Title	微生物由来フェニル乳酸誘導体からのバイオベースポリエステルの合成と物性評価
Author(s)	Nguyen, Hieu Duc
Citation	
Issue Date	2015-09
Type	Thesis or Dissertation
Text version	ETD
URL	http://hdl.handle.net/10119/12974
Rights	
Description	Supervisor:金子　達雄，マテリアルサイエンス研究科，博士

Syntheses and characterization of bio-based polyesters from microbial phenyllactate derivatives

by

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Submitted to

Japan Advanced Institute of Science and Technology

In partial fulfillment of the requirements

For the degree of

Doctor of Philosophy

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Japan Advanced Institute of Science and Technology

September 2015

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Chapter 1

General Introduction

Forewords: Plastics are responsible for countless aspects of the modern ultilities we need today for health and wellbeing, nutrition, shelter and transportation to safety and security, communication, leisure activities and innovations of industry. Plastics improve our lives; bring us joy, convenience, efficiency and connection to others. Sometimes these materials even save our lives. In short, plastics flexibility and adaptability enable them to provide many different solutions in an increasingly complex world.

At every face of our daily lives, when we awaken to the sound of our alarm clock/radio, we make coffee, our use your toothbrush and hairbrush, we put on clothing, we fill your reusable lunch bag, we drive your car (putting your toddler in a child safety seat) or ride your bicycle (don’t forget that helmet!), we listen to our iPod, we make a few calls on our smart phone, we work at your computer, we enjoy a bottle of water, we remove the protective film from around the meat and vegetables we’ll prepare for dinner. And that only begins to touch the surface of the importance of plastics in your everyday life. However, as any material we used on earth, plastics resources are explored from nature which is certainly limited; therefore plastics are also too valuable to be wasted. In future we have to change the ways those we are exploring, using plastics, as we have been, to avoid wasting plastics for a sustainable society.

1.1 Plastics resources and environmental problems

A plastic material is any of a wide range of synthetic or semi-synthetic organic solids that are moldable. Plastics are typically organic polymers of high molecular mass, but they often contain other substances. They are usually synthetic, most commonly derived from petrochemicals, but many are partially natural. Most plastics contain organic polymers [1-6]. The vast majority of these polymers are based on chains of carbon atoms alone or with oxygen, sulfur, or nitrogen as well [2-12]. The backbone is that part of the chain on the main "path" linking a large number of repeat units together. To customize the properties of a plastic, different molecular groups "hang" from the backbone (usually they are "hung" as part of the monomers before the monomers are linked together to form the polymer chain) [13-21]. The structure of these “side chains” influences the properties of the polymer. This fine tuning of the repeating unit's molecular structure influences the properties of the polymer. Most plastics contain other organic or inorganic compounds blended in. The amount of additives ranges from zero percentage (for example in polymers used to wrap foods) to more than 50% for certain electronic applications. The average content of additives is 20% by weight of the polymer.Many of the controversies associated with plastics are associated with the additives, solvent and toxic catalysts.

Today almost man made plastic and fibers are produced from synthetic polymers. Synthetic polymers, made from petroleum which took million years to form. Worldwide plastics production rose to 280 million tons in 2011, according to first rough estimates published by PlasticsEurope [12, 15, 20-22]. This represents around

4% increase from 2010, when 270 million tons of plastics were produced. It also confirms the return of long term growth after the economic crisis. From 2010 to 2016, global plastics consumption is expected to grow by an average of about 4 % each year. The five largest plastics types (polyolefin, polyvinyl chloride, polystyrene, and polyethylene terephthalate) account for about 70% of the total global demand, i.e. 200 million tons. According to Wilfried Haensel, executive director of the association, "these estimates give us confidence as to the market growth and stability over the months to come" [22]. However, the world's resources are depleting at an amazing speed. Fossil fuels are exhaustible and due to the increasing population, these will not be sustainable in the future. Plastics are produced from byproducts of the fossil fuel processing. Fossil fuels are limited and are non-renewable [23].

While a small part of total plastic used is recycled (3% approximately) the main problem with plastic besides there being so much of it is that it doesn't biodegrade when human dumb them into ecosystem. No natural process can break it down. (Experts point out that the durability that makes plastic so useful to humans also makes it quite harmful to nature.) Instead, plastic can be photo-degraded [20, 24-26]. A plastic cigarette lighter cast out to sea will fragment into smaller and smaller pieces of plastic without breaking into simpler compounds, which scientists estimate could take hundreds of years. The tiny plastic particles can get sucked up by filter feeders and damage their bodies. Other marine animals eat the plastic, which can poison them or lead to deadly blockages [25]. Nurdles also have the insidious property of soaking up toxic chemicals. Over time, even chemicals or poisons that are widely diffused in water can become highly concentrated as they're mopped up by nurdles. These poison-filled masses threaten the entire food chain, especially when eaten by filter feeders that are then consumed by large creature [23, 24].

Plastic has acutely affected albatrosses, which roam a wide swath of the northern Pacific Ocean. Albatrosses frequently grab food wherever they can find it, which leads to many of the birds ingesting and dying from plastic and other trash. On Midway Island, which comes into contact with parts of the Eastern Garbage Patch, albatrosses give birth to 500,000 chicks every year [27]. Two hundred thousand of them die, many of them by consuming plastic fed to them by their parents, who confuse it for food. In total, more than a million birds and marine animals die each year from consuming or becoming caught in plastic and other debris[28].

Overall, world plastic industry now faces three main problems in order to maintain sustainability in future: (1) Depletion of resources; (2) Biodegradability after wasted

1.2 Bio-based plastic: concept and trends

Bio-based plastics are plastics produced from biomass sources. Biomass sources are renewable and are more environmental friendly than the current sources for plastic production. Bioplastics and normal plastics have many thing in common, the only difference is that, bioplastics use renewable resources in their manufacturing and are mostly biodegradable and recyclable [29 - 31].

For detail, bioplasticsare produced from plant sources as renewable materials and the products could be further recycled as new' biomass sources, thermal, organic or chemical recycling. Additionally, the production of bioplastics are more environmental friendly, as less CO_2 are produced, thus not contributing much to the global warming [32].

Moreover, many bioplastics are biodegradable and can be made from a wide range of different plants or microorganisms. Going along with developing biotechnology, in future, genetically modified plants will need less water and reduce the costs. Bioplastics has the potential to reduce the petroleum consumption for plastic by 15 to 20 percent in 2025 [33]. Improved technical properties and innovations open new markets and applications with higher profit potentials in automotive, medicine and electronics [34 - 40].

Bioplastics Market Worldwide with high growth through Consumer Demands for Nontoxic Products. Nontoxic is the key image, sustainable and environmentally friendly products is the driving force. On the other hands, the world's resources are depleting rapidly, therefore, bioplastics could be a solution for the future.Bioplastics production companies are relatively small plants and are still in the early stages of the development and surely, with new developments in the future, the production will be more and more effective and will open new applications, and new opportunities [41, 42].

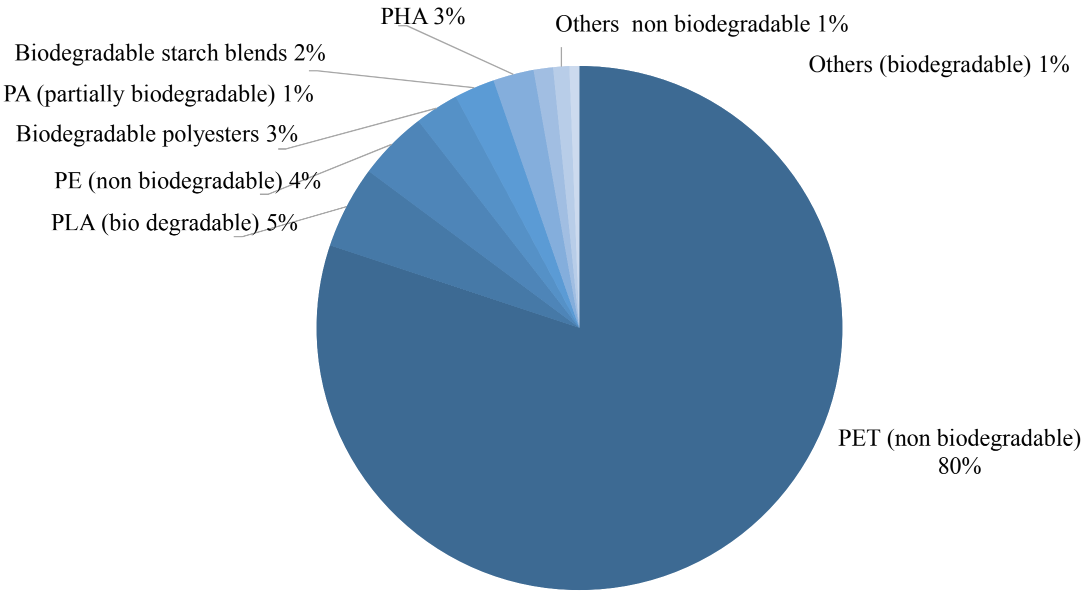


Figure 1.1 Production of bio-based plastic market by 2014

Following the recent estimation of European Bioplastics projects, the global bioplastic industry will experience a fivefold increase in production volumes between 2011 and 2016, from approximately 1.2 million metric tons to 5.8 million metric tons. The most significant growth is expected to be in nonbio-degradable plastics, such as bio-based polyethylene (PE) and polyethylene terephthalate (PET). The market forecast published by European Bioplastics estimates that PET will represent 4.6 million, or 80 percent of total bioplastic production capacity, by 2016. Asia had the most bioplastic production capacity online in 2011, with 34.6 percent. The relative capacity percentages for South America, Europe, North America and Australia were 32.8 percent, 18.5 percent, 13.7 percent and 0.4 percent. By 2016, Asia is expected to be home to 46.3 percent of capacity, with South America close behind at 45.1 percent. Europe, North America and Australia are expected to represent a relative 4.9 percent, 3.5 percent and 0.2 percent of production capacity [22, 23].Biodegradable Plastic markets with high growths to 2015 over 6 Billion US Dollar and 12.5 billion 2025. 65 percent of bioplastics in 2007 is Packaging and Food related and in 2025 still 40 Percent but Automotive and Electronics reach over 25 Percent by 2025 with higher profit potential. Innovations, Consumer demand and Legislation drive the markets. When bioplastics companies change their strategy from just replacing conventional today's plastic to new applications, product conceptions and production processes with the advantage of bioplastics the profitability and sale ability increase dramatically.In 2025 Europe will count for 31 percent, USA 28 percent and Asian for 32 percent of the total markets. Asian has the advantage that genetically modified plants are easier to realize and new outlets for agriculture are faster to build up. Bioplastics fast market growth of more than 8-10% per year. Bioplastics cover approximately 10-15% of the total plastics market and will increase its market share to 25-30% by 2020. The market itself is huge, it reached over 1billion US$ in 2007 and will be over 10 billion by 2020. More and more companies are entering and investing in this market. New applications and innovations in the Automotive and Electronics Industry lead to market expand. Over 500 bioplastics processing companies are already available, more than 5000 is expected by 2020 [24-26].

1.3. Bio-based polyester: application and advantages

Polyester is a category of polymers which contain the ester functional group in their main chain.Polyesters include naturally occurring chemicals, such as in the cutin of plant cuticles, as well as synthetics through step-growth polymerization such as polycarbonate and polybutyrate. Natural polyesters and a few synthetic ones are biodegradable, but most synthetic polyesters are not. Depending on the chemical structure, polyester can be a thermoplastic or thermoset, there are also polyester resins cured by hardeners; however, the most common polyesters are thermoplastics [26, 35].

Fabrics woven or knitted from polyester thread or yarn are used extensively in apparel and home furnishings, from shirts and pants to jackets and hats, bed sheets, blankets, upholstered furniture and computer mouse mats. Industrial polyester fibers, yarns and ropes are used in tire reinforcements, fabrics for conveyor belts, safety belts, coated fabrics and plastic reinforcements with high-energy absorption. Polyester fiber is used as cushioning and insulating material in pillows, comforters and upholstery padding. Polyesters are also used to make bottles, films,tarpaulin, canoes, liquid crystal displays, holograms, filters, dielectric film for capacitors, film insulation for wire and insulating tapes.

Polyesters are widely used as a finish on high-quality wood products such as guitars, pianos and vehicle/yacht interiors. They can quickly fill wood grain, with a high-build film thickness per coat. Some polyesters can be sanded and polished to a high-gloss, durable finish [38]. Polyester fabrics can provide specific advantages over natural fabrics, such as improved wrinkle resistance, durability and high color retention. Synthetic fibers also can create materials with superior water, wind and environmental resistance compared to plant-derived fibers. Liquid crystalline polyesters are among the first industrially used liquid crystal polymers. They are used for their mechanical properties and heat-resistance. These traits are also important in their application as an abradable seal in jet engines [19, 20].

1.4 Biodegradability of polyesters

Biodegradable polymers are defined as those which are degraded in biological environments not through thermal oxidation, photolysis, or radiolysis but through enzymatic or non-enzymatic hydrolysis. Polymer degradation takes place mostly through scission of the main chains or side-chains of polymer molecules, induced by their thermal activation, oxidation, photolysis, radiolysis, or hydrolysis [41]. Among those, polyester can undergo degradation in biological environments when living cells or microorganisms are present around the polymers. Such environments include soils, seas, rivers, and lakes on the earth as well as the body of human beings and animals [42].

Major application of biodegradable polymers is in plastic industries to replace biostable plastics for maintaining our earth environments clean. The first choice for processing of plastic wastes is reuse, but only some plastic products can be re-used after adequate processing, and many of them are very difficult to recycle. In these cases, wastes are processed by landfill or incineration, but these processes often pollute the environments [43]. If biodegradation by-products do not exert adverse effects on animals and plants on the earth, biodegradable plastics can be regarded as environment-friendly or ecological materials. Therefore, much attention has been focused on manufacturing biodegradable plastics which, however, should address several requirements. They are to be low in product cost, satisfactory in mechanical properties, and not harmful to animals and plants when biodegraded. The biodegradation kinetics is also an important issue of biodegradable plastics. Ideally, biodegradable polyester syntheses can be approach from renewable resources.

1.5 Synthesis and structural manipulation methods of bio-based polyesters

Synthesis of polyesters is generally achieved by a polycondensation reaction. See "condensation reactions in polymer chemistry". The general equation for the reaction of a diol with a diacid is:

$$(n+1)\ R(OH)_2 + n\ R'(COOH)_2 \rightarrow HO[ROOCR'COO]_nROH + 2n\ H_2O$$

1.5.1 Azeotropic esterification

In this classical method, an alcohol and a carboxylic acid react to form a carboxylic ester. To assemble a polymer, the water formed by the reaction must be continually removed by azeotropicdistillation.

1.5.2 Alcoholic transesterification

An alcohol-terminated oligomer and an ester-terminated oligomer condense to form an ester linkage, with loss of an alcohol. R and R' are the two oligomer chains, R" is a sacrificial unit such as a methyl group (methanol is the byproduct of the esterification reaction).

1.5.3 Acylation (HCl method)

The acid begins as an acid chloride, and thus the polycondensation proceeds with emission of hydrochloric acid (HCl) instead of water. This method can be carried out in solution or as an enamel.

1.5.4 Silyl method

In this variant of the HCl method, the carboxylic acid chloride is converted with the trimethyl silyl ether of the alcohol component and production of trimethyl silyl chloride is obtained

1.5.5 Ring-opening polymerization

Aliphatic polyesters can be assembled from lactones under very mild conditions, catalyzed anionically, cationically or metallorganically. A number of catalytic methods for the copolymerization of epoxides with cyclic anhydrides have also recently been shown to provide a wide array of functionalized polyesters, both saturated and unsaturated.

1.5.6 Enzymatic catalyzation

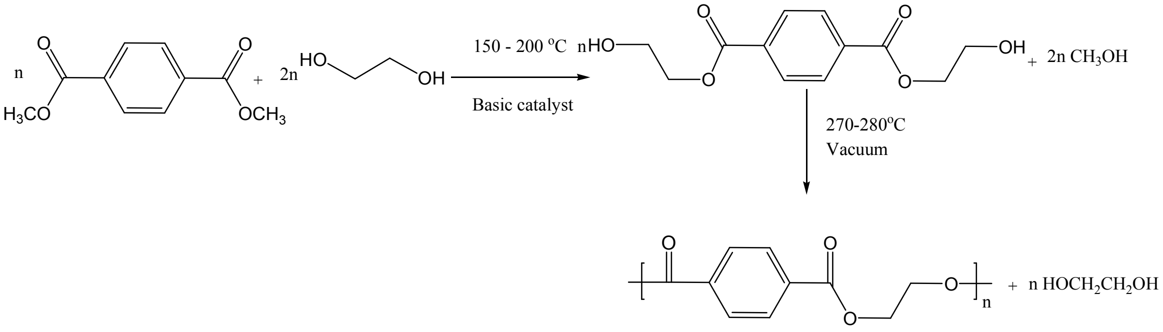
Immobilized enzymes have been developed to catalyze a wide array of reactions. These reactions include an expanding number of examples in which immobilized enzymes are used for bio-transformations conducted for esterification on an industrial scale [45, 46] Most enzymes are active at mild operational conditions of pH and temperatures (e.g., 30–50 °C). Furthermore, enzymes are produced from renewable resources and, in many cases, are free of heavy metals. These properties are attractive for the development of “sustainable processes” conducted with low-energy input, thereby resulting in safer processes. [47, 48]

1.5.7 Cross-linking

Unsaturated polyesters are thermosetting resins. They are generally copolymers prepared by polymerizing one or more diol with saturated and unsaturated dicarboxylic acids (maleic acid, fumaric acid...) or their anhydrides. The double bond of unsaturated polyesters reacts with a vinyl monomer, usually styrene, resulting in a 3-D cross-linked structure. This structure acts as a thermoset. The exothermiccross-linking reaction is initiated through a catalyst, usually an organic peroxide such as methyl ethyl ketone peroxide or benzoyl peroxide.

1.6 Polyethylene terephthalate

Commonly abbreviated PET, PETE, or the obsolete PETP or PET-P is a thermoplastic polymer resin of the polyester family and is used in synthetic fibers; beverage, food and other liquid containers; thermoforming applications; and engineering resins often in combination with glass fiber. The majority of the world's PET production is for synthetic fibers (in excess of 60%), with bottle production accounting for around 30% of global demand. In the context of textile applications, PET is referred to by its common name, polyester, whereas the acronym PET is generally used in relation to packaging. Polyester makes up about 18% of world polymer production and is the third-most-produced polymer; polyethylene (PE) and polypropylene (PP) are first and second, respectively.



Scheme 1.1 Synthesis of PET through Dimethyl terephthalate process

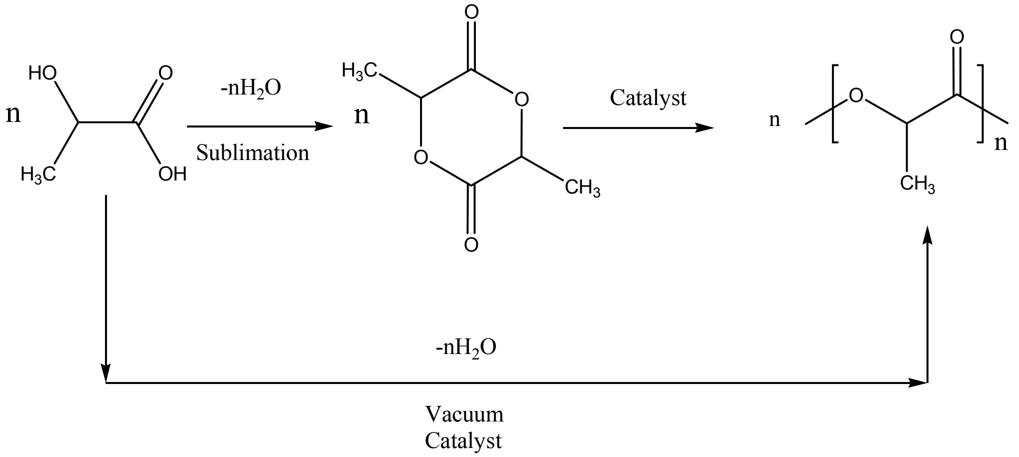
Depending on its processing and thermal history, polyethylene terephthalate may exist both as an amorphous (transparent) and as a semi-crystalline polymer. The semicrystalline material might appear transparent (particle size < 500 nm) or opaque and white (particle size up to a few microns) depending on its crystal structure and particle size. PET has T_m at 250 °C and T_g around 60-70 °C. Its monomer (bis-β-hydroxyterephthalate) can be synthesized by the esterification reaction between terephthalic acid and ethylene glycol with water as a byproduct, or by transesterification reaction between ethylene glycol and dimethyl terephthalate with methanol as a byproduct. Terephthalic acid and ethylene glycol – starting material of PET - can be obtained from bio mass [25]. Polymerization is through a polycondensation reaction of the monomers (done immediately after esterification/transesterification) with water as the byproduct.

1.6.1 Degradation of PET

PET is subjected to various types of degradations during processing. The main degradations that can occur are hydrolytic, thermal and, probably most important, thermal oxidation. When PET degrades, several things happen: discoloration, chain scissions resulting in reduced molecular weight, formation of acetaldehyde, and cross-links ("gel" or "fish-eye" formation). Discoloration is due to the formation of various chromophoric systems following prolonged thermal treatment at elevated temperatures. This becomes a problem when the optical requirements of the polymer are very high, such as in packaging applications. The thermal and thermooxidative degradation results in poor processability characteristics and performance of the material [50-55].

1.7 Poly(lactic acid)s

Poly(lactic acid) or polylactide (PLA) is a thermoplastic aliphatic polyester derived from renewable resources, such as corn starch (in the United States), tapioca roots, chips or starch (mostly in Asia), or sugarcane (in the rest of the world). In 2010, PLA had the second highest consumption volume of any bioplastic of the world [56-58].There are several industrial routes to usable (i.e. high molecular weight) PLA. Two main monomers are used: lactic acid, and the cyclic di-ester, lactide. The most common route to PLA is the ring-opening polymerization of lactide with various metal catalysts (typically tin octonoate) in solution, in the melt, or as a suspension. The metal-catalyzed reaction tends to cause racemization of the PLA, reducing its stereo-regularity compared to the starting material [1, 2, 3, 56].



Scheme 1.2 Synthesis of poly(lactic acid)

Another route to PLA is the direct condensation of lactic acid monomers. This process needs to be carried out at less than 200 °C; above that temperature, the entropically favored lactide monomer is generated. This reaction generates one equivalent of water for every condensation (esterification) step, and that is undesirable because water causes chain-transfer leading to low molecular weight material. The direct condensation is thus performed in a stepwise fashion, where lactic acid is first oligomerized to PLA oligomers. Thereafter, polycondensation is done in the melt or as a solution, where short oligomeric units are combined to give a high molecular weight polymer strand. Water removal by application of a vacuum or by azeotropic distillation is crucial to favor polycondensation over transesterification. Molecular weights of 130 kDa can be obtained this way. Even higher molecular weights can be attained by carefully crystallizing the crude polymer from the melt. Carboxylic acid and alcohol end groups are thus concentrated in the amorphous region of the solid polymer, and so they can react. Molecular weights of 128-152 kDa are obtainable [26].

Due to the chiral nature of lactic acid, several distinct forms of polylactide exist: poly-L-lactide (PLLA) is the product resulting from polymerization of L,L-lactide (also known as L-lactide). PLLA has a crystallinity of around 37%, a glass transition temperature between 60-65 °C, a melting temperature between 173-178 °C and a tensile modulus between 2.7-16 GPa. Heat resistant PLA can withstand temperatures of 110 °C [57]. PLA is soluble in chlorinated solvents, hot benzene, tetrahydrofuran, and dioxane[58]. PLA has similar mechanical properties to PETE polymer, but has a significantly lower maximum continuous use temperature.[59-60]Polylactic acid can be processed like most thermoplastics into fiber (for example using conventional melt spinning processes) and film. The melting temperature of PLLA can be increased 40-50 °C and its heat deflection temperature can be increased from approximately 60°C to up to 190 °C by physically blending the polymer with PDLA (poly-D-lactide). PDLA and PLLA form a highly regular stereo complex with increased crystallinity. The temperature stability is maximized when a 50:50 blend is used, but even at lower concentrations of 3-10% of PDLA, there is still a substantial improvement. In the latter case, PDLA acts as a nucleating agent, thereby increasing the crystallization rate. Biodegradation of PDLA is slower than for PLA due to the higher crystallinity of PDLA.

1.7.1 Degradation of PLAs

Similarly as degradation of PET, that of PLAscan processes chemically and physically under same factors such as pH, UV radiation, heating. Besides, owing to many screening on microorganism, there are revealing about majorbacteria species such as

Amycolatopsissps and Saccharotrix*sps*,which are able to degrade PLAs. A purified protease enzyme from *Amycolatopsis sp*., named PLA-depolymerase, was pointed out as factor to degrade PLA. Enzymes such as bromelain, pronase and most effectively proteinase K from *Tritirachium album*can also degrade PLA [1, 2, 3, 4, 55-59].

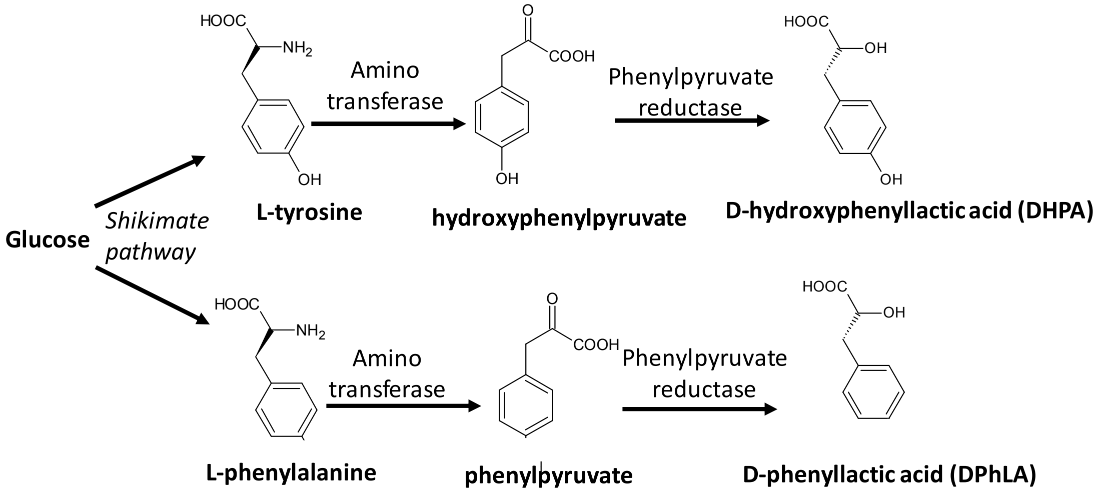
1.8 Use of aromatic bio-based compound in design and syntheses of bio-based polyesters

Biomolecules are very diverse in terms of their chemical structures most of which are dependent on group or species of living organisms. Among metabolic products, almost aromatic bio compounds are phenolic derivatives which are widely available in various environments as products via shikimate or polyketide biosynthetic pathway [30]. The aromatic component gives an important basis for chemo, bio or physico activity in living processes because aromatics bare many functions originated from chemical groups. Through the resonance of π-electrons induces not only rigidity and chemical stability of the aromatic ring but also photo-absorbance and fluorescence of a π-conjugated groups. Recently, researcher reveal vast number of aromatic compounds that play important role in living processes of organism. Such compounds usually exist as secondary metabolites of plants, fungi and bacteria, for example, several derivatives of cinamic acid such as 4HCA (*p*-coumaric acid), 3-methoxy-4HCA (feluric acid), 3,5-dimethoxy-4HCA (sinapic acid), 3,4-dihydroxycinnamic acid (caffeic acid; DHCA), and cinnamaldehyde. These bio-compounds have roles of allelopathy, anti-bacteria, and anti-herbivore. Many of those compounds have been isolated and produced massively through biotechnology to fulfill a human demand for fungicide, antimicrobial, anticancer, etc [61].

On the other hands, due to stability and chemical-modification ability of aromatic ring, many aromatic bio-compounds are employed as starting materials for novel valuable compounds through reachable chemistry techniques [62]. Conventional bio-based plastics are generally composed of aliphatic polyesters whose applications have been limited due to their poor thermal, mechanical properties. Therefore, aromatic biomolecules are an attractive resource for make more versatile by developing high performance bio-plastics with rigid-rod structures and π-conjugation functions [63-76].

1.9 Aims and scopes of this study

Recently, new microbial hydroxyl acids of phenyllacte family were biologically produced invitro by recombinant E. coli which expressing aminotranferase gene system of *Wikeramiafluorescene*. New hydroxyl acid which are massively produce with optical purity are D-phenyllactic acid DPhLA and D-hydroxyphenyllactic acid DHPA, contain multifunction structure including α-hydroxy, *p*-hydroxy phenyl and carboxylic acid (scheme 1.1). The main motivation of this work is scientific digging into syntheses and characterization new polyester with expectation to apply these materials in technical relevance to structure structure and chemical moiety of bio-derived aromatic hydroxy acid phenyllactic acids PhLAs and hydroxyphenyllactic acid DHPA[36].



Scheme 1.9 Biological process of producing phenyllactate derivatives [36]

The main aim of this research is to design polymer structure from natural structure of DHPA and PhLAs to prepare high-heat-deforming resistant polyester. The most important objective of study in this thesis is to study properties and analyzed new bio-based polymer structure. To achieve the goal, a detailed understanding of the chemistry and functionality of the different monomers and the resulting polymers was required. In order to develop suitable synthetic routes, in-depth studies into the chemistry of the several polymerization and modification reactions were performed. Fundamental polymer characteristics, chuch as functionality, glass transition temperature, molecular-weight (and distribution) as well as thermal decomposition was investigated to design polymeric architecture with a natural base.

This research project included a broad array of disciplines, ranging from polymer design, synthetic routes, controlling reaction equilibrium as well as material analytical and testing techniques. To achieve those objectives, a chain of approaching method was chosen, including the following step:

(1) To design molecular structure of bio-based polyester thought their monomer nature

(2) To polymerize of the bio-based polyester in suitable synthetic route with high efficiency and convenience

(3) To develop high-heat-resistance bio-based polyester

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Chapter 2

Syntheses and Characterization of High Molecular-weight Poly(phenyllactic acid)s under Lewis-acid Stable in Water Catalysts and Tin Metal Catalyst

Abstract

Optimization of a direct melt polycondensation in the presence of stable Lewis acids such as $HfCl_4.2THF$ resulted in poly(phenyllactic acid)s (PLPhLAs) with high molecular weight. As a result, the homo poly(L-phenyllactic acid), PLPhLA, with a number-average molecular weight (M_n) more than 100,000 g.mol^{-1} were obtained and showed specific optical rotation $\alpha_D^{25}$of 46° and the glass-transition temperature (T_g) of 55 °C whose absolute values were higher than the reported values and was comparable with T_g of chiral-homopolyester poly(L-lactic acid). The properties of resulted PPhLAs were characterized in relation with their structure by nuclear magnetic resonance analyses.

Keywords: phenyllactic aicd, lewis acid stable in water, polyester,

2.1 Introduction

Poly(L-lactic acid)s (PLAs), a biocompatible and biodegradable polyester, have drawn much attention in the chemical industry. PLAs have been majorly applied in medical tools, pharmaceutics, and agro-chemistry [1]. However, the application ranges of PLA-based polymers are limited because of the difficulty in controlling the hydrolysis rate, poor hydrophobicity, and brittleness. To overcome those problems, a series of lactic acid derivatives, where the hydrogen in a methyl group were substituted, were polymerized to give poly(butylglycolide), poly(hexylglycolide), and poly(ethylglycolide) [2]. Phenyllactic acids (LPhLA) are a lactic acids bio-derivatives produced by the bacteria such as *Lactobacillus plantarum*and fungus such as *Wikeramia fluorescene*[3]. The incorporation of a bulk aromatic group into lactic acid should be effective to increase the glass-transition temperature, T_g, of the corresponding bio-based plastics [4]. The bulky aromatic side group, however, brought about difficulty in solution polymerization; steric hindrance of the benzyl group could lower the polymerization rate [5]. Although direct polymerizations of LPhLA [6] and ring-opening polymerization (ROP) of LPhLA under phenyl sulfonic acid have been made [5], T_g values of poly(Lphenyllacticacid) (PLPhLA) were reported to be lower than the T_g of chiral homo PLA (55 °C), which might be attributed to low molecular weight less than 20,000 g.mol^{-1}.

In recent years, stable Lewis acids have been proven to overcome troubles of direct polycondensation of hydroxy acid. These catalysts are rare-earth metal triflate salts [7] and some other metals such as hafnium and vanadium. Stable Lewis acid catalysts have a resistance against inactivation by the water condensed in polymerization of hydroxy acids. The complex of hafnium(IV) chloride salt were reported to give a higher yield of conversionin direct polycondensation of diacids and diols than conventional catalysts and did not catalyze the transesterification atall [8]. Scandium trifluoromethanesulfonate and scandium trifluoromethanesulfonimide were also reported to have highactivity in polycondensation of several diacids and diols evenat room temperature [9].Here we conduct a one-step polymerization by a direct melt polycondensation of PhLA in the presence of the stable Lewis acids and obtain the T_g of PPhLAs.

2.2 Experiment part

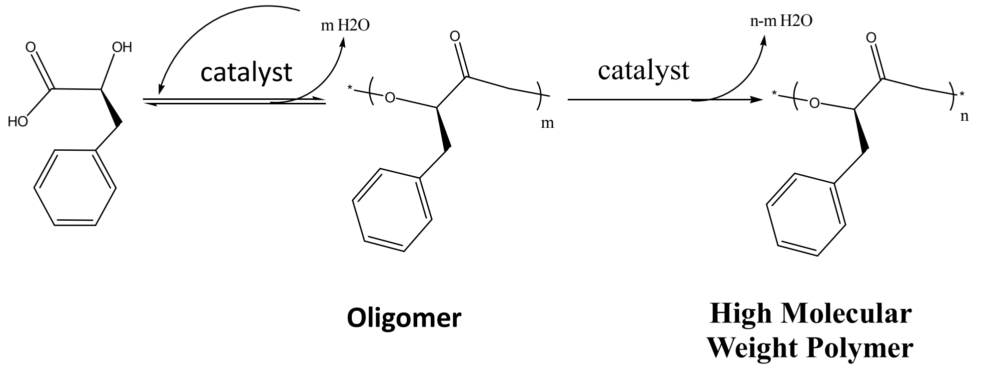
2.2.1 Material

L-phenyllactic acid purchased from Sigma-Aldrich purity 95%, D-phenyllactic acid is extracted from biomass of recombinant *E. coli* expressing *Wikeramia flourescene* aminotransferase gene.

Catalyst used are: hafnium(IV) chloride tetrahydrofuran complex - $HfCl_4$.2THF(purity 98%, Sigma-Aldrich); vanadium(III) chloride tetrahydrofuran complex - VCl_3.3THF (purity 98%, Sigma-Aldrich), ytterbium(III) trimethanesulfonate - $Yb(OTf)_3$(powder, purity 98%, Sigma-Aldrich);scandium(III) trimethanesulfonate - $Sc(OTf)_3$ (powder, purity 98%, Sigma-Aldrich); tin metal - Sn (powder, TCI)

2.2.2 Polymer syntheses

In order to find the Lewis acid most suitable for the polymerization of PhLAs, PPhLAs were synthesized in the presence of various stable Lewis acids such as $Sc(OTf)_3$,$Yb(OTf)_3$, VCl3x3THF (THF: tetrahydrofuran), and $Hf(OTf)_4$and Sn metal in the concentration of 0.5 wt%. The mixtures were stepwise heated at 130 and 150 °C for 3 h each with agitation under nitrogen gas flow and then at 180 °C for 6 h agitation under reduced pressure of 14 KPa. At the last stage, the pressure was reduced to 20 Pa while maintaining the temperature and the mixture was further agitated for 18 h.



Scheme 2.1 Syntheses method of high molecular-weight PhLAs polyester

The obtained polymer obtained initial step has a low molecular weight, because it is hard to remove water completely from the highly viscous reaction mixture; therefore at this step low molecular-weight polyester of a few ten thousands is obtained. So, this method is employed the polymer of low molecular weight is required then high molecular-weight polyester attained by transesterification at high temperature under bulk-polycondensation condition.

The polymer of low molecular weight and molecular-weight large distribution is the main disadvantage of direct polycondensation polymerization and it restricts its use. Moreover, the stereo-regularity is hard to be control during the course of polymerization.

However, a polymer of high molecular weight can be obtained by using controlled heating and pressure reducing process to improving methods polyester properties.

2.2.3 Structure confirmation

We confirmed the formation of the aimed structure of poly(phenyllactic acid)s from ^{1}H-NMR and ^{13}C-NMR spectra, solvent: DMSO-d6.

2.2.4 Molecular weight of polyester

The number average molecular weight (M_n), weight-average molecular-weight (M_w) and molecular weight distribution (PDI) were determined by gel permeation chromatography (GPC, conc. 5mg/mL, DMF as an eluent) after calibrating with the standard pullulan at 40 °C.

2.2.5 DSC measurement

Differential scanning calorimetry (DSC) was performed on DSC (Seiko instrument Inc., EXSTARX-DSC7000 SII) apparatus. Temperature and heat flow scales were calibrated using high purity indium. Sample weight was taken in the range 5-10 mg and samples were heated from 25 °C to 250 °C at the rate of 10 °C/min.

2.2.6 Thermogravimetric (TGA) Analysis

Polyesters were subjected to TGA using SSC/5200 SII Seiko Instruments Inc. equipment. Sample weighing 5-10 mg were heated from 25 °C to 700 °C at heating rate of 10°C/min in nitrogen atmosphere and corresponding weight loss was recorded.

2.3 Result and discussion

2.3.1 Optimization of polyesters syntheses

Since the polymerization did not proceed at room temperature, it was found that the heating was indispensable in this system. However, a sudden temperature jump to 180 °C induced rapid evaporation of the monomer, which reduced the yield of polymer products (fig. 2.1). On the other hand, the stepwise heating well restricted the monomer evaporation because of the stable oligomer formation through the initial lower-temperature treatment. The weight-average molecular-weight (M_w) and number-average molecular-weight (M_n) of the purified polymers were then analyzed and summarized in table 4.1.The obtained polymers were insoluble in water but soluble in various organic solvents (Table 4.2).

The mixture were first heated up to 130 ^{o}C in 3h under reduced pressure of 14kpa then heated up to 150 ^{o}C in 3h under the same pressure. In final step, the mixtures were heated up to 180 ^{o}C under the same vacuum for 6 hours and then extremely reduced to 20pa for last 48 hours. We select this route of heating for reaction to observe and compare the result of catalysts during time and at different temperatures. This route also helps conserving the best yield of reactions. The same procedure was applied for reaction with D-phenyllactic acid and racemic mixture of D-L-phenylactic acid. By this route of heat presented above the molecular weight value can reached 200,000 g/mol. After confirm the good reaction condition Using result above to assess activity of water stable Lewis acid for poly(phenyllactic acid)s, we expand this direct melt polycondensation with other well-known highly effective triflate and chloride salt such $Sc(OTf)_3$, $Yb(OTf)_3$, $VCl_3.(3THF)$, $Hf(OTf)_4$ in the same concentration 0.5 mol%.

Table 2.1 Catalyst screening test result of direct melt polycondensation of PhLAs

Entry	**Catalyst**	**Heating temperature (°C)**	**Concentration (mol%)**	**Time (h)**	**Yield (%)**	**M_w (g/mol)**	**M_w/M_n**
1	$Sc(OTf)_3$	130 + 150 + 180	0.5	24	85	150286	1.31
2	$Yb(OTf)_3$	130 + 150 +180	0.5	24	85	130695	1.01
3	$Hf(OTf)_4$	130 + 150 + 180	0.5	24	85	85528	1.48
4	$HfCl_4$	130 + 150 + 180	0.5	24	85	134261	1.17
5	Sn	130 + 150 + 180	0.5	24	90	219087	1.37

Table 2.1 which indicates thatall the catalysts used here were effective, and the yields were high except for vanadium chloride. VCl_3x3THF had the least activity but ytterbium and hafnium salts had sufficient activity to increase M_n to more than 50,000 $gmol^{-1}$. Especially for the polymerization in the presence of $Hf(OTf)_4$, M_n exceeded 100000 $g.mol^{-1}$. Fig.2.2 shows the time course of molecular weights of PLPhLA prepared under various amounts ofHfCl4x2THF. Molecular weights increased and almost saturated at around 40 hrs. If the polymerization was made for a longer time than 40 hrs, the molecular weight of the resulting polymers decreased a bit. The

reason for the molecular weight decrease may be due to negative effects of Lewis acid, which will be discussed later.

The concentration of Lewis acid still effectively on controlled the molecular weights; while the polymerization speed in the initial 6 hrs was almost independent of the catalyst content, the polymerization rates under 0.5 and 1wt% Lewis acid at 60 hrs were higher than those under 1.5 and 2.5 wt%.Excess catalyst was not good for polymerization. This may be similar to the above-mentioned long-time polymerization. The reason is discussed below. The polymerizing liquid became viscous at long reaction times, and condensed water was entrapped in the polymerization liquid. A small ratio of $HfCl_4$ may react with water vapor at over 130 °C for a long producing hydrochloride despite the catalyst being reported to be stable to water. Hydrochloride sometimes promotes the hydrolysis of PLPhLA chains, which may be the main reason for the lower molecular weight of PLPhLA prepared at higher $HfCl_4$ loading or at long reaction times (fig. 2.2).

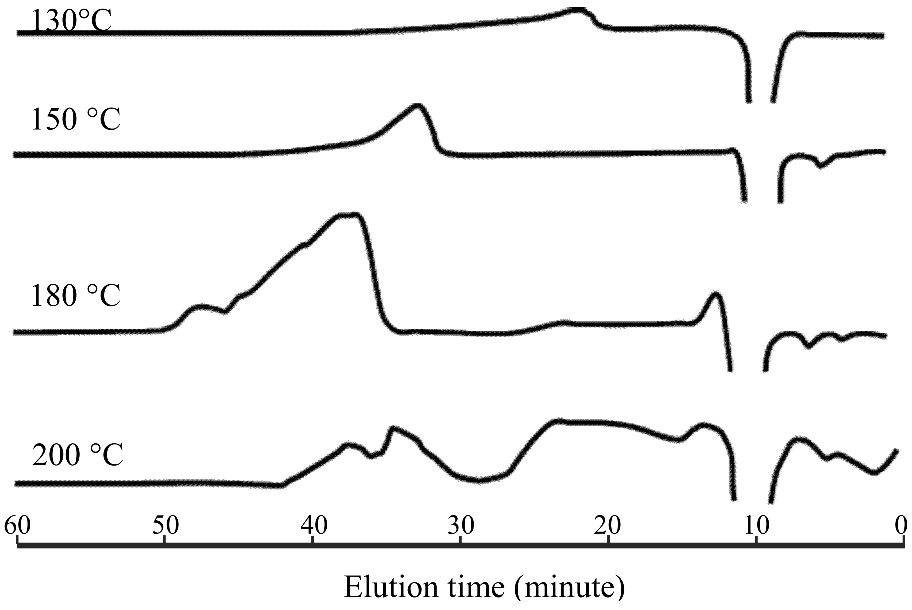


Figure 2.1 GPC spectra of PPhLA prepare at different temperature in 6h under $HfCl_4$ catalyst. Higher heating temperature caused chain breaking and produced low molecular mass polyesters

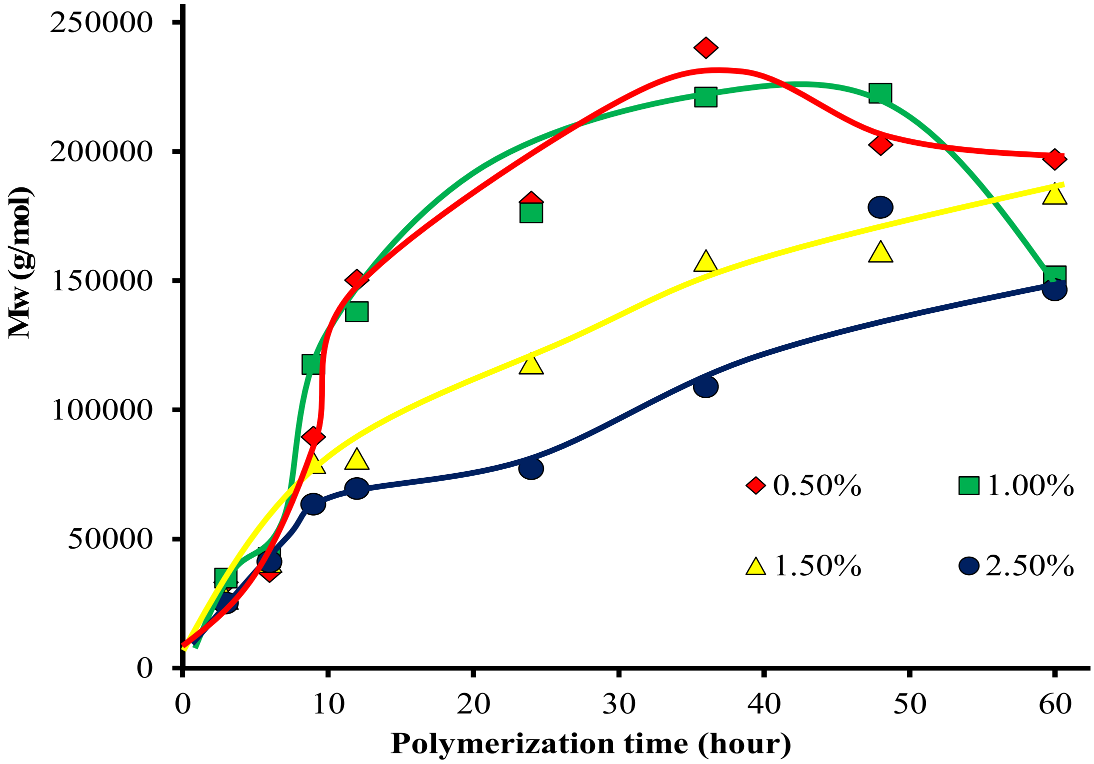


Figure 2.2 Time course of molecular weights of poly(phenyllactic acid)s prepared under various amounts of HfCl4. At higher concentration of acid catalyst loaded the chain back-breaking appeared after longtime reaction and heating.

Table 2.2. Solubility test of poly(L-phenyllactic acid) and its corresponding monomer

compounds	H_20	Acetone	MetOH	EtOH	DMF	THF	Hexane	$CHCl_3$	benzene
PLPhLA	-	+	-	-	+	+	-	+	+
PDPhLA	-	+	-	-	+	+	-	+	+
PDLPhLA	-	+	-	-	+	+	-	+	+

a) The mark "-" means insoluble in solvent while the marks "+" is soluble b) MetOH is methanol, EtOH is ethanol, DMF is dimethyl formamide, THF is tetra hydrofuran

2.3.2 Thermal properties of PPhLAs

2.3.2.1 Decomposing temperature

The decomposition temperature of polymer was recorded at 262 ^{0}C as 5% amount of polymers removed. The result is similar to previous study for low molecular weight poly(lactic acid) [4, 16, 17, 18]

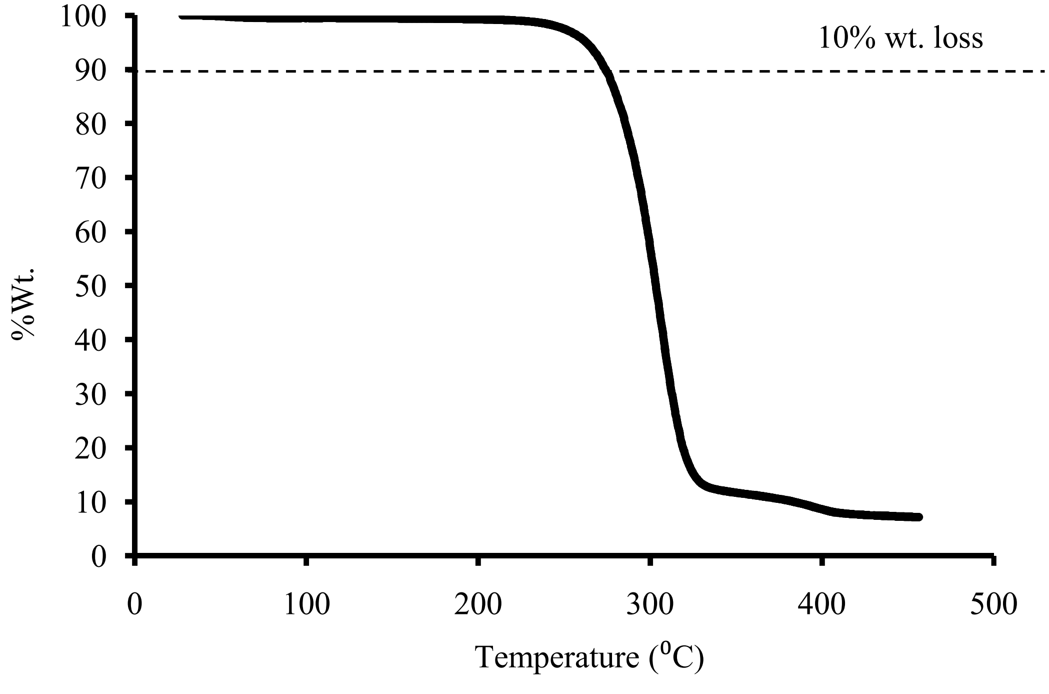


Figure 2.3.Thermal gravimetric spectrum of low molecular weight poly(L-phenyllactic acid) synthesized by melt polycondensation with HfCl4(THF)2

2.3.2.2 Heat-deforming resistance

Table 1 shows the glass-transition temperature, T_g, of PLPhLA under various polymerization conditions, where T_g was measured by differential scanning calorimetric (DSC). No melting peak was detected, but a flexion point typical of $T_g$appeared (Fig. 2.4). A representative relation curve is shown in fig2.5present the tendency ofT_g increased with an increase in Mn, which is not unexpected [10].Besides, the T_g leveled to show a maximum value of 57 °C (fig.2.4) which was higher than the Tg values of PLPhLA polymers reported in the literature (35 - 50 °C) [4, 5, 6].

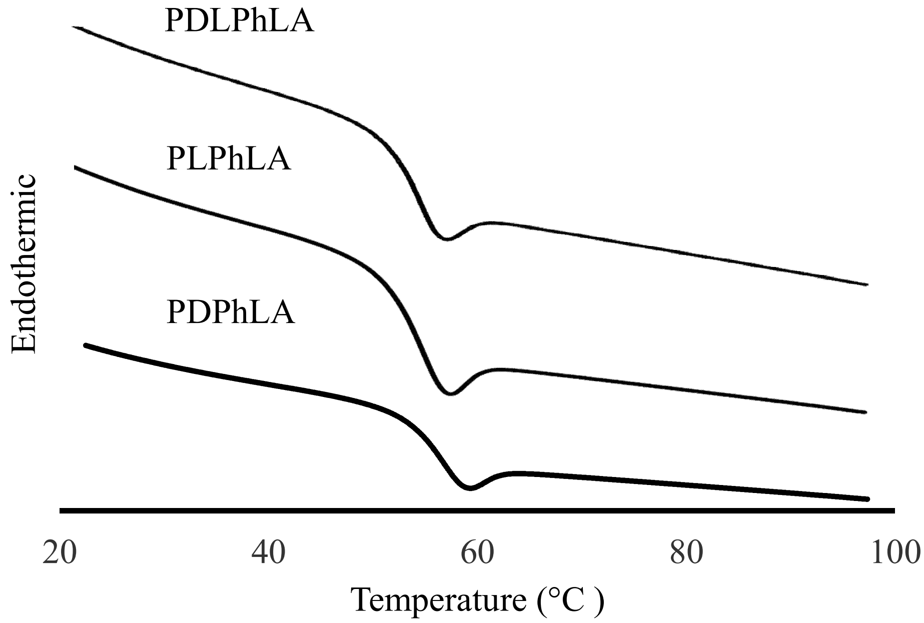


Figure 2.4 DSC thermogram of PPhLAs polyesters

The higher T_g of PPhLAs in the present study may be attributed to the high molecular weight, which was attained using special Lewis acid stable in water. However, the T_g of PPhLAs was comparable with that of poly(lactic acid) in spite of the presence of a bulky pendant group. The pendant phenyl group may hinder the main chain rotation but widen the inter-chain gap to weaken the inter-chain interaction [28, 29]. These opposite effects on the molecular mobility of PLPhLA chains can compete to result in little effect on T_g.

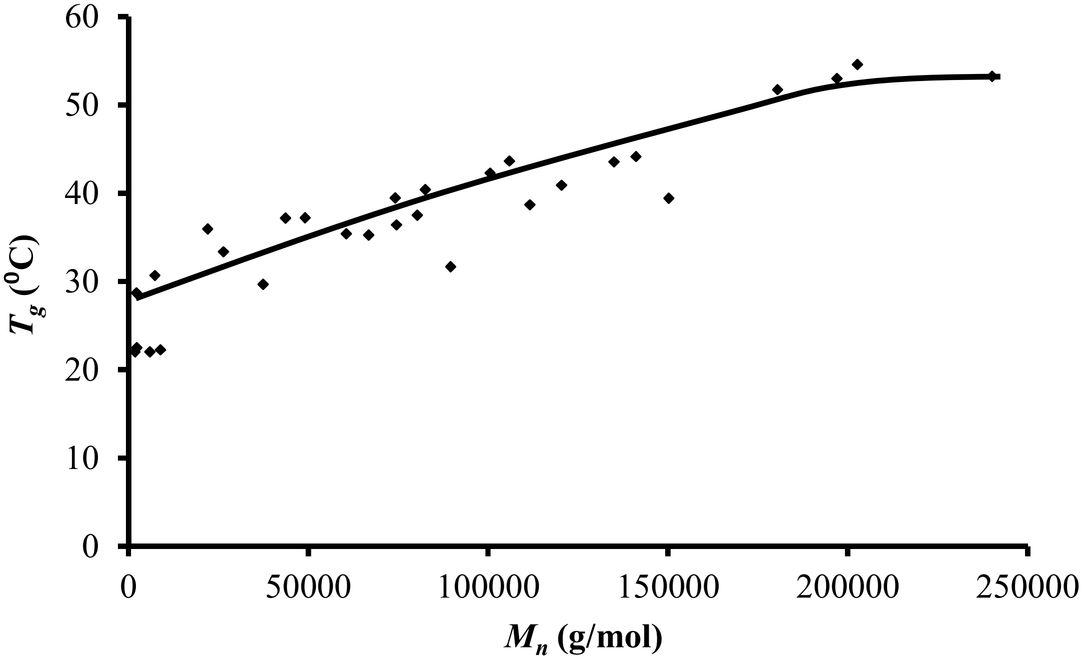


Figure 2.5 Relation between T_g and molecular-weight

2.3.3 Racemization and optical properties of PPhLAs

Due to racemization during heating of monomer under acid catalyst activity, the reverse configuration of monomer is produced and insert into polymer chain. The racemization reduced optical activity of polymer

In order to evaluate the racemization, we measured compare the region-regularity of PPhLAs on ^{13}C NMR spectra. Chemical shift of α-carbon at δ 165 ppm had the separation which corresponded to the region stereo-regularity. This separation, is produced by stereo configuration effect of α-carbon adjacent to each other, therefore each group of three adjacent carbon will generate different chemical shift on NMR. The low region-regularity group including LLD, LDL, DLD, DDL, LDD, DLL (character L is α-carbon of L configuration and character D is α-carbon of D configuration).

As seen in fig.2.6 the separation of chemical shift measured by J coupling parameter of 40 Hz. Low intensity of low region-regularity peak racemization comparing with high regio-regularity intensity of homo PPhLAs polyester noted that the racemization is suppressed in this melt polycondensation.

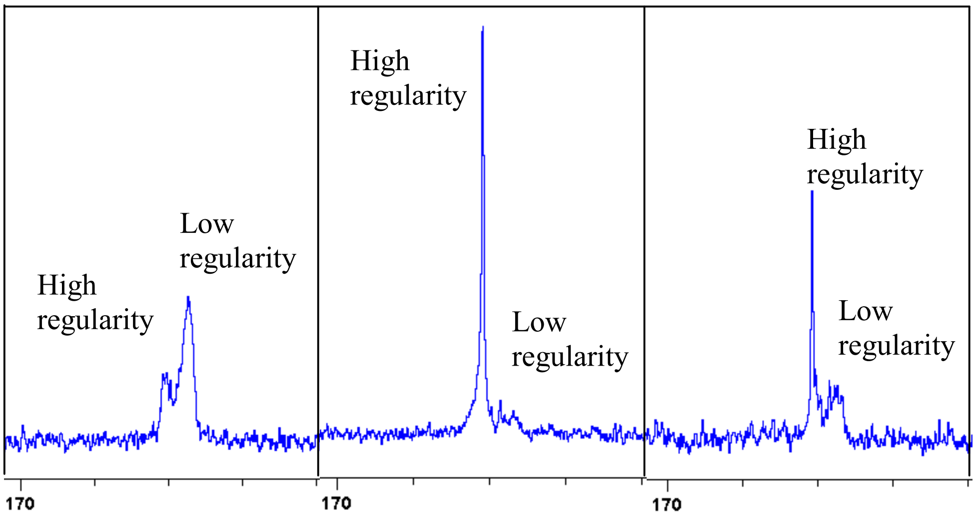


Figure 2.6 ^{13}C-NMR typical chemical sift peaks of α-carbon refer to stereo-regularity-depended property of internal-chain-α-hydrogen of poly(phenyllactic acid)s (a.

PDLPhLA; b. PDPhLA; c. PLPhLA) in different main configurations. (Low stereo-regularity: DLL (rmm) , DLD (rrm), LDD (rmr), LDL, DDL etc; high stereo-regularity LLLL, DDD (mmm).

On the other hand, we measured specific optical rotation α_D^{25} to confirm the optical activity of homo chiral PPhLAs After confirm the racemization, we plotted out the relation between molecular-weight of homo PPhLAs polyester. The obtained negative values from 32° of monomer increase to 47° of high molecular-weight polymer and saturated at near 150000 g/mol (Fig.2.7) The minimum value is lower than that reported [6, 15, 26, 27, 28] α_D^{25}= 38° of L and D lactic aid, α_D^{25}. Value of phenyllactic acid is lower than $\alpha_D^{25}$of lactic acid due to the bulky methyl-phenyl group binding to stereo center [14, 22, 23, 24. Increasing tendency of α_D^{25} indicating that the higher molecular weight increased the absolute values of α_D^{25}.Furthermore, we can emphasize that thermal racemization was restricted even in a high temperature condition at 180 °C for 6 h of last step polymerization.

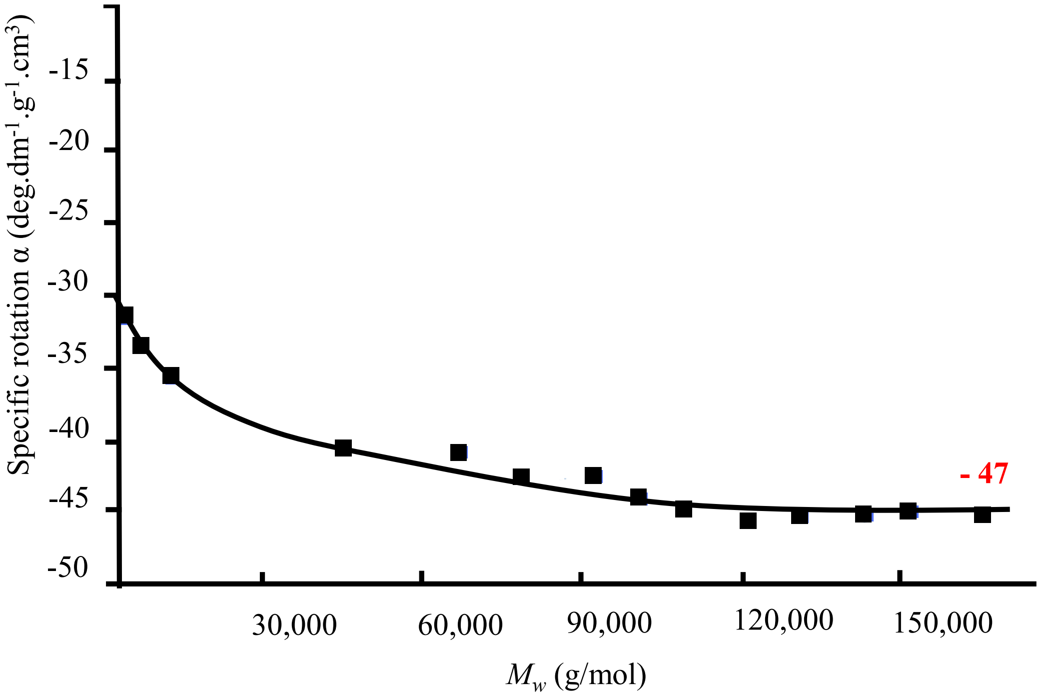


Figure2.7 Relation between α_D^{25}value and $M_w$of homo PPhLAs polyester

2.4 Conclusion

This chapter described the melt polycondensation reaction of phenylactic acids in presence some water-Lewis acids. On the other hand, a direct melt polycondensation normally gives low molecular weight polymers because of difficulties in controlling condensation equilibrium. To synthesized a bearing ring-side chain PLA derivative we have succeeded to use a direct melt polycondensation method under presence of some water stable Lewis acids to attain high molecular weight poly(phenyllactic acid)s.

In attempting to optimize the direct melt polycondensation for phenyllactic acids, this thesis employed well known high activity catalysts such as Hafnium (IV) Scandium

(III) Ytterbium (III) salts and Tin metal have set up the reactions under various conditions of pressures, temperatures.

As expected, these water-stable Lewis acids were marked to have high activity even in melt polycondensation earlier state. All the catalysts have resulted in high molecular weight polymers with high yield after extraction. The molecular weight value, i.e. the insertion of monomer and low molecular weight oligomers of direct melt polycondensation can be sensitively driven by the combination of vacuums, temperatures and catalyst activity to remove water residue and promote reaction. The extraction of catalysts from polymer mixtures is easy by two steps of reprecipitation with high volatility solvents such as methanol, acetone, and tetra hydro furan, etc.

High molecular weight poly(phenyllactic acid)s have similar maximum T_g value (around 55 ^{0}C) with PLAs, but as the result of this thesis that value is regardless of stereo regularity. The glass transition temperature of polymer shows an increase along with the increase of molecular weight. The dependence of T_g continue to near150000g/mol of molecular weight value. This limitation value of T_g dependence on molecular weight is far higher than the case of PLLA and PDLA (20000vg/mol).

In summary, the result from poly(phenyllactic acid)s under a direct melt-polycondensation has confirm activity of water-stable Lewis. In similarity to PLAs, PPhLAs indicated that the corporation of phenyl group to lactyl unit has low effect on polymer rigidity. By this result, poly(phenyllactic acid)s have potential of reduce brittleness of PLAs under co-polymerization as well as blending methods.

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Chapter 3

Syntheses and Characterization Of Bio-based Hyperbranched polyester-arylateFromMicrobialD-hydroxyphenyllacticAcidandGlycolic Acidby a Melt Polycondensation Reaction

Abstract

The synthesis method for high molecular weight polyester from hydroxy carboxylic acids is optimized in a melt polycondensation reaction with tin metal catalyst under reduced pressure. The copolyester of DHPA with glycolic acid resulted in significantly high T_g polyesters of maximum 110 ^{o}C. By spectroscopy study, we found that high T_g value of copolyester should be the result of hyperbranching property supported by trifunctional structure of DHPA as well as the addition of DHPA benzene ring. The copolymerization of DHPA and chiral lactic acids, chiral phenyllactic acids and hydroxy butyric acid are also conducted to obtain optically active polyester with significantly improved T_g. Branching process of polymer is observed through gel-permeation chromatograph and nuclear magnetic resonance analyses showed the role of DHPA as branching generating point at each course of polymerization time. Thermal properties of branched polymer were records in relation with branching degree and architecture development of polymer chain.

Keywords: D-hydroxyphenyllactic acid, glycolic acid, polyester, hyperbranching, glass-transition temperature.

Chapter 3

Syntheses and Characterization Of Bio-based Polyester-arylate From Microbial D-hydroxyphenyllactic Acid With Glycolic acid in a Melt Polycondensation Reaction

3.1 Introduction

Nowadays, bio-based polyesters are playing an important role in domestic and engineering plastic fields as renewable and bio-degradable materials. Several bio-based and partially bio-based polyesters such as polyglycolate, polylactates, polyhydroxybutyrates and polyethylene-terephthalate are synthesized and successfully become commercial plastics [1, 2, 3, 4]. However the application of those bio-based plastic is limited due to heat-deforming resistance barrier as they have glass-transition quite low [4], This problem make them disadvantage to conventional petroleum-based plastic like polyethylene while containing hot liquid such as water [5]. By a convenient synthetic method, this research reveal a possible way to improved T_g value of bio-based hydroxy carboxylic acid polyester by copolymerization with a microbial D-hydroxyphenyllactic acid (DHPA).

Branched polymers are characterized by the presence of branch points or the presence of more than two end groups and comprise a class of polymers between linear polymers and polymers networks [1]. Long-chain branched polymers offer significantly different physical properties such as melt rheology, mechanical behavior, and solvation properties, than linear polymer and polymers network [2, 3].

The importance of dendrimers and hyperbranched (HB) polymers has continuously drawn the attention of researchers during the last two decades due to their globular shape resulted from the branched backbone topology and their unique properties [4-6]. Although HB polymers have incomplete branched structures, they can be synthesized cost-efficiently and possess desirable properties similar to dendrimers. These attractive features have led to the development of novel synthetic routes for these polymers in this field [10-11]. Most HB polymers are prepared by the polycondensation of ABx type monomers, such as AB_2 [12, 13], AB_3 [12-14], AB_4 and AB_6 [17, 18], where the ABx play a role in the branching points. However, most of these ABx-monomers on a large scale for industrial are prepared via several steps, which include the rapid production of HB polymers on a large scale for industrial applications. Although many research groups have devoted themselves to develop facile, versatile, and cost-effective routes to synthesize HB polymers [19-20], only limited families of HB polymers have been prepared through the aforementioned methods,

On the other hand, there exist many ABxB'y- multifunctional biochemical such as glutamic acid, lysine, uronic acid, etc. We focused on D-hydroxyphenyllactic acid (DHPA), which is an ABB'-type (carboxyl-phenol-hydroxyl) biomonomer with a polymrizable dihydroxyl-acid group. DHPA is a secondary metabolite of L-tyrosine (the common amino acid exist in almost living organisms), produced under enzymatic activity of aminotransferase in *Wikeramia fluorescene*[21]. From DHPA and glycolic acid, we prepare high heat-deforming resistant polymers. However the effect of branching structure on on the thermal property had been primitively clarified.

Here we used a direct polymerization using appropriate acid catalyst stable in water and protic compound in melting condition to prepare bio-based polyester-arylate. We then investigate their hyper branching process and structure-thermal properties relationship in detail.

3.2 Experimental Section:

3.2.1 Materials

The wholly bio-derived DHPA was provided from cultivated biomass of Escherichia coli expressing pprA gene of Wickerhamia fluorescens, purity is over 99%. Glycolic acid purchased from TCI (98% purity). Sn metal powder purchased from TCI; hafnium (IV) chloride tetrahydrofuran complex - $HfCl_4.2THF$ (purity 98%, Sigma-Aldrich); vanadium (III) chloride tetrahydrofuran complex - $VCl_3.3THF$ (purity 98%, Sigma-Aldrich), ytterbium(III) trimethanesulfonate - $Yb(OTf)_3$ (powder, purity 98%, Sigma-Aldrich);scandium(III) trimethanesulfonate - $Sc(OTf)_3$ (powder, purity 98%, Sigma-Aldrich);

3.2.2 Structure characterization

1H NMR, spectra were determined by Bruker Biospin AG 400 MHz, 54 mm spectrometer using DMSO-d6 as solvent. Melting temperature was recorded with Mettle FP90 central processor and Mettler FP82HT hot stage. FTIR spectra were recorded with Perkin Elmer, spectrum one. Mass spectra were measured using Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS, Solarix), scanned from m/z 150 to 1000.

3.2.3 Molecular weight of polyester

The number average molecular weight (Mn), weight average molecular weight (Mw) and molecular weight distribution (PDI) were determined by gel permeation chromatography (GPC, conc. 5mg/mL, DMF as an eluent) after calibrating with the standard pullulan at 40 ^{o}C.

3.2.4 DSC measurement

Differential scanning calorimetry (DSC) was performed on DSC (Seiko instrument Inc., EXSTARX-DSC7000 SII) apparatus. Temperature and heat flow scales were calibrated using high purity indium. Sample weight was taken in the range 5-10 mg and samples were heated from 25 oC to 250 oC at the rate of 10 oC/min.

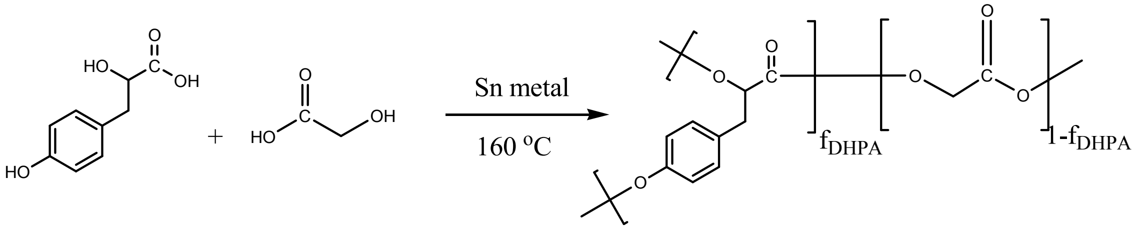
3.2.5 Thermogravimetric (TGA) Analysis

Polyesters were subjected to TGA using SSC/5200 SII Seiko Instruments Inc. equipment. Sample weighing 5-10 mg were heated from 25 oC to 700 oC at heating rate of 10oC/min in nitrogen atmosphere and corresponding weight loss was recorded.

3.3 Result and discussion

3.3.1 Chain propagation and molecular-weight

Polyester-arylate of DHPA were synthesized in melt polycondensation using Yb(OTf)3 acid catalyst stable in water. DHPA take the role of ABB’ monomer and glycolic acid (GLY) was taken the role of AB monomer (scheme 3.1). First we used a conventional catalyst such as HfCl4xTHF for the preparation of the P(DHPA-co-GLY), which showed multimodal GPC peaks indicative of a heterogeneous structure, presumably due to difficulty in control transesterification an. Next, we tried to find suitable catalysts for the production of copolyester with well-defined structures, such as $Yb(OTf)_3$, $ScOTf)_3$, $Hf(OTf)_4$, VCl3x5THF and yield dark color polyester with brittleness and widen range molecular-mass after 4 hours of polymerization from 160 ^{o}C, However Sn metal efficiently catalyzed polycondensation reaction and generated polymer with Mw of 100000 g/mol and mono unity peak in GPC spectra, low molecular-weight distribution, which increased gradually during time. As a result it was conclude that Sn metal can be attributed to its suitable acidity [22] and long duration during heating in presence of protic compound.



Scheme 3.1 Synthesis of copolymer of P(DHPA-*co*-GLY)

Using Sn metal as catalyst a serie of copolymer with feeding ratio of DHPA 80, 65, 50, 30 and 20 percent were prepare the Mn value were in a narrow range of 21000-50000, whereas the the M_w value were over a wide range of 70000 to 150000. As a result the polydispersed index for C_{DHPA} of 80 and 20 mol% were more than 4 and were higher than the other copolymers with medium C_{DHPA} composition of 65, 50, 30 (table 3.1). While a large number of DHPA unit could raise the number of branching points ABB' to increase PDI value, large number of DHPA unit also provided very rigid bulky structure and hard to be agitated.

Table 3.1 molecular weights of poly(DHPA-*co*-GLY) with different in feed composition of DHPA [a]

C_{DHPA} [b] (mol%)	M_w *g/mol*	M_n *g/mol*	PDI[b]	Yield (%) [c]
20	95300	23200	4.03	75

35	86800	21000	3.50	72
50	156600	45700	3.42	85
65	103500	25800	4.01	85
80	232400	56000	4.15	86
100	351100	66000	5.32	87

[a] reaction were carried out in the presence of Sn at 160 °C, pressure 24 KPa for 4 hours then same temperature at 100 Pa four 24 hours till the reaction mixture became solid.[b] DHPA molar composition to the total feeding monomers Calculated after precipitation in methanol and drying

3.3.2 Structural analyses

3.3.2.1 ^{1}HNMR spectra assignment

The structure of these hyperbranching copolyesters were characterizaed by 1HNMR. A representative 1H-NMR spectrum from a poly(DHPA-co-GLY) (DHPA composition: 50%) is shown in figure 3.1. A series of 1H-NMR peaks marks of chemical shifts are: singlet of carboxylic **-k-** δ ppm 13 (s, 1H), singlet of of hydoxyl-aryl **-i -** δ ppm 9.20 (s, 1 H)   multiplet of methanediyl **-b-** (-CH2-) δ ppm 2.76 (dtd, *J*=8.53, 5.27, 5.27, 2.51 Hz, 1 H), multiplet methine **-a-**δ ppm 4.15 (dt, *J*=7.91, 5.58 Hz, 1 H, doublet of 2-hydroxy **-d-** δ ppm 5.50 (d, *J*=6.27 Hz, 1 H) , phenyl have two triplet **e-**δ ppm 6.98 (d, *J*=8.53 Hz, 2.26 Hz, 1 H)and **f-**δ ppm 6.65 (d, *J*=8.53, 2.26Hz, 1H), doublet of α-hydroxyl **-i–** δ ppm 5.5 (d, *J* = 6.0 Hz, 1H) . The double multiplet of methine, doublet α-hydroxy and double multiplet methanediyl is corresponded to chiral effect. Singlet of GLY –OH is **g**δ ppm 5.2; α proton of GLY are **h** - singlet at δ ppm 3.9 (s, 2H)

The peak at **d** hydoxy aryl δ ppm 9.00 of DHPA unit corresponded to the end group B', the peaks at **i** δ ppm 5.5 of α-hydoxyl of DHPA and **g**δ ppm 5.2 hydroxyl of GLY corresponded to the end group B of HB architecture. The integral strength ratio in the realistic copolymer structure showed no side reaction such as oxidization at the phenyl group. The integral strength of the hydroxy aryl and was estimated at δ ppm 9.35 and the integral strength of the two hydroxyls was estimated at δ ppm 5.50 and at δ ppm 5.15 Ratio of these group in comparison to monomer feeding ratio showed agreement

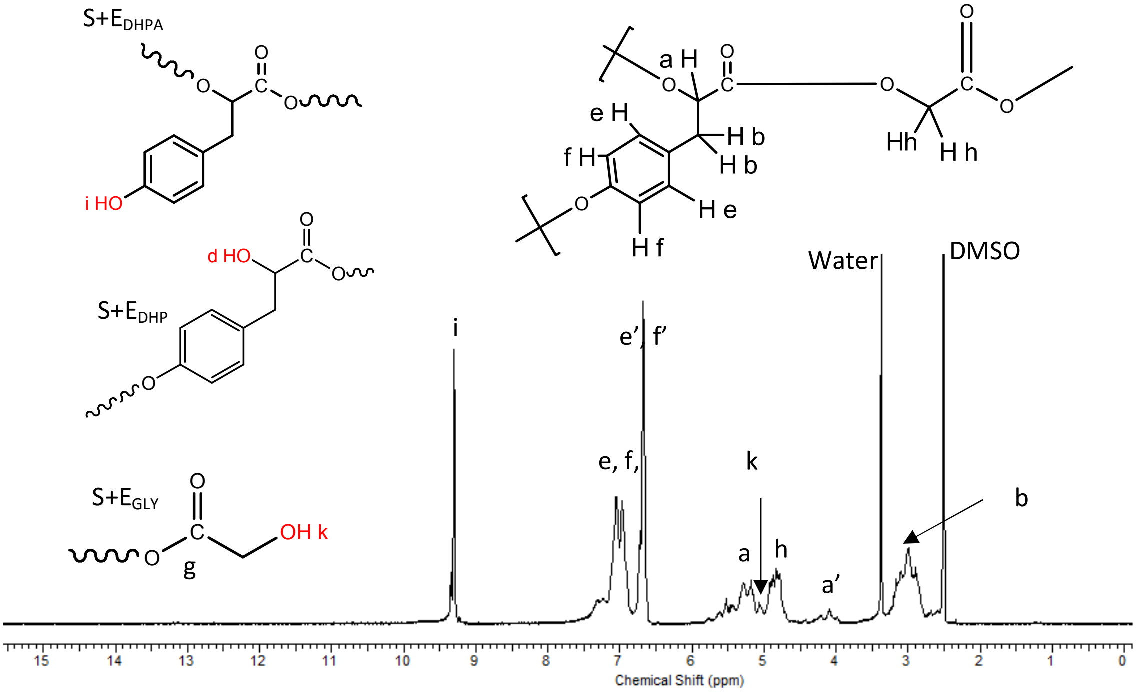


Figure 3.1 ^{1}H-NMR spectrum of poly(DHPA-co-GLY) with monomer feeding ratio of DHPA/GLY = 1:1 after 24 hours polymerization.

3.3.2.2 Chain propagation effect to hyperbranching property of poly(DHPA-GLY)

As we know GLY and DHPA have multifunction chemical moiety. Thus the development of copolymer architecture is the integrated dependence of: (i) The competition of functional group in monomer joining (ii) Steric hindrance of developing polymeric architecture; (iii) Monomer-feeding ratio (iv); Synthesis kinetic which refer to transesterification, temperature, pressure, and time of polymerization.

GPC analyses for samples collected at certain reaction time were used as the tracking information for polymerization degree, Molecular weight value obtained over time will be used as important parameter to investigate the effect of chain propagation to the HB property in the copolymerizations of poly(DHPA-co-GLY) with different feeding ratio and homo polymer P(DHPA). The evolution of molecular-weight and branch architecture showed relations which give convenient understanding about the role of monomer and branching architecture to the mechanistic hypothesis [25-36].

In polymerization of homo polymer P(DHPA), at initial time some minors peak appear together with the main peak (Mw=100000 g/mol, PDI = 3.6) in the chromatogram of oligomeric samples prepare for 4 hours; after 12 hours these minor peak disappeared, and only one broaden peak was detected. The mono peak broadens and shifts to shorter retention time, meaning increasing in the molecular weight. After 18 hours of polymerization, a very high molecular weight peak appeared (more than 350000 g/mol), and increase it size with an increase in polymerization time from 8 to 16 hours. In addition, the M_w/M_n increased slightly, which could be attributed to an increase in the branching degree. Based on this consideration, the appearance of multimodal GPC peaks suggests the presence of different components with different branching forms in the HB polymers [37].

In preparation copolymer poly(DHPA-*co*-GLY) with feeding ratio of 50% glycolic acid into reaction, at initial step 6 hours, M_w obtained value of 30000 g/mol, this value increase to 60000 g/mol after 12 hours and stable up to 18 hours. After 24 hours M_w of P(DHPA-co-gly) obtained value of 950000 g/mol. The difference in the kinetic of reaction between copolymer and homopolymer could be the result of differences in reactivity of functional group in formation of ester bond due to steric hindrance as well as competition of functional group when joining to main chain. In the case of P(DHPA) the bulky side chain of DHPA give a larger steric hindrance at the stereo center, while glycolic acid have no side chain. Thus in further time reaction the branching process support by ABB' structure of DHPA significantly increase the reaction site which favors for P(DHPA-*co*-GLY)'s M_w value. Together with increase of reacting site on oligomeric back-bone of P(DHPA-*co*-GLY) is the significant decrease of GLY precursor concentration and and gradually decrease of DHPA precursor concentration . As a consequence, the PDI value of P(DHPA) also increase significantly when more DHPA were added into polymer frame owing to higher randomness in reacting probability of phenol and alpha hydroxy of DHPA. The branch development also appeared more frequently after 12 hours. Because phenol and alpha hydroxy present in the same reactant DHPA, therefore their probability to collide with carboxylic is quite similar.

NMR is used for determining the branching degree value which gave direct information of dynamic changes over the course of the branching polymerization. Considering that the hydroxy group of GLY have higher reactivity than alpha hydroxy of DHPA due to low steric hindrance and alpha hydroxy have much higher reactivity than phenol; $-OH_{GLY}$>> $-OH_{DHPA}$> $-PheOH_{DHPA}$. In addition, number of carboxylic moiety is lower than hydroxy, we can hypothesize that after long course of polymerization the number of non-reacted end-groups of all unit ($E_{\sum}$) is equal to the number of side-chain DHPA (S_{DHPA}) plus end-chain DHPA (E_{DHPA}) on hyper-branch polymers. As a whole, the DHPA molar fraction ($f_{DHPA,}$) of all unit in poly(DHPA-co-GLY) was almost the same as the content of end-chained groups. Content estimated from the integral ratio of the proton signals from copolymers with different C_{DHPA} values (Fig 3.2)

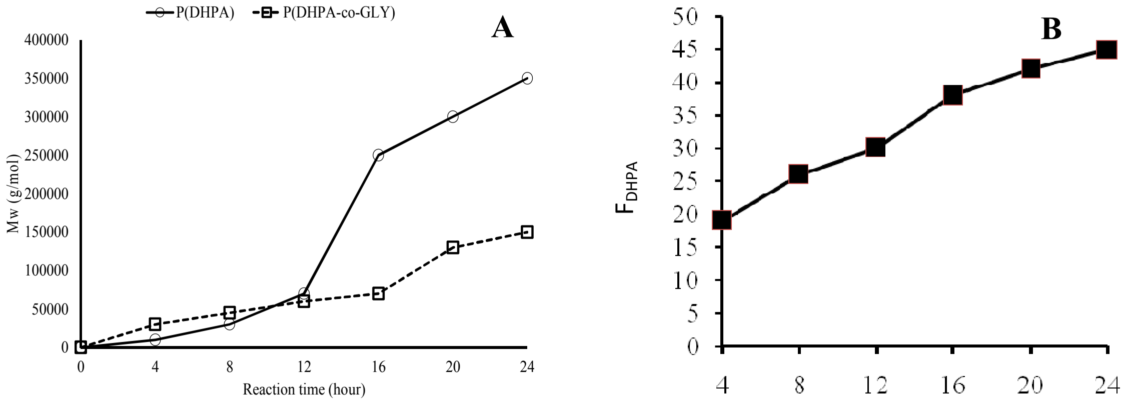


Figure 3.2. A. change in the M_w value showing polymerization time in copolymer poly(DHPA-*co*-GLY) with a DHPA feeding ratio of 50 mol% and homo polymer P(DHPA); **B.** Influence of the reaction time on the percentage of DHPA in poly($DHPA_{50\%}$-*co*-GLY)

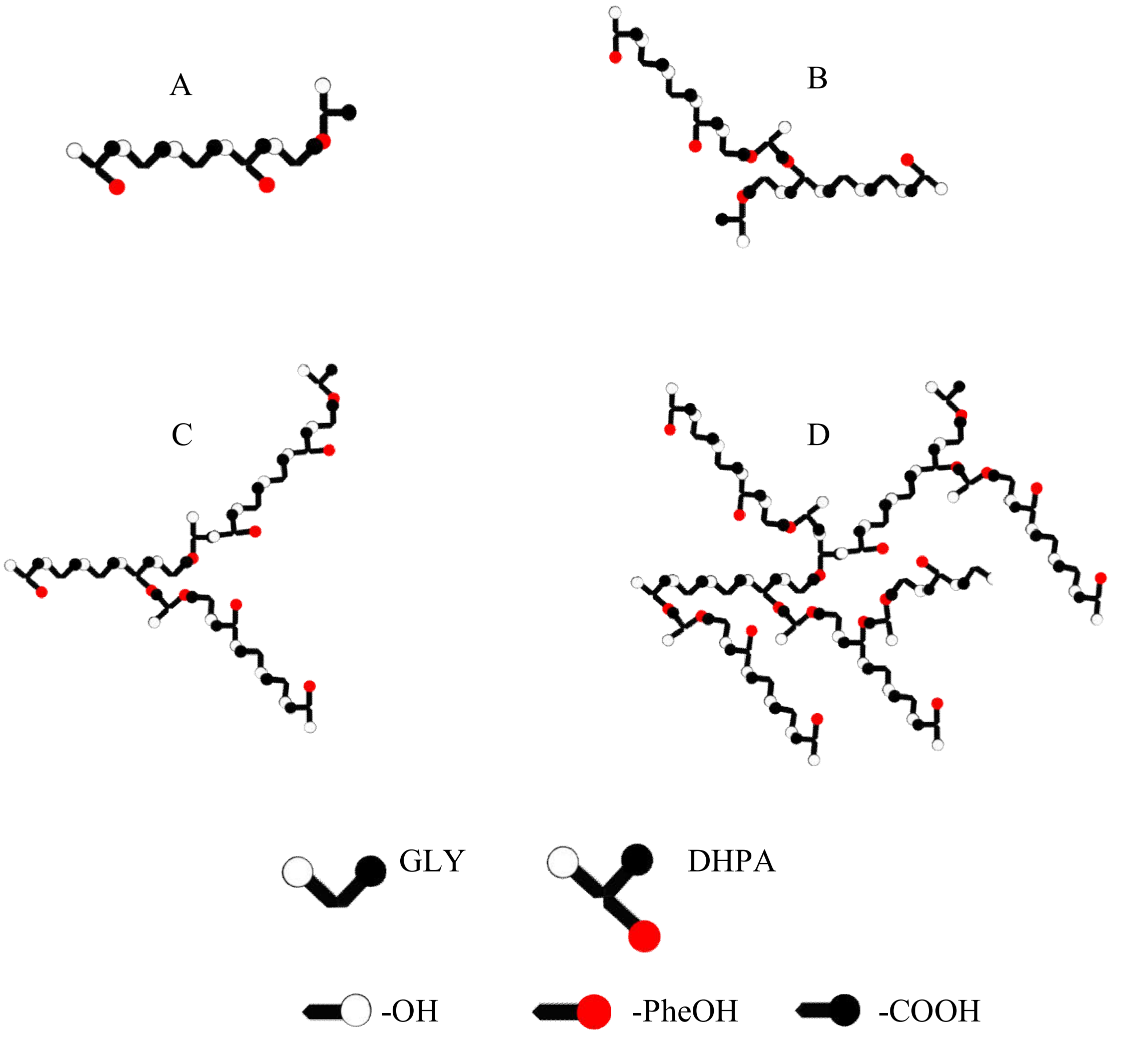
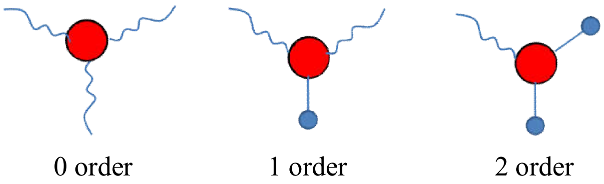


Figure 3.3. Proposed hypothesis for the polymer hyperbranching development by copolymerization of DHPA with GLY. **A**. short chain polyester was formed with higher joining ratio of alpha hydroxy to carboxylic; **B**. Side-chain appeared randomly at phenol site; **C**. The branching architecture start with one more side chain propagated at other functional group site; **D**. Hyper-branching polymer was developed

For an insight look of the polymer M_w in relation with structure, the branching degree (BD) is one of the most important molecular parameters of hyper-branched polymers, because the BD generates differences in the molecular structure from the linear analogs. In order to investigate the process of branched polymer chain formation, we focused on various signal of hydroxy groups (-OH, -PheOH) in DHPA with hydroxy group in GLY. If only one hydroxy or aryl hydroxy group of DHPA reacted at a site, then a branching would be not considered, and the residual -OH or -PheOH groups became side chain, S. Therefore, there are two kind of non reacted -OH signal is shown in illustrations S_{DHPA}. Furthermore, one can confirm two -OH end groups in DHPA (illustration E_{DHPA} in Fig. 3.1) and one end group in glycolic acid (Illustration E_{GLY} in Fig. 3.1). In total, three -OH signals should be detected in the H-NMR of the copolymers. Fig. 3.1 also shows a close-up view focusing on the hydroxy and phenol groups of poly(DHPA-co-GLY) with a C_{DHPA} of 50 %. The end -OH of GLY could be easily recognized by comparison to the assignment of the DHPA homopolymer. The DHPA related -OH could be assigned using the proton signals of the monomer proton.

Calculation of unit composition of P($DHPA_{50\%}$-co-GLY) based on the close-up view of the ^{1}H-NMR spectrum which focuses on signals of the $DHPA_{OH}$, $DHPA_{–PheOH}$, and GLY_{OH} group. The aromatic proton marked as “**e**”, “**f**” (in-chained aryl) and **“e’”**, **“f’”** (side and end-chained phenol) suggesting the in-chained and end-chained phenyl ratio. “**a**” marked for methine shift or in-chained DHPA, “**h**” is α-proton of GLY, “**g**” is side-chained and enchained -OH GLY, “**d**” is end and side-chained α-hydroxyl, “**i**” is aryl-hydroxyl or end-chained side chained DHPA.

An exact calculation of branching degree of polymer by signal of each non-reacted group is impossible. Considering each DHPA unit as branch generating point as scheme 3.1 each 0-order point create a branch, each 1-order point belong to a chain and each 2-order point end the chain (including HDPA unit with two non-reacted functional group and non-reacted –OH or –COOH of GLY unit)



Based on the integration of total assignment, total non-reacted –PheOH (i, e', f'), assignments, integration of DHPA reacted methine (δ 5.5 ppm) and non reacted methine (δ4.4 ppm) average branching degree $\overline{BD}$ is calculated following equation (3.1)

$$\overline{BD}=\sum_{0\text{-order}} / \{\sum-(\sum_{1\text{-order}}+\sum_{2\text{-order}})\} \tag{3.1}$$

Where $\sum_{0\text{-order}} = a+(e+f)/2$

$\sum_{1\text{-order}} = \{\sum - a' - g - (e'+f')/2\} + (\sum - k)$

$\sum_{2\text{-order}} = (e'+f')/2 + k$

$\sum = a + a'$ (the whole DHPA unit) = h (the whole GLY unit)

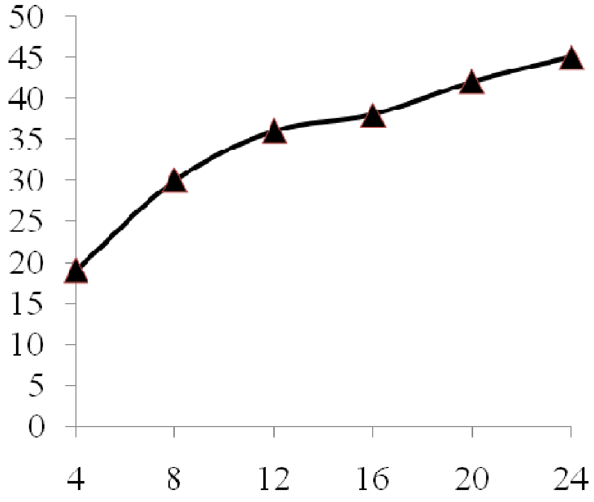


Fig. 3.4 is a representative plot of BD in a copolymer with a C_{DHPA} of 50 mol%. During the initial 12 hrs of polymerization, the BD decreased with reaction time from 0.19 at 4 hours to 0.30 at 8 hours, and the ratio increased most dramatically during the initial 8 hours. The end chain ratio decrease partially implies decrease of branching. On the other hand, with a further increase in the reaction time from 12 to 16 hrs, the BD increased slightly and then almost kept constant.

The BD decreased with the reaction time from 8 to 12 hrs, but increased from 16 to 24 hrs. During the first 4 hrs of polymerization, the BD was low at 0.19 because the α hydroxyl, which has a higher reactivity than -PheOH, was consumed preferentially at the beginning of polymerization to create small hyperbranching oligomers (from A to B in Fig. 3.3). However, the BD increased drastically from 0.38 at 16 hrs. Further branching of the small oligomers may have been difficult due to steric hindrance, and thus main chain propagation was favored, causing the rapid branching decline. The degree of branching decreased as the reaction time increased from 12 to 20 hrs, and then became almost constant at 0.43 up to 20 hrs, because further polymerization branching reactions occurred frequently but in random site due to addition of trifunction DHPA unit (from B to D in Fig. 3.3). After 14 hrs, the BD increased again to make the chain ends highly dense, presumably due to the HB reaction caused by a monomer or polymer chain reaction with the residual acetyl groups to create large polymers with M_w values more than 350000 g/mol (from D to E in Fig. 3.2). It should be noted that the branching degree of the copolymer was not high, even at the last stage of polymerization, which may be attributed to the following reasons. The αOH of DHPA and GLY reacted with carboxylic acid higher efficiently than the -PheOH of DHPA; and once if one OH reacted to form ester bond the reactivity of the other might be remarkably reduced by steric hindrance of the already-formed backbone.

3.3.3 Thermal properties of branched polymer

Hypothesizing for branched polymer, the glass transition temperature depends on not only back-bone structure but also side chain number content in the entire architecture. Longer side chain increase free volume fraction of polymeric materials [37]. The increased density of polymer chain ends restricting molecular mobility to decrease T_g. However when the chain obtained a certain length, effect of chain length and M_w will be saturated and structure of chain play the important role to determine the T_g Therefore, the T_g value is not only dependent on the molecular weight and flexibility of the polymer chains. The relationship between the T_g vs reaction time and the T_g vs DHPA unit ratio in poly(DHPA$_{50\%}$-co-GLY)s summarized in Fig. 3.6 and Fig. 3.7, respectively.

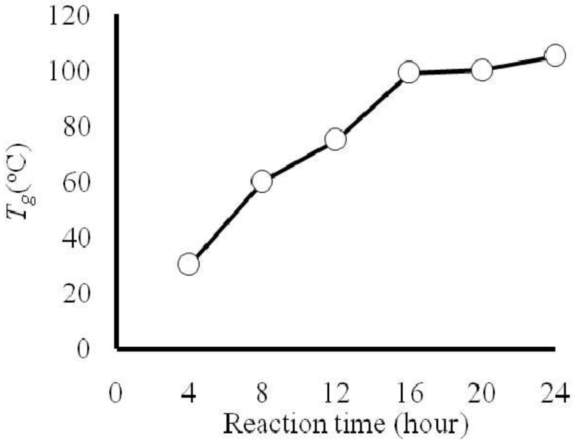


Figure 3.6 Relationship between the T_g versus reaction time

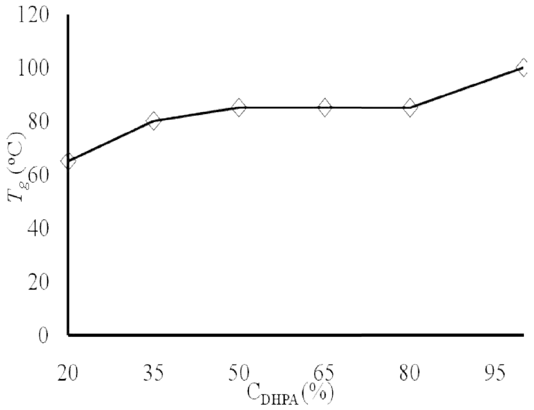


Figure 3.7. Relationship between the T_g versus DHPA unit ratio

Fig. 3.6 shows a phenomenom that T_g of branch polymer increased with a in the reaction time to 8 h, became almost constant from 8 h to 12 h. On the other hand, as seen from Fig. 3.1., the M_w increased with increasing reaction time, although the GPC peaks showed a broaden range of peak and contributed strongly to the increased T_g.

However, the further T_g increase with the reaction times over 12 h could not be explained simply by an increase of M_w. The value of the f_{DHPA} increased throughout the entire polymerization reaction as shown in Fig. 3.2, which contributed to the increase in the T_g in both copolymer systems with input C_{DHPA} of 50 mol% (Fig. 3.7), Because of the reduced flexibility in the copolymer with an increased of phenyl ring composition. Therefore, it was difficult to postulate that the content of phenyl ring and side chain volume which have the dominant effect on the T_g increase. At the beginning BD is not high, the content of GLY unit is more dominant, M_w of polymerization may also be effective in causing the T_g to change. Consequently, the further T_g increase in the polymerization range over 12 h was mainly due to the BD increase together with the increase of DHPA unit.

The thermal stability of poly(DHPA-*co*-GLY)s was also estimated from the weight loss change upon increasing the temperature by thermogravimetric. A thermogravimetric curve of poly(DHPA-*co*-GLY) with a C_{DHPA} of 60 mol% is shown in Fig. 3.8. The T_{10} decrease might be due to a decrease in the f_{DHPA} because the homopolymer poly(DHPA) showed a higher degradation temperature than the poly(glycolicacid) [30]. However, a higher thermal stability at found in the polymerization of poly(DHPA-*co*-GLY) with a C_{DHPA} of 35 mol% (a T_{10} of 353 ^oC) and a C_{DHPA} of 50 mol% (a T_{10} of 361 ^oC) as obviously observed from Fig. 3.8, which could be mainly attributed to a very high BD leading to an increased inter-branch force and thermal-decomposition temperature. Furthermore, thermal stability of poly(DHPA-*co*-GLY) was compared with non-branched homopolymer poly(glycolic acid), which showed a lower degradation temperature, T_{10} of 310 ^oC than those of poly(DHPA-co-GLY)s with BD of around 0.1 prepared by polymerization for more than 8 h. Although BDs in copolymers were not high, T_{10} values were much higher than that of non-branching homopolymer, which suggests that appropriate branching in poly(DHPA-co-GLY)s played important role in increasing thermal decomposition.

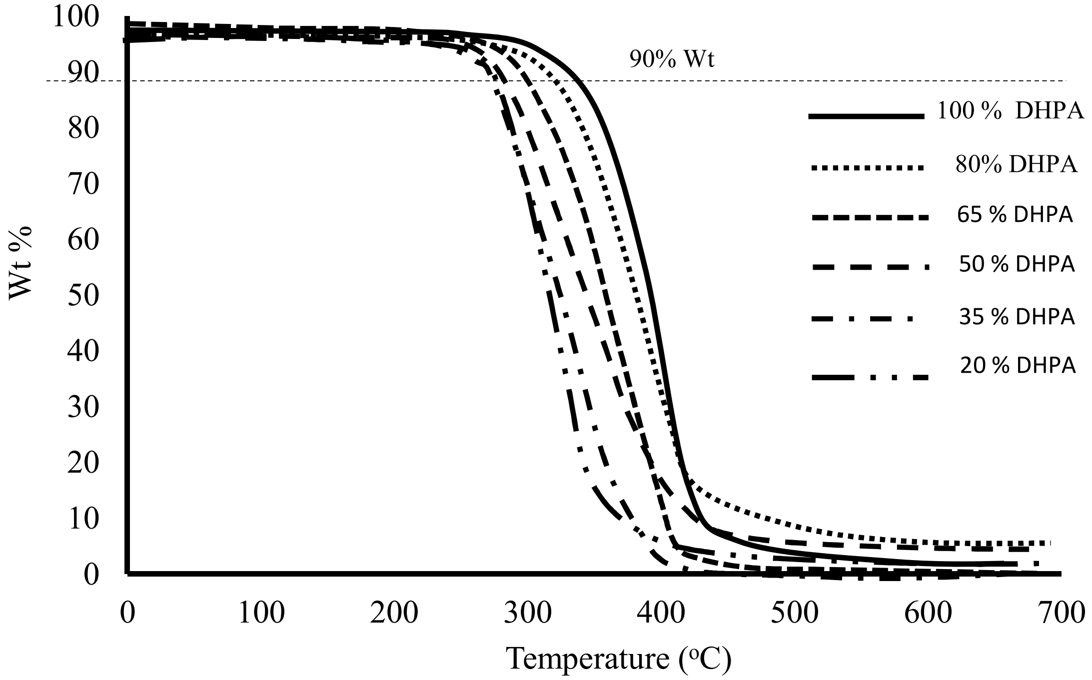


Figure 3.8 Weight-loss versus heating temperature behavior of polyester with different DHPA composition

3.4. Conclusion

We described a facile method for the one-pot synthesis of well-defined biodegradable hyperbranched polyesters, poly(DHPA-*co*-GLY)s, in the presence of Sn metal as an effective catalyst, from bio-available monomers of D-hydroxyphenyllactic acid as a multifunctional ABB’ unit and glycolic acid as an ordinal AB unit. The polymer structures such as the copolymer composition and branching degree were investigated by ^{1}H-NMR spectroscopy. Three stages of the hyperbranching process are proposed; in the first stage, GLY-rich oligomers with short branches and a high branching degree were formed, the main chain was preferentially propagated. During the second stage, accompanied by a gradually increase in the BD. In the third stage, a preferential branching reaction was caused by monomer reactions with the addition of DHPA unit to the polymer the polymers. This hyperbranching together with addition of phenyl ring affected the glass transition temperature and degradation temperature of the polymers. The glass transition temperature increased with an increasing in the molecular weight, and saturated at a threshold polymerization time, but then increased again following further polymerization for over 12 h due to phenyl ring conten. The degradation temperature also showed an increasing with the branching degree in the consistence with the DHPA composition.

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Chapter 4

Syntheses and Thermal Property of Bio-based Polyester From Microbial D-hydroxyphenyllactic Acid

Abstract

the trifunctional characteristic of DHPA is focused increase with the aim to increase thermostability of bio-based polylactate derivatives. I used modified microbial D-hydroxyphenyllactic acid (DHPA) as a monomer to produced polyester having benzene ring in its backbone. Several surveys of polymerization of DHPA's precursor were conducted with aliphatic and aromatic diacyl chloride. Firstly a reactivity of methylated DHPA in polycondensation with a series of aliphatic diacid chloride was confirmed to give semi-aromatic polymers. Next I prepared thermally-stable DHPA-based Polymers by polycondensation with aromatic diacylchlorides such as terephthaloyl chloride and isophthaloyl chloride. As a consequence, the polylactate derivatives showing a glass-transition temperature (T_g) as high as 130 ^{o}C without any additives was synthesized.

Keywords: D-hydroxyphenyllactic acid, microbial, polyester, heat-deforming resistant, aromatic

Chapter 4

Syntheses and Thermal Property of Bio-based Polyester From Microbial D-hydroxyphenyllactic Acid

4.1 Introduction

Studies about thermal behavior aspect in relation to structure of polyesters are important issues for polyester applications, especially, control the thermal behavior of bio-based polyester with certain natural structures that make them more useful as substitutes for conventional fossil-based plastic. In recent year, several polyesters which are partially and fully bio-available have been remarkably attended in plastic synthesis because of their bio-degradable and eco-compatible characteristics [1 - 10]. In addition, due to high-strength and high-modulus, they have been applied in some commodities, medical tools and agro-chemistry [5-19]. However the application ranges of the bio-based plastics are limited because of their low heat-deforming resistance (i.e. glass transition temperature -T_g), for example polyglycolate (37 °C), polylactates (60 ^{o}C), polyhydroxybutyrate (0 ^{o}C) and poly ethylene-terephthalate (70 ^{o}C) [20]. To overcome that problems, the flexibility of polyester main chain should be reduced by incorporating a rigid component, for example, an incorporated benzene group dramatically increases T_g value from below 0^{o}C in the case of poly(ethylene oxide) [4] to higher than 250^{o}C in the case of poly(p-phenylene oxide) [21, 23]. By mean of green chemistry, the alternation of bio-based polyesters structure by other aromatic bio-derivatives is one of the best methods to modify and manipulate heat-deforming resistance property of bio-based polyesters.

In this chapter, I aimed to use benzene-containing microbial D-hydroxy phenyllactic acid (DHPA) in polycondensations with several bio-derived aliphatic and aromatic diacylchlorides and characterized the thermal behavior of the bio-based polyesters over their polymer degree. DHPA is produced massively from glucose by a strain of *Escherichia coli* expressing pprA gene of fungus*Wickerhamia fluorescens* [26]. In these polyester syntheses, carboxylic group of DHPA was protected by a methylation, then an aliphatic-aromatic diol derivative of DHPA was used to obtain polyester in the optimized anhydrous solvated polycondensations with diacyl chlorides. The result aromatic-aliphatic co polyesters were synthesized successfully with molecular-weight values which were around 10,000 g/mol. In the cases of aliphatic diacyl chlorides, such as succinyl, glutaryl, adipoyl, pimeloyl, suberoyl, azelaoyl and sebacoyl dichlorides, the glass-transition temperature (T_g) of polyester decrease from 40^{o}C to subzero when the number of carbon in cooperated aliphatic chain increase as a result of flexibility of aliphatic chain.

On the other hand, microbial DHPA was effectively cooperated with the recent bioavailable terephthalic acid chloride derivative [28-35] suggest that the true bio-derived polyesters can be more applicable in plastic field. The other aliphatic diacylchlorides used in this research, such as succinyl, glutaryl, adipoyl, pimeloyl, suberoyl, azelaoyl, and sebacoyl chlorides are also available from corresponded bio-derived diacids [36] through severalpopular chlorination methods [37]. Properties of resulted bio-based polyesters which have remarkably improved T_g values in comparison with several commercial bio-based polyesters will be discussed.

4.2 Experimental Section:

4.2.1 Materials

The wholly bio-derived DHPA was provided from cultivated biomass of Escherichia coli expressing pprA gene of *Wickerhamia fluorescens*, purity is over 99%. Terephthaloyl chloride (TPC) and isophthaloyl chloride (IPC) (from TCI) was purified by recrystallization in anhydrous hexane (from TCI), anhydrous methanol (from Sigma-Aldrich), anhydrous pyridine (from TCI), anhydrous 1-methyl-2-pyrolydol – NMP, anhydrous N-dimethyl-acetamide - DMAc (Sigma Aldrich), trimethylsilyl chloride - TMSCl (from TCI), succinyl chloride (4C), glurtaryl chloride (5C), adipoyl dichloride (6C), pimeloyl chloride (7C), suberoyl chloride (8C), azelaoyl (9C) chloride, sebacoyl chloride (9C) (from TCI, 98%) were used after distillation.

4.2.2 Structure characterization

1H NMR, spectra were determined by Bruker Biospin AG 400 MHz, 54 mm spectrometer using DMSO-d6 as solvent. Melting temperature was recorded with Mettle FP90 central processor and Mettler FP82HT hot stage. FTIR spectra were recorded with Perkin Elmer, spectrum one. Mass spectra were measured using Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS, Solarix), scanned from m/z 150 to 1000.

4.2.3 Molecular weight of polyester

The number average molecular weight (Mn), weight average molecular weight (Mw) and molecular weight distribution (PDI) were determined by gel permeation chromatography (GPC, conc. 5mg/mL, DMF as an eluent) after calibrating with the standard pullulan at 40 ^{o}C.

4.2.4 DSC measurement

Differential scanning calorimetry (DSC) was performed on DSC (Seiko instrument Inc., EXSTARX-DSC7000 SII) apparatus. Temperature and heat flow scales were calibrated using high purity indium. Sample weight was taken in the range 5-10 m gand samples were heated from 25 ^oC to 250 ^oC at the rate of 10 ^oC/min.

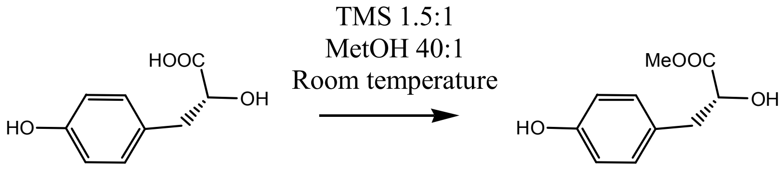
4.2.5 Thermogravimetric (TGA) Analysis

Polyesters were subjected to TGA using SSC/5200 SII Seiko Instruments Inc. equipment. Samples weighing 5-10 mg were heated from 25 ^oC to 700^oC at heating rate of 10 ^oC/min in nitrogen atmosphere and corresponding weight loss was recorded.

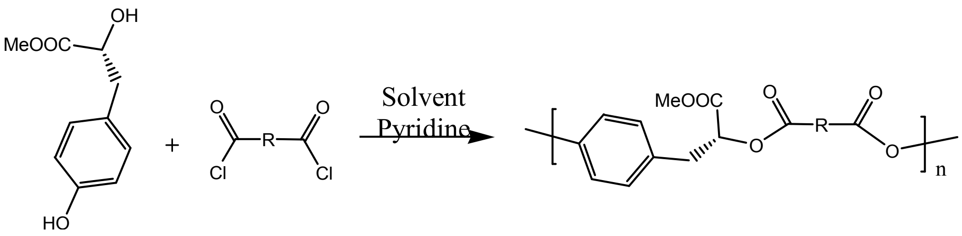
4.2.6 Syntheses

Firstly, the carboxylic group of DHPA was protected by a methylation. Methylation was performed by reaction of DHPA with excessive anhydrous methanol (Sigma-Aldrich) in trimethylsilyl chloride of 1.5 molar ratio (scheme4.1a). Reaction mixture was neutralize by $NaHCO_3$ in methanol, then methanol was removed in evaporator. Diol derivatives of DHPA was extracted and recrystallized in diethylether, the purity DHPAand DHPA diol derivative was confirmed by ^{1}H-NMR spectrum(Fig. S 4.1, S 4.2, Sup. info.).

Bio-based polyesters polymerization were performed by reaction of DHPA's diol derivative with acylchlorides in 1:1 molar ratio in DMAc, and 2.5:1 molar ratio of pyridine (scheme4.1b). Liquid aliphatic diacylchlorides were dropped wise into sealed flask of anhydrous diol solution 1M for30 minutes at room temperature, whereas solution of aromatic diacylchlorides were prepare and drop into sealed and refluxing1M solution of diol at room °C for 12 hours.



Scheme4.1a Diol synthesized from DHPA



Scheme4.1b Synthesis of bio-based polymer from D-*p*-hydroxyphenyllactic acid

After reaction, the liquid mixture were dropped to equivalent 20.0 water volume and stirred to wash out pyridine chloride salt, monomers, solvent and precipitate the solid polymer. Solution trace in polymer was evaporated under reduced pressure at 60 °C.After polymerization, weight average molecular-weight (M_w) and number average molecular weight (M_n) was measured in Shodex KD-803 and KD-807 gel permeable chromatography (GPC) system, using dimethyl formamide as elution solvent, Varian polyethylene calibration kit PG-10. 10%-decomposed temperature (T_{10}) of polymer was observed by thermal gravimetric analysis (TGA) from room temperature to 700°C. Thermal behavior of polymer was characterized in differential scanning calorimetric analysis (DSC) to observe T_g.The polymers structure was confirmed by BRUCKER 400 MHz proton nuclear magnetic resonance (^{1}H-NMR).

4.3Result and discussion

4.3.1 DHPA-derived aliphatic-cooperated polyester

4.3.1.1 Polymerization

In ^{1}H-NMR spectra of DHPA-derived diol (Fig. S 4.2), the methylation result is confirmed by taking signal integration of methyl group to compare with signal integration of hydroxyphenyl, and the ratio of 3:4 is consistent to the number of protons confirmed that no side reaction occurred at methyl ester group. The DHPA-derived diol has typical peak of methyl at 3.68 ppm, methanediyl from 2.96 to 3.2 ppm, methine at around 4.44 ppm, 2-hydroxy at around 5.35 ppm, benzene at 6.7 and 7.12 ppm, *p*-hydroxy at 11.0 ppm. The doublet of methine at around 4.44 ppm, 2-hydroxy at around 5.35 ppm is corresponded to chiral effect. Polymerizations with aliphatic chlorides were performed in several temperature and solvent condition to reduced oxidization during time. With succinyl dichloride, glutaryl, and adipoyl dichloride, reaction temperature was keep at -20 ^{o}C for 30 minutes in THF, whiles reaction of pimeloyl, suberoyl, azelaoyl and sebacoyl diacyl chlorides were performed in DMAc at room temperature with rapid magnetic stirrer agitation. Polymerization of DHPA-derived diol with aliphatic diacylchlorides resulted in dark-brown to light yellow polymeric materials.

In this polymerization the maximum value of polyester M_w is 1.6x10^4 (g/mol) and M_n is 9x10^3 (g/mol), corresponded poly-dispersed index is 1.33 (table 1). In ^{1}H-NMR spectra, the typical chemical shifts of methanediyl of aliphatic chain appear as triplet from δ 1.52 to δ 2.8 ppm (for -CH_2- adjacent to carbonyl) and quartet from δ 1.4 to δ 1.7 ppm (for middle -CH_2-) (Fig S 4.5 to S 4.10 sup. info.). There are occurrences of changes from δ 4.44 to δ 5.5 ppm in chemical shift of DHPA methanediyl and changes in chemical shift from δ 2.92 to δ 3.3 ppm in DHPA methine (Fig. S 4.5 to S 4.10 sup. info.). Therefore, we could confirm the formation of ester bonds by those chemical shift changes. These changes are already reported in references [10], [11] and [12]. The sharp and coupling peaks appeared in almost ^{1}H-NMR spectra of DHPA with aliphatic diacyl chlorides co-operations indicated for the low molecular weight polymer. Because in such these solvent polymerizations, we should notice that when the number of carbon in aliphatic acyl chlorides increase the molecular flexibility of oligomer will increase. Therefore, the M_w of almost polyester in these solvent polymerization of aliphatic diacyl chlorides attained low values (table 1), those may due to cyclic product formation enhanced by the flexibility of aliphatic component.

4.3.1.2 Thermal properties of aliphatic-cooperated DHPA-derived polyesters

As a result of chain flexibility, when the number of carbon in back-bond of aliphatic chain increase the T_g of polyesters also decrease (Fig. S 4.1). With PDHPA-4C and PDHPA-5C, we can processed them to fiber shape by hot-stretching, shape are stable in room temperature. Polyesters from 6C to 10C at room temperature were sticky materials, while 4C and 5C were obtained as powder.10% weight-loss of aliphatic-cooperated polyester were made from 290 to 330 ^{o}C (Fig.S 4.2),indicated the pyrolysis corresponded to decomposing of ester bonds [2].

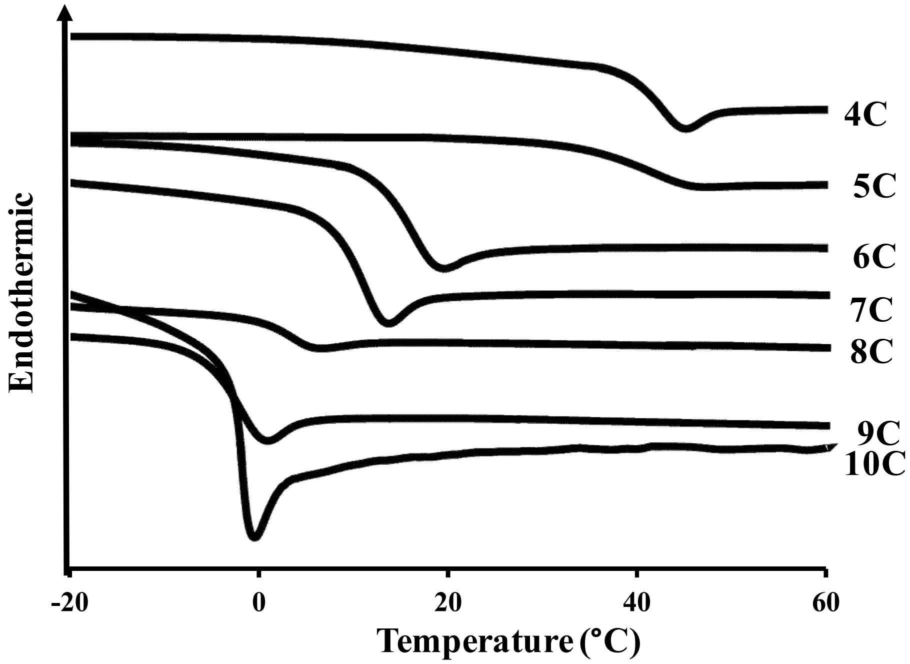


Figure 4.1a. DSC thermo-gram of aliphatic-cooperated DHPA-derived polyesters

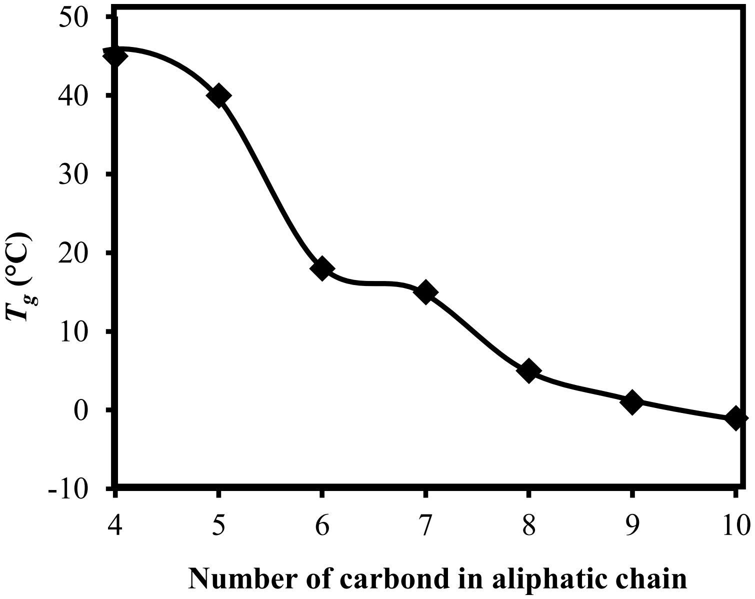


Figure 4.1b. Relation of carbon number in cooperated aliphatic chain and T_g of DHPA-derived polyesters.

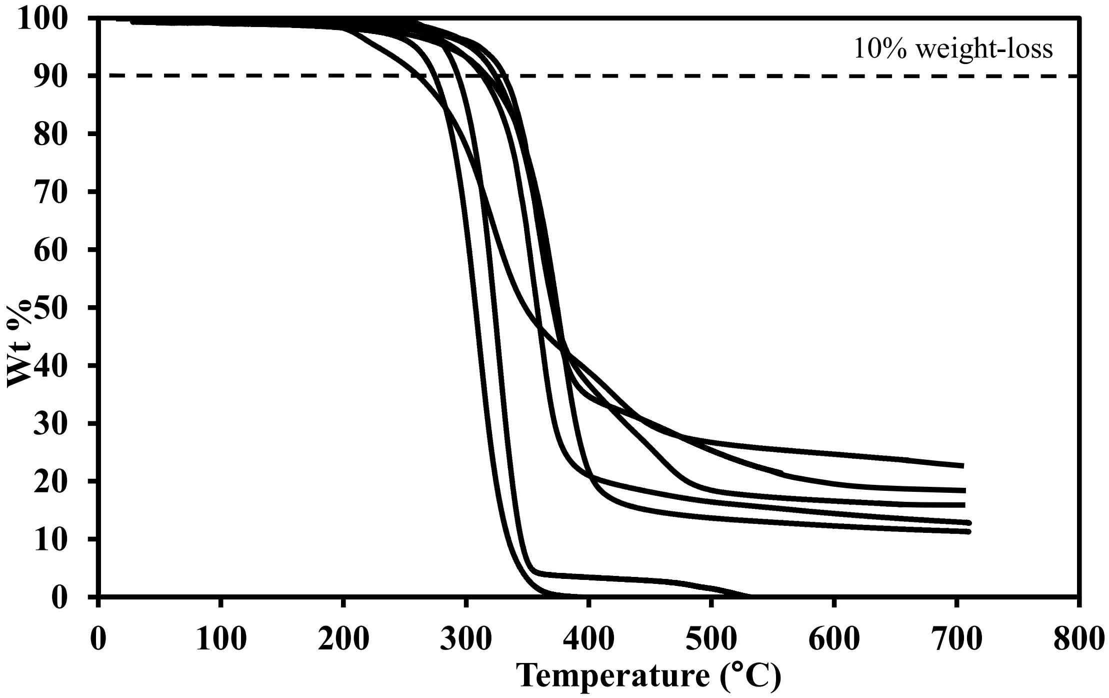


Figure 4.2TGA curves of aliphatic-cooperated DHPA-derived polyesters

4.3.1.3 Optical properties of aliphatic-cooperated DHPA-derived polyesters

The aliphatic-cooperated DHPA-derived polyesters were dissolved in acetone and coated on a glass plate to expose their transparency, as shown in Fig. 4.3 all those polyester thin coating are transparent.

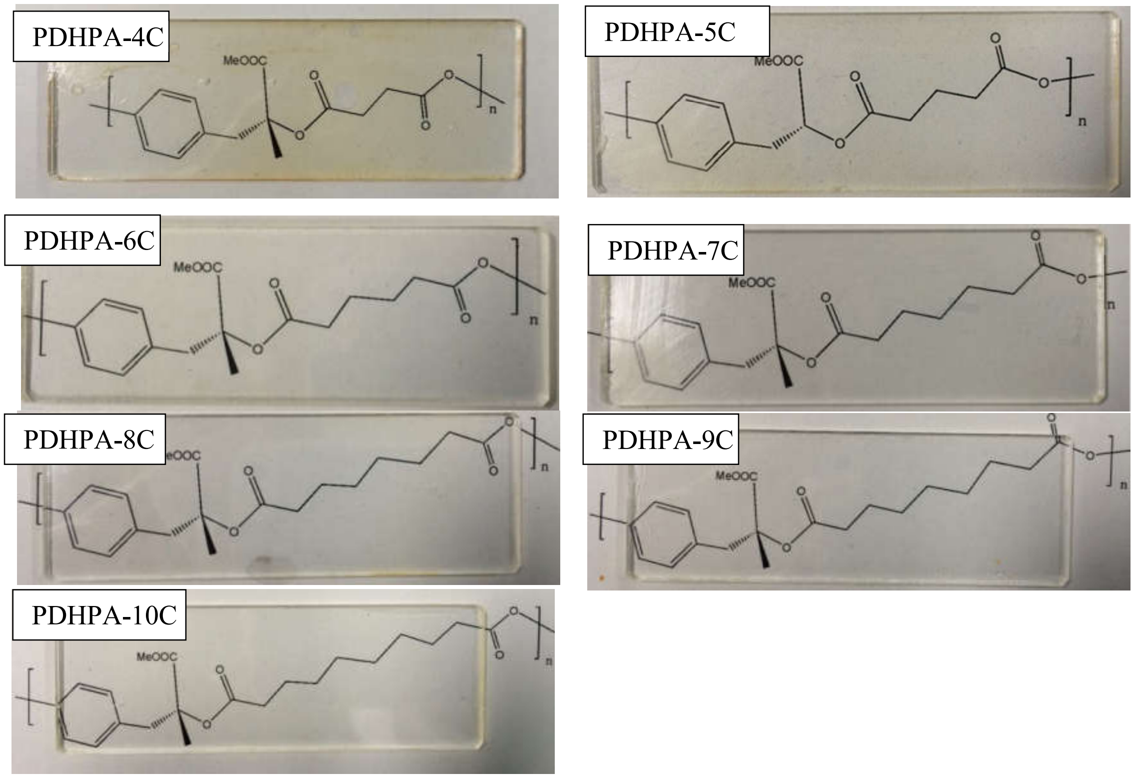

PDHPA-4C
PDHPA-5C
PDHPA-6C
PDHPA-7C
PDHPA-8C
PDHPA-9C
PDHPA-10C

Figure 4.3. Transparency of of aliphatic-cooperated DHPA-derived polyesters shown by coating on glass plate

Tabe 4.1: Properties of resulted DHPA-derived polyesters

PEs	T_g (°C)	T_{10} (°C)	M_w	M_n	*PDI*	*T%*	R_f
4C	45	270	12,000	8,000	1.51	93	1.591
5C	40	296	14,000	8,200	1.71	91	1.591
6C	25	310	16,000	7,000	2.24	92	-
7C	15	300	16,000	6,500	2.46	92	-
8C	10	310	16,000	6,500	2.13	92	-

9C	0	320	11,000	4,500	1.83	93	-
10C	0	320	8,000	4,000	1.98	93	-
IP	115	300	15,000	9,800	1.53	95	1.584
TP	130	350	16,000	9,800	1.56	98	1.584

PEs stand for polyesters: 4C is poly(DHPA-*co*-succinyl), 5C is poly (DHPA-*co*-glutaryl), 6C is poly(DHPA-*co*- adipoyl), 7C is poly(DHPA-*co*-pimeloyl), 8C is poly(DHPA-*co*-suberoyl), 9C is poly(DHPA-*co*-azelaoyl) 10C is poly(DHPA-*co*-sebacoyl), TP is poly(DHPA-*co*-terephthaloyl), IP is poly(DHPA-*co*-isophthaloyl); T_g is glass transition temperature; T_{10} is the temperature that 10% weight-loss occur; $M_w$is weight-average molecular-weight; M_n is average molecular-weight; PDI is poly-disperse index = M_w / M_n.; *T% is* % light transmission through polyesters layer at 450 nm wavelength, R_f is refractive index measured at 589 nm wavelength.

4.3.2DHPA-derived aromatic-cooperated polyesters

4.3.2.1 Polymerization of DHPA-derived diol with aromatic diacylchlorides

Polymerization of DHPA-derived diol with aromatic diacylchlorides resulted in solid, white polymers powder, poly(hydroxyphenyllactate-*co*-isophthalate) -PDHPA-IP and poly(hydroxyphenyllactate-*co*-terephthalate) - PDHPA-TP.PDHPA-IP and PDHPA-TP can be processed into transparent films by mixing with DMAc to a homogeneous sticky solution and followed by gradual heating to remove solvent (Fig.4.4). In ^{1}H-NMR spectra,the typical chemical shifts of isophthaloyl appear at 7.76 ppm, 8.22 ppm and 8.52 ppm, chemical shifts of terephthaloyl are 8.01 ppm and 8.26 ppm. The change of peak shape from sharp to broaden, and disappearing of coupling effect of every typical peaks also consistent with the high molecular-weight polymers properties.

4.3.2.2 Thermal properties of aromatic-cooperated DHPA-derived polyesters

In TGA curve, 10% weight loss of PDHPA-IP was made at 300 °C, whereas that of PDHPA-TP was made at 350 °C(Fig. 4.6).PDHPA-IP is softens at 115°C, PDHPA-TP is softens at 130 °C as the reduce of heat absorbent rate in DSC thermogram(Fig. 4.5), this T_g value is higher than those of other bioavailable terephthalate polyester (above 60 ^{o}C) such as polybuthylene terephthalate (PBT),polyethylene terephthalate (PET) [8], as well as and other lactide-derived polyesters such as poly(lactic acid)s (PLLAs) and poly(phenyllactic acid)s (PPhLAs) (above 50 ^{o}C) [2, 26, 43, 44, 45]. This T_g value indicates that PDHPA-IP and PDHPA-TP have significant heat-deforming resistivity. Therefore, we can consider the remarkable contribution of benzene component in DHPA to the rigidity of bio-based PDHPA-IP and PDHPA-TP.

4.3.2.3 Optical properties of aromatic-cooperated DHPA-derived polyesters

Polyesters which have T_g higher than room temperature (PDHPA-4C, PDHPA-5C, PDHPA-IP and PDHPA-TP) were processed into film shape and measured refractive index. As a result of benzene ring cooperated, those have refractive indices higher than PLAs and similar to PET at selected 589 nm wavelength, PDHPA-IP and PDHPA-TP have refractive index value comparable to polycarbonate [14].Transparency of 10 μm polyesters layer coated on glass were taken shown more than 90% light transmittance at 450 nm wavelength. Summary of polymers thermal properties over their structure and properties were summarized in table 1. Results of solubility tests are summarized in table 4.2.

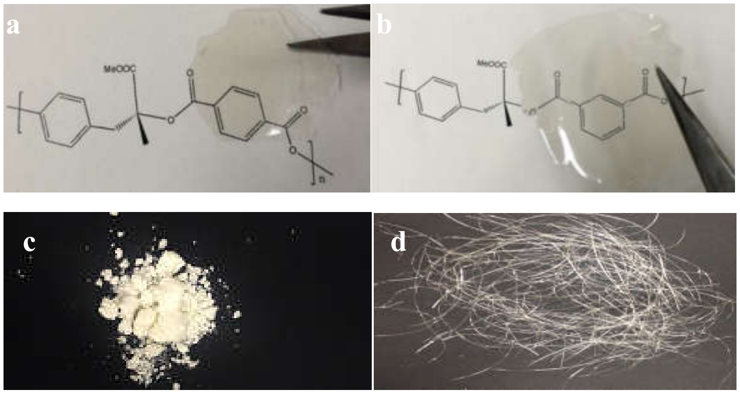


Figure 4.4. Pictures of aromatic polyester films derived from microbial DHPA-derived polyester of (a) PDHPA-TP film, (b) PDHPA-IP film, (c) PDHPA-TP powder, and (d) PDHPA-TP fiber.

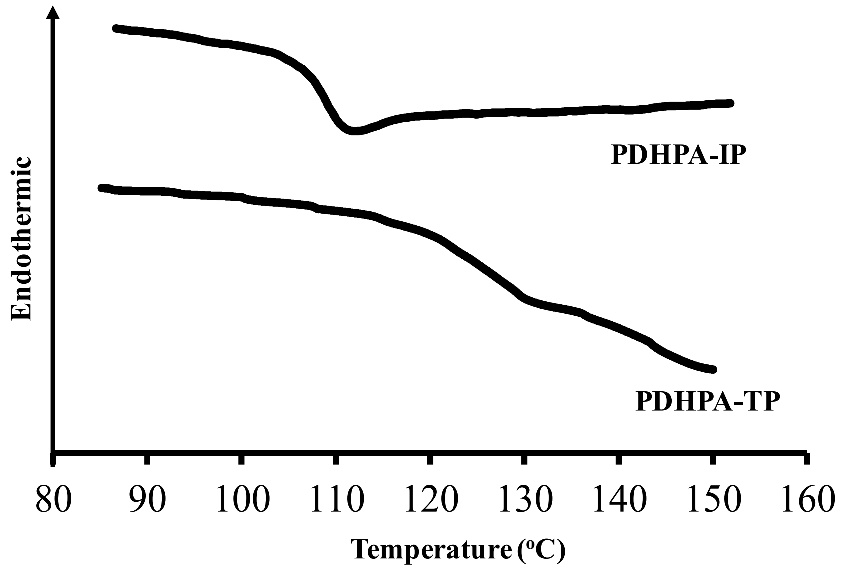


Figure 4.5. DSC thermo-gram of aromatic-DHPA-derived polyesters

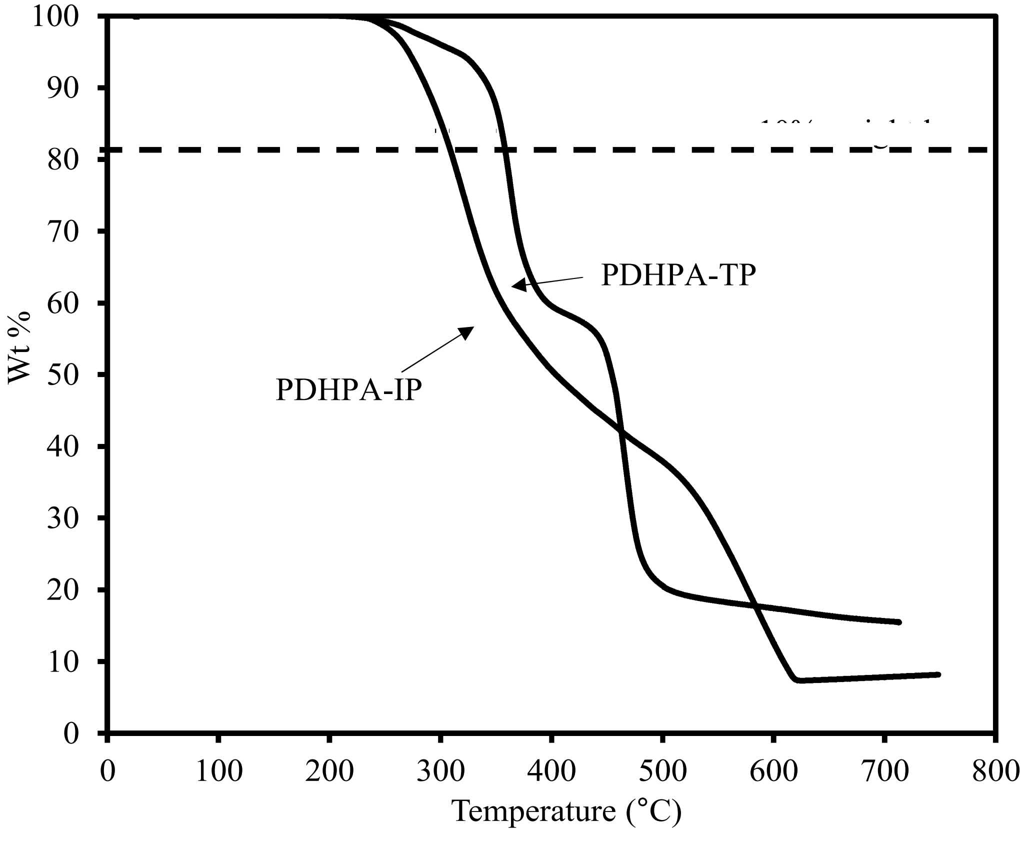


Figure 4.6. TGA curve of PDHPA-*co*-IP and PDHPA-co-TP

Table 4.2: Solubility of DHPA-derived polyesters

PEs	Solvent									
	DMF	DMAc	NMP	Pyridine	THF	Acetone	CH_2Cl_2	EtOH	MetOH	Water
4C	+	+	+	+	+	+	+	-	-	-
5C	+	+	+	+	+	+	+	-	-	-
6C	+	+	+	+	+	+	+	-	-	-
7C	+	+	+	+	+	+	+	-	-	-
8C	+	+	+	+	+	+	+	-	-	-
9C	+	+	+	+	+	+	+	-	-	-
10C	+	+	+	+	+	+	+	-	-	-
TP	+	+	+	+	+	+	+	-	-	-
IP	+	+	+	+	+	+	+	-	-	-

(+) soluble, (-) insoluble.

4.3.2.4. Thermomechanical behavior of DHPA-derived polyesters

In order to avoid solvent residues and heat shock which may make the polymer fiber brittle, we used the roll-pulling method as described in the experimental section in accordance with the method detailed in reference. [17] We processed PDHPA-4C into fibers at temperature above the T_g of each polymer. The other PDHPA-nCs from n=5 to n=10 behaved as sticky liquids at room temperature. Therefore, only solid fibers of PDHPA-4C, PDHPA-TP and PDHPA-IP were chosen for mechanical testing, under the temperature a little higher than T_g, polymer fibers were pulled out gradually in a steady speed using a speed-controlling roller. Polymer fibers had uniformity in thickness along their whole length. **Fig. 7** shows the tensile curves of DHPA-derived polyesters and in Table 3 the results of tensile tests of DHPA-derived polyesters are summarized. The PDHPA-4C had the highest stress-at-break and strain-ratio of 8% because the polymer structure had a good balance of flexibility and rigidity compared to those of PDHPA-IP and PDHPA-TP, although all the fibers showed low mechanical strength due to their low molecular weight. PDHPA-IP and PDHPA-TP had higher Young's moduli as a result of their rigid molecular architecture. Polymer chain micro-motion was reduced by the rigidity of the phenyllene ring in terephthalate structure of PDHPA-TP leading to the highest Young's modulus value as well as T_g of all the polymers prepared here.[17]

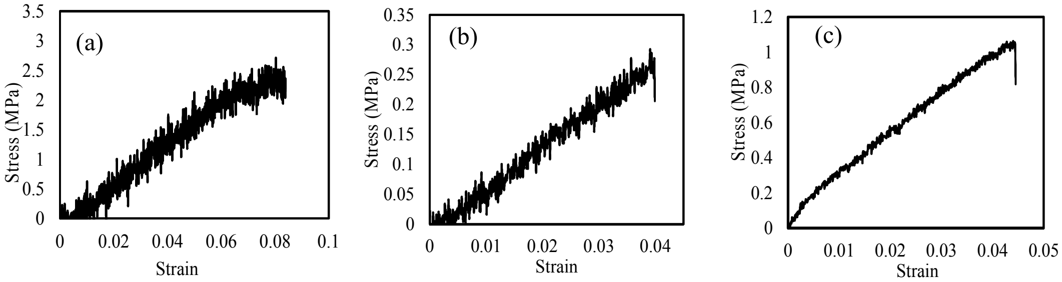


Figure 4.7. Stress-strain curves of PDHPA-PEs, (a) PDHPA-4C; (b) (PDHPA-IP; (c) (PDHPA-TP).

Table 4. 3. Tensile strength, Young's modulus, and elongation at break of DHPA-derived polyesters

polyesters	E (MPa)[a]	ε^b (%)
PDHPA-C4	3.47	8.0
PDHPA-IP	6.25	4.1
PDHPA-TP	14.46	4.5

a) Young's moduli, E (MPa) were determined by initial slope of stress-strain curve. b) elongations at break, ε, (strain x 100%, initial length = 10 mm) were determined by stress-strain curve.

4.3.2.5. Hydrolytic degradation of DHPA-derived polyester

Hydrolysis screening test were conducted with representative polyesters such as PDHPA-4C, PDHPA-IP, and PDHPA-TP because these polyesters had M_n value appropriate for evaluation, and they were solid and easy to rinse after hydrolysis. We used emulsion of the tested polymers by dispersing them in water/DMAc solution, which created conditions favorable for more rapid degradation. **Fig. 4.8** shows the weight-loss (A) and molecular weight distribution (B) curves of PDHPA-4C, PDHPA-TP and PDHPA-IP before and after hydrolytic degradation at 80 °C for 4 days. Table 4 show M_n, and polydispersed index DPI (M_w/M_n) of PDHPA-4C, PDHPA-TP, and PDHPA-IP before and after hydrolytic degradation. The degradation rate was calculated according to the following equation:

$$\ln DP(t_2) = \ln DP(t_1) - k(t_2 - t_1) \quad (1)$$

where DP is the degree of polymerization estimated from M_n, and *DP(t2)* and *DP(t1*) are *DP* values at degradation times t_2 and t_1, respectively.[18] The *k* values of the polymers were 0.519, 0.301 and 0.230 day^{-1} for DHPA-4C, DHPA-IP and DHPA-TP respectively. The *k* values decreased in the following order: PDHPA-4C > PDHPA-IP > PDHPA-TP. This trend is consistent with the solubility of the polymers (PDHPA-4C < PDHPA-IP <PDHPA-TP). According to the literature,[19, 20] the *k* value of poly(lactic acid) (PLA) is 0.506 and the k value of PDHPA-4C is higher than that PLA. On the other hand, the *k* value of PDHPA-IP and PDHPA-TP were lower than that of PLA with similarity in initial M_n value.

Fig. 4.8C shows the GPC chromatogram of PDHPA-TP before and after hydrolysis at pH = 7.4 and 80 °C; the longer hydrolysis time resulted in longer retention time of polymer facture in GPC, which indicated that the lower molecular weight polymer fractures were caused by the hydrolysis of ester bonds in the presence of water. After 4 days, oligomeric fractions remained around 2000~4000 g/mol. The differences in the hydrolysis results could be due to differences in the initial molecular weight or in sample preparation.

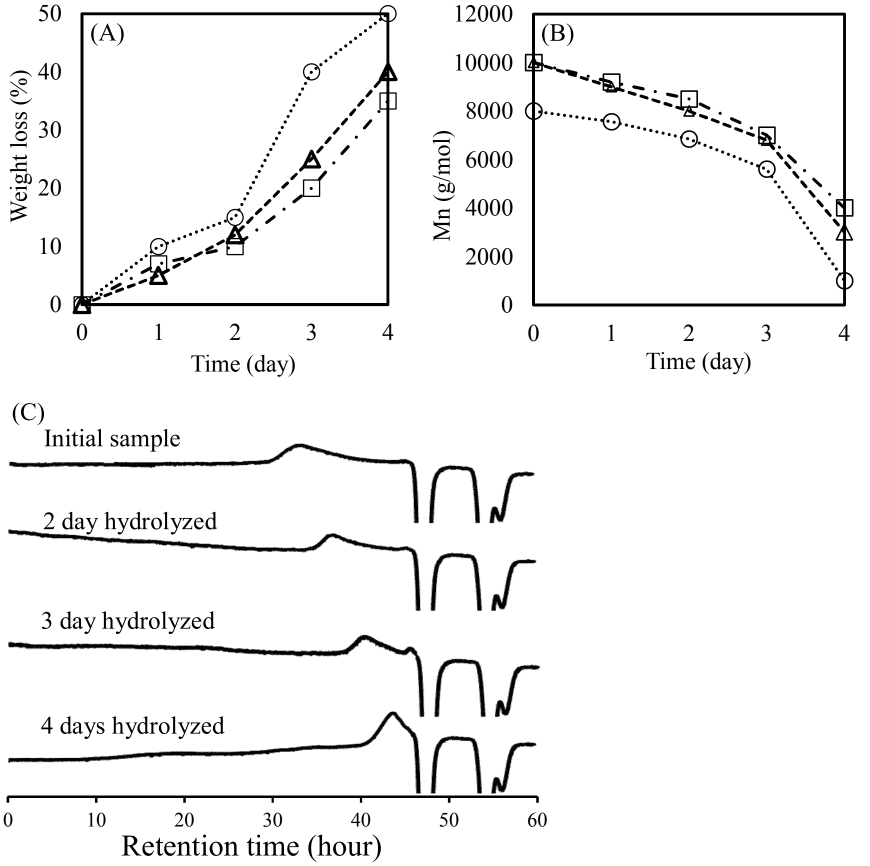


Figure 4.8. Weight loss (A) and M_n (B) of PDHPA-4C (circle marker), PDHPA-IP (triangle marker), PDHPA-TP (square marker) during hydrolytic degradation at 80 °C, as a function of degradation time. (C)GPC curves of PDHPA-TP before and after hydrolysis under pH = 7.4 at 80 °C.

Table 4.4. M_n and PDI values of DHPA-PEs before and after hydrolytic degradation

DHPA-PEs	**Before hydrolytic degradation**		**After hydrolytic degradation**	
	M_n (g/mol)	***PDI***	***M_n*** (g/mol)	***PDI***
DHPA-4C	8,000	1.51	1800	2.33
DHPA-IP	9,800	1.53	3500	2.12

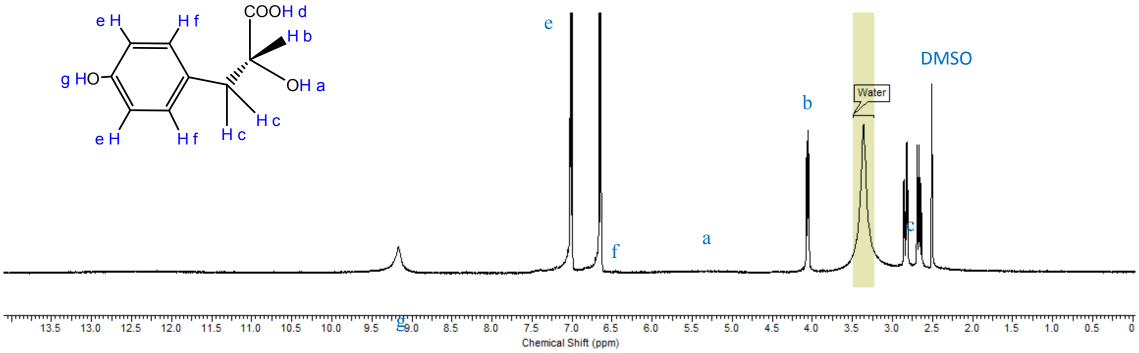
DHPA-TP	9,800	1.56	4200	1.97

4.4 Conclusion

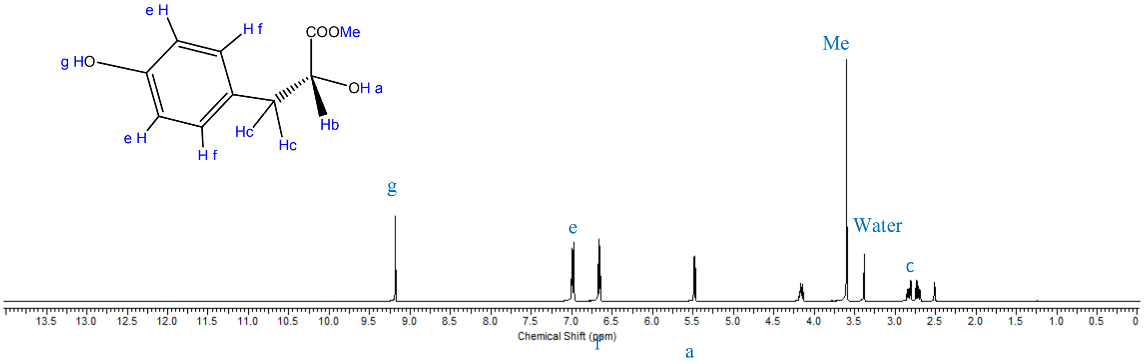
Poly(lactic acid) derivatives containing benzene ring in the main chains were synthesized from microbial D-p-hydroxyphenyllactic acid (DHPA) with various diacid chloride and transparent film of the resulting polymers were processed. The transparency of the film were very high and refractive index values were also high comparable with that of polycarbonates.

Although the polyesters prepared by using aliphatic diacid dichloride showed T_g lower than poly(lactic acid),The polyesters prepared by using aromatic acid chloride showed T_g higher than those of poly(lactic acid), poly(phenyllactic acid), poly(ethylene terephthalate) and poly(butylenes terephthalate), owing to the benzene ring in the backbone. Microbial DHPA was effectively cooperated with terephthalic acid recently got bioavailable [25] suggesting that the fully bio-derived aromatic polyesters prepared here can be widely applicable transparent film.

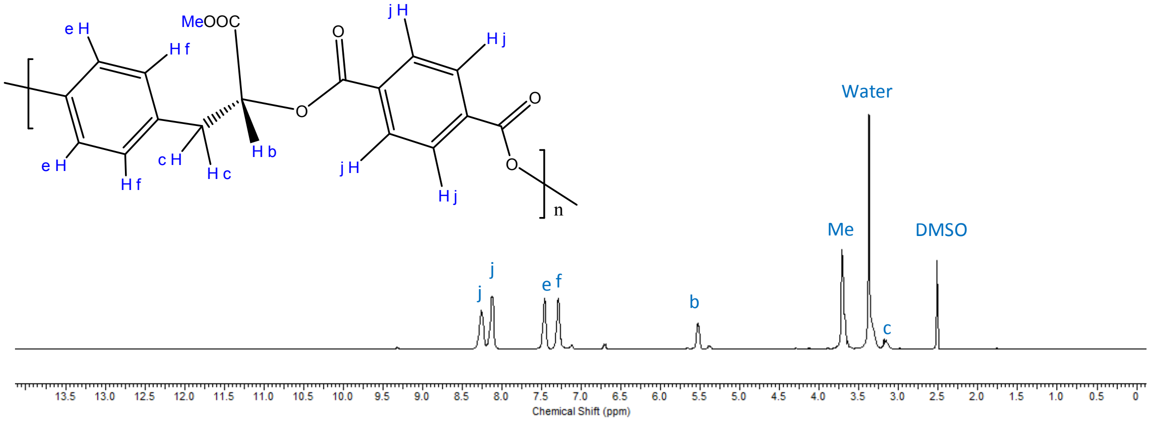
SUPPORTING INFORMATION FOR CHAPTER 4



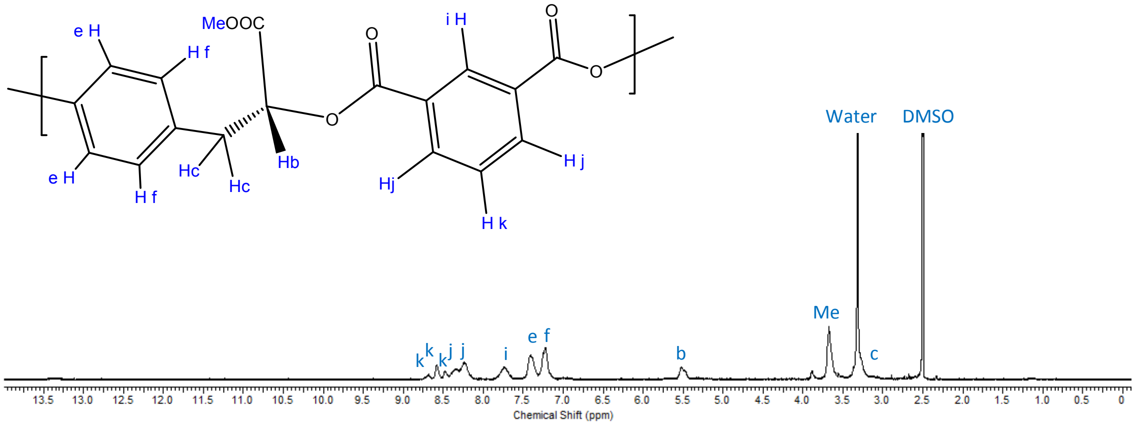
S 4.1a ^{1}H-NMR spectrum of DHPA (because of shielding effect, the hydroxyl peak (a) and carbonyl peak (d) is very broaden)



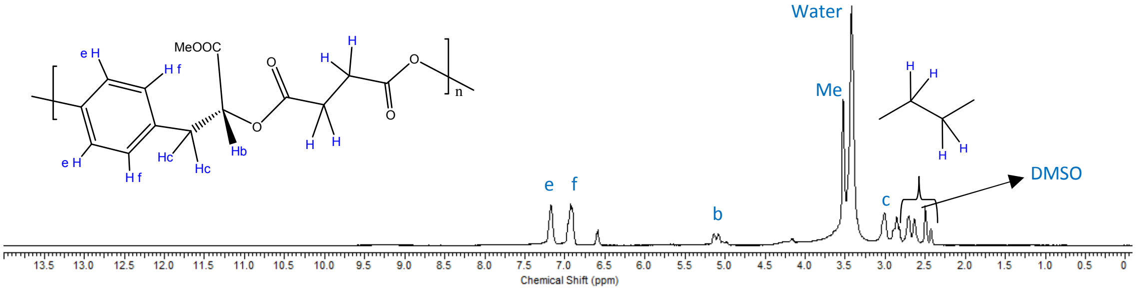
S 4.1b ^{1}H-NMR spectrum of methylated DHPA



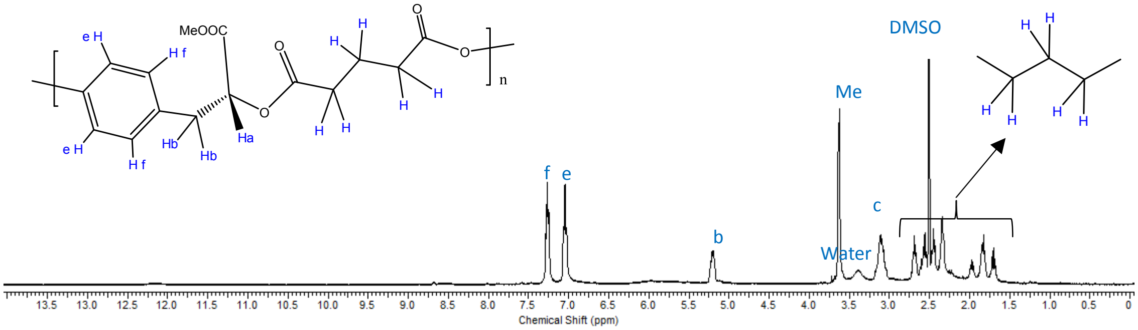
S 4.1c. ^{1}H-NMR spectra of monomer and PDHPA-co-TP



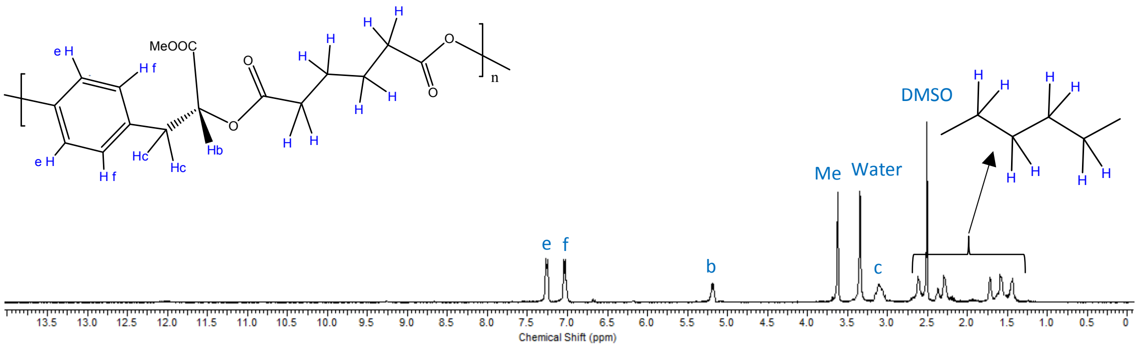
S 4.1d ^{1}H-NMR spectra of monomer and PDHPA-co-IP



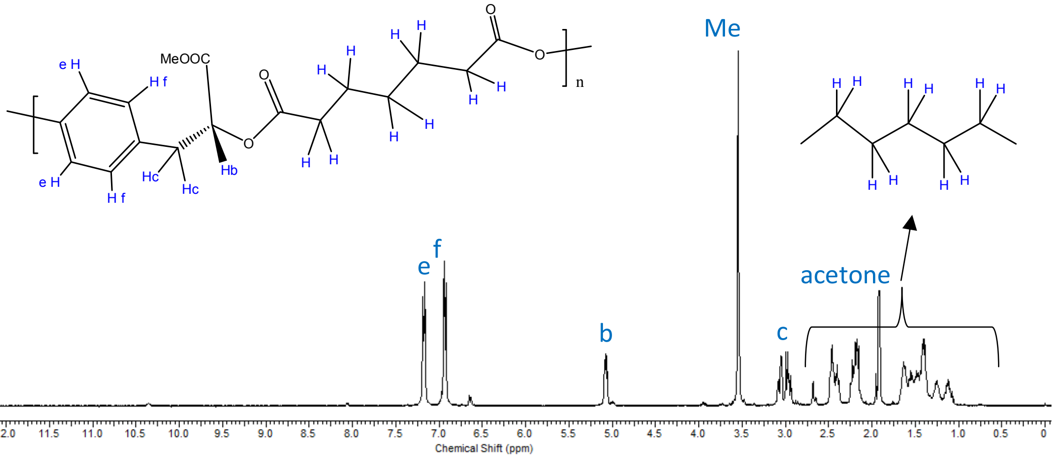
S 4.1e ^{1}H-NMR spectrum of monomer and poly(methyl-4-hydroxyphenyllactate-co-succinate) - PDHPA-4C



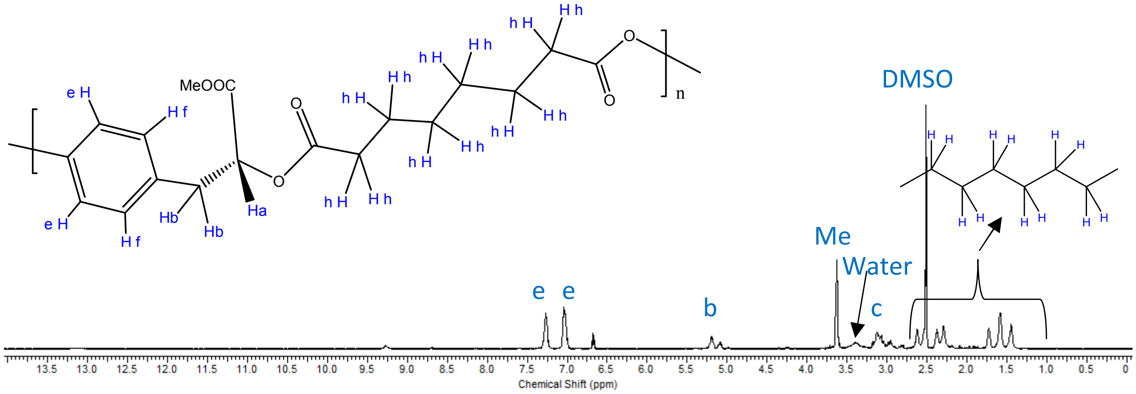
S 4.1f ^{1}H-NMR spectrum of monomer and poly(methyl-4-hydroxyphenyllactate-co-glutarate) - PDHPA-5C



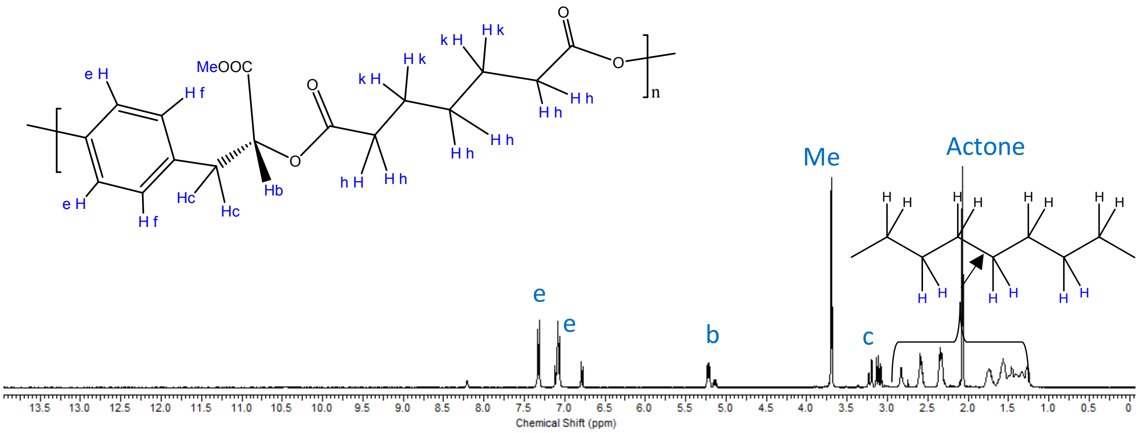
S 4.1g ^{1}H-NMR spectrum of monomer and poly(methyl-4-hydroxyphenyllactate-co-adipoate) - PDHPA-6C



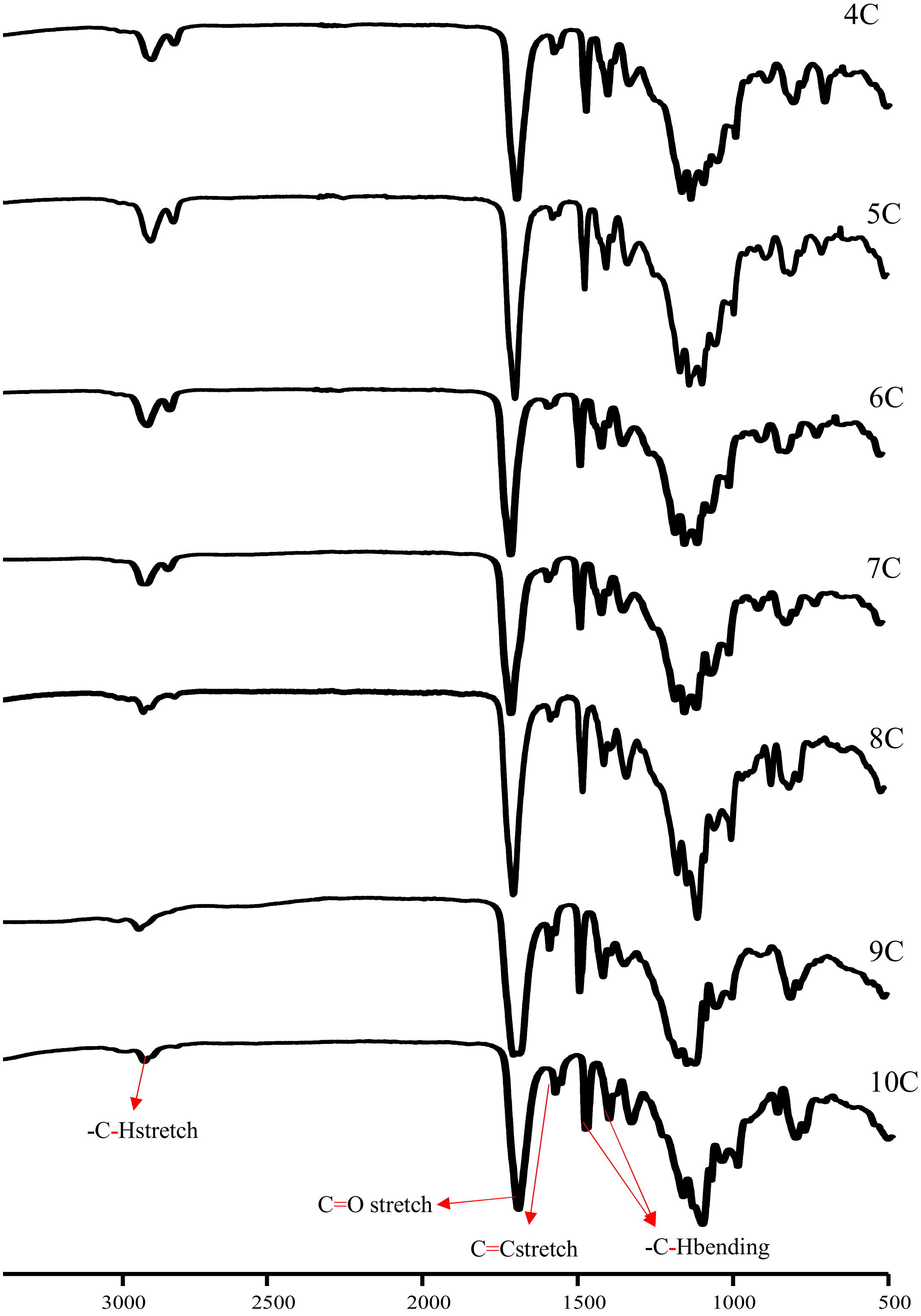
S 4.1h ^{1}H-NMR spectrum of poly(methyl-4-hydroxyphenyllactate-co-pimeloate) - PDHPA-7C



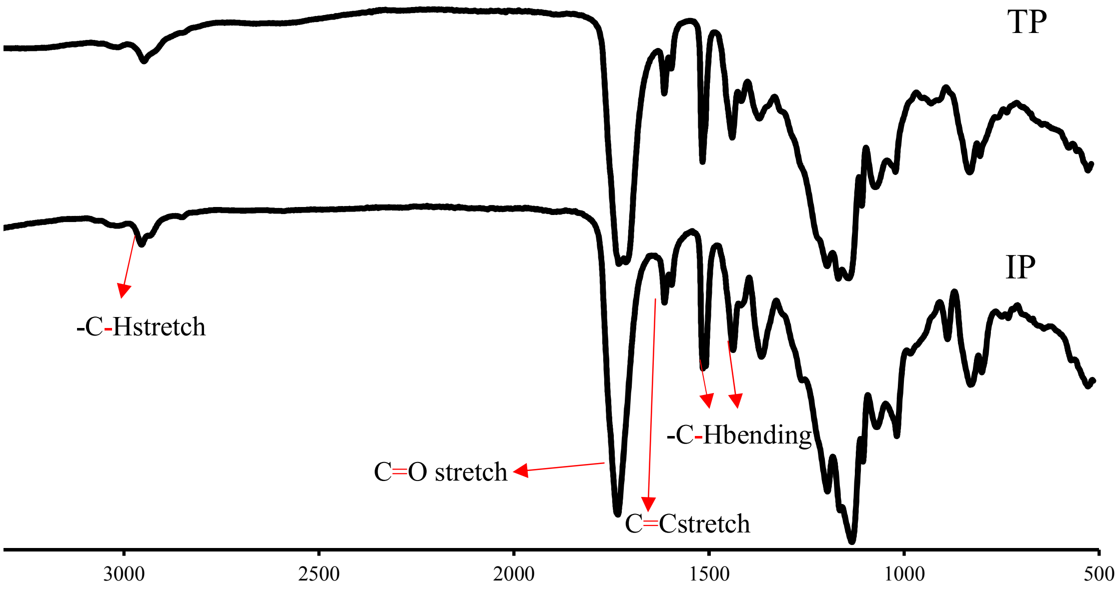
S 4.1i ^{1}H-NMR spectrum of poly(methyl-4-hydroxyphenyllactate-co-suberate) - PDHPA-8C



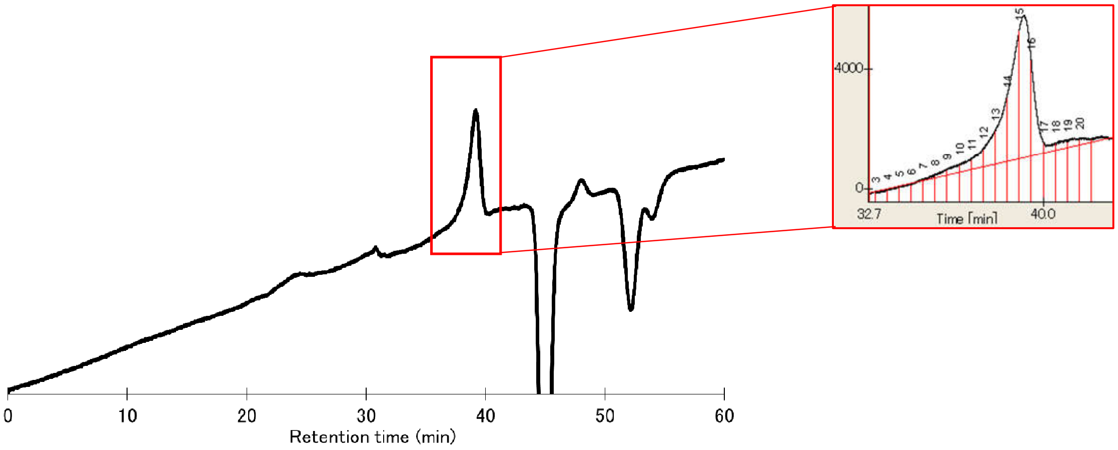
S 4.1k ^{1}H – NMR spectrum of poly(methyl-D hydroxyphenyllactate-co-azeloate) - PDHPA-9C



S 4.2a FT-IR spectra of aliphatic-coperated DHPA polyesters



S 4.2b FT-IR spectra of aromatic-coperated DHPA polyesters



S 4.3 GPC curve of DHPA-co-TP (the slide is calculated peak)

4.5 References

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Chapter 5

General Conclusion

The research in this thesis described preparation of bio-based polyesters from microbial phenyllactate derivatives, with high heat-deforming resistance. Polymers structures based on design concept of natural structure for sustainable polymer in green chemistry. The important and interesting results throughout this study are summarized as following:

In **Chapter 2**, the optically active polyesters as well as racemic polyester with methyl phenyl side chain were prepared from microbial chiral phenyllactic acids PhLAs. The syntheses were conducted under optimized melt polycondensation reaction with several catalysts stable in water such as hafnium(IV) chloride tetrahydrofuran complex - $HfCl_4.2THF$, vanadium(III) chloride tetrahydrofuran complex - $VCl_3.3THF$, ytterbium(III) trimethanesulfonate - $Yb(OTf)_3$, scandium(III) trimethanesulfonate - $Sc(OTf)_3$ tin metal - Sn. Among those, $HfCl_4.2THF$ complex and Sn metal had the highest reactivity and durability in this melt condition, resulted in high M_w value over 100000 g/mol. In syntheses of optically active polester, conservation of chiral monomer structure is important because chiral monomer can be racemized and reduce optical activity of polymer in this melt heating condition. However, those catalyst show very little racemization activity in ^{13}C-NMR spectra, therefore, we can proposed the dependence of glass-transition temperature - T_g of PPhLAs on M_w and dependence of specific rotation on M_w. The glass-transition temperature of PPhLAs strongly depended on M_w value and showed a saturation at 55

°C (150000), this result is comparable to optically pure high molecular-weight poly(lactic acid). The specific rotation value of optically active PPhLAs also showed a saturation point at 47° (150000 g/mol). However PPhLAs showed no dependence of glass-transition temperature on specific optical properties as PDPhLA, PLPhLA and PDLPhLA have similar T_g value.

Chapter 3 described the syntheses of bio-based hyperbranched polyester-arylate from microbial DHPA and glycolic acid in the presence of Sn metal catalyst, under direct melt condition. Multifunctional structure of DHPA acted as the branch-generating point, ABB'. Three stage of branching process are proposed; in the first stage DHPA in melt condition rich DHPA oligomers contain short branch and rich side-functional free group appear. During the second stage, the branched structure is propagated through functional end group, accompanied by increase in branching degree but slowed down the branching generation. In the third stage. The branches are propagated preferentially through both aryl-OH and α-OH. Hyper branching strongly affected the glass-transition temperature and degradation temperature of polymer. The glass-transition temperature increase with the increase in the molecular weight, and saturated at a threshold polymerization time, but then increase again following further polymerization for over 16 hours. The results obtained here can lead to new application in various fields such as biomedical and environmental materials with biodegradable properties.

In **Chapter 4**, we described the polyester design and syntheses based on multifunctional structure of microbial D-hydroxyphenyllactic acid DHPA. DHPA, had the potential of mass production by fermentation of recombinant E. coli expressing aminotransferase gene of *Wikerahmia fluorescene*. The copolymer of DHPA-derived diol was successfully synthesized in the polycondensation with several aliphatic and aromatic diacid chlorides in solvent condition. The DHPA-derived diol was first synthesized by protection of carboxylic group. After carboxylic group of (DHPA) was protected by methylation to give a diol derivative, the polymerization of was conducted by an esterification reaction between diol derivative with several aliphatic and aromatic diacylchlorides. The polyester of DHPA with aromatic diacylchlorides have maximum glass-transition temperature (T_g) value at 130 ^{o}C in the case of terephthaloyl-co-DHPA, whereas polymerization of DHPA with other aliphatic diacylchlorides resulted in polyesters with low T_g values, ranging from 40 ^{o}C to subzero.

Intro of benzene ring into microbial D-hydroxyphenyllactic acid (DHPA) into main chain in molecular design is an effective method to improve heat-deforming resistance of bio-based polyester. In comparison to bio-based and partially bio-based polyesters that have similar components, structure of DHPA-derived diol monomer that contains benzene ring component gave DHPA-derived polyesters improved $\mathrm{T_g}$ values. The multiple benzene ring unit also give DHPA-derived polyester high refractive index property which is comparable to polycarbonate – the optical plastic.

Overall, in this thesis, author designed some novel polyester based on microbial phenyllactate derivatives through convenient polymerization methods. The prepared polyester showed good heat-deforming resistant, and optical activity. This study has afforded interesting insights into the chemistry, functionality and applicability of natural structure to synthesized applicable polyesters from renewable resources. We hope that these bio-based polyesters can be developed to be useful for application as biomedical and environmental material.

Acknowledgements

First of all I would like to express my sincere thanks to my supervisor, Prof. Tatsuo Kaneko for his active valuable guidance and kind support during my stay in Japan for doctoral course and this research activity. All my achievememnt of my study would not be possible without his creative mind and ethuasitic guidance.

Second I would like to express my sincere gratitude to Prof. Naoki Takaya of University of Tsukuba who give me a careful teaching in technique and valuable understanding about biology experiment as well as his very kind support for my staying in Tsukuba University.

I also would like to express my special thanks to Prof. Minoru Terano and Prof Kohki Ebitani of JAIST for their kind supervision and encouragement during my research activities and lifestyle in Japan

Last but not least, I would like send my great thanks to Dr. S. Tateyama, Dr. M. Okajima of JAIST and Kaneko Laboratory's members, Dr. K. Oinuma of Tsukuba University, and Takaya Laboratory's members for their great helps as well as discussions of my research.

This research was granted by ALCA project: Generation of Super-engineering Plastics Using Microbial Biomass.

Achievements

Articles

1. **Hieu Nguyen Duc**, Seiji Tateyama, Naoki Takaya, Tomoya Fujita, Takashi Ito and Tatsuo Kaneko, Fermentation of aromatic lactate monomer and its polymerization to produce highly thermoresistant bioplastics (Online accepted by *Polymer Journal*)
2. **Hieu Nguyen Duc**, Seiji Tateyama, Naoki Takaya, and Tatsuo Kaneko, Syntheses of optically active phenol resin from microbial D-hydroxyphenyllactic acid (in preparation)

Other Articles

1. **Hieu Nguyen Duc**, Xin Jin, Daisaku Kaneko, Tatsuo Kaneko, "Syntheses of High Molecular Weight Poly(L-Phenyllactic acid)s by a Direct Polycondensation in the Presence of Stable Lewis Acids", *Chemistry Letters*, 40, 584-585, (2011).
2. Development of high performance bioplastics from aromatic biomolecules, **N. D. Hieu**, S. Tateyama, S. Wang, K. Kan, Y. Katsuaki, T. Kaneko, Vietnam J. Chem., 50, 68, 135-141, 2012
3. Microbial monomers custom-synthesized to build true bio-derived aromatic polymers, T. Fujita, **H. Nguyen**, T. Ito, S. Zhou, L. Osada, S. Tateyama, T. Kneko, N. Takaya, Appl. Microbiol. Biotechnol., 97, 20, 8887-8894, 2013

Attended international conferences

1. "New poly(lactic acid) derivatives derived from microbial D-hydroxyphenyllactic acid".

   Nguyen Duc Hieu, Seiji Tateyama, Akio Miyasato, Naoki Takaya and Tatsuo Kaneko *(oral presentation).*

   IUPAC 8th International Conference on Novel Materials and Syntheses (NMS-VIII) & 22nd International Symposium on Fine Chemistry and Functional Polymers (FCFP-XXII), Shanghai, China 17-24 October, 2013.
2. "Syntheses and properties of polyesters from D-hydroxyphenyllactic acid".

   Nguyen Duc Hieu, Seiji Tateyama, Akio Miyasato, Naoki Takaya and Tatsuo Kaneko *(poster presentation).*

   8th International Colloquium on Heterogeneous Ziegler-Natta Catalysts, Kanazawa, Ishikawa, Japan 27th -30th March 2012.
3. "Development of high performance bioplastics from aromatic biomolecules".

   Hieu Nguyen Duc, Seiji Tateyama, Siqian Wang, Kai Kan, Yasaki Katsuaki, Tatsuo Kaneko *(oral presentation).*

   1st International Workshop on Corrosion and Protection of Materials 4th -7th December 2012, Hanoi, Vietnam.

Abstract for Minor Research Theme

Minor Research Title: **Isolation of Natural Phenyllactates-Consuming Microorganisms from Soil**

Abstract

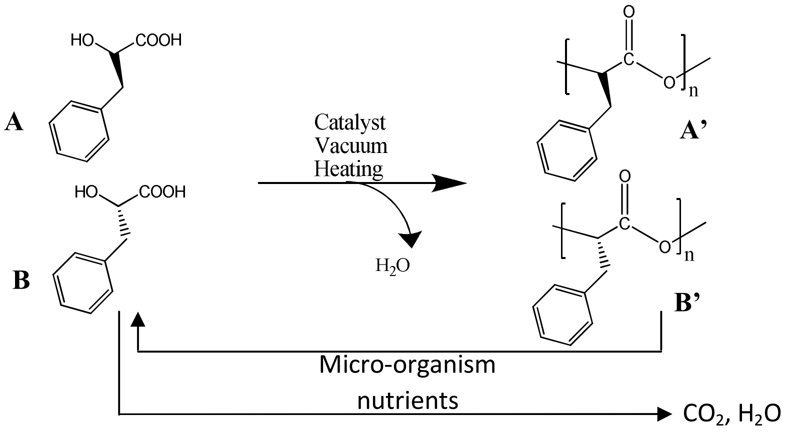
Poly(phenyllactate)s are polyester prepared from microbial D- and L-phenyllactic acid. A screening of microorganisms which have degradation and consumption activities against poly(phenyllactate)s is conducted with natural soil sampling. By in-vitro cultivation of microorganisms we confirmed that poly(phenyllactate)s can be naturally degrade and metabolized in ecosystem through bio-degradation processes of soil-inhabiting microorganisms. The degradation activities are evaluated by two steps: polymer degradation and monomer consumption. The microorganism species that have degradation effect to poly(phenyllactate)s are identified, including bacteria and fungus. The biological classification for highest biodegrading-active bacteria are conducted using 16s-Ribosome rRNA genetic material analyses, revealing some species belong to *Bacilus sp*.,*Burkhodilus sp.,*and*Lactobacilus sp*..

Keywords: polyester**,** poly(phenyllactate)s, bio-degradation, biological classification

1. **Aim of research**

Among lactic acid derivatives, D-phenyllactac acid (DPhLA) and L-phenyllactic acid (LPhLA) are aromatic compounds. Because having a stereo-center, PhLAs and have two enantiomers configurations. The nature of containing multifunctional groups such as phenol, hydroxyl and carboxyl makes PhLAs high potential of modification and cooperation by means of organic chemistry. Recently, full syntheses of PhLA from petroleum are very complicated. The racemic mixture of PhLAs can be produced less expensively from hydroxyphenyl pyruvic acid (a bio compound). The D configuration of PhLA is produced in-vitro from an isolated *Lactobacilus sp.*by Takaya Laboratory (Tsukuba University, Japan) [2]. These compounds have been investigated in antibiotic activity among some phenyl secondary metabolites [3]. In order to reveal the nature of environmental-compatibility PhLAs polymers we investigate the biodegradability of them by soil-bacteria in nature. In a certain extent, polymers of PhLAs and PhLAs may cause poisonings to bacteria [4]. When using polymer as carbon nutrient, some microorganism hydrolyze ester bonds or metabolize size chain of polymer gradually by their enzyme system to produce energy, and simple compounds for their functionality [5]. Organism can also metabolize monomers to other simple product for their carbon resource by specific enzyme system [6]. However, while decomposing polymer, the activity of ester-hydrolysis enzymes is also affected by optical properties of cooperated units because of their stereo-specificity. In addition, by the stereo-specificity of enzymes, some micro-organism can only produce enzyme to metabolize one type of enantiomers [7]. Therefore, one species of microorganism can hydrolyze one type of polymer (i.e. D/L, L or D) and/or can metabolite one type of monomer (i.e. L or D). Besides, though some microorganisms not use PPhLAs as their carbon source but their enzyme activity, as mention above, during their life cycle, may cause degradations to polymer [8].

With respect to observe a full cycle of environmental benign polymers based on PhLA derivatives, I conduct my minor research including: (1) Isolate the bacteria in soil that use D-phenyllactic acid as feed stock, (2) Isolate the bacteria that can use poly(phenyllactic acid)s as feed stock, (3) Isolate the population of bacteria that can degrade the polyester from PhLAs



Scheme 1. A cycle of environmentally benign polymers based on phenyllactic acid derivatives

2. Results

By a series of soil samples screening to collect microorganism, we can confirmed that phenyllactes and poly(phenyllactate)s polyester can be degraded in natural environment by some species of bacteria and fungus. Four species of bacteria with high monomer consumption were isolated and bio-classified belong to three genus of bacteria (Bacilus, Lactobacilus, and Burkholderia. Some species expressed a high monomer consumption with metabolic transformation 100% (a *Burkholderia sp.*). The existence of microorganism which can hydrolyze poly(polyphenyllactate)s were also detected and isolated but the classification were not done yet. By this screening we can confidently confirmed the environmentally friendly aspect of polyphenyllactates polyester, such as their bio-hydrolysis and metabolic ability.

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