rich blends, the ones of neat PLA and neat PHBV also included.



Note:

- The thermogram label: Blend ID (wt. fraction of PHBV/PLA/PLA-g-MA).
- Thermograms of the blends with PLAGMA-Type A are in green.
- Thermograms of the blends with PLAGMA-Type B are in pink.
- Thermograms of the blends with PLAGMA-Type C are in teal.

Figure 4-10 DSC thermograms of PLA-Rich blends, the ones of neat PLA and neat PHBV also included.

ΔH_c and ΔH_m were used to calculate the degree of crystallinity of the neat polymer and polymer blends. Overlapped melting peaks of PLA and PHBV portions in the blend samples were deconvoluted to get the ΔH_m specific to each phase. In Figure 4-11 and 4-12, the calculated X_c of PLA and PHBV component in the blends are plotted against the PHBV content and grouped by the PLAGMA-Type that

was used in the blends. The negative value of calculated X_c of PLA in PLA-riched blends (BR4, BR10, BR13, BR12, BR17, BR3, BR9, and BNR2) could be observed from Figure 4-11. ΔH_c obtained from these samples are higher than their ΔH_m obtained from the peak deconvolution. This could be because some of PHBV also crystallized during the cold crystallization of PLA. An increase of X_c of PLA with increasing PHBV content was observed in the blends with a weight fraction of PHBV ≥ 0.45 (Figure 4-11). This could be ascribed to the interface-induced nucleation or interface-assisted crystallization where the nucleation of one polymer occurs on the surface of pre-existing crystals of a different polymer [15]. In our study, most of the crystalline structures of PHBV pre-existed in the blend after its cold crystallization during the cooling cycle before the 2nd heating cycle where the data of ΔH_c and ΔH_f were retrieved. It is possible that the interface-induced nucleation occurring in PLA caused the X_c of PLA to increase. Regarding the effect of the compatibilizer, no significant difference in X_c of PLA between non-compatibilized and compatibilized blends with 0.75 - PHBV weight fraction was observed. For the X_c of PHBV, Figure 4-12 shows in the non-compatibilized materials that when the weight fraction of PHBV decreased from 1 to 0.75 and 0.25, the X_c of PHBV decreased from around 80% to 55% and 40%. This decline seems to be slowed down when the compatibilizer was included into the system. It could be seen from samples BR6, BR1, BR15, BR8, BR11, and BR15, that even though the weight fraction of PHBV was reduced to 0.45 – 0.70, X_c of PHBV is still above 55%. Also, at 0.75 - PHBV weight fraction, the X_c of PHBV in compatibilized blends (BR8, BR16, and BR14) was slightly higher than in the non-compatibilized one (BNR1).

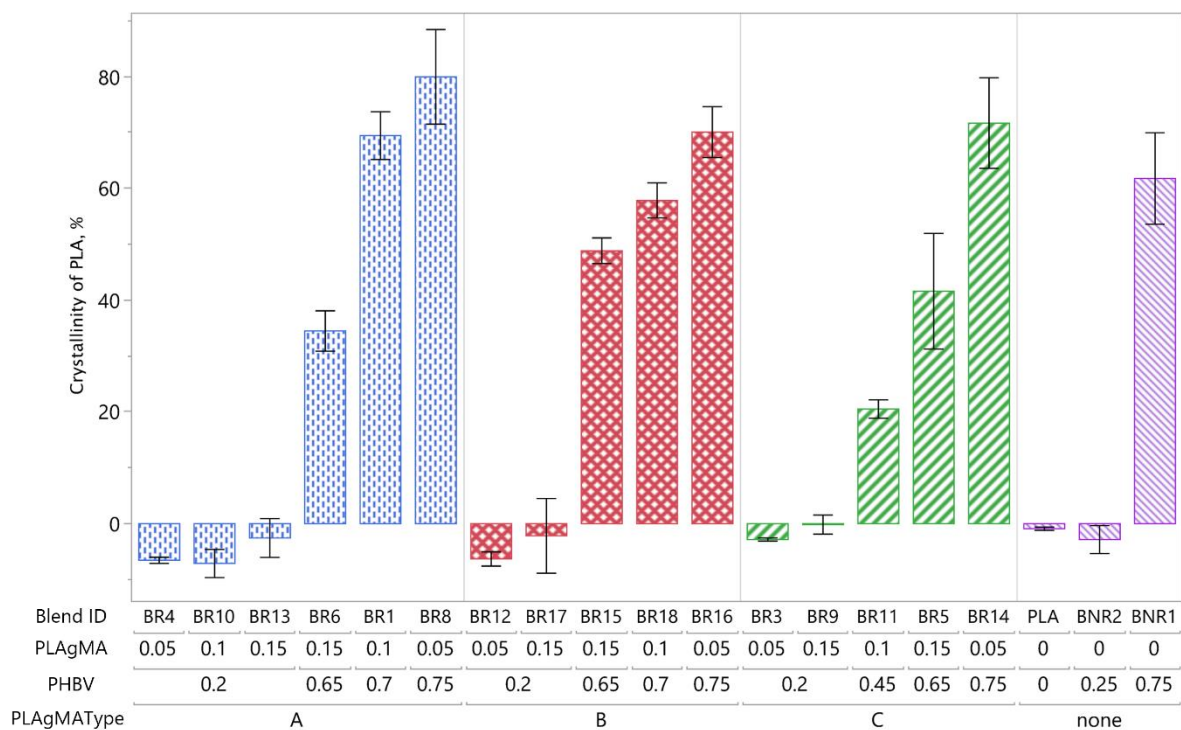


Figure 4-11 Crystallinity of PLA (%) in the blend samples

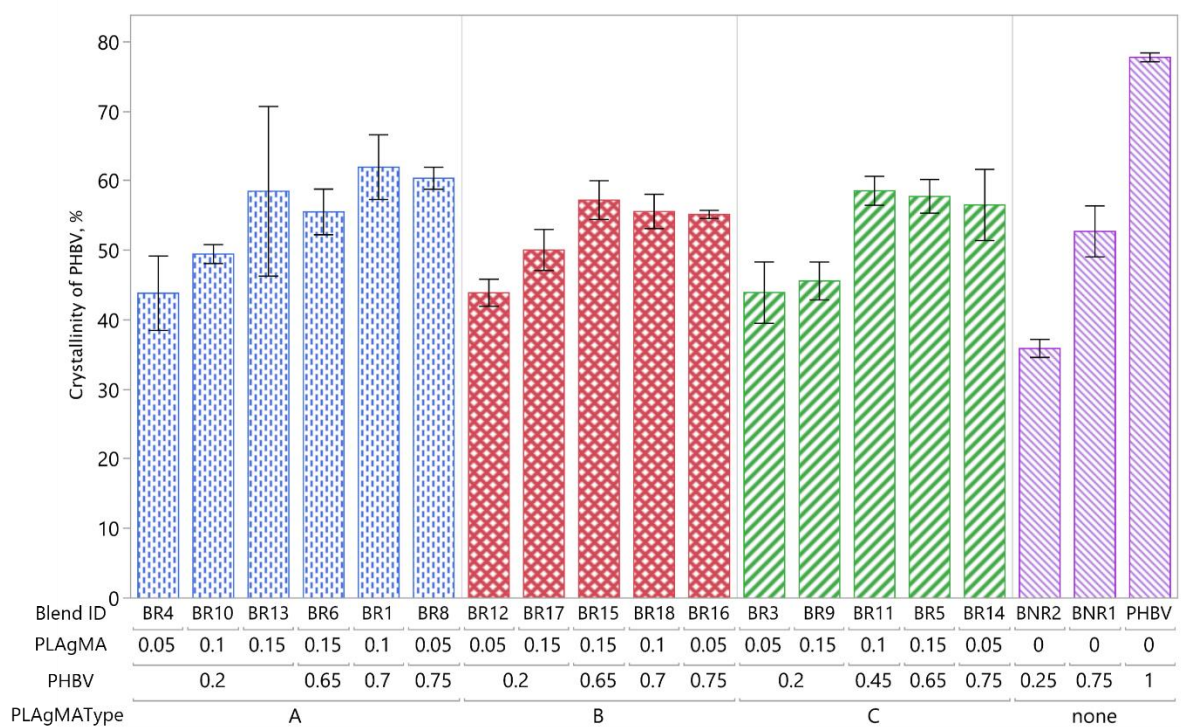


Figure 4-12 Crystallinity of PHBV (%) in the blend samples and neat PHBV

Figure 4-13 depicts the crystalline fraction in the whole material of all samples. Neat PHBV contains the highest crystalline fraction while pure PLA is mostly amorphous. About 10% crystallinity was obtained by including 25% of PHBV in the non-compatible blend, BR2. A comparable level of crystallinity was achieved with about 20% of PHBV in compatible blends regardless of the type of PLAgMA. At 75% PHBV, addition of PLAgMA also resulted in higher crystallinity compared to the non-compatible blend (BNR1). According to Fenni et al. [15], in general, reactive compatibilization, in which a molecule which can react with one or both phases is added during the extrusion process, reduces the crystallinity in polymer blends since the crystallization can be affected by chain scission or reduction of chain mobility due to cross-linking. In our case, even though PLAgMA compatibilizer that contains active anhydride functional groups was added to the polymer blends and the aforementioned reactions could be expected, increases of crystallinity in most compatible blends were still observed. This could be because the change in the crystallization behavior that occurred in our blend system was dominated by surface-induced nucleation, causing a significant increase in the crystallinity of PLA and of the overall material. This generally happens in systems where the addition of compatibilizer results in a large decrease in the size of the dispersed phase [15].

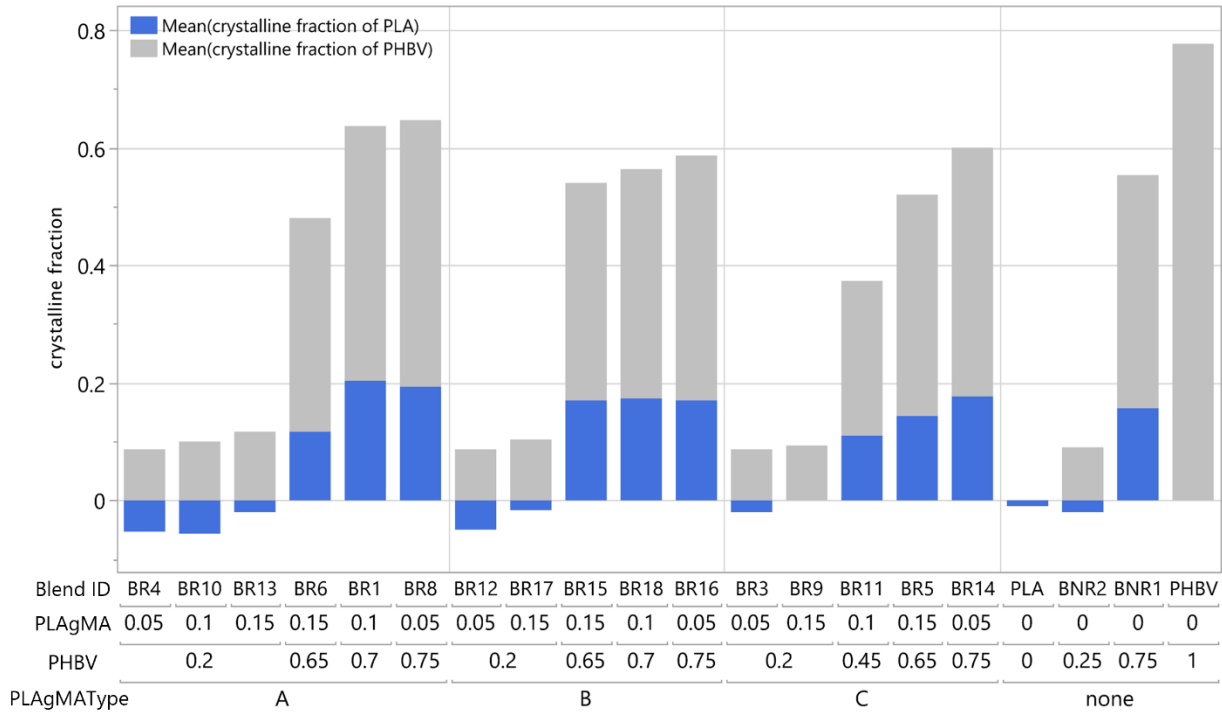


Figure 4-13 Crystalline fraction in the whole material of all samples. The solid blue and solid grey parts of the bar represents the crystalline of PLA and PHBV, respectively.

The glass transition temperature provides information regarding the miscibility of the polymer blend. Inward shifting of T_g s of the blend components is an important indicator of improved miscibility in polymer blending. In this study, the T_g of the neat PHBV sample and the ones of the PHBV phase in most of the blend samples could not be detected by the DSC. For four blends; BR3, BR9, BR12, and BR17, the T_{g-PHBV} can be reported at 0.03 ± 0.19 °C, 0.38 ± 0.09 °C, 0.06 ± 0.40 °C and -1.01 ± 0.27 °C, respectively. BR3 and BR9 contained PLAGMA-Type C with the content ratio PHBV/PLA/PLA-g-MA of 0.2/0.75/0.05 and 0.2/0.65/0.15, respectively. BR12 and BR17 had the same formulation as BR3 and BR9 but contained PLAGMA-Type B. Since the T_g of neat PLA was 61.67 ± 0.22 °C, T_{g-PLA} of all blends decreased as PHBV was blended in. This roughly suggests improved compatibility between the blend components. The degree of miscibility was also investigated through the Gordon-Taylor equation (G-T equation) by applying it to T_{g-PLA} in different compositions. The simplified G-T equation is as follows:

$$T_{g-mix} = \frac{W_1 T_{g1} + k W_2 T_{g2}}{W_1 + k W_2} \quad \text{Eq. 4-11}$$

where W_1 and W_2 are the weight fraction of PHBV and PLA. T_{g1} , and T_{g2} are the glass transition temperature of the neat PHBV and PLA, respectively. k is an adjustable fitting parameter indicating the miscibility of the system where $k = 1$ represents high miscibility of the blend components. Figure 4-14 illustrates the plots of the observed T_g with the smooth curves of GT-equation with the k parameters that were estimated using the least squares method. To evaluate the effect of different compatibilizers, T_g data of non-compatibilized materials, and the blends with PLAGMA-Type A, B, and C were analyzed separately to give the estimated k parameter specific to each group. The k of these groups of materials are 24.11, 16.14, 18.23, and 16.86, respectively. The obtained values of k of all four groups are considerably away from 1, indicating poor miscibility of the blends. However, the lower k of the blends with PLAGMA-Type A, B and C compared to the one of the non-compatibilized material indicates improvement due to the addition of these PLAGMA compatibilizers. PLAGMA-Type A shows the highest impact, followed by C and B.

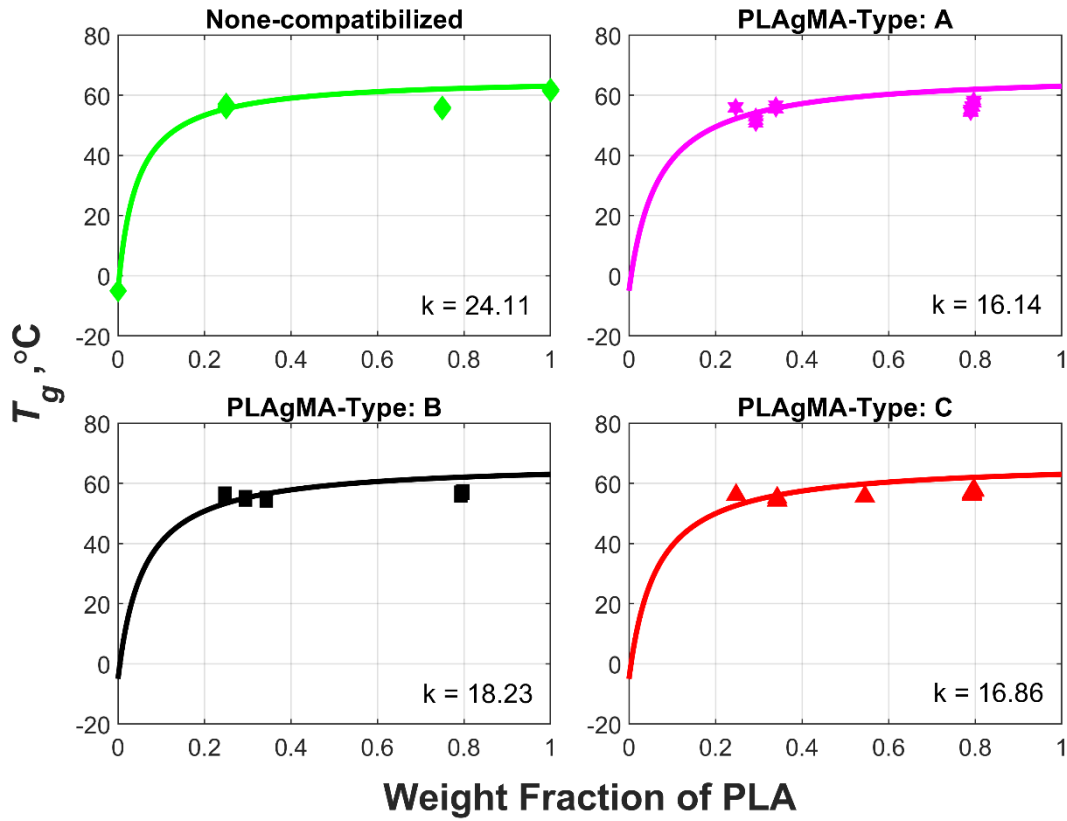


Figure 4-14 Observed T_g at different weight fraction of PLA with the curve of GT-equation with k parameters obtained using the least squares method

4.3.3 Mechanical properties

Tensile strength, % elongation at break, and elastic modulus of the neat PHBV and PLA, the non-compatible and compatibilized blends are depicted in Figure 4-15. In one graph, the data of the blends are grouped by PLAgMA-Type of A, B, C, and None. From Figure 4-15a, neat PLA has the highest tensile strength among all samples while PHBV has the lowest. In non-compatible blends, by including 25 and 75 wt. % of PLA (BNR1 and BNR2), the tensile strength of PHBV was improved by twofold to threefold. The compatibilized blends: BR8 (0.75/0.2/0.05A)², BR18 (0.7/0.2/0.1B), BR11 (0.45/0.45/0.1C), and BR5 (0.65/0.2/0.15C), even though having PLA content equal to or lower than 0.45, have comparable tensile strength to the non-compatible blend, BNR2 with the PLA weight fraction of 0.75. This indicates the effect of the compatibilizers on improving the interfacial adhesion between the blend components that resulted in an increase of the tensile strength. Addition of PLA-*g*-MA could also induce random chain scission in the polymer blend component backbone that results in reduction of the molecular weight. We observed this impact on the tensile strength of the polymer blends in two different ways; first, in the blends with the weight fractions PHBV/PLA/PLAgMA: 0.2/0.65/0.15, BR13, BR17, and BR9 and the blends with PHBV/PLA/PLAgMA: 0.65/0.2/0.15 which are BR6, BR15, and BR5, the tensile strength of BR9 and BR5 that contain PLA-*g*-MA type C is significantly higher than that of BR13 and BR6 with PLA-*g*-MA type A. According to the initial properties of PLA-*g*-MA type A, B, and C (refer to Table 4-1), a high % of MA grafting yield of PLA-*g*-MA type A could increase the chance of chain scission of PLA in the blending process, causing the overall molecular weight of the blended material to decrease resulting in lower tensile strength compared to the blends that contain PLA-*g*-MA with lower grafted MA. Second, in the group of BR8, BR16, and BR14 that have PHBV/PLA/PLAgMA: 0.75/0.2/0.05, BR8 with PLA-*g*-MA type A had a higher tensile strength compared to BR14 with PLA-*g*-MA type C. Reduced-molecular weight polymer chains due to the random chain scission that occurred at the interface between the blend components can promote the solubility and miscibility that resulted in better interfacial adhesion and improved tensile strength [2].

² The content ratio of the blend component is expressed as PHBV/PLA/PLA-*g*-MA where PHBV, PLA or PLA-*g*-MA represents the weight fraction of each component. The letter A, B, or C indicates the type of the compatibilizer PLA-*g*-MA.

The elongation at break of neat PLA sample was the highest among all samples, as shown in Figure 4-15b. Inclusion of 0.25 weight fraction of PHBV, sample BNR2, resulted in more than 50% reduction of the elongation at break compared to neat PLA. Addition of PLA-*g*-MA seemed unable to slow down this reduction as no significant difference can be observed in the samples BR4, BR10, BR13, BR12, BR3, and BR9 which have comparable content of PHBV and PLA compared to BNR2. The sample BR17 (0.2/0.65/0.15B) showed significantly higher elongation at break compared to BR13 and BR9, which both had the same content of PHBV and PLA with different types of PLA-*g*-MA of A and C, respectively. This could be attributed to the higher molecular weight of PLA-*g*-MA type B compared to the other two.

Regarding the elastic modulus, which indicates the stiffness of a material, our results did not demonstrate significant difference between samples that could suggest a clear pattern of an influence of the blend component contents, the addition of PLA-*g*-MA compatibilizer, and PLA-*g*-MA type on this characteristic. This could be because the impacts of these factors were quite weak, for example, the modulus of neat PLA and PHBV were not significantly different, therefore changing of their contents in each blend sample did not make a pronounced difference in the modulus of these blends. Additionally, the specimens we used in measuring the modulus were shorter than what is recommended in ASTM D882-18; this might cause our result to be affected by grip slippage occurring during the measurement.

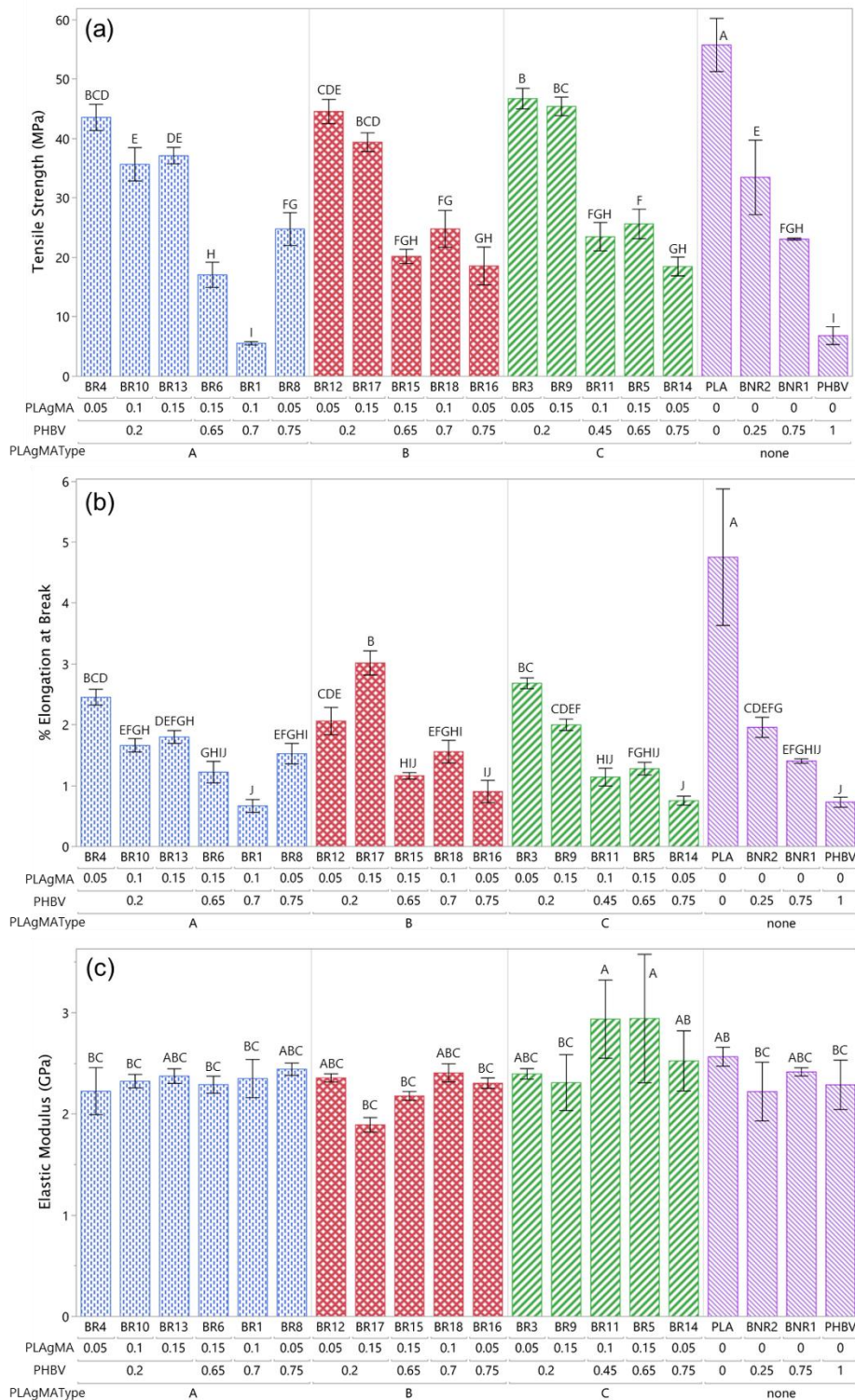


Figure 4-15 a) tensile strength, b) % elongation at break, and c) elastic modulus vs weight fraction of PHBV. The data are grouped by PLAGMA-Type and the error bar depicts one standard deviation. Levels not connected by same capital letter (above each bar) are significantly different ($\alpha=0.05$)

4.3.4 Factorial-Mixture Model of Tensile Properties

The result of least squares fit of the factorial-mixture model, Eq. 4-10, on the tensile strength, % elongation at break, and elastic modulus are shown in Table 4-3. ANOVA results indicate the significance of the model on all responses which means at least one parameter has a significant relationship to the responses. The R^2 suggests the predicted values of elastic modulus did not fit the experimental data as well as the ones of tensile strength and % elongation. Even though the lack of fit test of these responses appeared significant, this prediction model could be further used due to a good fit of R^2 .

As all mixture components need to sum to 1, the model itself is influenced by an increase or decrease of any component in the mixture. The coefficient of each variable indicates the magnitude of an influence that variable could have on a response of interest when there is a change of its fraction in the mixture. Weight fractions of PHBV, PLA, and PLAGMA or the mixture terms of these components are highly significant ($P < 0.01$) for all response predictions. Higher coefficients of PLA main effect in the tensile strength and % elongation evidently supported that increasing the fraction of PLA in the blend resulted in increased ductility of the blended materials. This is attributed to high tensile strength and elongation of the neat PLA compared to PHBV. The significance of both main effect and the interaction with PHBV and PLA, PLA-g-MA affected the change of the tensile strength and elongation.

While the interaction of *PLAGMA-type A* and *C* and their concentration showed a significant effect on the tensile strength, the concentration of *PLAGMA-type A* and *B* could affect the elongation. This agrees with our discussion earlier in 4.3.3 based on charts of the average of tensile strength and elongation at break, Figure 4-15a and b, that focused on the difference of the blend containing PLAGMA-type A and C for the tensile strength and the significant high elongation of the blend containing PLAGMA-type B. The coefficients of PHBV, $(PHBV-0.2)/0.55$, and PLA, $(PLA-0.2)/0.55$, content terms of the model predicting elastic modulus are similar; this reasonably explains why the difference of the modulus between samples was not very evident.

Table 4-3 Parameter estimates and summary of fit of factorial-mixture model for tensile properties

Term	Tensile Strength, MPa	%Elongation	Elastic Modulus, GPa
(PHBV-0.2)/0.55	20.572***	1.085***	2.425***
(PLA-0.2)/0.55	44.976***	2.375***	2.325***
(PLAgMA-0.05)/0.55	501.975***	24.000**	-3.504
PLAgMAType[A]*PHBV	-0.718	0.304***	-0.128
PLAgMAType[B]*PHBV	2.043	-0.167	-0.052
PLAgMAType[C]*PHBV	-1.325	-0.137	0.180**
PLAgMAType[A]*PLA	0.054	-0.027	0.010
PLAgMAType[B]*PLA	-1.025	-0.086	-0.001
PLAgMAType[C]*PLA	0.971	0.113	-0.009
PLAgMAType[A]*PLAgMA	-28.338***	-2.584***	0.171
PLAgMAType[B]*PLAgMA	1.617	3.119***	-1.408***
PLAgMAType[C]*PLAgMA	26.722***	-0.535	1.237**
PHBV*PLA	-26.462**	-1.521	1.645**
PHBV*PLAgMA	-586.045***	-26.896**	7.562
PLA*PLAgMA	-587.456***	-27.324**	6.223
ANOVA, Prob>F	<.0001***	<.0001***	<.0001***
R²	0.91	0.83	0.53
Lack of fit, Prob>F	<.0001***	<.0001***	0.0887

Note: ***, and ** indicate statistical significance at type I error (α) of 0.01, and 0.05, respectively.

Based on the obtained prediction models for our responses of interest, we could find the point of the mixture of the blend components, the content of PLA, PHBV, and PLAGMA-type with specific PLAGMA-type that provides the desired level of responses using the desirability function where a desirability function of a single response is a three-part piecewise smooth function, consisting of interpolating cubics between the user-defined control points, low, middle, and high, and exponentials in the tails. The overall desirability function that combines the desirability functions of multiple responses together can be used to maximize or minimize all responses of interest simultaneously. A detailed explanation regarding desirability functions can be found in section 3.3.5. In this part of our study, we considered the tensile strength together with the % elongation at break. The control points of the desirability function for each response are listed in Table 4-4.

Table 4-4 Control points of the desirability functions for tensile strength and % elongation at break responses

	<i>Tensile Strength, MPa</i>	<i>% Elongation at Break</i>	<i>Desirability</i>
<u>Control points</u>			
High	50	3.5	0.9819
Middle	25	2	0.50
Low	0	0.5	0.066

Table 4-5 and Figure 4-16 show the points of the mixture content of PLA, PHBV, and PLAGMA of different PLAGMA types with maximum desirability. The first three columns of Figure 4-16a, b, and c demonstrate the progression of the responses in relation to the content of PLA, PHBV, and PLAGMA, respectively; tensile strength and % elongation at break tend to be maximized with high content of PLA and low content of PHBV. Considering the confidence interval of the responses at all three points with maximum desirability, there are overlaps of the confidence intervals of different PLAGMA-types; this suggested comparable performance of different types of PLAGMA. Therefore, to make a decision to choose among PLAGMA types, other factors such as other properties relating to target applications of the resultant material can facilitate and scope down the selection criteria. Optimization of multiple responses can also be done using overlapped contour plots of the responses. This method was demonstrated in section 4.3.6.

Table 4-5 Mixture points with maximum desirability for tensile strength and % elongation at break with PLAGMA type A, B, and C

<u>Weight Fractions</u>										
PHBV	PLA	PLAGMA	PLAGMA Type	Tensile Strength (MPa)	Tensile Strength (MPa) Lower CI	Tensile Strength (MPa) Upper CI	% Elongation at Break	% Elongation at Break Lower CI	% Elongation at Break Upper CI	Desirability
0.2	0.75	0.05	A	45.030	41.497	48.564	2.348	2.077	2.619	0.689
0.2	0.65	0.15	B	40.132	36.492	43.772	2.739	2.460	3.018	0.752
0.2	0.75	0.05	C	45.947	42.160	49.734	2.489	2.198	2.779	0.728

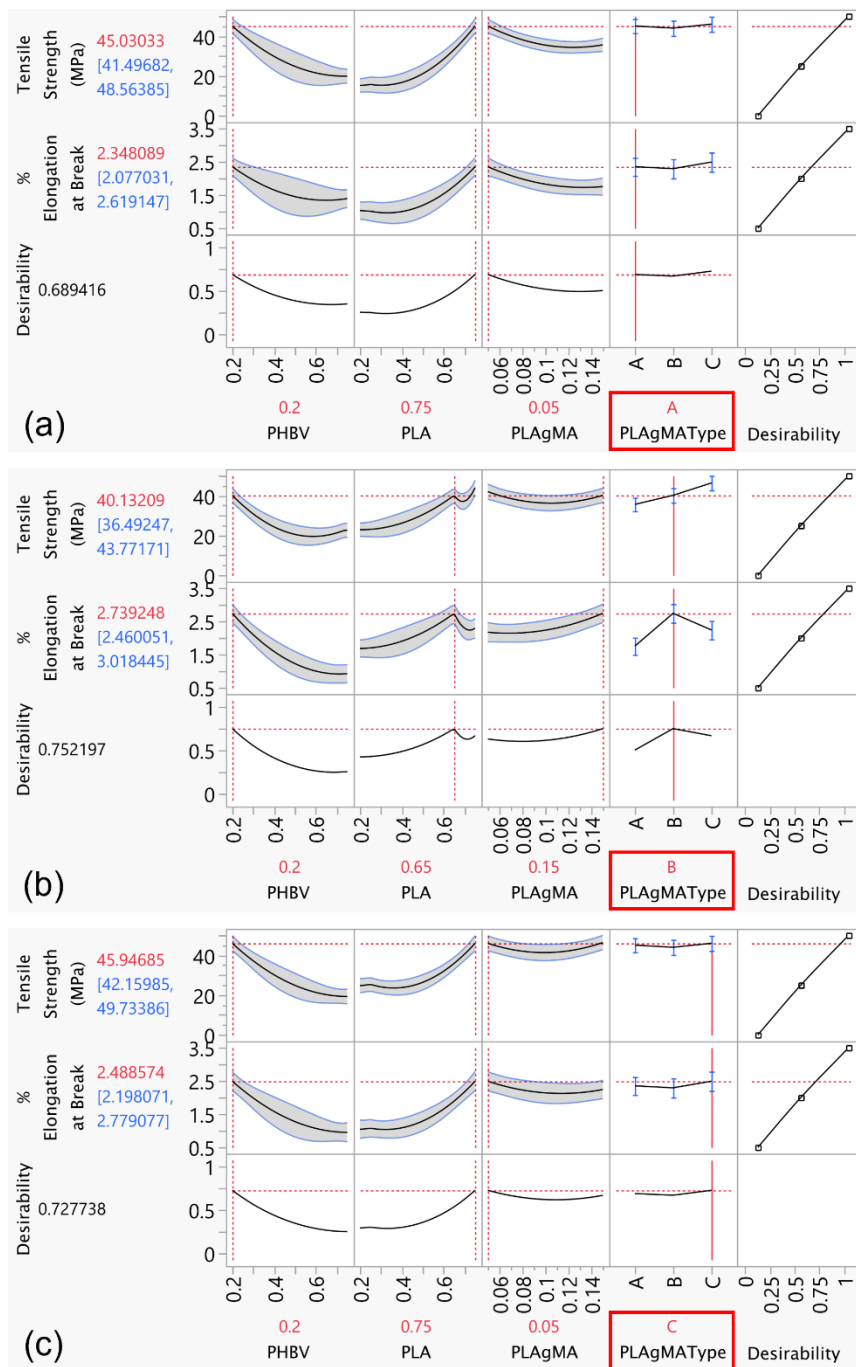


Figure 4-16 Prediction profiler based on the desirability of tensile strength and %elongation at break; (a) PLAgMAType=A, (b) PLAgMAType=B, and (c) PLAgMAType=C

4.3.5 Barrier Properties

Higher barrier performance is one of the advantages of PHBV compared to PLA. The barrier properties to water and O_2 of neat PHBV, neat PLA, and all PHBV/PLA blends are summarized in Figure 4-17 and the values of $P'O_2$ and $P'H_2O$ are listed in Table 4-12. PHBV shows the best barrier performance, whereas incorporation of more than 45% of PHBV contributed to more than 50% decrease of the permeability of water and O_2 compared to PLA. Inclusion of 25% of PHBV in the non-compatible blend, BNR2, resulted in the reduction of $P'O_2$ and $P'H_2O$ of PLA by 65 and 74%, comparatively higher than the reduction that occurred in compatibilized blends. For a comparable level of permeability reduction in compatibilized blends, about 45 to 65 wt % of PHBV needed to be added into the blends regardless of PLAGMA type. The better barrier performance of non-compatible blends compared to the compatibilized ones has also been reported in the studies of Zembouai et al [10], Jost and Kopitzky [2], and González-Ausejo et al. [16]. Interpenetrating spherulites that lead to interlamellar crystallization of one phase into the other as well as the change in the mobility of gases in the amorphous phases have been discussed as the reasons for this behavior [2].

Regarding the connection between the barrier properties and the crystallinity, the inverse relationship between the crystalline fraction and the permeability of water and O_2 can be observed (Figure 4-13 and Figure 4-17). In the big picture, a high crystalline fraction resulted in low permeability. However, considered closely, the trend of permeability change of each blend formulation does not draw a close tie to the crystallinity one on one; for example, among the three blends, BR11, BR5, and BR14, of PLAGMAType C, BR11 with the lowest crystalline fraction does not correspond to the highest permeability among the three, and BR14 with the highest crystallinity does not show the lowest permeability either. This indicates that there are a number of factors besides the crystallinity that partially influence the barrier properties of the blended materials – the blend and crystalline morphologies, polymer chain mobility, to name a few.

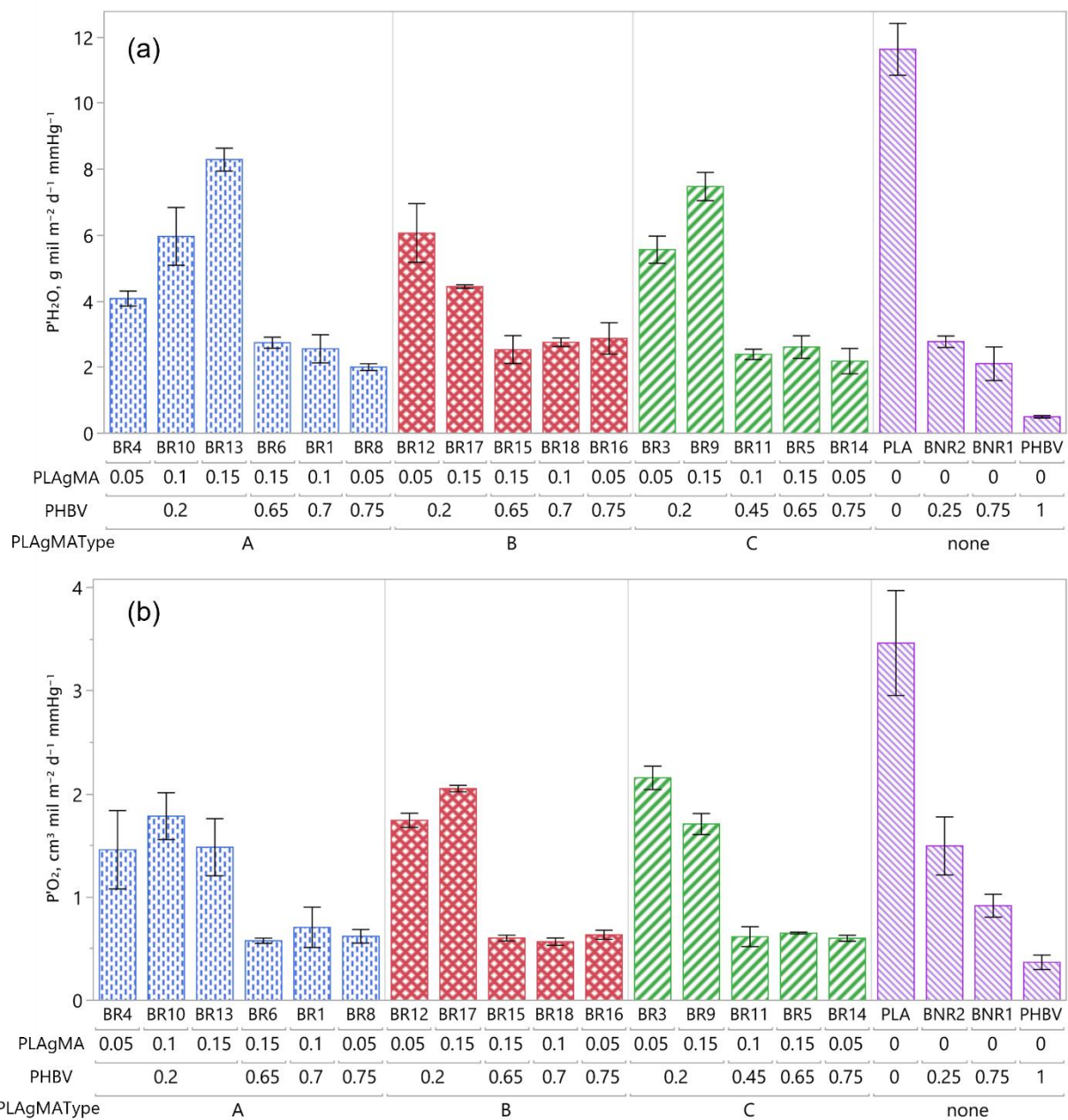


Figure 4-17 The means of a) $P'H_2O$ and b) $P'O_2$ grouped and ordered by PLAGMA-Type, PHBV and PLAGMA weight fractions. The data of neat polymer samples and non-compatible blends are also included.

4.3.6 Factorial-Mixture Model of Barrier Properties

The result of regression analysis according to Eq. 4-10 on the data of $P'H_2O$ and $P'O_2$ are in Table 4-6. The ANOVA indicates the significance of the relationship between at least one parameter in the model to the responses. The R^2 close to 1 on both responses suggests a good fit of the experimental data to the prediction of the model. At the same time, the lack of fit test of the model on both responses appeared to be significant. To solve the significance of lack of fit, the data of $P'H_2O$ and $P'O_2$ were transformed by log and reciprocal transformation, respectively. Parameter estimates from the regression of the transformed data and the summary of fit are also shown in Table 4-6.

The mixture terms of the weight fractions of PHBV and PLA, as well as their interaction, appear to be significant to both responses whereas the weight fraction of PLAGMA is not significant. The interaction terms between the factorial variable, *PLAGMA-Type*, and the mixture terms of the weight fractions of PHBV, PLA, and PLAGMA, are significant only to Log ($P'H_2O$) when the *PLAGMA-Type* is A or B.

Table 4-6 Parameter estimates and summary of fit of factorial-mixture model for barrier properties

Term	$P'H_2O$	Log ($P'H_2O$)	$P'O_2$	Reciprocal ($P'O_2$)
(PHBV-0.2)/0.55	2.381***	0.840***	0.600***	1.631***
(PLA-0.2)/0.55	5.210***	1.635***	1.806***	0.577***
(PLAGMA-0.05)/0.55	5.426	-2.333	-11.013	7.401
PLAgMAType[A]*PHBV	-0.769***	-0.188***	0.008	-0.031
PLAgMAType[B]*PHBV	0.984***	0.251***	-0.111	0.056
PLAgMAType[C]*PHBV	-0.215	-0.063	0.102	-0.025
PLAgMAType[A]*PLA	-0.776***	-0.187***	-0.276***	0.093
PLAgMAType[B]*PLA	0.403	0.113**	0.026	-0.040
PLAgMAType[C]*PLA	0.373	0.074	0.250***	-0.053
PLAgMAType[A]*PLAGMA	7.145***	1.500***	-0.053	0.198
PLAgMAType[B]*PLAGMA	-7.966***	-1.696***	0.842	-0.073
PLAgMAType[C]*PLAGMA	0.821	0.196	-0.788	-0.125
PHBV*PLA	-9.562***	-2.464***	-3.755***	3.056***
PHBV*PLAGMA	-1.822	4.716	14.110	-6.909
PLA*PLAGMA	11.754	6.766	15.432	-8.327
ANOVA, Prob>F	<.0001***	<.0001***	<.0001***	<.0001***
R²	0.92	0.94	0.94	0.94
Lack of fit, Prob>F	0.0043***	0.3355	0.0063***	0.0547

Note: ***, and ** indicate statistical significance at type I error (α) of 0.01, and 0.05, respectively.

Figure 4-18 depicts the mixture profiler where the contour plots of $P'H_2O$ and $P'O_2$ predicted by the models are displayed on the ternary mixture plot of the three components of the polymer blends. The plots with different PLAGMAType are presented separately. While the blue lines, blue dots, and blue shaded area represent the values of $P'H_2O$, the contour plot components of $P'O_2$ are in red. As dots next to the contour lines indicate the direction of higher value of the prediction, we can see that preferable low value of $P'H_2O$ and $P'O_2$ tend to point to the combination of low concentration of PLAGMA and high fraction of PHBV in all types of PLAGMA. The mixture profiler can be utilized in tailoring a polymer blend with desirable properties. For example, based on the mixture profiler we obtained, in the situation that we have only PLAGMA type B and the $P'H_2O$ and $P'O_2$ values of below $2.30 \text{ g}\cdot\text{mil}\cdot\text{m}^2\cdot\text{d}^{-1}\cdot\text{mmHg}^{-1}$ and $0.55 \text{ cm}^3\cdot\text{mil}\cdot\text{m}^2\cdot\text{d}^{-1}\cdot\text{mmHg}^{-1}$, respectively, are the desirable levels of barrier properties, the possible range of the blend component contents is the overlapped blue and red shaded area, B1 as highlighted with a green border in Figure 4-18, PLAGMAType = B. Green points, B1a, B1b, and B1c, represent examples of the blend contents, PHBV/PLA/PLAGMATypeB, that could give the aforementioned desirable barrier properties: B1a (0.5/0.45/0.05), B1b (0.55/0.40/0.05), and B1c (0.60/0.325/0.075).

The bullseye on the mixture profiler of PLAGMAType = A shows the optimum point obtained by the desirability method that results in the lowest combination of $P'H_2O$ and $P'O_2$. The result of the desirability analysis is shown in Figure 4-19. The content of PHBV/PLA/PLAGMATypeA at 0.55/0.40/0.05 results in the desirability closest to 1 which corresponds to the values of $P'H_2O$ and $P'O_2$ at $1.45 \text{ g}\cdot\text{mil}\cdot\text{m}^2\cdot\text{d}^{-1}\cdot\text{mmHg}^{-1}$ and $0.51 \text{ cm}^3\cdot\text{mil}\cdot\text{m}^2\cdot\text{d}^{-1}\cdot\text{mmHg}^{-1}$, respectively.

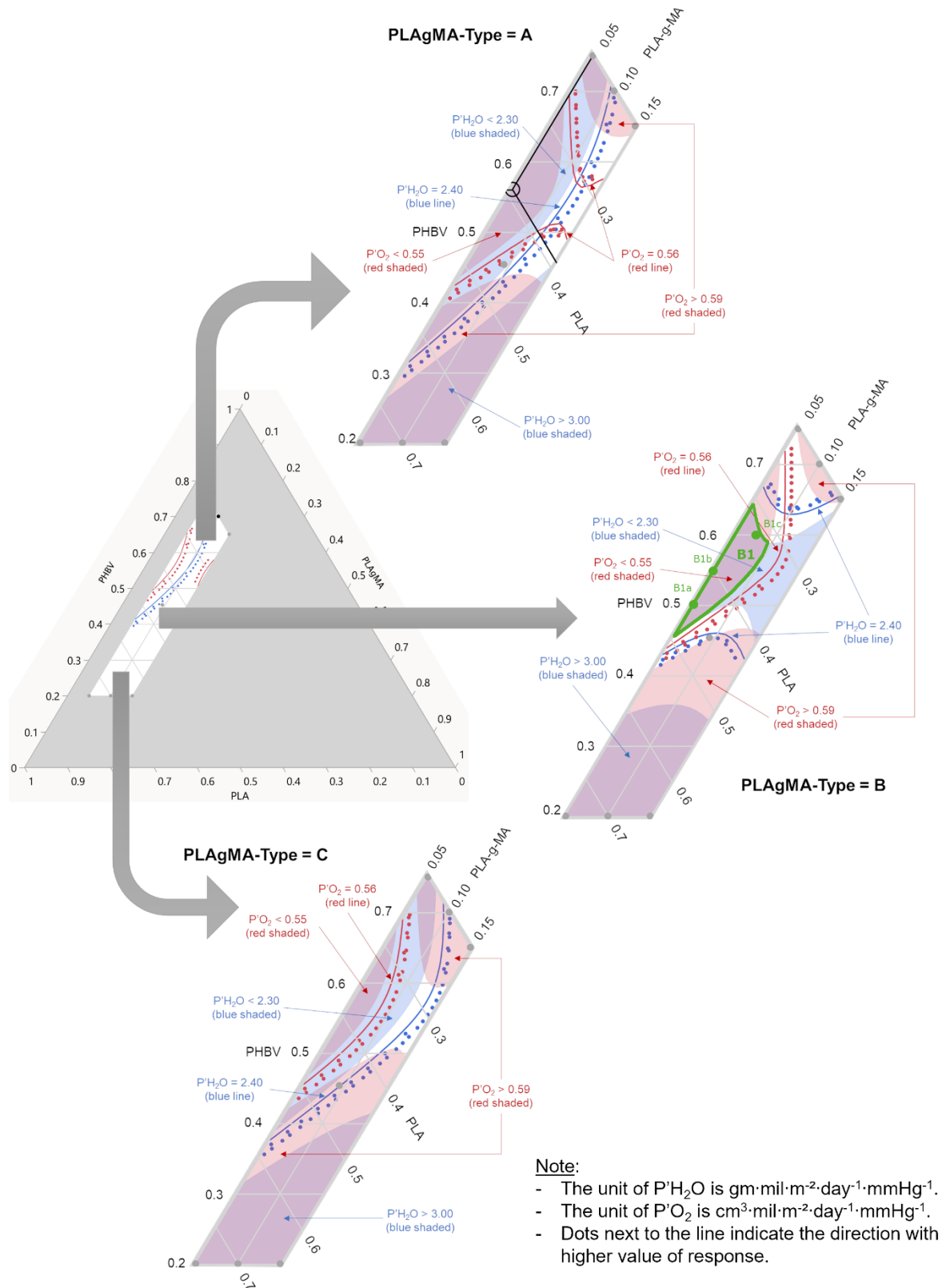


Figure 4-18 Mixture profiler of $P'H_2O$ and $P'O_2$ responses separated by PLAGMA-Type variable.

The results of an optimization $P'H_2O$ and $P'O_2$ responses using the desirability functions with the control points as shown in Table 4-7 are demonstrated in Table 4-8 and Figure 4-19.

Table 4-7 Control points of the desirability functions for $P'H_2O$ and $P'O_2$ responses

	$P'H_2O$	$P'O_2$	Desirability
<u>Control points</u>			
High	9.0	2.5	0.066
Middle	5.0	1.5	0.50
Low	1.0	0.5	0.9819

Note:

- The unit of $P'H_2O$ is $g \cdot mil \cdot m^2 \cdot d^{-1} \cdot mmHg^{-1}$.

- The unit of $P'O_2$ is $cm^3 \cdot mil \cdot m^2 \cdot d^{-1} \cdot mmHg^{-1}$.

Table 4-8 Mixture points with maximum desirability for $P'H_2O$ and $P'O_2$ with PLAGMA type A, B, and C

Weight Fractions			PLAgMA Type	P'O ₂	P'O ₂		P'H ₂ O	P'H ₂ O		Desirability
PHBV	PLA	PLAgMA			Lower CI	Upper CI		Lower CI	Upper CI	
0.562	0.388	0.050	A	0.508	0.600	0.440	1.448	1.118	1.875	0.944
0.554	0.396	0.050	B	0.505	0.596	0.439	2.139	1.656	2.762	0.886
0.577	0.373	0.050	C	0.519	0.594	0.462	1.714	1.396	2.105	0.920

Note:

- The unit of $P'H_2O$ is $g \cdot mil \cdot m^2 \cdot d^{-1} \cdot mmHg^{-1}$.

- The unit of $P'O_2$ is $cm^3 \cdot mil \cdot m^2 \cdot d^{-1} \cdot mmHg^{-1}$.

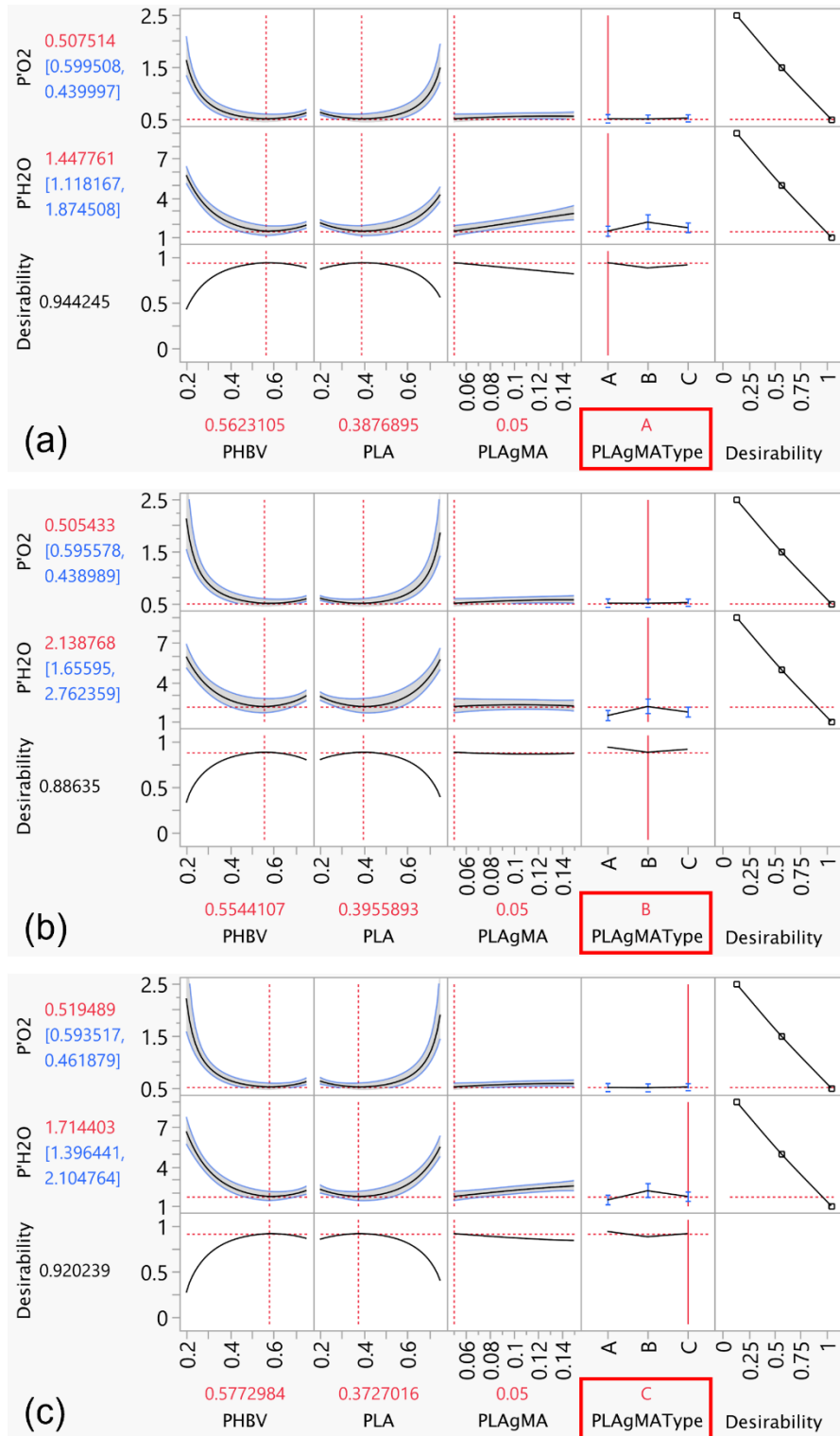


Figure 4-19 Prediction profiler based on the desirability of $P'O_2$ and $P'H_2O$; (a) PLAGMAType=A, (b) PLAGMAType=B, and (c) PLAGMAType=C

4.4 Conclusions

This study was performed to investigate the properties of PHBV/PLA polymer blends, which were compatibilized using maleated PLA or PLAGMA. The compatibilized blends were produced based on the mixture experimental design with a three-component mixture of PHBV, PLA, and PLAGMA where the weight fractions of pure PHBV and PLA were varied between 0.20 – 0.75, and PLAGMA was between 0.05 – 0.15. The chemical properties assessed using FTIR analysis as well as the enthalpies of fusion obtained from the DSC demonstrated significant increase in the crystalline fractions in the polymer blends compared to neat PLA. The % crystallinity of PLA increased with increasing weight fraction of PHBV in the blends. The miscibility between the blend components was exhibited through the shifting of T_g of PLA and the decrease of k constants based on the Gordon-Taylor equation of the compatibilized blends compared to the one obtained from the non-compatibilized blended materials. According to the TGA results, the thermal stability of PHBV was improved as PLA was included in the blends.

Tensile strength and elongation at break of the polymer blends were influenced by the original properties of the base polymers, PLA and PHBV, where neat PLA had the highest tensile strength and elongation at break, and neat PHBV had the lowest values. In non-compatibilized polymer blends, inclusion of 0.25 and 0.75 weight fraction of PLA resulted in 200 to 300% increase of the tensile strength compared to neat PHBV. A comparable level of improvement could be achieved with less than 0.45 weight fraction of PLA together with an addition of PLAGMA regardless of PLAGMA-type used in the blends. This was attributed to the effect of PLAGMA that promoted the interfacial adhesion between the blend components. The impact of PLAGMA addition on elongation at break did not exhibit clearly, as most of the compatibilized blend samples did not show significant differences compared to the non-compatibilized ones. Significant differences of elastic modulus between the blend samples were not observed from the average measurement results; the pattern of an influence of component contents, and PLAGMA type was difficult to determine. However, the factorial-mixture model, that takes into account the effect of PLAGMA type and the mixture terms of PHBV, PLA, and PLAGMA contents, to predict the tensile strength, % elongation at break, as well as the elastic modulus of the polymer blends indicated validity of these factors on the responses. The overall desirability function based on the prediction model demonstrated an optimization of multiple responses and suggested the points of maximum desirability for

maximizing tensile strength and elongation at break at the PHBV/PLA/PLAgMA weight fractions of 0.2/0.75/0.05, 0.2/0.65/0.15, and 0.2/0.75/0.05 for PLAGMA-type A, B, and C, respectively.

Regarding the barrier properties, the water and O₂ permeability were decreased more than 60% with an inclusion of PHBV at 25 wt. % in the non-compatibilized blend and at 45 – 60 wt. % in compatibilized blends. Overall improvement in the barrier properties compared to pure PLA was related to the increase in the crystalline fraction in the polymer blends. Modelling of barrier properties using the combination of the mixture variables of the blend components and the factorial variable of PLAGMA types indicated valid influences of PHBV and PLA content, as well as their interaction on both water and O₂ permeability. The impact of different types of PLAGMA was significant only in the model predicting water permeability. The contour plots of the prediction of water and O₂ permeability could be utilized to generate polymer blends with desirable barrier properties.

APPENDIX

Table 4-9 Temperature of decomposition at 5%, 20%, 50%, 80%, and 95% weight loss of neat polymer samples, and PHBV/PLA blends

PLAgMA Type	Blend ID	PHBV	PLA	PLAgMA	$T_5, ^\circ\text{C}$		$T_{20}, ^\circ\text{C}$		$T_{50}, ^\circ\text{C}$		$T_{80}, ^\circ\text{C}$		$T_{95}, ^\circ\text{C}$	
					Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
none	nPLA	0	1	0	329.4	4.1	348.0	3.8	363.0	3.3	373.9	2.8	380.5	2.8
none	nPHBV	1	0	0	241.8	0.3	257.0	0.6	267.0	0.5	272.7	0.5	275.7	0.8
none	BNR1	0.75	0.25	0	253.0	2.1	266.4	3.0	276.6	2.2	303.3	2.0	318.9	0.1
none	BNR2	0.25	0.75	0	258.4	4.2	279.8	3.8	321.4	6.6	343.3	7.9	369.0	29.2
A	BR8	0.75	0.2	0.05	244.4	2.3	258.1	1.9	269.4	1.0	286.9	2.9	306.5	1.2
A	BR1	0.7	0.2	0.1	232.3	3.2	248.2	3.4	260.9	3.1	278.1	4.0	295.4	4.4
A	BR6	0.65	0.2	0.15	249.0	1.5	262.4	1.7	273.6	2.1	298.1	3.7	313.4	4.8
A	BR4	0.2	0.75	0.05	274.1	2.0	290.0	1.7	320.4	4.0	343.5	1.0	355.5	1.4
A	BR10	0.2	0.7	0.1	275.2	3.7	290.6	2.5	318.7	7.1	341.6	5.4	353.8	4.6
A	BR13	0.2	0.65	0.15	272.7	6.2	287.8	5.2	311.8	5.3	332.0	8.4	344.4	8.5
B	BR16	0.75	0.2	0.05	242.8	1.6	256.7	1.4	267.7	1.2	283.2	0.9	299.6	1.6
B	BR18	0.7	0.2	0.1	246.1	6.5	260.2	5.6	272.0	4.0	290.5	4.9	309.2	4.1
B	BR15	0.65	0.2	0.15	260.2	3.7	271.4	2.4	281.1	2.2	309.6	4.2	324.0	3.7
B	BR12	0.2	0.75	0.05	274.1	1.8	289.6	1.1	317.7	1.0	332.8	0.8	341.2	1.7
B	BR17	0.2	0.65	0.15	278.3	1.0	292.8	0.6	317.5	4.1	333.7	4.1	341.5	4.0
C	BR14	0.75	0.2	0.05	256.4	4.0	267.6	3.2	277.1	2.5	299.5	3.3	319.8	4.4
C	BR5	0.65	0.2	0.15	267.3	1.3	276.4	1.3	286.0	1.5	322.4	4.8	335.7	4.5
C	BR11	0.45	0.45	0.1	268.7	6.7	280.8	4.4	310.3	8.0	345.1	7.0	354.0	10.9
C	BR3	0.2	0.75	0.05	275.1	3.0	290.0	1.5	317.3	0.6	331.2	0.2	339.0	0.9
C	BR9	0.2	0.65	0.15	285.9	1.2	302.8	1.0	345.6	5.5	362.3	5.4	370.4	6.0

Table 4-10 T_g , T_c , and temperature at melting peaks (T_m) of neat polymer samples, and PHBV/PLA blend samples

PLAgMA Type	ID	Weight Fraction			T_g , °C	T_c , °C	Melting		
		PHBV	PLA	PLAgMA			T_{m-PLA} , °C	$T_{m-PHBV-1}$, °C	$T_{m-PHBV-2}$, °C
none	nPHBV	1	0	0	n/a	n/a	n/a	155.2 ± 0.2	163.6 ± 0.1
none	nPLA	0	1	0	61.7 ± 0.2	117.7 ± 0.1	150.8 ± 0.1	n/a	n/a
C	BR11	0.45	0.45	0.1	55.7 ± 0.0	119.2 ± 0.1	148.9 ± 0.0	165.1 ± 0.0	171.2 ± 0.1
PHBV-rich blends									
none	BNR1	0.75	0.25	0	56.3 ± 0.6	119.2 ± 0.3	149.2 ± 0.2	160.3 ± 0.3	166.2 ± 0.3
A	BR8	0.75	0.2	0.05	56.0 ± 0.1	118.8 ± 0.2	148.2 ± 0.1	165.6 ± 0.1	
	BR1	0.7	0.2	0.1	51.8 ± 1.1	114.2 ± 0.3	145.8 ± 0.3	159.6 ± 0.3	167.2 ± 0.4
	BR6	0.65	0.2	0.15	55.9 ± 0.6	118.5 ± 0.3	148.6 ± 0.4	165.7 ± 0.4	
B	BR16	0.75	0.2	0.05	56.0 ± 0.6	118.7 ± 0.5	148.4 ± 0.2	165.3 ± 0.4	171.2 ± 0.1
	BR18	0.7	0.2	0.1	54.9 ± 0.6	117.8 ± 0.3	148.0 ± 0.3	165.4 ± 0.4	170.6 ± 0.2
	BR15	0.65	0.2	0.15	54.6 ± 0.6	116.3 ± 0.3	147.6 ± 0.2	164.7 ± 0.5	170.5 ± 0.3
C	BR14	0.75	0.2	0.05	56.2 ± 0.0	117.4 ± 0.2	148.1 ± 0.3	164.3 ± 0.2	170.6 ± 0.2
	BR5	0.65	0.2	0.15	54.6 ± 0.5	117.1 ± 0.5	147.6 ± 0.0	164.8 ± 0.2	170.7 ± 0.1
PLA-rich blends									
none	BNR2	0.25	0.75	0	55.8 ± 0.3	118.2 ± 0.2	149.6 ± 0.3	162.5 ± 0.1	171.2 ± 0.1
A	BR4	0.2	0.75	0.05	57.8 ± 0.4	125.8 ± 0.5	151.0 ± 0.0	166.2 ± 0.5	
	BR10	0.2	0.7	0.1	56.3 ± 0.2	120.7 ± 0.3	150.6 ± 0.2	163.5 ± 0.2	171.8 ± 0.1
	BR13	0.2	0.65	0.15	54.8 ± 0.4	117.7 ± 0.5	149.7 ± 0.2	161.9 ± 0.2	170.8 ± 0.1
B	BR12	0.2	0.75	0.05	57.2 ± 0.4	124.8 ± 0.3	150.8 ± 0.2	164.5 ± 0.2	
	BR17	0.2	0.65	0.15	56.3 ± 0.6	122.2 ± 1.1	140.0 ± 0.2	163.2 ± 0.4	170.7 ± 0.1
C	BR3	0.2	0.75	0.05	57.7 ± 0.2	125.8 ± 0.5	150.6 ± 0.2	165.4 ± 0.5	171.0 ± 0.1
	BR9	0.2	0.65	0.15	56.6 ± 0.4	122.0 ± 0.8	150.5 ± 0.2	164.4 ± 0.2	171.3 ± 0.1

Table 4-11 ΔH_c , ΔH_m , and X_c of neat polymer samples, and PHBV/PLA blend samples

PLAgMA Type	ID	Weight Fraction			TA analysis integration		Fityk – Pearson 7A: Melting Peak Deconvolution			X_c , %	
		PHBV	PLA	PLAgMA	ΔH_c , J g ⁻¹	ΔH_m , J g ⁻¹	ΔH_{m-PLA} , J g ⁻¹	$\Delta H_{m-PHBV1}$, J g ⁻¹	$\Delta H_{m-PHBV2}$, J g ⁻¹	PLA	PHBV
none	nPHBV	1	0	0	n/a	84.8 ± 0.7	n/a	n/a	n/a	n/a	58.1 ± 0.5
none	nPLA	0	1	0	25.2 ± 0.7	24.4 ± 0.5	n/a	n/a	n/a	-0.9 ± 0.3	n/a
C	BR11	0.45	0.45	0.1	13.6 ± 0.2	52.7 ± 4.9	24.0 ± 1.1	26.7 ± 0.8	2.0 ± 0.2	20.5 ± 1.7	43.7 ± 1.5
PHBV-rich blends											
none	BNR1	0.75	0.25	0	5.5 ± 0.4	63.0 ± 4.6	19.9 ± 1.6	41.6 ± 2.6	1.5 ± 0.7	62.1 ± 8.2	39.4 ± 2.7
A	BR8	0.75	0.2	0.05	7.2 ± 0.4	75.0 ± 0.4	25.6 ± 1.6	47.7 ± 2.1	1.6 ± 0.8	80.4 ± 8.5	45.1 ± 1.2
	BR1	0.7	0.2	0.1	6.7 ± 0.3	73.0 ± 3.0	25.8 ± 1.1	35.6 ± 4.1	11.7 ± 0.6	69.9 ± 4.3	46.2 ± 3.5
	BR6	0.65	0.2	0.15	10.0 ± 0.8	60.3 ± 3.7	21.0 ± 1.4	37.2 ± 1.9	2.2 ± 0.4	34.7 ± 3.6	41.5 ± 2.5
B	BR16	0.75	0.2	0.05	6.4 ± 0.4	67.7 ± 1.1	22.6 ± 0.7	42.5 ± 1.0	2.6 ± 0.7	70.5 ± 4.6	41.2 ± 0.4
	BR18	0.7	0.2	0.1	8.0 ± 0.4	64.4 ± 1.8	24.0 ± 0.9	41.1 ± 1.8	1.3 ± 0.1	58.2 ± 3.1	41.5 ± 1.9
	BR15	0.65	0.2	0.15	8.7 ± 0.6	64.9 ± 2.4	24.4 ± 1.3	37.8 ± 1.9	2.7 ± 0.2	49.1 ± 2.3	42.7 ± 2.1
C	BR14	0.75	0.2	0.05	5.4 ± 1.1	68.2 ± 4.9	22.0 ± 0.8	40.1 ± 1.9	6.1 ± 2.8	71.6 ± 8.1	42.2 ± 8.8
	BR5	0.65	0.2	0.15	10.4 ± 1.3	64.6 ± 3.6	23.7 ± 2.0	38.6 ± 2.0	2.3 ± 0.3	41.6 ± 10.3	43.1 ± 1.8
PLA-rich blends											
none	BNR2	0.25	0.75	0	28.1 ± 2.3	35.9 ± 1.0	26.1 ± 0.6	5.1 ± 0.3	4.7 ± 0.1	-2.8 ± 2.5	26.8 ± 0.9
A	BR4	0.2	0.75	0.05	16.3 ± 1.1	21.0 ± 2.6	11.4 ± 1.5	7.8 ± 1.1	1.8 ± 0.2	-6.6 ± 0.6	32.7 ± 4.0
	BR10	0.2	0.7	0.1	28.6 ± 0.8	34.1 ± 1.6	23.3 ± 1.3	6.4 ± 0.1	4.4 ± 0.4	-7.1 ± 2.6	36.9 ± 1.0
	BR13	0.2	0.65	0.15	29.4 ± 1.5	40.2 ± 3.7	27.5 ± 1.0	6.9 ± 2.3	5.8 ± 0.7	-2.6 ± 3.5	43.7 ± 9.1
B	BR12	0.2	0.75	0.05	18.8 ± 1.9	23.6 ± 1.3	14.0 ± 1.0	7.4 ± 0.4	2.2 ± 0.2	6.4 ± 1.3	32.8 ± 1.5
	BR17	0.2	0.65	0.15	19.2 ± 2.6	28.5 ± 3.1	17.6 ± 2.4	7.2 ± 0.2	3.7 ± 0.6	-2.2 ± 6.8	37.4 ± 2.2
C	BR3	0.2	0.75	0.05	13.8 ± 1.1	21.3 ± 2.2	11.7 ± 1.2	7.5 ± 0.7	2.1 ± 0.2	-2.8 ± 0.3	32.8 ± 3.3
	BR9	0.2	0.65	0.15	22.3 ± 2.3	32.1 ± 3.2	22.7 ± 2.8	6.1 ± 0.2	3.8 ± 0.6	-0.2 ± 1.7	34.0 ± 2.0

Table 4-12 Average O_2 and H_2O permeability coefficients ($P'O_2$ and $P'H_2O$) of the blend samples

PLAgMA Type	Blend ID	Content Ratio			$P'O_2$		$P'H_2O$	
		PHBV	PLA	PLA-g-MA	$cm^3 \text{ mil m}^{-2} \text{ d}^{-1} \text{ mmHg}^{-1}$		$g \text{ mil m}^{-2} \text{ d}^{-1} \text{ mmHg}^{-1}$	
					Mean	Std Dev	Mean	Std Dev
A (MA:DCP 7:0.5)	BR1	0.70	0.20	0.10	0.7	0.2	2.6	0.4
	BR4	0.20	0.75	0.05	1.5	0.4	4.1	0.2
	BR6	0.65	0.20	0.15	0.6	0.0	2.7	0.2
	BR8	0.75	0.20	0.05	0.6	0.1	2.0	0.1
	BR10	0.20	0.70	0.10	1.8	0.2	6.0	0.9
	BR13	0.20	0.65	0.15	1.5	0.3	8.3	0.4
B (MA:DCP 4.5:0.5)	BR12	0.20	0.75	0.05	1.7	0.1	6.1	0.9
	BR15	0.65	0.20	0.15	0.6	0.0	2.5	0.4
	BR16	0.75	0.20	0.05	0.6	0.0	2.9	0.5
	BR17	0.20	0.65	0.15	2.0	0.0	5.3	1.5
	BR18	0.70	0.20	0.10	0.6	0.0	2.8	0.1
C (MA:DCP 2:0.5)	BR3	0.20	0.75	0.05	2.2	0.1	5.6	0.4
	BR5	0.65	0.20	0.15	0.6	0.0	2.6	0.3
	BR9	0.20	0.65	0.15	1.7	0.1	7.5	0.4
	BR11	0.45	0.45	0.10	0.6	0.1	2.4	0.2
	BR14	0.75	0.20	0.05	0.6	0.0	2.2	0.4
none	BNR1	0.75	0.25	0.00	0.9	0.1	2.1	0.5
	BNR2	0.25	0.75	0.00	1.2	0.5	2.7	0.1
	PHBV	1.00	0.00	0.00	0.4	0.1	0.8	0.6
	PLA	0.00	1.00	0.00	3.5	0.5	11.6	0.8

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5 IMPACT OF COMPATIBILIZER ON THE BIODEGRADATION OF POLY(LACTIC ACID) AND POLY(3-HYDROXYBUTYRATE-CO-HYDROXYVALERATE) BLENDS

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5.1 Introduction

Renewable biobased, biodegradable, and compostable polymeric materials have been gaining interest from consumers and product manufacturers due to rising environmental awareness regarding conventional petroleum-based polymers and the use of single-use plastics [1,2]. Poly(lactic acid) (PLA) and polyhydroxyalkanoates (PHAs) are well-known biobased-biodegradable materials. While PLA is a plant-based polymer derived from renewable sources, such as corn sugar, potato, and sugar cane, and normally biodegraded in thermophilic condition with high humidity, 58 °C with 58% RH to be specific [3]; PHA is a family of biobased microbially synthesized polymer materials with a reputation of superior biodegradability at mesophilic conditions. From the PHA family, poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) are the most popular. PHBV has properties comparable to utility polymers such as polypropylene (PP). However, due to its high production cost, the use of PHBV is currently limited mostly to biomedical applications [2,4,5].

Polymer blending is a feasible approach to lower costs and improve properties, broadening the field of applications to more general markets such as food and medical packaging and agriculture. PLA can be blended with PHBV, bringing down the net cost of resulting blend while improving properties such as mechanical and thermal, among others. Improvement in performance such as reduction in the crystallinity of PHBV and improvement in the gas barrier properties of PLA can also be anticipated. Moreover, the compostability of both materials is preserved.

Reactive compatibilization of polymers is an inexpensive method to generate a new polymeric material combining outstanding properties of the polymers being blended [6]. Properties of the polymer blend generally depend on the intrinsic properties and the concentration of the blend components. Moreover, they are influenced by the miscibility of the blend components. Blends of PLA and PHBV are

generally immiscible [7–12]. Compatibilization by adding maleated PHBV or adding diisocyanates as a reactive initiator in the blending process have both been reported to improve the miscibility of PLA/PHBV blends [13,14]. The use of the compatibilizer enhances the overall mechanical and barrier properties of the blends. Maleated PLA (PLA-*g*-MA) can likewise potentially be used to compatibilize PLA/PHBV blends. The process of reactive maleation of PLA to optimize the amount of grafted maleic anhydride and molecular weight of PLA-*g*-MA has been well studied [15]. Since these factors influence the degree of compatibility between the blend components that consequently affect the performance of the final blended materials, knowing how to control them facilitates tailoring the properties in blends that use PLA-*g*-MA as a compatibilizer. PLA-*g*-MA has been studied in the blending of PLA and other biobased materials such as thermoplastic cassava starch [16,17].

In the development and modification of biodegradable polymer materials like the blending of PLA and PHBV, to improve the overall properties for meeting specific applications, it is also critical to verify the biodegradability of the final blend. Even though the biodegradation of PLA and PHBV as a single polymer, and the modified materials based on either of them were widely studied, there is still a gap of knowledge in the biodegradation mechanism of the PHBV/PLA blend, as well as the impact of compatibilization on the biodegradation process. Each of these blend components is known to follow different pathways during its biodegradation process. While the biodegradation of PLA is initiated mainly by abiotic hydrolytic chain scission, PHBV relies on microbial enzymatic hydrolysis [1,18–20]. Therefore, the biodegradation process in the blend structure and in the effect of the PLA-*g*-MA compatibilizer should be further understood to create blends for different end of life scenarios such as home and industrial composting or agriculture applications. Hence, this study aims to elucidate the impact of the PLA-*g*-MA used as a compatibilizer in the blend of PLA and PHBV on the aerobic biodegradation process through a comparison study based on the neat polymers, compatibilized, and non-compatibilized blends.

5.2 Experimental

5.2.1 Material Preparation

Materials used in this experiment included: 1) neat PLA (PLA), 2) neat PHBV (PHBV), 3) non-compatibilized PLA/PHBV blend (75:25) (PLA/PHBV), and 4) PLA/PHBV blend compatibilized with maleic

anhydride-grafted PLA (PLA/PLA-*g*-MA/PHBV). The material composition is listed in Table 5-1. PLA (Ingeo™ Biopolymer 2003D) was obtained from NatureWorks LLC. (Minnetonka, MN, USA). PHBV (TianAn ENMAT™ Y1000P resin) was from TianAn Biologic Materials Co., Ltd. (Beilun Port, Ningbo City, PRC). Maleic anhydride (MA), and dicumyl peroxide (DCP) were purchased from MilliporeSigma (St. Louis, MO, USA) and used as received. Cellulose (powder) was used as a positive control for the biodegradation studies and obtained from MilliporeSigma (St. Louis, MO, USA).

Table 5-1 Component contents of tested materials.

Sample ID	Blend component content (wt. %)			PLA- <i>g</i> -MA component content (wt. %)		
	PLA	PHBV	PLA- <i>g</i> -MA	PLA	MA	DCP
PLA	100	-	-	-	-	-
PHBV	-	100	-	-	-	-
PLA/PHBV	75	25	-	-	-	-
PLA/PLA-<i>g</i>-MA/PHBV	65	20	15	93	6.5	0.5

The compatibilizer, maleic anhydride-grafted PLA (PLA-*g*-MA), was pre-produced by compounding in a Century ZSK-30 twin-screw co-rotating extruder (Traverse City, MI, USA). Before processing, PLA was dried at 60 °C for 24 h to remove excess moisture. PLA was then manually tumble-mixed with DCP and MA. The mixture was fed into the extruder using a mechanical feeder set at a rate of 70 g min⁻¹. Ten temperature zones of the barrel were set using the following temperature profile: 140/150/160/160/160/170/170/170/170/160 °C. The screw speed was set at 120 rpm. The extrudate was cooled in a water bath, then pelletized in a BT 25 pelletizer (Scheer Bay Co., Bay City, MI, USA). The pellets were dried at 60 °C for 24 h then stored in a freezer (c. -18 °C) for further processing.

In the blending process, PLA, PHBV and PLA-*g*-MA pellets were dried for 24 h at 65 °C before processing. The Century ZSK-30 twin-screw co-rotating extruder was also used for blending with the feeder rate of 160 rpm, barrel temperature profile of 95/140/160/170/170/170/170/185/165/160 °C, and screw speed of 100 rpm. The extrudate was cooled and pelletized as described in the processing of PLA-*g*-MA, then dried to remove excess water before storage. The PLA sample was compounded using the same processing parameters as the blend samples. The samples PLA, PHBV, PLA/PHBV, and PLA/PLA-*g*-MA/PHBV were fabricated into a thin sheet form using an M CARVER compression molder (Carver Laboratory Press, Menomonee Falls, WI). The average thickness of each sample was: PLA (0.227 ±

0.012 mm), PHBV (0.146 ± 0.033 mm), PLA/PHBV (0.191 ± 0.014 mm), and PLA/PLA-*g*-MA/PHBV (0.190 ± 0.025 mm).

5.2.2 Biodegradation Test

Carbon content of the test samples was determined by a Perkin Elmer CHN analyzer model 2400 (Waltham, Mass., USA) followed a method described elsewhere [18]. Samples were later cut into small squares of 1 cm x 1 cm to be used in the biodegradation tests.

The biodegradation test was conducted using an in-house built Direct Measurement Respirometric system (DMR) where the CO₂ evolved from the biodegradation of the material was measured. Detailed information about the system and calculation method can be found in the work of Castro-Aguirre [21]. Briefly, the main components of the DMR system include: 1) *environmental chamber* controlling the temperature surrounding the bioreactors, 2) *relative humidity generator* mixing dry and water-saturated air to maintain the desired relative humidity (RH), 3) *scrubbing system* reducing the CO₂ concentration of the air feeding into the bioreactors from 400 to below 30 ppm so that essentially only the CO₂ released from the biodegradation process of the test material is measured, 4) *bioreactors* containing test materials and the biodegradation media, 5) *gas analyzer system* measuring the CO₂ concentration of the air from each bioreactor by using a non-dispersive infrared gas analyzer (NDIR), and 6) *control software* controlling the air valve switching system, and recording measured CO₂ concentration, temperature, and RH.

In this work, the system was maintained at 58 ± 2 °C and $50 \pm 5\%$ RH to simulate controlled composting conditions following ASTM D5338 and ISO 14855 standards [REF]. An inoculated vermiculite was used as a medium for the biodegradation test, with each bioreactor containing a mix of the inoculated vermiculite and the test material. The inoculation was done by mixing vermiculite premium grade (Sun Gro Horticulture Distribution Inc., Bellevue, WA, USA) with an inoculum solution at the ratio of 1:4 (wt.) where the inoculum solution was a mix of compost extract and a mineral solution (Table 5-2 in the APPENDIX) at a ratio of 1:1. The compost extract was prepared by mixing dry compost (manure-straw compost obtained from the MSU Composting Facility, East Lansing, MI) with deionized water (20% wt./vol.), stirring, settling for 30 min, then filtering using a sieve with 1 mm mesh.

Each bioreactor contained 400 g (wet wt.) of inoculated vermiculite thoroughly mixed with 8 g of the test material. The measurement of evolved CO₂ was run in triplicate for each test material, so three bioreactors were used per material. One additional bioreactor of each material was prepared and stored in the studied condition; samples from this bioreactor were collected at pre-determined periods of time for further characterization. Triplicates of blank bioreactors (with inoculated vermiculite only) and triplicates of the positive standard (bioreactor containing a mix of inoculated vermiculite and 8 g of cellulose) were also analyzed. The airflow, temperature, and RH were monitored throughout the duration of the experiment. Approximately 20 cm³ of deionized water was injected into each bioreactor twice a week to maintain the humidity inside the bioreactor. All bioreactors were shaken after water was injected to avoid clumps, channels, and non-uniform distribution of samples. The method to calculate evolved CO₂ and mineralization of the samples is provided in the APPENDIX.

5.2.3 Material Characterization

Tested samples at the beginning of the test and at days 5, 10, 15, 20, 25, and 30 of the experiment were collected to be characterized by the following methods:

5.2.3.1 Differential Scanning Calorimetry (DSC)

Thermal analysis of the samples was performed using a TA instrument Q100 (TA Instruments, New Castle, DE, USA), with a refrigerated cooling system under nitrogen flow of 70 mL min⁻¹. Samples (5-10 mg) were placed in a standard non-hermetic aluminum DSC pan and encapsulated using a sample press with an empty pan used as reference. The heating cycle run was set from -20 °C to 200 °C at a heating rate of 10 °C min⁻¹. The DSC thermograms were analyzed using the TA universal analysis software to determine the glass transition temperature (T_g), crystallization temperature (T_c), melting temperature (T_m), and enthalpy of fusion (ΔH_f). The deconvolution of the overlapped endothermic melting peaks obtained from the blend samples, PLA/PLA-*g*-MA/PHBV and PLA/PHBV, was conducted using Fityk 1.3.1 software with Pearson 7Area peak shape [22].

5.2.3.2 Size Exclusion Chromatography (SEC)

Samples of 20 ± 5 mg were dissolved in 10 mL of HPLC grade tetrahydrofuran (THF) for 24 hr at ambient temperature. The solution was then filtered using a 0.45 µm PTFE filter. Approximately 1 mL of the filtrate was placed in a glass vial that was later loaded in the autosampler (Waters 717) of the SEC

system from Waters (Milford, MA, USA) equipped with an isocratic pump (Waters 1515), a refractive index detector (Waters 2414) and a series of Styragel GPC columns (Styragel HR-4, HR-3, HR-2). The measurement was carried out at a controlled temperature of 35 °C with a flow rate of THF as the mobile phase of 1 mL min⁻¹. The weight average and number average molecular weights (M_w and M_n) and molecular weight distribution (MWD) were analyzed using Waters Breeze™ 2 Software with the Mark-Houwink constants (K and α values) of PLA in THF at 35 °C obtained from the values of the PS standard of 0.000174 mL g⁻¹ and 0.736, respectively. The *molecular weight*, M_w , properties of only the PLA fraction of the sample were reported, as PHBV is insoluble in THF.

5.2.3.3 Scanning Electron Microscopy (SEM)

The surface morphology of the samples was observed using a scanning electron microscope SEM JEOL 6610LV (JEOL, Tokyo, JP). The samples were mounted on aluminum stubs using carbon adhesive tape and then were gold coated using an Emscope SC500 sputter coater (Emscope Laboratories, Ashford, UK). The samples were imaged using an accelerating voltage of 10 kV with a magnification range of 10X to 20,000X.

5.3 **Results and discussion**

5.3.1 **Evolution of CO₂ and Mineralization**

Figure 5-1 shows the average cumulative CO₂ and % mineralization of each test material plotted as a function of the incubation time in days. The tests on the neat polymers, PLA and PHBV, were conducted to observe their baseline influence on the biodegradation behavior of the blended material. Differences between the results obtained from the non-compatible sample, PLA/PHBV, and the compatible sample, PLA/PLA-*g*-MA/PHBV, yielded information regarding the effects of PLA-*g*-MA used as a compatibilizer. Cellulose as a positive control was also evaluated.

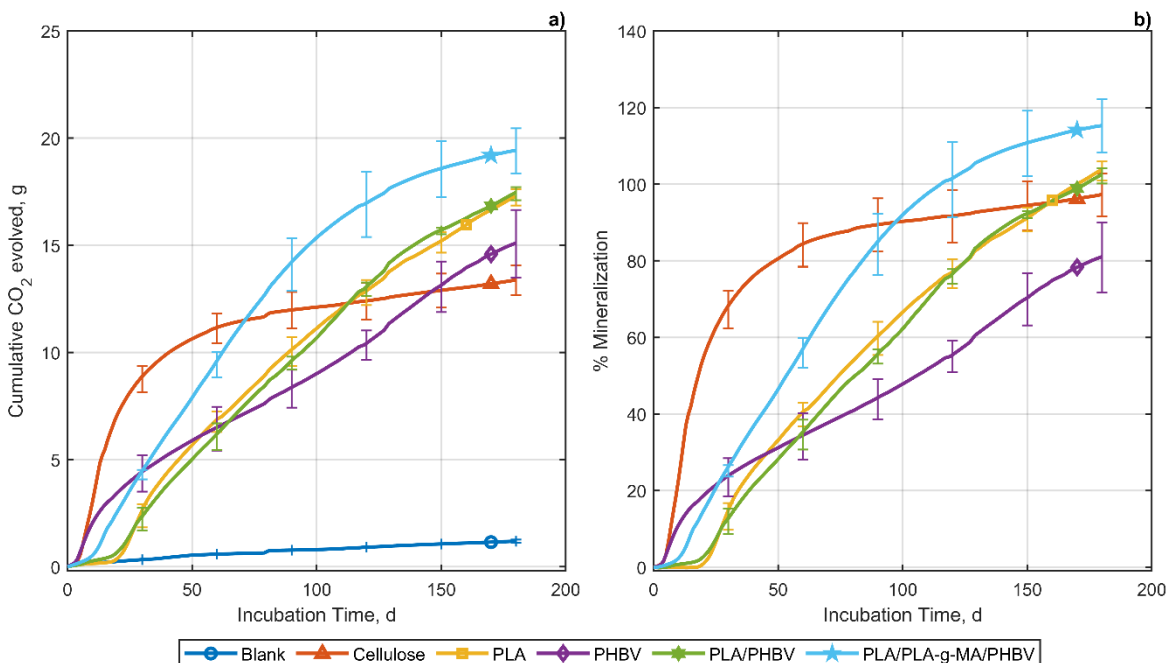


Figure 5-1 a) CO₂ evolution and b) % Mineralization of the blank, cellulose, PLA, PHBV, PLA/PHBV, and PLA/PLA-g-MA/PHBV in inoculated vermiculite media.

Figure 5-1 shows the typical two-stage biodegradation mechanism of PLA from the % mineralization plot [23]. PLA films showed a lag phase of c. 25 days corresponding to the first dominant step of the chemical hydrolysis of ester linkages that normally occurs without large involvement of microorganisms. This was also indicated by the lack of CO₂ evolution during this period [18,23]. The second step of microbial enzymatic degradation of low molecular weight polymer chains resulting from the first step appeared on the % mineralization plot after 25 days through the end of the test at 180 days where the % mineralization was above 100%. The start of the biotic phase shows a good correlation with the M_w of c. 10 kDa necessary to start the biotic phase of degradation (Table 5-5) as previously reported [18]. The % mineralization of PHBV had a very brief lag phase of about 5 days, similar to that of cellulose. PHBV and cellulose generally degrade by a single-step process involving microbial degradation of the polymers themselves [23]. A short lag time, in general, is for the microorganisms to adjust to the surface of the material before starting the enzymatic process. This process releases CO₂ as a byproduct.

The % mineralization at the end of the test (day 180) of the samples containing PLA (PLA, PLA/PHBV, and PLA/PLA-g-MA/PHBV) ended up over 100%, whereas that of PHBV was about 81%

(Table 5-3 and Table 5-4 in the APPENDIX). Mineralization that reaches above 100% is indicative of the priming effect, the enhanced degradation of organic materials in the biodegradation matrix being used, which is usually reported when testing glucose, starch, and cellulose [24]. Use of inoculated vermiculite as a matrix instead of compost has been reported to reduce the priming effect [24]. However, the priming effect could be seen in the tests with highly degradable material that vermiculite has been used as a medium [25]. In our case, even though inoculated vermiculite was used, mineralization above 100% could still be seen. Our hypotheses regarding this finding are: 1) carbon-containing organic materials left in the compost extract that was mixed in the inoculum were sufficient to generate a significant amount of CO₂, 2) DCP, which is an oxidation initiator used in the functionalization of the MA on PLA, could still remain in PLA/PHBV and could induce oxidation of the organic substances in the compost extract, 3) the microorganisms present in the bioreactors could have captured CO₂ from the air supplied to the system as a carbon source for growth, causing later release of that of CO₂ when the microorganisms decomposed [26]. PLA with lower molecular weight (72.6 kDa compared to the ones of 92.5 kDa and 82.9 kDa) has been found to reach over 100% mineralization in the test in compost in the study of Castro-Aguirre [18], so we anticipated that our PLA-containing samples with the molecular weight maximum at 64.4 kDa probably could induce the priming effect. Detailed molecular weight data for the samples can be found in Table 5-5.

PLA and PLA/PHBV had a similar lag phase of c. 25 days, indicating that the extra step of processing, leading to M_w reduction for the PLA fraction in PLA/PHBV of c. 11% was not a significant factor for accelerating the biodegradation process. On the other hand, PLA/PLA-*g*-MA/PHBV samples showed the shortest lag phase of about 10 days, showing an improvement of the compatibilized blend by increasing the rate of abiotic degradation. The shortest lag phase for PLA/PLA-*g*-MA/PHBV blend is associated with an extra step of processing during its production, that led to an initial M_w of c. 82 kDa when adding PLA-*g*-MA, and also due to an effect of the compatibilizer, increasing the rate of the chemical hydrolysis, since the reduction from day 0 to day 5 is c. 70% of M_w for PLA/PLA-*g*-MA/PHBV, while for PLA and PLA/PHBV the reduction of M_w in the same period of time was c. 40 and c. 44%, respectively. PLA/PLA-*g*-MA/PHBV also had the fastest increase in both CO₂ evolution and % mineralization. The presence of anhydride, in the PLA-*g*-MA compatibilizer used in the PLA/PLA-*g*-

MA/PHBV sample, could facilitate the hydrolysis of PLA [27]. This caused the M_w of the PLA/PLA-*g*-MA/PHBV sample to decrease to a level that was suitable for faster digestion by microorganisms, so the mineralization reached the biodegradation phase earlier than in the PLA and PLA/PHBV samples. A faster chemical hydrolysis of the PLA fraction, deteriorating the bulk structure of PLA and the overall structure of the PLA/PLA-*g*-MA/PHBV blend, allowed easy availability of the PHBV fraction in the blend for microbial assimilation, resulting in a faster biotic phase as compared to the PLA/PHBV blend. Likewise, the main effect of the compatibilizer seems to be related to the PLA fraction of the PLA/PLA-*g*-MA/PHBV blend since its proportion is higher than the PHBV. On the other hand, the CO₂ evolution and % mineralization of PLA/PHBV was similar to the pattern of PLA. This is expected since PLA was the major component in the blend with a weight content of 75%. It has been reported that the presence of anhydride groups in general increases the chemical hydrolysis due to the susceptibility of this group to chemical hydrolysis, even faster than the ester group [28]. This effect is mostly observed during the chemical hydrolysis of the reactive blend PLA/PLA-*g*-MA/PHBV.

5.3.2 Thermal Properties

Figure 5-2 shows overlaid DSC heat flow curves of the samples through the biodegradation until day 30 of the test. The curves of the neat polymer samples, PLA and PHBV, show the melting peak ranges of PLA between about 130 °C and 160 °C, while that of PHBV was between 155 °C and 175 °C. When analyzing the heat flow curves of the blend samples, the melting peak(s) at lower temperature belonged to the PLA part of the blend, and vice versa. Since the amount of PHBV in the blend samples was low at 25 wt.%, the melting peaks are quite hard to recognize from the overlay curves. A summary of the temperature at peak of the heat flow curves is provided in Table 5-6 in the APPENDIX.

As shown in **Figure 5-2**, the endothermic crystallization could be seen only in the initial sample of PLA, and the blends, PLA/PHBV and PLA/PLA-*g*-MA/PHBV. The average enthalpy of fusion, ΔH_f of the samples from day 0 of these materials is shown in **Figure 5-3** they were close to 0 J·g⁻¹. This indicates that initially, before biodegradation, these samples did not contain any significant PLA crystallinity; the PLA part of the samples was amorphous. The melting transition shown in the heat flow curves of the samples of the later days, day 5, 10, 15, 20, 25, and 30, was due to the melting of the PLA crystallites that formed as a result of the hydrolysis of PLA occurred during the incubation. Several of the melting

peaks show the formation of the α and δ crystal forms characteristic of PLA. The melting peak of PLA shifted to lower temperature with increasing incubation time. The slight decrease of T_m was observed beginning on day 15. A similar movement of the melting peak was also clearly seen in PLA/PHBV samples with the T_m above 150 °C until day 15 and decreasing to below 150 °C at day 20. PLA/PHBV showed slightly faster movement at day 20, though the T_m at day 30 of both samples was equivalent. The curves of PLA/PLA-*g*-MA/PHBV also demonstrate this melting peak shifting, however, with faster progress, starting from day 10, and a larger decrease over the incubation time. The T_m of PLA/PLA-*g*-MA/PHBV samples at day 5, 10, 15, 25, and 30 also appeared to be lower than those of PLA and PLA/PHBV. As discussed in section 5.3.1, within the incubation time of 25 days, PLA and PLA/PHBV samples were in the first stage of the biodegradation mechanism where the abiotic hydrolytic degradation was the main reaction occurring. The chain-scission degradation resulted in increasing of smaller molecular chains that allowed more segmental mobility and lower onset melting temperature. The crystals formed as a result of the hydrolysis process under the biodegradation test condition at 58 °C, which is quite low compared to the typical crystallization temperature of PLA, could result in less uniform crystal formation with less stable (thinner) lamellae and lower melting temperature [29].

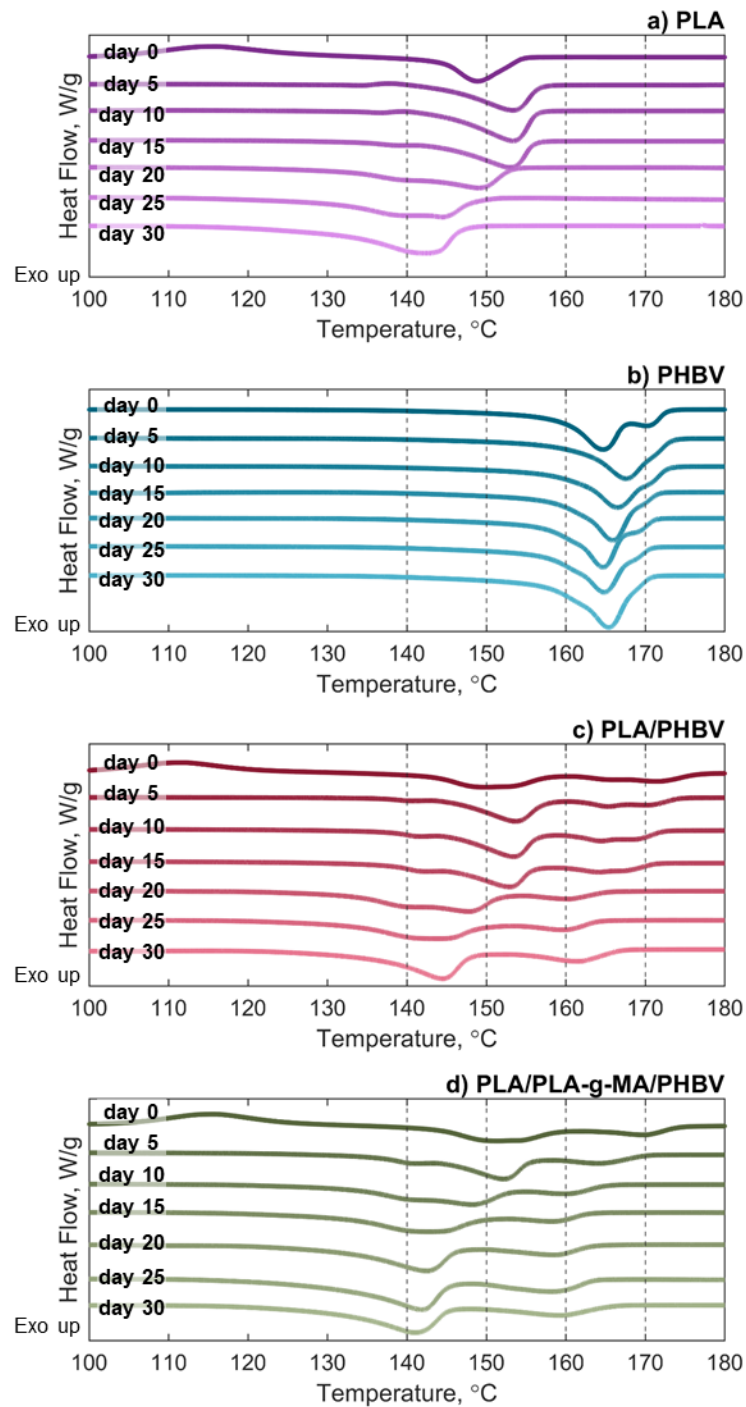
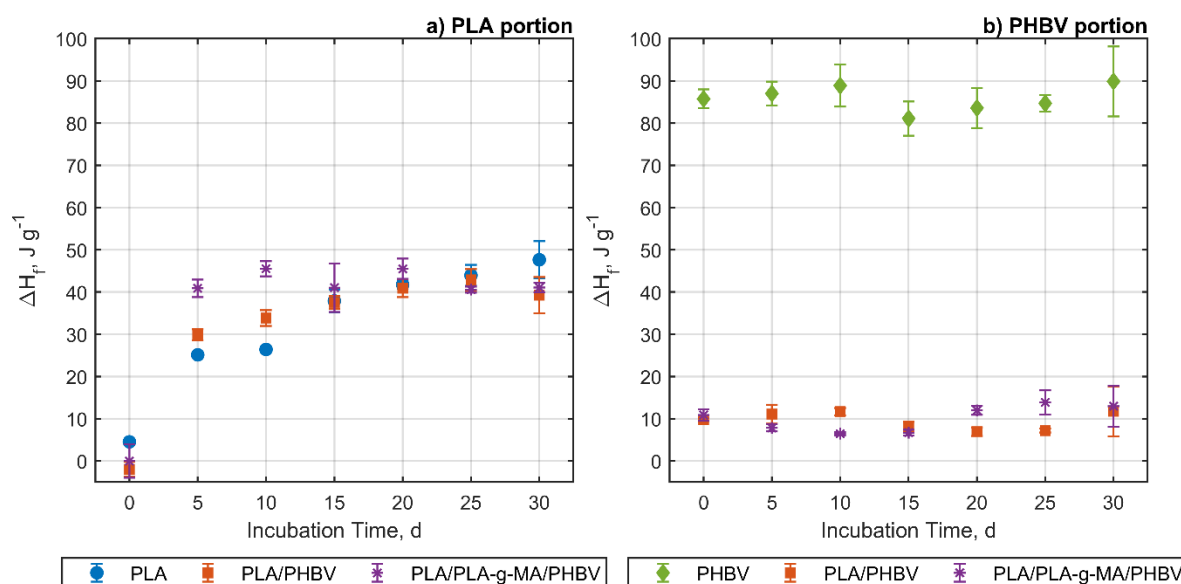


Figure 5-2 DSC heat flow curves of a) PLA, b) PHBV, c) PLA/PHBV, d) PLA/PLA-g-MA/PHBV samples at different incubation times. Thermograms are shifted along the vertical axis to ease the comparison.

The heat flow curves of PHBV, unlike the samples containing PLA, did not show any exothermic crystallization, indicating that the initial sample at day 0 originally had a certain amount of crystallinity and **Figure 5-3b** shows the ΔH_f of about 85 J·g⁻¹. In **Figure 5-2b**, PHBV did not exhibit the shifting of melting peaks to lower temperature as occurred in PLA and the blend samples. The T_m tended to be constant with increasing incubation time. However, in the blend samples PLA/PHBV (day 20 and after) and PLA/PLA-g-MA/PHBV (day 10 and after), the melting peaks of the PHBV part of the blend showed shifting to lower temperatures following the shift of the PLA component since the composition of PHBV in the blend was much lower than PLA. The morphology of PHBV in PLA could be in the form of very small droplets in the continuous phase of PLA. In that case, the crystallization of PLA, influenced by hydrolysis during the incubation, occurred surrounding the PHBV droplet phase, and segmental movement and transition of low molecular weight PLA chains might initiate movement in PHBV chains as well.



*Figure 5-3 Enthalpy of fusion obtained from the DSC heat flow curves of PLA, PHBV, PLA/PHBV, and PLA/PLA-g-MA/PHBV samples where **a) PLA portion** was from the melting peaks of PLA, and **b) PHBV portion** was from the melting peaks of PHBV*

Figure 5-3 demonstrates the average enthalpy of fusion, ΔH_f from the heat flow curves of each sample at different incubation times. This results from changes in the crystallinity of the samples. The percent crystallinity was not calculated since we could not identify how much of each blend component

remained in the sample after a certain incubation time. In the blend samples, PLA/PHBV and PLA/PLA-*g*-MA/PHBV, the melting peaks of PLA and PHBV overlapped. Deconvolution was done to estimate the area of the peaks belonged to each material. In the figure, a) shows the ΔH_f from the melting range of PLA, marked as ΔH_{f-PLA} and b) shows the ΔH_f from the melting range of PHBV, marked as ΔH_{f-PHBV} . ΔH_{f-PLA} of PLA, PLA/PHBV, and PLA/PLA-*g*-MA/PHBV samples all increased from 0 J·g⁻¹ on day 0 to 25.12 ± 0.40, 29.86 ± 1.33, and 40.88 ± 2.08 J·g⁻¹, respectively on day 5. This supports the discussion in Section 5.3.1 that the presence of anhydride in the PLA/PLA-*g*-MA/PHBV sample helped accelerate the hydrolysis of ester bonds in PLA, producing smaller molecular chains that crystallized faster. While ΔH_{f-PLA} of PLA/PLA-*g*-MA/PHBV remained above 40 J·g⁻¹ from day 0 until day 30, ΔH_{f-PLA} of PLA and PLA/PHBV gradually increased with the incubation time. During the first 25 days, PLA and PLA/PHBV still underwent the process of hydrolytic degradation. Smaller hydrolyzed molecular chains allowed more segmental mobility and formation of local crystalline structures, facilitating the nucleation and crystal growth that in turn required more enthalpy of fusion in the melting process [30].

ΔH_{f-PHBV} of PHBV as well as of the blend samples, PLA/PHBV and PLA/PLA-*g*-MA/PHBV, did not show a significant change during the first 30 days of the test. This indicated that the crystallinity of these samples was likely stable during this period of time. This is possibly due to the fact that PHBV is generally much less sensitive to hydrolytic degradation than PLA, so the chance of crystallinity increasing as a result of increasing low molecular weight chains was low. At the same time, the biotic degradation that already started after 5 days of incubation also did not cause a significant reduction of the crystallinity since the process tended to occur in the amorphous regions rather than in the crystalline structures that are more stable. This kept the ΔH_{f-PHBV} of PHBV and the blends from drastic changes during the first 30 days.

The thermal properties results demonstrated different degradation mechanisms occurred in PLA and PHBV during the first 30 days of the biodegradation test. While PLA experienced the changes in bulk properties including molecular weight reduction due to the hydrolysis that resulted in the shifting of T_m to lower temperature and the increase in ΔH_f , PHBV tended to be impacted by the change that occurred on the surface rather than the change in bulk properties of the material as stable T_m and ΔH_f were observed. Surface erosion by microbial enzymatic activity has been reported by Salomez et al. [1] as a major

process occurring in PHBV that has been clearly assessed from an exponential increase in the mass loss of the material during the beginning of the degradation. Even though the change in mass of the test materials over the biodegradation time and the molecular weight change of PHBV were not measured in our study, the visual appearance and the observation on the surface morphology using the scanning electron microscopy that are discussed in the following section evidenced the occurrence of surface erosion on PHBV.

5.3.3 Molecular Weight

The molecular weight properties of PLA in PLA, PLA/PHBV, and PLA/PLA-*g*-MA/PHBV at different incubation times were measured in this study. As was mentioned earlier, typically PLA experiences a two-stage biodegradation mechanism where the hydrolysis of the ester linkages has a major role in the first stage. The change of the molecular weight is the key evidence of this process. Figure 5-4 shows the molecular weight distribution (MWD) of the PLA-containing samples: a) PLA, b) PLA/PHBV, and c) PLA/PLA-*g*-MA/PHBV collected at different incubation times. The right-most curve of each plot is the distribution with the highest weight average molecular weight (M_w), moving to the next on the left, the decreasing of M_w of the distribution could be observed. The molecular weight of all samples decreased as a function of biodegradation time. It is clearly seen that the molecular weight of the initial sample of PLA/PLA-*g*-MA/PHBV was lower than those of PLA, and PLA/PHBV (detailed values of the molecular weight of the samples can be found in **Table 5-5**). The addition of PLA-*g*-MA as a blend compatibilizer resulted in reduction of the molecular weight of PLA. The reduction of molecular weight of PLA/PLA-*g*-MA/PHBV sample progressed the fastest among the three samples (the molecular weight reduction rates of the samples are demonstrated in Figure 5-7 in the APPENDIX). This is attributed to the presence of anhydride that facilitated the hydrolytic degradation, as previously discussed. Also, maleic acid released from the degradation of PLA/PLA-*g*-MA/PHBV sample could generate acidic conditions that accelerate the hydrolysis of PLA [31]. Considering only the plot of PLA/PLA-*g*-MA/PHBV sample in Figure 5-4c, the curve of day 10 shows more than half of its population was below the molecular weight of 10 kDa with a considerable part of the curve below 3 kDa, reaching the level of molecular size that is suitable for microorganisms to begin the degradation process. This supported the result of % mineralization of PLA/PLA-*g*-MA/PHBV in **Figure 5-1b** that showed the biodegradation phase beginning approximately

after day 10 of the test. Regarding the PLA/PHBV sample, even though there was 25 wt.% of PHBV in the blend, the MWD of PLA/PHBV is quite similar to the ones of PLA. This is probably because without any chemical reaction influence, adding PHBV did not affect the molecular weight properties of the PLA in the blend.

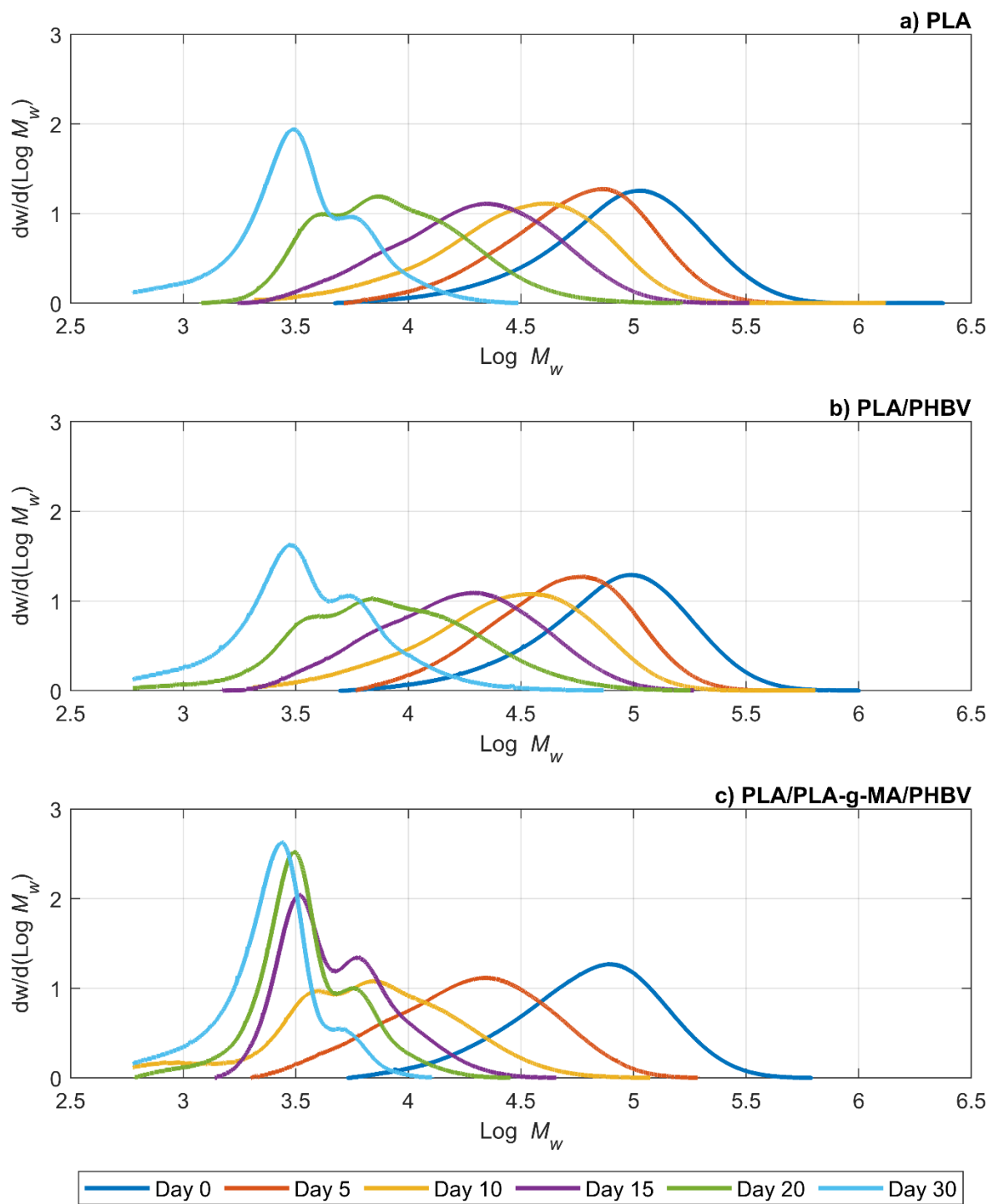


Figure 5-4 Molecular weight distribution of the PLA portion of a) PLA, b) PLA/PHBV, and c) PLA/PLA-g-MA/PHBV samples at different incubation time (days 5, 10, 15, 20, 30)

5.3.4 Visual Appearance and Surface Morphology

The visual appearance of the samples during the biodegradation test is shown in Figure 5-5. Rows a), c), and d) from day 0 to day 5 show the samples PLA, PLA/PHBV, and PLA/PLA-*g*-MA/PHBV which contained PLA became opaque with development of a white color. This is because of the rapid increase of crystallinity that could also be seen in the increase of ΔH_{f-PLA} of these samples in Figure 5-3a. The sample PLA/PLA-*g*-MA/PHBV (Figure 5-5d) was the earliest to start breaking, as can be clearly recognized from the samples collected on day 10 of the test. This is the time with a drastic drop of molecular weight of the sample to under 10 kDa. The breakage of PLA and PLA/PHBV samples could be seen clearly happening on day 25 where their molecular weight also dropped to under 10 kDa. The change of visual appearance of the PHBV sample was different from those of the samples containing PLA. No change of opacity was found in PHBV and the onset of breakage of the samples could not be clearly identified. The samples became recognizably thinner over time from simple observation. However, the thickness of the collected samples was not measured in this study. Black stains (Figure 5-5b day 10, 15, 20, 25, 30) could be seen on some samples in the bioreactor that was used for sample collection. This was possibly because fungi are major microorganisms degrading PHBV [1]. Some traces of fungal filaments were found on the PHBV samples collected. Based on observation of fungi producing the filaments during the degradation process, it can be anticipated that the mineralization of PHBV, even if it entered the plateau phase, would not reach 100% as some of the carbon the microbes obtained from the polymer may be used to produce the filaments instead of being released as CO₂.

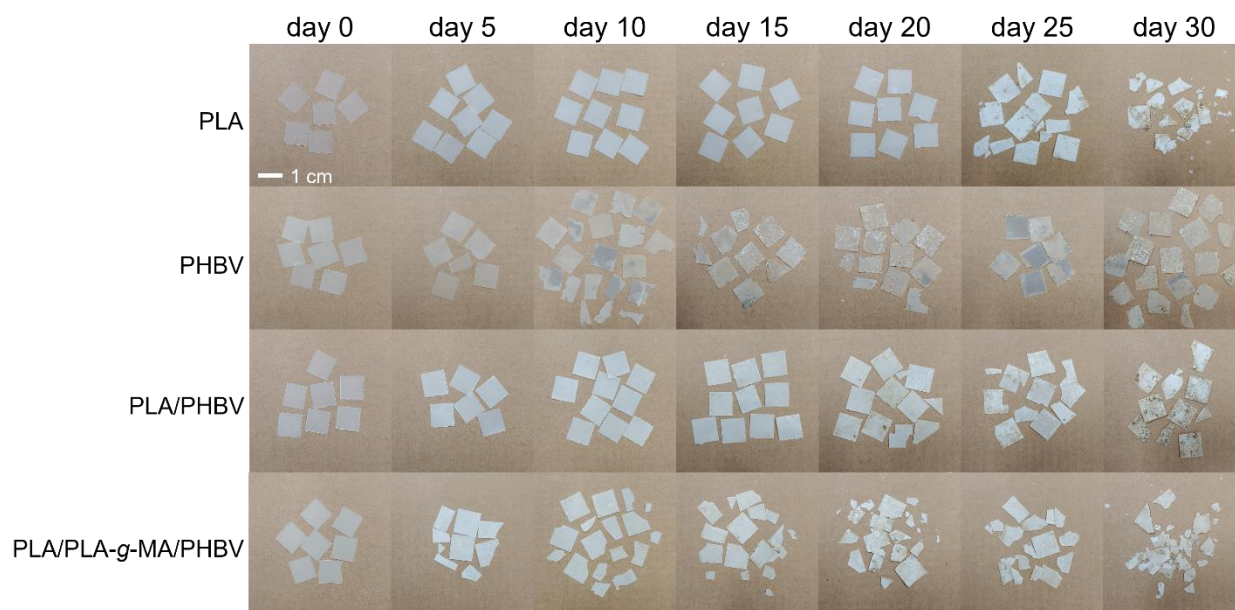


Figure 5-5 Visual appearance of PLA, PHBV, PLA/PHBV, and PLA/PLA-g-MA/PHBV samples at different incubation time (days 0, 5, 10, 15, 20, 25 and 30)

Figure 5-6 shows micrographs of the surfaces of collected samples from days 0, 10, and 20. The PHBV sample on day 10 showed a unique pattern of the crystal lamellae compared to the surface on day 0, which was relatively smooth. This revealed the typical biodegradation behavior of PHBV that occurred mainly due to the enzymatic process starting on the surface of the material. From the micrographs of PHBV, the smooth surface from day 0 could be removed by the microbial process revealing the structure of the crystalline region, and as the degradation progressed further on the crystals, the layers of lamellae could be seen becoming duller in the micrograph from day 30. The fine and uniform crystalline structures are clearly seen in **Figure 5-6c**-day 20 and **Figure 5-6d**-day 20 of PLA/PHBV and PLA/PLA-g-MA/PHBV samples. These structures did not appear in the micrographs of day 0 of the samples. Thus, they could be visual evidence of the crystallization occurring as a result of the hydrolytic degradation during the biodegradation of PLA in PLA/PHBV and PLA/PLA-g-MA/PHBV samples. At day 30, holes could be seen in the PLA/PLA-g-MA/PHBV and PLA/PHBV samples, which could be attributed to an initial degradation of the PHBV phase and amorphous PLA.

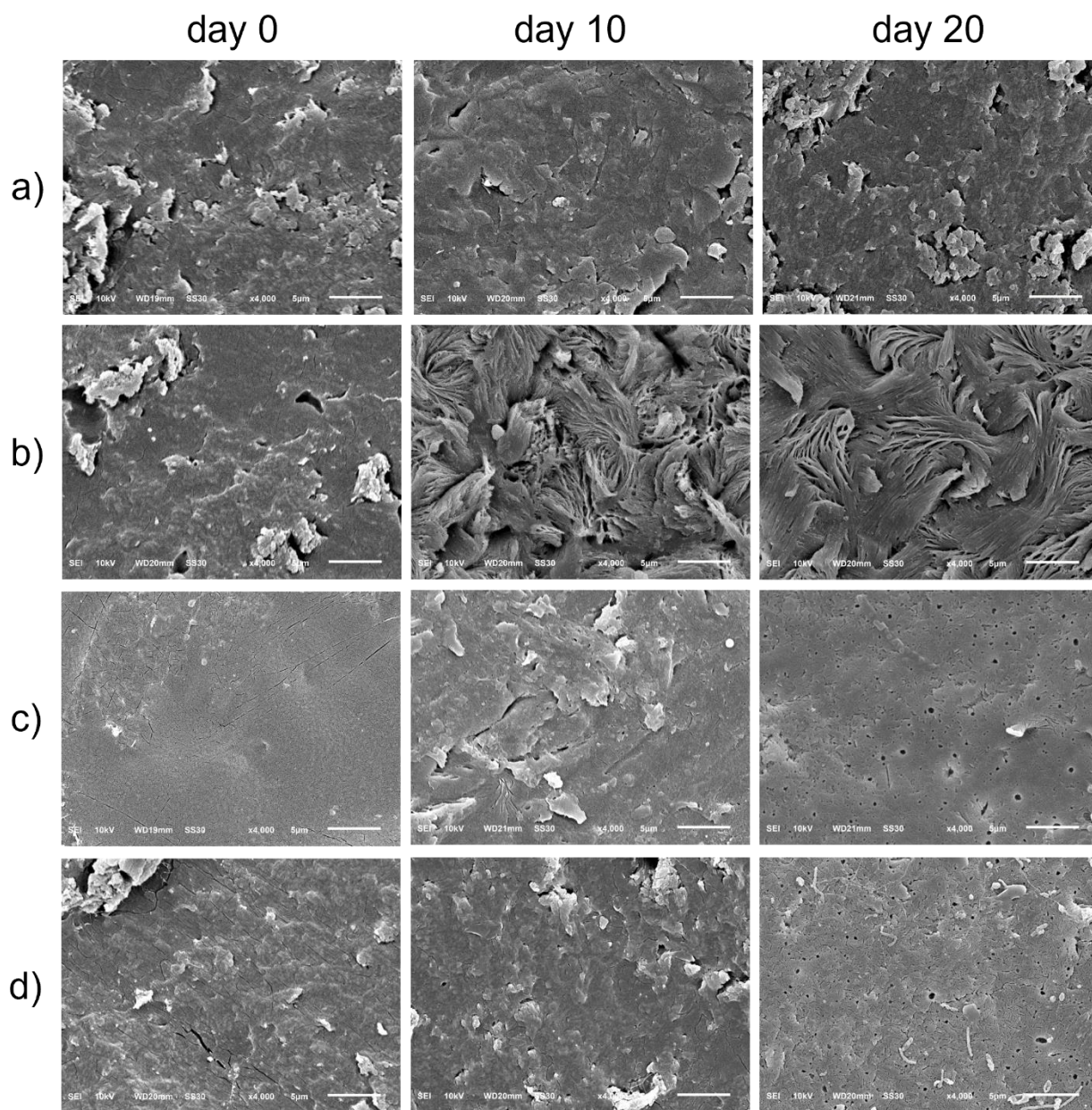


Figure 5-6 Surface morphology of a) PLA, b) PHBV, c) PLA/PHBV, and d) PLA/PLA-g-MA/PHBV samples at different incubation time (days 0, 10, and 20) by SEM with 4000X magnification

5.4 Conclusion

The aerobic biodegradation of PLA, PHBV, a non-compatibilized blend, PLA/PHBV, and a compatibilized blend using PLA-g-MA as a compatibilizer, PLA/PLA-g-MA/PHBV was evaluated so that the impact of PLA-g-MA on the biodegradation could be understood. The CO₂ evolution and % mineralization showed that PLA/PLA-g-MA/PHBV was the fastest to reach 100% mineralization, followed

by PLA and PLA/PHBV samples, whereas PHBV reached only 81% mineralization at the end of the test of 180 days. The accelerated biodegradation of PLA/PLA-*g*-MA/PHBV could be attributed to the facilitation of the hydrolysis of PLA in the blend due to the presence of anhydride from PLA-*g*-MA added to the material. This conclusion was supported by the drastic increase in enthalpy of fusion, ΔH_f as well as a fast reduction of the molecular weight of PLA/PLA-*g*-MA/PHBV compared to PLA and PLA/PHBV. The PHBV sample showed the biodegradation was barely affected by abiotic hydrolytic degradation; the main mechanism was enzymatic microbial degradation as the thermal properties result did not show any shifting of the melting transition and the ΔH_f remained stable until 30 days of the test. The scanning electron micrographs also revealed the biodegradation of PHBV that initially occurred was from the surface and later showed the degradation of the crystalline structure. The PLA crystals formed during the biodegradation of PLA/PHBV and PLA/PLA-*g*-MA/PHBV samples could be seen from SEM photos.

APPENDIX

Cumulative Evolved CO₂ and Mineralization Calculation

The cumulative evolved CO₂, in grams, was calculated using Eq. 12 where gCO_2 is the cumulative mass of evolved CO₂ (g), $C(t)$ is the average CO₂ concentration (ppm) during the measurement time (30 s), $F(t)$ is the flow rate in standard cubic centimeters per minute (sccm), t is the experimental time (days), 44 is the molecular weight of CO₂, 22414 is the standard gas volume in cubic centimeters per mole, and 10^6 is the conversion factor for ppm. The time integral to calculate cumulative CO₂ was evaluated using the trapezoidal method of numerical integration.

$$gCO_2 = \int_0^t \frac{C(t) \times F(t) \times 44}{22414 \times 10^6} dt \quad \text{Eq. 12}$$

Percent mineralization ($\%Mineralization$) was calculated using Eq. 13 based on the carbon content of the test materials where sCO_2 is the cumulative mass of CO₂ (g) evolved from a sample bioreactor, bCO_2 is the average cumulative mass of CO₂ (g) evolved from the blank bioreactor, W is the mass of the test material (g), $\%C$ is the percent carbon in the sample obtained from the CHN analysis, 44 is the molecular weight of CO₂, and 12 is the atomic weight of carbon.

$$\%Mineralization = \frac{sCO_2 - bCO_2}{W \times \frac{\%C}{100} \times \frac{44}{12}} \times 100 \quad \text{Eq. 13}$$

Table 5-2 Detailed composition of 1 L of mineral solution.

1 L of Mineral solution	
KH ₂ PO ₄ , g	1
MgSO ₄ , g	0.5
CaCl ₂ (10% sol), mL	1
NaCl (10% sol), mL	1
Trace-element solution, mL	1

1 L of trace-element solution	
H ₃ BO ₃ , mg	500
KI, mg	100
FeCl ₃ , mg	200
MnSO ₄ , mg	400
(NH ₄) ₆ Mo ₇ O ₂₄ , mg	200
FeSO ₄ , mg	400

Table 5-3 Cumulative CO₂ evolved (g) of cellulose (positive control), neat PLA (PLA), neat PHBV (PHBV), non-compatible PLA/PHBV blend (PLA/PHBV), and PLA/PHBV blend compatibilized with maleated PLA (PLA/PLA-g-MA/PHBV).

Experimental Time (d)	Cumulative Evolved CO ₂ (g)				
	cellulose	PLA	PHBV	PLA/PHBV	PLA/PLA-g-MA/PHBV
30	8.9 ± 0.6	2.6 ± 0.5	4.4 ± 0.9	2.4 ± 0.6	4.5 ± 0.2
60	11.2 ± 0.7	6.9 ± 0.5	6.5 ± 1.0	6.2 ± 0.6	9.6 ± 0.6
120	12.4 ± 0.8	12.9 ± 0.6	10.4 ± 0.7	13.1 ± 0.3	17.0 ± 1.5
180	13.4 ± 0.7	17.3 ± 0.4	15.1 ± 1.6	17.5 ± 0.3	19.4 ± 1.0

Table 5-4 Percent mineralization of cellulose (positive control), neat PLA (PLA), neat PHBV (PHBV), non-compatible PLA/PHBV blend (PLA/PHBV), and PLA/PHBV blend compatibilized with maleated PLA (PLA/PLA-g-MA/PHBV).

Experimental Time (d)	Percent Mineralization (%)				
	cellulose	PLA	PHBV	PLA/PHBV	PLA/PLA-g-MA/PHBV
30	68.3 ± 5.1	14.9 ± 3.5	23.9 ± 5.1	12.7 ± 3.8	26.2 ± 1.4
60	84.4 ± 5.7	40.6 ± 0.5	34.5 ± 6.0	35.4 ± 3.9	57.1 ± 4.1
120	91.7 ± 6.9	77.2 ± 3.8	55.4 ± 4.1	76.6 ± 1.9	101.5 ± 9.8
180	97.3 ± 5.5	103.7 ± 2.4	81.1 ± 9.1	102.5 ± 2.0	115.3 ± 6.9

Table 5-5 Molecular weight of PLA, PLA/PHBV, and PLA/PLA-g-MA/PHBV samples at different incubation time (day 0, 5, 10, 15, 20, and 30).

Sample	Incubation Time (d)	M_n (kDa)	M_w (kDa)	Dispersity
PLA	0	64.4 ± 3.9	119.7 ± 3.4	1.9 ± 0.1
	5	41.4 ± 0.7	71.4 ± 0.1	1.7 ± 0.0
	10	19.6 ± 1.8	41.1 ± 1.2	2.1 ± 0.2
	15	13.7 ± 0.7	26.0 ± 1.4	1.9 ± 0.0
	20	6.6 ± 0.5	11.3 ± 0.5	1.7 ± 0.1
	30	2.7 ± 0.1	4.0 ± 0.1	1.5 ± 0.0
PLA/PHBV	0	59.7 ± 2.2	105.5 ± 1.2	1.8 ± 0.0
	5	36.1 ± 0.5	59.2 ± 0.2	1.6 ± 0.0
	10	19.5 ± 3.5	38.9 ± 3.6	2.0 ± 0.2
	15	11.4 ± 0.8	22.1 ± 0.4	1.9 ± 0.1
	20	4.7 ± 0.7	11.7 ± 0.6	2.5 ± 0.3
	30	2.8 ± 0.0	4.7 ± 0.1	1.7 ± 0.0
PLA/PLA-g-MA/PHBV	0	45.4 ± 0.7	82.4 ± 3.6	1.8 ± 0.1
	5	13.1 ± 0.9	25.3 ± 0.3	1.9 ± 0.2
	10	4.4 ± 0.1	9.6 ± 0.1	2.2 ± 0.0
	15	4.2 ± 0.0	5.8 ± 0.0	1.4 ± 0.0
	20	3.1 ± 0.1	4.1 ± 0.1	1.3 ± 0.0
	30	2.2 ± 0.1	2.9 ± 0.1	1.3 ± 0.0

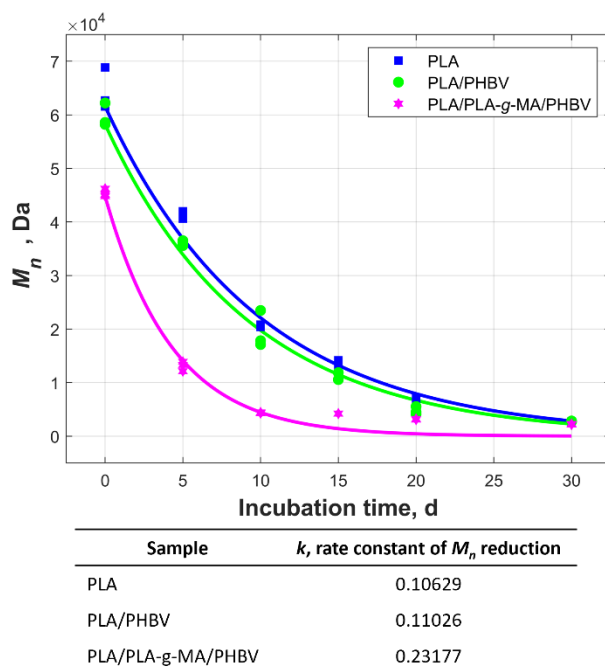


Figure 5-7 Molecular weight reduction as a function of time for PLA, PLA/PHBV, and PLA/PLA-g-MA/PHBV. Lines indicate fitting of a first order reaction of the form $M_n = M_{n0} \exp(-kt)$, where M_{n0} is the initial M_n , k is the rate constant and t is the time.

Table 5-6 Temperature at peaks obtained from the melting transition of the DSC heat flow curves of PLA, PHBV, PLA/PHBV, and PLA/PLA-g-MA/PHBV samples at different incubation time.

Sample	Time (d)	Temperature at peaks (°C) obtained from melting transition			
		PLA melting range		PHBV melting range	
		1 st peak	2 nd peak	1 st peak	2 nd peak
PLA	0		148.6 ± 0.2		
	5		153.4 ± 0.2		
	10		153.8 ± 0.4		
	15	138.6 ± 0.2	153.0 ± 0.4		
	20	139.0 ± 0.3	148.8 ± 0.5		
	25	139.3 ± 0.4	145.0 ± 0.6		
	30		142.4 ± 0.7		
PHBV	0			165.0 ± 0.4	170.4 ± 0.1
	5			167.1 ± 0.1	
	10			166.2 ± 0.3	
	15			165.7 ± 0.8	
	20			165.1 ± 0.7	
	25			165.1 ± 0.2	
	30			165.3 ± 1.0	
PLA/PHBV	0		149.6 ± 0.3	162.5 ± 0.1	171.2 ± 0.1
	5	140.1 ± 0.2	153.3 ± 0.3	164.8 ± 0.6	169.9 ± 0.4
	10	141.2 ± 0.1	153.4 ± 0.1	164.1 ± 0.2	168.7 ± 0.1
	15	141.3 ± 0.3	152.4 ± 0.6	163.2 ± 0.7	166.7 ± 0.6
	20	140.5 ± 0.5	148.3 ± 0.4	160.9 ± 0.4	
	25		143.7 ± 0.8	159.4 ± 0.5	
	30		145.0 ± 0.3	162.2 ± 0.5	
PLA/PLA- <i>g</i> -MA/PHBV	0		149.7 ± 0.2	162.9 ± 0.2	170.8 ± 0.1
	5	140.6 ± 0.2	152.5 ± 0.4	164.8 ± 0.3	
	10	140.3 ± 0.2	148.1 ± 0.4	160.3 ± 0.4	
	15		142.5 ± 0.6	158.4 ± 0.3	
	20		142.3 ± 0.5	158.6 ± 0.1	
	25		142.5 ± 0.6	159.4 ± 0.7	
	30		142.2 ± 1.1	159.8 ± 0.9	

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6 CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

6.1 Conclusions

This research aimed to investigate polymer blends of two biobased, biodegradable polymers, PLA and PHBV, with the hope to expand the usage of PHBV in more general applications such as packaging, at the same time preserving the compostability of both polymers. Maleated PLA or PLA-*g*-MA was selected to be used as a compatibilizer based on its good performance in other biodegradable polymer blends. The research was organized into three parts: First, the compatibilizer, PLA-*g*-MA, was produced by a reactive melt functionalization of maleic anhydride, MA on the PLA backbone in a twin-screw co-rotating extruder using dicumyl peroxide, DCP as a reactive initiator. Second, the samples of PLA/PHBV blends were prepared by the melt blending method and evaluated for their tensile properties and barrier properties. Third, a biodegradation study was performed to evaluate the compostability and comparatively observe the biodegradation process of the base polymers, PLA and PHBV, the non-compatibilized PLA/PHBV blend, and the blend compatibilized with PLA-*g*-MA.

Response surface methodology (RSM) was utilized for experimental design and development of the model in determining the factors affecting the quality of PLA-*g*-MA and PLA/PHBV polymer blends. In the preparation of PLA-*g*-MA, the experiment was planned based on the response surface central composite design, CCD, considering the contents of MA and DCP as the factors affecting the quality responses of PLA-*g*-MA including MA grafting yield and the molecular weight properties. In the polymer blending between PHBV and PLA using PLA-*g*-MA as a compatibilizer, the mixture design of experiments in combination with factorial design was used to develop the model evaluating the relationship of the mixture of contents, PHBV, PLA, and PLA-*g*-MA, and the type of PLA-*g*-MA on the tensile and barrier properties of the polymer blends.

While the FTIR analysis of all produced PLA-*g*-MA demonstrated characteristic absorption bands indicating the grafting of MA on PLA, the model regression of MA grafting yield obtained from the titration as well as the molecular weight properties suggested a significant influence of DCP content. Increase of DCP tended to decrease MA-grafting yield, and resulted in the reduction of M_n , M_w , and IV with the increase of dispersity. The optimization of these responses could be done through the desirability method

that suggested the optimum point with the content of DCP = 0.1 wt. % and MA = 3.94 wt. % (PLA basis) when both responses were equally important.

In PLA/PHBV blending, the increase in the crystalline fraction in the polymer blends compared to neat PLA was clearly observed from FTIR analysis and the enthalpies of fusion obtained from DSC. This resulted in an improvement of the barrier properties of the PLA/PHBV blends compared to neat PLA. Increased miscibility between the blend components of the compatibilized blends compared to the non-compatibilized blended materials was exhibited through the shifting of T_g of PLA and the decrease of k constants based on the Gordon-Taylor equation. As a result, the tensile strength of the polymer blend equivalent to the level of 75 wt. % of PLA could be achieved by less than 45 wt. % of PLA in the blend with the addition of a compatibilizer, PLA-*g*-MA, regardless of the type of PLA-*g*-MA. This could be attributed to the increased interfacial between the blend components. While the factorial-mixture model regression suggested the validity of the mixture variable of PLA, PHBV, and PLA-*g*-MA contents on the tensile strength and % elongation at break, the barrier properties were influenced by only the content of PLA and PHBV. The overlapped contour plots as well as the desirability functions could be used to optimize the mixture of the PLA/PHBV blend components to result in desired tensile and barrier properties.

Biodegradation testing of neat PLA and PHBV, the non-compatibilized PLA/PHBV blend, and the blend compatibilized with PLA-*g*-MA (PLA/PLA-*g*-MA/PHBV), was conducted using the direct measurement respirometric (DMR) system following ASTM 5338 and ISO 14855 standards, with the aim to understand the impact of PLA-*g*-MA on the biodegradation. PLA/PLA-*g*-MA/PHBV had accelerated biodegradation compared to PLA and PLA/PHBV samples according to the CO₂ evolution and % mineralization. The presence of anhydride from PLA-*g*-MA facilitated the hydrolysis of PLA in the blend, supported by the rapid increase of enthalpy of fusion, ΔH_f as well as fast reduction of the molecular weight of the PLA fraction in PLA/PLA-*g*-MA/PHBV compared to PLA and PLA/PHBV samples. PHBV showed a different biodegradation pattern from PLA-containing samples; little shifting of melting transitions and change of ΔH_f that are the evidence of abiotic hydrolytic degradation were observed. Erosion of PHBV occurring from the surface and then progressing to the crystalline structures could be seen from the scanning electron micrographs. These suggested the biodegradation of PHBV relies mostly

on enzymatic microbial degradation rather than the hydrolytic degradation that is the main trigger for the degradation of PLA.

6.2 Recommendations for future work

Based on this research of the blending of PLA and PHBV using PLA-*g*-MA as a compatibilizer, suggestions for future study include:

- Expanding the study to PHBV with higher molecular weight or other polyhydroxyalkanoate (PHA) polymers might yield materials that could be fabricated into packaging forms and studied for various packaging applications.
- Multilayering of PHBV or other PHA polymers and PLA tend to create interesting high barrier property materials that at the same time preserve the compostability of the polymers.
- In the preparation and characterization of PLA-*g*-MA, the amount of grafted maleic anhydride on PLA can also be measured using a quantitative FTIR technique. This possibly indicates the form of anhydride grafts that could affect the compatibilizing performance of PLA-*g*-MA.
- Detailed observation of polymer blend morphology using microscopy techniques can contribute to better understanding of their properties, including thermal properties and mechanical properties.
- The crossed factorial-mixture experimental design adopted in this research can also be applied to combining the mixture problems with the process variables. This will be useful when the factors relating to the process such as temperature, time, etc., are of interest.
- The effect of the blend components on the compostability can be investigated using a mixture design approach where the biodegradation test needs to be expanded to cover all the experimental design points. However, other experimental designs may be considered to reduce the size of the experiment.