NOVEL APPROACHES TO BIODEGRADABLE POLYMERS: SYNTHESIS AND BIODEGRADATION STUDIES

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CERTIFICATE

This is to certify that the thesis entitled **"NOVEL APPROACHES TO BIODEGRADABLE POLYMERS: SYNTHESIS AND BIODEGRADATION STUDIES"** is an authentic record of the research work carried out by Mrs. Gisha Elizabeth Luckachan, M.Sc., under my supervision in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** of the **Cochin University of Science and Technology**, Kochi, and further that no part of this thesis has been presented before, for any other degree.

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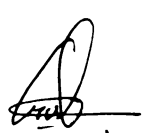
DECLARATION

I, Gisha Elizabeth Luckachan, do hereby declare that this thesis entitled **"NOVEL APPROACHES TO BIODEGRADABLE POLYMERS: SYNTHESIS AND BIODEGRADATION STUDIES"** is a bonafide record of research work done by me, and that no part of this thesis has been submitted earlier for the award of any other Degree, Diploma, Title or Recognition.



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PREFACE

Biodegradable polymers have opened an emerging area of great interest because they are the ultimate solution for the disposal problems of synthetic polymers used for short time applications in the environmental and biomedical field. The biodegradable polymers available until recently have a number of limitations in terms of strength and dimensional stability. Most of them have processing problems and are also very expensive. Recent developments in biodegradable polymers show that monomers and polymers obtained from renewable resources are important owing to their inherent biodegradability, biocompatibility and easy availability. The present study is, therefore, mostly concerned with the utilization of renewable resources by effecting chemical modification/copolymerization on existing synthetic polymers/natural polymers for introducing better biodegradability and material properties.

The thesis describes multiple approaches in the design of new biodegradable polymers: (1) Chemical modification of an existing non-biodegradable polymer, polyethylene, by anchoring monosaccharides after functionalization to introduce biodegradability. (2) Copolymerization of an existing biodegradable polymer, polylactide, with suitable monomers and/or polymers to tailor their properties to suit the emerging requirements such as (2a) graft copolymerization of lactide onto chitosan to get controlled solvation and biodegradability and (2b) copolymerization of polylactide with

cycloaliphatic amide segments to improve upon the thermal properties and processability.

The results of the present investigation have been published/presented/ under publication as described below:

Publications:

1. Sugar end-capped polyethylene: Ceric ammonium nitrate initiated oxidation and melt phase grafting of glucose onto polyethylene and its microbial degradation. **Gisha Elizabeth Luckachan**, Lissy Jose, V.S. Prasad, C.K.S. Pillai. *Polym. Degrad. Stab.* **2005**, 91(7), 1484-1494.
2. Chitosan/oligoL-lactide graft copolymers: Effect of hydrophobic side chains on the physico-chemical properties and biodegradability. **Gisha Elizabeth Luckachan**, C.K.S. Pillai. *Carbohydr. Polym.* **2006,** 64(2), 254-266.
3. Random multiblock poly(ester amide)s containing poly(L-lactide) and cycloaliphatic amide segments: Synthesis and biodegradation studies. **Gisha Elizabeth Luckachan**, C.K.S. Pillai. *J. Polym. Sci: Part A. Polym. Chem.* **2006,** 44(10), 3250-3260.
4. Polylactides and their copolymers: A review of their present status and future prospects. **Gisha Elizabeth Luckachan**, C.K.S. Pillai. *Progr. Polym. Sci.* (to be communicated)
5. Functionalization of polyethylene by different oxidizing agents. **Gisha Elizabeth Luckachan**, C.K.S. Pillai. *J. Apply. Polym. Sci.* (to be communicated)

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1. Biodegradable polyethylene by grafting of highly hydrophilic natural monomers using melt reaction techniques. **Gisha, E.L.**; Prasad, V.S.: Lissy, J.; Pillai, C.K.S. National conference on "Tissue Replacement

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3. Functionalization of polyethylene by novel methods. **Gisha, E.L.**; Prasad, V.S.; Lissy, J.; Pillai, C.K.S. International seminar on "Frontiers of Polymer Science and Engineering, 2002," IIT, Kharagpur, India.
4. Biodegradable sugar grafted LDPE: Effect of *in vitro* enzymatic degradation. **Gisha, E.L.**; Lissy, J.; Pitunia, C.M.M.; Prasad, V.S.; Pillai, C.K.S. "1st IUPAC International Conference on Biobased Polymers, 2003," Riken Institute, Japan.
5. Synthesis of biodegradable chitosan/polylactide graft copolymer. **Gisha, E.L.**; Pillai, C.K.S. The International Conference on "Polymers for Advanced Technologies, MACRO 2004", Thiruvananthapuram, India.
6. A SEM study on the biodegradation of chitosan/oligoL-lactide graft copolymers. **Gisha, E.L.**; Pillai, C.K.S. National Conference on "Electronic Microscopy, 2006," Thiruvananthapuram, India.

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CHAPTER 1

INTRODUCTION

1.1. Relevance of Biodegradable Polymers

Lord Alexander Todd (Nobel laureate in Chemistry, 1957) once said that polymers are the biggest contribution of chemistry to mankind[1]. They have become indispensable to our life with their wide range of applications in diverse fields such as packaging, agriculture, consumer products, medical appliances, building materials, industry, aerospace materials etc. However, the resistance of synthetic polymers to chemical, physical and biological degradation has become a serious concern when used in areas such as surgery, pharmacology, agriculture and the environment, and as a consequence time-resistant polymeric wastes are becoming less and less acceptable[2]. Naturally, the necessity for polymeric materials satisfying the conditions of biodegradability, biocompatibility and release of low-toxicity degradation products, as an alternative to these existing polymers is apparent.

The severe environmental problems, including the increasing difficulties of waste disposal and the deepening threat of global warming (due to carbon dioxide released during incineration) caused by the non-biodegradability of polyethylene (used in packaging and agriculture field) have raised concerns all over the world. We must confront them in order to build a new society and

economy free of plastic pollution in the 21st century. The use of biodegradable counterparts as an alternative to non-biodegradable polymers is, therefore, evoking considerable interest lately. Unfortunately, the degradable polymers available until recently have inferior physical properties in terms of strength and dimensional stability and most of them are very expensive and are technically difficult to process. As a result, attempts have been made to solve these problems by including biodegradability into polymers in everyday use through slight modifications of their structure. Polyethylene can be made to an environmentally friendly polymer by introducing sugar moieties that will increase the hydrophilicity and the number of active sites for the microbial attack on polyethylene backbone.

In recent years, biodegradable polymers from renewable resources have attracted much interest for environmental and medical applications because of their desirable properties of biodegradability, biocompatibility and natural abundance. Polylactides (PLA) is the leading candidate among these biopolymers. Due to its high capital cost and slow degradation rate as compared to the waste accumulation rate, the focus of PLA has been mainly on the biomedical field. However, certain limitations of PLA such as low hydrophilicity and degradation rate, poor soft tissue compatibility, low thermal and physical properties, lack of processability limits their wide utilization. These limitations of PLA along with its increased use in medicine generated more research interest for new materials by copolymerization of PLA with suitable monomers and/or polymers, so that some of the problems associated

with them can be solved for wider applications. A controlled solvation and degradation could be achieved by graft copolymerization of lactide onto chitosan, an amino polysaccharide present in the nature. The local toxicity due to the acid byproducts of PLA can be neutralized by the alkalescence of chitosan to get better biocompatibility. Copolymerization of PLA with cycloaliphatic amide segments would be expected to compliment the defects of PLA by the formation of intramolecular and intermolecular hydrogen bonds between ester and amide groups. Cycloaliphatic monomers are expected to be a better alternative to aliphatic counter parts as they are biocompatible and their incorporation increases the thermal and physical properties of copolymers at lower molecular weight in poly(ester amide)s.

1.2. What is Biodegradation?

There are a number of definitions on biodegradation, which depend on the field of application of the polymers (biomedical area or natural environment)[3-5]. Van der Zee[6] and Seal[7] reviewed all of the definitions found in different standards. The definition given by Albertsson and Karlsson appears to give a broad outlook on biodegradation. According to them, biodegradation can be defined as an event that takes place through the action of enzymes and/or chemical decomposition associated with living organisms and their secretion products. It is also necessary to consider abiotic reactions like photodegradation, oxidation and hydrolysis, which may alter the polymer before or during biodegradation because of environmental factors[8,9].

1.3. Categories of Biodegradable Polymers

Biodegradable polymers can be divided into three categories based on the origin of the raw materials and the process used in their manufacture[3,10-15].

1.3.1. Natural Biodegradable Polymers

The natural polymers or polymers from renewable resources fall in four broad groups:

- Polysaccharides (starch, cellulose, lignin, chitin and chitosan)
- Proteins (wool, silk, collagen, gelatin and casein)
- Bacterial polyesters (polyhydroxyalkanoates (PHAs))
- Others (lignin, shellac, natural rubber etc.)

1.3.2. Synthetic Biodegradable Polymers

Polymers produced from feed stocks derived from petrochemical or biological resources such as polyesters[16,17], polycaprolactone (PCL), polyamides, polyurethanes, polyureas, polyanhydrides, poly(vinyl alcohol) (PVA), poly(vinyl esters) etc. generally fall in this classification. The higher cost of production is the main barrier for the wide utilization of these polymers.

1.3.3. Biodegradable Polymer Blends

The blending of biodegradable polymers from renewable and non-renewable resources is a method of reducing the overall cost of the material and offers a method of modifying both properties and degradation rates[18,19]. Melt processability, humidity resistance and mechanical properties of starch can be improved by blending starch with synthetic polymers such as PVA[20].

PLA[21], PCL, PHAs etc. to make these materials suitable for the production of biodegradable films, injection-molded items and foams[4].

1.4. Factors Affecting Biodegradation

Factors on which the biodegradation of a polymer depends are[3,22]:

1.4.1. Polymer Characteristics

- **Chemical structure**

Polymers containing hydrolysable linkages along the chain or easily oxidisable functional groups on the chain are susceptible to biodegradation by microorganisms and hydrolytic enzymes. Most of the biodegradable polymers, therefore, have easily hydrolysable linkages. Furthermore the polymer chains must be flexible enough to fit into the active sites of the enzymes[23].

- **Degree of hydrophilicity**

Polymers containing hydrophilic segments (such as PVA, poly(acrylic acid), polyether) are seemed to be more biodegradable than hydrophobic polymers of comparable molecular weight. The biodegradability of a polymer could be promoted by increasing the hydrophilicity of the polymer by chemical modifications. Hydrophilicity in a polymer not only helps in better penetration of microorganism in the polymer but also induces the production of necessary enzymes within the microorganism relatively more easily compared to hydrophobic polymer.

- **Stereochemistry**

Stereochemistry has a role in biodegradation of polymers. Huang et al. reported that when the poly(ester-urea)s synthesized from D-, L-, and D,L-

phenylalanines subjected to enzyme catalyzed degradation, the pure L-isomer was degraded much faster than the D,L-isomers[24].

- **Degree and type of branching**

The main factors affecting the enzymatic degradation of synthetic polymers are the steric configuration of the polymer itself and the nature of the substituents adjacent to the susceptible bond. Branching of the molecular chain inhibits biodegradation.

- **Crystallinity**

Crystalline regions of a polymer are difficult to penetrate and are inaccessible to microbes as well as to abiotic agents. Amorphous regions are selectively degraded prior to the degradation of the crystalline regions in synthetic polymers as well as in biopolymers. The size, shape and number of crystallites have a pronounced effect on the chain mobility of the amorphous regions and thus affect the rate of degradation.

- **Molecular size**

Initiation process of biodegradation takes place due to the presence of end groups in the form of unsaturation, hydroperoxide or any other functional groups in the polymer structure. High molecular weight polymer has lesser concentration of these chain end functionality and thus is more resistant to biodegradation. However, biopolymers (such as cellulose or starch) as well as some synthetic polymers (e.g. polycaprolactone) are readily biodegradable despite the long chain structure. Degradation of these polymers depends on the

presence of hydrolysable linkages and hydrophilic functional groups present in their chemical structure.

1.4.2. Environmental Factors

In biological environment, certain factors such as temperature, moisture, salts, pH and oxygen are essential for the biodegradation process. The most significant element is the moisture factor[5].

1.5. Mode of Biodegradation

1.5.1. Microorganisms

The term microorganism covers a heterogeneous group of living beings (e.g. bacteria and fungi), which have a microscopic size and are, for the most part, unicellular. The degradative action of microorganisms is chiefly a result of the production of enzymes, which breakdown nonliving substrates in order to obtain nutrient materials present in polymer compositions. Certain environmental conditions such as optimal ambient temperature, the presence of nutrient materials and high humidity are essential for optimum growth and degradative activity of microorganisms[3,5].

1.5.2. Enzymes

Enzymes are essentially biological catalysts, with the same action as chemical catalysts. The activity of enzymes is closely related to the conformational structure, which creates certain regions at the surface forming an active site. At the active site the interaction between the enzyme and substrate takes place leading to chemical reaction, giving a particular product. The active sites are specific for a given substrate or a series of substrates. For

optimal activity certain enzymes must associate with cofactors that can be metal ions (e.g. Na, K, Mg, Ca and Zn) or organic cofactors such as NAD^+, $NADP^+$, FAD^+ and ATP called coenzymes[3,5].

Different enzymes have different actions, some enzymes change the substrate through a free radical mechanism while others follow alternative chemical routes typically of biological oxidation and biological hydrolysis (Figure 1.1)[3].

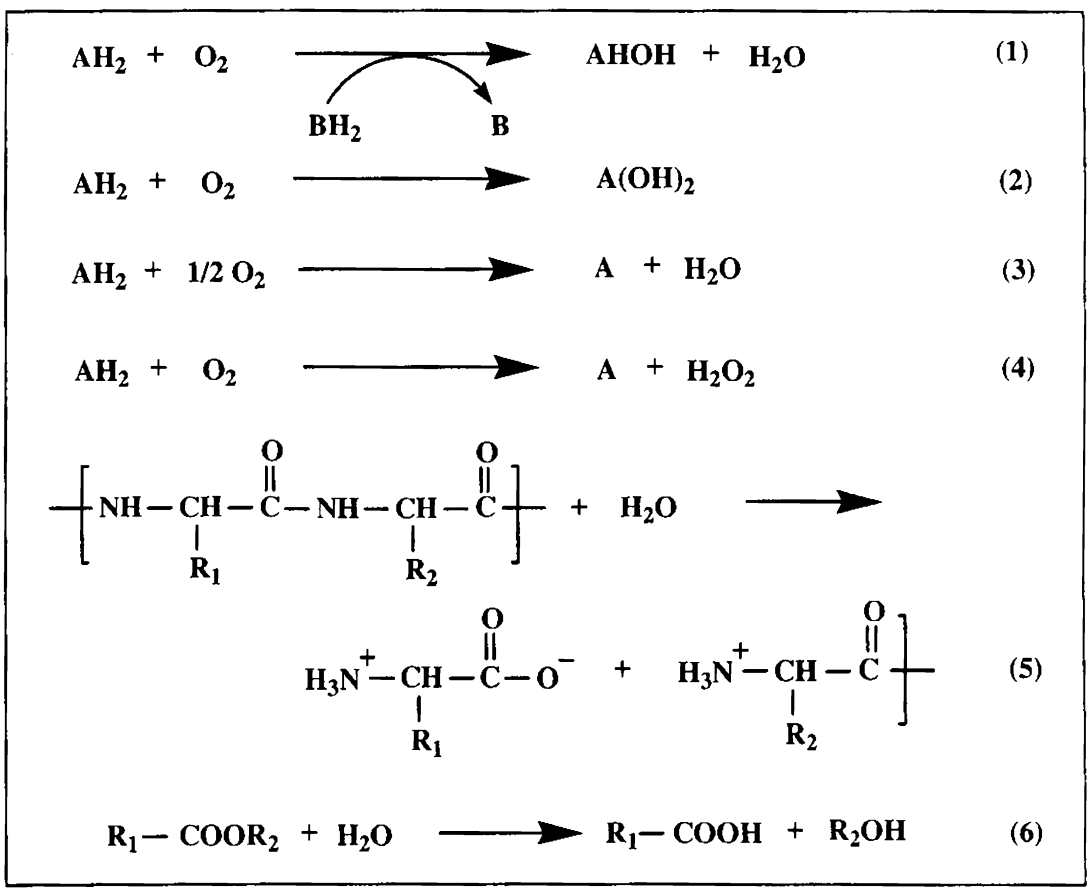

Figure 1.1. Biological oxidation and hydrolysis by enzymes.

- **Biological oxidation**

Certain enzymes react directly with oxygen (e.g. cytochromoxidase) (Figure 1.1. eq. 1). In many cases oxygen is directly incorporated into the substrate (e.g. hydroxylases or oxygenases) (Figure 1.1. eq. 2). In another type of biological oxidation the oxygen molecule function as a hydrogen acceptor (i.e. electron acceptor) (e.g. oxidases) (Figure 1.1. eq. 3 & 4).

- **Biological hydrolysis**

Several different hydrolysis reactions occur in biological organisms. Proteolytic enzymes (proteases) catalyze the hydrolysis of peptide bonds (Figure 1.1. eq. 5) and also the related hydrolysis of an ester bond (Figure 1.1. eq. 6).

1.6. Chemistry of Biodegradation

Biodegradation is the only degradation path way that is able to completely remove a polymer or its degradation products from the environment[5]. Biodegradation takes place in two stages (Figure 1.2): The first stage is the depolymerization of the macromolecules into shorter chains. This step normally occurs outside the organism due to the size of the polymer chain and the insoluble nature of many polymers. Extra-cellular enzymes (endo or exo-enzymes) and abiotic reactions are responsible for the polymeric chain cleavage. During this phase the contact area between the polymer and the microorganisms increases[3,25].

The second step corresponds to the mineralization. Once sufficient small size oligomeric fragments are formed, they are transported into cells where

they are bioassimilated by the microorganisms and then mineralized[5,26]. Biodegradation takes place in two different conditions depending upon the presence of oxygen; aerobic biodegradation (in the presence of oxygen) and anaerobic biodegradation (in the absence of oxygen). Complete biodegradation or mineralization occurs when no residue remains, i.e., when the original product is completely converted into gaseous products and salts.

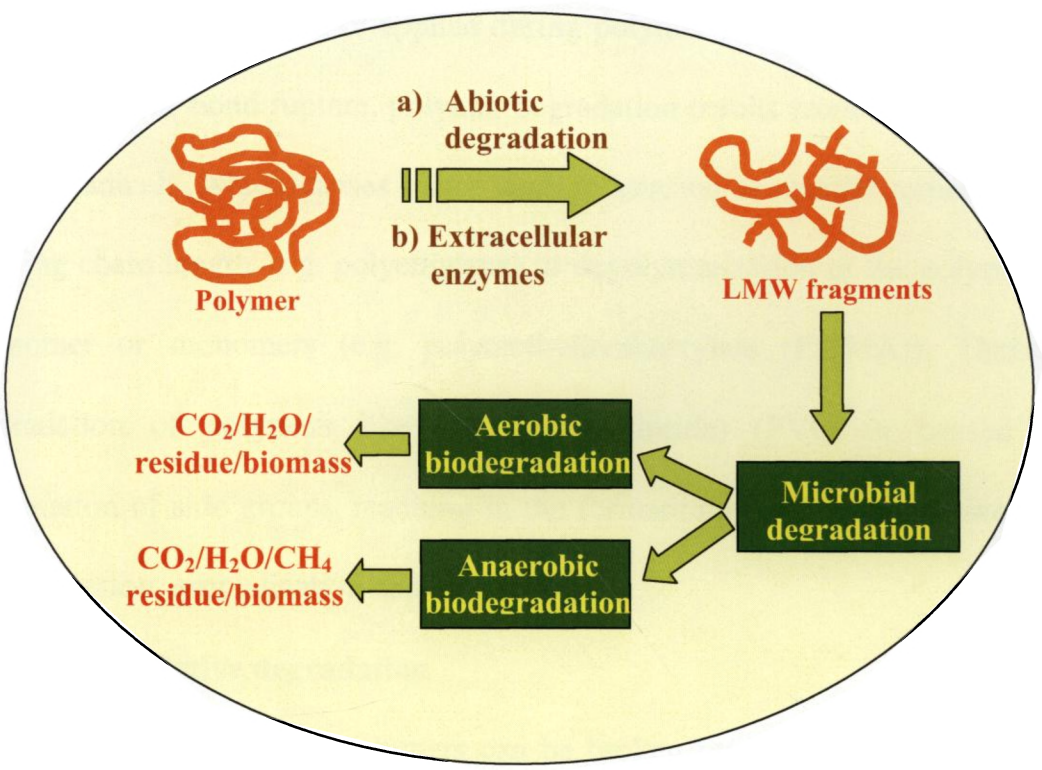

Figure 1.2. Diagrammatic representation of the chemistry of biodegradation.

1.7. The Way of Polymer Degradation

Polymer degradation in natural environment occurs in two stages. The first stage is mainly abiotic and the second stage is purely biotic[25,27].

1.7.1. Abiotic Degradation

As microbes cannot act upon high molecular weight polymers, they have to be fragmented into smaller chains before microbial degradation. The environmental factors, causing chemical degradation of polymers under natural conditions are given below[25,27] .

a) Thermal degradation

All polymers can be degraded by the influence of heat, whether present at the service temperature or applied during polymer processing. When heated to the extent of bond rupture, polymer degradation results from the production of free radicals, which causes either random scission to smaller molecules of varying chain length (e.g. polyethylene) or depolymerization of the polymer to monomer or monomers (e.g. polymethylmethacrylate (PMMA)). Thermal degradation of polymers like poly(vinyl chloride) (PVC) is caused by elimination of side groups, resulting in the formation of polyenes followed by chain scission, aromatization and char formation.

b) Photo-oxidative degradation

Chemical bonds in polymers can be broken by the highest energy UV waves of the solar spectrum, leading to their photodegradation[28,29]. Photo-oxidative degradation is a radical-based auto-oxidative process as given in Figure 1.3[30]. Chromophoric species such as carbonyl groups, hydroperoxides, unsaturation, metallic impurities such as iron and titanium, polynuclear aromatic compounds (PNA) such as anthracene, phenanthrene and naphthalene are responsible for the UV absorption[31]. They are incorporated into polymers

during processing or introduced intentionally into the polymer structures either by copolymerization or by mixing with polymers.

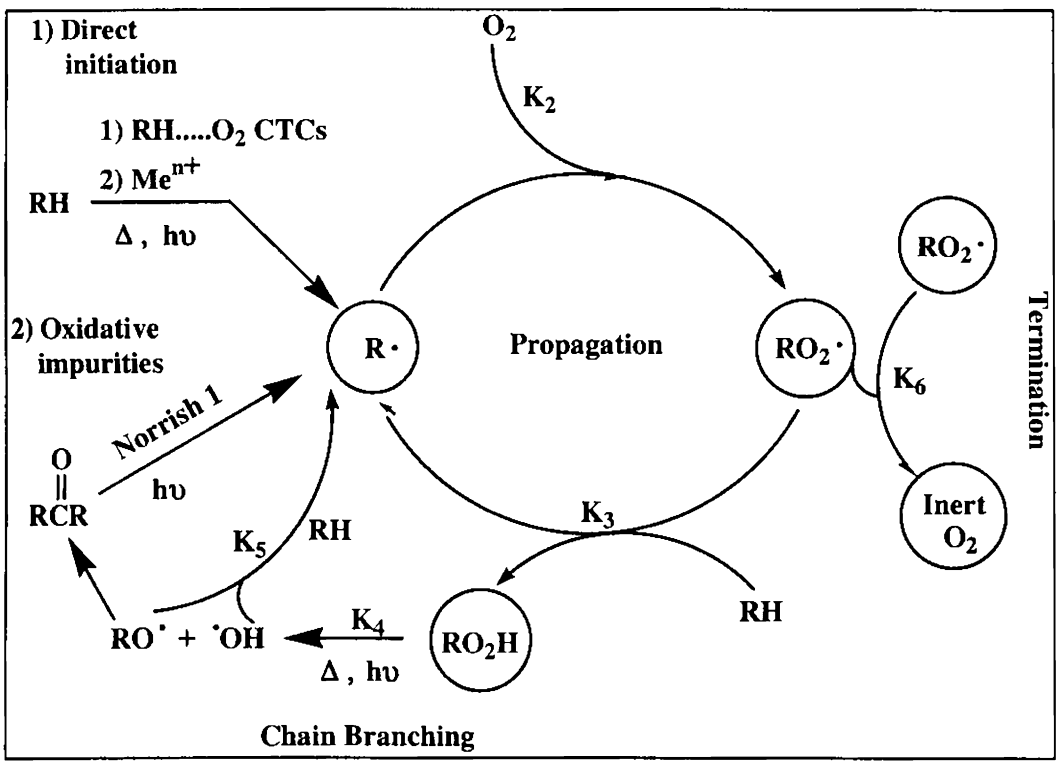

Figure 1.3. Auto-oxidative mechanism for all polymers (R- polymer chain, H- most labile hydrogen, X^0 - any radical, k_i- reaction rate).

c) Hydrolytic degradation or Bioerosion

Hydrolysis is the most important mode of degradation of biodegradable polymers especially synthetic polymers[11,32]. Depending on the erosion mechanism degradable polymers are classified into surface (or heterogeneous) and bulk (or homogeneous) eroding materials as illustrated in Figure 1.4[33]. The way a polymer matrix erodes depends on the diffusivity of water inside the matrix (V_{Dif}), the degradation rate of the polymer's functional groups (V_{Deg}) and the matrix dimensions. When $V_{Deg}>V_{Dif}$; the surface erosion dominates,

when $V_{Deg}<V_{Dif}$; the bulk erosion dominates. Since bulk erosion is not confined to the surface of the device, the size of a device will remain constant for a considerable portion of time during its application. Surface eroding polymers lose material from the surface only. They get smaller but keep their original geometric shape and bulk integrity. There are several factors that influence the velocity of this degradation: the type of chemical bond, pH, copolymer composition, chain stiffness, formation of crystalline domains and water uptake are the most important. Hydrolysis can be catalyzed by acid or basic compounds and by enzymes[33-35].

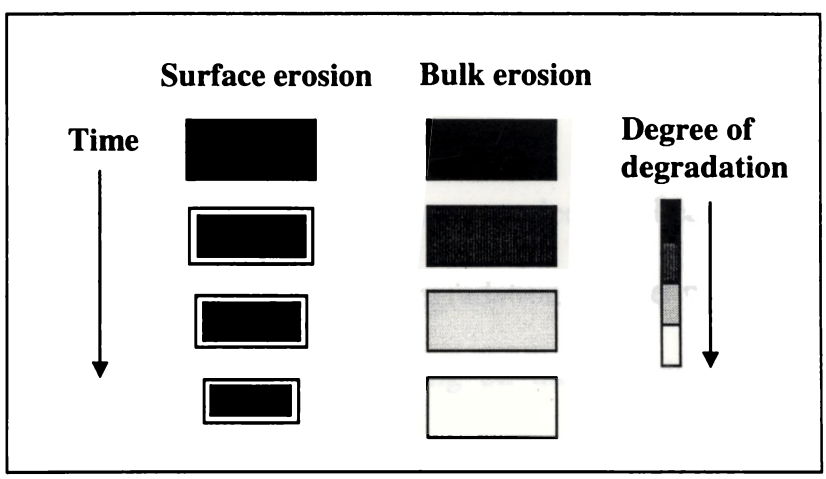

Figure 1.4. Schematic illustration of the changes a polymer matrix undergoes during surface erosion and bulk erosion.

d) Degradation due to environmental stress cracking (ESC)

ESC is defined as the catastrophic failure of a material at a stress much lower than its ultimate strength, due to combined effects of stress and environment. The stress-cracking agents that occurred in the environment are detergents, alcohols, oils, solvents, chemicals and vapors of polar liquids.

e) Chemical degradation

Chemical degradation of corrosive gases and liquids can affect most polymers, except PTFE (polytretrafluoroethylene) and PEEK (polyether ether ketone). Ozone, atmospheric pollutants (such as nitric and sulfuric oxides) and acids like sulfuric, nitric and hydrochloric will attack and degrade most polymers.

f) Mechano-chemical degradation

Owing to their length, polymers have the ability to convert mechanical energy applied in shear into main-chain bond energy resulting in bond scission. When polymers are subjected to shear, during processing in a screw extruder, macro alkyl radicals are formed that lead to accelerated oxidation.

g) Radiation-induced degradation

Transfer of energy by gamma ray or electron beam irradiation onto polymer backbone results in severe degradation of the polymer. Irradiation produces free radicals, which, depending on the chemistry of the polymer, initiates degradation (e.g. polyethylene, polypropylene).

h) Degradation due to weathering

All plastics subjected to long-term exposure to weather degrade to different extent, depending on their composition. "Weathering" implies the action of individual; or a combination of various environmental factors on polymers: heat, light, ionizing radiation, oxygen, ozone, humidity, rain, wind, dust, bacteria, and chemical pollutants (SO_2 and nitric oxides etc.).

1.7.2. Biotic Degradation

In this stage the low molecular weight products formed by the abiotic degradation are consumed by microorganisms, leaving CO_2, water, and other harmless substances at the end of the metabolic process.

1.8. Test Methods and Standards for Biodegradable Polymers

American Standard Testing Methods (ASTM) and the Indian Standard Organization (ISO) have proposed several test methods to assess the biodegradability and compostability of polymers[3,5,36,37]. The Biodegradable Products Institute (BPI) and the U.S. Composting Council (USCC) have launched a labeling program so that consumers can recognize and choose certified compostable products.

The use of this compostable logo signifies that the plastic products meet the ASTM D6400-99 specification for compostable plastics, as defined by the BPI

1.9. Applications of Biodegradable Polymers

For economic reasons, even for health and safety reasons, biodegradable plastic products are establishing themselves as viable alternatives in a number of important niche markets. Some of these lie in the fields of medicine, textiles,

hygiene and agriculture, as well as some specific packaging applications[3,27]. Few of the commercially available biodegradable polymers are listed in Table 1.1.

Table 1.1. Commercially available biodegradable polymers[11,12,38]

Trade Name	Composition	Use
Dexon & Medifit	Polyglycolic acid	Absorbable sutures
Vicryl	PLLA (8%)-co-PGA (92%)	Absorbable sutures
PDS II	Poly(p-dioxanone)	Absorbable sutures
Lactomer	Poly(LLA/GA 70/30)	Ligating clips & bone pins
Monocryl	Poly(GA-co-ε-CL)	Absorbable sutures
Maxon	Poly(GA-co-trimethylene carbonate)	Absorbable sutures
Lupron Depot	PLA/PLGA	Drug delivery products
Zoladex	PLA/PLGA	Drug delivery products
Biopol	Poly(3-HB-co-3-HV)	Packaging & Agriculture
Tone & Enviroplastic-C	Poly(ε-caprolactone)	Agricultural planting Container
Bionolle	Poly(butylene succinate)	Agriculture & Packaging
	Poly(ethylene succinate)	Agriculture & Packaging
Aqua-NOVON & Vinex	Starch/PVOH	Agricultural mulch film
BIOCETA	Cellulose acetate	Agricultural mulch film
Biomax	PET modified	Disposable items, bottles
Cereplast	Corn & potato starch	Biodegradable utensils
Mater-Bi	Starch & Vegetable oil	Disposable items, packaging, personal care & hygiene

The task of making commercially available synthetic polymers "environmentally friendly", which is one of the main goals to be achieved in the field of biodegradable polymers, appears to be surmounted by lots of technical difficulties. It would, therefore, be interesting to discuss initially the cases of highly hydrophobic polymers such as polyethylene, which are the main causes for the environmental problems of the present world.

1.10. Biodegradation of Polyethylene

Plastics, made from petrochemical feed stocks are portrayed by the "green" lobby as ecologically undesirable, because they are made from fossil carbon resources and it is assumed that they are very resistant to biodegradation in the environment. It is ironical that the very physical property of durability, that have made the polyolefin so commercially successful in packaging and other agricultural applications, is a disadvantage, when the material appears in the waste stream[39,40]. The environmental impact of persistent plastic wastes is growing more global concern, and alternative disposal methods are limited. Incineration may generate toxic air pollution, and satisfactory landfill sites are limited. Recycling is, at present, only viable for high cost, low volume specialty plastics[41,42]. The high cost or the lack of commercially acceptable performance of fully biodegradable polymers available until recently has meant that they have never replaced traditional non-degradable plastics in the mass market[43,44]. Hence, it is increasingly felt that the best solution for rectifying the

environmental pollution caused by the non-biodegradability of existing commercial plastics would be making them biodegradable.

1.10.1. Historical Perspective

The potential degradability and ultimate biodegradability of polyethylene (PE) have been started to consider in the early 1970s as specific attributes for applications in packaging and agricultural market segment. Since that time several techniques in which starch is used as a biodegradable additive have been patented[45-54]. In 1978, Griffin had first presented the idea of using native or modified starch such as thermoplastic starch and plasticized starch as filler in polyethylene in order to increase the biodegradability of the resulting material[55]. The incompatibility between starch and LDPE affected the mechanical properties of the blend negatively[56,57]. Later Dennenberg et al.[58], Henderson et al.[59] and Fanta and Doane[60] have made extensive studies on the modification of starch with vinyl monomers to increase the compatibility between starch and LDPE. A percolation threshold concentration of 31.17% was established by Peanasky et al. for the continues accessibility of starch in PE–starch blends[61]. Later, Goheen and Wool came to a similar conclusion in the biodegradation of PE–starch blends in soil over a period of eight months[62].

Several teams have reviewed the progresses made in the synthesis of biodegradable polyethylene[3,63-66]. In a series of works, Bikiaris et al. used ethylene-co-acrylic acid (EAA) as compatibilizer in LDPE/plasticized starch (PLST) blends[67-69]. They reported that EAA increases the thermal oxidative degradation of LDPE, where as PLST inhibits it. Later, the same group has

prepared LDPE/PLST blend containing PE-g-MAH copolymer as a compatibilizer[70,71]. Even though, compatibilized blends have retained the tensile strength of LDPE to a great extend at high amounts of starch (up to 30 wt %), the biodegradation rate showed a decrease compared to their uncompatibilized counterparts. So far, PE-g-MAH was considered as the most effective compatibilizer between starch and polyethylene and only very small amount of MAH is needed to attain a large improvement in mechanical properties[72-77]. Sastry et al. synthesized films of polyethylene–starch blends containing vegetable oil as a compatibilizer[78]. Kiatkamjornwong et al. used cassava starch-g-poly(acrylic acid) prepared by γ-ray irradiation, which was further modified by esterification and etherification with poly(ethylene glycol) 4000 and propylene oxide respectively, for a biodegradable LDPE sheet[79].

The thermo-oxidative ageing studies done by Sharma et al. revealed that the incorporation of pro-oxidant, which consisted of metal salts (Manganese stearate) and unsaturated elastomer (SBR and ENR-50), enhanced the degradation rate of sago starch filled LLDPE composites[80]. Where as Khabbaz et al. reported that starch have an inhibiting effect on the thermal degradation of LDPE containing pro-oxidant and photosensitizers[31,81]. Abd El-Rehim et al. observed an accelerating effect in biodegradation of LDPE/starch blend subjected to soil burial treatment after UV-irradiation[82]. These studies indicated that an exposure of the material to sunlight is necessary to stimulate the photo-oxidative action on PE, which further reduces the application scope of these polymers. Whereas, the temperature at landfill sites are suitable for the thermo-

oxidative degradation of PE, that attracts many researchers to carry out biodegradation of thermally oxidized PE to evaluate their ultimate biodegradation (e.g. mineralization) in soil[39,83-87]. They observed bioassimilation of PE films after its thermo-oxidative degradation into low molar mass products. More recently, Raghavan et al.[88] prepared starch/PE/poly(lactic acid) composites and Sedlarik et al.[89] prepared lactose-filled composites of metallocene linear low-density polyethylene.

This brief history on the synthesis and properties of biodegradable polyethylene reveals that the existing techniques are not a satisfactory solution for the environmental problems arising from the non-biodegradability of polyethylene. Even though, in almost all reported works starch was considered as the best additive for the pre-biotic degradation of PE, it raises secondary environmental problems after starch degradation. Polyethylene modifications with monosaccharides, disaccharides and other hydrophilic molecules are actively pursued to eliminate the drawbacks of starch and minimize the environmental problems.

1.10.2. Polyethylene Modifications to Facilitate Biodegradation

The methods adopted to facilitate polyethylene disintegration and subsequent biodegradation are as follows[3]:

i) Insertion of week links into vinyl polymer

Particular emphasis on this approach of insertion of 'weak links' on non biodegradable polymers has been placed on two types of polymer modifications: namely the insertion of functional groups in the main chain,

especially ester groups (Figure 1.5), which can be cleaved by chemical hydrolysis, and the insertion of functional groups in or on the main chain that can undergo photochemical chain-cleavage reactions, typically carbonyl groups (Figure 1.6). On irradiation with ultraviolet light, the activated ketone groups present can take part in two different types of free radical bond-breaking reactions referred to as Norrish I and Norrish II reactions (Figure 1.7).

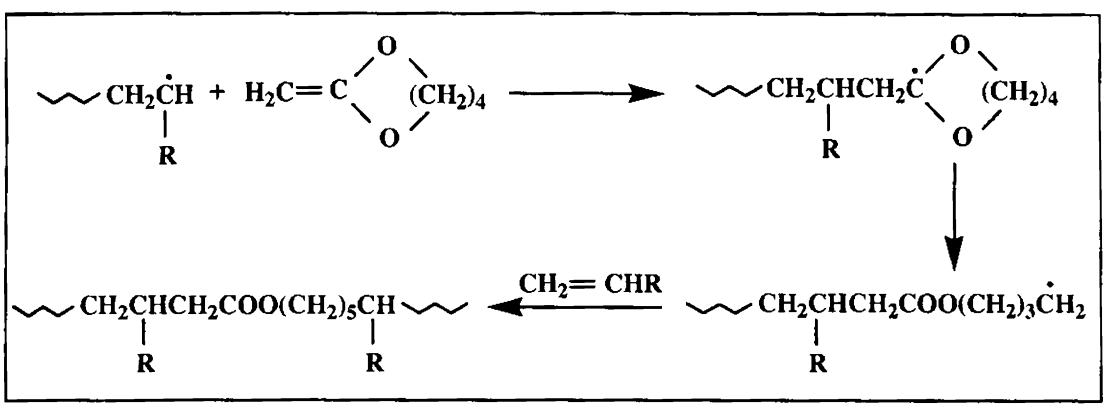

Figure 1.5. Insertion of ester group into vinyl polymer.

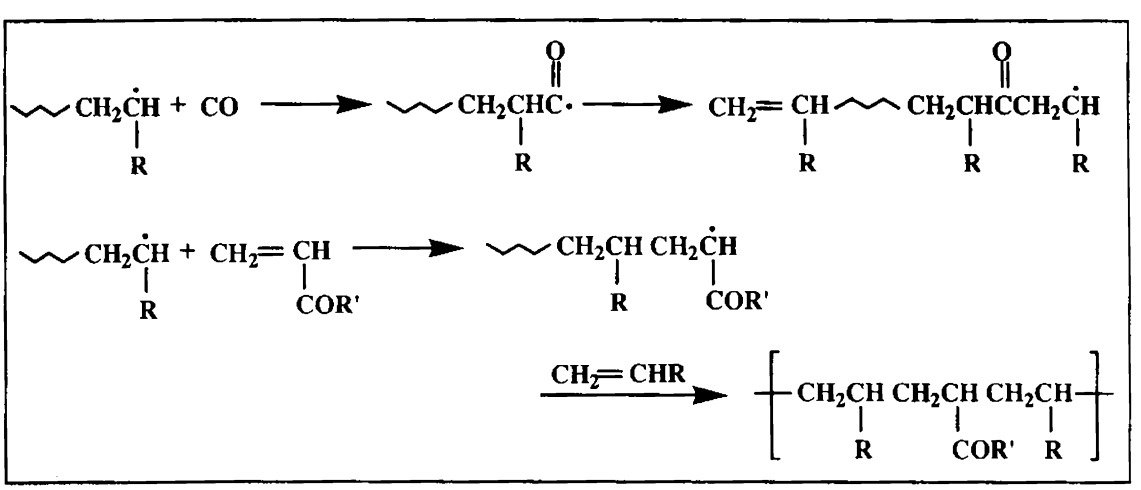

Figure 1.6. Insertion of ketone group into vinyl polymer.

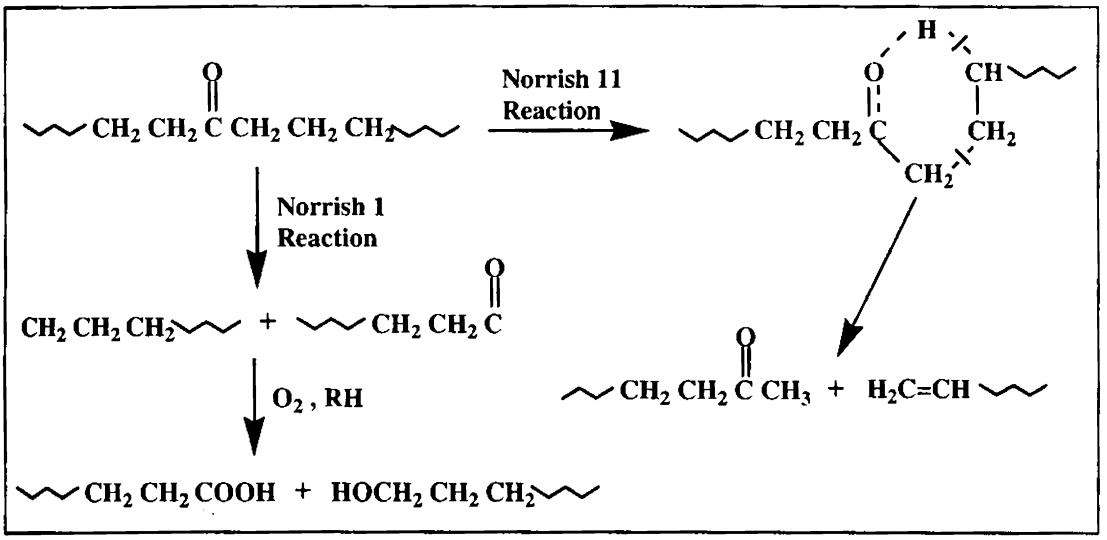

Figure 1.7. Norrish I and Norrish II reaction mechanisms for the degradation of copolymers of ethylene.

ii) Compounding of polymer with pro-oxidants and photosensitizers

The thermal (Δ) and/or photolytic (hυ) pre-biotic treatment, which constitutes the major route for promoting the eventual biodegradation of polyethylene, could be enhanced by using pro-oxidant additives[68,69]. The pro-oxidants most generally used for this purpose are divalent transition metal salts of higher aliphatic acids such as stearic acid, unsaturated elastomers as auto-oxidizable substances and transition metal complexes such as dithiocarbonates as photosensitizers or photoinitiators[80,90,91]. During photo- and thermo-oxidative degradation, the material degrades by a free radical chain reaction, involving the formation of hydroperoxides, carbonyl groups and finally to low molecular mass oxidation products such as carboxylic acids, alcohols, ketones, esters and low molecular mass hydrocarbons (Figure 1.3)[92]. Peroxidation and

carbonyl group formation also lead to hydrophilic surface modification friendly to microorganisms, that are thus able to bioassimilate the low molar mass oxidation products[93,94]. Transition metals catalyze the hydroperoxide decomposition step of the oxidation mechanism according to the reactions given in Figure 1.8[69,95].

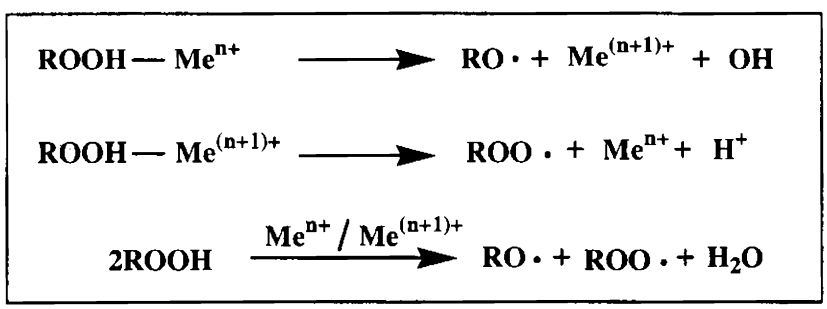

Figure 1.8. Reaction of metal catalysts with hydroperoxides.

Unsaturated compounds, which are very prone to oxidation, generate free radicals much more readily than saturated groups. These free radicals catalyze the initiation step of LDPE degradation by transforming LDPE from RH into free radical R·[69].

iii) Blends of biodegradable and non-biodegradable polymers

The most frequently adopted approach to degradability design of LDPE has been the introduction of pro-degradant additives such as starch and cellulose into synthetic polymers[3]. When these blends are deposited in the environment, various microorganisms consume the biodegradable component, leaving the polymer in a form full of holes. This form enables easier disintegration of the polymer into small pieces and also increases the total surface area accessible to microorganisms. Recent studies on this blends have

shown that even after starch consumption molecular weight of the polymer remain unaffected that will further evoke environmental problems[69].

1.10.3. Chemistry of Polyethylene Biodegradation

Biodegradation of polyethylene involve a complex interaction of abiotic and biotic mediated oxidative processes[96,97]. The carboxylic acids formed by the abiotic reactions undergo β-oxidation, where two carbon fragments are removed from the carboxylic molecule by reaction with coenzyme A, leaving behind a still activated acid. The activated acid repeatedly participates in the same process until the whole chain is split into two carbon fragments (Figure 1.9). The two carbon fragments enter the citric acid cycle, from which carbon dioxide and water are released[98,99].

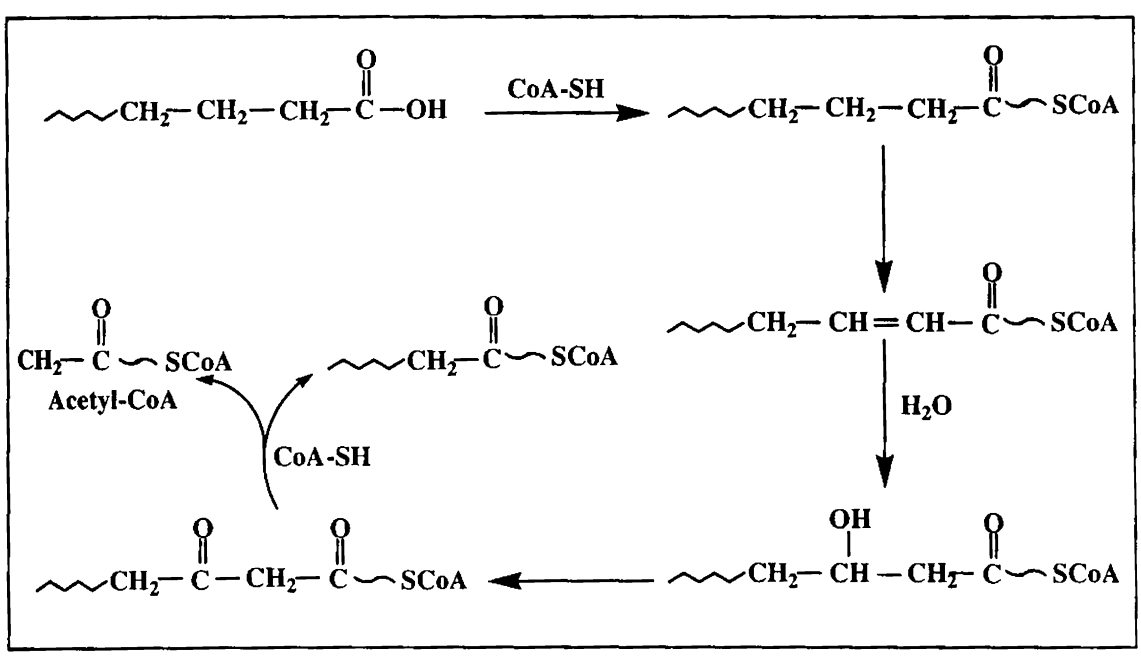

Figure 1.9. Biotic conversion of carboxylic acids (β-oxidation)[100,101]

An analysis of the existing biodegradable plastics from polyethylene as discussed above indicates inadequacies in terms of technology, cost of production and extent of degradability. It appears that a comprehensive approach is required to attain the realistic goals in the development of biodegradable polymers. In this connection, it would be interesting to look for biodegradable polymers from renewable resources.

1.11. Polylactide and Its Copolymers

1.11.1. Polylactide a Dominating Biopolymer

Polylactides (PLA) are considered as the most versatile material among biodegradable polymers because of its inherent biodegradability, biocompatibility and the easy availability from renewable agricultural sources[102]. These attributes make them a leading candidate in biomedical and pharmaceutical industries as a resorbable implant material, wound closure, bone fixation devices and a vehicle for controlled drug delivery[103-105]. It is also used as an environment friendly plastic, although their market is still limited due to its higher cost and slow degradation rate as compared to the waste accumulation rate[106,107]. However, their clinical applications are sometimes affected by the high hydrophobic and consequent poor water uptake, which results in a slow hydrolytic degradation rate[35,108]. Another potential disadvantage is the complications resulting from the accumulation of lactic acid produced in the process of PLA degradation, and its poor processability[109,110]. Ultra high molecular weight is required for processing PLA into strong fibers. PLA is belonging to the group of thermally less stable polymers and have poor

physical properties and high cost of production[111]. Copolymerization of lactide with other comonomers and/or polymers is used to modify the properties of PLA and to control its degradation behaviour suitable for the specific applications in the field.

1.11.2. PLA based Copolymers: A Literature Review

In 1932, Carothers demonstrated the synthesis of poly(L-lactide) (PLLA) by ring opening polymerization of lactide[112]. Since that time PLA has been used as a bioabsorbable material in the medical and pharmaceutical fields[113-116]. However, the application scope of PLA is limited because of the certain weaknesses mentioned above. Copolymerization of L-lactide with other monomers has been recognized as an important tool to modify PLLA suitable for specific applications in the field. The frequently employed comonomers are D-lactide[117], meso-lactide[118-121], glycolide[122-125], caprolactone (ε-CL)[126] and trimethylene carbonate (TMC)[127]. Grijpma and Pennings have done a detailed study on the synthesis, thermal properties and hydrolytic degradation of these copolymers[127]. Glycolide copolymers are much more hydrophilic than PLLA, while ε-CL and TMC reduce the glass transition temperature of the L-lactide copolymer. All comonomers decrease the crystallinity of the polylactide and provide optimal mechanical properties to the materials.

Another approach for increasing the hydrophilicity and degradation rate of polylactide was the copolymerization with other polymers such as polyethylene glycol[128-135]and chitosan[136-140]. In 1998, Kim et al. prepared biodegradable nanospheres composed of methoxy poly(ethylene glycol) and

D,L-lactide block copolymers as novel drug carriers[141]. In 2001, Otsuka et al. gave a review article regarding the self assembly of poly(ethylene glycol)-PLA block copolymers for biomedical applications[142].

Albertsson et al. prepared a pH sensitive physically cross linked hydrogel by grafting D,L-lactic acid onto amino groups in chitosan without using a catalyst[136]. Later Dutta et al. reported that the molecular mechanism of gelation involves interaction between chitosan and lactic acid[143]. Yao et al. reported the *in vitro* fibroblast static cultivation on a cytocompatible poly (chitosan-g-L-lactic acid) film and the cell growth rate on the copolymer film was found to be much faster than that of the chitosan film[137]. In another work, Liu et al. have reported the synthesis of a brush like copolymer of polylactide grafted onto chitosan[138]. Later, Wu et al. studied the amphiphilic properties of a graft copolymer of water-soluble chitosan and polylactide prepared by using triethyl amine as catalyst[144]. In 2005, Peesan et al.[145] prepared hexanoyl chitosan/PLA blend films and in 2006, Wan et al.[146] prepared a biodegradable polylactide/chitosan blend film. The biodegradability of these chitosan/polylactide graft copolymers was, however, not studied in these works.

The introduction of functional groups in polylactide for the modification of its properties has been investigated since 1990s. Barrera et al. synthesized a copolymer of poly(lactic acid-co-lysine) containing lysine residues[147]. The free amino group in the lysine residue was used to chemically attach a biologically active peptide GRGDY[147,148]. Gonsalves et al.[149]and Ouchi et al.[150] reported the

introduction of hydroxyl and carboxylic acid groups on the surface of polylactide by copolymerization with the amino acids and aspartic acid (P(Lac-Asp)), respectively. Recently, Wang and coworkers synthesized a series of polylactide- polyurethanes (PLAUs) having the property of shape–recovery temperature near to the body temperature from poly(L-lactide) diols, hexamethylene diisocyanate and 1,4-butanediol[151].

In 1992 Simone et al.[152] and Castaldo et al.[153] introduced the idea of random multiblock PEAs containing poly(L-lactide) blocks as hydrolytically degradable segments. They reported that the presence of amide functions in the polymer chain might lead to better physical properties than aliphatic polyesters usually have. Qian et al. reported a decrease in the degradation rate of PEAs with increase of amide content, macromolecular weight and thickness of the test samples, and increase with incubation temperature and pH of the degradation medium[154-158]. Angelo et al. prepared poly(ether-ester-amide)s based on poly(L,L-lactide) macromer for the delivery of bioactive compounds[159]. They reported that an enhancement of biodegradability of PEAs could be achieved by the incorporation of flexible hydrophilic oligo(ethylene glycol) segments into the main chain.

1.11.3. Synthetic Methods of PLA

Lactic acid polymers are most commonly prepared by ring opening polymerization (ROP) of lactides by means of cationic, anionic, enzymatic and co-ordination insertion mechanisms[102,160,161]. Extremely strong acids or carbenium ion donors are capable of initiating a cationic polymerization of

lactides. The anionic polymerization of lactides is initiated by alkali metal alkoxides, phenoxides and carboxylates. Racemization is an unavoidable side reaction of both cationic and anionic polymerizations.

Co-ordination insertion mechanism is based on metal alkoxides having a covalent metal-oxygen bond and the character of weak Lewis acids. The lactide plays temporarily the role of a ligand co-ordinated with the metal atom via the carbonyl O-atom (Figure 1.10)[102]. This co-ordination enhances the electrophilicity of the CO-group and the nucleophilicity of OR-group, so that an "insertion" of the lactone into the metal-oxygen bond may occur. Typical initiators of this mechanism are the alkoxides of Mg, Al, Zn, Sn, Zr and Ti. An advantage of the covalent initiators is the easy control of the molecular weights via the monomer/initiator ratio. Furthermore, the risk of side reactions is much lower compared to ionic initiators, so that much higher molecular weights can be obtained. Moreover, the covalent nature of these initiators significantly reduces the risk of racemization even at high temperatures.

Enzymatic polymerization is an eco-friendly process of polymer synthesis and is based on using easily renewable resources as starting materials. Enzyme catalyzed polymerizations have several advantages over conventional chemical methods: (a) mild reaction conditions, i.e., temperature, pressure, pH and absence of organic solvents, (b) high enantio and regioselectivity and (c) recyclabiliy of catalysts.

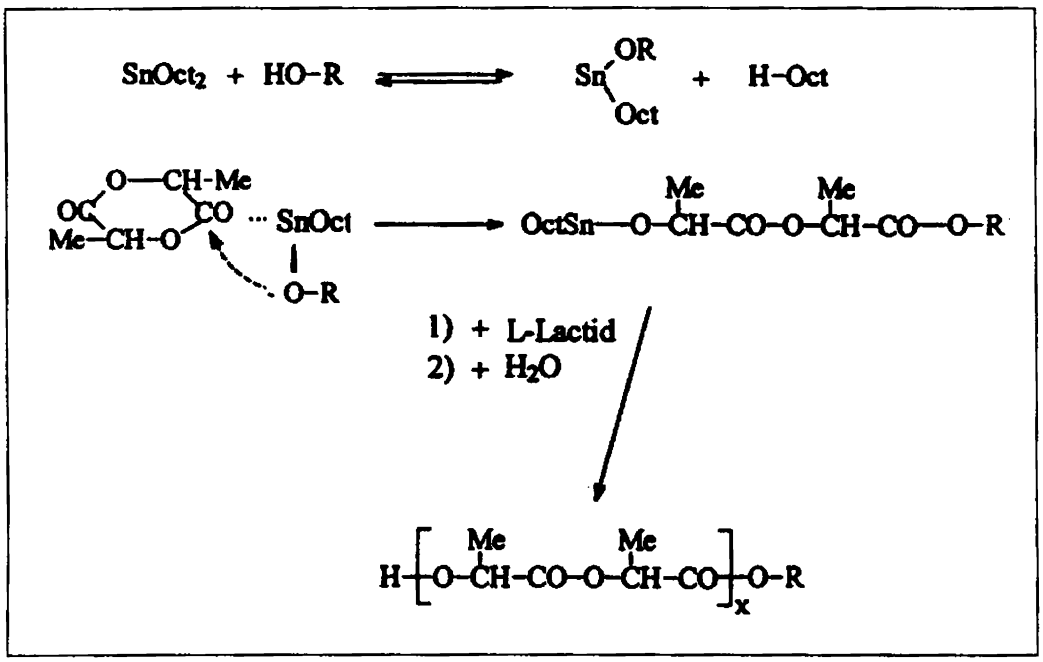

Figure 1.10. Ring opening polymerization of lactides.

1.11.4. Properties of PLA

The presence of asymmetric carbon atoms generates structural particularities, which make lactic acid-derived polymers rather special when compared with other polymers. Because of the chirality of the lactyl unit, lactide exists in three diastereoisomeric forms, i.e. L-lactide, D-lactide and meso-lactide, the latter containing a L-lactyl unit and a D-lactyl one in the ring. An equimolar ratio of L- and D-lactide is referred to as racemic D,L-lactide[35]. The thermal, mechanical and biodegradation properties of lactic acid polymers are known to depend on the choice and distribution of stereoisomers within the polymer chains. High purity L- and D-lactides form stereoregular isotactic poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA), respectively. These are

semicrystalline polymers with a high melting point (T_m ~ 180°C) and a glass transition temperature in the range of 55-60°C. PLLA can be used as a hard and tough engineering plastic for a broad variety of applications, for instance, in medicine for the internal fixation of bone fractures. The meso- and D,L-lactide, on the other hand, form atactic poly(D,L-lactide) (PDLLA), which are amorphous. It can be used for the production of transparent films, glues and for drug delivery applications. The presence of $-CH_3$ side groups imparts a hydrophobic nature to PLLA. The steric shielding effect of the ester groups by methyl substituents, reduces the hydrolytic degradation rate of PLLA on comparing with that of other polylactones[11].

1.11.5. Mechanism of Degradation of PLA

The mechanism of hydrolytic degradation of PLA polymers is quite unusual. In general, a bulk erosion mechanism has been considered as the main degradation pathway for PLA and its copolymers such as PLGA; random chain scission on the linkage of ester bonds in the polymer backbone proceeds homogeneously throughout the device[103]. Recently, it was reported that massive devices (>1mm thick) of PDLA, PLA and PLGA degrade via a heterogeneous mechanism i.e. the degradation proceeds more rapidly in the center than at the surface. This was attributed to the autocatalytic action of the carboxylic acid end groups of degrading products which were trapped in the matrix[162,163]. If the surrounding tissues cannot eliminate the acid by products of a rapidly degrading implant, then an inflammatory or toxic response may result[35,164].

Recently, there has been an increasing number of reports on the enzymatic hydrolysis of PLA[165-169]. Enzymatic hydrolysis of PLA proceeds mainly via the surface erosion mechanism and the surface area of polymeric materials would have a great influence on the enzymatic degradation. Abiotic hydrolysis is the initial stage of microbial degradation of PLA in nature. The released degradation products could be consumed by microorganisms[106,170].

Although PLA has many admirable properties, it is shown that it has serious disadvantages that can be overcome by materials copolymerization techniques. In this connection copolymerization with chitosan and cycloaliphatic amides appears to be gaining promise.

1.12. Chitosan as a Biomaterial

There is a growing interest in utilizing renewable resources as a functional biopolymer in a broad range of scientific areas such as biomedical, agricultural, food, cosmetics, wastewater management and environmental field[101]. Among them, chitosan, a unique amino polysaccharide has attained great interest because of its special structure, properties and its inexpensive abundant resources[171-175]. The specific properties of chitosan such as biocompatibility, biodegradability, bioactivity, low immunogenicity and multifunctionality increase its potential as a beneficial material for various applications in biomedical field[176-188]. Although, the area of possible applications is broad, a break-through has not yet been achieved. This is related to the insufficient property-profiles of chitosan: insolubility in water and most organic solvents, slow *in vivo* degradation and the unsatisfactory mechanical

and processing properties[189-192]. Intensive research and a concentration of effort will be needed to improve the properties of the biopolymer in bioplastic and biomedical applications. Chemical derivatisation of chitosan with various types of branches was reported as one of the strategies for tailor-made applications of chitosan in biomedical field[193-195].

1.12.1. Structure and Properties of Chitosan

Chitosan is a linear amino polysaccharide composed of β-(1,4)-linked 2-amino-2-deoxy-D-glucopyranose (GlcN, D-unit) and 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc, A-unit). This polycationic biopolymer is generally obtained by alkaline deacetylation of chitin (β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc)), which is the main component of the exoskeleton of crustaceans, such as crabs and shrimps[184,193]. Chitin, in fact, is the second abundant polysaccharide next to cellulose, making chitosan a plentiful and relatively inexpensive product[196]. The main parameters influencing the characteristics of chitosan are its molecular weight and its degree of acetylation (DA). Moreover, DA influences the physicochemical properties and biological properties of chitosan[196]. Chitosan can be digested *in vivo* by lysozomal enzymes according to the amount of N-acetyl groups and their distribution in the backbone[187,197]. The aminosugars resulted from the degradation of chitosan can be incorporated into glycosaminoglycans and glycoproteins metabolic pathways, or excreted. It is a haemostatic agent, which presents antithrombogenic properties. Since chitosan provided bacteriostatic and fungistatic activities it appears to be a good candidate for wound dressing

and for soft tissue regeneration[198-200]. Chitosan in hydrogel form have been considered to be advantageous in their application as a wound dressing material, in controlled drug delivery systems and in tissue engineering[179,201-204]. Since chitosan has a capacity of forming film it has been suggested as a biopolymer of choice for the development of contact lens (soft and hard contact lenses) and artificial kidney membranes[183]. Chitosan is insoluble in neutral water and soluble in dilute acids when the degree of acetylation is lower than 0.5 (p*K*a value is 6.5) and it behaves as a cationic polyelectrolyte[205-207].

1.12.2. Chemical Modifications of Chitosan

Chemical derivatisation of chitosan with hydrophilic moieties such as carboxymethylation[208,209], quaternarization[210], acetylation[211] etc. was reported as one of the methods to improve the solubility of chitosan in water. Since the chemical modifications change the fundamental skeleton of chitosan, the modified chitosan loose the original physicochemical and biochemical activities. Thus, as an alternative strategy to improve the water solubility but at the same time keeping the fundamental skeleton of chitosan intact, the covalent conjugation of hydrophilic polymers such as polyethylene glycol is preferable[212-217].

Introduction of carbohydrate branches onto chitosan as a branched polysaccharide analogue is another interesting modification to induce new chemical and biological functions[192,218,219]. Since the specific recognition of cell, virus, and bacteria by sugars has been discovered, this modification has generally been used to introduce cell-specific sugars into chitosan.

In order to solve the problem of insolubility in organic solvents, some chemical modifications to introduce hydrophobic nature to chitosan such as phthaloylation, tosylation and acylation reactions were done[193,220]. The introduction of hydrophobic branches generally endows the polymers with a better soluble range than chitosan itself, and also with other new physico-chemical properties such as the formation of some polymeric assemblies including gels, polymeric vesicles, Langmuir–Blodgett films and liquid crystals. Recently, there has been a growing interest in grafting vinyl monomers onto chitosan for biomedical applications[221,222]. The enzymatic approaches to modification of chitin and chitosan is interesting, owing to its specificity and environmental impact compared with chemical modifications[184,194].

1.13. Significance of Aliphatic Poly(ester amide)s

Aliphatic poly(ester amide)s (PEAs) has been the object of extensive investigation during the last few decades as an emerging class of biodegradable condensation copolymers. Aliphatic polyesters have poor thermal, mechanical and processing properties. Aliphatic polyamides, on the other hand, have better thermal and mechanical properties than aliphatic polyesters, but their degradation rate is too low to classify them as biodegradable polymers[223,224]. Therefore, by combining the favorable properties of these two classes of polymers, it could be possible to produce new polymeric materials possessing not only good biodegradability but also good materials and processing properties. Another advantage of PEAs over other biodegradable polymers is

that they can be suitably tailored to the needs of their applications by varying the nature of the monomers and/or their composition and architecture[225]. Recently, cycloaliphatic systems are considered as a better alternative to aliphatic counterparts in poly(ester amide)s[226-229]. This is because the conformational restrictions arising from the rigidity of cyclic ring structures largely influence the thermal and physical properties of the copolymers than their homopolymers.

1.13.1. Structure -Property Relation of Poly(ester amide)s

Poly(ester amide)s are a class of biodegradable condensation polymers having regular enchainment of ester and amide groups as the integral part of polymer chain[230]. According to the distribution law of ester and amide groups along the chain, PEAs can be grouped into three fundamental classes: alternating copolymers, random copolymers and block or segmented copolymers[231-233]. Two types of hydrogen bonds are involved in PEAs, amide-amide and amide-ester hydrogen bonds[234]. Hydrogen bonding has a strong influence on the biodegradability of PEAs: increased hydrogen bonding leads to lower biodegradability[235]. Meanwhile, the chemical structure, crystallinity degree, chain flexibility and a balance of hydrophilic and hydrophobic properties also affect the enzymatic degradation of PEAs. The ester component, in fact, reduces the crystallinity of polyamide segments and increases the chain flexibility of PEAs needed to fit into the active sites of the enzymes. Since amide groups are polar, their incorporation into the ester main chain leads to an increase of hydrophilicity[236]. The enhanced hydrophilicity increases the amount

of water sorption in the amorphous region followed by degradation. The hydrolytic degradation rate of PEAs are determined by ester moiety content, since λ_{ester} is much larger than λ_{amide}(Figure 1.11)[34,158]. So the chemical composition must have a great effect on the biodegradation of these copolymers[237-239].

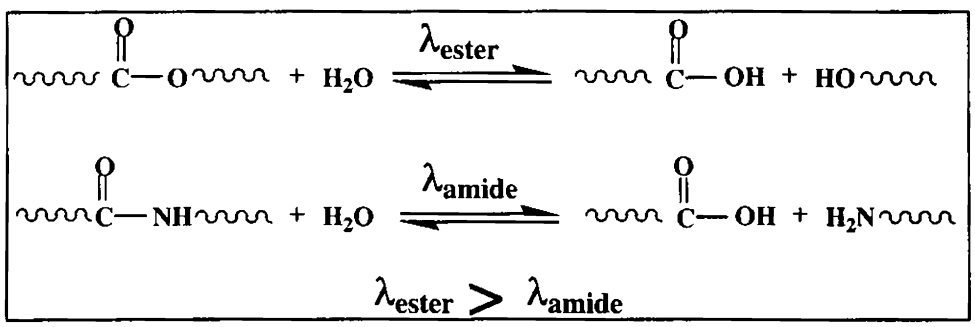

Figure 1.11. Hydrolytic degradation of PEAs.

Enhanced degradability of poly(ester amide)s could be achieved by incorporating into the chain both hydrophilic, flexible segments consisting of short sequences of ethylene glycol groups and/or linkages built up by α-amino acid sequences, that can be degraded by specific enzymes[240]. Therefore, the combination of good physical properties, biocompatibility and controlled degradability makes PEAs a valuable material for biomedical applications as absorbable sutures or temporary implants and drug delivery systems[241-248].

1.14. Scope and Objectives of the Present Work

It can be noted from the preceding discussions that the biodegradable polymers for short time applications have attracted much interest all over the world in different sectors such as surgery, pharmacology, agriculture and the

environment. The reason behind this growing interest is the incompatibility of the polymeric waste with the environment where they are disposed after the usage. The recovery of polymeric waste as a solution to this problem is not easy or feasible, like in surgery for obvious reasons, or in the environment in the case of litter. The development of novel biodegradable polymers satisfying the requirement of degradability, compatibility with the disposed environment and the release of low-toxicity degradation products are the ultimate solution to these issues. An analysis of the existing biodegradable plastics indicates inadequacies in terms of either technology or cost of production especially in the case of applications in environmental pollution. So, there is a need to look for novel methods for introducing biodegradability in existing polymers for use in the environmental areas. Alternately, one can consider modifying suitably the natural polymers obtained from the renewable resources, by tailoring their properties by altering the composition and structure with a view to solving some of the existing problems associated with them for the synthesize of new biodegradable polymers. The present work, therefore, has adopted a dual strategy of (1) modification of polyethylene, a commodity plastic largely used in packaging materials, using a novel technique for anchoring mono/disaccharides, which being carbohydrates can act as a nutrient source for microorganisms to enhance biodegradation and (2) design of copolymers based on polylactide, using either chitosan or cycloaliphatic amide segments, which are gaining importance as a polymer of great opportunities.

The mass production and consumption of polyethylene for the various applications in packaging and agricultural fields, has resulted in mounding global concerns over the environmental consequences of such materials, when they enter the waste stream after their intended uses. The degradable polymers available until recently have inferior physical properties in terms of strength and dimensional stability and most of them are very expensive and are technically difficult to process. These inadequacies persisting for the eco-friendly applications of the existing biodegradable plastics indicate the need for novel approaches for the design of the material. So, the strategy employed here for minimizing the environmental pollution of polyethylene was to increase its biodegradability by oxidation and grafting of simple sugars such as monosaccharides onto polyethylene chain ends. The increased hydrophilicity and the easy accessibility of chain ends will attract microbes towards polyethylene, leading to fast degradation. Anchoring of simple sugars instead of starch on polyethylene can reduce the negative effects on physical properties observed in the case of polysaccharide grafting.

The world scenario of biodegradable polymers shows that the polymeric materials generated from renewable resources are growing in interest due to their inherent biodegradability, biocompatibility and easy availability. Among these biopolymers, polylactides are the leading candidates for various applications in biomedical field and in the environment. Due to the high capital cost and slow degradation rate as compared to the waste accumulation rate, the focus of PLA has been mainly on the biomedical field. However, certain

limitations of PLA such as low hydrophilicity and degradation rate, poor soft tissue compatibility, low thermal and physical properties, lack of processability, high cost of production limits their wide utilization. These limitations of PLA along with its increased use in medicine generated the necessity in search for new materials by copolymerization with suitable monomers and/or polymers, so that some of the problems associated with them can be solved for wider applications. Graft copolymerization of lactide onto chitosan was one of the strategies adopted to explore their full potential and control the functions suitable for the applications in biomedical and pharmaceutical fields. The interest in chitosan stems from the fact that they are the only amino polysaccharide present in the nature. Further more, they are biocompatible, biodegradable, have antimicrobial activity, low immunogenecity and more over low production cost. Controlled solvation and degradation could be achieved by controlling the ratio of chitosan:L-lactide in the graft copolymer to obtain an optimum hydrophobic-hydrophilic balance. In addition, the alkalescence of chitosan can neutralize the acidic degradation products of polylactide, so that the local toxicity due to the acid byproducts can thus be alleviated to get better biocompatibility. Again a controlled degradability of chitosan by various enzymes present in the human body could be achieved after lactide grafting. Another strategy employed for the synthesis of a new biodegradable copolymer based on PLA was: copolymerization with cycloaliphatic amide segments, so that the resulting poly(ester amide)s would be expected to compliment the defects of PLA by the formation of

intramolecular and intermolecular hydrogen bonds between ester and amide groups. The combination of the favorable properties of both classes of materials may lead to new materials that combine good end-use and processing properties and biodegradability. The advantage of cycloaliphatic structures are that the incorporation of these rings in the amide segments increases the thermal properties of PEAs even at relatively low molecular weight than that of its linear aliphatic counter parts. Again, as cycloaliphatic systems are nontoxic the synthesized PEAs should be biocompatible.

Accordingly the objectives of the present work can be formulated as:

(1) Effect of different oxidizing agents on the functionalization of polyethylene.

(2) Synthesis and biodegradation studies of polyethylene modified with sugar grafting, using ceric ammonium nitrate as an oxidant and initiator by a "one pot" melt phase reaction in Brabender plasti-corder.

(3) Synthesis and characterization of chitosan/oligoL-lactide graft copolymers and study of the effect of hydrophobic side chains on their physico-chemical properties and biodegradability.

(4) Synthesis and biodegradation studies of random multiblock poly(ester amide)s containing poly(L-lactide) macromer and cycloaliphatic amide segments.

1.6. References

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CHAPTER 2

SUGAR END-CAPPED POLYETHYLENE: CERIC AMMONIUM NITRATE INITIATED OXIDATION AND MELT PHASE GRAFTING OF GLUCOSE ONTO POLYETHYLENE AND ITS MICROBIAL DEGRADATION

2.1. Abstract

Low density polyethylene (LDPE) was modified to introduce biodegradability by grafting highly hydrophilic monomers (which can act as nutrients for microorganisms) such as glucose by a novel melt phase reaction in Brabender plasti-corder in the presence of ceric ammonium nitrate (CAN) to obtain 4-O-hydroxymethyl D-arabinose (Sugar) end-capped LDPE (**Su-g-LDPE**). A maximum grafting of 16% was observed at 70:30 LDPE:Glucose feed ratio and at 0.1% CAN concentration. The grafted polymers were characterized by FTIR, thermal analysis, WAXD and mechanical property measurements. The biodegradability of **Su-g-LDPE** was carried out by soil burial test and by optical density measurements in presence of an aerobic bacterium *Pseudomonas sp.*. The degraded polymers showed changes in weight, crystallinity and inherent viscosity. Optical density of the medium registered an increase with degradation. FTIR spectra of the degraded samples

showed 70% decrease in the ketone carbonyl index (ν_{1719}/ν_{1465}) of **Su-g-LDPE,** indicating microbial degradation of LDPE matrix, which was further confirmed by SEM micrographs. The present data support a microbial oxidation process where the carbonyl is oxidized to carboxylic acid and effects cleavage of the LDPE chain at the ends.

2.2. Introduction

The environmental pollution caused by the non-biodegradability of synthetic hydrocarbon polymers especially low density polyethylene (LDPE) used in packaging, agriculture and industry has been created and continue to create large concern all over the world. These polymers are characterized by properties such as high molecular weight, chemical inertness, hydrophobicity, relative impermeability to oxygen etc., which make them resistant to microbial attack and at the same time present enormous restrictions in the design and development of biodegradable polymers[1,2]. However, several design techniques have been developed for introducing biodegradability in polyethylene[3-9]. Blending of polyethylene with biodegradable polymers such as starch and cellulose has been practiced as a viable methodology[3-5]. It has, however, been noted that the starch gets enzymatically hydrolyzed in a biologically active environment leaving the polymer in a disintegrated form[1]. A fairly large amount of starch is needed to get a reasonable degradability even at the cost of its mechanical property[6]. It is also now well understood that the molecular weight of LDPE remains unaffected even after the complete degradation of

starch causing further environmental pollution[1]. Chemical modification or grafting of starch and starch-derived products onto LDPE provides another feasible approach for incorporating starch into the polymer for effecting degradation by microbial consumption of starch followed by chain scission[7-9]. Yet another methodology reported for the development of biodegradable LDPE is the modification of polymer back bone by introducing some weak links such as >C=O groups that may permit controlled degradation of LDPE into low molecular weight oligomers which can then be utilized and consumed by microorganisms[10]. The many inadequacies of the existing biodegradable plastics for eco-friendly applications indicate the need for novel approaches for the design of the material.

A combined methodology of *in situ* oxidative modification of polymer backbone by introducing >C=O groups followed by grafting of simple sugars is expected to yield added advantage in polyethylene degradation. Anchoring of simple sugars such as monosaccharides or disaccharides instead of starch on polyethylene can reduce the negative effects on physical properties observed in the case of polysaccharide grafting. Glucose, a naturally abundant, renewable monosaccharide that can be chemically modified by oxidative ring opening reactions is having great potential as a hydrophilic modifier to induce biodegradation. The utilization of ceric ammonium nitrate (CAN) for chemical modification of polymers by ring opening of glucose is reported[11]. There is no available literature on reactive grafting of glucose onto polyethylene in presence of CAN. Hardly any successful research has been done for designing

new polymers or effecting minor chemical modifications of polyethylene with attachment of simple sugar molecules. Recent literature shows novel possibilities for grafting of monomers in a melt phase reaction at high shear conditions when free radicals are generated *in situ*[12]. Various attempts have been reported in the functionalization of LDPE[13-15]. The use of CAN has not been mentioned in any work on the oxidation of LDPE. As oxidation is reported to be the first step in the biodegradation of polyethylene, the use of oxidized LDPE might enhance the microbial oxidation followed by chain scission[16].

In this chapter, oxidation of LDPE followed by grafting of glucose by melt phase reaction in Brabender plasti-corder is studied in presence of CAN which is acting as an oxidant as well as an initiator. The grafting of highly hydrophilic sugar molecule, glucose onto polyethylene functionalized by oxidation is expected to make polyethylene more susceptible to microbial attack by increasing the number of active sites and the hydrophilicity of LDPE. The modified LDPE was characterized by spectroscopic, thermal and mechanical evaluation. Biodegradation of grafted LDPE was studied by soil burial test and in presence of an aerobic bacterium *Pseudomonas sp.*. Degradation was followed by weight loss, optical density measurements, thermal evaluation, FTIR spectroscopy, WAXD and changes in surface morphology by SEM.

2.3. Experimental Section

2.3.1. Materials

Film grade polyethylene (Indothene 24FS040; melt flow index 4.0gm/10min and density 0.922gm/cc.) was supplied by Indian Petrochemical Corporation Limited, Vadodara. CAN was purchased from Aldrich Chemical Company, USA. Glucose, $K_2Cr_2O_7$, $KMnO_4$, toluene and benzene were obtained from S.D.Fine-Chem Ltd, Mumbai. Solvents were of AR grade and purified by standard methods. A commercial soil-based compost produced from municipal solid waste was collected from a biotreatment plant at Velappinsala, Kerala, India.

2.3.2. Oxidation of Polyethylene

Oxidation of LDPE was carried out using three methodologies to study the effect of various oxidizing agents on LDPE.

Method 1: **Oxidation in solution state**

$K_2Cr_2O_7/H_2O/H_2SO_4$ mixture and $KMnO_4/H_2O/H_2SO_4$ mixture was used for the oxidation of LDPE in solution state. 1g of polyethylene was dissolved in minimum quantity of benzene at 90°C. To this solution, appropriate amount of oxidizing agents were added followed by the addition of adogen (Methyltricapryl ammonium chloride) as a phase transfer catalyst. After half an hour oxidation, the product was precipitated in water, dried in vacuum oven at 60°C to a constant weight.

Method 2: **Oxidation in melt state**

Oxidation of LDPE using CAN as oxidizing agent was carried out in melt phase in Brabender plasti-corder. After melting of LDPE granules (25g) at 130°C, CAN (0.1%) were added. Oxidation was done for 10 minutes. Then, the product was taken out from the mixer, dissolved in toluene and washed with water and acetone. Dried in vacuum oven at 60°C to a constant weight.

Method 3: **Oxidation in solid state**

Photo oxidation of LDPE was carried out by placing thin films of LDPE under UV lamp (230V 50Hz) for half an hour.

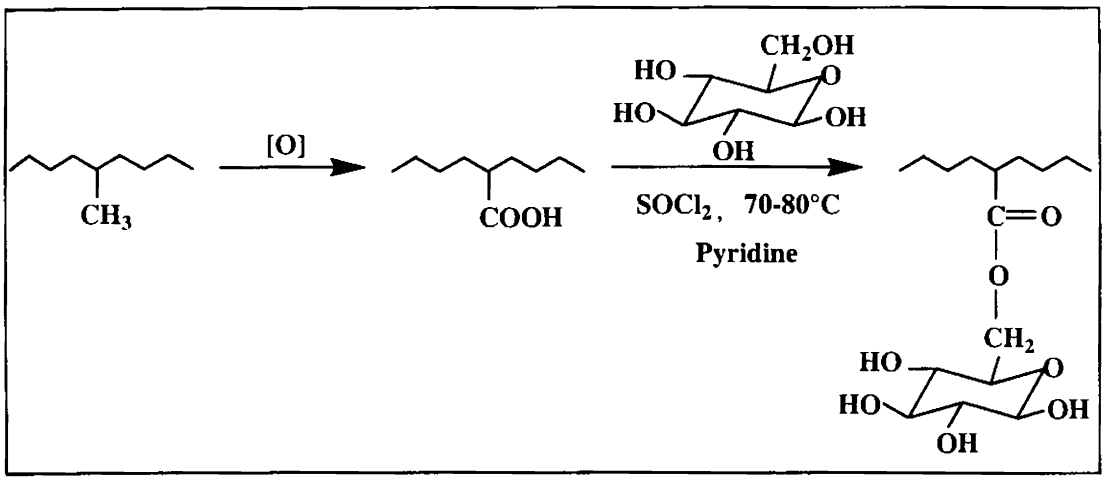
The extent of oxidation was studied by treating the oxidized samples of LDPE with NaOH/EtOH mixture for one hour[13]. The product was then washed with ethanol and dried in vacuum oven.

2.3.3. Synthesis of Sugar Grafted Polyethylene

Different methods were used for the grafting of glucose onto carboxyl functionalized LDPE (LDPE-COOH) and ketone carbonyl functionalized LDPE (OxiLDPE).

Method 1: **Solution grafting by Acid chloride method**

Grafting of glucose onto carboxyl functionalized LDPE was done *in situ* by using $SOCl_2$ as given in Scheme 2.1. 1g of LDPE-COOH dissolved in toluene were taken in a RB flask fitted with a guard tube. 2g of glucose was taken and dissolved in 4ml of pyridine. This solution was added to the LDPE-COOH/toluene mixture followed by the slow addition of 3ml of thionyl chloride. The contents were stirred for 12h at 70-80°C. The product was precipitated in water and dried in vacuum oven at 60°C to a constant weight.



Scheme 2.1. Synthetic procedure of the grafting of glucose onto LDPE by acid chloride method.

Method 2: **Melt grafting in Brabender plasti-corder**

Sugar grafting onto ketone carbonyl functionalized LDPE by the ring opening of glucose in presence of CAN was carried out by melt phase reaction in Brabender plasti-corder fitted with a measuring mixer (W-50) preset at 130°C and a rotor speed of 60rpm in air. The advantage of melt grafting over solution method is that in this case both oxidation and grafting could be done *in situ*. Torque generated during grafting was recorded with time. A general procedure for the melt grafting of glucose onto LDPE is given below:

25g of LDPE were put into the mixer preset at 130°C. After the melting of LDPE for 2 minutes, CAN was added. Oxidation of LDPE as described earlier was carried out for the first 10minutes followed by the addition of glucose. Reaction continued until the increase of torque after the flux got stabilized. Un-reacted glucose and CAN were removed by dissolving the

sample in toluene and washed thoroughly with water by using mechanical stirrer. The product was filtered through a sintered glass crucible and dried at 60°C in vacuum oven to a constant weight. The percentage grafting and grafting efficiency were determined as per the equations given below[17].

$$\%\text{Grafting} = [(W_g - W_o) / W_o] \times 100$$

$$\text{Grafting efficiency} = [(W_g - W_o) / (W_t - W_o)] \times 100$$

where W_g and W_o denote the weights of the grafted LDPE and initial weight of LDPE, respectively. W_t is the weight of the resulted product after melt reaction. Experiment was repeated at different percentages of CAN and glucose and also at different temperature conditions. **Su-g-LDPE** having a maximum grafting of 16% was chosen for further studies.

Hydroxyl values of the grafted LDPE samples were analyzed by adding a standard solution of pyridine-acetic anhydride to the toluene solution of the sample and refluxing for 4h. Excess of acetic acid was back titrated with standard KOH. Hydroxyl value was calculated as the number of milligram of KOH per gram of the sample[18].

2.3.4. Water Absorption

Water absorption test was carried out according to ASTM D750-95. Compression molded specimens (5x5cm) of **Su-g-LDPE**, CAN oxidized LDPE (OxiLDPE) and LDPE were prepared, dried in vacuum oven at 60°C for 24h and cooled in a desiccator until it attained a constant weight. The conditioned samples were placed in a container of distilled water maintained at 30°C. The samples were taken out from the water at regular intervals, gently blotted with

a tissue paper, weighed immediately, and replaced in the water. The percentage increase in weight during the immersion in water was calculated according to the equation.

$$\% \ W_f = [(W_w - W_d) / W_d] \times 100$$

where W_f is the final increased weight percentage, W_d and W_w are the weights of the samples when dry and after moisture uptake, respectively. The average of three values was recorded.

2.3.5. Soil Burial Test

Biodegradation was studied by soil burial method as per ASTM D5338-98. Soil-based compost was taken in 3 compost chambers. The first was for control sample (LDPE alone) and the other two for **Su-g-LDPE** and OxiLDPE respectively. Humidity of the soil was maintained at 40-45% by sprinkling water. The chambers were stored at about 30-35°C. Rectangular polymer films (10x2cm) prepared by compression molding were buried completely into the wet soil at a depth of 10cm. Samples were removed from the soil at constant time intervals and washed gently with distilled water and dried in vacuum oven at 60°C to a constant weight. Weight ratio of the samples with respect to time was recorded as a measure of biodegradation.

2.3.6. Exposure to Aerobic Microorganism

In vitro enzymatic degradation of **Su-g-LDPE** was carried out using a pure soil bacterial culture *Pseudomonas sp. Pseudomonas* culture was routinely maintained in potato dextrose agar (PDA). The culture was inoculated into basal salt medium containing K_2HPO_4 ($0.5gl^{-1}$); $MgSO_4.7H_2O$ ($0.02gl^{-1}$);

$CaCl_2.2H_2O$ (0.01gl^{-1}); NH_4NO_3 (3gl^{-1}); $FeSO_4.7H_2O$ (0.01gl^{-1}); $MgSO_4.H_2O$ (0.5mgl^{-1}); $ZnSO_4$ (0.5mgl^{-1}); $CuSO_4.5H_2O$ (0.5μgl^{-1}); $CoCl_2.6H_2O$ (0.5μgl^{-1}). The pH of the medium was adjusted to 7.2 prior to sterilization. The medium was sterilized at 121°C for 20minutes. LDPE and **Su-g-LDPE** samples were surface sterilized with 0.5% ethanol for 2 hour and then added separately to the sterilized medium in conical flasks. A conical flask containing the sterilized medium without any sample was taken as control. These were incubated at 37°C. The optical density of the medium was monitored as a measure of bacterial growth after five days and continued over a period of four weeks at 388nm.

2.3.7. Measurements

The FTIR spectra of compression-molded films were recorded with a Nicolet Magna 560 IR instrument. Tensile properties were determined according to ASTM D638 using a universal testing machine, Instron Model 1193. Rectangular strips of specimens were conditioned at ambient temperature (22.0±2.0°C) and relative humidity (50±5%) before testing. A crosshead speed of 100mm/min, gauge length of 32.0mm and a width of 5.0mm were used. Differential scanning calorimetry (DSC) was performed with a TA instrument DSC 2920 connected with thermal analyst 2100 system under N_2 at a heating rate of 10°C/min in the temperature range of 50-200°C. The instrument was calibrated using indium. Every thermogram was repeated at least twice and the second heating run was taken as the result. Thermo gravimetric analysis (TGA) was conducted with a Du Pont TGA 951 connected with thermal analyst 2000

system under N_2 at a purge rate of 90ml/min. Samples were heated from room temperature to 600°C at a heating rate of 20°C/min. The inherent viscosity (η_{inh}) was measured with an Ubbelhode viscometer thermostated at 90±0.5°C at a concentration of 0.05dL/g with toluene as solvent. The WAXD patterns were recorded on a Rigaku D_{max} powder diffractometer with Ni-filtered Cu K_α radiation (2θ = 0 - 45°). Contact angle measurements of the samples were done using contact angle goniometer (Kernco Instruments Co. Inc., Texas, USA). The surface morphology of **Su-g-LDPE** samples before and after biodegradation was observed by means of a Scanning Electron Microscopy (Hitachi 2403A, Japan) after coating the surface by sputtering with gold (10-20nm thick). Optical density measurements of the medium were done using a UV-VIS spectrophotometer, Spectronic Model 21.

2.4. Results and Discussion

2.4.1. Functionalization of Polyethylene by Oxidation

Functionalization of LDPE is expected to enhance the anchoring of carbohydrate moieties to the highly hydrophobic polymer, which in turn can increase the active sites for microbial attack and biodegradation of polyethylene. In the present work mainly three methodologies were compared to study the effect of various oxidizing agents on LDPE. LDPE in benzene solution was treated with $K_2Cr_2O_7/H_2O/H_2SO_4$ mixture as explained in the experimental part and the product was analyzed by FTIR. A strong absorption observed at 1715cm^{-1} in the FTIR spectra of the product, correspond to

carbonyl stretching mode indicating the formation of -COOH group (Figure 2.1a). In order to confirm the formation of carboxyl group, the product was deprotonated by treatment with NaOH/EtOH mixture and the resulting product was analyzed by FTIR. A decrease in intensity of absorbance at 1715cm^{-1} and appearance of a new peak at 1566cm^{-1} was observed (Figure 2.1b). The formation of carboxyl groups (LDPE-COOH) during oxidation was confirmed by the carboxylate peak (LDPE-COO^-Na^+) at 1566cm^{-1} formed by the deprotonation of carboxyl group of oxidized LDPE. Bergbreiter et al. reported that on deprotonation, oxidized LDPE prepared by oxidation with CrO_3-H_2SO_4 mixture showing the disappearance of the acid carbonyl peak at 1710cm^{-1} and appearance of carboxylate peak at 1560cm^{-1} [13].

The experiment was further repeated in presence of $KMnO_4/H_2O/H_2SO_4$ mixture and the product was analyzed by FTIR spectroscopy. A strong absorption observed at 1715cm^{-1} with increased intensity compared to that in the case of $K_2Cr_2O_7/H_2O/H_2SO_4$ oxidized LDPE, correspond to carbonyl stretching mode indicating the formation of -COOH group (Figure 2.1c). The results indicated that in the case of LDPE oxidation, $KMnO_4/H_2O/H_2SO_4$ system is more effective than that of $K_2Cr_2O_7/H_2O/H_2SO_4$ system. This observed result might be attributed to the corresponding increase in oxidation/reduction potential of MnO_4^- [19]. The formation of carboxylic group (LDPE-COOH) was further confirmed by deprotonation with NaOH/EtOH mixture and the FTIR of resulting product showed a decrease in intensity of peak at 1715cm^{-1} and the appearance of new carboxylate peak at 1566cm^{-1}

(Figure 2.1d). Oxidation was also carried out in the solid state by UV irradiation. FTIR spectra of the product showed a strong absorbance at 1715cm^{-1} indicating the formation of COOH group (Figure 2.1e). Kim et al. also reported the formation of carboxyl groups on the surface of HDPE oxidized by low energy ion beam irradiation[20].

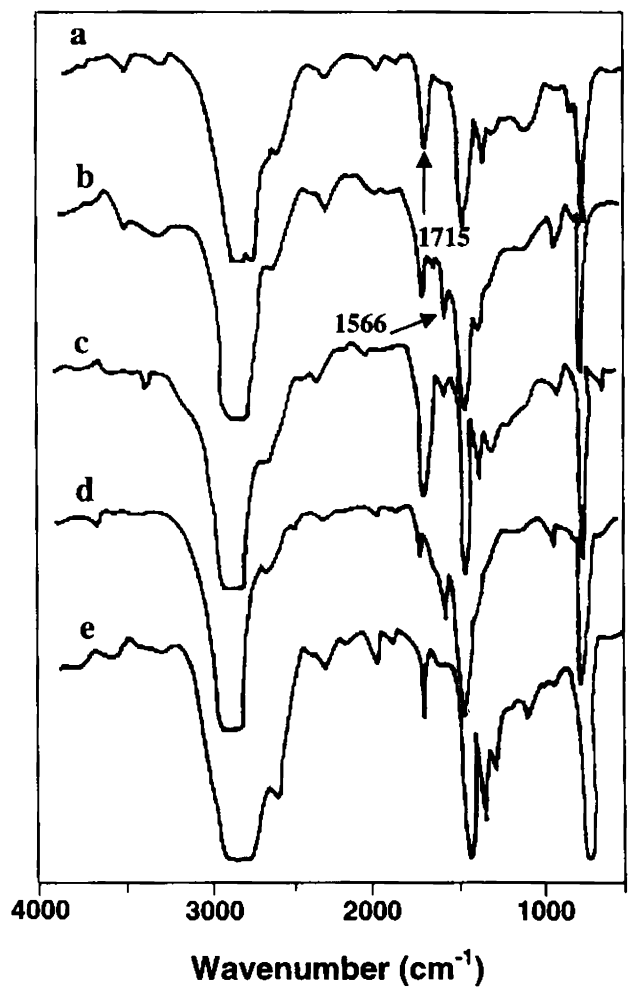


Figure 2.1. FTIR spectra of (a) $K_2Cr_2O_7/H_2O/H_2SO_4$ oxidized LDPE (b) Deprotonated $K_2Cr_2O_7/H_2O/H_2SO_4$ oxidized LDPE (c) $KMnO_4/H_2O/H_2SO_4$ oxidized LDPE (d) Deprotonated $KMnO_4/H_2O/H_2SO_4$ oxidized LDPE (e) UV oxidized LDPE.

Oxidation of LDPE in melt in the presence of CAN was carried out in Brabender plasti-corder. FTIR of the CAN oxidized LDPE (OxiLDPE) showed an absorption at 1719cm^{-1} corresponding to ketonic carbonyl stretching mode (Figure 2.2a & 2.2b). In order to confirm the formation of ketonic carbonyl groups rather than the formation of carboxyl groups during CAN oxidation, the product was treated with NaOH/EtOH mixture. The FTIR spectra of the resulting product showed no change in the intensity of absorbance at 1719cm^{-1} and no formation of new carboxylate peak at 1566cm^{-1}.The formation of ketonic carbonyl groups can affect the chain length probably due to the chain breaking of LDPE at branching positions during functionalization as indicated by a decrease in inherent viscosity of LDPE from 0.87 to 0.74dL/g of OxiLDPE. These results confirmed that there is no -COOH group formation in the case of LDPE when CAN is used as an oxidant unlike $KMnO_4/H_2O/H_2SO_4$ system, $K_2Cr_2O_7/H_2O/H_2SO_4$ system and UV irradiation.

2.4.2. Sugar Grafted Polyethylene

2.4.2.1. Esterification of LDPE-COOH by Acid Chloride Method

The anchoring of glucose on to LDPE was tried by condensing the carboxyl functionalized LDPE with glucose by an *in situ* acid chloride route in toluene (Scheme 2.1). No considerable yield was obtained by esterification in solution. The low yield observed may be attributed to the inhomogeneity of glucose and LDPE in toluene medium.

2.4.2.2. Sugar Grafting of OxiLDPE using CAN

As solution grafting was found to be cumbersome, grafting of sugar on to OxiLDPE was done by melt phase reaction in presence of CAN in Brabender plasti-corder at high shear conditions. CAN based reactions of glucose and starch, in general, use an acid in the presence of water for increasing the concentration of Ce^{4+} species by favoring the reverse reaction[11,21].

$$Ce^{4+} + H_2O \rightleftharpoons [Ce(OH)]^{3+} + H^+$$

The present method of reactive grafting in melt avoids the use of any acid and water in the grafting reaction in presence of CAN for the first time. The presence of moisture in the system could also be completely eliminated as grafting was conducted at a temperature above 100°C.

The %grafting was studied at various LDPE:Glucose feed ratios and at different initiator concentrations. The results obtained are shown in Tables 2.1 & 2.2. It was observed that on increasing monomer concentration in the feed upto 75:25 LDPE:Glucose, %grafting increased initially, and then reached a plateau condition with further increase in monomer concentration. A similar increase in percentage grafting was observed in the case of initiator concentration also upto 0.1% CAN. With further increase in initiator concentration, no considerable increase in grafting was observed in the melt reaction. A maximum grafting of 16% was obtained at a feed ratio of 75:25 LDPE:Glucose and at 0.1% CAN as initiator. The grafting reaction was repeated at different temperatures in order to optimize the grafting conditions.

It was found that as the temperature increased above 150°C, the yield was quite low and the product obtained was slightly brown in colour, which may be due to the onset of degradation of glucose. When the temperature was reduced, the %grafting decreased with decrease in temperature upto 110°C below which no considerable grafting was observed. The optimum temperature for the effective grafting was found to be at 130°C. As both the glucose and LDPE have totally different polar character, they are generally immiscible. But, under high shearing conditions in Brabender plasti-corder at 130°C, it was possible to obtain a homogeneous mix of glucose and LDPE leading to better grafting within short duration. The hydroxyl values of **Su-g-LDPE**s given in Table 2.1 shows an increase with increase of %grafting and at 16% grafting the value is 316mg of KOH per gram of sample indicating the presence of hydroxyl groups on LDPE.

Table 2.1. Effect of glucose concentration on the synthesis of Su-g-LDPE

LDPE:Glucose	%Grafting	Grafting efficiency (%)	Hydroxyl value (mg of KOH/gm of sample)
90:10	7.50	32.58	158
80:20	13.14	49.33	189
75:25	16.00	72.98	316
70:30	15.58	64.23	270
60:40	15.84	69.76	291
50:50	15.70	60.76	254

Initiator (CAN):0.1%, Temperature: 130°C, rpm: 60, Atmosphere: Air.

Table 2.2. Effect of initiator concentration on the synthesis of Su-g-LDPE

Initiator (CAN) (%)	%Grafting	Grafting efficiency (%)
0.05	4.14	10.33
0.10	16.00	72.98
0.50	15.90	69.89

LDPE:Glucose feed ratio 75:25, Temperature: 130°C, rpm: 60, Atmosphere: Air.

2.4.3. FTIR Analysis

The FTIR spectra of **Su-g-LDPE** given in Figure 2.2c showed additional absorptions at 3376, 1036 and 1076cm^{-1} compared to that of OxiLDPE (Figure 2.2b) corresponding to $\nu_{O\text{-}H}$, $\nu_{C\text{-}Osym}$ and $\nu_{C\text{-}Oasym}$ respectively, indicating the presence of sugar molecules in the grafted LDPE. The absence of peaks at 1235, 910 and 800cm^{-1} corresponding to the cyclic ether structure of glucose and the appearance of peaks at 1146 and 940cm^{-1} corresponding to aliphatic ether may be attributed to the ring opening of glucose during reactive grafting in presence of CAN (Figure 2.2c & 2.2d). The FTIR spectra of the product obtained in the absence of CAN did not show significant changes in absorptions on comparing with FTIR spectra of LDPE indicating the absence of sugar grafting when CAN was not used as a catalyst.

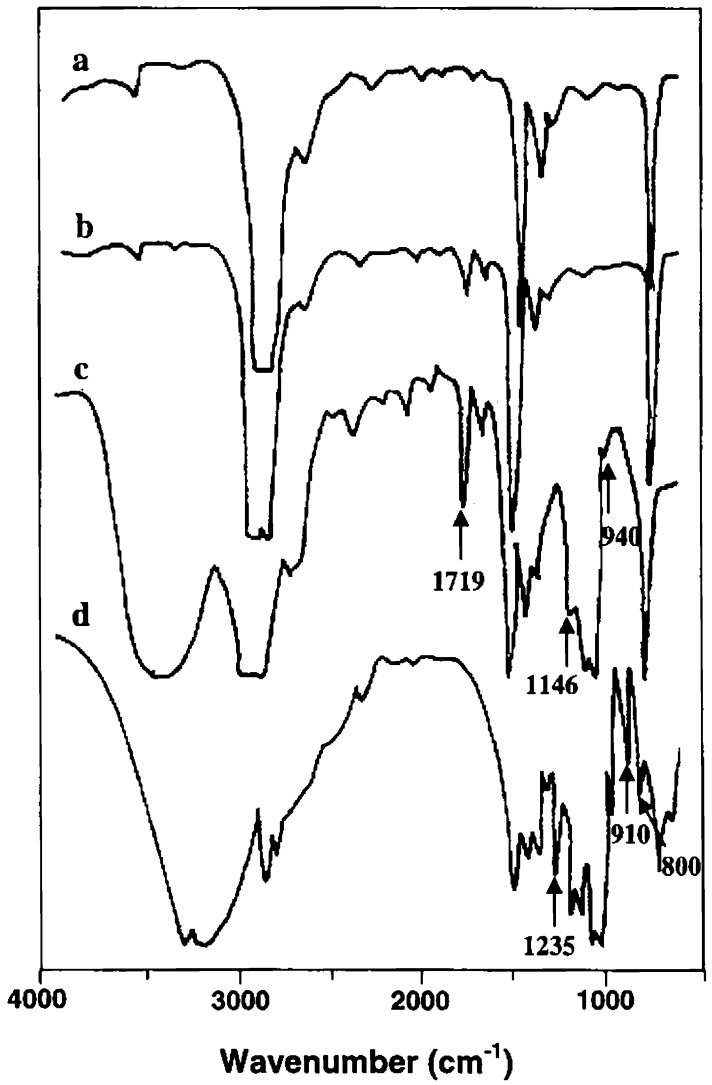
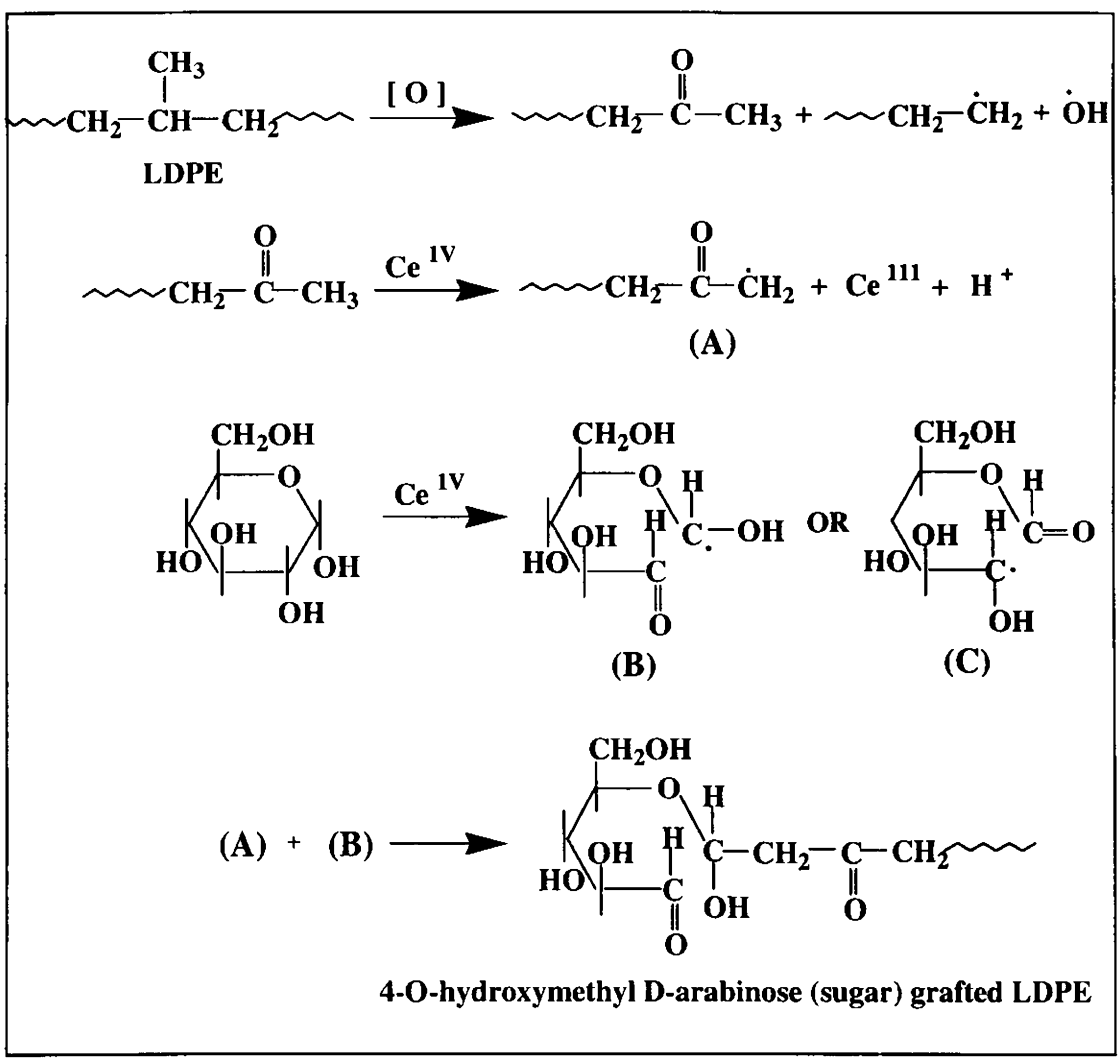


Figure 2.2. FTIR spectra of (a) LDPE, (b) OxiLDPE, (c) Su-g-LDPE, (d) Glucose.

CAN is understood to act both as an oxidizer and as an initiator in the events that lead to the formation of the grafted products. Carbonyl groups are formed initially during oxidation of LDPE in presence of CAN at the tertiary carbons. The high temperature and the oxidizing conditions can generate CH_2 radicals in the OxiLDPE at the α-carbon of the carbonyl groups[22].

Simultaneously, CAN initiates ring opening of glucose between C_1 and C_2 positions resulting in the formation of 4-O-hydroxymethyl D-arabinose free radical that gets grafted through a radical mechanism to give 4-O-hydroxymethyl D-arabinose (Sugar) grafted LDPE (**Su-g-LDPE**)[11]. These events can be depicted in Scheme 2.2.



Scheme 2.2. Synthetic procedure of Su-g-LDPE in presence of CAN.

The increase in absorption observed at 1719 cm^{-1} in the FTIR of **Su-g-LDPE** compared to OxiLDPE may be attributed to the contribution of $\nu_{C=O}$ from -CHO group of 4-O-hydroxymethyl D-arabinose further confirming the grafting mechanism (Figure 2.2b & 2.2c). An increase in inherent viscosity of OxiLDPE was observed from 0.74 to 0.78 dL/g after grafting. This may be as a result of the hydrogen bonding effect of hydrophilic moieties grafted onto OxiLDPE, further confirmed the formation of **Su-g-LDPE**.

2.4.4. Torque Behavior as a Measure of Grafting

All the compositions of **Su-g-LDPE** exhibited an increase in torque with time and finally got stabilized within 20minutes after the flux. This increase in torque observed with time could be attributed to the increase in the melt viscosity by incorporation of glucose onto LDPE, indicating the successful grafting of sugar moieties onto LDPE by melt phase reaction. Increase in torque during processing and increase in the melt viscosity are often quoted as evidence for the grafting of polar functionality[12]. This increase in shear stress of **Su-g-LDPE** may be explained in terms of the strong hydrogen bond interactions between hydroxyl groups of grafted sugar moieties and with ketonic carbonyl groups formed on polymer backbone by oxidation. Figure 2.3 shows the effect of CAN as an initiator in the torque–time behavior of **Su-g-LDPE**. It is observed that during melt reaction between glucose and LDPE, the torque after the flux got increased only in presence of CAN while in the absence of CAN, there was no increase in torque after the flux. This showed

that CAN play a significant role in the grafting of glucose and in the absence of CAN there was no reaction taking place.

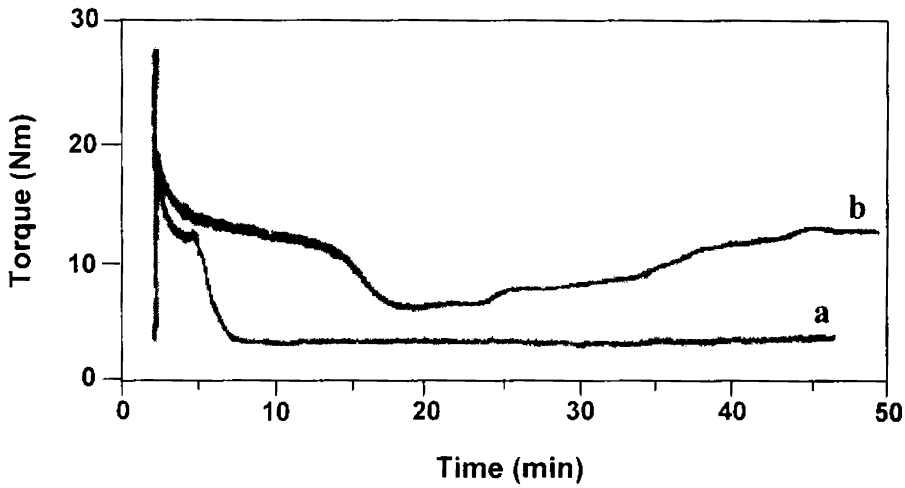


Figure 2.3. Effect of CAN as an initiator in the torque–time behavior of Su-g-LDPE: (a) in the absence of CAN, (b) in the presence of CAN.

2.4.5. Effect of Sugar Grafting on Mechanical Properties

The variation of tensile strength and Young's modulus with %grafting is shown in Figure 2.4. There is an initial decrease in tensile strength (36%) observed upto 7.5% sugar grafting whereas on further increase in percentage grafting, tensile strength of the grafted LDPE showed a steady increase. At the maximum grafting of 16%, tensile strength of grafted LDPE reached a value 7.82MPa, which is nearer to 8.46MPa of LDPE alone. The observed initial decrease in the mechanical properties of LDPE at lower %grafting may be attributed to the decrease in non-polar interactions (Van der Waals) between the polyethylene chains. As the sugar particles are highly hydrophilic, the strong hydrogen bond interaction between LDPE chains through grafted sugar

moieties at higher %grafting might have increased the stress needed for chain alignment, chain slippage and ultimately to chain breakage resulting in increased tensile strength[23]. The percentage reduction in tensile properties of LDPE, when grafting of sugar was carried out in presence of CAN is only 7.5 % in comparison to that of 20% of starch-polyethylene blend with 15wt% of starch as reported by Danjaji et al.[4]. This indicates that **Su-g-LDPE** has advantages over starch-LDPE composites for applications where mechanical properties are critical. Young's modulus of LDPE, which is a measure of the stiffness of the material, also showed a similar trend.

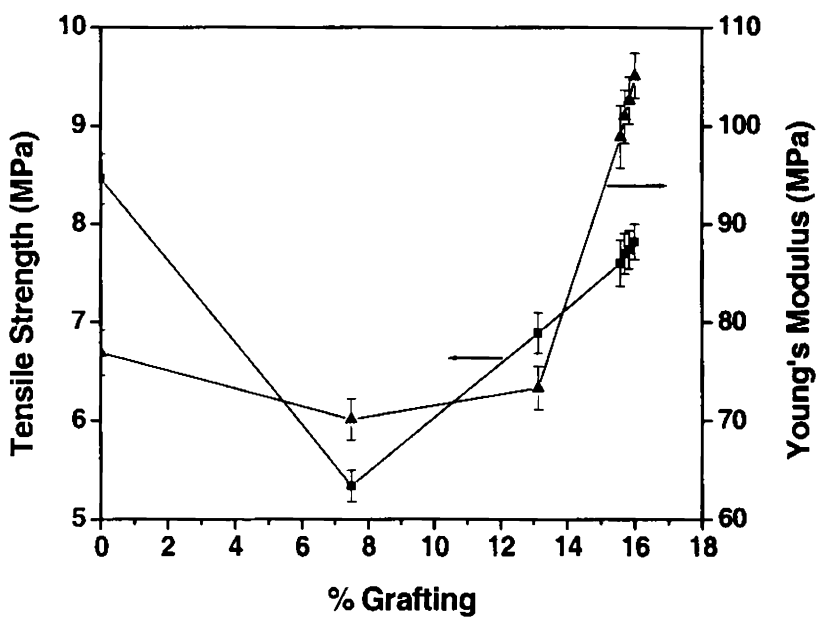


Figure 2.4. Effect of sugar grafting on mechanical properties of LDPE.

2.4.6. Thermal and WAXD Measurements

DSC thermograms of LDPE and **Su-g-LDPE** given in Figure 2.5a & 2.5b showed endotherms corresponding to the melting transition of the samples

at 109.5 and 111.3°C respectively. Data are given in Table 2.3. The single endotherm observed in DSC thermogram of **Su-g-LDPE** confirms the existence of sugar moieties as grafted particles. The enthalpy changes observed corresponding to first-order transitions were 127.5 and 112.8J/g respectively for LDPE and **Su-g-LDPE**. The melting temperature (T_m) increased slightly but the heat of melting ($\triangle H_m$) showed a decrease in the case of grafted LDPE. The observed slight increase in T_m of **Su-g-LDPE** may be due to the hydrogen bonding interactions between the grafted LDPE chains.

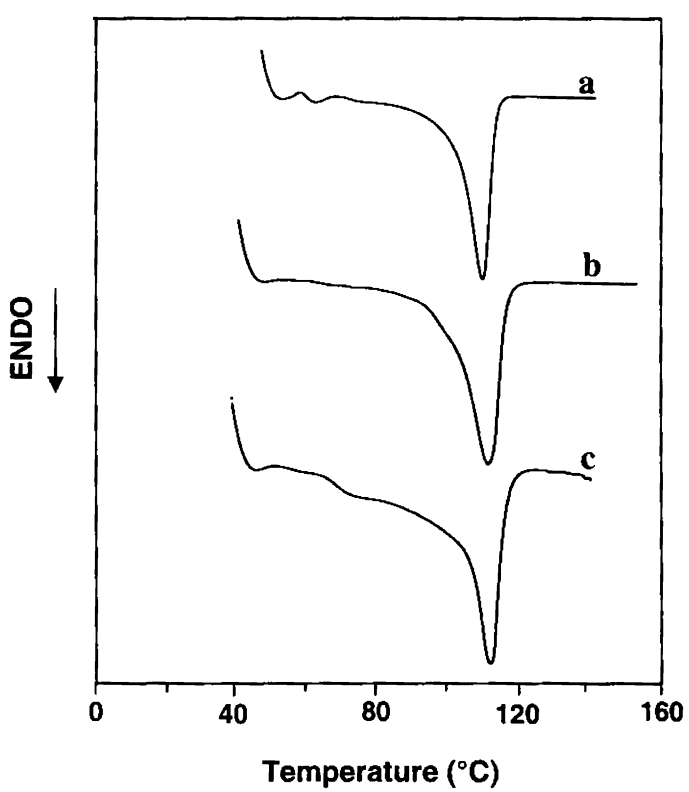


Figure 2.5. DSC thermograms of (a) LDPE, (b) Su-g-LDPE, (c) Biodegraded Su-g-LDPE.

This was further confirmed by WAXD measurements, which showed a broad peak at 2.23° 2θ (d spacing 39.6Å) compared to that of LDPE, indicating

the long range ordering in **Su-g-LDPE** due to the hydrogen bonding interactions between LDPE chains through grafted sugar moieties (Figure 2.6a & 2.6b). Bault et al. reported a similar observation of a d spacing of 37.9Å in a non-intercalated bilayer structure of 6-O-decyl-D-galactitols formed by hydrogen bonding interaction between carbohydrate residues[24]. The observed decrease in the $\triangle H_m$ of **Su-g-LDPE** indicated that crystallinity of LDPE was disturbed by sugar grafting although the melting point showed slight increase possibly due to the hydrogen bonding interaction of the grafted sugar moieties (Table 2.3).

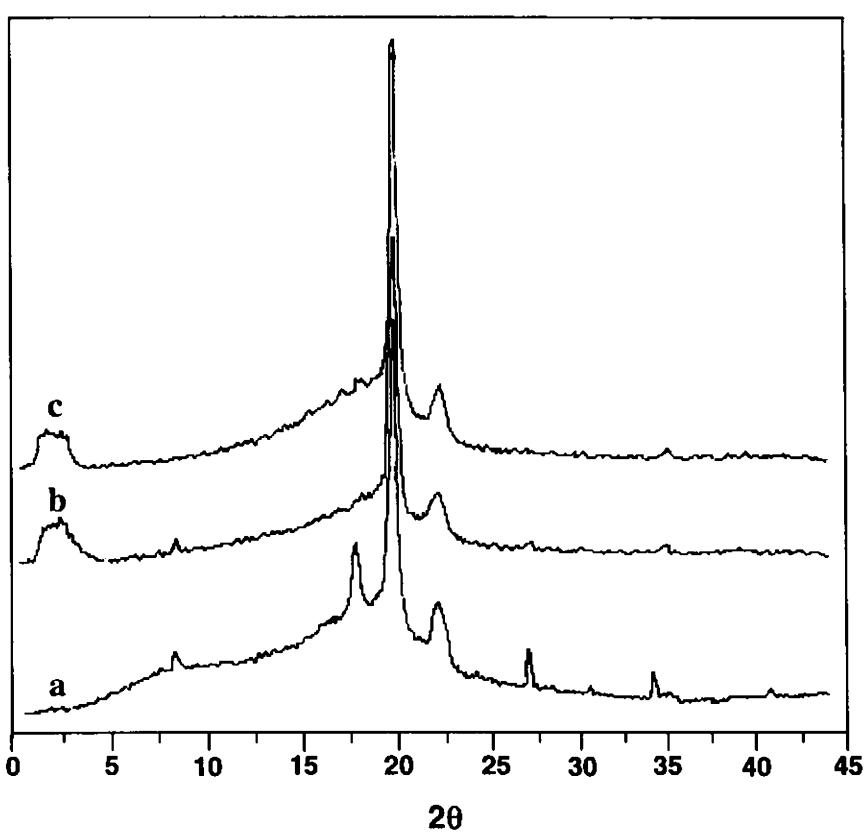


Figure 2.6. WAX-ray patterns of (a) LDPE, (b) Su-g-LDPE, (c) Biodegraded Su-g-LDPE.

Table 2.3. DSC and WAXD data of LDPE samples.

	T_m (°C)	ΔH_m (J/g)	%Crystallinity
LDPE	**109.5**	**127.5**	**66.67**
Su-g-LDPE	**111.3**	**112.8**	**53.85**
Su-g-LDPE (Degraded)	**112.5(70.0)**	**159.6**	**58.65**

TGA thermograms of the LDPE and modified LDPE given in Figure 2.7 indicate a gradual decrease in T_5 (temperature at 5% decomposition) value observed from 348 to 278°C in the thermogram of grafted LDPE compared to LDPE showing the decreasing trend in thermal stability with increase in % grafting of **Su-g-LDPE**.

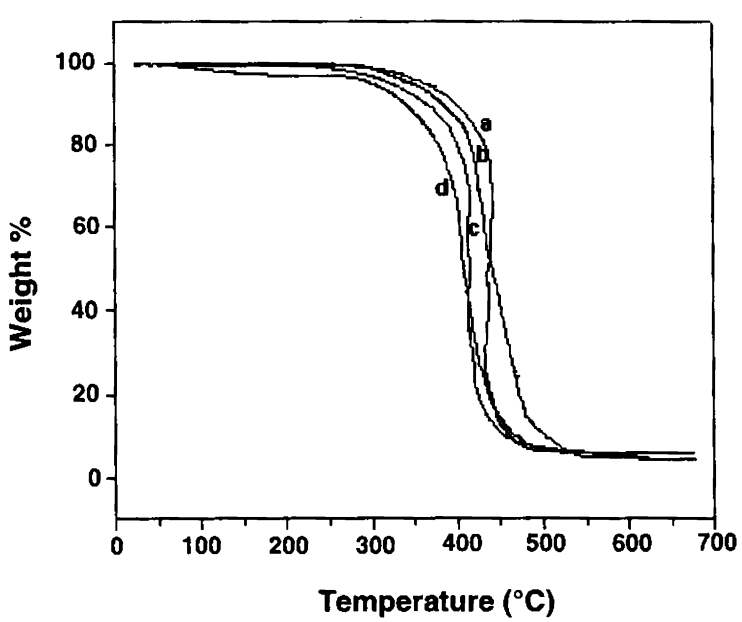


Figure 2.7. TGA thermograms of (a) LDPE, (b) 90:10 LDPE:Glucose, (c) 60:40 LDPE:Glucose, (d) 75:25 LDPE:Glucose.

2.4.7. Effect of Grafting on Water Absorption

The extend of hydrophilicity of grafted LDPE was established by percentage water absorption and contact angle measurements. A 5.2% of water absorption observed after 60 days of immersion in water, for the grafted LDPE over LDPE alone indicated the presence of highly hydrophilic sugar moiety derived from glucose grafted onto LDPE (Figure 2.8). The observed decrease in contact angle of LDPE from Θ_a=93° to Θ_a=74° after grafting confirmed further the hydrophilicity of **Su-g-LDPE**.

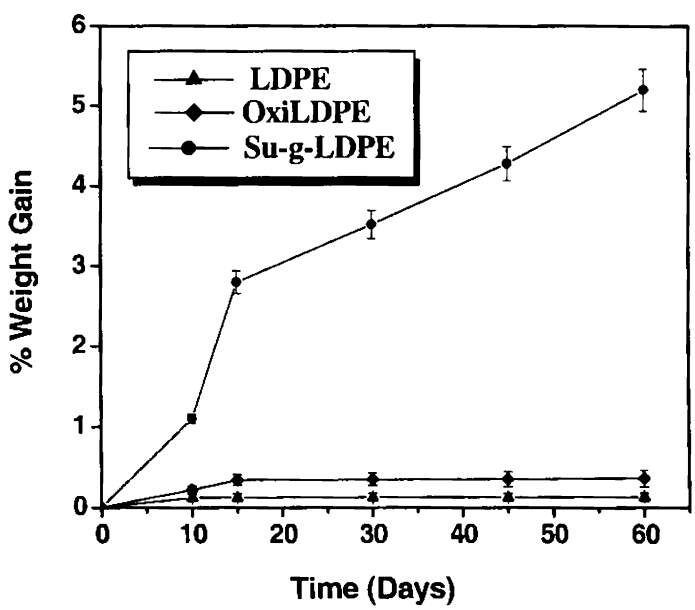


Figure 2.8. Percentage weight gain of LDPE samples during water absorption.

2.4.8. Biodegradation Studies – Soil Burial Test

Biodegradation studies of **Su-g-LDPE**, OxiLDPE and LDPE alone were carried out by soil-burial test as per ASTM D5338-98.

2.4.8.1. FTIR Spectroscopy

FTIR analysis is a valuable tool for biodegradation study of LDPE. The remarkable fact observed in the biodegradation studies of **Su-g-LDPE** was the indication of the initiation of polyethylene degradation, which was reported to be not observed in the starch/LDPE blends[1]. FTIR spectra of biodegraded **Su-g-LDPE** given in Figure 2.9 showed a decrease in the ratio of the absorbance of $\nu_{C\text{-}Osym}$ with $\nu_{C\text{-}H}$, A_{1036}/A_{1465} from 0.94 to 0.77 and $\nu_{C\text{-}Oasym}$ with $\nu_{C\text{-}H}$, A_{1076}/A_{1465} from 0.93 to 0.73 compared to that of the undegraded **Su-g-LDPE**. Similarly a lowering in the ratio of the absorbance of $\nu_{O\text{-}H}$ with $\nu_{C\text{-}H}$, A_{3376}/A_{1465} from 0.93 to 0.75 was also observed.

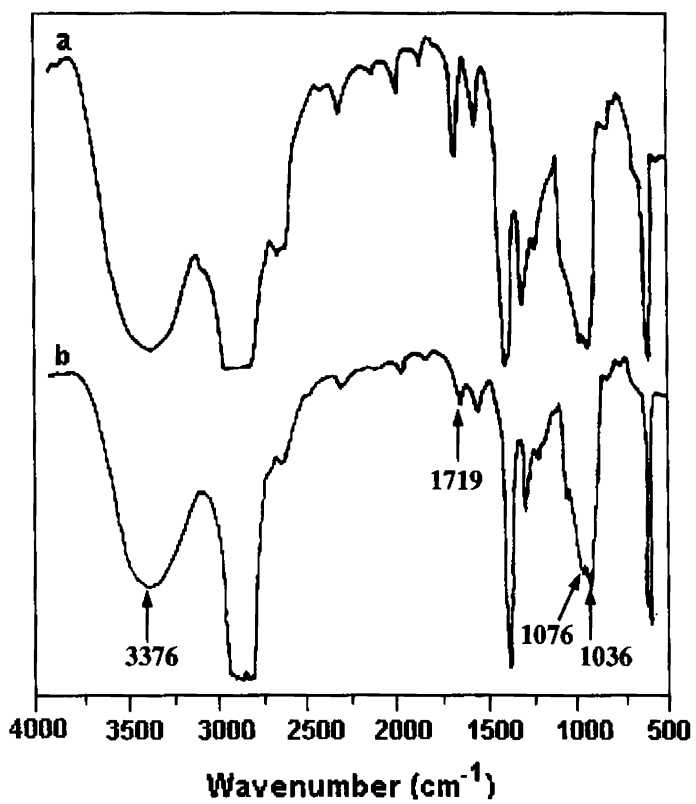


Figure 2.9. FTIR spectra of Su-g-LDPE: (a) before biodegradation, (b) after biodegradation.

This trend, which was observed in **Su-g-LDPE** with biodegradation, indicated that sugar has been continuously removed with time of degradation. Changes in the polyethylene matrix with degradation time were analyzed by two indices calculated from FTIR spectra as used by Albertsson et al. to monitor degradation rates in polyethylene: the 1719/1465 ketone carbonyl index (-C=O) and the 1640/1465 double bond index (-C=C-)[25]. In the case of degraded **Su-g-LDPE**, the double bond (-C=C-) index calculated from the FTIR spectra showed a decreasing trend with time of degradation indicating LDPE degradation.

The carbonyl index (CI) values as a function of time for samples are presented in Figure 2.10. The CI value of OxiLDPE showed an increase of 13% with 12 months of soil burial indicating the microbial oxidation of short chains[26]. In contrast, the grafted polyethylene showed a decrease of 70% in CI at 12 months exposure to soil.

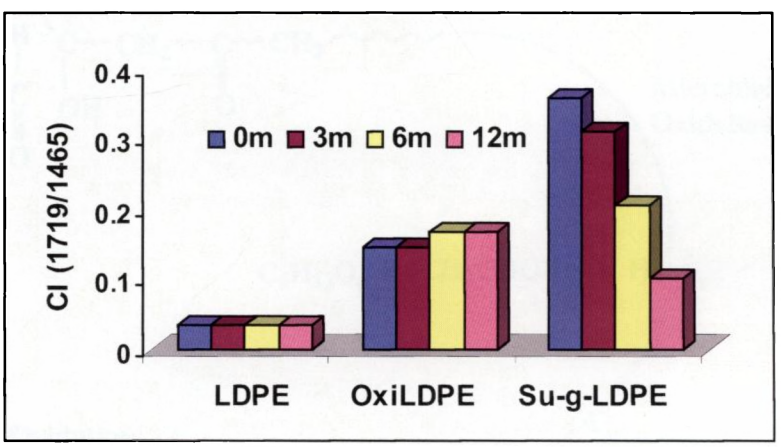
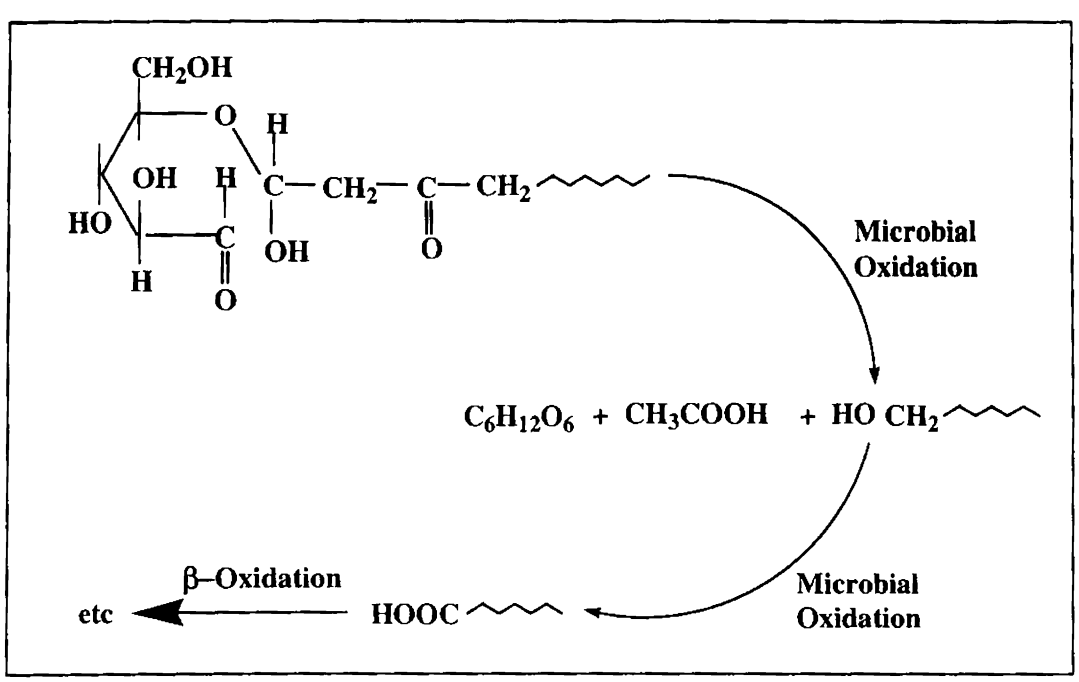


Figure 2.10. Carbonyl index of LDPE samples subjected to biodegradation.

Similar decrease in carbonyl index was also observed for photo irradiated LDPE and thermally treated LDPE under composting conditions and in fungi environment respectively[26,27].

According to Albertsson et al. the initial step of biodegradation of LDPE is oxidation followed by microbial attack[16]. In **Su-g-LDPE**, as the oxidation of LDPE was already done as a part of grafting reaction the first step of the microbial degradation is expected to be over, which can facilitate further biodegradation. Ratledge proposed that microbial oxidation of alkanes can take place at the terminal alkane carbon or at sub terminal position to give ultimately carboxylic acids[28]. Scheme 2.3 depicts a probable route to biodegradation of **Su-g-LDPE** involving the microbial consumption of sugar units followed by the cleavage of LDPE at the chain ends so that the observed decrease in the CI of **Su-g-LDPE** with degradation time can be explained.



Scheme 2.3. Microbial degradation of Su-g-LDPE.

Microbial oxidation thus involves the oxidation of the carbonyl groups to carboxylic acid leaving behind a hydroxyl terminated LDPE. Further degradation can take place through the route of β-oxidation as proposed by Albertsson et al.[25]. It should be noted here that because of the high molecular weight and hydrophobic nature, the biodegradation of LDPE is extremely slow and it may take hundreds of years in normal circumstances[2,29]. The increased hydrophilicity and sugar capped chain ends in the **Su-g-LDPE** might have facilitated the increased degradation observed in the present case. The microbial oxidation of LDPE chains was further confirmed by a decrease in inherent viscosity of **Su-g-LDPE** in comparison to no change in the case of LDPE (Figure 2.11).

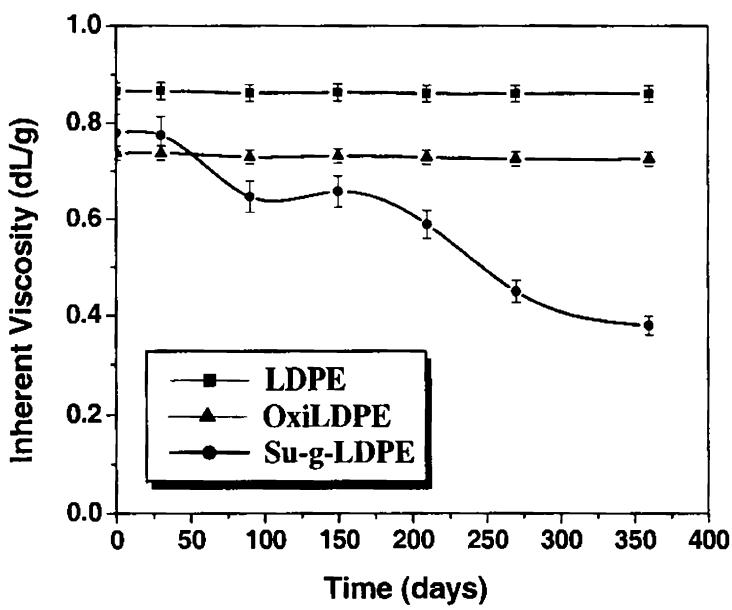


Figure 2.11. Changes in the inherent viscosity of LDPE samples with time of exposure to soil environment.

2.4.8.2. Thermal and WAXD Studies

After twelve months of exposure to compost soil environment, the biodegraded **Su-g-LDPE** was analyzed for crystallinity by DSC and WAXD measurements. DSC trace of degraded **Su-g-LDPE** exhibited an additional broad endotherm at 70.0°C indicating the distribution of different lamellar sizes (Figure 2.5c). A similar observation was reported by Albertsson et al. in the case of biotically degraded LDPE[16]. Enthalpy of fusion of **Su-g-LDPE** showed an increase from 112.8 to 159.6J/g after degradation (Table 2.3). It is generally understood that during biodegradation, the amorphous fraction of the polyethylene, which has higher mobility and hence higher accessibility to microorganisms, is exposed to microbial attack[7]. Therefore, the microbial degradation results in an increase in the overall degree of crystallinity of the polyethylene sample[30]. This observation is further confirmed by WAXD measurements, which show an increase in crystallinity after biodegradation as seen in Figure 2.6c & Table 2.3.

2.4.8.3. Morphological Changes

Figure 2.12 shows the SEM micrographs of the **Su-g-LDPE** exposed to soil environment for 4 and 12 months. SEM micrograph of **Su-g-LDPE** sample after 4 months soil burial shows whitened parts with small and large cavities (Figure 2.12B). Otake et al has reported that the characteristic feature of the biodegradation of LDPE is the whitening[2]. This whitening is brought about by surface erosion due to biodegradation of polyethylene. The occurrence of the small and large cavities on the surface may be due to the absence of a uniform

distribution of short branches or biodegradable products in the polymer matrix[26,27]. Extensive surface erosion of **Su-g-LDPE** caused by microorganisms was observed on studying the micrograph obtained after 12 months soil burial (Figure 2.12C).

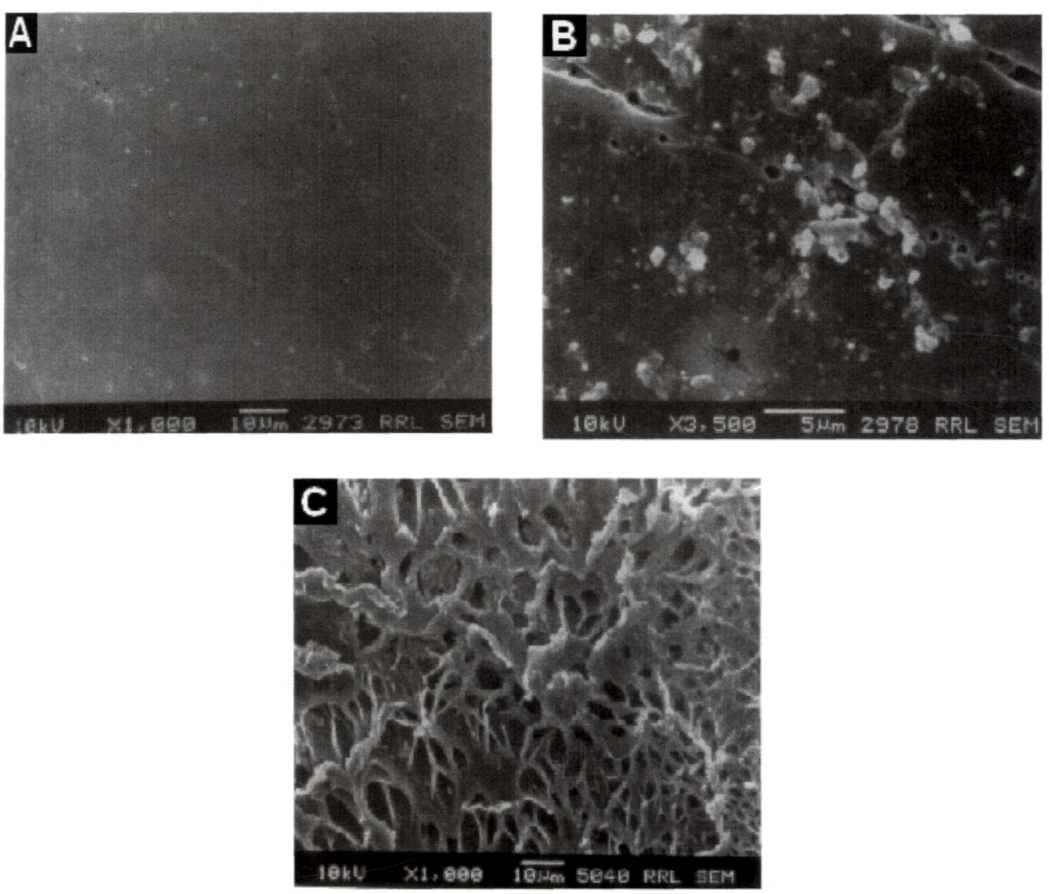


Figure 2.12. SEM micrographs of Su-g-LDPE: (A) before biodegradation, (B) after 4 months biodegradation, (C) after 12 months biodegradation.

2.4.8.4. Weight Loss Studies

Weight ratio of the samples during soil burial test was monitored with time of degradation as shown in Figure 2.13. The curve showed a constant

decrease in weight up to 90 days and after that a sharp decrease in weight was observed up to 270 days in the case of **Su-g-LDPE** where as LDPE and OxiLDPE didn't show any significant change in weight during soil burial test within the same period. These results indicated that the presence of sugar molecules acts as a nutrient source for microbial growth resulting in the initiation of degradation of Su-g-LDPE. In a computer simulation study of the degradation of starch/PE blends, Peanasky et al. showed that removal of starch from the starch/PE blends takes place only above 30vol% starch[6]. In the present case, only 16% glucose has been grafted and 5.2% glucose was lost in 360 days indicating removal of glucose from the surface layer only. As sugar is chemically bound in the present case, the release of sugar could indicate a C-C bond cleavage initiated by microbial attack unlike in starch/PE blends.

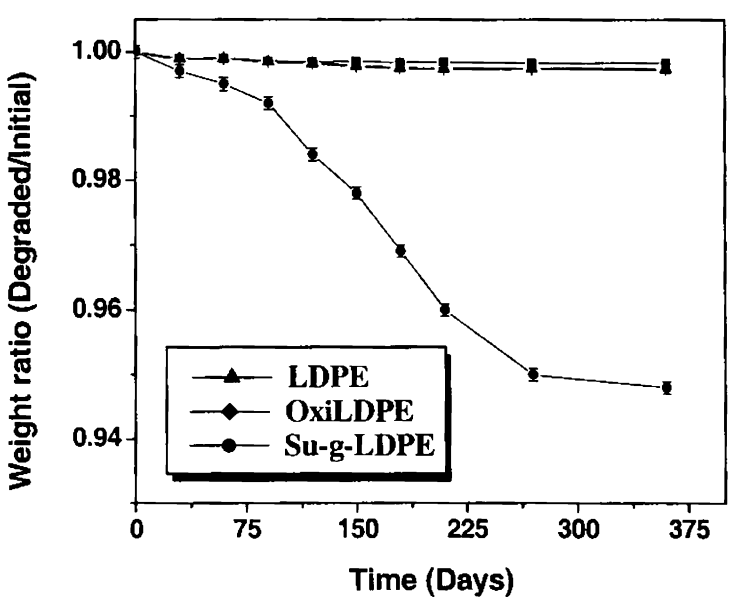


Figure 2.13. Changes in the weight ratio of LDPE samples during biodegradation.

2.4.9. Microbial Degradation by Optical Density Measurements

Degradation of **Su-g-LDPE** was also studied in presence of *Pseudomonas sp.* by following the optical density of the medium with time for 25 days. The rate of increase of optical density for **Su-g-LDPE** in comparison to that of the control and LDPE is shown in Figure 2.14. The increase in optical density with time observed in the case of **Su-g-LDPE** showed the growth of microorganisms in the medium by utilizing highly hydrophilic sugar moieties, which can be used as a nutrient source. Based on these findings, it can be said that the highly hydrophilic moieties on the surface provide a suitable active growth medium for the action of microorganisms on **Su-g-LDPE**.

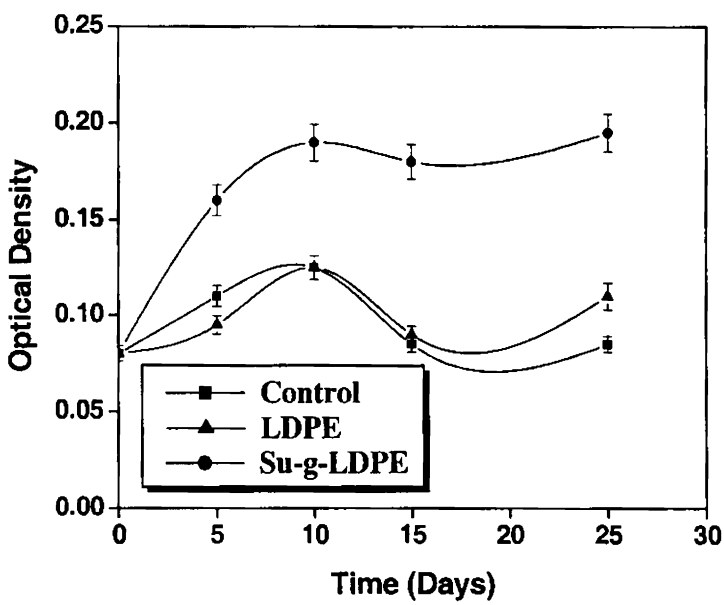


Figure 2.14. Growth pattern of *Pseudomonas sp.* in the medium containing LDPE samples.

2.5. Conclusions

LDPE was modified by sugar grafting to get 4-O-hydroxymethyl D-arabinose end-capped LDPE by the ring opening of glucose during melt phase reaction in Brabender plasti-corder in presence of CAN at 130°C. The role of CAN as an oxidant as well as an initiator in the grafting of the sugar onto LDPE was established. A maximum grafting of 16% was observed at 75:25 LDPE:Glucose feed ratio, at 0.1% CAN concentration. The FTIR spectra of **Su-g-LDPE** showed the characteristic peaks of sugar, confirming grafting. The observed initial decrease in tensile strength and Young's modulus of **Su-g-LDPE** at lower grafting percentage was compensated at higher grafting percentages. The increase in the amount of grafted sugar moieties in the polymer enhances intermolecular interactions such as hydrogen bonding at higher grafting percentages giving rise to improved mechanical properties. The presence of hydrogen bonding was further confirmed by observing a slight increase in melting transition of LDPE after grafting in DSC thermogram and a long range ordering of grafted LDPE chains in WAXD measurements. TGA analysis indicated that the thermal stability of LDPE was not affected deleteriously on sugar grafting. Water absorption and contact angle measurements showed the hydrophilic nature of grafted LDPE. During biodegradation in soil environment, the microbial oxidation of LDPE backbone was established by observing a decrease in CI of **Su-g-LDPE** with time of degradation. A stepwise cleavage of LDPE from the chain ends can be envisaged with microbes consuming the sugar units on the surface of **Su-g-**

LDPE and further oxidizing the ketonic carbonyl to carboxylic acid. The microbial degradation was further supported by the increase in crystallinity and the decrease in viscosity of the degraded samples. SEM showed surface erosion caused by microbial degradation on **Su-g-LDPE**. *In vitro* degradation of **Su-g-LDPE** studied by monitoring the increase in optical density of the media indicating the growth of microbes using grafted sugar as a nutrient source. This suggests that sugar provided not only a condition suitable for the growth of microorganisms but also a favorable environment for the action of microbes on polyethylene.

2.6. References

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CHAPTER 3

CHITOSAN/OLIGO L-LACTIDE GRAFT COPOLYMERS: EFFECT OF HYDROPHOBIC SIDE CHAINS ON THE PHYSICO-CHEMICAL PROPERTIES AND BIODEGRADABILITY

3.1. Abstract

Graft copolymerization of L-lactide (LLA) onto chitosan (CS) was carried out by ring opening polymerization, using $Ti(OBu)_4$ as catalyst in DMSO at 90°C in nitrogen atmosphere, to obtain chitosan/oligoL-lactide graft copolymers (**CL**). Grafting studies indicated that the lactide content in the feed molar ratio influenced the grafting percentage and the amount of lactide in the graft copolymer. The graft copolymers were characterized by FTIR, ^1H NMR, WAXD and thermal methods. Unlike chitosan, all **CL** graft copolymers were converted to hydrogels in aqueous environment. As expected, the swelling ratio was found to be decreasing on increasing the amount of hydrophobic side chains in the graft copolymers. Similarly, the LLA content of the graft copolymers was found to influence their biodegradation, carried out *in vitro* by hydrolytic and enzymatic means. DSC analysis and SEM micrographs of the hydrolytically degraded samples showed a decreasing trend in degradation rate with increase of LLA content. Enzymatic degradation was studied by exposing

the samples to two types of enzymes such as papain from *Carica Papaya* and lipase from *Candida Cylindracea*. Examinations by SEM, weight loss studies, spectroscopic and DSC analysis showed that the biodegradation of the graft copolymers could be controlled by the LLA content. The grafting of LLA onto CS results in **CL** graft copolymers having increased hydrophilicity and controlled degradation rate that may have wide applications in wound dressing and in controlled drug delivery systems.

3.2. Introduction

Biodegradable polymers derived from renewable resources have recently generated much interest in biomedical and environmental applications because of their inherent biodegradability, biocompatibility and easy availability. Chitosan, a biodegradable copolymer of glucosamine and N-acetylglucosamine, is a versatile material of interesting structure and extraordinary properties that gave rise to applications ranging from health care to agriculture to dyes for fabrics to medicine[1]. Additionally, the special properties of chitosan such as multifunctionality, biocompatibility, low immunogenicity, biological activities and structural similarity to natural glycosaminoglycans make it a very valuable material for many applications in biomedical field[2-6]. The extended applications of chitosan, however, are frequently limited by its insolubility in water and neutral pH due to its rigid crystalline nature[7,8]. The *in vivo* degradability of chitosan is possible by lyzosomal enzymes, depending upon their degree of acetylation. It is expected

that the biological and physiological potential of chitosan would increase dramatically with the easy availability of water-soluble and/or water swelling chitosan[9]. Graft copolymers of chitosan and polylactide (PLA) appear to be potential materials in the development of water swelling chitosan.

PLA are the most widely used biopolymer for various applications in the biomedical and environmental fields, because of their resorbability in the human body with the release of non-toxic degradation products and mechanical properties comparable with that of hydrocarbon based polymers such as polyethylene and polystyrene[10-13]. Due to the high capital cost, the focus of PLA has been mainly on the biomedical field. Even though, PLA is considered to be biodegradable, their low hydrophilicity and high crystallinity reduce its rate of degradation, especially in the case of poly(L-lactide)(PLLA)[14,15]. Copolymerization of PLLA with poly(glycolic acid) (PGA) is a possible method to enhance the rate of degradation by disturbing the crystallinity of PLLA[16-18]. But the hydrophobic property of PLGA reduces its applications in drug delivery systems[19]. The accumulation of acidic degradation products, because of the fast degradation of PLA by the autocatalytic action of carboxylic acid end groups, results in poorer soft tissue compatibility[20-23]. The lack of these properties along with its increased use in medicine have attracted much research interest in synthesizing new materials that have better properties for specific applications in the field.

The physico-chemical properties and biodegradability of CS and PLLA could be enhanced substantially by graft copolymerization of L-lactide onto

chitosan. Controlled solvation and degradation could be achieved by controlling the ratio of chitosan:L-lactide in the graft copolymer to obtain an optimum hydrophobic-hydrophilic balance. In addition, the alkalescence of chitosan can neutralize the acidic degradation products of polylactide[24-26]. The local toxicity due to the acid byproducts can thus be alleviated to get better biocompatibility. Chitosan/L-lactide graft copolymers are thus expected to have improved applications in biomedical and pharmaceutical fields than CS and PLLA. Since CS being the second most abundant biopolymer in the nature, the overall cost of the graft copolymers should be economically feasible.

Only very limited work has been reported on the copolymerization of lactide and chitosan. Yao et al. reported the *in vitro* fibroblast static cultivation on a cytocompatible poly (chitosan-g-L-lactic acid) film and the cell growth rate on the copolymer film was found to be much faster than that of the chitosan film[25]. In another work, Liu et al. have reported the synthesis of a brush like copolymer of polylactide grafted onto chitosan[24]. Later, Wu et al. studied the amphiphilic properties of a graft copolymer of water-soluble chitosan and polylactide prepared by using triethyl amine as catalyst[27]. The first attempt to synthesize a pH sensitive physically cross linked hydrogel by grafting D,L-lactic acid onto amino groups in chitosan without using a catalyst was reported by Albertsson et al.[28]. The biodegradability of these chitosan/polylactide graft copolymers was, however, not studied in these works. One can use a ring opening polymerization catalyst to build up the polylactide chains and simultaneously anchor the chains onto chitosan by

grafting. The ring opening polymerization of L-lactide using a covalent initiator would significantly reduce the risk of racemization even at high temperatures in comparison to other polymerization methods[29].

This chapter deals with the efforts undertaken to explore the potential of CS and PLLA by synthesizing chitosan/L-lactide graft copolymers, so that some of the problems associated with them can be minimized. Preliminary experiments indicated that $Ti(OBu)_4$ as ring opening catalyst in DMSO gives a graft copolymer having the natural polysaccharide chitosan as the main chain and the artificial biopolymer oligoL-lactide (OLLA) as the side chain. The effect of hydrophobic side chains on the physico-chemical properties of graft copolymers were analyzed by FTIR, ^{1}H NMR, WAXD, TGA and DSC. *In vitro* biodegradation studies were done, for the first time, to study the effect of lactide side chains on the biodegradation of **CL** graft copolymers. Degradation was monitored by weight loss and further confirmed by SEM, FTIR, ^{1}H NMR and DSC analysis.

3.3. Experimental Section

3.3.1. Materials

L-Lactide and $SnOct_2$ were purchased from Aldrich Chemical Company, USA and were used as received. Chitosan ($\bar{M}_v$= 1.7 x 10^5, DD = 90%) was purchased from India Sea Foods Pvt Ltd Kochi, Kerala. The viscosity average MW of the chitosan was calculated by the Mark-Houwink equation: $[\eta] = K_m M^a$, where K_m = 3.5 × 10^{-4}, a = 0.76. OLLA ($\bar{M}_n$= 800) was prepared by

ring opening polymerization of L-lactide using $SnOct_2$ at 130°C in N_2 atmosphere. DMSO, DMF, DMA, and pyridine were obtained from S.D.Fine-Chem Ltd, Mumbai and were used after distillation. $Ti(OBu)_4$, $SnCl_4$, LiCl, ethyl acetate, NaH_2PO_4, Na_2HPO_4, cystein HCl and EDTA were collected from S.D.Fine-Chem Ltd, Mumbai and were used as received. Lipase was purchased from Fluka Biochemika, Sigma-Aldrich, USA and papain from Sisco Research Laboratories Pvt Ltd, Mumbai.

3.3.2. Synthesis of Graft Copolymers

Chitosan (2g) in 10ml of DMSO was taken in a two-neck RB flask. Nitrogen was purged for half an hour followed by the addition of appropriate amount of L-lactide and $Ti(OBu)_4$ (1.2×10^{-5} mol). Reaction was carried out for 24h at 90°C in N_2 atmosphere. After cooling down, the mixture was precipitated in ice-cold acetone and then soxhlett extracted with ethyl acetate for 12h. Dried in vacuum oven at 40°C for 48h. Grafting percentage and the amount of lactide in graft copolymer was calculated.

3.3.3. Swelling Studies

The water uptake capacity of **CL** graft copolymers was determined by the hydration of graft copolymers in deionized water at room temperature. At regular intervals the hydrated samples were taken out from deionized water and weighed immediately on an electronic balance after blotting the surface water with a filter paper. Weighing was continued until it reaches a constant weight. The percentage water content of the **CL** graft copolymer was calculated as follows:

$$\%W_c = [(W_w - W_d) / W_d] \times 100$$

where W_c is the percentage water content of **CL** graft copolymer at equilibrium. W_d and W_w are the weights of the samples at dry and at equilibrium respectively. Average of three values was recorded.

3.3.4. Hydrolytic Degradation

The hydrolytic degradation of **CL** graft copolymers was carried out *in vitro* by incubating the samples in pellet form (prepared by hot pressing) in deionized water in vials. The vials were placed in an oven at 60°C. At predetermined time intervals, the samples were taken from the medium and dried in vacuum oven to a constant weight. The weight loss of **CL** graft copolymers with time was monitored as a measure of degradation.

$$\%\text{Weight loss} = [(W_i - W_d) / W_i] \times 100$$

where W_i and W_d are the weights of samples before and after degradation. Average of three values was recorded. Variations on the surface morphology of samples after degradation were examined by SEM micrographs. Further confirmation of hydrolytic degradation was done by DSC analysis.

3.3.5. Enzymatic Degradation

Enzymatic degradation studies were conducted at 40°C by using two types of enzymes; a proteolytic enzyme papain from *Carica Papaya* (1Anston u/g) and an esterase enzyme lipase from *Candida Cylindracea* (LCC) (2.06u/mg). Enzymatic media, 10ml, consists of a sodium phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.00) containing sodium azide (0.02wt%) and 3mg of the appropriate enzyme. In the case of papain, the buffer solution was activated

with cystein HCl and EDTA (Enzyme in 50mM buffer was mixed with 50mM cystein HCl and 3mM EDTA incubated for 30minutes at 30°C). Polymer samples weighing about 25mg were prepared in pellet form by hot pressing and taken in glass vials containing 10ml of sodium phosphate buffer solution. Samples were taken from the enzymatic media at regular intervals and dried in vacuum oven to a constant weight. Weight loss of the samples with respect to time was taken as a measure of biodegradation. DSC, FTIR, ^{1}H NMR and SEM micrographs were used for the confirmation of biodegradation.

3.3.6. Measurements

The FTIR spectra of the samples were taken using Nicolet-Magna 560 spectrophotometer. The ^{1}H NMR spectra were recorded with a 300MHz Brucker NMR spectrophotometer in TFA/$CDCl_3$ containing a small amount of TMS as internal standard. The WAXD measurements were carried out in the reflection mode on a Rigaku Miniflex X-ray diffractometer with a Ni-filtered Cu K_α radiation (2θ = 0-45°). Differential scanning calorimetry (DSC) studies were conducted with a TA instrument DSC 2920 connected with thermal analyst 2100 system under N_2 (90ml/min). The instrument was calibrated using indium. Samples of 2-3mg were sealed in aluminium pans and subjected to heating at a rate of 10°C/min in the temperature range of 40-300°C. Thermogravimetric analysis (TGA) and Differential thermogravimetry (DTG) was conducted with a Shimadzu DTG-60 connected with TA-60WS thermal analyzer under N_2 (30ml/min). Samples were heated from room temperature to 600°C at a heating rate of 10°C/min. The surface morphology studies were

done by means of a Scanning Electron Microscopy (Hitachi 2403A, Japan) after coating the surface by sputtering with gold (10-20nm thick).

3.4. Results and Discussion

3.4.1. Synthesis of Chitosan/oligoL-lactide Graft Copolymers

The grafting of L-lactide onto chitosan was carried out in a number of solvents, catalysts and at various temperatures to identify the most effective ring opening catalyst and to optimize the conditions of grafting. Table 3.1 show that a substantially good amount of grafting was observed in DMF, DMA, and DMSO. But, no significant grafting was observed in pyridine. Among the different ring opening catalysts studied for the grafting reaction, metal alkoxides were found to be better catalysts than Lewis acids. Metal alkoxides are known to be involved in a co-ordination insertion mechanism and the -OH and $-NH_2$ groups of chitosan will act as co-catalysts for the lactide ring opening polymerization[30]. It was observed that the percentage grafting was quite low and the product was brown in colour above a temperature of 100°C. A maximum grafting of 99.50% was observed in $Ti(OBu)_4$/DMSO mixture at 90°C in the feed ratio of 1:5 CS:LLA. So, further experiments for the preparation of **CL** graft copolymers were done in $Ti(OBu)_4$/DMSO mixture at 90°C. This is the first report, wherein a ring opening catalyst that has an advantage over other catalysts in reducing the risk of racemization was used to get L-lactide grafted onto chitosan.

Table 3.1. Effect of various reaction conditions on the synthesis of CL graft copolymer

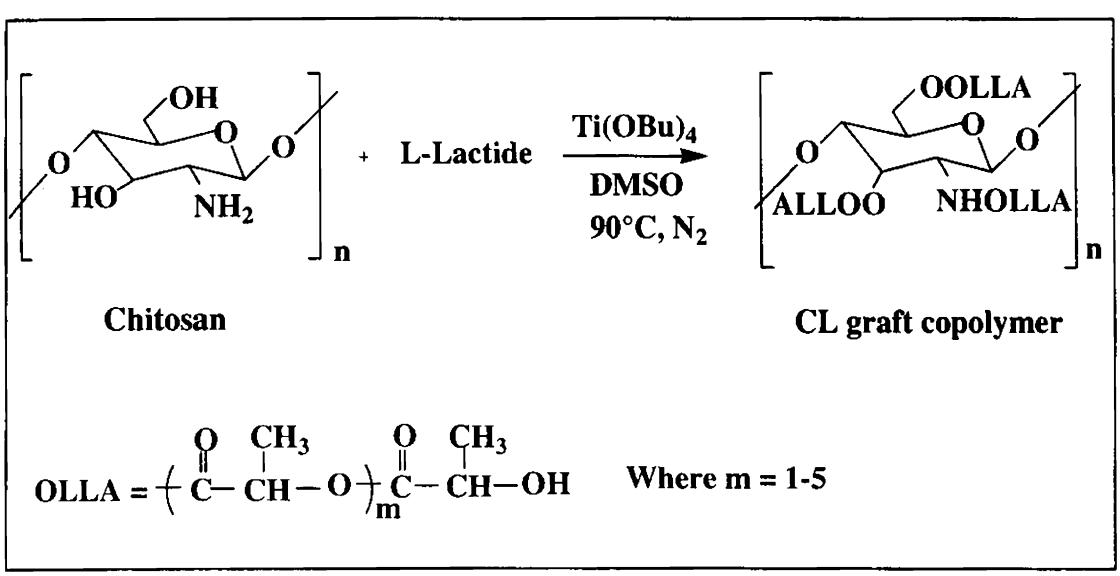
Solvent	Temperature (°C)	Catalyst	% Grafting
DMF	90	$SnOct_2$	83.00
DMA	"	"	82.00
DMSO	"	"	93.00
Pyridine	"	"	8.90
DMSO	110	$SnOct_2$	76.00 (browning)
"	130	"	34.47 (browning)
DMSO	90	$SnCl_4$	52.50
"	"	$Ti(OBu)_4$	99.50
"	"	LiCl	32.35

The effect of L-Lactide concentration on the percentage of grafting of L-lactide onto chitosan is given in Table 3.2. It can be seen from Table 3.2 that the grafting percentage and the molar composition of lactide in graft copolymer increase with the increase of lactide content in the feed molar ratio. Thus, when the molar ratio of the CS:LLA in the feed increased from 1:2 to 1:30, the grafting percentage rose from 45.04 to 224.05. Meanwhile, the molar ratio of lactide to chitosan in the graft copolymer also rose from 1.00 to 5.01. This indicates that the higher the concentration of lactide, the higher is the reactivity of lactide with chitosan. A schematic representation of grafting of LLA onto CS in presence of $Ti(OBu)_4$ is shown in Scheme 3.1.

Table 3.2. Effect of L-lactide concentration on CL graft copolymer synthesis

	CS:LLA (Feed molar ratio)	%Yield	% Grafting	$F_{LLA}/F_{chitosan}$ [a]	Graft branch length[b]
CL-2	1:2	47.56	45.04	0.85	1.00
CL-5	1:5	31.57	99.50	1.65	2.01
CL-7	1:7	37.10	117.50	2.21	2.62
CL-10	1:10	35.17	122.06	2.55	2.73
CL-20	1:20	34.51	172.17	3.74	3.85
CL-30	1:30	32.24	224.05	4.95	5.01

[a] Molar composition of LLA in graft copolymer = % Grafting × 161/72
[b] From ^{1}H NMR.



Scheme 3.1. Synthesis of CL graft copolymers.

3.4.2. FTIR Analysis

Structural changes of chitosan brought about by LLA grafting were studied by FTIR spectroscopy (Figure 3.1). In comparison to the FTIR spectra of CS, **CL** graft copolymers have a new absorption at 1758cm^{-1} assigned to the ester carbonyl group of the branched oligoL-lactide (OLLA) existing as side chain. On increasing the weight fraction of LLA in **CL** graft copolymer an increase in the intensity of absorption at 1758cm^{-1} was observed.

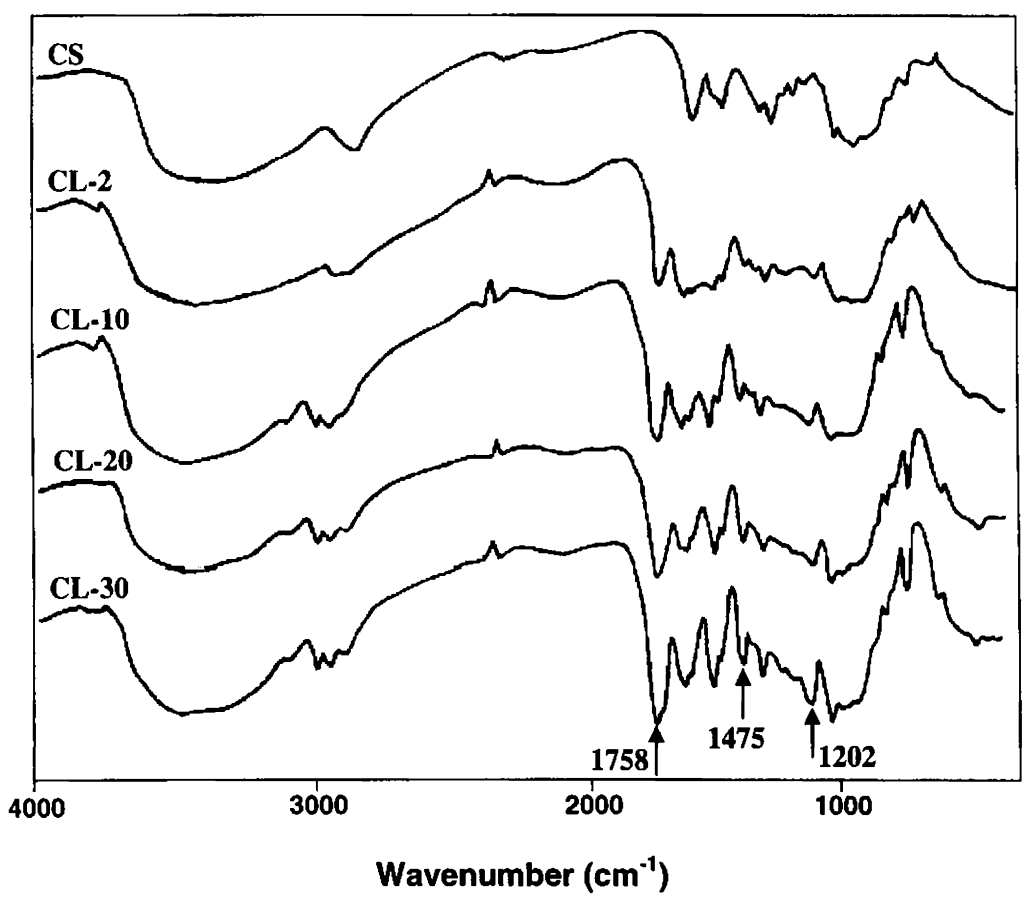


Figure 3.1. FTIR spectra of CS and CL graft copolymers.

The methyl asymmetric deformation of OLLA appears at ~1475cm^{-1}. The ~1202cm^{-1} singlet observed in the copolymer is assigned to the symmetric C-O-C stretching modes of the ester groups. There are two other peaks at ~1131 and ~1046cm^{-1} attributed to the methyl rocking and C-CH_3 stretching vibration, respectively[24].

3.4.3. ^{1}H NMR Studies

The ^{1}H NMR spectra of chitosan and **CL-30** graft copolymer are shown in Figure 3.2. The ^{1}H NMR spectra of chitosan showed peaks at δ7.73 (s, 2H, NH_2), 3.58(H2), 4.08(H6), 4.23(H3), 4.73(H5), 4.98(H1) and 5.26(H-4). The graft copolymer not only showed the original signals of chitosan, but also has new peaks at δ4.3 and 5.45. These peaks can be assigned to the terminal methine protons of the branched OLLA and its repeat units in the chain, respectively. The peak at δ1.68 is attributed to the methyl protons of OLLA[30]. A similar result has been observed by Liu et al. in a brush like graft copolymer of poly(D,L-lactide) grafted onto chitosan[24]. The integral intensity ratio (the ratio of the methine amount of OLLA in the chain and that in the end of the chain) between the peaks at δ5.45 and 4.3 is determined by the graft branch length[30]. In the samples of **CL-10**, **CL-20** and **CL-30** graft copolymers; the integral intensity ratio between the peaks at δ5.45 and 4.3 is 2.55, 3.74 and 4.95 respectively, which correlate well with the results of gravimetric method (Table 3.2). These results indicate that the **CL** graft copolymers contained oligoL-lactide side chains and the amount of branched polymer increases with increase of lactide content in the feed molar ratio.

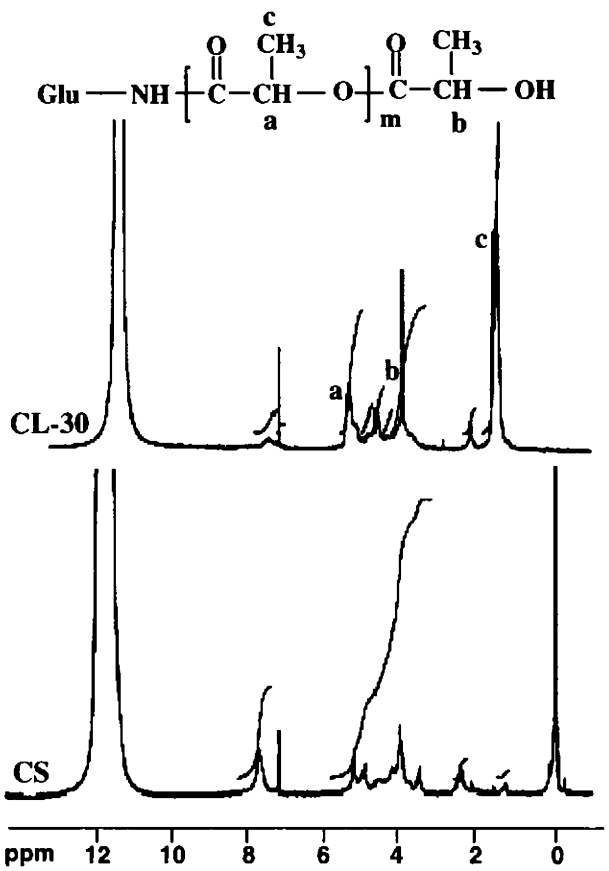


Figure 3.2. ^{1}H NMR spectra of CS and CL-30 graft copolymer.

3.4.4. Physical Properties

WAX-ray diffraction profiles of CS and **CL** graft copolymers are shown in Figure 3.3. The X-ray diffraction pattern of native chitosan showed hydrated polymorphism with a 020 reflection at 10° 2θ, a characteristic of "tendon" form and 100 and 110 reflections at 20° 2θ. The reflection at 020 is associated with the most ordered regions formed through hydrogen bonding between acetamido groups, which facilitate the incorporation of water molecules forming a hydrated crystal[31]. It is interesting to note that the grafting of LLA onto chitosan results in the broadening of the peak at 20° 2θ with increased

intensity. Whereas, the peak at 10° 2θ showed a considerable decrease in intensity and got merged with the main broad peak. These changes in the peak intensity suggest different packing of chains and/or different hydrogen bonding network in the graft copolymers[31]. A comparison of the X-ray diffraction profiles of CS and **CL** graft copolymers indicates that the grafting has destroyed the original crystallinity of chitosan. This shows that the grafting of lactide onto chitosan chain takes place at random along the chain, giving rise to a random copolymer[25,27]. The highly grafted **CL-30** showed a weak absorption at 14° 2θ in addition to the main broad absorption. This may be attributed to the ordering of chitosan chains as a consequence of the self-assembling of longer OLLA side chains by hydrogen bonding and dipole dipole interactions.

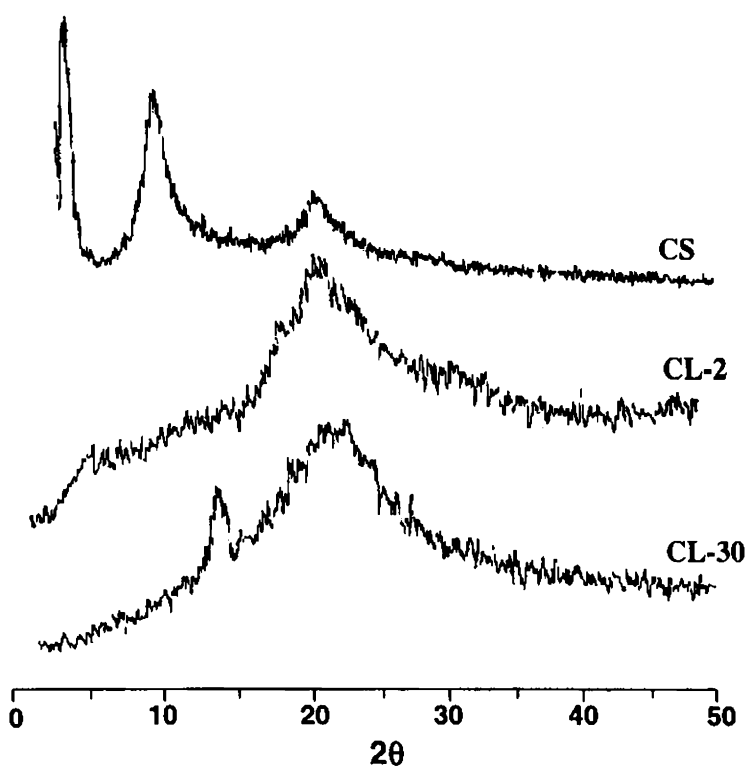


Figure 3.3. WAX-ray diffraction patterns of CS and CL graft copolymers.

A similar observation on cytocompatible poly(chitosan-g-L-lactic acid) was reported by Yao et al.[25].

3.4.5. Thermal Properties

Thermal properties of CS and its graft copolymers were studied by TGA/DTG and DSC analysis. It can be seen from Figure 3.4 and Figure 3.5 that both CS and its graft copolymers have a two-stage degradation pattern in TGA and DTG thermograms, respectively. This may be attributed to the thermal evaporation of bound water (which could not be removed completely on drying) and thermal degradation of the sample, respectively[32]. Table 3.3 shows that the graft copolymers have a higher T_{max1} (maximum temperature of decomposition of the first stage) than that of chitosan itself, indicating that the removal of water in the case of graft copolymers in the first stage of degradation take place at a higher temperature than that of chitosan.

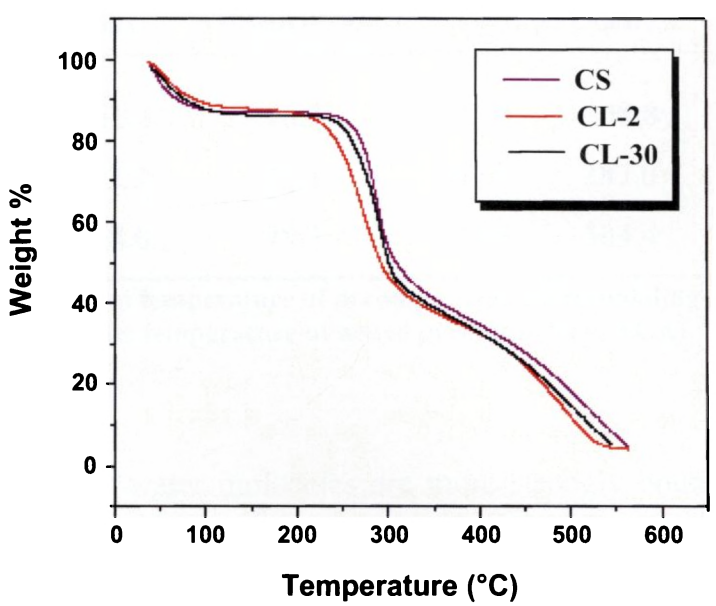


Figure 3.4. TGA thermograms of CS and CL graft copolymers.

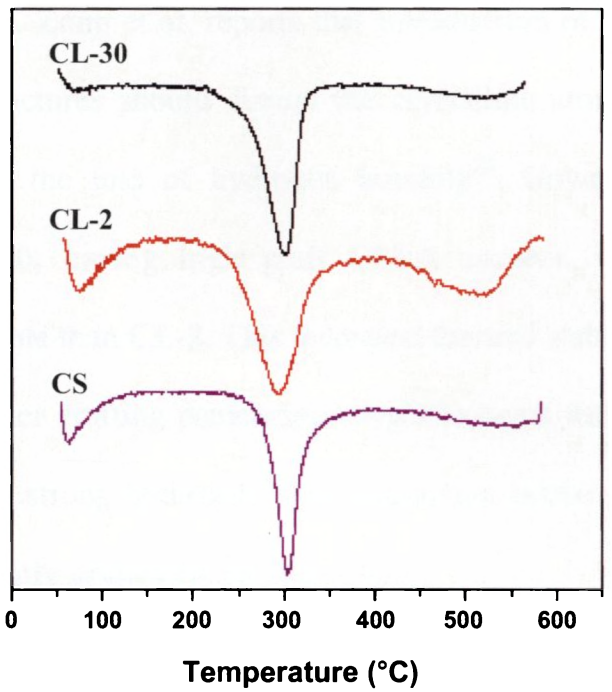


Figure 3.5. DTG curves of CS and CL graft copolymers.

Table 3.3. Thermal degradation data of CS and CL graft copolymers

	T_{max1}	Wt_{max1}(%)	T_0	T_{max2}	Wt_{max2} (%)
CS	61.4	96.2	247.0	308.8	67.0
CL-2	72.7	95.3	209.9	287.0	60.7
CL-30	68.6	99.3	234.5	304.4	62.4

T_{max}- maximum temperature of decomposition corresponding to each stage(from DTG), T_o - onset temperature of active pyrolysis (from TGA).

This reveals that water molecules are more strongly bound to the copolymer than to chitosan alone. The grafted LLA chains might reduce the hydrogen bonding between chitosan chains and increase the interaction between water and chitosan chains[27]. T_0 correspond to the onset temperature of active

pyrolysis of graft copolymers (T_0 = <240.0°C) was found to be less than that of CS (T_0 = 247.0°C). Zong et al. reports that introduction of flexible units into polysaccharide structures should disrupt the crystalline structure of chitosan, especially through the loss of hydrogen bonding[33]. However, among graft copolymers, **CL-30,** having high graft OLLA content, was found to be thermally more stable than **CL-2**. This increased thermal stability of the grafted copolymers at higher grafting percentages might indicate the possibility of the formation again of strong hydrogen bond interaction between chitosan chains through the covalently grafted OLLA side chains.

The DSC thermograms given in Figure 3.6 shows that all samples have a wide endothermic peak centered between 102.5-124.0°C with an onset at 49.0-55.4°C, which may be attributed to the evaporation of bound water present in the sample[34,35]. The presence of bound water has a strong influence on the overall polymorphic nature of the macromolecule. Therefore, the endotherm related to the evaporation of bound water is expected to reflect the chemical and molecular changes taken place on chitosan during LLA grafting. Values for the transition temperatures and their associated enthalpies are given in Table 3.4. In agreement with the TGA/DTG data, the DSC thermograms also show that the bound water in the graft copolymers has higher evaporation temperatures and ΔH values than that in chitosan. On the basis of these results it can be deduced that these macromolecules differ in their water holding capacity and strength of water-polymer interaction.

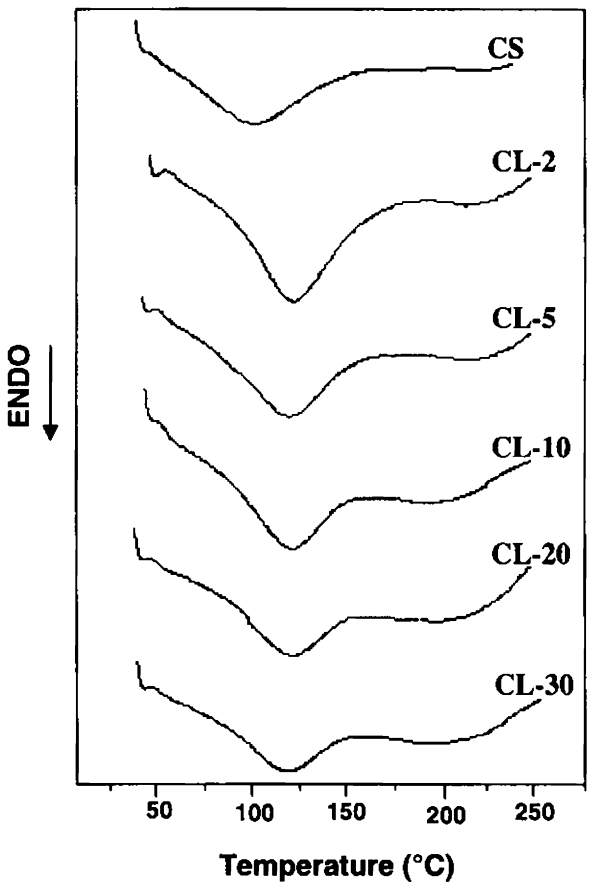


Figure 3.6. DSC thermograms of CS and CL graft copolymers.

Table 3.4. Thermal transitions of CS and CL graft copolymers

Polymer	Endotherm 1 (°C)			
	T_0	T_p	T_c	ΔH(J/mg)
CS	49.0	102.5	160.9	159.7
CL-2	55.4	124.0	164.2	228.8
CL-5	55.0	123.6	155.7	210.9
CL-10	53.1	123.0	163.2	198.8
CL-20	50.0	122.1	162.5	170.5
CL-30	51.9	120.9	161.0	162.6

T_o. onset temperature, T_p - peak temperature, T_c - completion temperature, ΔH-enthalpy

Grafting of hydrophobic side chains results in the decrease of chitosan crystallinity by loosening the hydrogen bonds and increasing the number of free hydrophilic hydroxyl groups and amino groups of chitosan, which in turn can hold water molecules more strongly[34-37]. Furthermore, the decrease in ordered structure due to chemical modification observed in WAXD may also contribute significantly towards increase in the content of sorbed water. The evaporation temperature of bound water in **CL** graft copolymers increase by about 18-22°C compared to those in chitosan. Among **CL** graft copolymers, the endothermic peak area and ΔH decreased with increase of percentage grafting, indicating a possible correlation between the water holding capacity and the chemical and supramolecular structure of these polymers[34,35]. The presence of bound water in the grafted sample was further confirmed when it was noted that during a second run of the DSC, the first endotherm vanished.

3.4.6. Swelling Studies

The effect of CS:LLA feed ratio on equivalent water uptake of **CL** graft copolymers is shown in Figure 3.7. It can be seen from Figure 3.7 that unlike chitosan, all graft copolymers are converted to hydrogels in deionized water. This property is of special interest in biomedical applications like wound dressings and controlled drug release systems[1,28,36,38]. In the case of chitosan, even though it is hydrophilic, the strong hydrogen bonding and crystallinity reduces the infiltration and water diffusion[26]. Grafting of LLA onto chitosan separates chitosan backbones and drastically reduces its hydrogen bonding and crystallinity and increases its affinity towards water[39]. This results in swelling

of graft copolymers in water in spite of the hydrophobicity of OLLA side chains.

Maximum swelling was shown by **CL** graft copolymers having lower lactide content. This is because, as these samples have low molar ratio of lactide to chitosan in the copolymer as shown in Table 3.1, it will form a loose physically cross linked copolymer through hydrogen bonding and dipole-dipole interactions between neighboring ester groups and chitosan chains[25,36,40]. So, these samples have the highest swelling among the samples investigated. However, on increasing LLA content in the feed ratio, a decrease in swelling ratio was observed. At higher lactide content, due to aggregation, the hydrophobic nature of the side chains becomes dominant. The number of hydrophilic sites on the chitosan backbone also got decreased at higher lactide content. It is also possible that the strong hydrogen bond interaction between

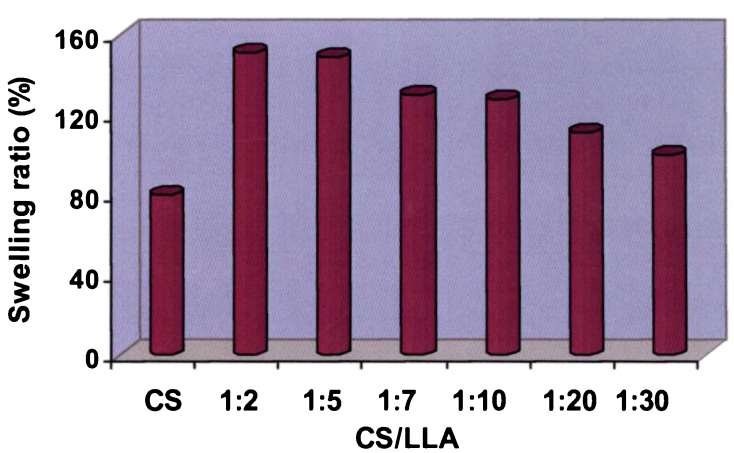


Figure 3.7. Swelling studies of CS and CL graft copolymers.

chitosan chains through their covalently grafted groups might lead to a decrease of the amount of freezing and non-freezing bound water that gives a lower swelling of the samples[36]. Yao et al. observed a similar behaviour in a pH sensitive swelling studies of poly(chitosan-g-L-lactic acid)[25]. These results indicate that the graft copolymers exhibit better swelling in the neutral medium than that of CS and PLA individually.

3.4.7. Hydrolytic Degradation

The changes in weight loss of CS and **CL** graft copolymers subjected to hydrolytic degradation in deionized water is shown in Figure 3.8. All **CL** graft copolymers showed a higher weight loss in water than that of chitosan. Similarly, on increasing the lactide content in graft copolymers a decrease in weight loss was observed. Indeed, this can be clearly explained by noticing the hydration properties of **CL** graft copolymers. In the case of graft copolymers, those having lower LLA content have better accessibility to water as shown in Figure 3.7 and thus prone to more degradation. At higher LLA content, the hydrophobic property of side chains and hydrogen bonding of graft copolymer may become the dominant factor, which will lead to lower degradation. After 100days of hydrolytic degradation, the **CL-2** graft copolymer was observed to have solubility in water, indicating the fast degradation of **CL-2** graft copolymer into water soluble fragments.

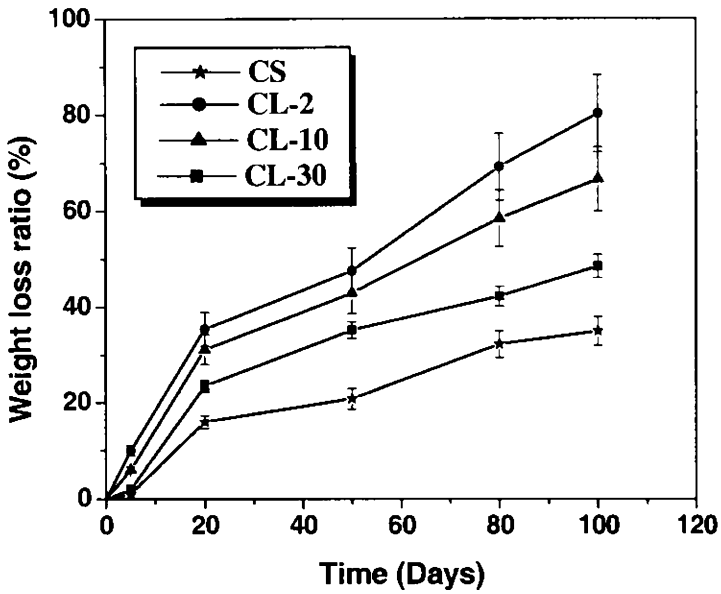


Figure 3.8. Changes in weight loss of CS and CL graft copolymers with time of immersion in deionized water.

The extent of degradation was assessed by studying the SEM micrographs of CS and **CL** graft copolymers taken after 80 days of exposure to deionized water (Figure 3.9). Hydrolytic degradation results in surface erosion of chitosan with the formation of small pores (Figure 3.9B). Hydrolytically degraded **CL-2** and **CL-10** graft copolymers given in Figure 3.9C and Figure 3.9D, respectively showed more pores and cracks on the surface than degraded chitosan. This can be understood from the fact that the grafting results in the formation of amorphous copolymer and hydrolytic degradation takes place preferentially on the amorphous portion of graft copolymer and the resulting short chains are dissolved out into water by creating pores on the surface. But in the case of **CL-30** graft copolymer (Figure 3.9E), the SEM micrograph showed less surface erosion compared to that of **CL-2** and **CL-10** graft

copolymers. This may be attributed to the decreased hydration of **CL-30** graft copolymer that results in lower hydrolytic degradation.

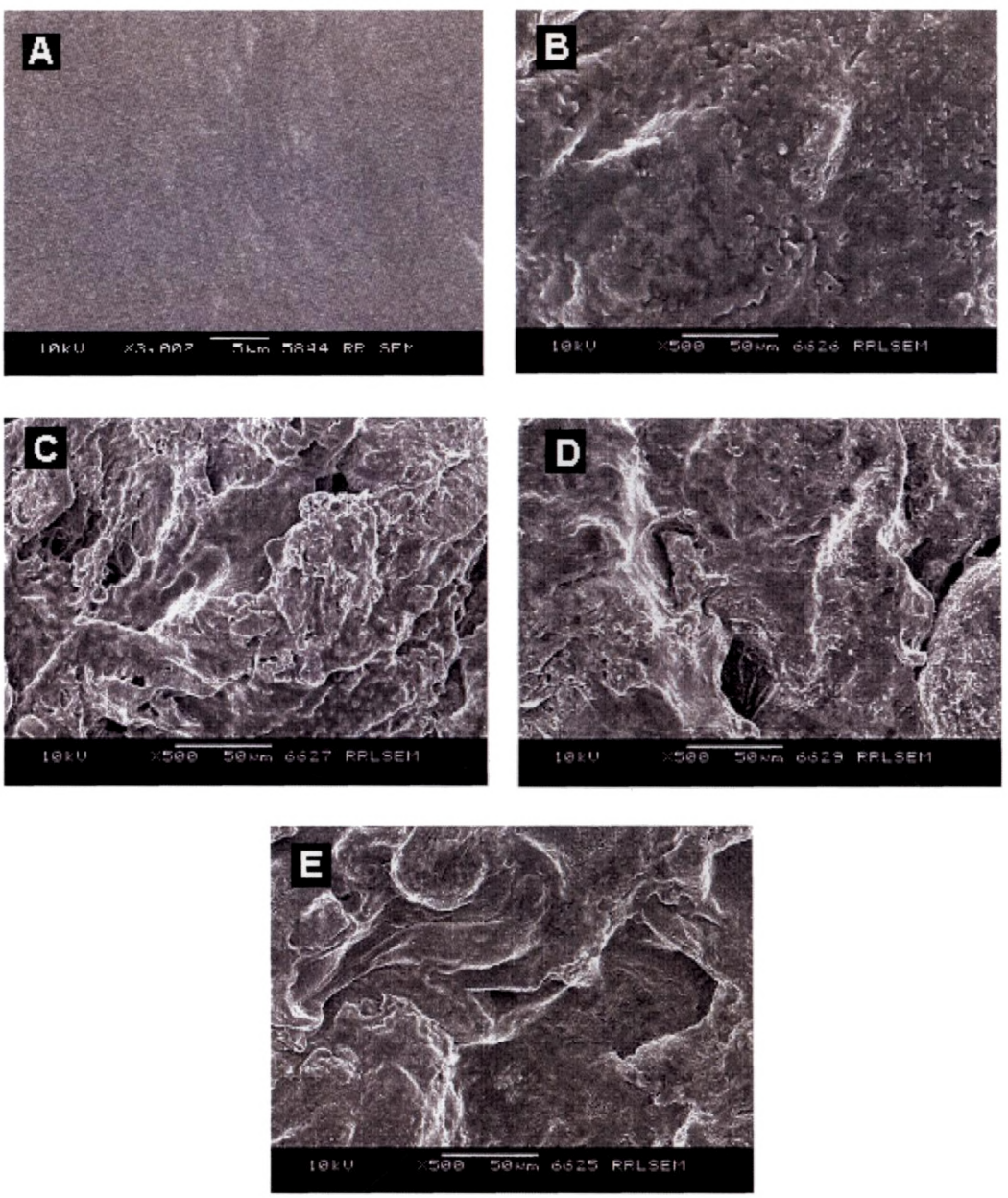


Figure 3.9. SEM micrographs of hydrolytically degraded samples taken after 80 days of immersion in deionized water: (A) CL graft copolymer before degradation, (B) Chitosan, (C) CL-2, (D) CL-10 and (E) CL-30 graft copolymers.

Hydrolytic degradation was further confirmed by DSC analysis (Figure 3.10). DSC thermogram of **CL-2** graft copolymer after 80 days of hydrolytic degradation did not show the endotherm corresponding to the evaporation of bound water that were observed originally in it before degradation, indicating that the polymer has lost its molecular structure by degradation. Whereas, **CL-30** graft copolymer showed a decrease in the peak area and peak position of the endotherm when compare with the non-degraded **CL-30** graft copolymer, which indicated that the polymer has degraded but at a slower rate than that of **CL-2** graft copolymer (Figure 3.6 & Figure 3.10 and Table 3.5). These findings reveal that the degradation rate of **CL** graft copolymers can be controlled by changing the amount of LLA content in graft copolymers.

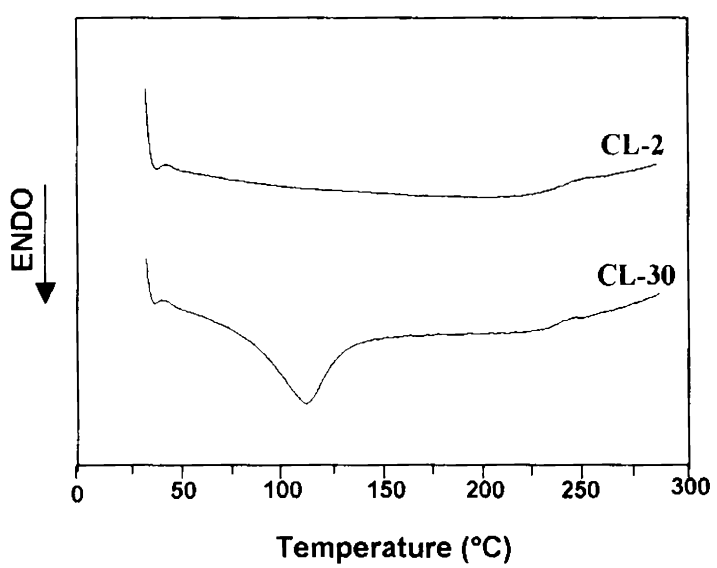


Figure 3.10. DSC thermograms of CL-2 and CL-30 graft copolymers after 80 days of hydrolytic degradation.

Table 3.5. Thermal data of degraded CL graft copolymers

Polymer	Endotherm 1 (°C)			
	T_0	T_p	T_c	ΔH(J/mg)
(Hydrolytically Degrd)				
CL-2	-	-	-	-
CL-30	50.0	118.1	147.9	139.1
(Lipase Degrd)				
CL-2	55.7	120.3	150.0	211.0
CL-30	47.6	113.0	148.3	149.9

T_0 - onset temperature, T_p - peak temperature, T_c - completion temperature, ΔH-enthalpy

3.4.8. Enzymatic Degradation

Fundamental information regarding the enzymatic degradation of **CL** graft copolymers should be required for the *in vitro* and *in vivo* biomedical applications. Therefore, the enzymatic degradation of **CL** graft copolymers was studied by using two types of enzymes, proteolytic enzyme papain from *Carica Papaya* and esterase enzyme lipase from *Candida Cylindracea*. Selection of enzymes was based on its activity on chitosan. Papain was reported to be one of the more efficient hydrolytic agents for chitosan and lipases depolymerize chitosan to a limited extent[41,42]. Certain authors sustain the view that the unspecific activity of lipases is due to the presence of chitosanases as impurities[43]. Chitosan is easily hydrolysable by chitosanases that are

completely absent in mammals[44]. Lipase being one of the main enzymes present in the human body, it is more relevant to study the susceptibility of **CL** graft copolymers to lipase that would increase its biomedical significances. Lipases are good hydrolytic agents for esters. As the ester side chains are grafted onto chitosan, **CL** graft copolymers would expect to be susceptible to enzymatic attack by lipases. The effect of lactide side chains on the enzymatic degradation of **CL** graft copolymers was also studied.

3.4.8.1. In Presence of Papain from *Carica Papaya*

The action of papain on CS, **CL** graft copolymers and OLLA was studied and the weight loss compared with the initial weight of the samples is shown in Figure 3.11. CS and **CL** graft copolymers showed a sharp increase in weight loss during the initial period of degradation. After 24h, the increase in weight loss was observed to be slow. Compared to CS, **CL** graft copolymers showed a decrease in weight loss in papain medium. A similar decrease in weight loss was also observed on increasing the lactide content in **CL** graft copolymers. Eventhough, **CL** graft copolymers have an ability to form hydrogels in neutral pH, chitosan showed a higher degradability in papain medium than that of graft copolymers. This is because, during degradation, the addition of cystein HCl and EDTA (to activate papain) changes the pH of the solution to be 5.4, which resulted in the swelling of chitosan to form entangled hydrogels[40]. Papain is very specific for the β $1\rightarrow 4$ glycosidic bond cleavage of chitosan[42]. Since the pure OLLA degradation by papain is very less, it is

assumed that the weight loss of **CL** graft copolymers may be mainly caused or at least initiated from the degradation of chitosan.

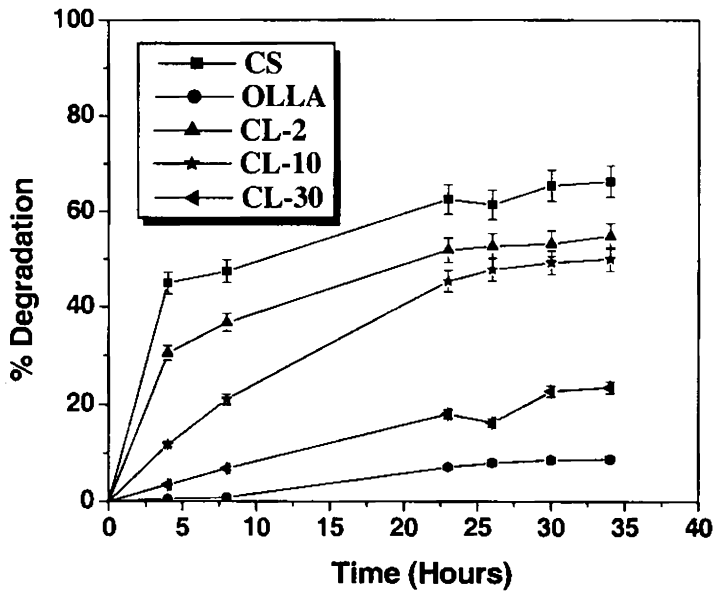


Figure 3.11. Changes in % degradation of CL graft copolymers with time of exposure to papain medium.

Further investigation from the FTIR of the degraded **CL-2** graft copolymer showed in Figure 3.12, indicates evidence of the removal of OLLA by showing a decrease in the ratio of the absorbance of $\nu_{C=O}$ with ν_{O-H}, A_{1758}/A_{3449} from 0.75 to 0.28. Therefore, it is more plausible to assume that some of the grafted side chains, especially the short ones, are easily dissolved out into water accompanied with the degraded chitosan fragments. This would increase the weight loss of the copolymer samples having shorter graft branch length. In addition, on increasing the monomer feed, the number of OLLA side chains increases and the chances of forming short grafted side chains become less. The dissolution of degraded chitosan fragments having longer OLLA side

chains into water is not possible. Therefore, the weight loss of copolymers having longer graft branch length should be smaller as can be seen in Figure 3.11. Don et al observed a similar behaviour on the lysozyme promoted degradation of chitosan-g-poly(acrylic acid) copolymers[45].

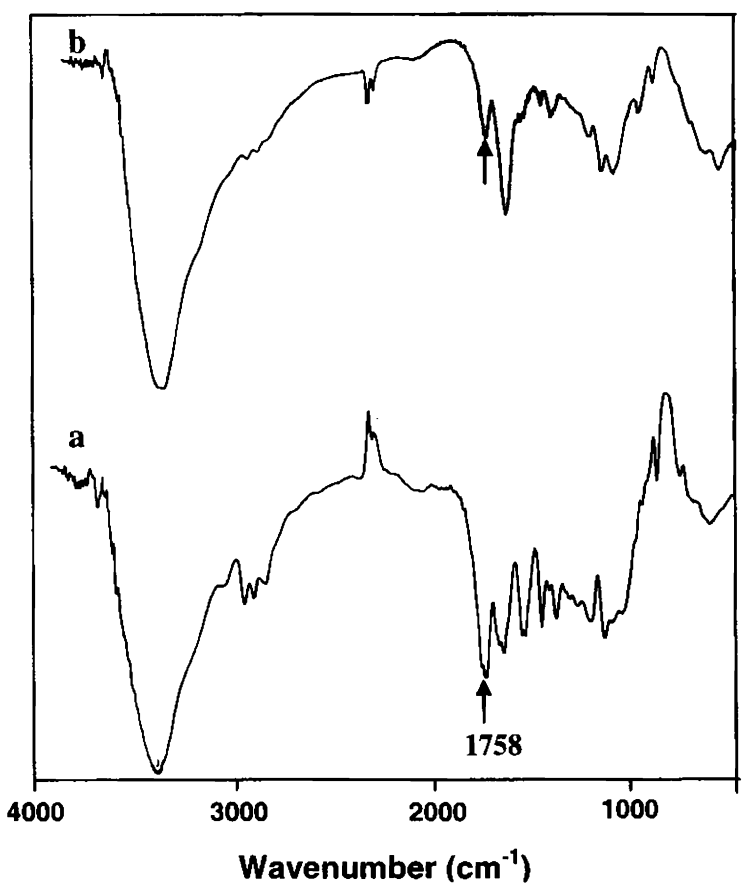


Figure 3.12. FTIR spectra of CL-2 graft copolymer (a) before and (b) after enzymatic degradation by papain.

Degradation was further confirmed by SEM micrographs of **CL** graft copolymers taken after 8h immersion in papain medium (Figure 3.13). In the case of graft copolymers, **CL-2** degraded severely and **CL-10** showed less surface erosion than that of **CL-2** (Figure 3.13B & 3.13C), whereas **CL-30** was

observed to be almost maintaining its physical form even after 8h exposure to papain medium (Figure 3.13D). These observations clearly indicate the role of lactide in papain promoted degradation of **CL** graft copolymers. An increase in the lactide content decreases the degradation rate of **CL** graft copolymers in papain medium.

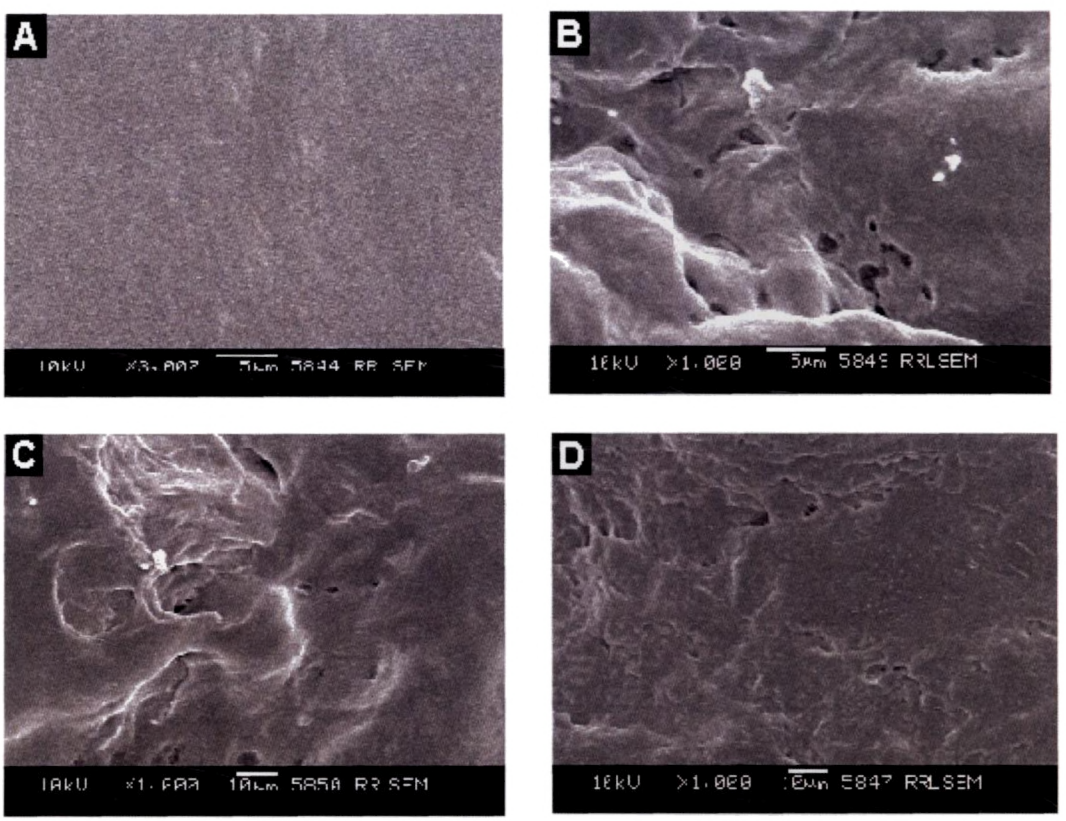


Figure 3.13. SEM micrographs of enzymatically degraded samples taken after 8h immersion in papain medium: (A) CL graft copolymer before degradation, (B) CL-2, (C) CL-10 and (D) CL-30 graft copolymers.

3.4.8.2. In Presence of Lipase from *Candida Cylindracea*.

Figure 3.14 shows the % degradation of CS, **CL** graft copolymers and OLLA with time of immersion in esterase enzyme lipase from *Candida*

Cylindracea. OLLA and **CL** graft copolymers showed a constant increase in weight loss with time and it goes on increasing after the desired period of degradation. Muzzarelli et al. reported that the lipases of various origins have different activity on the depolymerization of chitosan[43,44]. In the present study the digestibility of chitosan by lipase from *Candida Cylindracea* was found to be very negligible. But the **CL** graft copolymers were observed to be susceptible to lipase. It was also found that **CL** graft copolymers degrade in faster rate than OLLA in lipase medium. This indicated that the weight loss of **CL** graft copolymers in lipase medium is due to the escape of both CS and OLLA side chains. It was reported by Muzzarelli et al. that the substitution of chitin and chitosan with hydrophobic groups confers degradability to a modest extent[43,46]. In addition, lipase susceptibility on graft copolymers was observed to be increased with increase of grafting percentage.

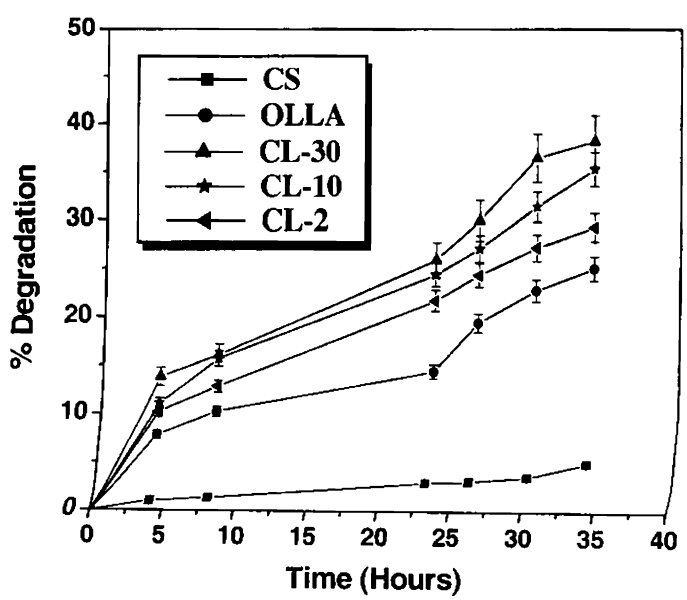


Figure 3.14. Changes in % degradation of CL graft copolymers with time of exposure to lipase medium.

The ^{1}H NMR spectra of degraded **CL-30** graft copolymer given in Figure 3.15 showed almost complete vanishing of peaks corresponding to lactide side chains at δ5.45 and 1.68. This indicated that the action of lipase on **CL** graft copolymers has been started from the lactide grafted sites. These results imply that the chitosan segment consisting of glucosamine residues is not accessible to the lipase active site and the random distribution of OLLA side chains is at least required to be adsorbed to the active site of lipase[47]. Lee et al. observed a similar behaviour in the case of enzymatic degradation of N-acyl chitosan by lysozyme[48]. Moreover, the action of lipases is on insoluble substrates particularly at hydrophilic-hydrophobic interfaces[46]. In **CL** graft copolymers, as the LLA is grafted onto a hydrophilic polymer, the accessibility of water to lactide side chains and chitosan active sites will be higher, thus getting better degradation than OLLA and chitosan alone.

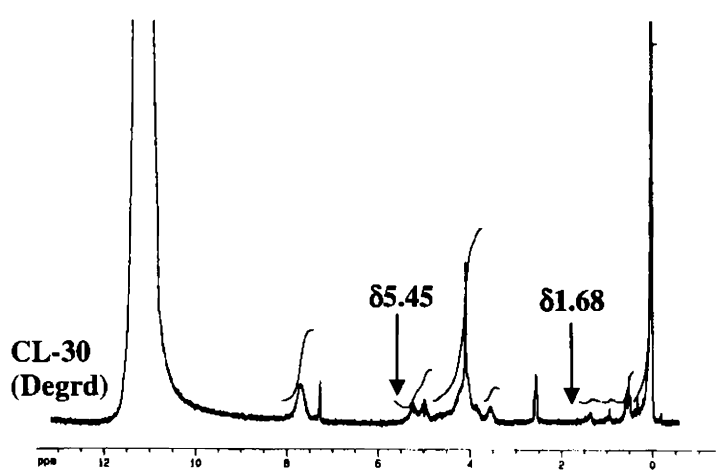


Figure 3.15. ^{1}H NMR spectra of degraded CL-30 graft copolymer.

These hypotheses were confirmed further by DSC thermograms of degraded **CL-2** and **CL-30** graft copolymers taken after 30h exposure to lipase medium (Figure 3.16). In comparison to the non-degraded **CL-2** and **CL-30** graft copolymers, the degraded samples are showing a decrease in peak area and peak position of the endotherm corresponding to the evaporation of bound water (Figure 3.6 & Figure 3.16 and Table 3.5). These changes indicate that the exposure of **CL** graft copolymers to lipase leads to a remarkably more ordered structure, that is presumably the result of the removal of more disordered portion of graft copolymer by enzymatic hydrolysis.

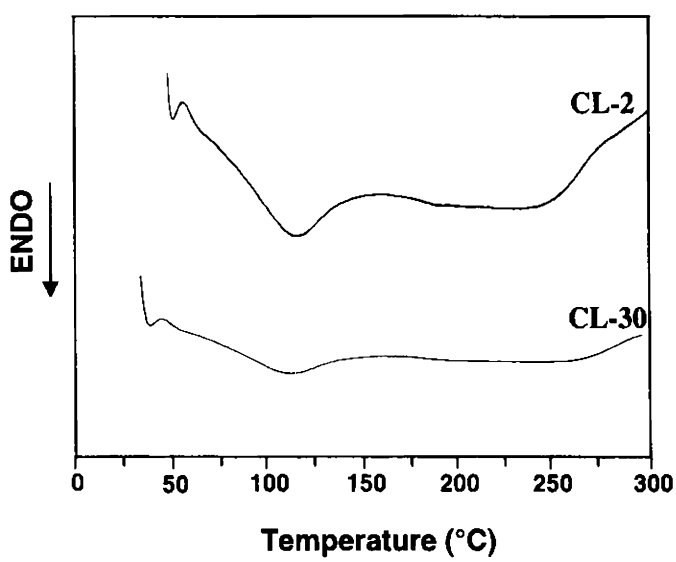


Figure 3.16. DSC thermograms of CL-2 and CL-30 graft copolymers subjected to 30h enzymatic degradation in lipase medium.

On comparing the activity of papain and lipase on **CL** graft copolymers, it can be concluded that the highly grafted chitosan was less susceptible to hydrolysis than chitosan in papain medium. On the contrary, the highly grafted

chitosan is more prone to hydrolysis in lipase than the original chitosan and OLLA. Lipase being one of the main enzymes present in the human body, the increased susceptibility of **CL** graft copolymers to lipase would increase its applications in biomedical and pharmaceutical fields. These *in vitro* degradation studies indicate that the degradation rate of **CL** graft copolymers as a biomaterial can be controlled by changing the amount of LLA content in graft copolymer.

3.5. Conclusions

Chitosan/oligoL-lactide graft copolymer was synthesized in DMSO at 90°C in presence of $Ti(OBu)_4$ as ring opening catalyst. Grafting percentage and the molar composition of lactide in graft copolymer increased with increase of lactide content in the feed molar ratio. FTIR and ^1H NMR studies established the formation of OLLA as side chain in the graft copolymer. The disturbance in hydrogen bonding and crystallinity of chitosan brought about by LLA grafting results in the formation of amorphous copolymer. DSC and TGA thermograms showed that the water holding capacity of chitosan was increased by LLA grafting. The graft copolymers were converted to hydrogels on exposure to deionized water. At higher grafting percentages, the longer OLLA side chains have a tendency to self-assemble with each other by hydrogen bonding and dipole dipole interactions between oligoester side chains, which results in the lower swelling of graft copolymers. A decrease in hydrolytic degradation was observed with increase of lactide content in **CL** graft copolymers. Even though,

CL graft copolymers were susceptible to both papain and lipase, the highly grafted chitosan was less susceptible to hydrolysis in papain medium, whereas it was more prone to hydrolysis in lipase than the original chitosan and OLLA. These results indicate that the physico-chemical properties and the rate of degradation of graft copolymers as a biomaterial can be controlled by adjusting the amount of LLA in the **CL** graft copolymers, which may find wide applications in wound dressing and in controlled drug delivery systems.

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CHAPTER 4

RANDOM MULTIBLOCK POLY(ESTER AMIDE)S CONTAINING POLY(L-LACTIDE) AND CYCLO ALIPHATIC AMIDE SEGMENTS: SYNTHESIS AND BIODEGRADATION STUDIES

4.1. Abstract

Novel multiblock poly(ester amide)s containing poly(L-lactide) and cycloaliphatic amide segments were synthesized from telechelic oligomer of α,ω-hydroxyl terminated poly(L-lactide), 1,3-cyclohexylbis(methylamine) and sebacoylchloride by the "two-step" interfacial polycondensation method. The blocky nature of PEAs was established by FTIR and ^{1}H NMR spectroscopies. The effect of relative content of ester and amide segments on the crystallization nature of PEAs was investigated by WAXD and DSC analysis. PEAs having lower content of PLLA, **PEA 1** and **PEA 2**, showed a crystallization pattern analogous to polyamides where as **PEA 3**, those having higher content of PLLA, showed two crystalline phases characterized by polyester and polyamide segments. Random nature of PEAs was observed from single T_g values. Biodegradation studies using the enzyme lipase from *Candida Cylindracea* showed higher degradation rate for **PEA 3** than that of **PEA 1** and

PEA 2. FTIR, ^{1}H NMR and DSC analysis of the degraded products showed the involvement of ester linkages in the degradation process.

4.2. Introduction

In recent years, considerable attention has been devoted to the preparation of biocompatible, biodegradable, reabsorbable synthetic polymers with controlled life times, because of their wide utilization in medicine as sutures, temporary implants, drug delivery systems etc. Aliphatic polyesters (PE) mainly poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA) and their copolymers are the best known biodegradable, biocompatible polymers used intensively in biomedical field[1-4]. However, the lack of certain optimum properties such as thermal, mechanical and processing limits their wider use[5]. Ultra high molecular weight is required for processing PLLA to strong fibers. The cost of PLLA is very high because of the tedious process involved in its production[6]. These limitations of PLLA along with its increased use in medicine stimulate research interest in synthesizing new materials having unique properties for specific applicability in the field. Aliphatic polyamides (PA), on the other hand, have better thermal and mechanical properties than aliphatic polyesters, but their hydrolytic degradation rate is too low to classify them as biodegradable polymers[7-9].

Poly(ester amide)s, having properties in between that of polyesters and polyamides, are an emerging class of biodegradable condensation copolymers of great promise. The presence of hydrolysable ester linkages in the main chain

and the potential lowering of the crystallinity can enhance the degradability of these polymers in comparison with aliphatic polyamides. On the other hand, the presence of amide functions in the polymer chain might lead to better thermal and physical properties than aliphatic polyesters usually have[10,11]. The rigidity due to the double bond character of the amide groups, coupled with the extensive hydrogen bonding between amide-amide and amide-ester, can enhance the mechanical properties of the PEAs[12,13]. Moreover, they can be suitably tailored to the needs of their applications by varying the nature of the monomers and/or their composition and architecture[8]. Therefore, by incorporating polyamide segments into the PLLA main chain, it could be possible to synthesize a polymeric material possessing good material and processing properties and biodegradability than their homopolymer counterparts. Simone et al. reported that the hydrolytic degradation rate of block poly(ester amide)s containing poly(L-lactide) segments is influenced by PE content and to a lesser extent by crystallinity[14]. Similarly Zhiyong et al. observed a direct relation in the hydrolytic degradation of a copolymer of lactic acid and aminoundecanoic acid with the ester content[15]. Angelo et al. synthesized biocompatible poly(ether-ester-amide)s based on poly(L,L-lactide), characterized by the presence of both hydrolytically degradable ester groups and enzymatically degradable peptide bonds[8]. In all these works the thermal stability and the processability of the copolymers as well as the susceptibility towards enzymatic degradation were not studied.

It was reported quite extensively in the literature that the crystal structure and physical properties of polyesters and polyamides could be increased by the incorporation of cycloaliphatic monomers[16-18]. The rigidity, arising from the conformational restriction in the cyclic ring, makes them has relatively high thermal properties in comparison with their linear aliphatic counterparts. Therefore, it would be interesting to modify PLLA with a polyamide containing rigid cycloaliphatic monomers, to produce rapidly crystallizable copolyesteramide with good thermal properties at lower molecular weight. Since the cycloaliphatic systems are nontoxic the prepared polymers should expected to be biocompatible. We could not locate any reports on the synthesize of poly(ester amide)s based on PLLA and cycloaliphatic amide segments. In the present work, we have synthesized, new random multiblock poly(ester amide)s of PLLA blocks as hydrolytically degradable segment and polyamide blocks constituted by 1,3-cyclohexylbis(methylamine) (CHMA) and sebacic acid as hard segment. Polymers were characterized by FTIR, ^{1}H NMR, WAXD, DSC and TGA analysis. The preliminary investigations of the *in vitro* biodegradation process of PEAs were carried out using an enzyme lipase from *Candida Cylindracea.*

4.3. Experimental Section

4.3.1. Materials

L-Lactide, sebacoylchloride, 1,3-cyclohexylbis(methylamine) (isomer mixture) and stannous 2-ethylhexanoate ($SnOct_2$) were purchased from Aldrich

Chemical Company, USA and were used as received. $CHCl_3$, 1,4-butanediol, n-hexane, benzene, KOH, NaH_2PO_4, Na_2HPO_4 and sodium azide were collected from S.D.Fine-Chem Ltd, Mumbai. The solvents were used after purification with standard procedures. Lipase was purchased from Fluka Biochemika, Sigma-Aldrich, USA.

4.3.2. Synthesis of PLLA based Macromer (HO-PLLA-OH)

α,ω-Hydroxyl terminated poly(L-lactide) (HO-PLLA-OH) macromer was synthesized by reacting in bulk appropriate amounts of L-lactide (14.4g; 100mmol), using 1,4-butanediol (BDL) (0.602g; 6.68mmol) as initiator and $SnOct_2$ (0.021g; 0.052mmol) as a ring opening catalyst at 130°C for 24 h in a vacuum sealed RB flask. After cooling to room temperature the reaction product was dissolved in $CHCl_3$ and precipitated in ice-cold ether. The product was filtered and dried in vacuum oven until it attains a constant weight.

White powder. Yield 76%. Mn calcud. by ^{1}H NMR ≅ 1500. FTIR (KBr, ν_{max} cm^{-1}): 3345 (-OH), 1756 (-COO-), 1234 (-CH_3). ^{1}H NMR ($CDCl_3$, δ in ppm): 1.50 (d, -CO-CH(C**H**$_3$)-OH), 1.52 (d, -CO-CH(C**H**$_3$)-O-), 1.63 (broad, -CH_2(C**H**$_2$)$_2$$CH_2$-, 4H), 4.08 (broad, -$CH_2$C**H**$_2$O-, 4H), 4.32 (q, -CO-C**H**(CH_3)-OH), 5.16 (q, -CO-C**H**(CH_3)-O-). DSC: T_m 112°C, ΔH_m 140.4mJ/g, T_c 60°C.

4.3.3. Synthesis of Poly(ester amide)s

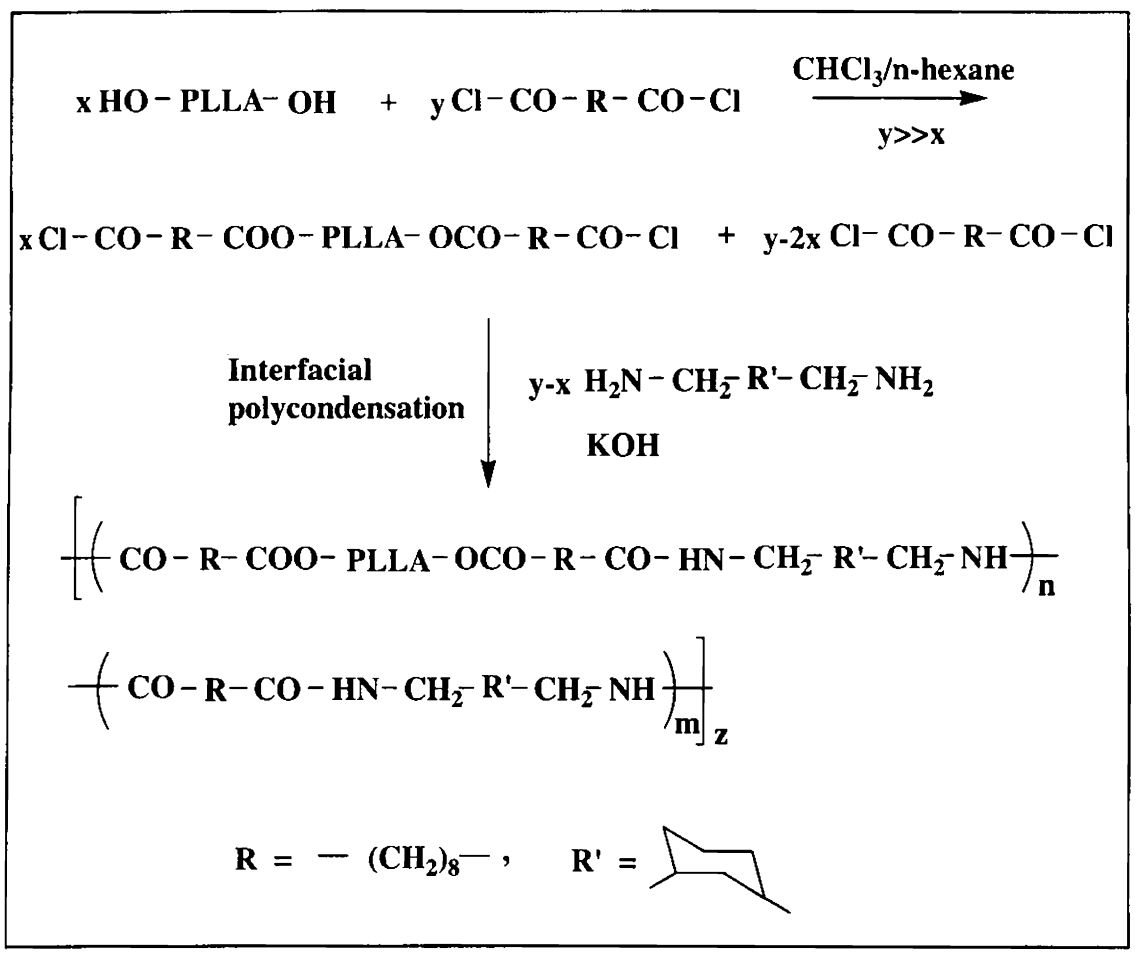
Random multiblock poly(ester amide)s was prepared by a "two-step route" outlined in Scheme 4.1. A typical procedure for the synthesis of **PEA 1** is given below:

(a) Preparation of COCl end-capped HO-PLLA-OH oligomer: A solution of the HO-PLLA-OH macromer (1.5g; 1mmol) in 5ml of anhydrous $CHCl_3$ was added drop wise at 50°C under stirring to an excess of sebacoylchloride (2.391g; 10mmol) dissolved in 2ml of anhydrous $CHCl_3$/n-hexane mixture (1:1 by vol). After 15min, 5ml of anhydrous n-hexane were added and the solution was kept at this temperature for 2h.

(b) Interfacial polycondensation: The $CHCl_3$/n-hexane solution containing COCl end-capped HO-PLLA-OH oligomers and unreacted sebacoylchloride was quickly poured under vigorous stirring into a stochiometric amount of CHMA (1.28g; 9mmol) in 100ml of water containing KOH (1.01g) as acid acceptor at 0-5°C. After 10min, the resulting polymer was collected, washed repeatedly with distilled water and dried in vacuum oven at 40°C for 24h. The product was extracted with boiling benzene for 8h and dried to give 4.34g of **PEA 1**, 84% yield, white powder.

FTIR (KBr, ν_{max} cm^{-1}): 3296 and 3086 (secondary amide N-H stretching), 2922 and 2854 (CH stretching of cyclohexane ring and CH_2), 1756 (C=O stretching of ester), 1643 (secondary amide C=O stretching), 1552 (secondary amide N-H bending and C-N stretching), 1192 (-CO-O stretching of ester), 1000-1180 (two to three bands due to C-O and C-N stretching of ester and amide linkages), 700-750 (amide V band). ^{1}H NMR (TFA/$CDCl_3$, δ in ppm): 1.73 (b, -CO-CH_2-($\mathbf{CH_2}$)$_6$-CH_2-CO-) and methyl protons of PLLA block), 2.13-1.96 (m, **ring-H**, 10H), 2.42 (b, -O-CH_2($\mathbf{CH_2}$)$_2$$CH_2$-O-, 4H), 2.84 (t, -$\mathbf{CH_2}$-COO-, 4H), 3.07 (b, -

CH_2-CONH-, 4H), 3.78-3.83 (2b, -CH_2-NHCO-, 4H), 4.68 (b, -CH_2CH_2O-), 5.73 (q, methine protons of PLLA block), 9.08 (b, -CH_2-NHCO-, 2H).



Scheme 4.1. Synthetic route of PEAs.

4.3.4. Synthesis of PLLASe and PCHMASe

Oligopolyester of PLLA (PLLASe) and oligopolyamide of CHMA (PCHMASe) was synthesized as model compounds for comparison with the copolymers, which were constituted by these homopolymers.

PLLASe was synthesized by treating HO-PLLA-OH with sebacoylchloride (1:1 ratio) in anhydrous $CHCl_3$/n-hexane mixture (1:1 by vol)

at 80°C for 12h. The product was precipitated in ice-cold water and dried in vacuum oven at 50°C for 24h.

^{1}H NMR (TFA/$CDCl_3$, δ in ppm): 1.38 (b, end methyl protons of PLLA block), 1.72 (b, -CO-CH_2-($\mathbf{CH_2}$)$_6$-CH_2-CO-) and methyl protons of PLLA block), 1.89 (b, -O-CH_2($\mathbf{CH_2}$)$_2$$CH_2$-O-, 4H), 2.67 (t, -$\mathbf{CH_2}$-COO-, 4H), 4.55 (b, -$CH_2$$\mathbf{CH_2}$O-, 4H), 5.04 (q, -CO-C**H**(CH_3)-OH, 1H), 5.59 (q, methine protons of PLLA block). DSC: T_m 120°C, ΔH_m 138.4 mJ/g.

PCHMASe was prepared by pouring sebacoylchloride dissolved in anhydrous $CHCl_3$/n-hexane mixture (1:1 by vol) to a water solution of 1,3-cyclohexylbis (methylamine) (1:1 ratio) containing KOH as acid acceptor with vigorous stirring. After 10min, the precipitated product was filtered and washed thoroughly with water. Dried in vacuum oven at 60°C for 24h.

^{1}H NMR (TFA/$CDCl_3$, δ in ppm): 1.45 (b, -CO-CH_2-($\mathbf{CH_2}$)$_6$-CH_2-CO-), 12H), 1.95-1.84 (m, **ring-H**, 10H), 2.56 (t, -$\mathbf{CH_2}$-COOH, 2H), 2.77 (b, -$\mathbf{CH_2}$-CONH-, 4H), 3.58-3.49 (2b, -$\mathbf{CH_2}$-NHCO-, 4H), 8.75 (b, -CH_2-N**H**CO-, 2H). DSC: T_m 179°C, ΔH_m 148.7mJ/g, T_g 80°C.

4.3.5. Enzymatic Degradation

Enzymatic degradation studies were conducted at 40°C by using an esterase enzyme lipase from *Candida Cylindracea* (LCC) (2.06 u/mg). Enzymatic media, 10ml, consists of a sodium phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.00) containing sodium azide (0.02wt %) and 3mg of the appropriate enzyme. Polymer samples weighing about 3mg were taken in glass vials containing 10ml of sodium phosphate buffer solution. Samples were

taken from the enzymatic media at regular intervals and dried in vacuum oven to a constant weight. Weight loss of the samples with respect to time was taken as a measure of biodegradation. FTIR, ^{1}H NMR and DSC analysis were used for the confirmation of biodegradation.

4.3.6. Measurements

The inherent viscosity of the polymers was measured using Cannon-Ubbelohde viscometer in m-cresol at a concentration of 0.5dL/g. FTIR spectra of KBr mixed samples were recorded with a Shimadzu IR Prestige-21 (200VCE) instrument in the spectral region between 4000 and 500cm^{-1}. The KBr pellets of 1mm thickness and 10mm diameter were prepared by mixing 1-2mg of the solid sample with oven dried KBr. The nuclear magnetic resonance (^{1}H NMR) spectrum was recorded with a 300MHz Brücker NMR spectrophotometer in $CF_3COOH/CDCl_3$ containing a small amount of TMS as internal standard. 10 to 15mg of the polymer samples were dissolved by adding 0.5ml of TFA and then added 3-4 drops of $CDCl_3$. Wide angle X-ray diffractions (WAXD) of the finely powdered polymer samples were measured in the reflection mode on a Rigaku Miniflex X-ray diffractometer with a Ni-filtered Cu K_α radiation. Differential scanning calorimetry (DSC) studies were conducted with a TA instrument DSC 821^{e} (Mettler Toledo) calibrated with indium. 3-9mg of samples was subjected to two heating runs at a rate of 10°C/min, under 90ml/min purge of dry nitrogen. First heating was done from -30 to 180°C, annealed at this temperature for 1h followed by cooling to -30°C. Second heating was carried out from -30 to 200°C. The thermal stability of the

polymers were determined using Shimadzu DTG-60 connected with TA-60WS thermal analyzer at a heating rate of 10°C/min under nitrogen at a purge rate of 30ml/min. Heating was done from room temperature to 600°C.

4.4. Results and Discussion

4.4.1. Synthesis of Telechelic Oligomer of PLLA

Telechelic oligomer of PLLA bearing hydroxyl groups as chain ends were prepared by reacting in bulk appropriate amounts of LLA, using BDL as initiator and $SnOct_2$ as a catalyst. Figure 4.1 depicts the ^1H NMR spectra of dihydroxylated PLLA.

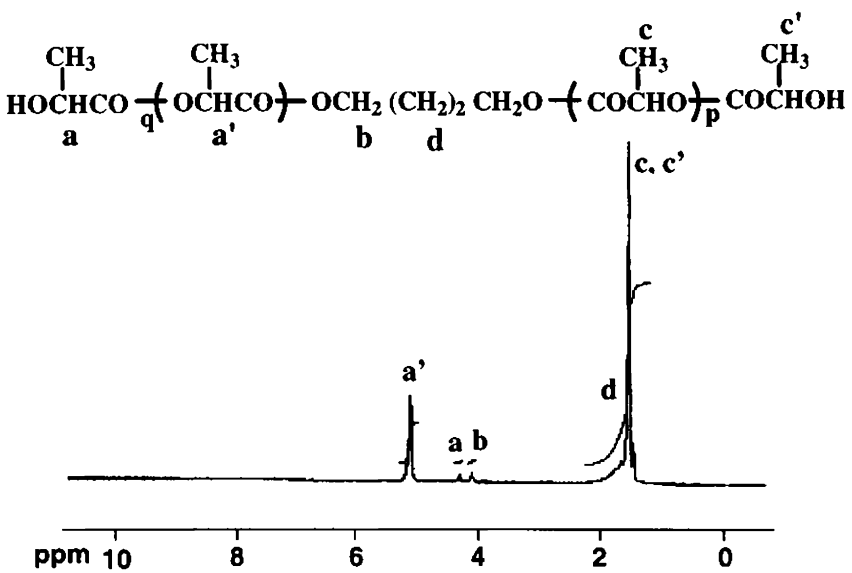


Figure 4.1. ^1H NMR spectra of HO-PLLA-OH.

The number average molecular weight of the oligomer determined by ^1H NMR spectroscopy, from the integration ratio of methine resonances of PLLA at

δ5.16 and δ4.32 with those of -CH_2CH_2O- resonances of BDL at δ4.08 as per the eq[19] given below, was ≅ 1500.

$$\mathbf{Mn = [S_a + S_{a'} / S_b] \times 4 \times 72 + 90}$$

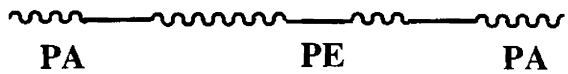
Where S is the integrated area of the corresponding protons. It was reported by Espartero et al. that the CH contribution of both hydroxyl and carboxyl end units of oligomeric PLLAs are appearing at δ4.20 and δ4.99 respectively[20]. In the present case, the ^{1}H NMR spectra of PLLA macromer show no contributions from carboxyl end units, revealing the hydroxyl end capping of PLLA. Melting temperature of PLLA macromer at 112°C, which is lower than that of high molecular weight PLLA, 175°C, is in agreement with the low molecular weight of the PLLA segments in the macromer.

4.4.2. Synthesis of Polymers

Poly(ester amide)s with different sequence length of polyamide segments were prepared from the preformed polyester segments (derived from telechelic oligomers of PLLA), CHMA and sebacoylchloride by following the "two-step" interfacial polycondensation method (Scheme 4.1).

In the first step, the α,ω-hydroxyl terminated PLLA was reacted with an excess of sebacoylchloride to obtain a sebacoyl-COCl end-capped intermediate, together with a variable amount of unreacted sebacoylchloride. In the second step, the chain extension with a diamine was performed following the stirred interfacial polycondensation technique, by dissolving the diamine and an acid acceptor in water. The stoichiometric molar ratio of macromer/diamine/sebacoylchloride is 1:2:3. The expected copolymer

architecture is random multiblock type that can be schematically represented as follows.



Poly(ester-amide)s having different compositions was prepared by varying the molar ratio of PLLA macromer and CHMA in the feed. The prepared polymers are soluble in m-cresol, Con. H_2SO_4 and insoluble in $CHCl_3$, THF and sparingly soluble in DMAc. The inherent viscosity values determined in m-cresol in the range of 0.37-0.48dL/g indicate a moderate degree of polymerization (Table 4.1).

Table 4.1. Yield, inherent viscosity and solubility data of PEAs

Polymer	Yield	η_{inh}	Solubility				
	(%)	(dL/g)	$CHCl_3$	m-Cresol	THF	Con.H_2SO_4	DMAc
PEA 1	84	0.44	-	+	-	+	+
PEA 2	85	0.40	-	+	-	+	+
PEA 3	85	0.37	-	+	-	+	±

- insoluble, + soluble, ± sparingly soluble

4.4.3. Polymer Structure

The molecular structure of the resulting polymers was ascertained by FTIR and ^1H NMR spectroscopies.

4.4.3.1. FTIR Studies

The FTIR spectra of PEAs given in Figure 4.2 showed the characteristic amide bands at 3296, 3086, 1643 and 1552cm^{-1} correspond to amide A (N-H stretching), amide B, secondary amide I band (C=O stretching) and secondary amide II band (N-H bending, C-N stretching) respectively.

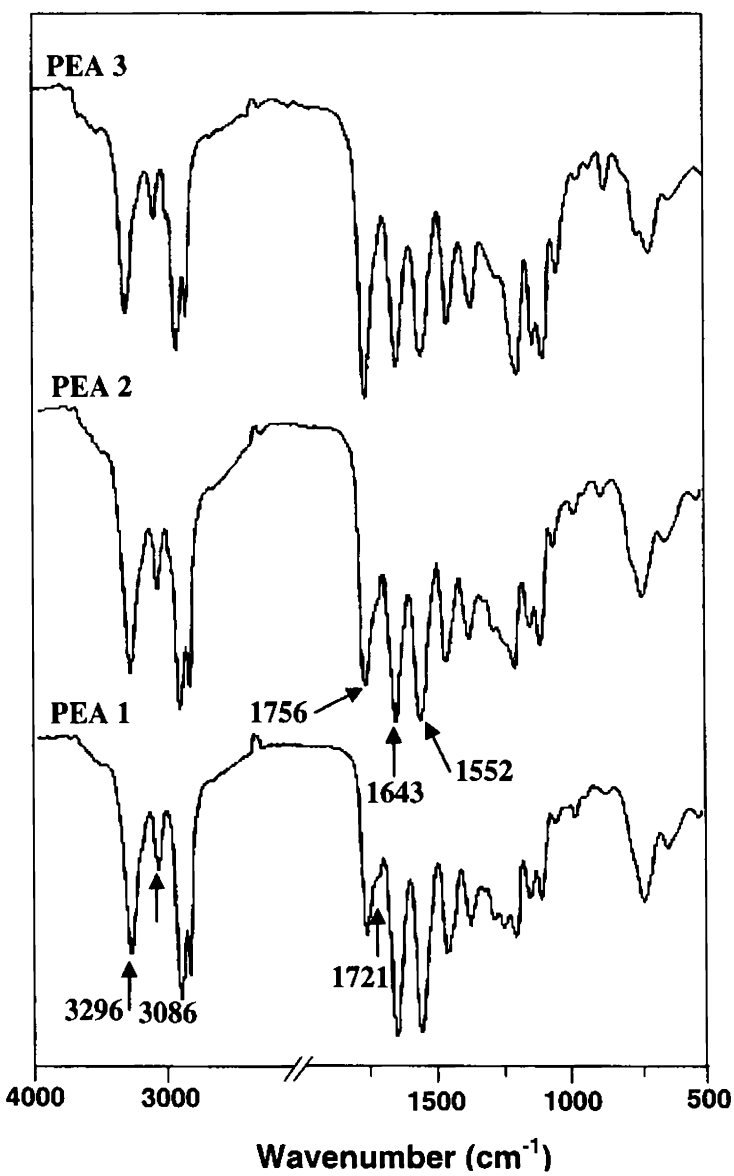


Figure 4.2. FTIR spectra of PEAs.

It should be pointed out that the N-H stretching mode appears as a single broad band at 3296cm^{-1}, indicating the existence of strong hydrogen bond interactions between amide-amide and amide-ester groups in PEAs. The characteristic ester carbonyl peak of PLLA was observed at 1756cm^{-1} with a shoulder peak at around 1721cm^{-1}, indicating the existence of free and hydrogen bonded ester carbonyl groups. The expected increase in the PLLA content on going from **PEA 1** to **PEA 3** was confirmed by taking the ratio of intensities of amide I and ester carbonyl groups. With the increase in PLLA content, amide I/C=O ester ratio decreased from 1.44 for **PEA 1** to 1.39 for **PEA 2** and 0.80 for **PEA 3**. The peaks at 2922 and 2854cm^{-1} are attributed to the ν_{CHsym} and ν_{CH2sym} respectively.

4.4.3.2. ^{1}H NMR Studies

Further evidence for the molecular architecture of the poly(ester amide) copolymers is provided by the analysis of ^{1}H NMR spectra. The typical ^{1}H NMR spectrum of **PEA 2** is given in Figure 4.3 along with PLLASe and PCHMASe to assign the peaks made on it. The resonances of PE fragments corresponding to the methine protons of PLLA, -CH_2**C**H_2O- protons of butanol and -**C**H_2-COO- protons of sebacic acid are observed at δ5.73, 4.68, and 2.84 respectively. Similarly, -**C**H_2-NHCO- and -**C**H_2-CONH- resonances of PA fragments are observed at δ3.77-3.92 and 3.05 respectively. It is interesting to note that the signal at δ5.04 corresponding to -CO-C**H**(CH_3)-OH of PLLASe was not present in **PEA 2**. This indicated that the end methine protons of HO-

PLLA-OH are added to the ester main chain. The two broad peaks at δ3.92 and 3.77 are due to the *cis*- and *trans*- isomeric protons respectively of the amide linkage -CONH-CH_2 in PEA. The intensity ratio of these isomeric peaks suggests that *cis*- and *trans*-isomers of PEA are present in the ratio of 18:82. Cyclohexane ring of diamine was observed at δ2.02-1.92 and PLLA methyl protons at δ1.72.

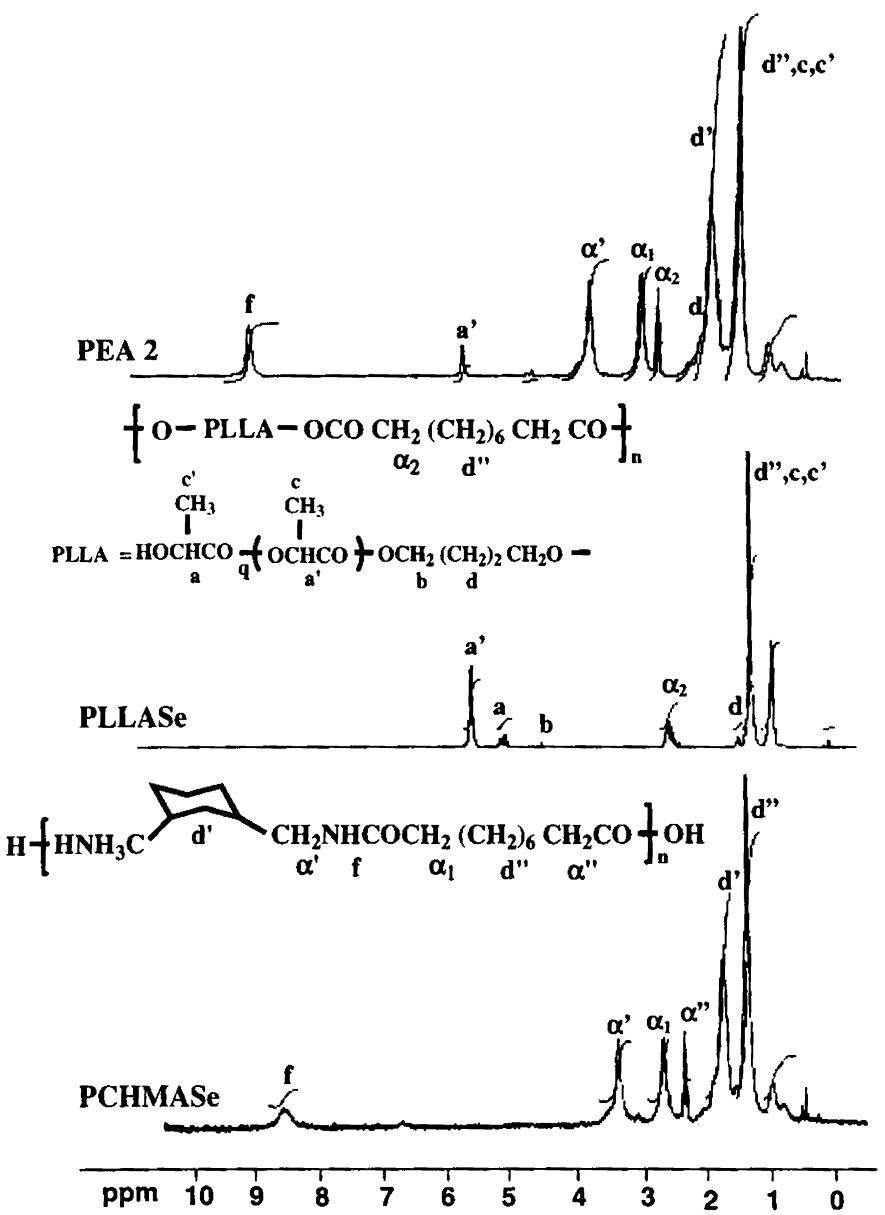


Figure 4.3. ^{1}H NMR spectra of PCHMASe, PLLASe and PEA 2.

The presence of the two distinct resonances of the -C**H**$_2$CO- protons of sebacic acid unit at δ3.05 (α_1) inserted in polyamide block and δ2.84 (α_2) belong to acid unit linking two heterotype blocks confirmed the blocky nature of the copolymers (Figure 4.4)[8,14].

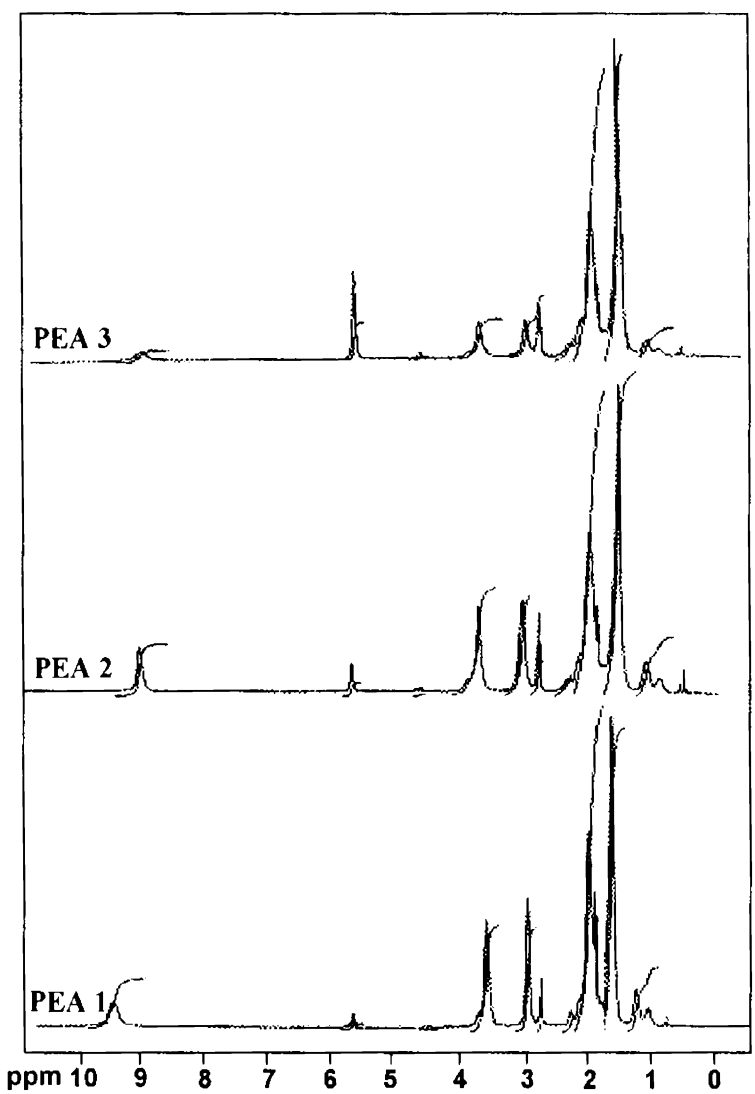


Figure 4.4. ^{1}H NMR spectra of PEAs.

Table 4.2 shows the chemical compositions determined by comparing the integration of methine resonance of PLLA (a') with those of α_1, α_2 and α`

resonances[21]. The ester/amide ratio of the polymers was evaluated by taking the area ratio of resonances corresponding to methine protons of PLLA (a') and -CH_2-NHCO- of PA segment (α')[22]. The results given in Table 4.2 are further confirming the increased incorporation of ester component in PEAs with increase of PLLA molar ratio in the feed. The ratio of the integrated α_1 and α_2 peaks represents the average number of repeat units, n, of the polyamide block[14].

Table 4.2. Data from ^{1}H NMR

Polymer	Molar ratio; Seb/CHMA/PLLA		Ester/Amide	Mn of PA
	Feed	Exp		
PEA 1	**1.00/0.90/0.10**	**1.08/0.87/0.05**	**5.4:94.6**	**1421.7**
PEA 2	**1.00/0.80/0.20**	**1.03/0.85/0.12**	**12.1:87.9**	**1232.2**
PEA 3	**1.00/0.60/0.40**	**0.92/0.56/0.52**	**48.0:52.0**	**882.9**

4.4.4. Thermal Properties and Morphology of Polymers

Thermal behaviour of PEAs was studied by DSC and TGA analysis. Morphology was confirmed by WAXD studies.

4.4.4.1. Thermal Studies

Representative DSC thermograms of the two runs performed for the three PEAs are displayed in Figure 4.5 and T_g, T_m and ΔH_m values recorded from these experiments are listed in Table 4.3. All the PEAs prepared are semi crystalline, as shown by the presence of broad melting endotherms in their

DSC traces. Samples coming directly from synthesis display a complex profile containing multiple endotherms, arising from melting of a heterogeneous population of crystallites. Villuendas et al. reported a similar observation of multiple endotherms in the case of copoly(ester amide)s derived from amino alcohols and L-tartaric and succinic acids[23]. It was reported that these multiple fusion peaks constitutes a common characteristic of poly(ester amide)s and are usually associated with different populations of lamellar crystals having different thickness[24]. Annealing at a temperature near to its high melting transition for 1h followed by slow cooling gives rise to more perfect crystals, which was observed in the second run as a single broad endotherm with a melting point lower than the higher melting crystals in the first run. The lowering of T_m can be attributed to the partial isomerization of the *trans*-cyclohexane residues to *cis*-cyclohexane residues during annealing at 180°C[16]. Since *cis*- isomer introduces a kink in the polymer chain, fewer cyclic monomers are available for incorporation into the crystals[16].

The T_m (taken from the second heating run) of the poly(ester amide)s, **PEA 1** and **PEA 2**, fall in a temperature range of 166 to 164°C, close to the T_m of PCHMASe (179°C). This result indicates that the PA component is responsible for the high melting transition, hinting that the ordering of the amide units takes place first and that of ester units next. In the case of **PEA 3**, those having higher content of PLLA, in addition to the high temperature melting endotherm, a low temperature melting endotherm at 101°C, close to the T_m of HO-PLLA-OH was also appeared, revealing the blocky nature of

poly(ester amide)s. It was reported that copolymers having block like structure present two crystalline phases characterized by melting temperatures typical of linear polyesters and polyamides[25,26]. Gonsalves et al.[9] and Castaldo et al.[26] reported a similar observation for analogous poly(ester amide)s. These observations are fully consistent with WAXD results given below.

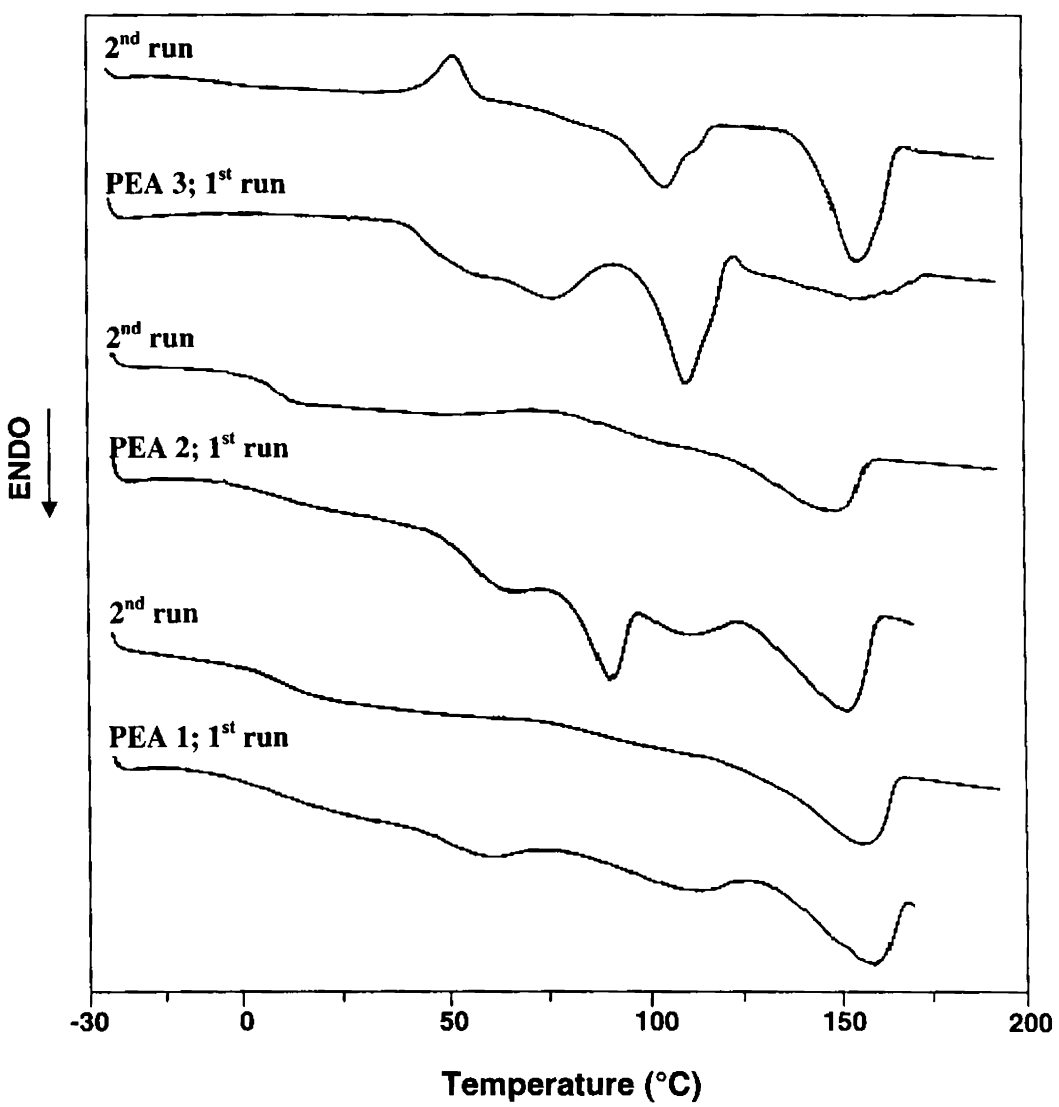


Figure 4.5. DSC thermograms of PEAs.

Table 4.3.Thermal properties of PEAs

Polymer	T_m (°C)		ΔH_m (mJ/g)		T_g (°C)
	PE	PA	PE	PA	
PEA 1	-	166	-	195.9	-
PEA 2	-	164	-	170.7	65
PEA 3	101	159	77.3	140.9	51

In **PEA 3** the fusion peak in the second heating run is preceded by an exothermic transition at 53°C, which can be ascribed to recrystallization. At higher content of cycloaliphatic structures, the rigidity of the chains increases, that will lead to lower mobility of the polymer chains to recrystallization[16]. This might explain the less pronounced exothermal transitions in **PEA 1** and **PEA 2.**

4.4.4.2. WAX-ray Diffraction Studies

The WAXD spectra of **PEA 1** and **PEA 2** given in Figure 4.6 showed a strong diffraction peak at 20.8° 2θ, indicating an amide-adjacent crystallization of copolymers. This diffraction signal (d spacing 4.26Å) yields information about the interchain distance within a hydrogen bonded sheet. Vanhaecht et al. reported a similar observation in the copolyamides 4.14/1,4-DACH.14 derived from 1,12-dodecanedicarbonyl dichloride and a variable amount of 1,4-diaminobutane and 1,4-diaminocyclohexane[18]. It was very interesting to note that in the case of **PEA 3,** two new peaks at 16.7 and 18.8° 2θ were formed, in

addition to the major peak with decreased intensity and multiple reflections at 20.8° 2θ. The new reflections closely correspond to those found for high molecular weight PLLA (16.2 and 18.6° 2θ), indicating the development of a crystalline structure of the PLLA type[8].

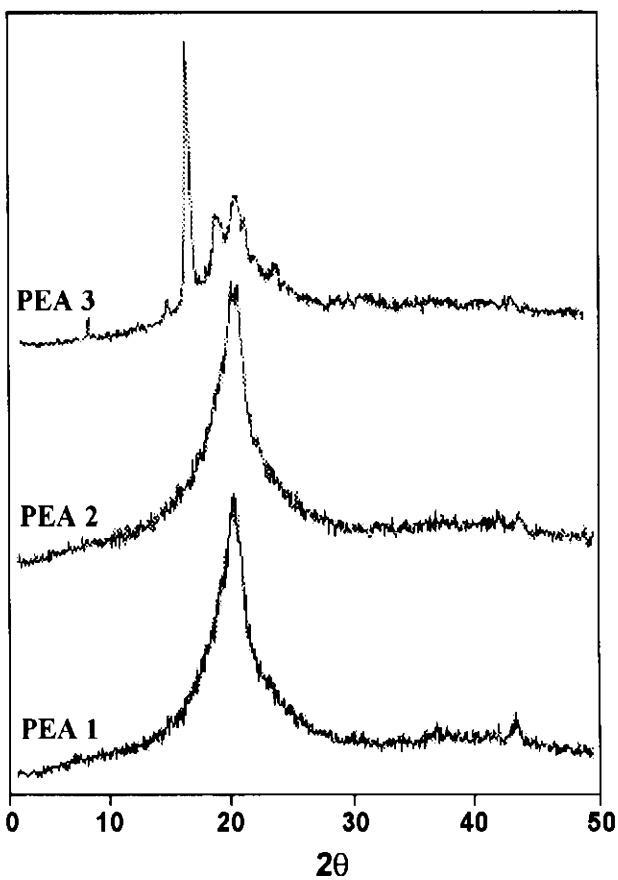


Figure 4.6. WAX-ray diffraction patterns of PEAs.

4.4.4.3. Glass Transition Temperature (T_g)

Second order transition was evaluated from the first heating run, as T_g decreases after a heating/cooling cycle. All PEAs showed a single T_g value (Figure 4.5 & Table 4.3). It is more remarkable that **PEA 3** having two melting transitions corresponding to polyamide and polyester blocks showed a single

T_g, indicating the miscibility of ester and amide units in the amorphous phase. Nijenhuis et al. reported that the single T_g is an indication of the miscibility of two polymers in a blend based on his study on PLLA/PEO blends[27]. In addition, this results seems to support the view that our synthesized copolymers have random distribution in comonomer units[7]. The T_g values also are dependent in a relevant way on the copolymer composition, showing a drastic decrease with an increase in ester content. **PEA 3** shows a T_g value of 51°C, which is lower than that of **PEA 2** at 65°C. This effect is due to the decrease of amide groups and hydrogen bond concentrations in the amorphous phase with a consequent increase of overall chain flexibility[26].

4.4.4.4. Thermal Stability Studies

Thermal stability of PEAs was studied by TGA analysis. TGA thermograms of the polymers given in Figure 4.7 showed a two-stage degradation pattern. The First stage decomposition start at around 230°C (T_o) may be attributed to the decomposition of ester linkages and the second stage decomposition initiate at around 430°C contributes to the decomposition of amide linkages. Even though, on increasing PLLA content a decrease in T_0 was observed, all synthesized PEAs are thermally stable upto a temperature of 230°C. This value is higher than the melting temperature, and consequently the polymer is stable through fusion and can be processed from the melt state. These results indicate that, by the introduction of cycloaliphatic amide segments into the PLLA macromer (Mn ≅ 1500), it could be possible to

synthesize a new polymeric material with a thermal stability comparable to that of PLLA (2.1dL/g, T0=200°C)[28], at the lower molecular weight.

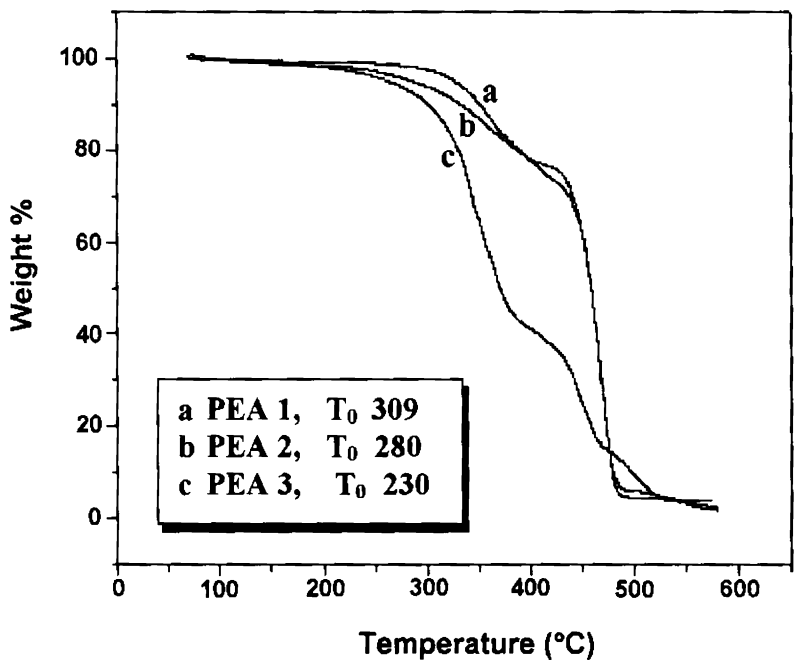


Figure 4.7. TGA thermograms of PEAs (T_o- onset temperature of first stage decomposition).

From these thermal studies it can be concluded that the prepared PEAs have a low melting transition and high thermal stability at lower molecular weight. Since, the thermal properties, comparable to that of PLLA could be attained at lower molecular weight, the applicability of these new poly(ester amide)s are expected to be not impaired.

4.4.5. Enzymatic Degradation

Enzymatic degradation of PEAs was carried out with lipase from *Candida Cylindracea* for a period of 48h, in order to achieve a reliable assessment of the influence of composition on biodegradation.

4.4.5.1. Weight Loss Studies

The enzymatic degradation was studied by monitoring the changes in weight loss of the samples as a function of immersion time. The data from these measurements are represented graphically in Figure 4.8, revealing that for all copolymers the loss of mass took place from the very early stages of incubation and continued steadily with time.

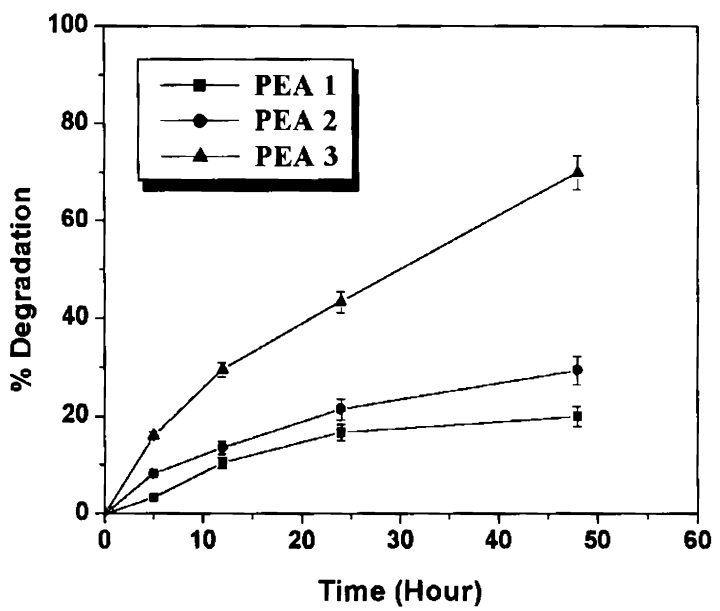


Figure 4.8. Enzymatic degradation of PEAs.

The highest biodegradation ratpwas found for **PEA 3**, which underwent a weight loss of about 69.9% after 48h of degradation. On the other hand, **PEA 1** and **PEA 2** showed only 20.0 and 29.3% weight loss after a similar period of time. These results indicate, therefore, that the PEAs are biodegradable and their biodegradability is strongly dependent upon their ester content. It was reported by Achim Gopferich that since the λ_{ester} (rate of degradation of ester

bond) is much higher than that of λ_{amide} (rate of degradation of amide bond), the rate of degradation of the PEAs is determined by the content of ester moiety[29,30]. Further studies regarding the degradation evolution of the PEAs were monitored by analyzing the degradation products of **PEA 3**.

4.4.5.2. Spectroscopic Analysis: Compositional Changes

The changes in the chemical constitution of typical **PEA 3** copolymer brought about by enzymatic degradation were analyzed by FTIR and ^{1}H NMR spectroscopies, for investigating the mechanism of degradation of PEAs. According to the FTIR spectra shown in Figure 4.9, as the degradation proceeded, the intensity of absorbance at 1756cm^{-1} decreased steadily, but the intensity of absorbance at 1643cm^{-1} decreased very slowly. The higher susceptibility of polyester segments towards enzymatic degradation was confirmed further by taking the amide I/C=O ester intensity ratio. Before degradation, the amide I/C=O ester intensity ratio was 0.80. When this copolymer was incubated in enzymatic media for 12h, it increased to be 1.12 and became 1.69 after 48h of incubation. These results indicate that, during degradation, ester content decreased quickly but amide content decreased slowly. These results are in good agreement with those reported by Marten et al.[31] and Chandra et al.[32]. In addition to these changes enzymatic degradation results in the formation of a new absorption at 1709cm^{-1}, corresponding to $\nu_{C=Ostret}$ of carboxyl groups. This result clearly indicates that the degradation of PEAs takes place mainly through ester linkages and to a limited extent through amide linkages.

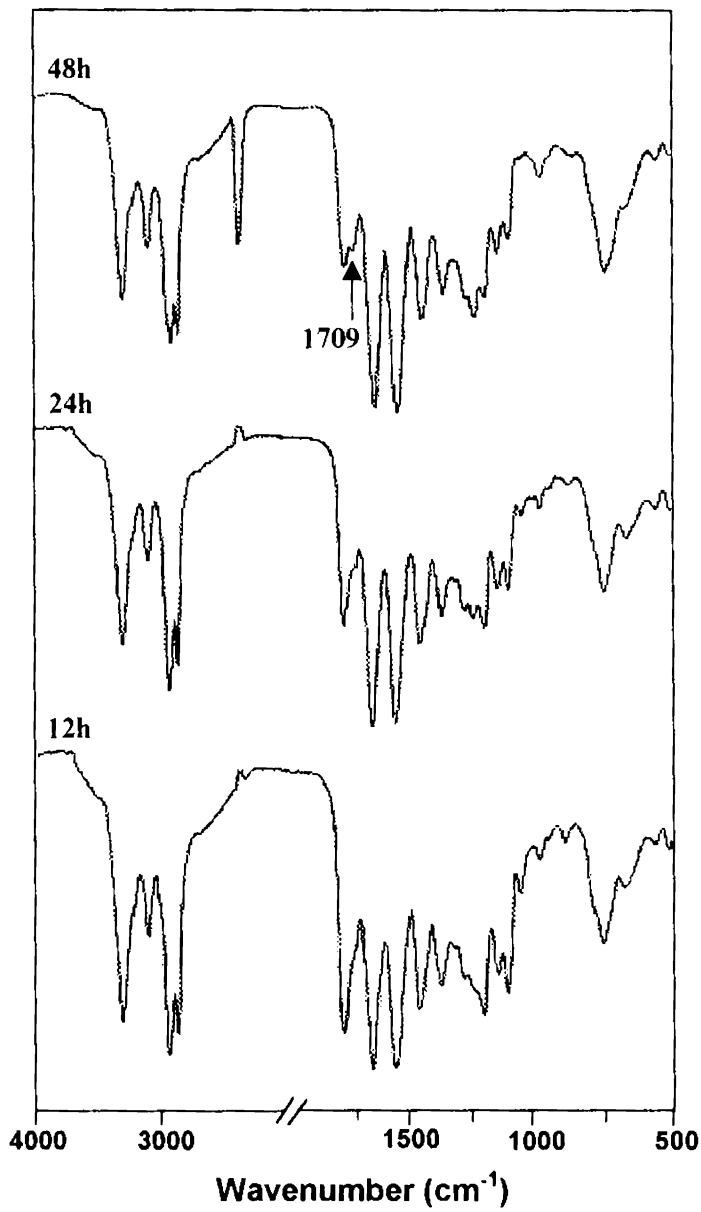


Figure 4.9. FTIR spectra of degraded PEA 3.

The ^{1}H NMR spectra of degraded **PEA 3** given in Figure 4.10 corroborated the scission of the ester linkages. The signals at δ5.73 and 2.84, corresponding to the methine protons of PLLA unit and -CH_2-COO- protons of sebacic acid respectively, showed a decrease in intensity with degradation. This

result may be attributed to the fact that ester linkages of PEAs are preferentially cleaved during enzymatic degradation. Some evidences for ester bond hydrolysis by lipase from *Candida Cylindracea* on structurally related PEAs were previously reported and are in agreement with our report[33]. It is interesting to note that the two new signals, assigned to the resonances of methine protons attached to the hydroxyl end groups of PLLA and to the methylene protons attached to the carboxylic groups of sebacic acid, appeared at δ4.8 and 2.8 respectively. From these results it can be concluded, therefore, that degradation of **PEA 3** take place through cleavage of LLA-SA labile bonds and LLA-LLA labile bonds.

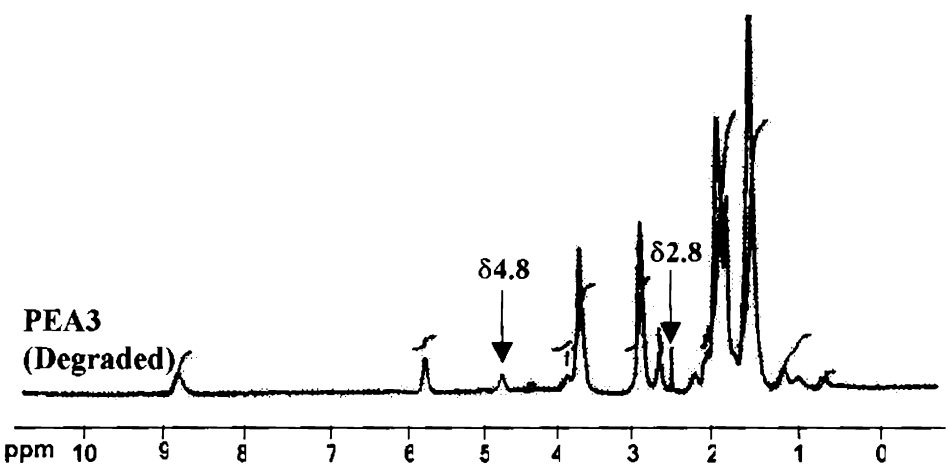


Figure 4.10. ^{1}H NMR spectra of PEA 3 after 48h of degradation in lipase.

These observations are fully consistent with FTIR results reported above. On the contrary, no changes concerning the signals arising from the -NH protons of amide linkages and no formation of signals corresponding to -NH_2 end groups are detected, revealing that the backbone amide bonds are not

involved in the degradation process. Additionally, it was noted that the ester/amide ratio decreased from 48.0:52.0 to 14.1:85.9 after 48h degradation.

4.4.5.3. Changes in Thermal Properties

Changes in the thermal properties of **PEA 3** accompanying their enzymatic degradation are given in Figure 4.11 and thermal data are tabulated in Table 4.4. It is noticeable that after 12h degradation, the T_m values of the low and high melting endotherms, initially present in the thermogram of **PEA 3**, got increased. Additionally, two T_g values one at 40°C and another at 57°C also appeared. This indicates that the enzymatic degradation of **PEA 3** have been started from the amorphous regions. On further degradation, the T_m and T_g correspond to PLLA was completely vanished and the T_m of high melting endotherm was increased to 173°C, approaching the melting temperature of polyamide segments.

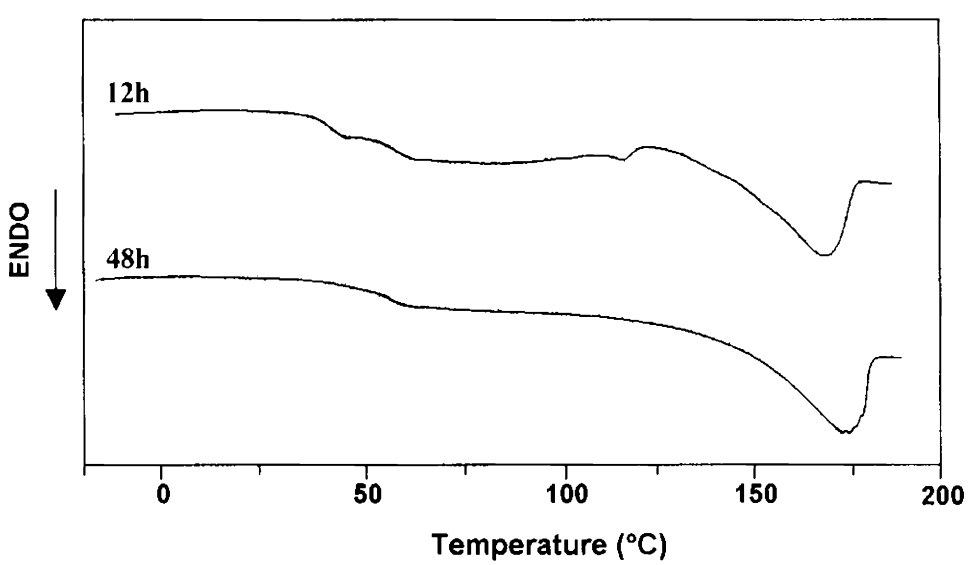


Figure 4.11. DSC thermograms of degraded PEA 3.

It can be concluded from these results that most of the PLLA segments are degraded leaving behind a polymeric residue similar to the homopolymer of polyamide.

Table 4.4. Thermal properties of degraded PEA 3

Time	T_m (°C)		ΔH_m (mJ/g)		T_g (°C)
	PE	PA	PE	PA	
12h	116	167	10.1	149.7	57 (40)
48h	-	173	-	172.6	56

4.5. Conclusions

Random multiblock poly(ester amide)s based on PLLA macromer and cycloaliphatic amide segments were synthesized from α,ω-hydroxyl terminated PLLA macromer, 1,3-cyclohexylbis(methylamine) and sebacoylchloride. FTIR and ^{1}H NMR spectroscopy confirmed the formation of block PEAs. **PEA 1** and **PEA 2** showed a crystallization pattern analogous to PA. This was observed in WAXD with a single diffraction signal in the range of PA. DSC thermograms showed a single melting transition at a temperature near to PA. In **PEA 3**, the diffraction signals and melting transitions characteristic of PE segments were also appeared. The random nature of PEAs was confirmed by the appearance of single T_g values. Since no evidence of thermal degradation was observed below a temperature of 230°C, the prepared polymers can be processed from the melt

state. Biodegradation studies indicated that degradation of PEAs is influenced by the PLLA content. Biodegradation rate of PEAs increased with increase in PLLA content. Spectroscopic investigations and thermal analysis of degraded products showed the breakage of ester linkages. These results indicate that by the incorporation of cycloaliphatic amide segments into the PLLA macromer, it could be possible to synthesize PEAs having good thermal properties and biodegradability at the lower molecular weight that would increase its potential in the biomedical applications.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Synthetic polymers are considered to be an invaluable gift of modern sciences and technology to mankind. They have occupied a major part of our life with their wide range of applications in diverse fields such as packaging, agriculture, food, consumer products, medical appliances, building materials, industry, aerospace materials etc. However, the positive properties of these polymers such as resistance to chemical, physical and biological degradation present disposal problems when their usefulness ceases. In this respect, polymeric materials satisfying the requirement of biodegradability, biocompatibility and release of low-toxicity degradation products have become important. The design of biodegradable polymers requires application of modern approaches to obtain realistic goals. In the present work, multiple approaches are adopted to identify methods to introduce biodegradability in polymeric materials and to improve upon the performance of some existing biodegradable polymers. In the first approach, novel methods of chemical modification of existing non-biodegradable polymers are attempted, taking polyethylene as a model polymer used worldwide as a commodity plastic. Second approach adopted pertains to the design of novel biodegradable

polymers based on renewable resources such as polylactides, which are gaining importance as biodegradable polymers of great opportunities.

(A) The environmental pollution caused by the non-biodegradability of polyethylene used in packaging and agricultural applications has become a serious problem causing concern throughout the world. As, there are many inadequacies persisting for the eco-friendly applications of the existing biodegradable plastics, attempts have been made to solve these problems by incorporating biodegradability into polyethylene through modifications of their chemical structure. Polyethylene could be made to an environmentally friendly polymer by introducing sugar moieties that will increase the hydrophilicity and the number of active sites for the microbial attack on polyethylene backbone.

(B) Polylactides (PLA), which are the leading candidates among the recently developed biodegradable polymers from renewable resources are gaining importance, owing to their inherent biodegradability, biocompatibility and easy availability. There are, however, some limitations such as low thermal and physical properties, low hydrophilicity and degradation rate, poor soft tissue compatibility, poor processability and high cost of production in their wide utilization. Copolymerization of lactide with appropriate comonomers and/or polymers is expected to solve some of these problems and to get new polymeric materials with unique properties for specific applications in the field. **(B1)** Graft copolymerization of lactide onto chitosan, an aminopolysaccharide present in the nature, might deliver graft copolymers with controlled solvation and degradation. The natural abundance of chitosan will reduce the overall cost

of the copolymer. **(B2)** Copolymerization of PLA with cycloaliphatic amide segments would be expected to compliment the defects of PLA by the formation of intramolecular and intermolecular hydrogen bonds between ester and amide groups. The combination of the favorable properties of both classes of materials may lead to new materials that combine good end-use and processing properties and biodegradability. The advantage of cycloaliphatic structures are that the incorporation of these rings in the amide segments increases the thermal properties of PEAs even at relatively low molecular weight than that of its linear aliphatic counter parts. Again, since cycloaliphatic systems are nontoxic, the synthesized PEAs should be biocompatible. So, the objectives of the present work were:

(1) Effect of different oxidizing agents on the functionalization of polyethylene.

(2) Synthesis and biodegradation studies of polyethylene modified with sugar grafting, using ceric ammonium nitrate as an oxidant and initiator by a "one pot" melt phase reaction in Brabender plasti-corder.

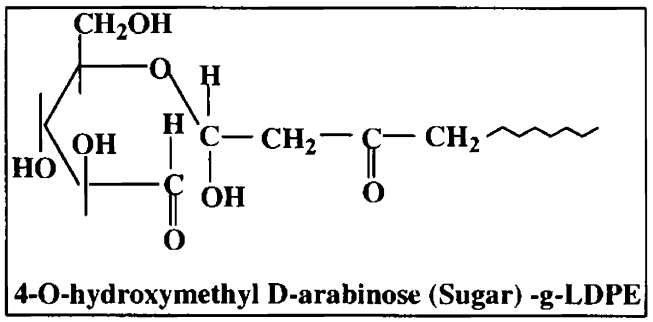
(3) Synthesis and characterization of chitosan/oligoL-lactide graft copolymers and study of the effect of hydrophobic side chains on their physico-chemical properties and biodegradability.

(4) Synthesis and biodegradation studies of random multiblock poly(ester amide)s containing poly(L-lactide) macromer and cycloaliphatic amide segments.

Chapter 1 out of five chapters gave a brief review on biodegradable polymers giving emphasis to polyethylene, polylactide, chitosan and aliphatic poly(ester amid)s.

Chapter 2 described the functionalization of LDPE under different oxidizing conditions and the modification of oxidized LDPE by the grafting of highly hydrophilic monomers such as glucose to enhance the biodegradability of LDPE. Biodegradation studies in composting conditions and in a pure soil bacterial culture *Pseudomonas sp.* was also included. In the present work, mainly three methodologies for oxidation (in solution, in melt and in solid state) were compared to study the effect of various oxidizing agents on LDPE. Oxidation of LDPE in presence of $KMnO_4/H_2O/H_2SO_4$ system, $K_2Cr_2O_7/H_2O/H_2SO_4$ system and UV irradiation resulted in the formation of -COOH groups on LDPE backbone. It was found that only ketonic carbonyl groups are formed when CAN is used as an oxidant. Grafting of glucose onto carboxyl functionalized LDPE (LDPE-COOH) was done by acid chloride method. Since this method was found to be cumbersome, grafting of glucose on to ketonic carbonyl functionalized LDPE (OxiLDPE) was done by a novel "one pot" melt phase reaction in presence of CAN in Brabender plasti-corder at high shear conditions. The present method of reactive grafting in melt avoids the use of any acid and water in the grafting reaction in presence of CAN for the first time. CAN played a dual role both as an oxidant as well as an initiator in the grafting of glucose onto LDPE. Glucose underwent a ring opening reaction to get 4-O-hydroxymethyl D- arabinose (Sugar) end-capped LDPE (**Su-g-**

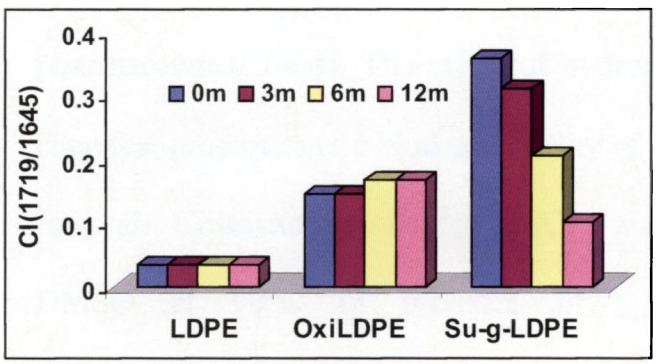
LDPE) in presence of CAN. The ring opening of glucose during reactive grafting in presence of CAN was confirmed by FTIR

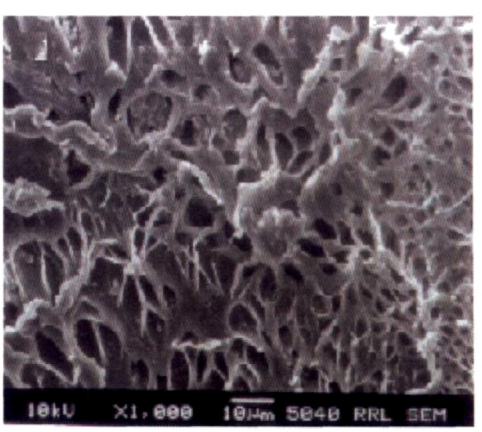


4-O-hydroxymethyl D-arabinose (Sugar) -g-LDPE

spectroscopy. FTIR spectra of **Su-g-LDPE** showed complete vanishing of peaks corresponding to cyclic ether structure of glucose at 1235, 910 and 800cm^{-1} and the appearance of new peaks corresponding to aliphatic ether structure at 1146 and 940cm^{-1}. The mechanical properties of **Su-g-LDPE** (Tensile strength: 7.82MPa) were observed to be comparable with that of LDPE alone (Tensile strength: 8.46MPa) at higher grafting percentages. This may be attributed to the strong intermolecular interactions such as hydrogen bonding between LDPE chains through their grafted sugar moieties at higher grafting percentages. This was further confirmed by WAXD measurements, which showed a broad peak at 2.23° 2θ (d spacing 39.6Å) compared to that of LDPE, indicating the long range ordering in **Su-g-LDPE** resulted by the hydrogen bonding interactions between LDPE chains through grafted sugar moieties. Though LDPE is highly hydrophobic, **Su-g-LDPE** showed a water absorption of 5.2%, indicating hydrophilic nature of the grafted LDPE that was further confirmed by observing a decrease in contact angle of LDPE from

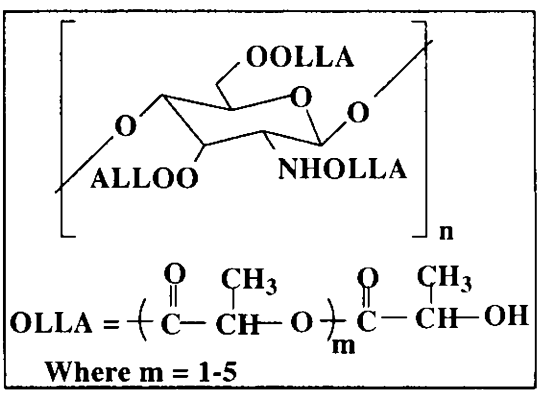
Θ_a=93° to Θ_a=74° after grafting. During biodegradation studies in compost soil environment a decrease in ketone carbonyl index (CI (ν_{1719}/ν_{1465})) of **Su-g-LDPE** with time of degradation was observed, indicating the initiation of LDPE chain degradation. A stepwise cleavage of LDPE from the chain ends can be envisaged with microbes consuming the sugar units on the surface of **Su-g-LDPE** and further oxidizing the ketonic carbonyl to carboxylic acid. The microbial degradation resulted in the increase in crystallinity and decrease in viscosity of the degraded samples. SEM showed surface erosion caused by microbial degradation on **Su-g-LDPE**. *In vitro* degradation of **Su-g-LDPE** studied by monitoring the increase in optical density of the media indicating the growth of microbes using grafted sugar as a nutrient source. The increased hydrophilicity and sugar capped chain ends in the **Su-g-LDPE** might have facilitated the enhanced degradation observed in the present case. These results suggested that sugar, grafted onto LDPE provided not only a condition suitable for the growth of microorganisms but also a favorable environment for the action of microbes on polyethylene.





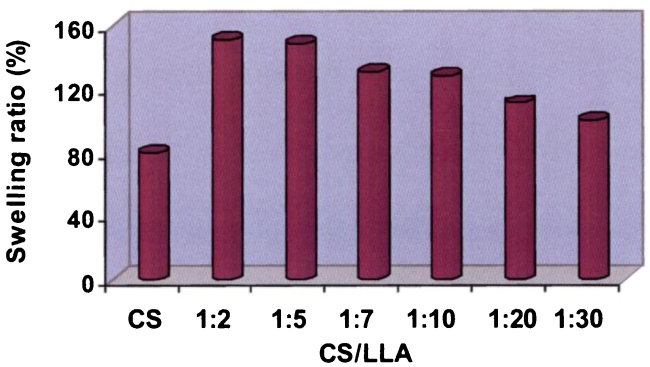
12 month Degraded Su-g-LDPE

Chapter 3 dealt with the graft copolymerization of L-lactide (LLA) onto chitosan (CS) to solve some of the inherent problems of these renewable resource based polymers for their wide utilization in biomedical and pharmaceutical fields. The effect of hydrophobic side chains on the physico-chemical properties and biodegradability of chitosan derivatives were described in detail. **Chitosan/oligoL-lactide (CL)** graft copolymers were synthesized in DMSO at 90°C in presence of $Ti(OBu)_4$ as ring opening catalyst. Grafting percentage and the molar composition of lactide in graft copolymer increased with increase of lactide content in the feed molar ratio.



FTIR and ^1H NMR studies showed the formation of oligoL-lactide (OLLA) as side chain in the graft copolymer. The disturbance in hydrogen bonding and crystallinity of chitosan brought about by LLA grafting resulted in the formation of amorphous copolymer. The DSC and TGA thermograms of grafted copolymers showed a higher evaporation temperature for the bound water than that in chitosan. Grafting of hydrophobic side chains resulted in the decrease of chitosan crystallinity by loosening the hydrogen bonds and increasing the number of free hydrophilic hydroxyl groups and amino groups

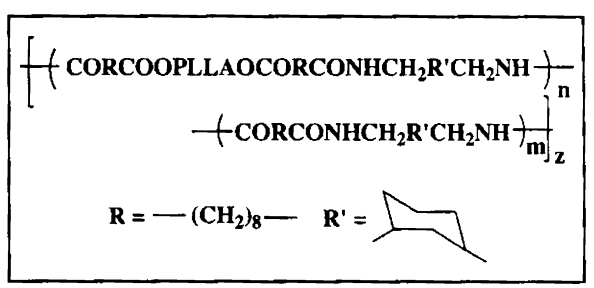
of chitosan, which in turn can hold water molecules more strongly. The graft copolymers were converted to hydrogels on exposure to deionized water. This



property is of special interest in biomedical applications such as wound dressing and in controlled drug delivery systems. At higher grafting percentages due to the self-assembling of longer OLLA side chains by the hydrogen bonding and dipole dipole interactions, the hydrophobic nature of the side chains became dominant, which resulted in the lower swelling of graft copolymers at higher grafting percentages. A decrease in hydrolytic degradation was observed with increase of lactide content in **CL** graft copolymers. *In vitro* enzymatic degradation was studied by using two types of enzymes, proteolytic enzyme papain from *Carica Papaya* and esterase enzyme lipase from *Candida Cylindracea*. Even though, **CL** graft copolymers were susceptible to both papain and lipase, the highly grafted chitosan was less susceptible to hydrolysis in papain medium whereas it was more prone to hydrolysis in lipase than the original chitosan and OLLA. The higher susceptibility of **CL** graft copolymers towards lipase compared to that of chitosan (showed no significant degradation in lipase) indicated that the chitosan segment consisting of glucosamine residues is not accessible to the lipase active site and the random distribution of OLLA side chains is at least required to be adsorbed to the active sites of the lipase. This was further

confirmed by the ^{1}H NMR spectra of degraded **CL-30** graft copolymer that showed almost complete vanishing of peaks corresponding to OLLA side chains grafted onto CS. As lipase is one of the main enzymes present in the human body, the higher susceptibility of **CL** graft copolymers towards lipase than that of chitosan and OLLA would increase its potential in biomedical field. These results indicated that the physico-chemical properties and the rate of degradation of graft copolymers as a biomaterial could be controlled by adjusting the amount of LLA in the **CL** graft copolymers.

Chapter 4 presented the synthesis and characterization of random multiblock poly(ester amide)s containing PLLA macromer and cycloaliphatic amide segments. Detailed *in vitro* biodegradation studies of PEAs using lipase from *Candida Cylindracea* were made. Poly(ester amide)s (**PEA 1**, **PEA 2** & **PEA 3**) with different sequence length of polyamide segments were prepared from the preformed polyester segments (derived from telechelic oligomers of PLLA), 1,3-cyclohexylbis(methylamine) and sebacoylchloride by the "two-step" interfacial polycondensation method. **PEA 1** and **PEA 2** showed a single diffraction signal at 20.8° 2θ and a single melting transition at 166 and 164°C respectively, indicating an amide-adjacent crystallization of copolymers. In **PEA 3**, the diffraction signals (16.7 and 18.8° 2θ) and melting transition (101°C) characteristic of PLLA macromer were also appeared. This was attributed to the development of a crystalline structure of



the PLLA type at higher ester content, revealing the blocky nature of PEAs. The random nature of PEAs was confirmed by the appearance of single T_g values. Even though the prepared PEAs were in low molecular weight (0.35 to 0.44dL/g), they showed a thermal stability upto a temperature of 230°C. This may be attributed to the presence of strong hydrogen bond interactions between ester and amide groups and the presence of rigid cyclic ring structures in the polymer backbone. Since no evidence of thermal decomposition was observed below a temperature of 200°C, they can be processed from the melt state. All PEAs were susceptible to lipase promoted degradation and the rate of degradation increased with increase in PLLA content. Spectroscopic investigations and thermal analysis of degraded products showed the breakage of ester linkages. The results indicated that by the incorporation of cycloaliphatic amide segments onto the PLLA macromer it could be possible to synthesize PEAs having good thermal properties and biodegradability at the lower molecular weight that would increase its potential in the biomedical applications.

Future Directions:

The design of biodegradable polymers with tailor made physico-chemical properties and biodegradability have opened enormous research interest because of the serious problems raised by the incompatibility of the polymeric wastes with the environment. Modification of polyethylene by grafting of highly hydrophilic sugar moieties described in Chapter 2 were interesting because of the improved mechanical properties (compared to that of

LDPE/starch blend) and enhanced degradability of **Su-g-LDPE** that will contribute to minimize the environmental pollution caused by the non-biodegradability of polyethylene. The work could be extended to various other sugars and oligomers to obtain a comparative data on their behaviour. The "one shoot" process provides the films directly and so, the cost of production can be brought down.

Chitosan/oligoL-lactide graft copolymers given in Chapter 3 were capable of forming hydrogels in neutral water and have higher susceptibility in lipase media. The variation of these properties with lactide content in graft copolymer would increase its potential in biomedical and pharmaceutical fields. The preparation and characterization of the gels and their biomedical applications will form an interesting piece of work.

Copolymerization of PLLA with cycloaliphatic amide segments described in Chapter 4 were interesting because of their higher thermal stability at lower molecular weight and biodegradability that would expected to increase its potential in biomedical field as a better alternative to PLLA. Polymers discussed in Chapter 4 require some more modifications to improve the degradability of polyamide segments to be considered as a potential material for biomedical applications. As the degradability of polyamide segments are low due to the rigidity of cycloaliphatic structure, it would be worthwhile to study the biodegradability of PEAs by incorporating flexible hydrophilic oligo(ethylene glycol) segments or enzymatic recognition sites such as amino acids into the main chain.