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Synthesis and characterization of a brush-like copolymer of polylactide grafted onto chitosan

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Abstract—A brush-like poly(DL)-lactide grafted onto chitosan as the backbone was investigated. The graft copolymerization was carried out with triethylaluminum as catalyst in toluene at 70 °C. It was found that a greater lactide content in the feeding ratio results in a higher grafting percentage. FTIR spectrometry, ^{1}H NMR, DSC scanning, and wide-angle X-ray scattering, respectively, are used to characterize these branch copolymers. A copolymer has a definite melting point when the molar feeding ratio of lactide to chitosan is more than 10:1, and the ΔH of the copolymers increases with the feed ratio of lactide to chitosan in feeding.

Keywords: Chitosan; Polylactide; Graft copolymer

1. Introduction

Biodegradable materials are becoming increasingly more important in the biomaterials field, particularly for wound healing, tissue reconstruction, and controlled drug delivery. The main advantage of biodegradable over nonbiodegradable materials is the disappearance of implanted foreign materials from the body as a result of their biodegradation. Most synthetic biodegradable materials such as polylactide and its copolymers with polyglycolide and polycaprolactone have high initial strengths, while natural products like chitin and its derivatives are low in mechanical strength but exhibit excellent cell adhesion.[1]

Chitosan, the fully or partially deacetylated form of chitin, the principal component of living organisms such as fungi and crustaceans, contains 2-acetamido-2-deoxy-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose groups. This polymer is known to be nontoxic, odorless, and biocompatible in animal tissues, as well as being enzymatically biodegradable. Much attention has been paid to its biomedical, ecological, and industrial application in the past decades.

Chitosan and its derivatives have been reported to be useful for biomedical applications such as wound healing and dressings, drug delivery agents, anti-cholesterolemic agents, blood anti-coagulants, anti-tumor agents, and immunoadjuvants.[2]

Polylactide and its copolymers form a family of biogradable and biocompatible polymers that have been widely used in biomedical applications, such as absorbable sutures, sustained drug delivery systems, implants for orthopedic devices and absorbable fibers.[4,5] Early studies on polylactide showed that it was readily resorbed by living tissues.[5] However, the high crystallinity and low hydrophilicity of polylactide reduce its degradation rate, which results in poorer soft tissue compatibility.[5,6] A possible way to solve the problem is by copolymerizing the lactide with other biological materials such as chitin and its derivatives. By inducing the molecular chains of chitin and its derivatives, the copolymer may be able to be degraded faster than polylactide, yet retain its initial strength.

Chitosan is degraded via the cleavage of the β-glycosidic bond between the *N*-acetylglucosamine units.[3] Since chitosan is more hydrophilic than polylactide, the chitosan:polylactide copolymers may exhibit different

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degrees of hydration depending on the ratio chitosan:polylactide in the copolymers. In particular, hydration plays an important role in polymer degradation via hydrolysis of the ester backbone.

Very little has been reported about the copolymerization of polylactide and chitin or its derivatives. In this work, we synthesized a kind of novel graft copolymer with the natural polysaccharide chitosan as the main chain and the artificial biopolymer poly(DL)-lactide as the side chains using Et_3Al as the catalyst in toluene. The relationships of comonomers and the resulting grafting copolymer microstructure and thermal properties are reported. A mechanism for the copolymerization reaction is also advanced.

2. Experimental

2.1. Materials

DL-Lactide was synthesized by condensation polymerization of DL-lactic acid *in vacuum* firstly, and then decomposing of condensed oligomer into lactide at high temperature, using nanometer ZnO powder as catalyst. The raw lactide was purified by recrystallization from dried EtOAc. Its melting point was between 124 and 126 °C. Triethylaluminum in toluene (15%) was purchased from Tokyo Kasei and used without further purification. Chitosan was obtained by refluxing chitin in 40% NaOH and for 4 h at 110 °C, and then washing to neutral with distilled water. The degree of deacetylation (94%) and viscosity average molecular weight (1×10^6) were determined by elemental analyses and $[\eta] = 6.589\times10^{-3}M_v^{0.88}$, respectively. Toluene and EtOAc were dried by refluxing over CaH_2 and purified by distillation.

2.2. NMR spectroscopy

^{1}H NMR spectra of grafted copolymers were recorded with a Bruker AM 400 400 MHz spectrometer. The spectrum of chitosan was recorded on a Gemini-2000 at 300 MHz. In both cases, CF_3COOD–D_2O were used as solvent.

2.3. FTIR spectroscopy

FTIR spectra were obtained with a Bruker 55 spectrometer, using KBr pellets.

2.4. Differential scanning calorimetry

The melting temperature and the melting enthalpy (ΔH_m) were determined by differential scanning calorimetry. The melting points were defined as the peak of the endothermal curve. The DSC measurements were carried out with a Perkin–Elmer DSC7 differential scanning calorimeter under N_2 purge, at a heating rate of 10 °C/min. Temperature and heating flow calibration were performed with indium and gallium. The scanning range was from −50–200 °C.

2.5. Wide-angle X-ray scattering (WAXS)

WAXS was recorded on a Rigaku Dmax-rC diffractometer, in which the high-intensity monochromatic Ni-filtered CuKα radiation was generated at 40 kV and 100 mA.

2.6. Synthesis of lactide grafted to chitosan

Polymerizations were carried out under stirring for 24 h in toluene at 70 °C. DL-Lactide does not dissolve below the temperature in toluene. Chitosan (5 mmol, 100 mesh) was degassed for 1 h in vacuo below 1 mm Hg, and added to the reactor. The chitosan was suspended in toluene (40 mL). Et_3Al (3.8 g, 5 mmol 15% solution in toluene) was added dropwise via a syringe through a rubber septum under vigorous stirring at ambient temperature in an apparatus equipped with an oil valve to a gas buret. After ethane evolution ceased, the reaction was kept for one additional hour under stirring. The lactide with various molar ratios to chitosan (2:1, 5:1, 6:1, 10:1, 20:1, 40:1) was transferred into the reactor after it was placed under a vacuum below 1 mm Hg for 1 h. The reactor was placed into a preheated oil bath at 70 °C, and the reaction was allowed to proceed for 24 h. When the setting reaction time of 24 h elapsed, the reactor was cooled to room temperature. The mixture was precipitated in alcohol (300 mL). The precipitated product was filtered out with a sintered glass funnel. The grafting copolymers were twice washed with EtOAc, and then extracted in a Soxhlet extractor with the same solvent for 8 h. All samples were dried in an oven at 40 °C for 48 h in vacuo.

3. Results and discussion

3.1. Synthesis and characterization

Functional aluminum alkoxides such as $(C_2H_5)_{3-\rho}$-$Al(ORX)_\rho$ where X can be halogen atoms, tertiary amines, and double bonds have been proven to be very efficient in the ring-opening polymerization of end-reactive lactones, such as caprolactone[7] and lactide.[8] Compounds with a hydroxyl group reacting with Et_3Al could be used to obtain the alkoxides. There is a primary hydroxyl group (C-6) and a secondary one (C-3) in chitosan molecules, respectively. The Et_3Al can react with the hydroxyl groups and can also initiate the polymerization of lactide.

Table 1. The lactide grafted onto chitosan copolymer in toluene at 70 °C with Et_3Al as catalyst

Molar ratio (lactide:chitosan)	Total yield (%)	Grafting percentage (%)[a]	$F_{LA}/F_{chitosan}$[b]	ΔH (cal/g)	T (°C)
2:1	50.4	43	0.96		
5:1	38.1	109	2.44		
6:1	32.8	136	3.76		
10:1	43.8	338	7.56	2.3359	150.65
20:1	26.7	408	9.12	3.1186	149.76
40:1	15.2	462	10.33	3.3055	138.95

[a] $\text{Grafting percentage (\%)} = \dfrac{\text{graft copolymer (g)} - \text{chitosan (g)}}{\text{chitosan (g)}} \times 100\%$.

[b] $\text{Molar composition in graft copolymer} = \text{grafting percentage} \times \dfrac{161}{72}$.

The polymerization of lactide onto chitosan in various molar ratios took place easily under the designated conditions (see Experimental section). The results are shown in Table 1.

The grafting percentage and the amount of lactide introduced to chitosan increase with the feeding molar ratio are shown in Table 1. When the feeding molar ratio of lactide to chitosan increased from 2:1 to 40:1, the grafting percentage rose from 43% to 462%; meanwhile, the molar ratio of polylactide to chitosan in the copolymer also rose from 0.96 to 10.33. This indicates that the higher the concentration of the lactide in toluene, the higher the opportunity for the lactide to react with chitosan reactive centers. The FTIR spectra for some samples and chitosan itself are compared in Figure 1.

The band that appeared in the copolymers at $\sim$1758 cm^{-1} was assigned to the carbonyl group of the branched polylactide. The methyl asymmetric deformation of polylactide appears at $\sim$1475 cm^{-1}. The $\sim$1188 and $\sim$1215 cm^{-1} doublets observed in the copolymer are assigned to the symmetric C–O–C stretching modes of the ester group. There are two other peaks at $\sim$1130 and $\sim$1045 cm^{-1} attributed to the methyl rocking and $C–CH_3$ stretching, vibration, respectively. This evidence suggests that the lactide can indeed react with chitosan with Et_3Al as catalyst. Increasing of the ratio of lactide to chitosan in the feed made the band at $\sim$1188 cm^{-1} rise, which means that the more lactide had been grafted to chitosan.

The peak positioned $\sim$3440 cm^{-1} belonging to the hydroxyl group, the amino group, and the amide of chitosan did not move remarkably after the copolymerization, but its intensity became less with the increase of lactide in the feeding ratio. We added an equal molar ratio of Et_3Al with chitosan, and did not hydrolyze the terminal products; thus this band is attributed to the corresponding unreacted groups of chitosan. This means that there are still a certain number of hydrogen bonds that survived in the samples. It should be noticed that the peaks $\sim$1635 and $\sim$1595 cm^{-1}, which are ascribed to the amino groups in chitosan, changed into a singlet and shifted to $\sim$1630 cm^{-1} after the reaction. The same phenomenon has also been observed in our experiments in which chitin and hydropropylchitin react with Et_3Al, and then polymerize with lactide under the same conditions as in this study.

Dubois et al. have reported that primary amines and Et_3Al can initiate the polymerization of PCL, but the final product is a secondary amide.[9] When polycaprolactone terminated with a primary amino group initiates the ring-opening copolymerization of the lactide, the block copolymer also has a secondary amide structure.[11] In our study, no obvious evidence for the secondary

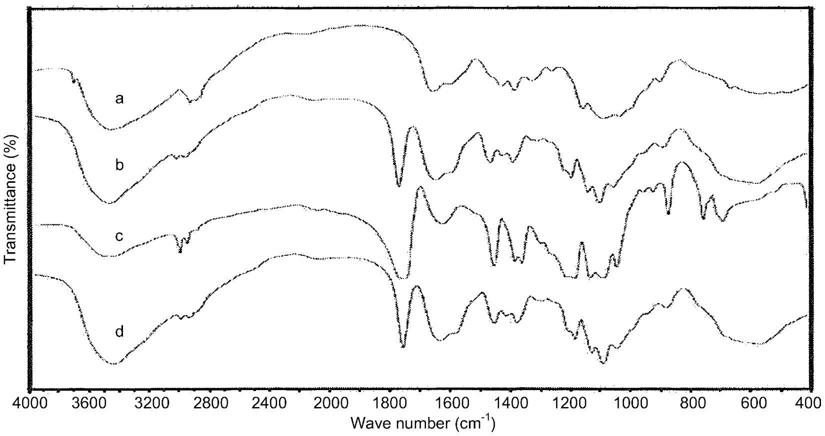


Figure 1. The FTIR spectra for chitosan and graft copolymers: (a) chitosan, (b) 5:1, (c) 10:1, (d) 20:1.

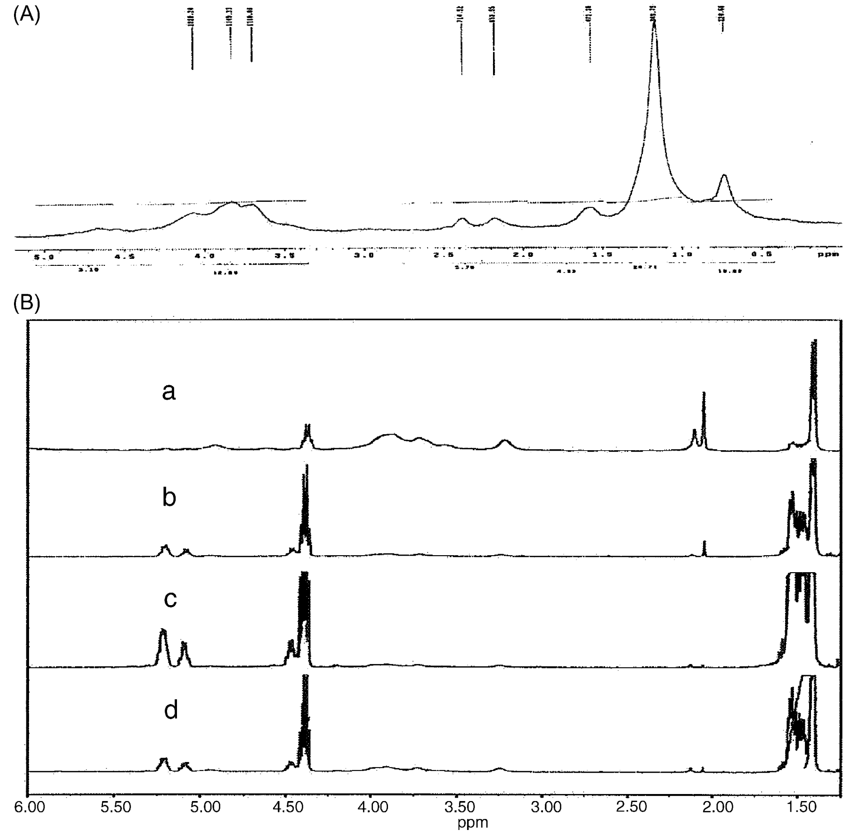


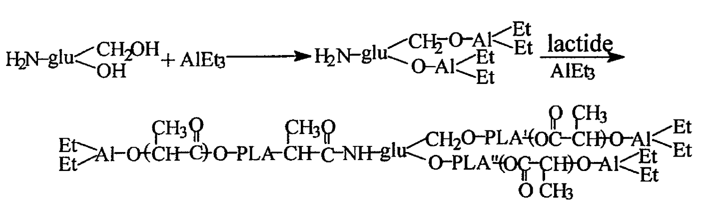
Figure 2. The ^{1}H NMR spectra of chitosan and graft copolymers: (A) chitosan; (B) (a) 5:1, (b) 10:1, (c) 20:1, (d) 40:1.

amide could be found in the FTIR spectrum. Owing to the fact that Et_3Al cannot react with primary amines,[9] the reacting mechanism needs to be further studied.

The ^{1}H NMR spectra of the original chitosan in 1:1 $CF_3COOD–D_2O$ and grafted copolymer of 5:1, 10:1, 20:1, and 40:1 in the same solvent are compared in Figure 2A and B, respectively. Chitosan shows a singlet at δ 3.15 and mutiplets at δ 3.55–3.80 (H-3, H-4, H-5, 2H-6) and a small signal at δ 4.4 (H-1) corresponding to the ring methine protons. The singlet at δ 1.95 is due to the survival of the *N*-acetylglucosamine units of chitin. The copolymers not only show the original signals of chitosan, but also have the new peaks at δ 4.3 and δ 5.3 assigned to the terminal methine protons of the branched polylactide and repeat units of it in the chain, respectively. Both signals are clearly separated into two groups of protons. This implies that there are two different kinds of atoms bonding to the methine. The peak at δ 1.56 is attributed to the methyl of polylactide.[10]

Very different from that which other authors have reported,[10] the intensity of the peak at δ 4.3 is stronger than that of that peak at δ 5.3. In the common ^{1}H NMR spectra of polylactide, the signal at δ 5.3 is much greater than that at δ 4.3, and the molecular weight of polylactide can be calculated from the ratio of the two signal intensities. Indeed, in the reaction of Et_3Al initiating the ring-opening polymerization of caprolactone, the Et_3Al cannot only react with a hydroxyl group of ω-primary amines, but it can also catalyze the reaction between a primary amine and caprolactone to get a secondary amide.[9] When polycaprolactone terminated with a primary amino group initiates the ring-opening copolymerization of the lactide, the block copolymer has also a secondary amide structure.[11] According to the same mechanism, when the lactide is grafted onto chitiosan using Et_3Al as catalyst, Et_3Al cannot only react with the hydroxyl group in chitosan, but it can also catalyze the reaction between a primary amine and lactide. Although the two methyls existing in the lactide increase steric hinderance, which makes its reactivity lower than that of caprolactone, in some cases, they can initiate ring-opening polymerization mutually to produce diblock and triblock copolymers.[12,13] So we assume lactide can act with chisotan as in Scheme 1 to produce **I**, which has a secondary amide structure under Et_3Al as catalyst.

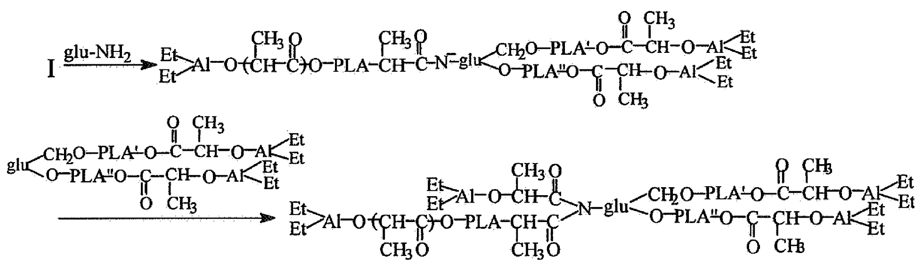
As we know, chitosan is a weakly basic polymer with a $pK_b = 6.3–7.0$.[14] The amide in **I** can carry out a transfer reaction like that in Scheme 2, which is shown under the amino of chitosan that survived as catalyst, which also take place in PCL polymerization initiated by an ω-primary amine.[15] This hypothesis can explain why the intensity of the methine proton peak at δ 4.3 is stronger than the one at δ 5.3 and why the singlet peak appeared at $\sim$1630 cm^{-1} in the FTIR spectra. This



(1)

I

Scheme 1.



II (2)

Scheme 2.

assumption is supported by the nucleophilicity of the amide and its reactivity toward the carbonyl group of the ester under basic conditions.[16]

The integral intensity ratio between peak δ 4.3 and δ 5.3, that is the ratio of the ending methine amount and that in the chain of the polylactide, is determined by the graft branch length. If the length of the polylactide is shorter, the amount of methine in the chain is less, and the peak intensity of the resonance at δ 4.3 would be stronger than that at δ 5.3. In this study, owing to the methine peak intensity, the δ 4.3 resonance is much greater than the peak of δ 5.3; therefore, the graft copolymerization between chitosan and lactide produces predominantly short-branched polylactide. In the samples of 10:1, 20:1, and 40:1, the integral intensity ratio between peak δ 4.3 and δ 5.3 is 2.24, 2.45, and 3.05, respectively. The amount of short-branched polymer increases with an increase in lactide content in the feeding ratio.

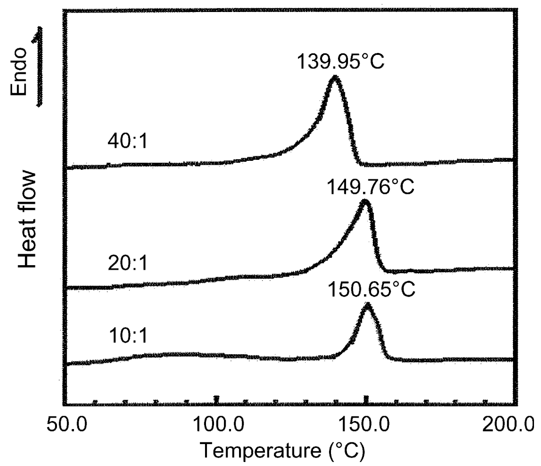


Figure 3. The DSC curves of graft copolymers: (a) 10:1, (b) 20:1, (c) 40:1.

3.2. Thermal properties of the grafting copolymers

It was found that samples with a feeding ratio of lactide to chitosan of >10:1 have a melting transition in their DSC curves. The melting enthalpies and peak temperatures of each of them are also shown in Table 1. The DSCs are the curves illustrated in Figure 3. The melting points of the copolymers decrease from 150.65 to 139.95 °C with the content of polylactide increasing. The enthalpies of the copolymers rise slowly from 2.3359 to 3.3055 cal/g. Namely the melting point (T) gradually shifted to lower temperature as a greater content of lactide is bonded in the copolymers, but the enthalpy (ΔH) is raised.

There are a considerable number of intermolecular and intramolecular hydrogen bonds that existed in chitin and chitosan molecules, which makes their melting points higher than their decomposition points. If chitosan were to be bonded with branched chains, the integrity of its molecular structure will be broken. As all hydroxyl and amino groups in chitosan were fully substituted with alkyl groups, that is, all hydrogen bonds in it were destroyed, it still does not have a melting transition.[17] This phenomenon is different with cellulose. In

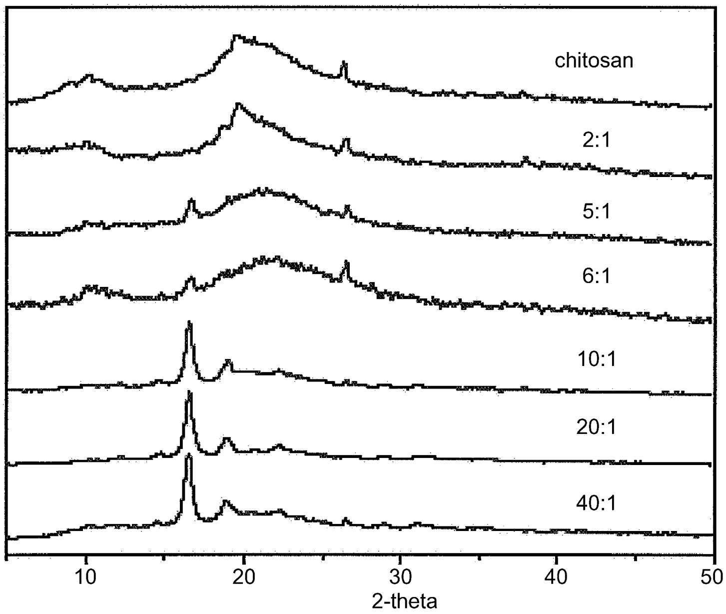


Figure 4. WAXS patterns of chitosan and its graft copolymer: (a) chitosan, (b) 2:1, (c) 5:1, (d) 10:1, (e) 20:1, (f) 40:1.

the same case, its alkyl derivatives have clear melting points.[18] This suggests that chitosan has a more rigid structure than cellulose, which is supported by the fact that the persistence length of chitosan of 22 nm is much greater than that of cellulose (11 nm) in LiCl (5%) + *N*,*N*-dimethylacetamide mix solution.[19] When chitosan was grafted with polylactide, its original integrity was also destroyed. The hydrogen bonds that existed in it were broken to a certain extent, which makes intermolecular interactions decrease. On the other hand, the ester bond is more flexible than that of a hydrocarbon. When it is used as the branched chain of chitosan, it can plasticize internally the rigid main chain of chitosan. Meanwhile, the branched ester linkage is more polar than that of a hydrocarbon, so there is a stronger repulsive force among molecules of the branched ester than that existing among hydrocarbons, which can be in favor of chitosan retaining its spatial configuration.

The melting point of a polymer is determined by the ratio between melting enthalpy and entropy, namely $T = \Delta H/\Delta S$. The ΔH differences among the samples 10:1, 20:1, 40:1 are not significant, but they have the tendency to increase. Meanwhile this melting point decreases. This means that ΔS must be increasing. With the content of lactide increasing in the feeding ratio, the amount of short-branched chain rises in the copolymer (see discussion in ^{1}H NMR sections); therefore, the conformation entropy must also increase, and the melting points have a tendency to drop. The dropping range for short side chains from 10:1 to 20:1 is less than from 20:1 to 40:1; meanwhile, the decreasing extent of the melting point of the copolymer obeys the same trend.

3.3. Wide-angle X-ray scattering

Figure 4 shows the WAXS patterns as compared with that of chitosan. The strongest reflection of chitosan appears at $2\theta = 19.84°$. In the feeding ratio, the ratio of lactide to chitosan is 2:1, the strongest reflection shifts to $2\theta = 19.74°$. In the case of 5:1 and 6:1, it is $2\theta = 21.66°$ and 21.44°, respectively. These results indicate the crystalline patterns were different with chitosan, when lactide was grafted onto it; the original crystallinity was destroyed.

The strongest peak appears at about $2\theta = 16.7°$ for samples of 10:1, 20:1, 40:1, which changes more remarkably than the ones mentioned above. In addition to that, these three samples have almost the same *d*-spacing, which means that graft copolymers have a similar packing mode when the graft percentage is >338% (in the feeding ratio 10:1).

4. Conclusions

A new type of copolymer lactide grafted onto chitosan was synthesized, and the grafting copolymerization could be carried out well in toluene at 70 °C. The derivatives of chitosan have clear melting transitions in the DSC scans when the molar feeding ratio lactide to chitosan is >10:1.

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