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Chitosan/oligo L-lactide graft copolymers: Effect of hydrophobic side chains on the physico-chemical properties and biodegradability

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Abstract

Graft copolymerization of L-lactide (LLA) onto chitosan (CS) was carried out by ring opening polymerisation using $Ti(OBu)_4$ as catalyst in DMSO at 90 °C in nitrogen atmosphere to obtain chitosan/oligo L-lactide graft copolymers (CL). The ring opening polymerisation of L-lactide using a covalent initiator would significantly reduce the risk of racemization even at high temperatures in comparison to other polymerization methods. It is also expected to provide copolymers having better physico-chemical properties and biodegradability than the homopolymers for applications in biomedical and pharmaceutical fields. 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, ^{1}H 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 variations in degradation depending on the amount 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, FTIR and DSC analysis showed that the biodegradation of the graft copolymers could be controlled by the LLA content. In conclusion, 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.

Keywords: Chitosan; L-Lactide; Biodegradation; Hydrogen bonding

1. Introduction

Biodegradable polymers derived from renewable resources have recently generated much interest in biomedical applications such as wound dressing, tissue regeneration, and drug delivery systems. Chitosan, a biodegradable copolymer of glucosamine and *N*-acetylglucosamine, is a versatile material of interesting structure and extraordinary properties that give rise to applications ranging from health care to agriculture to dyes for fabrics to medicine. Additionally, the special properties of chitosan such as biocompatibility, biodegradability, low immunogenecity, and biological activities, make it a very valuable material for many applications in biomedical field (Jin, Song, & Hourston, 2004; Mi, Tan, Liang, & Sung, 2002; Ravikumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Zhao, Mitomo, Nagasawa, Yoshii, & Kume, 2003). The extended applications of chitosan, however, are frequently limited by its insolubility in water and neutral pH due to its rigid crystalline nature (Majeti & Kumar, 2000; Park, Cho, Chung, Kwon, & Jeong, 2003). 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 and its copolymers are widely used in the field of biomedical applications especially in drug delivery systems, because of their inherent degradability and biocompatibility (Hollinger, 1995; Scoot & Gilead, 1995; Tsuji & Miyauchi, 2001a,b; Vert, Schwach, Engel, & Coudane, 1998). Even though PLA is considered to be biodegradable, their low hydrophilicity and high crystallinity reduce its rate of degradation (Bergsma et al., 1995; Hasirci, Lewandrowski, Gresser, Wise, & Trantolo, 2001; Middleton & Tipton,

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2000). Copolymerization of PLA with poly(glycolic acid) (PGA) is a possible method to enhance the rate of degradation by disturbing the crystallinity of PLA (Holy, Dang, Davies, & Shoichet, 1999; Lewis, 1990; Lu et al., 2000; Park, 1995; Tracy et al., 1999). But the hydrophobic property of PLGA reduces its applications in drug delivery systems. (Liu, Guo, Huang, & Deng, 2003). The accumulation of acidic degradation products formed by PLA degradation results in poorer soft tissue compatibility (Chen, Mccarthy, & Gross, 1997; Grijpm, Kroeze, Wijenhuis, & Pennings, 1993; Liu, Tian, & Hu, 2004; Yao et al., 2003). The physico-chemical properties and biodegradability of CS and PLA 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 (CS:LLA) 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 (Li, Ding, & Zhou, 2004; Yao et al., 2003). The local toxicity due to the acid byproducts can thus be alleviated to get better biocompatibility. CL graft copolymers are thus expected to have better applications in biomedical and pharmaceutical fields.

Only very limited work has been reported on the copolymerization of lactide and chitosan. Yao et al., (2003) 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. In another work, Liu et al., (2004) have reported the synthesis of a brush like copolymer of polylactide grafted onto chitosan. Later, Wu et al., (2005). studied the amphiphilic properties of a graft copolymer of water-soluble chitosan and polylactide prepared by using triethyl amine as catalyst. 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. (Qu, Wirsen, & Albertsson, 1999). 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 (Kricheldorf, 2001). 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 oligo L-lactide as the side chain. The effect of hydrophobic side chains on the physico-chemical properties of graft copolymers was analysed 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 spectroscopy and DSC analysis.

2. Experimental part

2.1. Materials

L-Lactide and $Sn(OCt)_2$ were purchased from Aldrich Chemical Company, USA and were used as received. Chitosan ($\bar{M}_v = 1.7 \times 10^5$, degree of deacetylation 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 $K_m = 3.5 \times 10^{-4}$, $a = 0.76$. OLLA ($\bar{M}_n = 800$) was prepared by ring opening polymerization of L-lactide using $Sn(OCt)_2$ 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 and Na_2HPO_4 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.

2.2. Synthesis of chitosan/oligo L-lactide graft copolymers

Chitosan in 10 ml of DMSO was taken in a two-neck RB flask. Nitrogen was purged for half an hour followed by the addition of L-lactide and $Ti(OBu)_4$ (1.2×10^{-5} mol). Reaction was carried out for 24 h at 90 °C in nitrogen atmosphere. After cooling down, the mixture was precipitated in ice-cold acetone and filtered. The graft copolymer was washed twice with ethyl acetate, and then soxhlett extracted with the same solvent for 12 h. The samples were dried in vacuum oven at 40 °C for 48 h. Grafting percentage and the amount of lactide in graft copolymer was calculated. This reaction conditions were optimized by doing grafting at various solvents, catalysts and at various temperature conditions. Experiment was again repeated at different CS:LLA feed molar ratios to study the effect of L-lactide concentration on CL graft copolymer synthesis.

2.3. Swelling studies

The water uptake capacity of each CL graft copolymer 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

$$\%W_c = \left[\frac{W_w - W_d}{W_d}\right] \times 100$$

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

2.4. 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 with known weights. 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 weight of samples before and after degradation. Average of three values were 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.

2.5. Enzymatic degradation

Enzymatic degradation studies were conducted at 40 °C by using an esterase enzyme lipase from *Candida Cylindracea* (LCC) (2.06 U/mg) and a proteolytic enzyme papain from *Carica Papaya* (1 Anston U/g). Enzymatic media, 10 ml, consists of a sodium phosphate buffer (NaH_2PO_4/Na_2HPO_4, pH 7.00) containing sodium azide (0.02 wt%) and 3 mg of the appropriate enzyme. In the case of papain, the buffer solution was activated with cystein HCl and EDTA (Enzyme in 50 mM buffer was mixed with 50 mM cystein HCl and 3 mM EDTA incubated for 30 min at 30 °C). Polymer samples weighing about 25 mg were prepared in pellet form by hot pressing and taken in glass vials containing 10 ml 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 spectroscopy and SEM were used for confirmation of biodegradation.

2.6. Measurements

The FTIR spectra of the samples were recorded with a Nicolet Magna 560 IR instrument in the spectral region between 4000 and 500 cm^{-1}. The ^1H NMR spectra of chitosan and CL graft copolymers were recorded with a 300 MHz Brucker NMR spectrophotometer in TFAc/$CDCl_3$ 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 ($2\theta = 0 - 45°$). DSC studies were conducted with a TA instrument DSC 2920 connected with thermal analyst 2100 system under N_2 atmosphere (90 ml/min). The instrument was calibrated using indium. Samples of 2–3 mg 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 at a purge rate of 30 ml/min. Samples were heated from room temperature to 600 °C at a heating rate of 10 °C/min. The surface morphology of biodegraded CL graft copolymers was observed by means of a Scanning Electron Microscopy (Hitachi 2403A, Japan) after coating the surface by sputtering with gold (10–20 nm thick).

3. Results and discussion

3.1. Synthesis of chitosan/oligo L-lactide graft copolymers

The grafting of L-lactide onto chitosan was carried out in a number of solvents and catalysts and at various temperatures to identify the most effective ring opening catalyst and to optimize the conditions of grafting. Table 1 shows 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 are found to be better catalysts than Lewis acids. Metal alkoxides are known to be involved in a co-ordination insertion mechanism and the presence of –OH and $–NH_2$ groups of chitosan will act as co-catalysts for the lactide ring opening polymerization (Arvanitoyannis, Nakayama, Kawasaki, & Yamamoto, 1995). 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 recemization was used to get L-lactide grafted onto chitosan (Kricheldorf, 2001).

The effect of L-lactide concentration on the percentage of grafting of L-lactide onto chitosan is given in Table 2 It can be seen from Table 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

Table 1
Effect of various reaction conditions on the synthesis of CL graft copolymer

Solvent	Temperature (°C)	Catalyst	% Grafting
DMF	90	$Sn(OCt)_2$	83.00
DMA	90	$Sn(OCt)_2$	82.00
DMSO	90	$Sn(OCt)_2$	93.00
Pyridine	90	$Sn(OCt)_2$	8.90
DMSO	110	$Sn(OCt)_2$	76.00 (browning)
	130	$Sn(OCt)_2$	34.47 (browning)
DMSO	90	$Sn(OCt)_2$	93.00
	90	$SnCl_4$	52.50
	90	$Ti(OBu)_4$	99.50
	90	LiCl	32.35

Table 2
Effect of L-lactide concentration on CL graft copolymer synthesis

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

[a] %Grafting $=[W_g - W_0/W_0] \times 100$. W_g and W_0 denote the weight of the grafted CS and initial weight of CS, respectively.
[b] Molar composition of LLA in graft copolymer = %grafting × 161/72.
[c] From ^{1}H NMR.

higher the concentration of lactide, the higher the reactivity of lactide with chitosan. A schematic representation of grafting of LLA onto CS in the presence of $Ti(OBu)_4$ is shown in Scheme 1.

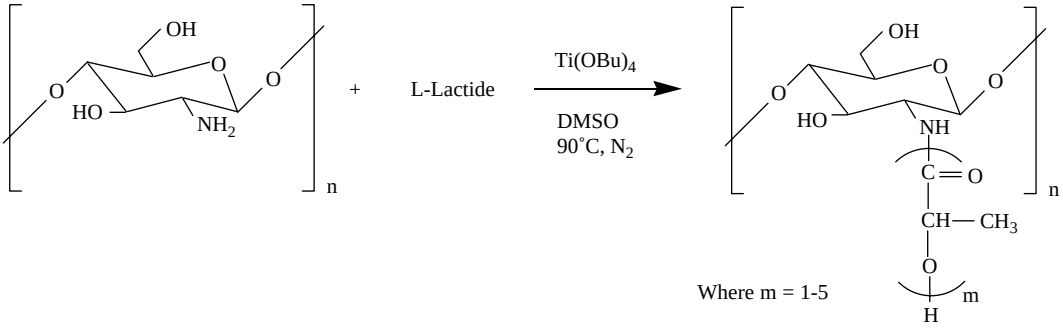
3.2. FTIR analysis

Structural changes of chitosan brought about by LLA grafting were studied by FTIR spectroscopy (Fig. 1). In comparison to the FTIR spectra of chitosan, CL graft copolymers have a new absorption at 1758 cm^{-1} assigned to the ester carbonyl group of the branched oligo L-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. 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 (Liu et al., 2004). The observed increase in the intensity of amide-1 peak (1665 cm^{-1}) of chitosan after LLA grafting indicates formation of the amide linkage during grafting. A similar observation has been reported by Qu et al. who have used D,L-lactic acid for grafting without any catalyst (Qu, Wirsen, & Albertsson, 2000a,b). The amino group at C2 is expected to be more reactive than the hydroxyl groups at C3 and C6 position due possibly to their high nucleophilicity according to (Dutta, Ravikumar, & Dutta, 2002). But at the same it is not possible to totally neglect the reactivity of –OH groups because this was the main coinitiator used by several research teams in the ROP of lactides. So, there is a possibility that esterification might also take place partly, the main product being the amide.

3.3. ^{1}H NMR studies

The ^{1}H NMR spectra of chitosan and CL-30 graft copolymer are shown in Fig. 2. The ^{1}H NMR spectra of chitosan showed peaks at δ 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 δ 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 δ1.68 is attributed to the methyl protons of OLLA (Arvanitoyannis et al., 1995). A similar result has been observed by Liu et al., (2004) in a brush like graft copolymer of poly D,L-lactide grafted onto chitosan. These results indicate that the CL graft copolymers contained oligo L-lactide side chains.

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 δ 5.45 and 4.3 is determined by the graft branch length (Arvanitoyannis et al., 1995). In the samples of CL-10, CL-20 and CL-30 graft copolymers, the integral intensity ratio between the peaks at δ 5.45 and 4.3 is 2.55, 3.74 and 4.95, respectively. The results correlate well with the results of gravimetric method (Table 2). The amount of branched polymer increases with increase of lactide content in feed molar ratio. The amine peak at δ 7.73 observed in CS was



Scheme 1. Schematic representation of the synthesis of CL graft copolymers.

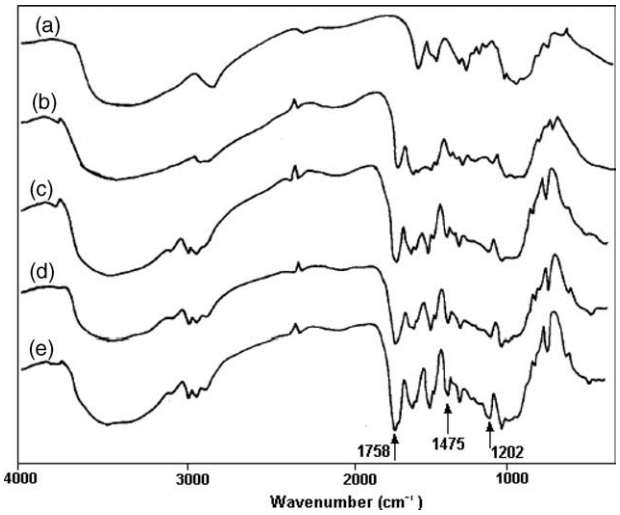


Fig. 1. FTIR spectra of (a) CS, (b) CL-2, (c) CL-10, (d) CL-20 and (e) CL-30.

shifted to at δ 7.47 in graft copolymer indicating the involvement of amine groups of CS in LLA grafting.

3.4. Physical properties

X-ray diffraction profiles of CS and CL graft copolymers are shown in Fig. 3. The X-ray diffraction pattern of native chitosan showed hydrated polymorphism with a 020 reflection

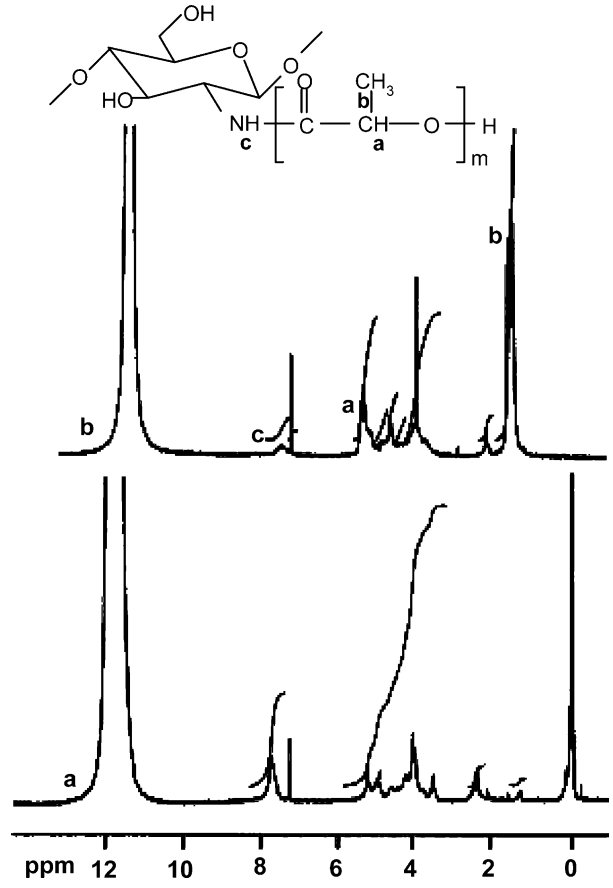


Fig. 2. ^{1}H NMR spectra of (a) CS and (b) CL-30.

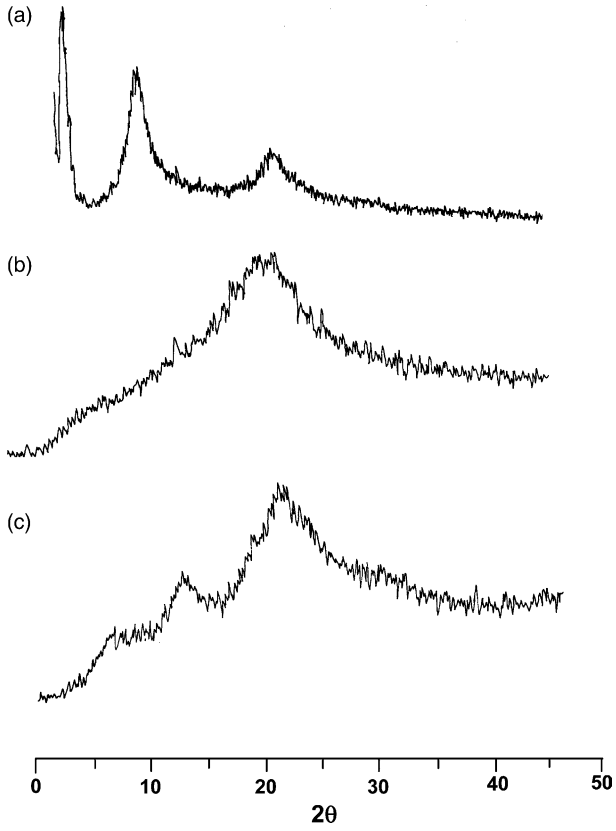


Fig. 3. WAX-ray diffraction patterns of (a) CS, (b) CL-2 and (c) CL-30.

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 (Kittur, Acharya, Kumar, & Tharanathan, 2003). It is interesting to note that the grafting of LLA onto chitosan results in almost complete removal of the peak at 10° 2θ whereas the intensity of the peak at 20° 2θ increased considerably. These changes in the peak intensity suggest different packing of chains and/or different hydrogen bonding network in the graft copolymer (Kittur et al., 2003). This indicates that the crystalline patterns of chitosan and its graft copolymers are different. 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 (Wu et al., 2005; Yao et al., 2003). 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 crystallization of longer OLLA side chains that might self assemble by hydrogen bonding and dipole–dipole interactions between oligo ester side chains. A similar observation on cytocompatible poly(chitosan-*g*-L-lactic acid) was reported by Yao et al. (2003). Another peak of L-lactide oligomer reported in literature at 19° 2θ may be merged with the main broad peak.

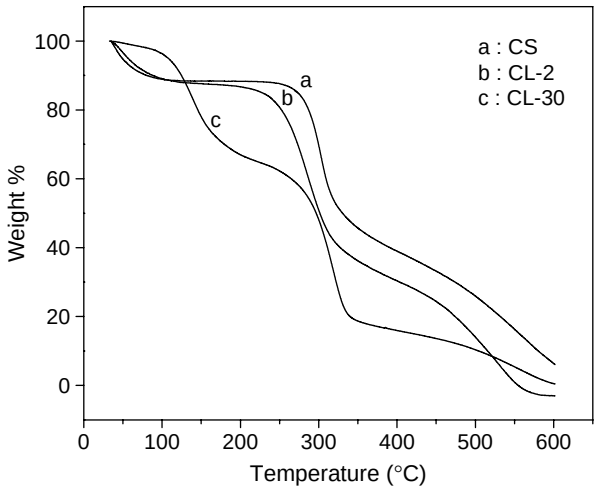


Fig. 4. TGA thermograms of CL graft copolymers.

3.5. Thermal properties

Thermal properties of CS and its graft copolymers were studied by TGA/DTG and DSC analysis. It can be seen from Figs. 4 and 5 that both CS and its graft copolymers (except at high LLA content such as CL-30) 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 (Qin et al., 2003). Table 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 takes place at a higher temperature than that of chitosan. This reveals that

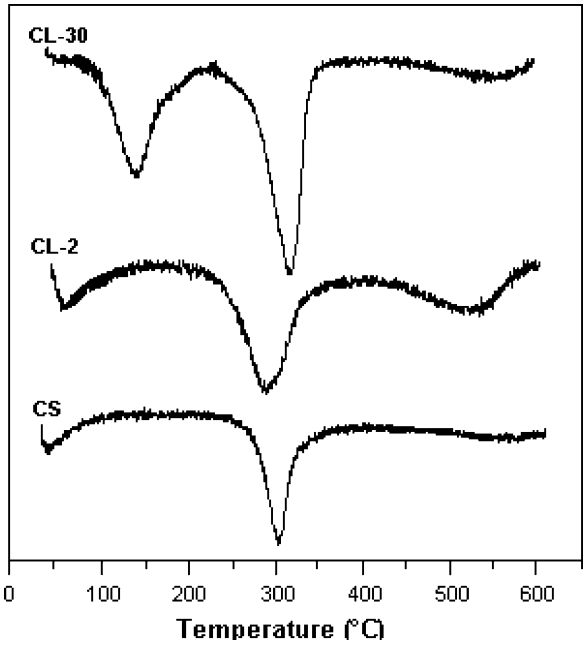


Fig. 5. DTG curves of CL graft copolymers.

Table 3
Thermal degradation data of CL graft copolymers

	T_{max1}	$Wt_{max1(\%)}$	T_{0LLA}	T_{max2}	$Wt_{max2(\%)}$	T_0	T_{max3}	$Wt_{max3(\%)}$
CS	44.1	96.2	–	–	–	252.4	303.8	67.0
CL-2	53.7	95.3	–	–	–	209.4	287.0	60.7
CL-30	51.7	99.3	90.0	140.9	82.4	240.0	319.4	33.7

T_{oLLA}, onset temperature of LLA degradation; T_o, onset temperature of active pyrolysis; T_{max}, maximum temperature of decomposition corresponding to each stage.

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 (Kittur et al., 2003; Wu et al., 2005). In the case of CL-30 graft copolymer, weight loss takes place in three stages. LLA being aliphatic, the weight loss during the second stage might attribute to the degradation of LLA side chains (T_{0LLA} = 90 °C, T_{max2} = 140.9 °C). The aliphatic nature of LLA also affects the thermal stability of the graft copolymers (T_0 = < 240 °C), which was found to be less than that of CS (T_0 = 252 °C). Zong, Kimura, Takahashi, and Yamane (2000). report 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 a 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 LLA chains.

Close examination of DSC thermograms in Fig. 6 reveals that CL graft copolymers have two thermal transitions compared with chitosan, which have only one thermal transition. The first thermal event registered in all the samples was a wide endothermic peak centred between 102.5 and 127.2 °C with an onset at 49.0–55.4 °C, which may be attributed to the evaporation of bound water present in the sample (Zeng, Fang, & Xu, 2004). Values for the transition temperatures and their associated enthalpies are given in Table 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. 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. 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. (Kittur et al., 2002; Prashanth, Kittur, & Tharanathan, 2002; Qu, Wirsen,

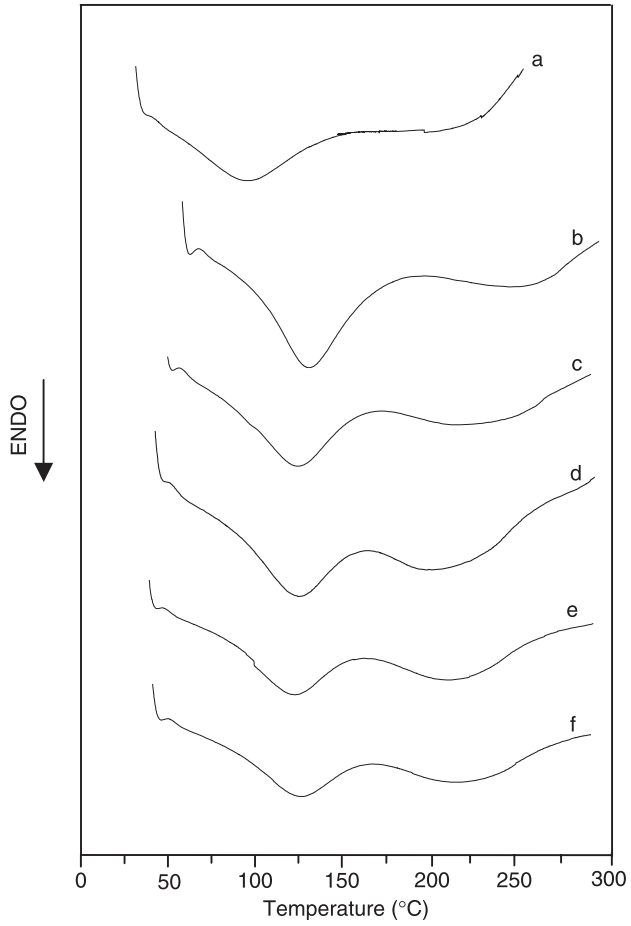


Fig. 6. DSC thermograms of (a) CS, (b) CL-2, (c) CL-5, (d) CL-10, (e) CL-20 and (f) CL-30.

& Albertsson, 2000a,b; Sato et al., 1997). The evaporation temperature of bound water in CL graft copolymers increase by about 20–25 °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 (Kittur et al., 2002; Prashanth et al., 2002). 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.

The second thermal event registered for all CL graft copolymers was a wide endothermic peak centred between 203.5 and 237.1 °C (Fig. 6). This may be attributed to the melting transition of the samples. In the case of chitosan, the strong intermolecular and intramolecular hydrogen bonds make its melting point higher than its decomposition temperature. When lactide was grafted onto chitosan, the hydrogen bonds that existed in chitosan were disturbed to a certain extent, which forces intermolecular interactions to decrease (Liu et al., 2004). It is interesting to note that melting peak temperature (T_p) and the enthalpy (ΔH) of CL graft copolymers initially decreases with increase of grafting percentage up to CL-10 and then increases with increase of grafting percentage above CL-10. The observed increase in T_p and ΔH values of the grafted copolymers at higher grafting percentages confirming further the formation of strong hydrogen bond interaction between chitosan chains through the covalently grafted LLA chains.

3.6. Swelling studies

The effect of CS:LLA feed ratio on equivalent water uptake of CL graft copolymers is shown in Fig. 7. It can be seen from Fig. 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 (Kamath, Kincaid, & Mandal, 1996; Lee, Kim, & Lee, 2000; Qu, Wirsen, & Albertsson, 1999, 2000a,b; Singh & Ray, 1994). In the case of chitosan, even though it is hydrophilic, the strong hydrogen bonding and crystallinity reduces the infiltration and water diffusion (Li et al., 2004). Grafting of LLA onto chitosan separates chitosan backbones and drastically reduces its hydrogen bonding and crystallinity and increases its affinity towards water (Gorochovceva & Mukuska, 2004). 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 1, it will form a loose physically cross linked copolymer through hydrogen bonding and dipole–dipole interactions between neighbouring ester groups and chitosan chains (Berger et al., 2004; Qu et al. 2000a,b; Yao et al., 2003). So, these samples have the highest swelling among the samples

Table 4
Thermal transitions of CL graft copolymers

Polymer	Endotherm 1 (°C)				Endotherm 2 (°C)			
	T_o	T_p	T_c	ΔH (J/mg)	T_o	T_p	T_c	ΔH (J/mg)
Chitosan	49.0	102.5	160.9	259.8	–	–	–	–
CL-2	55.4	127.2	164.2	398.9	184.9	237.1	294.5	123.0
CL-5	55.0	126.5	155.7	325.9	169.2	222.9	281.1	118.2
CL-10	53.1	125.1	163.2	298.1	169.6	203.5	270.4	90.7
CL-20	50.0	123.6	162.5	270.5	169.6	211.5	273.4	102.9
CL-30	51.9	121.9	161.0	262.1	169.5	216.3	279.6	112.4

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

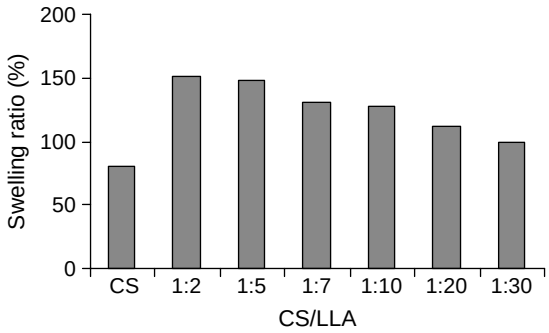


Fig. 7. Swelling studies of CL graft copolymers.

investigated. However, on increasing LLA content in feed ratio, a decrease in swelling ratio was observed. At higher lactide content, due to aggregation, the hydrophobic nature of the side chains become 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 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 (Qu et al., 2000a,b). Yao et al. (2003) 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. As many enzyme assays are performed in the neutral pH, the biological and physiological potential of CL graft copolymers would increase dramatically.

3.7. Hydrolytic degradation

The changes in weight loss of CS and CL graft copolymers subjected to hydrolytic degradation in deionized water is shown in Fig. 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 Fig. 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.

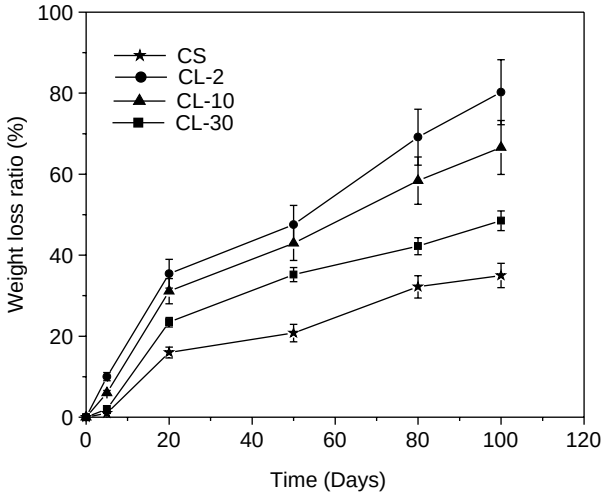


Fig. 8. Changes in weight loss of CL graft copolymers with time of immersion in deionized water.

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 (Fig. 9). Hydrolytic degradation results in surface erosion of chitosan with the formation of small pores (Fig. 9B). Hydrolytically degraded CL-2 and CL-10 graft copolymers given in Fig. 9C and D, 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 (Fig. 9E), the SEM micrograph showed less surface erosion compared to that of CL-2 and CL-10. This may be attributed to the decreased hydration of CL-30 that results in lower hydrolytic degradation.

Hydrolytic degradation was further confirmed by DSC analysis (Fig. 10). DSC thermogram of CL-2 graft copolymer after 80 days of hydrolytic degradation did not show the two endotherms that were observed originally in it before degradation indicating that the polymer has already degraded (Figs. 6b and 10a and Table 5). In comparison, CL-30 copolymers showed a decrease in the peak area and peak position of first endotherm and complete vanishing of the second endotherm indicating that the polymer has degraded, but at a slower rate than that of CL-2 (Figs. 6f and 10b and Table 5). These findings reveal that the degradation rate of CL graft copolymers can be controlled by changing the amount of LLA content in graft copolymer.

3.8. 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 *C. Papaya* and esterase enzyme lipase from *C. Cylindracea*. Selection of enzymes was based on its activity on chitosan. Papain was reported to be more efficient hydrolytic agents for chitosan and lipases depolymerize chitosan to a limited extent (Kumar, Varadaraj, Lalitha, & Tharanathan, 2004; Muzzarelli, Terbojevich, Muzzarelli, & Francescangeli, 2002). Certain authors sustain the view that the unspecific activity of lipases is due to the presence of

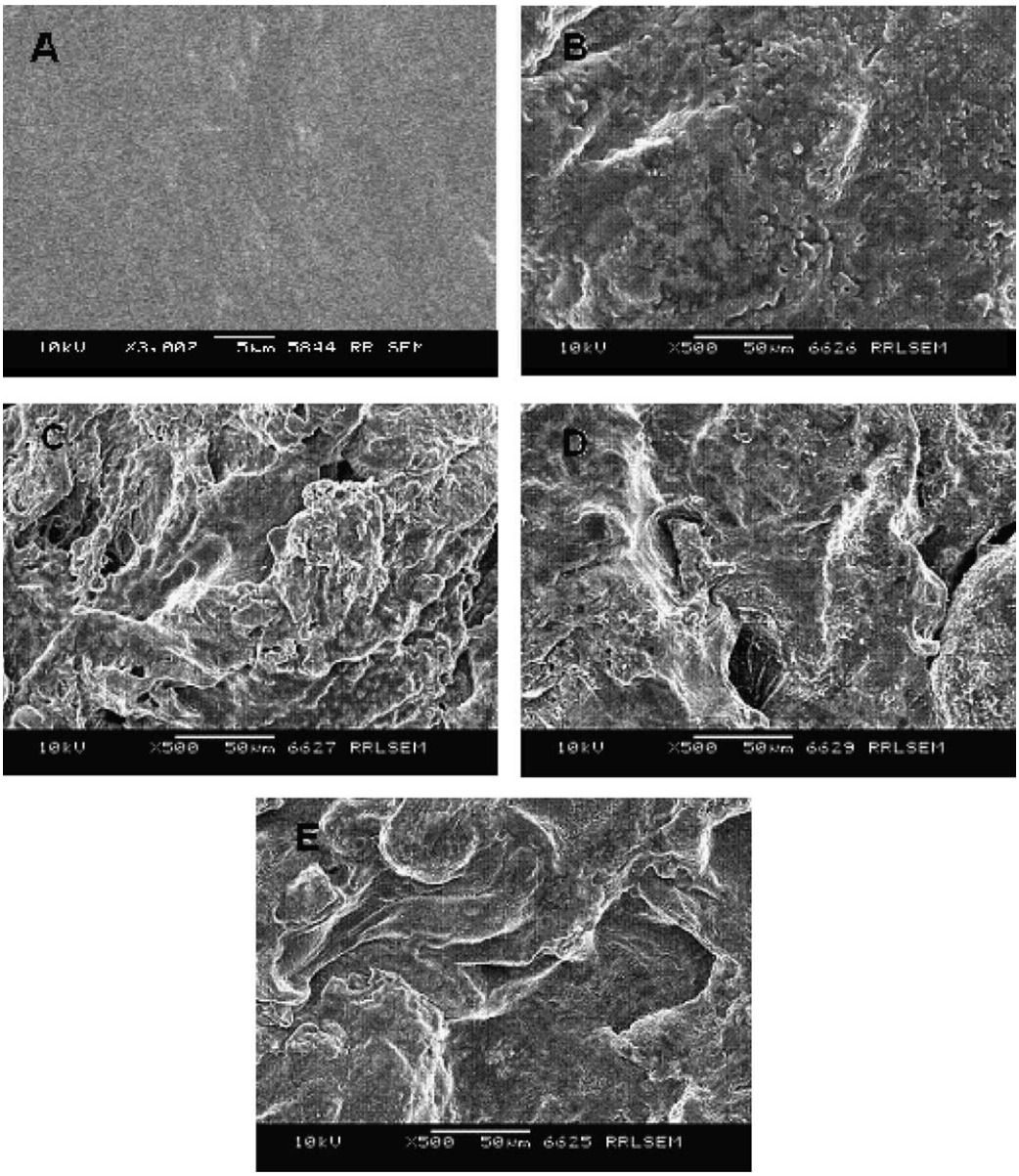


Fig. 9. SEM micrographs of hydrolytically degraded samples taken after 80 days of immersion in deionized water: (A) CL graft copolymer before degradation, (B) CS, (C) CL-2, (D) CL-10 and (E) CL-30.

chitosanases as impurities. Chitosan is easily hydrolysable by chitosanases that are completely absent in mammals (Muzzarelli, Francescangeli, Tosi, & Muzzarelli, 2004; Tomihata & Ikada, 1997). Since 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.

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 Fig. 11. CS and CL

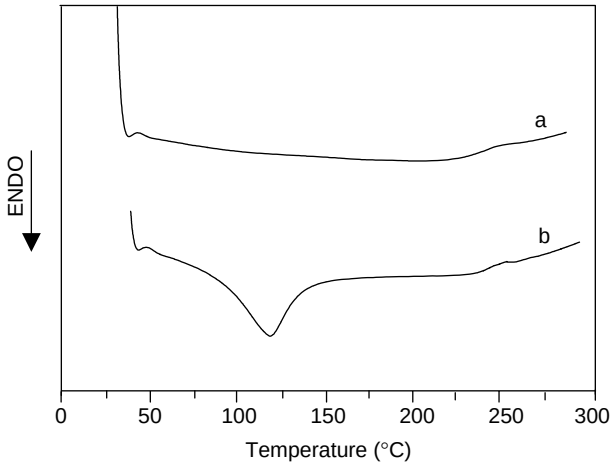


Fig. 10. DSC thermograms of (a) CL-2, and (b) CL-30 after 80 days of hydrolytic degradation.

Table 5
Thermal transitions of CL graft copolymers after degradation

Polymer	Endotherm 1 (°C)				Endotherm 2 (°C)			
	T_o	T_p	T_c	ΔH (J/mg)	T_o	T_p	T_c	ΔH (J/mg)
CL-2 (Hydroly degr)	–	–	–	–	–	–	–	–
CL-30 (Hydroly degr)	50.0	119.9	147.9	239.1	–	–	–	–
CL-2 (Lipase degr)	52.7	120.3	150.0	291.0	–	–	–	–
CL-30 (Lipase degr)	49.6	119.0	148.3	200.9	–	–	–	–

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

graft copolymers showed a sharp increase in weight loss during the initial period of degradation. After 24 h, 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. Papain is very specific for the β 1→4 glycosidic bond cleavage of chitosan (Kumar et al., 2004). 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. Further investigation from the FTIR of the degraded CL-2 graft copolymer showed in Fig. 12 indicates evidence of the removal of OLLA by showing a decrease in the ratio of the absorbance of $\nu_{C=O}$ with ν_{OH}, 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 Fig. 11. Don, Chuang, and Chiu (2002). observed a similar behaviour on the lysozyme promoted degradation of chitosan-*g*-poly(acrylic acid) copolymers

Degradation was further confirmed by SEM micrographs of CL graft copolymers taken after 8 h immersion in papain medium (Fig. 13). In the case of graft copolymers, CL-2 degraded severely and CL-10 showed less surface erosion than that of CL-2 (Fig. 13B and C) whereas CL-30 was observed to be almost maintaining its physical form even after 8 h exposure to papain medium (Fig. 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.

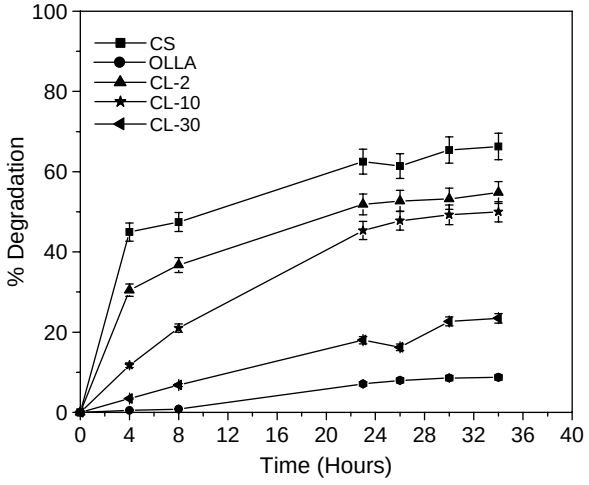


Fig. 11. Changes in percentage degradation of CL graft copolymers with time of exposure to papain medium.

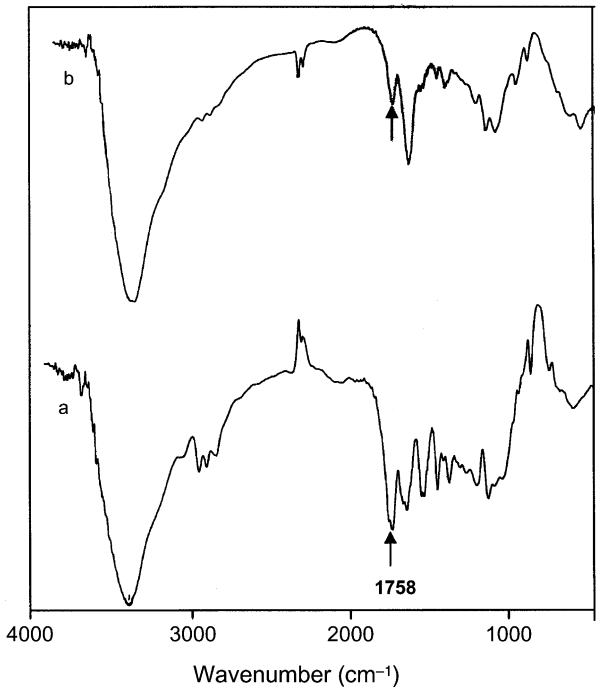


Fig. 12. FTIR spectra of CL-2 graft copolymer (a) before and (b) after enzymatic degradation by papain.

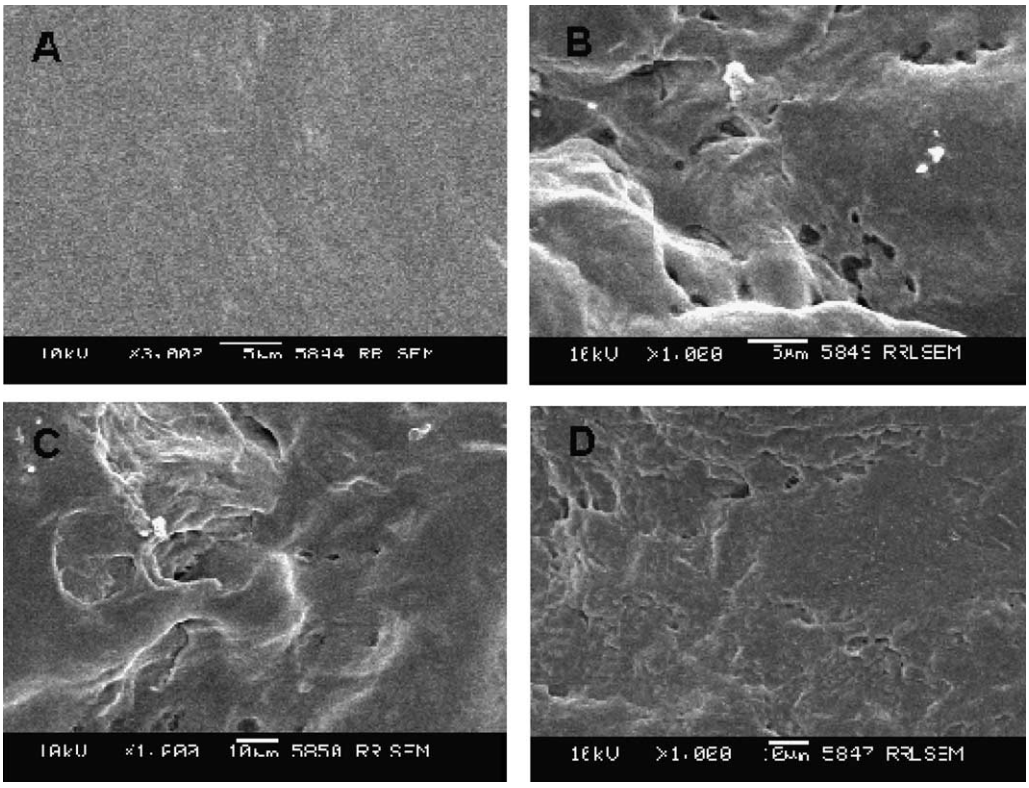


Fig. 13. SEM micrographs of enzymatically degraded samples taken after 8 h immersion in papain medium: (A) CL graft copolymer before degradation, (B) CL-2, (C) CL-10 and (D) CL-30.

Fig. 14 shows the percentage degradation of CS, CL graft copolymers and OLLA with time of immersion in esterase enzyme lipase from *C. 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. (2004) reported that the lipases of various origins have different activity on the depolymerization of chitosan. In the present study, the digestibility of chitosan by lipase from *C. 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 (Muzzarelli et al., 2004; Muzzarelli, Xia, Tomasetti, & Ilari, 1995). In addition, lipase susceptibility on graft copolymers was observed to be increased with increase of grafting percentage. 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 (Sashiwa, Saimoto, Shigemas, Ogawa, & Tokura, 1990) Lee, Ha, and Park (1995) 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 (Muzzarelli et al., 1995). 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. These hypotheses were confirmed further by DSC thermograms of degraded CL-2 and CL-30 taken after 30 h exposure to lipase medium. In comparison to the non-degraded CL-2 and CL-30, the degraded samples are showing a decrease in peak area and position of the first endotherm and complete vanishing of second endotherm (Figs. 6 and 15 and Table 5). These changes indicate that the exposure of CL graft copolymers to lipase leads to a remarkably

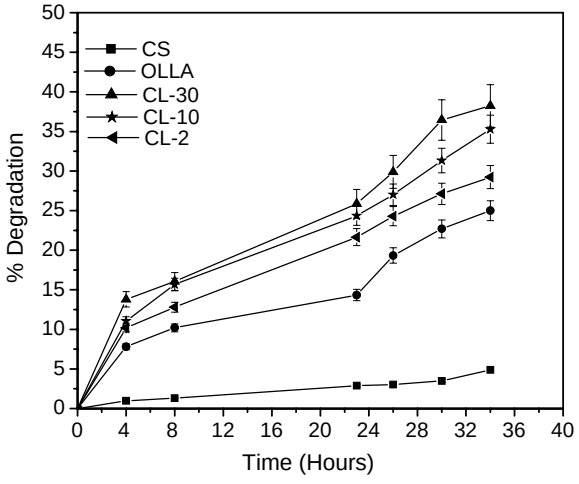


Fig. 14. Changes in percentage degradation of CL graft copolymers with time of exposure to lipase medium.

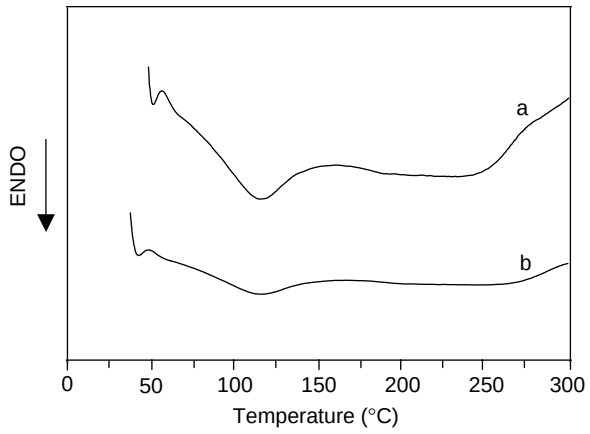


Fig. 15. DSC thermograms of (a) CL-2, and (b) CL-30 subjected to 30 h enzymatic degradation in lipase medium.

more ordered structure, that is presumably the result of the removal of more disordered portion of graft copolymer by enzymatic hydrolysis.

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 as an alternative to PLLA 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.

4. Conclusions

Chitosan/oligo L-lactide graft copolymer was synthesized in DMSO at 90 °C in the 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 having a melting transition with lower thermal stability than chitosan. 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. Thermal stability and the melting transition temperature also increased at higher grafting percentages. A decrease in hydrolytic degradation was observed with increase of lactide content in CL graft copolymer. 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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