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. 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. The extended applications of chitosan, however, are frequently limited by its insolubility in water and neutral pH due to its rigid crystalline nature. 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. 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. 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). Copolymerization of PLLA with poly(glycolic acid) (PGA) is a possible
method to enhance the rate of degradation by disturbing the crystallinity of
PLLA. But the hydrophobic property of PLGA reduces its applications in
drug delivery systems. 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. 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
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 alkalization of chitosan can neutralize the acidic degradation products of polylactide\textsuperscript{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 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\textsuperscript{25}. In another work, Liu et al. have reported the synthesis of a brush like copolymer of polylactide grafted onto chitosan\textsuperscript{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\textsuperscript{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.\textsuperscript{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, ¹H 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, ¹H NMR and DSC analysis.

3.3. Experimental Section

3.3.1. Materials

L-Lactide and SnOct₂ were purchased from Aldrich Chemical Company, USA and were used as received. Chitosan (M₀ = 1.7 x 10⁵, 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: [η] = KₘMᵃ, where Kₘ = 3.5 x 10⁻⁴, a = 0.76. OLLA (ₚMₙ= 800) was prepared by
ring opening polymerization of L-lactide using SnOct₂ at 130°C in N₂ atmosphere. DMSO, DMF, DMA, and pyridine were obtained from S.D.Fine-Chem Ltd, Mumbai and were used after distillation. Ti(OBu)₄, SnCl₄, LiCl, ethyl acetate, NaH₂PO₄, Na₂HPO₄, 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 x 10⁻⁵ mol). Reaction was carried out for 24h at 90°C in N₂ 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:
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.

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

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$_2$PO$_4$/Na$_2$HPO$_4$, 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$_\alpha$ radiation ($2\theta = 0$-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 -NH₂ groups of chitosan will act as co-catalysts for the lactide ring opening polymerization. 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

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Catalyst</th>
<th>% Grafting</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>90</td>
<td>SnOct₂</td>
<td>83.00</td>
</tr>
<tr>
<td>DMA</td>
<td>”</td>
<td>”</td>
<td>82.00</td>
</tr>
<tr>
<td>DMSO</td>
<td>”</td>
<td>”</td>
<td>93.00</td>
</tr>
<tr>
<td>Pyridine</td>
<td>”</td>
<td>”</td>
<td>8.90</td>
</tr>
<tr>
<td>DMSO</td>
<td>110</td>
<td>SnOct₂</td>
<td>76.00 (browning)</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>”</td>
<td>34.47 (browning)</td>
</tr>
<tr>
<td>DMSO</td>
<td>90</td>
<td>SnCl₄</td>
<td>52.50</td>
</tr>
<tr>
<td></td>
<td>”</td>
<td>Ti(OBu)₄</td>
<td>99.50</td>
</tr>
<tr>
<td></td>
<td>”</td>
<td>LiCl</td>
<td>32.35</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>CS:LLA</th>
<th>% Yield</th>
<th>% Grafting</th>
<th>F_{LLA}/F_{chitosan}</th>
<th>Graft branch length</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Feed molar ratio)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL-2</td>
<td>1:2</td>
<td>47.56</td>
<td>45.04</td>
<td>0.85</td>
</tr>
<tr>
<td>CL-5</td>
<td>1:5</td>
<td>31.57</td>
<td>99.50</td>
<td>1.65</td>
</tr>
<tr>
<td>CL-7</td>
<td>1:7</td>
<td>37.10</td>
<td>117.50</td>
<td>2.21</td>
</tr>
<tr>
<td>CL-10</td>
<td>1:10</td>
<td>35.17</td>
<td>122.06</td>
<td>2.55</td>
</tr>
<tr>
<td>CL-20</td>
<td>1:20</td>
<td>34.51</td>
<td>172.17</td>
<td>3.74</td>
</tr>
<tr>
<td>CL-30</td>
<td>1:30</td>
<td>32.24</td>
<td>224.05</td>
<td>4.95</td>
</tr>
</tbody>
</table>

* Molar composition of LLA in graft copolymer = % Grafting × 161/72
* From "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 1758 cm\(^{-1}\) 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 1758 cm\(^{-1}\) was observed.

![FTIR spectra of CS and CL graft copolymers.](image-url)

Figure 3.1. FTIR spectra of CS and CL graft copolymers.
The methyl asymmetric deformation of OLLA appears at ~1475 cm\(^{-1}\). The ~1202 cm\(^{-1}\) 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 ~1046 cm\(^{-1}\) attributed to the methyl rocking and C-CH\(_3\) stretching vibration, respectively\(^\text{24}\).

### 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 \(\delta 7.73\) (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 \(\delta 4.3\) 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 \(\delta 1.68\) is attributed to the methyl protons of OLLA\(^\text{30}\). A similar result has been observed by Liu et al. in a brush like graft copolymer of poly(D,L-lactide) grafted onto chitosan\(^\text{24}\). 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 \(\delta 5.45\) and 4.3 is determined by the graft branch length\(^\text{30}\). In the samples of CL-10, CL-20 and CL-30 graft copolymers; the integral intensity ratio between the peaks at \(\delta 5.45\) 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.
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. 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\textsuperscript{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\textsuperscript{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.\textsuperscript{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\textsuperscript{32}. Table 3.3 shows that the graft copolymers have a higher $T_{\text{max1}}$ (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.](image)
Figure 3.5. DTG curves of CS and CL graft copolymers.

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

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{max}1}$</th>
<th>$W_{t_{\text{max}1}}$ (%)</th>
<th>$T_{0}$</th>
<th>$T_{\text{max}2}$</th>
<th>$W_{t_{\text{max}2}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>61.4</td>
<td>96.2</td>
<td>247.0</td>
<td>308.8</td>
<td>67.0</td>
</tr>
<tr>
<td>CL-2</td>
<td>72.7</td>
<td>95.3</td>
<td>209.9</td>
<td>287.0</td>
<td>60.7</td>
</tr>
<tr>
<td>CL-30</td>
<td>68.6</td>
<td>99.3</td>
<td>234.5</td>
<td>304.4</td>
<td>62.4</td>
</tr>
</tbody>
</table>

$T_{\text{max}1}$ - maximum temperature of decomposition corresponding to each stage (from DTG), $T_{0}$ - 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\textsuperscript{37}. $T_{0}$ correspond to the onset temperature of active
pyrolysis of graft copolymers ($T_0 = <240.0^\circ C$) was found to be less than that of CS ($T_0 = 247.0^\circ C$). 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. 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$^\circ C$ with an onset at 49.0-55.4$^\circ C$, which may be attributed to the evaporation of bound water present in the sample. 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 $\Delta H$ 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

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Endotherm 1 (°C)</th>
<th>T₀</th>
<th>Tₚ</th>
<th>Tₑ</th>
<th>ΔH(J/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td></td>
<td>49.0</td>
<td>102.5</td>
<td>160.9</td>
<td>159.7</td>
</tr>
<tr>
<td>CL-2</td>
<td></td>
<td>55.4</td>
<td>124.0</td>
<td>164.2</td>
<td>228.8</td>
</tr>
<tr>
<td>CL-5</td>
<td></td>
<td>55.0</td>
<td>123.6</td>
<td>155.7</td>
<td>210.9</td>
</tr>
<tr>
<td>CL-10</td>
<td></td>
<td>53.1</td>
<td>123.0</td>
<td>163.2</td>
<td>198.8</td>
</tr>
<tr>
<td>CL-20</td>
<td></td>
<td>50.0</td>
<td>122.1</td>
<td>162.5</td>
<td>170.5</td>
</tr>
<tr>
<td>CL-30</td>
<td></td>
<td>51.9</td>
<td>120.9</td>
<td>161.0</td>
<td>162.6</td>
</tr>
</tbody>
</table>

T₀ - onset temperature, Tₚ - peak temperature, Tₑ - 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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{26}. Grafting of LLA onto chitosan separates chitosan backbones and drastically reduces its hydrogen bonding and crystallinity and increases its affinity towards water\textsuperscript{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. 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.](image-url)
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. Yao et al. observed a similar behaviour in a
pH sensitive swelling studies of poly(chitosan-g-L-lactic acid). 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
100 days 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.

![DSC thermograms of CL-2 and CL-30 graft copolymers after 80 days of hydrolytic degradation.](image)

**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

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Endotherm 1 (°C)</th>
<th></th>
<th></th>
<th>ΔH(J/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T₀</td>
<td>Tₚ</td>
<td>Tₖ</td>
</tr>
<tr>
<td>(Hydrolytically Degrd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL-2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CL-30</td>
<td>50.0</td>
<td>118.1</td>
<td>147.9</td>
<td>139.1</td>
</tr>
<tr>
<td>(Lipase Degrd)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL-2</td>
<td>55.7</td>
<td>120.3</td>
<td>150.0</td>
<td>211.0</td>
</tr>
<tr>
<td>CL-30</td>
<td>47.6</td>
<td>113.0</td>
<td>148.3</td>
<td>149.9</td>
</tr>
</tbody>
</table>

ₜ₀ - onset temperature, ₜₚ - peak temperature, ₜₖ - 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\textsuperscript{41,42}. Certain authors sustain the view that the unspecific activity of lipases is due to the presence of chitosanases as impurities\textsuperscript{43}. Chitosan is easily hydrolysable by chitosanases that are
completely absent in mammals\textsuperscript{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 \textit{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. Even though, 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\textsuperscript{40}. Papain is very specific for the $\beta 1\rightarrow 4$ glycosidic bond cleavage of chitosan\textsuperscript{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 νC=O with νO-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.](image)

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\textsuperscript{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\textsuperscript{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.](image-url)
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. Lee et al. observed a similar behaviour in the case of enzymatic degradation of N-acyl chitosan by lysozyme. Moreover, the action of lipases is on insoluble substrates particularly at hydrophilic-hydrophobic interfaces. 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.

![DSC thermograms of CL-2 and CL-30 graft copolymers subjected to 30h enzymatic degradation in lipase medium.](image)

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 $^1$H 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.
3.6. References


