# *Chapter I*

# *Introduction and literature survey*

# Chapter I

## Introduction and literature survey

Polymers are widely used in the manufacturing of products such as bottles, bags, toothbrushes, tires and supports for electronic components. Most polymers are extremely durable, requiring very long times for their degradation. This durability possesses a serious environmental problem because of the large amount of waste produced, especially in urban centers.

Industrial polymers are mainly petrochemical based materials. The problem of environmental pollution caused by indiscriminate dumping of plastic waste has assumed global proportions. Due to the synthetic and biologically inert nature of petroleum-derived plastics, the disposal of these solid wastes has been a focus of concern for waste management[1]. There are tow approaches that can be used for keeping the environment safe from these plastic wastes, the first one is the storage of wastes at landfill sites. But because of the very fast development of society, satisfactory landfill sites are also limited. On the other hand, burial of plastics wastes in landfill is a time bomb, with today's problem being shifted onto the shoulders of future generations. Another approach is the utilization, which can be divided into two steps, incineration and recycling. Incineration of plastic wastes always produces a big amount of carbon dioxide and creates global warming, and some times produces toxic gases, which again contribute to global pollution. On other hand recycling somehow solve the problem but its application is limited owing to a number of factors, recycling is expensive and synthetic waste is

difficult to sort according to origin, color and contained additives. A serious limitation of the range of possible applications of the recycled polymers is the "thermal memory" of the thermoplastic materials, nevertheless for many polymers, recycling is a useful method of recovering them from waste and it continues to be an object of interest for many scientists and practicians alike[2]. It is not always possible to recover all the used plastics. In addition, it is to be noted that recycling processes of waste plastics, whether it is material recycling or chemical recycling consume a considerable amount of energy, and that we cannot recycle plastics forever.

Based on these backgrounds, there is an urgent need for the development of green polymeric material that would not involve the use of toxic component in their manufacture and could be degraded in the natural environment. For these reason the development of biodegradable materials with controlled properties has been a subject of research challenge to the community of materials scientists and engineers. Biodegradable polymers are defined as those that undergo microbial induced chain scission leading to mineralization under specific conditions in terms of pH, humidity, oxygenation and the presence of some metals to ensure biodegradation of such polymers.

The so called biodegradable polymers can, therefore, be classified into natural polymers, chemically modified natural polymers, synthetic polymers composed from natural building blocks, and synthetic polymers from petrochemical building blocks.

Among the natural biodegradable polymers, the biologically produced polyesters, in particular poly3-hydroxybutyrate (PHB), or in general polyhydroxyalkanoates (PHA) have been drawing much attention as environmentally biodegradable and biocompatible polymers. PHAs are composed of 3-hydroxy fatty acid monomers, which form linear, head-to-tail polyester Fig. 1 PHA is typically produced as a polymer of around 100

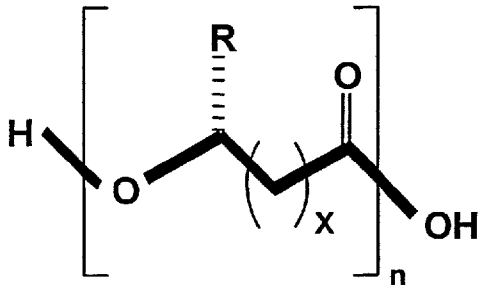



Figure 1: Chemical structure of PHA

R = Hydrocarbon (Up to C13) x = 1 to 3 or more

repeating units, which accumulate as inclusions of 0.2–0.5 μm in diameter. These inclusions or granules are synthesized and stored by both gram-positive and gram negative bacteria without hazardous effects to the hosts[3]. PHA accumulation occurs when the cells experience a nutrient imbalance such as excess carbon with limited nitrogen, phosphorus or oxygen[4-6]. The bacteria store the excess nutrients intracellularly by forming insoluble biopolymers from soluble molecules. The biopolymers become mobilized when conditions for normal growth return. The structure, physio-chemical properties, monomer composition and the number and size of the granules vary depending on the organism[4, 7]. Of all the characterized PHAs, alkyl groups, which occupy the R configuration at the C-3, vary from one carbon (C1) to over 14 carbons (C14) in length. PHAs can be subdivided into three broad classes according to the size of comprising monomers. PHAs containing up to C5 monomers are classified as short chain length PHAs (scl-PHA). PHAs with C6–C14 and above C14 monomers are classified as medium chain length (mcl-PHA) and long chain length (lcl-PHA) PHAs, respectively[8]. Scl-PHAs have properties close to conventional plastics while the mcl-PHAs are regarded as elastomers and rubbers. There are also reports on functional modifications of the monomers to improve the properties of the resulting bioplastic, such as the

introduction of unsaturated and halogenated branched chains. As well, heteropolymers can be formed by polymerization between more than one kinds of monomer. PHB is the most common type of scl-PHA and this homopolymer of 3-hydroxybutyric acid has been studied most extensively. Copolymers of PHA can be formed containing 3-hydroxybutyrate (HB), 3-hydroxyvalerate (HV), 3-hydroxyhexanoate (HH) or 4-hydroxybutyrate (4HB) monomers. Most of the microbes synthesize either scl-PHAs containing primarily 3HB units or mcl-PHAs containing 3-hydroxyoctanoate (HO) and 3-hydroxydecanoate (HD) as the major monomers[4, 5, 9]. Bacteria synthesize a wide range of PHAs and approximately 150 different constituents of PHAs have been identified[10]. PHAs extracted from bacterial cells have properties similar to conventional plastics, such as polypropylene[11]. PHAs can be degraded at a high rate (3– 9 months) by many microorganisms into carbon dioxide and water using their own secreted PHA depolymerases[12]. The PHAs can be produced from renewable resources, they are recyclable, and are considered natural materials. These properties make PHAs appropriate as suitable substituents to petrochemical thermoplastics[13]. The large diversity of monomers found in PHAs provides a wide spectrum of polymers with varying physical properties

## 1.1. Poly (R-3-hydroxybutyrate) homopolymer (PHB)

PHB is the first member of the PHAs to be discovered and is the most widespread in nature produced by many microorganisms[4, 14-16].The chemical structure of poly(3-hydroxybutyrate) is shown in Fig.2

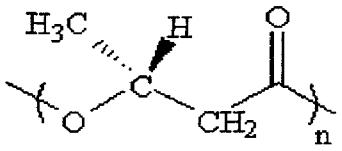



Figure 2: Chemical structure of PHB

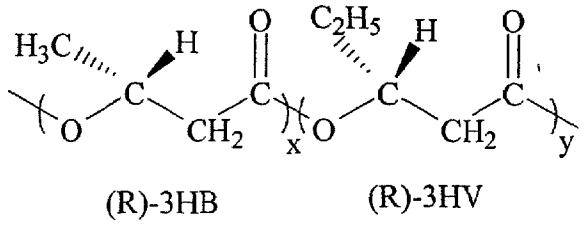
PHB has a perfectly isotactic structure with only the (R)-configuration, thus due to its absolute stereoregularity, isolated bacterial PHB crystallized readily from melt and achieves a high degree of crystallinity, typically about 70%. The glass transition temperature ($T_g$) is around 4°C, while the melting temperature is near 180°C, as measured by calorimetric analysis. The densities of crystalline and amorphous PHB are 1.26 and 1.18 g/cm$^3$, respectively. Mechanical properties like the Young s modulus (93.5 GPa) and the tensile strength (43 MPa) of PHB are close to those of isotactic polypropylene. The extension to break (5%) for PHB, however, is markedly lower than that of polypropylene (400%). Therefore, PHB appears as a stiffer and more brittle plastic material when compared with polypropylene. It is now well known that PHB suffers from several disadvantages that limit its application possibilities. Firstly, PHB homopolymer crystallizes slowly when cooled from the melt, and shows embrittlement caused by an aging process[15, 16]. Secondly, the melt of PHB is unstable and degrades at temperatures just above its melting point[17]. Much work has been carried out to understand further the reasons behind the brittle nature of PHB, and to improve the polymer's physical

properties. When spherulites of PHB homopolymer are grown from the melt, large-scale cracks are often visible in the spherulites. Barham and co workers[18-20] suggested that the formation of such cracks in PHB spherulites is one of the reasons that give to embrittlement. De Koning and Lemstra[16] reported that the embrittlement of PHB materials occurs during and after their initial crystallization from the melt. The secondary crystallization has been argued to result in the reorganization of lamellar crystals formed during the initial crystallization process, which tightly constrains the amorphous chains between crystals. They demonstrated that PHB materials could be toughened using an annealing treatment after initial crystallization[21].

## 1.2. Copolymers of PHB with hydroxyalkanoates.

The physical properties of PHB can be improved by incorporation of other hydroxyalkanoate (HA) units to form PHA copolymers. Random copolymers containing (R)-3HB as a constituent along other HA units of chain lengths ranging from 3-14 carbon atoms have been produced from various carbon substrates by a variety of bacteria.

Poly(R-3-hydroxybutyrate-co-R-3-hydroxyvalerate), PHBV, is now produced commercially by a fermentation process using glucose and propionic acid as a carbon sources for *Alcaligenes eutrophus* and is marketed under the trade name "Biopol". PHBV is a flexible and tough thermoplastic, while PHB is a rigid and brittle one. The chemical structure of PHBV is shown in Fig. 3.




(R)-3HB (R)-3HV

**Figure 3**: Chemical structure of P(HB-HV) )

PHBV is a statistically random and highly crystalline biodegradable copolyester[22, 23]. In general, co-monomer composition as well as the chemical structure of crystalline copolymer affects the degree of crystallinity and other properties, such as thermal behavior, both the mechanical strength, biodegradability and so on. In almost all crystalline copolymers, the minor comonomer unit would interrupt the crystallization behavior of major comonomer component and the former is excluded from the crystalline region, resulting in the decrease of the degree of crystallinity[24]. In case of bacterial PHBV, it is known that the minor comonomer component is included as the crystal constituent in the crystal lattice of the major comonomer unit[24-27]. This phenomenon is called cocrystallization. It has been reported that 3HB-rich and 3HV-rich PHBV copolymers form PHB and PHV homopolymer type crystalline lattice, respectively[23-28].The coexistence of both PHB and PHV homopolymer–type crystalline lattice has been observed by X-ray diffraction for PHBV samples in a relative broad comonomer composition range between ~36-56 mol % HV[29]. The mechanical properties of PHBV copolymers have been reported by Mitomo et. al[30] over a wide temperature range from –150 to 190°C. They showed that the modulus decreases as the 3HV content increases. The tensile strength (44-250 MPa) and strain (2-700%) increase with increasing 3HV content up to 28 mol % 3HV. Because of cocrystallization, PHBV maintains high degree of crystallinity of more than 50%, over the whole range of comonomer composition.Young's modulus is high at lower temperatures and decreases with increase in temperature.

The thermal degradation of PHBV copolymer has been studied by Kunioka et al[31] in the temperature range 100-200°C. The polyesters are thermally unstable above 170°C and their molecular weights decrease rapidly with time. The changes in molecular weight

follow the kinetic model of random chain scission at the ester groups. Koyama and Doi[32] prepared melt-crystallized films of various PHBV copolymers and studied the rates of enzymatic degradation using PHB depolymerase from *A. faecalis*. The erosion rates of PHBV films were several times higher than the rates of PHB homopolymer films with the same degree of crystallinity. Therefore, the significant difference in the erosion rates for melt-crystallized films of PHB and PHBV copolymers could not be explained only in terms of the degree of crystallinity and the average size of the crystal structures.

Soil degradation of PHBV films in microbial active soil composed of topsoil, sand, and decomposed manure or leaf, composted for 3 months to develop a natural microbial population at 30°C and 95 % relative humidity, pH 6.5-7.5, showed that solvent cast films containing 24 mol % 3HV lost about 35 % of its original weight. Meanwhile, extruded films containing 8 mol % 3HV lost 50 %[33]. PHB and PHBV samples incubated in soil at 15, 28, or 40°C are degraded by a wide range of microorganisms such as gram-negative bacteria, gram-positive bacteria, streptomycetes, and molds. Microbial degradation is accelerated at higher temperatures (40-55°C). Copolymer degradation is faster than the homopolymer and the degradation rates are dependent on the type of the soil used[34].

Rosa et al.[35] investigated the biodegradation of PHB, PHBV and poly(ε-caprolactone) PCL, in compost derived from municipal solid waste. The results showed that PHB degraded faster than the other two polymers, probably because the chemical structure of this polymer made the attack by microorganisms easier. The biodegradation of PHB occurred by three regimes that differ in their speed. The first regime involves hydrolysis of the polymer, with attack by water molecules and consequent polymer scission, and the formation of polymer fragments. The second regime involves action by

microorganisms, with enzymatic attack on the fragments generated in the first regime, while the third regime was characterized by stabilization of the values, indicating total biodegradation. The low biodegradation of PCL was attributable primarily to the structure formed by the $CH_2$ groups, which have a large bonding force, and to the absence of tertiary carbons.

Akmal et al.[36] investigated the biodegradation of PHB and its copolymer PHBV by using soil burial test and immersion test method at various places under the tropical environment in sea water. Their results showed that PHB biodegraded at a rate of 3.6% per week in activated sludge, 1.9% per week in soil, 1.5% per week in lake water and 0.8% per week in sea water. The degradation rates for PHBV were 17.8% per week in activated sludge, 6.7% per week in soil, and 3.2% per week in lake water and 2.7% per week in sea water. The biodegradation of both polymers were highest after burial into activated sludge with a half-life time (T½) of 14 weeks and the time for 100% degradation (T~100%) was 28 weeks for PHB, and a T½ of 3 weeks and (T~100%) was 6 weeks for PHBV.

A series of microbial copolyesters of R-3-hydroxybutyrate and R-4-hydroxybutyrate that show promising biodegradable properties[37] has been obtained from *A. eutrophus* grown in nitrogen-free culture media containing 4-hydroxybutyric acid and 3-hydroxybutyric acid[38]. The chemical structure of P (3HB-4HB) is represented in Fig. 4.

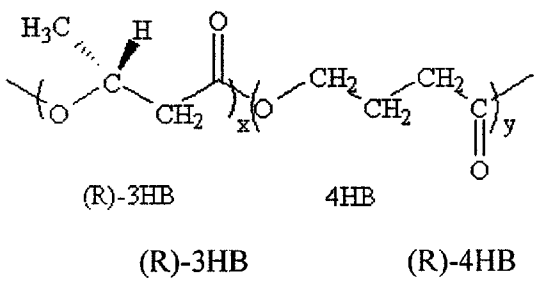



**Figure 4**: Chemical structure of P(3HB-4HB)

It has been reported that these bacterially copolyesters have some broad comonomer compositional distribution and have statistically random distribution of co-monomer units. Glass transition temperature decreases from 5 to –50°C as the mol % of 4HB increased from 0 to 100% and melting temperatures decreases from 180 to 54°C. During thermal degradation, at around 180°C, no appreciable weight loss takes place, and it follows the random scission at the ester groups. Crystallinity decreases from 59±5 to 42±5% with the increase in 4HB content up to 100 mol %. Only one crystalline form of the PHB lattice was observed for the X-ray diffraction patterns of P(3HB-4HB) copolymers with compositions of 0-29 mol % 4HB. In contrast, only the P(4HB) crystalline lattice was observed for P(3HB-4HB) copolymers with compositions of 78-100 mol % 4HB. The rates of crystal growth of PHB decreased with increasing the 4HB content, suggesting that the 4HB units are excluded from PHB crystalline phase[39, 40]. P(3HB-4HB) can not form isomorphic crystals because of the extent of the structural difference between 3HB and 4HB comonomer components[41]. Young's modulus, tensile strength, and % elongation at break for P(3HP-4HB), containing 94 mol % of 4HB are reported as 55 MPa, 39 MPa and 500%, respectively[42]. Enzymatic degradation studies of P(3HB-4HB) copolymer films in phosphate buffer of extracellular depolymerase from *A. faecalis* show an increase in weight loss as a function of exposure time. The rate of enzymatic degradation markedly increases with an increase in 4HB content, and the highest rate is for 28 mol % of 4HB content. For higher concentrations of 4HB (above 85 mol %), the rate of degradation is much slower than that of PHB[42]. Poly(3-hydroxybutyrate-co-3-hydroxypropionate), P(3HB-3HP), can be grown with *A. eutrophus* in nitrogen-free culture solution containing 3-hydroxypropionic acid, 1,5-

pentanediol, or 1,7-heptanediol[43]. The chemical structure of P(3HB-3HP) is given in Fig.5.

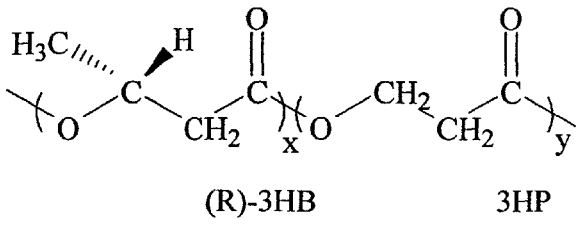



**Figure 5:** Chemical structure of P(3HB-3HP)

For solution cast films of P(3HB-3HP) copolymer, the melting temperature decreased from 180 to 44°C with increasing 3HP content and then increased to 77°C as the 3HP was increased from 67 to 100 mol % The glass transition temperature decreased from 4 to –19°C, as the 3HP fraction increased from 0 to 100 % mol[43]. Crystal structures and the degree of crystallinity for P(3HB-3HP) copolymers have been investigated from the X-ray diffractions of solution cast films having a wide range of compositions of 0-100 mol % 3HB[43] Only one crystalline form for the PHB lattice was observed for copolymers with compositions up to 43 mol% 3HP. The crystallographic parameters of the copolyesters with compositions up to 43 mol % 3HP were little influenced by the presence of the 3HP units, and the X-ray crystallinity decreased from 60 to 7 % as the 3HP fraction was increased from 0 to 67 mol %, suggesting that the 3HP units are excluded from the PHB crystalline phase. Cocrystallization of 3HB and 3HP units in the same crystal lattice does not occur in the P(3HB-3HP) copolymer[44].

Poly((R)-3-hydroxybutyrate-co-3-hydroxyhexanoate), P(3HB-3HH), is bacterially synthesized by *Ralstonia euteropha* from cheap renewable resources, such as coconut oil. P(3HB-3HH) has fascinating properties for practical use.

Its $T_g$ reduces from 4°C to – 4°C with increasing 3HH content up to 17 mol %. Tm also reduces from ~180 to 130°C. The percentage crystallinity also reduces from 59±5 to 29±5 % indicating that 3-hydroxyalkanoate units (3HH) cannot crystallize in the sequence of 3HB units, and act as defects in PHB lattice. The rates of crystal growth of P(3HB-3HH) were markedly reduced with an increase in 3HH fraction, indicating that randomly distributed 3HH units in P(3HB-3HH) lead to a remarkable decrease in the rate of deposition of the 3HB segments at the growing front of the PHB crystalline lamellae[45]. The tensile strength of the films decreased from 43 to 20 MPa as the 3HH fraction was increased from 0 to 17 mol %. In contrast, elongation at break point increased from 5 to 850 %. Thus the P(3HB-3HH) films become soft and flexible with an increase in 3HH fraction[46]. The chemical structure of P(3HB-3HH) is given in Fig.6.

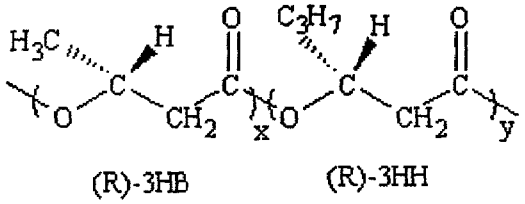



**Figure 6:** Chemical structure of P(3HB-3HH)

Enzymatic degradation studies of P(3HB-3HH) copolymer of the solvent cast films in phosphate buffer of extracellular depolymerase from *A. faecalis* show that the rate of degradation increases with an increase in 3HH content. The degradation occurs on the surface of the film and the rate of degradation of P(3HB-3HH) containing 17 mol % 3HH is 14 times higher than that of PHB film. This rapid erosion of copolymer film may be due to a decrease in the crystallinity[47, 49].

Wang et al.[49] investigated the biodegradability of novel thermoplastics poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) P(3HB-3HH) by subjecting films made of P(3HB-3HH) to degradation in activated sludge and compared with poly(3-hydroxybutyrate) (PHB) and Ecoflex biodegradable plastics were used for film preparations (BASF, Germany). After 18 days degradation, 40% of P(HB-co-12%-HH) and 20% of PHB were degraded, while Ecoflex only lost 5% of its weight. Also it was found that P(HB-co-12%-HH) was degraded faster as compared with PHB, P(HB-co-5%HH) and P(HB-co-20%-HH). Scanning electron microscopy (SEM) results revealed that P(HB-co-12%-HH) films had the most porous surface after degradation. All these indicate that surface morphology played an important role in degradation of P(3HB-3HH).. Combining the advantage of low crystallinity and rough surface of P(HB-co-12%-HH) leads to the fastest degradation.

### 1.3. PHB and PHBV-based polymer blends

Polymer blending is a well-used technique whenever modification of properties is required, because it uses conventional technology at low cost. Some of the drawbacks of PHB are overcome by blending PHB with polymers having very different molecular structures and characteristics, such as crystallinity, glass transition and melting temperatures. The microstructure of the blends, resulting from thermodynamic and kinetic factors, is regarded as an important factor in controlling the mechanical and the biodegradation behaviors. Moreover, some considerations upon the nature of the "driving force" of the miscibility have been made in order to explain miscibility behavior[50]. Polymer blends are physical mixtures of structurally different polymers. The mixture of two polymers forms either homogeneous or heterogeneous phases in the amorphous region on a microscopic scale.

Compared to copolymerization blending of PHB with other polymers may be more economical and more feasible to modify the physical properties, to improve the processabilities and to lower the cost. The physical properties of a mixture are strongly dependent on the phase structures. Blending of PHB may results in a decrease of the melting temperature that imply the possibility to process the materials at lower temperature, avoiding or limiting the degradation.

The low impact resistance of the PHB is due both to its relatively high glass transition temperature and its characteristic to form very large spherulities. Poly(3-hydroxybutyrate-hydroxyvalerate) copolymers partly fill the gap of toughness, however, they exhibit, lower melting points, with respect to PHB, narrowing the utilization temperature range[50]. Therefore, the miscibility of PHB–based polymer blends has been investigated extensively[19, 51-58]. Blending of PHB with its own PHBV copolymers retains the advantages of biodegradability and biocompatibility while offering potential improvement in mechanical properties and crystallization kinetics. Blends of PHB and PHBV containing 8 mol % HV obtained by co-dissolving the two polyesters in chloroform and precipitating the mixture in diethyl ether, showed one single sharp melting[51]. This suggests that the two components have been co-crystallized. The blends are highly crystalline and of the PHB type crystalline lattice. Blending of PHB and PHBV containing 76 mol % HV prepared by solution casting technique showed two $T_g$ values in DSC measurements, indicating immiscibility[52]. In addition, no depression of the apparent melting point was found for either polymer. Blends of PHB and PHBV containing 18.4 mol % HV were miscible over most of the composition range but separate into a two-phase system at high content of PHBVcopolymer[19, 53]. Abe et al.[54] studied the morphology of binary blends of PHB and atactic P[(R,S)HB], which was

prepared by the polymerization of racemic β-butyrolactone with $ZnEt_2/H_2O$ catalyst, and showed that the atactic P[(R,S)HB] was miscible with PHB in the melt and included under certain conditions in the amorphous regions between the lamellae of PHB. On the other hand, Kumagai et al[55] studied the enzymatic degradability of the blends of PHB with atactic P[(R,S)HB] in presence of PHB depolymerase from *A. faecalis*. The rates of enzymatic degradation of the blend films were higher than those of the corresponding PHB and atactic P[(R,S)HB] films. The highest rate was observed at about 50 % of P[(R,S)HB] component. It has been found that the presence of crystalline components such as PHB, P(HB-HV), poly(ε-caprolactone), poly(lactide) and poly(pivalolactone) by blending[56,57] or block-copolymerizing[58] with atactic PHB induce the enzymatic hydrolysis of the atactic P[(R,S)-HB] molecule by PHB depolymerase. These results suggest that the PHB depolymerase is liable to bind to the surface of stable crystalline lamellae, but it hardly binds to the surface of mobile polymer chains in an amorphous state above the glass transition temperature. The binding domain of PHB-depolymerase absorbs selectively to the crystalline phase on the film surface, and then catalytic domain hydrolyzes predominantly the PHB chains located in the amorphous regions on the surface.

The literature contains many other PHB blends including, PHB/Cellulose acetate[59], PHB/Poly(ethyleneoxide)[60], PHB/poly(methyleneoxide), PHB/poly(propyleneoxide). PHB/poly(epichlorohydrin)[61], PHB/poly (vinylacetate)-co-poly(vinyl alcohol)[62], PHB/poly(methyl methacrylate) [63, 64], PHB/(poly-ε-caprolactone)[65-67], PHB/ethylene-propylene rubber (PHB/EPR)[62], PHBV/poly(butylacrylate)[68], PHBV/polysaccharide blends [69, 70], PHBV/natural fibers polymer composites[71-73].

## 1.4. Copolymerization of PHB and PHBV with other biodegradable compounds

Another route that has been reported for the improvements of PHB is the copolymerization of PHB blocks with other flexible synthetic biodegradable polymeric components such as pol(ε-caprolactone), poly(butylene adiapate), poly(diethylene glycol adipate) (PDEGA) or poly(ethylene glycol), etc.[74-81] These studies have shown that the preparation of such copolymers allow to overcome the drawbacks inherent to PHB homopolymer.

## 1.5. Graft copolymerization of PHB and PHBV with vinyl monomers.

Graft polymerization is a well-known method to modify the chemical and physical properties of polymers for specific applications. The typical method of graft polymerization is a radical reaction of various monomers initiated by chemical initiators[82, 83], plasma, [84] electromagnetic and gamma radiation[85]. Out of these methods; chemical initiators have been extensively used for graft polymerization. Lee et al[86] studied graft copolymerization of acrylamide onto PHBV film to test the application of grafted film on its perm-selectivity. The effect of various polymerization conditions such as reaction time, monomer and initiator concentration on the graft% was studied. Graft% was increased initially with the increase of reaction time, monomer and initiator concentration, and leveled off at around 205 % G. Grafted films with different graft% and film thickness showed different internal structures[86]. To elucidate the morphology of grafted film, characterization methods such as Fourier transform infra-red and X-ray photoelectron spectroscopy and dimensional change analysis were used. Swelling behavior was also studied in various solvents mixtures. For the given graft %, thick film

showed a higher swelling ratio if water is used as a good solvent. In the case of the solvent system containing acetic acid as a good solvent, the swelling ratio was not affected by the film thickness. This is due to the very strong interaction between the amide group in the grafted chain and the acid group in acetic acid. The clear volume transition behavior was also observed. The swelling ratio is drastically changed at certain volume ratios (for example, 60% of methanol mixed with 40% acetic acid). This abrupt volume decrease is expected to be related to the internal structural variation[86].

Although PHB has inactive chemical structure, maleic anhydride was successfully grafted onto PHB chains by free-radical graft copolymerization[87]. The effects of various polymerization conditions on graft degree, such as solvents, monomer concentration, initiator concentration, reaction temperature, and time, were also investigated. The results show that the monomer and initiator concentrations play an important role in graft copolymerization, and graft degree initially increases with the increase in monomer and initiator concentrations, and then plateaus above a certain level. By changing the reaction conditions, graft degree can be controlled in the range from 0.2 to 0.85%. The crystallization behavior, the morphology, and the thermal stability of PHB and maleated PHB with various graft degrees were studied by DSC, WAXD, optical microscopy, and TGA. They reported[87] that the introduction of MA disturbs the regularity of PHB chains, intensifies the interaction among the chains, and greatly hinders their crystallization. On the other hand, the apparent Tg values of maleated PHB are not affected by the MA group. Although grafted MA does not affect the crystalline structure of PHB, as shown by the WAXD results, the relative intensities of the I(110)/I(020) plane for maleated PHB are enhanced with the increase in graft degree because of more restriction in the movement of segments for the (020) direction. In addition, it was found that the

morphology of maleated PHB isothermally crystallized at 100°C is strongly affected by the grafting degree. The banding textures of PHB become more clear and orderly after grafting with MA. The growth rate of PHB spherulite was decreased with the increase of extent of grafting. Thermal stabilities of PHB and maleated PHB were investigated and the results showed that the presence of a small amount of MA graft chains enhance the thermal stability of PHB. The biodegradability of PHB is promoted after grafting with MA because of the improvement in the wettability of PHB with the enzyme solution.

Polystyrene was introduced into PHB and its copolymer in grafting by a pre-irradiation method. It was found that the degree of grafting could be controlled by the pre-irradiation dose, time and temperature, and the maximum degree of grafting reaches 45%[88]. The results showed that the thermal stability of PHB and its copolymer PHBV was improved by a low degree of grafting of polystyrene. For instance, the time for 50% weight loss at 220°C was 76 min for control (ungrafted PHB) and 110 min for PHB having a degree of grafting of 9%. The glass transition temperature of a grafted PHB sample shifted to higher temperatures with the introduction of polystyrene chains[89].

Radiation-induced graft polymerization of vinyl acetate (VAc) onto PHB film was carried out and the difference in enzymatic degradability of PHB-g-VAc before and after saponification in methanol/NaOH mixture was investigated. As a result, the enzymatic degradability of saponified PHB-g-VAc was observed to increase with increasing the degree of saponification and incubation time. However, when the grafted PHB were saponified in alkali solution, there was the possibility not only of saponification of PVAc as graft chains, but also of degradation of the PHB substrate. At a degree of grafting higher than 5%, the grafted film was completely lost. This probably indicates that the

PVAc graft chains cover the surface of the PHB film and that the graft chains of the PHB-g-VAc film reacted selectively to become biodegradable polyvinyl alcohol (PVA)[90].

Graft copolymers of poly (methyl methacrylate) with poly (3-hydroxybutyrate), PHB, were prepared by the macromonomer method. PHB macromonomers were prepared from the esterification of oligomers with 2-hydroxyethyl methacrylate at their carboxylic acid end. Using free radical polymerization methods, the macromonomers were copolymerized with methyl methacrylate to yield graft (comb type) copolymers at different comonomer feed ratios. The single glass transitions observed for each copolymer composition suggested a random arrangement of the residues and that the macro domains were nanosized. The $T_g$ of the graft copolymers decreased from 100 to 3°C with increasing PHB blocks from o.5 to 14 %. The PHB grafts should be divided between small crystallites, which yielded the observed X-ray diffraction maxima, and blended with PMMA segments to form noncrystalline regions, responsible for the observed glass transitions. A gradual increase of crystallinity of the graft copolymers was observed with increasing PHB macromonomer content. These results reflect that the micro texture of the copolymers is relatively uniform, and the resistance to strain will depend on both noncrystalline and crystalline domains. The latter can act as bridges between chains, imparting continuity and pseudo-crosslinking to the overall structure[91].

PHB and PEG graft copolymers were prepared by two-step method. First, the PEG macromer (PEGM) was synthesized[92]; second, the PHB and PEGM were reacted under ultraviolet (UV) radiation to prepare PHB/PEG graft copolymer. Although graft polymerization onto PHB is rather difficult due to its high crystallinity and nonactive chemical structure, graft polymerization of PEG onto PHB was successively achieved[93]. It was found that the biodegradability of PHB increased with increasing PEG content.

## 1.6. Poly(N-vinylpyrrolidone)

Poly(N-vinylpyrrolidone), PVP, is a synthetically derived vinyl polymer with a unique combination of properties; solubility in both water and a range of organic solvents, non-toxicity, complexing ability towards several organic and inorganic compounds, good film forming ability, and biocompatibility[94-96]. PVP has been used in the biomedical field[97], for example, as blood plasma expander and in the cosmetic and food industrial sectors for decades. As a polymeric carrier, PVP has been shown to prolong the plasma circulation lifetime of bioconjugated drugs[98]. Given the featured properties of PVP, there is every reason to suspect that the presence of graft PVP chains upon the surface of a substrate would enhance the biocompatibility and hemocompatibility of that substrate when implanted into a biological system. Materials with bio- and hemocompatibility have been and are still sought after in a range of biomedical applications. The most common approach to achieve such materials so far has been the immobilization of biological molecules, typically heparin and albumin, on the biomaterials surface[99-102]. With a new non-destructive and solvent-free photo grafting technique, N-vinylpyrrolidone was covalently grafted onto the surfaces of degradable polymers; poly(L-lactide), poly(e-caprolactone), poly(lactide-co-glycolide), and poly(trimethylene carbonate). The wettability was markedly improved, as static contact angles changed from about 80° for the pristine substrates to around 30° after 30 min of grafting. Well-defined surface topographies, such as micro-patterns, are preserved in the process since the graft layers are thin. The biological response, measured as cytotoxicity, showed that the modified films provide good substrates, comparable with optimized cell culture plastics, for the adhesion and proliferation of normal human keratinocytes and skin fibroblasts[103].

## 1.5 Poly(N-isopropylacrylamide)

Poly(N-isopropylacrylamide) (PIPA) is one temperature-sensitive polymer that has been extensively studied in recent years. PIPA is well known for its novel temperature behavior in aqueous media. In aqueous solution, PIPA has inverse solubility and exhibits remarkable hydration–dehydration changes in response to relatively small changes in temperature. Aqueous solutions of PIPA show a lower critical solution temperature (LCST) near 32°C[104]. PIPA chains hydrate to form expanded structures in water when the temperature is below its LCST, but become compact structures by dehydration when heated above its LCST. As the volume-phase transition brings about dramatic changes in the physical properties of the PIPA gels, PIPA and its copolymer gels have been investigated for actuator[105], drug delivery[106], recovered and cultured cells[107], immobilized enzymes[108], isolated proteins[109], solute separations[110] and reversible surfaces[111, 112].

PIPA has been also used to modify polymer membranes. Grafted ethylene–vinyl alcohol copolymer membranes modified by PIPA exhibited temperature-responsive characteristics that were evaluated by measuring a dimensional change of the grafted membranes[113]. Significant temperature sensitivity of the polyvinyl alcohol membrane in the pervaporation process was observed after grafting PIPA onto it[114]. The pervaporation selectivity for the modified membranes can be adjusted by changing temperature; thus, the flexibility of the process is increased. The polyamide membranes grafted by PIPA and crosslinked poly(N-isopropylacrylamide-co-butylmethacrylate) membranes have been used to separate riboflavin and dextran, respectively, from water by dialysis[111-115]. After PIPA was grafted on the porous polyvinylidene fluoride membranes, the water filtration rate of the grafted membrane varied more than 10-fold between the temperature

above and below the LCST of PIPA[116]. PIPA has been extensively investigated for temperature-modulated drug delivery systems due to its thermosensitive properties Below the LCST, the hydrophilic PIPA chains interact with water. However, above the LCST, the hydrophobic PIPA chains collapse and such interactions do not occur anymore. In fact, the hydrophobic and collapsed PIPA chains actively interact with biocomponents, such as cells and proteins or other hydrophobic components, while the hydrophilic hydrated and flexible PIPA chains do not interact with them. It is this thermoresponsive nature of PIPA which is exploited for its biomedical applications, such as drug release[117-120].Copolymerization of NIPA with hydrophilic or hydrophobic monomers results in a shift of LCST to higher or lower values, respectively[121-123].