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Chemical modification of polyhydroxyalkanoates for multi-purpose applications

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Running head: *Chemical modification of polyhydroxyalkanoates*

Abstract

Polyhydroxyalkanoates (PHAs) have become an attractive biomaterial in research in the past few years due to their extensive potential industrial applications. Being long chain hydroxyl fatty acid molecules, the PHAs are hydrophobic in nature, and have less functional groups. These features limit their applications in various areas. To enhance their usage, these polymers may need to be modified including surface and chemical modifications. Such modifications may alter their mechanical properties, surface structure, amphiphilic character and rate of degradation to fulfil the requirements for their future applications. Chemical modifications allow incorporation of functional groups to PHAs that could not be introduced through biotechnological methods. These chemically reformed PHAs, with enhanced properties could be used for broad range of applications. This review presents different chemical modification approaches for PHAs as possible technologies for widening the range of product and application potentials.

Keywords: Chemical; PHAs; PHB; Polyhydroxyalkanoates; Polyhydroxybutyrates

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List of abbreviations

AAs = Alkanoic acids
ABCs = Amphiphilic block copolymers
AcHC = Acetylehomocysteine
ATBC = Amphiphatic triblock copolymer
bio-PE = Bio-polyethylene
bio-PET = Bio-polyethylene terephthalate
BCA = Butyl cyanoacrylate

BL = Butyrolactone
BuMA = Butyl methacrylate
CdS = Cadmium sulfide
CS = Chitosan
DMF = Dimethyl formamide
DEG = Diethylene glycol
EDMA = Ethylene dimethacrylate
EDS = Electron donating substituents
EG = Ethylene glycol
EO = Ethylene oxide
PHA-LE = Epoxidized linseed oil based PHA
2E4MI = 2-ethyle-4-methyleimidazole
ENR = Ethylene propylene rubber
3-HA = 3-hydroxyalkanoate
3-HB = Hydroxyl acids
HDP = Hydroxylated PHB
3-HV = 3-hydroxyvalerate
2-HP = 2-hydroxypropanoate
3-HP = 3-hydroxypropanoate
LDP = Low density polysaccharides derivatives
PHA-L = Linseed oil based PHA
MA = Malic acid
MeA = Methacrylate
MNPs = Metal nanoparticles
m-CPBA = Meta chloroperoxybenzoic acid
C1= Methyl
mcl = Medium chain length
mPEO = Monomethoxy polyethylene oxide
NPs = Nanoparticles
NNDCHC = 1,3-N,N- dicyclohexylcarbodiimid
PBS/A = Polybutylene succinate/Adipate
PBCA = Poly(butyle cyanaoacrylate
PECH = Polyepichlorohydrin
PHAS = PHA synthase
PHAs = Polyhydroxyalkanoates
PCL = Polyhydroxybutyrate
PHBV = Polyhydroxybutyrate-co- valerate
P(3HB-co-3HV)= Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
PE = Polyethylene
P(3HHx-co-3HO) = Poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate)

PECA = Poly(ethylene cyanoacrylate
PHH = Polyhydroxyheptanoate
PHOU = Polyhydroxy-octanoate-co-undecanoate
PHO-co-UD = Polyydroxyoctanoic-co-undecylenic acid
PHV = Polyhydroxyvalerate
PLA = Polylactic acid
PMMA = Poly(methyl methacrylate)
PNAS = Poly(N-acryloxsuccinimede
PTHF = Poly(tetrahydrofuran)
PP = Polypropylene
PS = Polystyrene
PEO = Polyethylene oxide
PET = Polyethylene terephthalate
PPO = Polypropylene oxide
PUs = Polyurethanes
PVA = Polyvinyl alcohol
PVAc = Polyvinyl acetate
PV DF = Polyvinylidene fluoride
scl = Short chain length
TBAOH = Tetrabutyl ammoniumhydroxide
C13 = Tridecyl
TPF = Tea plant fiber
ZnS = Zinc sulfide

1 Introduction

1.1 Plastics

Plastics are used in almost all industries across the spectra ranging from home, gardens, building, medical and transport. To obtain a wide range of strength and shapes, the structure of plastics can chemically be manipulated to achieve the required characteristics. Most of the polymers, such as polyethylene compounds (PE), polypropylene compounds (PP), nylon and polyvinyl chloride (PVC) are used as plastics in many industries. The molecular weight range of plastics range from 50,000 to 1,000,000 Dalton. The main disadvantage of plastics is their cost effectiveness as material easy to produce and dispose-off upon use. Plastics are basically xenobiotic and mostly resistant to biodegradation and can persist in soil for long period of time. In last few years, public apprehension has increased over the destructive effects of petroleum based plastic products on the environment (Reddy *et al.,* 2003).

Concerns over plastics synthetic petroleum based plastics therefore have giving impetus to ecofriendly bio-based and biodegradable plastics produced from renewable resources that can biodegrade in the environment. Biodegradable plastics are of different types, photodegradable, starch-linked, semi-biodegradable and completely biodegradable. Ultraviolet (UV) radiations are necessary for the degradation of photodegradable plastics because they have a light sensitive group which is directly assimilated into the structure of polymer. As plastics are thrown into landfills, due to nonexistence of sunlight in landfills, these plastics keep on non-degraded (Pillai *et al.,* 2011). Starch-linked polymers are semi-biodegradable; it is assumed that starch linked plastics are disposed of into landfills where starch is attacked by soil bacteria and released polymer segments that could be degraded by some bacteria. The eco-friendly plastics are novel and includes polyhydroxyalkanoates (PHAs), poly(lactic) acids (PLAs) and polysaccharides etc. (Reddy *et al.,* 2003).

1.2 Classification of plastics

Basically, there are four main classes of plastics depending upon degradability and source. Figure 1 shows the classification of plastics. These are bio-based bio-degradable, bio-based non-biodegradable, oil-based bio-degradable and oil-based non-biodegradable polymers. Typical oil based non-biodegradable plastics include polyethylene (PE), polypropylene (PP), polystyrene (PS) and polyethylene terephthalate (PET). However, poly(caprolacton) (PCL), poly(butylene succinate/adipate) PBS/A and PBS-co-terephthalate come under the category of oil-based bio-degradable polymers. The bio-based non-biodegradable plastics are high density polysaccharides derivative (HDP), bio-poly(ethylene) bio-PE, bio(polyethylene terephthalate) bio-PET and polyurethanes. Forth class of plastics include poly(lactic acid) PLA, low density polysaccharides derivatives (LDP) and polyhydroxyalkanoates (PHAs)

Among all the four classes bio-based biodegradable plastics are preferred as they are considered as eco-friendly plastics. PHAs are considered as more eco-friendly plastics than other bio-based plastics; they are produced by microorganism and could easily be degraded by them.

1.3 Polyhydroxyalkanoates (PHAs)

The PHAs are one of the biodegradable and biocompatible thermoplastics (Gamal *et al.*, 2013). These are larger molecules which are produced by Gram-negative and Gram-positive bacteria (Madison and Huisman, 1999). Basically, the PHAs have ester linkages in their structure which

are produced by many of different types of bacteria as an intra-cellular storing material for substantial amounts of carbon and energy (Tsuge, 2002).

PHAs possess unique properties one of these properties is their high biodegradability in various environments and are therefore of much interest as biodegradable polymer compounds. Without a doubt amongst biopolymers, the bio based polyesters represent an alternative for petrochemical thermoplastics. Most easily accessible PHAs have mostly been reported produced using microbial cultures grown on renewable materials in sterilized environments, however most current studies emphasis to use of waste materials for growth media (Bugnicourt *et al.*, 2014).

1.4 Chemical structure of PHAs /Isomerism

The PHAs that have been investigated to date are mainly linear polyesters containing 3-hydroxy fatty acid monomers. An ester bond is formed by the reaction between carboxyl group of one monomer and hydroxyl group of another monomer unit (Figure 2) (Madison and Huisman, 1999). Hence, most of the PHAs produced by various microorganisms are of rectus (R) configuration (Kemnitzer *et al.*, 1992). The most common representative of PHAs, polyhydroxybutyrate (PHB), is present mostly as in R configuration. However, sinister (S) PHB could also be synthesized by synthesizing copolymer with some stereo polymers. Kemnitzer *et al.*, (1992) synthesize a random stereocopolymer of PHB with butyraolacton (BL). All of R and S BL were successfully synthesized in high enantiomeric purity, after polymerization, small racemization (about 5%) was also observed. A study (Kemnitzer *et al.*, 1992), resulted in the stereocopolymer with enhanced properties such as crystallinity and thermal stability. In PHAs the hydroxyl substituted carbon atom is of R configuration except in a few cases where chirality is absent. Figure 2 represents general structure of PHAs, in this figure R represents alkyl group that may vary from methyl (C 1) to tridecyl (C 13) group and may also be positioned at β carbon (Madison and Huisman, 1999). There is great variation in the alkyl side chain which may be: aromatic, halogenated, epoxidized or other branched monomers (Choi and Yoon, 1994; Curley *et al.*, 1996; Kim *et al.*, 1992). These variations in the size and composition of side chain substituents are responsible for chemical modification of PHAs and for their wide diversification in applications.

1.5 Classification of PHAs

PHAs are grouped into three discrete classes according to the chain length. The first of these is short chain length (scl) polymers with maximum five carbon atoms chain, obtained from several bacteria, such as *Cupriavidus necator* and *Alcaligenes latus*. The second class includes medium chain length (mcl) polymers, containing 6 to 14 carbon atoms in the chain, obtained from several bacteria such as *Pseudomonas putida* and *Pseudomonas mendocina* (Akaraonye *et al.*, 2010). The third class include long chain PHAs having carbon atoms > 14 mostly produced by the bacteria such as *Shewanella oneidensis* and *Aureispira marina* (Suriyamongkol *et al.*, 2007) blends of PHAs in scl such as P(3HB-co-3HV) and in mcl-PHAs such as P(3HHx-co-3HO) are also found (Akaraonye *et al.*, 2010).

1.5.1 Short chain length (scl) PHAs

PHB, is known as crystalline and brittle polymer, but the blends of PHAs have low crystallinity and increased flexibility and are categorized as scl-PHAs and mcl-PHAs. These blends are manufactured in different plants such as *Arabidopsis* and in cotton, corn cell culture, *Brassica napus* and in tobacco by accumulating PHAS from *Ralstonia eutropha* or *Aeromonas caviae*. These scl-PHAs were found to emit the light at 590 nm wavelength while mcl-PHAs emit light of 575 nm (Arai et al., 2002; H. Wu et al., 2003; Sciences, 1998).

Arai, Nakashita et al. (2002) carried out gene modification to get a copolymer (PHB-co-HV-co-HH) by the addition of a signal encoder (which can target peroxisome) to the last 10 amino acid at –COO terminal of spinach glycolate oxidase, this modified gene was then transferred to *Arabidopsis thaliana* through agrobacterium regulated conversion. This resulted in the expression of transgenic gene along with its protein in Arabidopsis plant and in the accumulation of scl PHA in its tissues as detected by GC-MS analysis (Arai et al., 2002).

1.5.2 Medium chain length (mcl) PHAs

The mcl-PHAs are polyesters of hydroxyl acids (HAs) formed primarily by Pseudomonads during uneven growth conditions. These mcl-PHAs have high degree of biocompatibility and biodegradability and are obtained from renewable resources. As compared to scl PHAs, mcl PHAs has improved mechanical properties such as decreased brittleness, decreased crystallinity, low glass transition temperature, high melting temperatures and poor tensile strength

characteristics. Hence, these are more flexible materials. In short, these are elastomers, and due to these properties, these polymers may be suitable to biomedical applications e.g. cardiovascular applications along with controlled drug release. Functional moieties of mcl PHAs make them more versatile than other PHAs and could be modified more freely for particular applications (Byrom, 1987; H. Wu, Sheu, & Lee, 2003; Koning, 1995; Rai *et al.,* 2011).

The mcl-PHAs could be synthesized by three steps; in the first through increase in chain length by condensation of acyl-CoA with acetyl-CoA; in the second through degradation of fatty acid fatty acid through β-oxidation, this is the key alleyway at what time fatty acids are being used as substrates; and in the third through biosynthesis of fatty acids, which is the key step in biosynthesis of cheap carbon compounds (Klinke *et al.*, 1999).

Alkanoic acids (AAs) are the base materials for mcl PHA's synthesis their beta oxidation intermediates result in the production of mcl PHAs. To achieve the production of mcl-PHAs in plants, a strain, *Arabidopsis thaliana* was transmuted by its specific synthesizing enzyme *from Pseudomonas aeruginosa* with the accumulation of a specific enzyme (carboxyl 34 amino acid) from the *B. napus* with an enzyme isocitrate lyase (ICL) (Mittendorf *et al.*, 1998). Studies (Huijberts *et al.*, 1992; Javers and Karunanithy, 2012; Mittendorf *et al.*, 1998) proved that amended PHA synthase (PHAS) can be suitably directed to leaf form peroxisomes of the plants that are grown in light and to glyoxysomes of the plants that are grown in dark. Plants venting the PHAS have amassed electron additions in their glyoxysomes, leaf, peroxisomes and in vacuole also. The additions were analogous to additions of bacterial PHA. Spectrometric and gas chromatographic analysis proved that mcl PHAs are also present in transgenic plants. These demonstrations show that beta oxidation intermediates of AAs produce a wide assortment of hydroxy acyl acetyl-Co with R configuration which is the precursors for synthesis of mcl-PHA.

1.6 PHAs sources

PHAs are macromolecules which are produced by various species of microorganisms. More than 300 bacteria from family, *Halobactericeae* are some of the sources for PHAs synthesis (Suriyamongkol *et al.*, 2007). Synthesis of PHAs involves three genes and three enzymes and is the simplest biosynthetic pathway (Peoples and Sinskey, 1989). *Pseudomonas Oleovorans* and *Pseudomonas fragii* are the bacteria which can synthesize mcl PHAs through the beta oxidation of alkanoic acids by providing hydroxyalkanoyl-CoA substrate (Lageveen *et al.*, 1988; Peoples

and Sinskey, 1989). Plant's peroxisomes give Ac-CoA via beta oxidation of fatty acid and provide a compartment for the synthesis of PHAs (Hahn *et al.*, 1999)

Polyesters comprising 2-hydroxypropionate (2-HP), 3-hydroxypropionate (3-HP), 4-hydroxybutyrate (4-HB), 3-hydroxyvalerate (3-HV), and mcl 3-hydroxyalkanoate (3-HA) monomers can all be manufactured in *Escherichia coli* by assimilating exogenous or endogenous alleyways and/or genes (Wang *et al.*, 2013).

Bacterial production of PHAs is non-economical as compared to petroleum based plastics. Due to these reasons, there is interest in exploring production of PHAs in eukaryotic cells, specifically in crops. PHAs synthesis in insect and yeast cells explores the ideas that how these synthetic pathways can be introduced to plants. In a study, it was revealed that PHB is synthesized through the expression of PHB synthase gene in *saccharomyces cerevisiae* (Leaf *et al.,* 1996). But the accumulation of PHB was less than 5% may be due inadequate specific enzymes (Poirier *et al.*, 2001).

2 Properties of PHAs

The PHAs are attaining increased attention in the ecofriendly polymer market because of their favorable properties such as high biodegradability in various environs, not only in composting plants and processing versatility, Hence among biopolymers, these polyesters symbolize an future sustainable alternative for fossil fuel-based thermoplastics (Bugnicourt *et al.*, 2014).

2.1 Polyhydroxybutyrate (PHB)

A French microbiologist Maurice Lemoigne was first to isolate and characterize PHB in 1925 (Lemoigne, 1926). PHB has optical activity and is stereoregular homopolymer. Among PHAs, PHB is the most well-known biodegradable and biocompatible polymer as it gains significant interest by industries. It is used in agriculture, house hold and medical industries for broad range of applications (Ashby *et al.*, 2000). PHB is produced by remote type bacteria as linear, crystalline and amorphous forms. Its glass transition temperature is about 4 °C and its melting temperature is near 180 °C having density and specific gravity of 1.26 and 1.18 g/cm3 respectively. The mechanical possessions of this biopolymer are similar to isotactic polypropylene (Pfeiffer and Jendrossek, 2014; Sato *et al.*, 2015).

The molecular weight of PHB depends upon its source, growth situations and extraction method. Its molecular weight differ from 50, 000 to million (Rai *et al.*, 2011). Organically created, PHB is a semi crystalline isotactic stereo systematic polymer with 100% R configuration which permits enhanced degradability (Steinbüchel, 2005). Figure 3 shows general structure of PHB.

2.2 Crystalline behavior of PHB

PHB and other PHAs show crystallization when extracted from cell with some non-polar solvents while they remain as granules in cytoplasm. This crystallization behavior of PHB could be kinetically controlled and could be stopped by particles of submicron size or by coating of phospholipids and their proteins (Anderson & Dawes, 1990; Doi, 1995)

2.3 Structure-function relationship in PHAs

PHAs are composed of long chain fatty acid monomers mostly 3,000 to 40,000 monomer units in straight chain fashion. Fatty acids having hydroxyl group at carbon-3 undergo beta oxidation and give Ac-CoA residues which undergo condensation by the action of certain enzymes forming PHAs. Depending upon the type of monomers synthetic pathways vary from monomer to monomer indicating that synthetic enzymes are broadly dispersed and that the creation of a specific polyester is an enzyme specific incident (Luengo *et al.*, 2003).

When there is an inequity in the nutrients supply of a cell (increase in carbon concentration and limitation in N_2, P, and O_2), bacteria stock these surplus nutrients intracellularly and yield water insoluble biopolymers. When normal environment is restored, these biopolymers become militarized (Diez-Pascual and Diez-Vicente; Hahn *et al.*, 1997; Suriyamongkol *et al.*, 2007; Sato *et al.*, 2015).

The scl-PHAs have possessions in common with conservative plastics while, mcl PHAs are known as elastomers and rubber materials. The PHAs have some functionality through which monomers can be modified to give more versatile polymers such as introduction of unsaturation or halogenation of monomers. The properties of these polymers can be further improved. In addition homopolymers, hetropolymers can also be fabricated by the condensation of two or more than two different types of polymers. The most well-known representative of scl-PHAs is PHB. Blends of PHAs can also be manufactured such as polyhydroxybutyrate/polyhydroxyvalerate (PHB/PHV),

polyhydroxybutyrate/polyhydroxyheptanoate (PHB/PHH) (Steinbüchel, 2005; Suriyamongkol *et al.*, 2007; Kulkarni *et al.*, 2011).

Properties of bacterial PHAs are comparable to those of polypropylene (PP) conventional plastics (Byrom, 1987). The catabolism of PHAs to CO_2 and H_2O is facilitated by the enzyme PHA depolymerases (Jendrossek and Handrick, 2002). PHAs are also considered as natural green plastics as they can be recycled and produced from renewable resources which makes them substituents to petroleum based plastics (Poirier, 1999). The great variety of monomers presented by PHAs gives an extensive spectrum of polymers with different physical possessions. Their physcio-chemical properties are governed by their monomeric structure that is determined by manufacturing microorganisms and their nutrition (Zinn and Hany, 2005).

PHB is a homopolymer with high level of crystallinity and brittleness. These properties make it of less use. PHAs of long chain fatty acid monomers, are elastic and sticky in nature, rubber can be generated by modification of these long chain PHAs. Elasticity of PHAs can also be increased by making the blends pf PHB with long chain PHAs such as hydroxyvalerate (HV) and hydroxyhexanoate (HHx) etc. These blends present more flexibility and toughness and can be used for the manufacturing of various products such as bottles and food packaging materials (Griffin, 1994).

As PHAs are biodegradable and have biocompatibility, they are degraded into 3-HA (3-hydroxyacids) which are found in animal tissues. They have a number of applications in medical field such as in control drug release, implants and oesteosynthetic ingredients (Pouton and Akhtar, 1996; Sudesh, 2004) and can also be used as potential material in tissue engineering scaffolding (Williams *et al.*, 1999) .

3 Need for chemical modifications of PHAs

PHAs are comparatively new group of biodegradable polymers with high impact in future because of their different properties. They are considered as more attractive biomaterials in research since last few years due to their extensive applications. As PHAs, are long chain hydroxy fatty acid molecules, therefore, they are hydrophobic in nature, have low degradability under certain biological environments and have less functional groups. These features limit the space of their applications in various areas (Hu et al., 1999; Jamieson et al., 2007; Królikowska

et al., 2003; Mandal et al., 2006; Mohanpuria et al., 2008; Smith et al., 2008; Sweeney et al., 2004). To enhance their usage, these polymers are needed to be modified including surface and chemical modifications. These modifications may alter their mechanical properties, surface structure, amphiphilic character and rate of degradation to fulfil the requirements for their specific applications. Chemical modifications allow incorporating functional groups to PHAs that are not easily achievable through biological routes. These chemically reformed PHAs, with enhanced properties, can be used as multifunctional supplies. Different methods of chemical modifications includes epoxidation, carboxylation, chlorination, grafting (cationic, enzymatic, radiation based and free radical grafting), blending, esterification based copolymerization, incorporation of functional groups to PHAs through thermal degradation, metallization and thiolation (Bassas-Galià *et al.*, 2015; Hazer and Steinbüchel, 2007; Kai and Loh, 2013). To date several studies have been carried out on PHA modification including physical and biological modifications. Hence the functionalities of available PHAs have been broadly extended by chemical modification methods. Some chemical modification methods are discussed below and are presented in Table 1 which summarizes these chemical modification methods and properties achieved by each.

3.1 Chlorination

Chlorination is the process of introducing or adding chlorine to a substrate. In the case of polymers, unsaturated PHAs can undergo chlorination or saturated PHAs can undergo chlorination by substitution reaction. Arkin *et al.*, (2000) carried out chlorination of unsaturated PHA was carried out by producing chlorine gas and passing through the PHA solution in chloroform at room temperature and sunlight. This study revealed that as the extent of chlorination increased, properties of PHA changed from soft and sticky to crystalline, brittle and hard. The chlorinated PHA would serve as a very useful intermediate for blending of polymers and for further modifications such as carboxylation (Doi and Abe, 1990)**.**

In a different study, PHB and PHO chlorination was carried out by substitution reaction. In this study chlorine gas was passed through the polymers solution in chloroform. This resulted in molecular weight loss of polymers due to hydrolysis. Glass transition temperature and melting temperatures of chlorinated PHO was significantly increased while melting temperature of PHB was decreased from 170°C to 148°C and its glass transition temperature changed from -20 °C to

10 °C. when these chlorinated polymers were further subjected to modification such as PHA-quaternary ammonium salts, NaSO4 salts, phenyl derivatives were obtained. These chlorinated PHAs could also undergo Friedel-Crafts reactions with benzene to produce cross-linked polymers (Arkin and Hazer, 2002;Hazer and Steinbüchel, 2007). Hence chlorination is the simplest method of modification for PHAs.

3.2 Carboxylation

Carboxylation is the process of introducing carboxylate functional group in a substrate. PHAs with unsaturated side chains, chlorine groups or epoxidized side chains can be subjected to oxidation resulted in carboxylation of PHA with increased hydrophilicity. To bind target enzymes, hydrophilic constituents or biologically active molecule and carboxylic groups serve as the most active functional groups. Insertion of these functional groups to polymers resulted in increased hydrophilic character of the polymer. Almost 70 PHAs had been discovered that have such –COO group and OH end group (Kai and Loh, 2013; Zook and Kee, 2016).

Bacterial PHAs are hydrophobic in nature; their hydrophobicity can be reduced by some chemical methods. Stigers and Tew, (2003), reported carboxylation of an unsaturated copolymer PHO-co-PHU using OsO_4 and oxone. This oxidation was carried out in warm dimethylformamide (DMF) and resulted in some backbone degradation; this degradation was confirmed by NMR and GPC. This carboxylation was also confirmed by solubility of polymer before and after carboxylation in different solvents such as THF, H_2O and acetone.

Carboxylation of unsaturated PHOU was also carried out by using potassium permanganate as an oxidizing agent at 558^{o}C and resulted in loss of molecular weight of the polymer. IR and 1 HNMR spectroscopic technique was used to confirm carboxylation. The extent of carboxylation was varied with time, e.g. after 2 h, almost 50% carboxylation occurred. The hydrophilicity of polymer was increased and it was confirmed by its solubility in water (Lee and Park, 2000). In short, carboxylation could be utilized whenever there is a need to change hydrophobicity/hydrophilicity levels. In this regard, this method could be used for the preparation of hydrogels.

3.3 Epoxidation/Crosslinking

Epoxides are also known as oxiranes. The epoxy groups are very reactive functional moieties, and are important organic intermediate in industries. They could be used in many polymer reactions without degradation of polymer. Linseed oil based (PHA-L) mcl-PHA have low molecular weight and high degree of unsaturation in side chain. These are amorphous in nature and have considerable viscosity at room temperature. The unsaturated side chains of these PHAs were subjected to epoxidation by using meta-chloroperoxybenzoic acid (m-CPBA) as a cross-linker, to enhance the properties and applications of these PHAs. Approximately 37% olefinic the groups were converted to epoxy groups. These epoxy groups increase the chances of crosslinking for PHAs, when they are exposed to air. Analytical results revealed that the linseed oil based epoxidized PHA (PHA-LE) enhanced tensile strength and young's modulus than linseed based PHA (PHA-L) (Ashby *et al.*, 2000). Hence, epoxide function is highly reactive and could be successfully used for further reactions such as crosslinking which is defined as chemical joining of two molecules by covalent bond (Park *et al.*, 1998). . In the case of polymers, it could also be defined as formation of new chemical bond of one polymer with another polymer. The presence and extent of crosslinking in a material often have significant effects on its chemical and mechanical properties (Nicholson, 2012).

Arkin *et al.* (2000) subjected polyhydroxyoctanoate-co-10-undecanoates (PHOUs) to epoxidation due to the presence of vinyl group which easily undergo epoxidation reaction and copolymers were obtained by using (m-CPBA). The epoxidized copolymers contained up to 22 mol % repeating units. These epoxidized polymers were subjected for crosslinking with anhydride of succinic acid and 2-ethyl-4-methyleimidazole (2E4MI) used for crosslinking reaction initiation. Reversibility and degradation of reaction was avoided by using mild reaction conditions (temperature 90°C, for 1-4h). An increase in sol gel (involving alternation between sol and gel contents) contents and in glass transition temperature indicated Crosslinking. Ozawa and Kissinger methods were used for kinetic study of the epoxidation reaction and the values for the activation energy and frequency parameters were high obtained by Ozawa method than by Kissinger method.

A mcl-PHA obtained from palm oil was permitted to come in reaction with epoxidized natural rubber (ENR). The reaction did not occurr at normal temperature for a short period of time,

however with time as temperature was increased to 170°C, thermal degradation of mcl-PHA started and smaller units of polymer (carboxylic acid terminals) were obtained that resulted in the ENR ring opening (Lee *et al.*, 2010).

To increase the elastic properties of the two different types of PHAs (saturated and unsaturated) polyhydroxy-octanoate (PHO) and PHO-co-UD (polyhydroxyoctanoic-co-undecylenic acid), were subjected to peroxide crosslinking in the presence and absence of a different functional cross-linkers. The extent of crosslinking was determined by using sol-gel analytical technique; this technique verified crosslinking and demonstrated that variation in crosslinking was due to peroxide type and its concentration. DSC revealed that all the crystallinity in polymers was removed by crosslinking; in short crosslinking resulted in decreased tensile strength and also decreased tear resistance (Gagnon *et al.*, 1994).

In another study, composite of PHA with malic anhydride (PHA/MeA) crosslinked with tea plant fiber (PHA-g-MeA/t-TPF) was assessed; it was observed that PHA-g-MA/t-TPF has more enhanced mechanical properties than PHA/TPF due to high compatibility with TPF. It was also apparent that t-TPF had more dispersion homogeneity in the matrix of PHA-g-MeA due to ester linkage formation which resulted in creation of cross-linked and branched larger molecules among –COOH and OH groups of PHA/MeA and t-TPF respectively. This crosslinked composite had more polished properties such as increased water resistance, easy processing because of low viscosity and high biodegradability with an increase in TPF contents (Wu, 2013).

Similarly, a study was conducted to fabricate an unsaturated composite PHB/PHBU produced by *Escherichia coli* strain which was subjected to cross-linking through thiolene click chemistry and was analyzed for enhanced physical material goods and its biocompatibility with human mesenchymal stem cells. A significant increase in tensile strength was observed which was compatible to material with properties required for replacement of soft tissue. In that study (Levine *et al.*, 2015), it was also revealed that this chemically modified material did not possess toxicity towards human cells after cross-linking. Hence, crosslinking and epoxidation are linked with each other and considered more suitable and basic methods for the modification of PHAs.

3.4 Grafting

Grafting is a technique in which covalent bond exist between monomers and another polymer chain to obtain desired properties. Polymers properties are broadly different depending on their chain structure. Figure 4 shows general mechanism of grafting. A copolymer is comprised of two different polymer units; in case of graft copolymers one or more side chains are attached to the main chains as its blocks. Grafting is a convenient way to synthesize a copolymer with new properties with minimum loss of original properties of the polymer (Macit *et al.*, 2009). Different types of grafting methods are discussed below.

3.5 Radiation based grafting

Radiation based grafting follow the irradiation of a pure monomer through ionizing radiations or by high energy source. Carboxyl groups of PHAs are reactive sites and readily reacts with the amine groups of polysaccharides such as cellulose and chitosan (Yalpani *et al.*, 1991). As PHB is brittle, crystalline and moderately hydrophobic therefore, it often requires some chemical modifications for certain applications, one of these modifications is its graft copolymerization. In a study, grafting of two selected monomers methacrylate (MeA) and butyle methacrylate (BuM) on PHB was carried out by using radiation based graft copolymerization. This study revealed successful grafting on PHB. The grafted PHB was less crystalline because of the hindrance provided by BuM and MeA. Water uptake was also observed which indicates increased hydrophilicity (Gonzalez *et al.*, 2009)

As PHB is chemically inactive polyester, chitosan (CS) could be grafted onto PHB using gamma irradiation along with different solvents (acetone, ethyl acetate and acetic acid) as a suitable method. NMR analysis showed possible P(3HB-g-CS) structure. Per this study (Torres *et al.*, 2015 ethyl acetate was the most suitable solvent for grafting of CS on PHB while, acetone proved to be unsuitable solvent for this purpose. This study Torres *et al.*, 2015) concluded a direct relationship between crystallinity and grafting. However, radiation-based grafting polymerization reactions are not convenient at large scale, therefore chemically induced reactions are preferred (Allcock *et al.*, 2003) .

When PVA was grafted onto PHB using gamma rays irradiation with different solvents, the grafted PHB showed variation in different properties such as formation of cavities, surface roughness, and formation of waves. N-hexane was more suitable solvent for grafting. This study

concluded that biodegradability of grafted PHB can be enhanced with increased grafted PVA concentration (Torres *et al.*, 2015).

3.6 Free radical based grafting

In radical grafting, a growing polymer chain has an unpaired electron which becomes a radical site to react with the unsaturated part of coming monomer. In this way the unpaired electron gets transferred to the incoming monomer (Allcock *et al.*, 2003)

In chemical reactions, free radicals are generated by the initiators and is transferred to the substrate to react with the monomer resulting in a grafted copolymer. General mechanism for free radical generation by redox process through M^{n+}/H_2O_2, persulphates is presented as follows:

$$Fe^{+2} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\circ \quad (1)$$

$$Fe^{+2} + \text{-}O_3S\text{-}OO\text{-}SO_3 \rightarrow Fe^{3+}SO_4^{2-} + SO_4^{-\circ} \quad (2)$$

Regarding the activity of $SO_4^{-\circ}$, authors have different views, one suggests that the produced $SO_4^{-\circ}$ react with water to give OH° with subsequent production of free radicles on the backbone of polymer. An alternative view suggests that the $SO_4^{-\circ}$ directly reacts with polymer backbone to generate required the radicles as:

$$SO_4^{-\circ} + R_{polymer}\text{-}OH \rightarrow HSO_4 + R_{polymer}r\text{-}O^\circ \quad (3)$$

This radicle containing polymer reacts with incoming monomer to give grafted copolymer (Bhattacharya and Misra, 2004)

S. Nguyen and R.H. Marchessault (2006) successfully synthesized graft copolymer of PHB and poly(methyl methacrylate) (PMMA) using free radical based grafting mechanism. This synthesized copolymer had a comb shape and exhibited huge changes in its glass transition temperature (Tg) from 100°C to 3°C (Nguyen and Marchessault, 2004). Figure 5 shows general mechanism of radiation based grafting of PHB. Overall, free radical grafting is much better understood in fundamental and mechanistic sense, however this method of grafting is not specific.

3.7 Ionic graft polymerization

Ionic grafting is a specific ion based grafting which includes cationic and anionic polymerization reactions. In anionic polymerization, the growing polymer chain possesses a negative or positive

charge. In case of cation based grafting, stable cation could be generated if the initiator has electron donating substituents (EDS). These EDS stabilize the delocalization of positive charge in π-orbitals of a double bond. While, in anion based grafting the initiator should have an electron withdrawing substituent (EDS) in order to generate a stable carbanion, if the resonating and inductive structures were obtained simultaneously, the stability will be significantly increased (Cowie and Arrighi, 2007). Figure 6 shows general mechanism of cationic and ionic polymerization.

3.8 Anionic grafting

Anion graft polymerizations are those in which the growing polymer chain contains a negative charge. Anionic catalyst or initiators are required to start anionic graft polymerization reactions of which the most commonly used initiators includes alkali metal suspensions, Grignard reagents, Zieglar-Natta catalyst, metallocene initiators and organic radical anions. The graft polymerization process follow the typical procedure started from initiation step through dimerization step ending with a propagation step.

Kowalczuk et al (1994) successfully grafted beta-butyrolacton onto poly(methyl-methacrylate) PMMA by through anionic grafting. He observed that PMMA was partially sponified and it contained a carboxylate group. This carboxylate anion made a complex with 18-crown-6-potassium which acted as an initiator for the grafting of beta-butyrolacton onto PMMA. The grafted copolymer was successfully achieved at high yields (Kowalczuk *et al.*, 1994).

Anionic grafting offers some stereo control grafted copolymers; narrow molecular weight distribution and block copolymers are also accessible via “living polymers” (polymers which can propagate and grow to a desired size and their degree of termination or chain transfer is still negligible). Disadvantage of anionic grafting include requirements for low temperature and is only feasible for limited number of polymers (Allcock *et al.*, 2003)

3.9 Cationic grafting

Cation graft polymerization occurs in growing chain containing a positive charge. Cationic initiators or cationic catalyst plays major role in these polymerization reactions. Mostly, strong protonic acids, Lewis acids and their complexes are used for this purpose. The process includes initiation, propagation, chain transfer and an end termination step (Allcock *et al.*, 2003).

Combination of cationic and free radical based grafting was used to synthesize a multi-graft copolymer. In a study, polyhydroxyalkanoate-g-(poly(tetrahydrofuran)-b-poly(methyle methacrylate) (PHA-g-(PTHF-b-PMMA) multi-graft copolymer was achieved, carbonium cation was used for the initiation of cationic polymerization of THF onto PHA (PHA-g-PTHF). This graft copolymer was with hydroxyl end groups because the carbonium cation was generated from PHBV and PHBx. In the presence of cerium salts these hydroxyl end copolymers can start redox polymerization of polymethyle methacrylate (MMA) and PHA-g-(PTHF-b-PMMA) graft-copolymer would be achieved (Macit *et al.*, 2009). Cationic grafting has the main advantage of controlling molecular weight of a grafted copolymer. But this grafting process is limited only to the olefins and is moisture sensitive along with requirement of low temperature requirement to start the reaction (Allcock *et al.*, 2003).

3.10 Enzymatic grafting

Among physical and chemical grafting approaches, enzymatic grafting approach is more promising as it usually less hazardous when compared to the risky chemical approaches (Aljawish *et al.*, 2012). Laccases are blue color copper oxidases, capable to interactions with a wide diversity of appropriate substrates. Their commercial accessibility, wide oxidative abilities and potent abilities for surface amendments make them smart contenders for an extensive series of applications (Arora and Sharma, 2010Riva, 2006). Laccase based grafting of ethyl cellulose onto the surface of PHB was investigated using enzymatic grafting demonstrated that enzymatic grafting is ecofriendly and energy saving process for grafting providing a suitable environment for polymer grafting providing products with multiple functionalities (Iqbal *et al.*, 2014).

3.11 Thiolation

Thiolation is the process of introducing disulfide bond or sulfhydryl group to the substrates that may be a polymer such as PHAs. Jaffamines (polymer) are linear or unbranched chains of hydrocarbons with an ionizable amino group biocompatible and could be easily grafted on to other polymers. Thiolation was used for grafting of jaffamine onto PHOU which was first methylated and subjected for thiolation using acetylehomocysteine (AcHC) and thiolactone. This resulted in the conversion of amino group of jaffamine into thiol which was then grafted onto PHOU by photografting method. The resulted copolymer was amphiphilic in nature and thermosensitive (Azzam *et al.*, 2010; Caldwell *et al.*, 1997; Le Fer *et al.*, 2012).

Ethylene dimethacrylate (EDMA) was also grafted onto the surface of poly(N-acryloxsuccinimede) (PNAS) through thiol-ene-click chemistry. Thiol bearing ethylene glycol (EG) was used as thiol source. The reaction was initiated through UV irradiation and toluene was used as porogenic solvent. After polymerization allyl amine was grafted and an allyl containing, thiolation sensitive monolith poly(NAS-co-EDMA) was obtained. This monolith could be used as stationary phase in hydrophilic interaction chromatography and the thiolated copolymer can be further conjugated with other polymers to get more functional copolymers (Le Fer *et al.*, 2012; Tijunelyte *et al.*, 2012).

3.12 Blending

Blending of polymers gives exciting potentials to obtain economical and biodegradable materials with attractive mechanical properties. Many of studied have been carried out PHAs co-polymers especially blends of PHB with other different polymers. A systematic study was conducted to fabricate blends of PHB for biomedical applications (Yu *et al.*, 2006). The growth of fibroblast mice cells was observed on PHB and poly(hydroxybutyrate-co-polyhydroxyhexanoat) (PHBHHx) films and on their blends, were investigated. This study demonstrated that cellular growth was significantly increased on blends of PHB/PHBHHx than on independent PHB and PHBHHx which indicated better biocompatibility of blends (Yang *et al.*, 2002). A dramatic increase in the tensile strength in the films of blends of PHB/PHBHHx was also observed with the increase of PHBHHx contents (Deng *et al.*, 2002).

In a study blends of PHB/ethylene-propylene rubber (EPR) and PHB/PVAc it was reported that PHB/PVAc blends are compatible while that of PHB/EPR were immiscible, therefore PHB/PVAc blends showed single Tg value and exhibit a depression in their melting point. In case of PHB/EPR, PHB spherulites was observed with no change in their morphology (Kumagai and Doi, 1992; Paglia *et al.*, 1993) . Similar type of miscible and immiscible hybrids of PHB with some dissimilar polymers such as PHB/poly(epichlorohydrin) (PECH), PHB/polyvinylidene fluoride(PVDF), PHB/PEG, PHB/PMMA were also reported (Abbate *et al.*, 1991; Chiu *et al.*, 2001; Goh, 2014; Hay and Sharma, 2000; Kumagai and Doi, 1993; Paglia *et al.*, 1993; Sadocco *et al.*, 1993).

3.13 Esterification of PHAs

3.13.1 Triblock co polymers

Amphipathic block copolymers (ABCs) signify a unique group of efficient polymers that are exclusive building blocks aiding a number of applications principally linked with energetic and structural controller of materials interfaces (Forster and Antonietti, 1998). Two chains of methoxy-PEO- hydroxylated PHB (PHB-OH) sequence by using 1,3-N,N′-dicyclohexylcarbodiimid (NNDCHC) were linked together to make an amphipathic triblock copolymer (ATBC). PHB-OH was achieved by the reaction PHB with diethylene glycol (DEG) (transesterification reaction) using dibutyltin dilaurate as a catalyst (Andrade *et al.*, 2002). Thermogravimetric analysis indicated that the degradation of ATBC occurred in two distinct phases for PHB and PEO blocks. This analysis also revealed that the ATBC have enhanced thermal stability compared with the originators (Li *et al.*, 2003). A blend of epoxy resin (ER) (bisphenol type) and TBC PEO-PPO-PEO in ester linkage with ethylene oxide (EO) was synthesized properties of blend varied with the increasing EO concentration (Guo *et al.*, 2002).

Ring opening copolymerization of β-butyrolactone produced amorphous PHB-PEG-PHB ATBC. These ATBC nanoparticles (NPs) could be used for hydrophobic drug delivery. Release of pyrene was observed and it showed that release of pyrene from this ATBC showed second order kinetics behavior (Chen *et al.*, 2006).

3.13.2 Diblock copolymers

Non-cyclic polyesters with monomethoxy polyethyleneoxide (mPEO) have potential applications in drug release systems Polylactate (PLA) polymers are known as biodegradable and biocompatible polymers. When joined with mPEO they become potential supplements in drug delivery systems (Zhu *et al.*, 1989). A diblock copolymer PHB-b-mPEO was successfully synthesized and showed capability to self-assemble into nanoparticles and could be used in drug delivery systems and as carriers. Such type of carriers could have a longer lifetime in blood-streams (Zhu *et al.*, 2004).

A Diblock copolymer PHB-b-mPEG could be fabricated via catalytically transesterification in melt in one step procedure. Depolymerization of PHB can be carried out by two subsequent steps, pyrolysis and transesterification. Such Diblock copolymers were amphiphilic in nature and form colloidal of PHB crystalline (Ravenelle and Marchessault, 2002).

3.14 Functionalization of PHAs by thermal degradation

Thermal degradation of polymers is the deterioration of polymer because of overheating. At high temperature, the degradation of PHAs resulted in monomers, dimers and trimers which are volatile and these monomers readily reacts with one another or other polymers to change the properties of these polymers. This elimination mechanism was analyzed and found mainly dependent on thermal conditions and the oligomers obtained at low temperature, contained an unsaturated group and a carboxylic group linked through an ester linkage, this unsaturated group enhance the degradation of nearby ester linkages. In case of PHB thermal degradation, auto acceleration and auto catalytic effects were observed and followed random scission. PHB degradation at high temperature produced crotonic acid and butyrolactone (BL) (Kopinke *et al.*, 1996; Lehrle and Williams, 1994 Nguyen and Marchessault, 2004; Stromberg *et al.*, 195). At higher temperature, complete degradation of PHB resulted in the production of gases (CO_2 and CO) along with ketene and ethanol. In one previous concept, it was considered that unsaturated crotonate group of PHAs could not be subjected for free radical polymerization reaction. Nguyen and Marchessault (2004), suggested that PHB with carboxylic acid terminal group can be further subjected for modification, graft polymerization through free radical mechanism in acrylic backbone and PHB side chain copolymers. Poly methyl methacrylate (PMMA) grafted PHB was obtained by free radical grafting method, but it was a copolymer with undetermined molecular weight and high polydispersity; it could be controlled by using atom transfer radical polymerization method (Nguyen and Marchessault, 2005; Nguyen and Marchessault, 2006; Nguyen, 2008).

3.15 Regeneration of PHAs

Many cyclic compounds and derivatives of olefins resist polymerization reactions, analysis of this unpolymerization behavior lead to study of polymerization-depolymerization equilibrium studies (Allcock *et al.*, 2003). This behavior of materials lead to the idea of generation of molecular weight controlled polymers under specific conditions. As most of the PHAs are high molecular weight polymers Ryan and McCann (1996) were first to demonstrate that poly(butyl cyanoacrylate) (PBCA) could undergo depolymerization-repolymerization reactions in the presence of a base (THF) and gave monomer of butyl cyanoacrylate (BCA). On addition of tetrabutyle ammonium hydroxide, BCA first polymerize to a high molecular weight monomer

was followed by the depolymerization and formation of TBAOH which initiated low molecular weight daughter polymer formation (Ryan and McCann, 1996). Later on, it was demonstrated that base is not so much important for this depolymerization-repolymerization reaction and polymer degradation was observed at the solid state of polymer (Robello *et al.*, 1999). Moon et al., studied the degradation behavior of poly(ethyl-cyanoacrylate) (PECA) in basic solution. They concluded that this degradation behavior is because of an open depolymerization progression originated from chain terminus of the polymer. They also showed that polymer deprivation fashion can be controlled by change in reaction conditions (Han *et al.*, 2008). Hence, depolymerization-repolymerization reactions allows the syntheses of polymers or copolymers of desired molecular weight.

3.16 Metallization of PHAs

A polymer matrix with metal nanoparticles (MNPs) provides possibilities of fabricating useful materials for generating catalyst, sensors and electrical materials. It is demonstrated that during melt processing of blended/hybrid polymers MNPs could be used as an effective catalyst. In previous studies, it was also demonstrated that effect of MNPs vary from polymer to polymer. Such as increase or decrease in thermal stability of various polymers with MNPs. In a study (Lee *et al.*, 2006), palladium (Pd) NPs were introduced into different polymer matrix and thermal degradation of these polymers was investigated. This study concluded that thermal degradation of polystyrene (PS), polypropylene (PP) and methyle methacrylate (MMA) was decreased by Pd NPs, while thermal degradation of polyamide (PA) and poly(ethylene-terephthalate) (PET) was enhanced. In another study the effect of silver sulfide on thermal degradation of PHB was studied and this study concluded that PHB is degraded at lower temperature in the presence of silver sulfide (Yeo *et al.*, 2010). Similar type of effect of silver nanoparticles in starch/Ag nanocomposite was also reported (Božanić *et al.*, 2007).

Cadmium sulfide (CdS) and zinc sulfide (ZnS) are well-known, wide band gap compounds of group II-VI. Their nanoparticles (NPs) have been reported as effective materials for various applications than that of bulk CdS and ZnS. These applications include catalytic effect, electronic and optical effect (Cabot *et al.*, 2008; Pattabi and Uchil, 2000; Parvaneh *et al.*, 2015). In addition CdS NPs were successfully synthesized through biological rout and were encapsulated with PHB NPs and were proven for efficient immobilization. These encapsulated

CdS NPs were proven to be less toxic and could be efficiently used for various biomedical applications such as in treatment of cancer therapies, imaging and other related biological disorders (Pandian *et al.*, 2011). In another study, Zinc oxide reinforced polyhydroxybutyrate-co-valerate (PHBV) ZnO-PHBV nanocomposite were successfully fabricated and SEM images confirmed the even distribution ZnO NPs PHBV matrix. The ZnO NPs had increase thermal stability, crystallinity, stiffness and strength and the synthesized nanocomposite could be used in food packaging material (Diez-Pascual and Diez-Vicente). In short, metallization is the most effective way to modify polymers to obtain more functionalized PHAs.

Future perspectives

Chemical modification of PHAs explored functional PHAs which are suitable for a wide range of applications. Through depolymerization-repolymerization of PHAs molecular weights could be controlled while hydrophobicity could be reduced by carboxylation reactions of unsaturated PHAs. Among PHAs, PHBs are well-known to possess some objectionable properties such as brittleness and crystallinity which can be reduced by blocking or grafting reaction of PHB with other PHAs. Graft/block copolymerization is useful in controlled synthesis of polymer for desired and specific properties. PHAs are auspicious alternator to petroleum-based plastics, but they also have some drawbacks. The major disadvantage of PHAs is their high production cost and low yield of polymer. The purification methods of produced PHAs are also based on high cost purification techniques. Another complication is their proper disposal procedures which needs to be better managed and more cost effective and environmentally sound.

Bacterial PHAs possess a variety of beneficial physical properties making them useful potential materials for various applications. However, lack of functional groups makes them inconvenient for various fields. Alteration methods have been subject for research and discussed during the past few years mainly aiming to reduce the undesired properties, such as crystallinity and mechanical properties which can be altered during their biosynthesis or later by physical or chemical alteration methods. It is expected that more PHAs will be synthesized in the future, It is now possible to yield considerable amount of numerous PHAs, which will allow them to be tried for various applications and levels. The clarification of the PHA synthase mechanism will permit us to get more control over the design and synthesis of novel PHA in future which will aid in their successful use by many industries.

Concluding remarks

PHAs alteration is imperative in the different research zones for fabrication of novel polymer composites with diverse properties, such as thermal and mechanical flexibility of the resulting composite materials. Alteration of PHAs through chemical methods is an encouraging methodology to achieve different kinds of composites of PHAs, comprising of an inclusive variety of monomer units for graft/block copolymerization with man-made and further regular polymers that could not be achieved by biological method.

Conflict of interest disclosure

All the authors disclose no any potential sources of conflict of interest.

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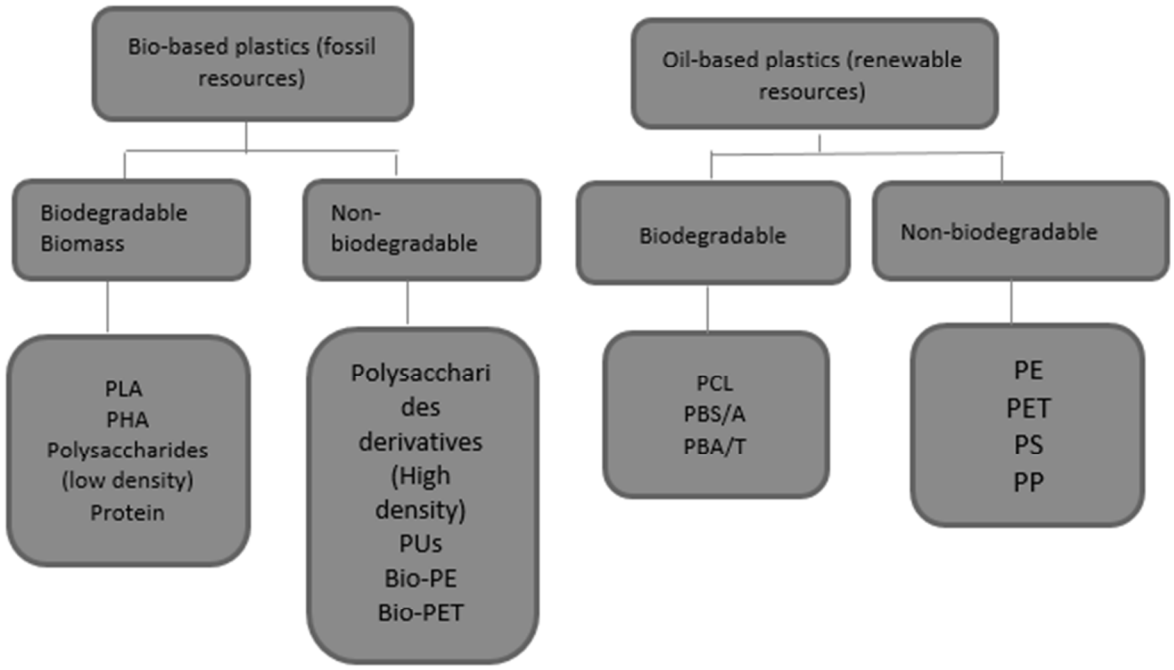

Figure 1. Classification of Plastics (Adapted from Iwata, 2015)

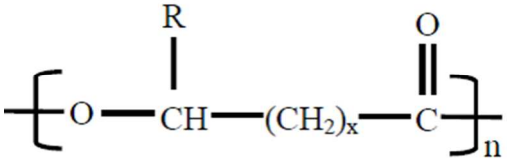

Figure 2. Chemical structure of PHAs. PHAs are generally composed of (R)-β-hydroxy fatty acids, where the pendant group (R) varies from methyl (C1) to tridecyl (C13) (Adapted from Madison and Huisman, 1999

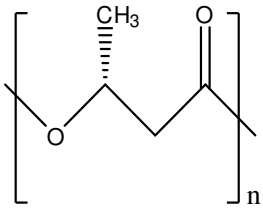

Figure 3. Structure of PHB

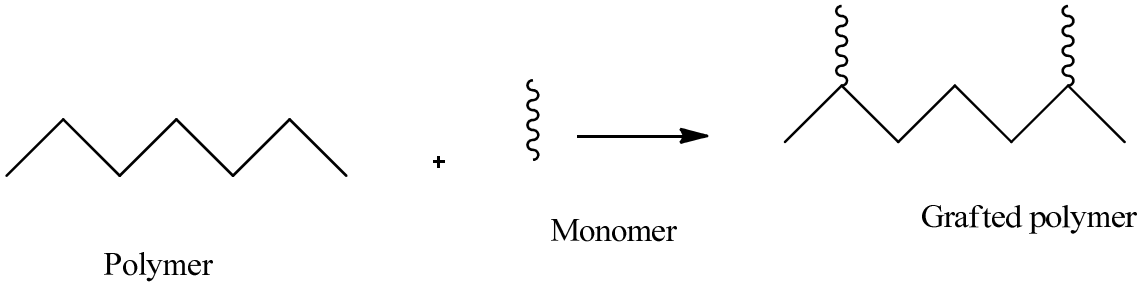

Figure 4. General mechanism of Grafting (Adapted from Bhattacharya and Misra, 2004)

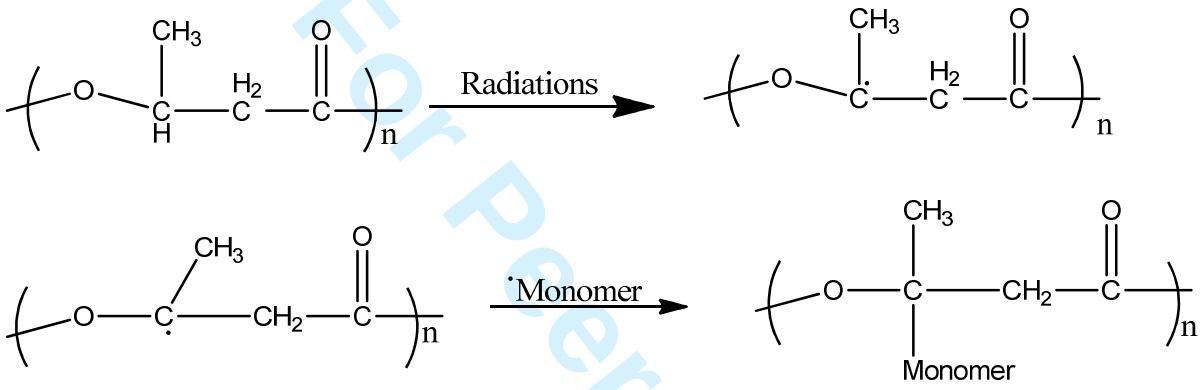

Figure 5. General mechanism of radiation based grafting of PHB (Grafting is also possible at C1 and C3) (Adopted fromTorres *et al.*, 2012)

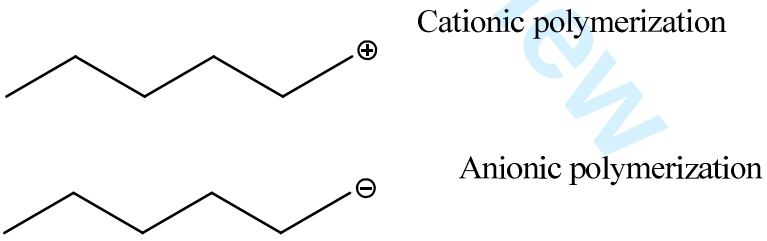

Figure 6. Cationic and anionic polymerization (Allcock et al., 2003)

Table 1. PHAs Chemical modification methods and properties achieved against each

Sr.No	Modification method	PHA/PHB based Substrate	Functional group	Product	Achieved properties	Reference
1	Epoxidation	Unsaturate-d linseed oil based PHA (PHA-L)	Epoxide group (E)	PHA-LE	Increase in tensile strength and Young's modulus	(Ashby *et al.*, 2000)
2	Epoxidation/Crosslinking	PHOU	Anhydride of succinic acid (ASA)	PHOU-ASA	Increase in sol-gel contents and glass transition temperature	(Arkin *et al.*, 2000).
3	Epoxidation/crosslinking	PHO-co-UD	peroxides	PHO-co-UD/Peroxides	Decrease in tensile strength and tear resistance	(Gagnon *et al.*, 1994)
4	Crosslinking	PHA-g-MA	TPF	PHA-g-MA/t-TPF	Increase in water resistance and biodegradability, lower viscosity	(Wu, 2013)
5	Crosslinking	PHB/PHBU	Thiolene	PHB/PHBU	Decreased toxicity and increased biocompatibility	(Levine *et al.*, 2015)
6	Carboxylation	PHO-co-PHU	OsO_4 and oxone	Carboxylated PHOU	Decrease in hydrophobicity	(Stigers and Tew, 2003)
7	Carboxylation	PHOU	$KMnO_4$	Carboxylated PHOU	Decrease in hydrophobicity, low molecular weight polymer	(Lee and Park, 2000)
8	Chlorination	PHA	Chlorine gas	PHA-Cl	Increase in crystallinity, brittleness and hardness	(Arkin *et al.*, 2000)

9	Chlorination	PHO	Chlorine gas	PHO-Cl	Increase in glass transition and melting temperature	(Arkin and Hazer, 2002)
10	Chlorination	PHB	Chlorine gas	PHB-Cl	Decrease in melting temperature and increase in glass transition temperature	(Arkin and Hazer, 2002)
11	Radiation based grafting	PHB	MA and BuMA	PHB-g-MA and PHB-g-BuMA	Increase in hydrophilicity and decrease in crystallinity	(Gonzalez *et al.*, 2009)
12	Radiation based grafting	PHB	PVA	PHB-g-PVA	Increase in biodegradability	(Torres *et al.*, 2015)
	Radiation based grafting	PHB	Chitosan (CS)	PHB-g-CS	Increase in crystallinity	Torres *et al.*, 2015
13	Free radical based grafting	PHB	PMMA	PHB-g-PMMA	Decrease in glass tranition temperature	(Nguyen and Marchessault, 2004)
14	Anionic grafting	PMMA	β-Butyrolacton (BL)	PMMA-g-BL	Saponification	(Kowalczuk *et al.*, 1994).
15	Cationic grafting	PHA	THF and PMMA	(PHA-g-(PTHF-b-PMMA)	Multi-graft copolymer and controlled molecular weight copolymer	(Macit *et al.*, 2009)
16	Enzymatic grafting	PHB	Ethylcellulose (EC) laccase enzyme	PHB-g-EC	Increase in mechanical properties and decrease in crystallinity	(Iqbal *et al.*, 2014)
17	Thiolation	PHOU	Jaffamine	PHOU-g-thiolated	Amphiphilic and thermosensitive copolymer	Le Fer *et al.*, 2012)

				jaffamine		
18	Thiolation	PNAS	EDMA and thiolated EG	poly(NAS-co-EDMA)	Increase in hydrophilicity	(Tijunelyte *et al.*, 2012)
19	Blending	PHB	PHBHHx	PHB/PHBHHx films	Increased biocompatibility and tensile strength	(Deng *et al.*, 2002)
20	Blending	PHB	EPR	PHB/EPR blend	Decrease in melting point	(Goh, 2014)
21	Esterification	PHB-OH	mPEO	ATBC	Increased thermal stability	(Li *et al.*, 2003)
22	Esterification	PHB	mPEO	PHB-b-mPEO	Increased life time	(Zhu *et al.*, 2004)
23	Esterification	PHB	mPEG	PHB-b-mPEG	Amphiphilic block co polymer	(Ravenelle and Marchessault, 2002)
24	Thermal degradation	PHB	-	Crotonic acid and butyrolactone	Molecular weight controlled polymer/monomer	(Kopinke *et al.*, 1996)
25	Regeneration	PBCA	THF (as base)	BCA	Molecular weight controlled polymer/monomer	Ryan and McCann, 1996)
26	Regeneration	PECA	THF	ECA	Molecular weight controlled polymer/monomer	(Han *et al.*, 2008)
27	Metallization	PS	Pd	PS-Pd	Thermal degradation decreased	(Lee *et al.*, 2006)
28	Metallization	PP	Pd	PP-Pd	Thermal degradation decreased	(Lee *et al.*, 2006

29	Metallization	MMA	Pd	MMA-Pd	Thermal degradation decreased	(Lee *et al.*, 2006
30	Metallization	PET	Pd	PET-Pd	Thermal degradation increased	(Lee *et al.*, 2006
31	Metallization	PA	Pd	PA-Pd	Thermal degradation increased	(Lee *et al.*, 2006