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Synthesis and Application of Chitosan-g-PLLA Copolymers

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The purpose of this paper is to study the synthesis and application of a new type of chitosan-g-poly(L-lactide) copolymer with different grafting percentage in the presence of triethylamine. FTIR and ^{1}H NMR results indicate that grafting percentage of graft copolymers increases with the molar feeding ratio of L-lactide to chitosan. The measurement of XRD and TG shows that graft copolymer exhibits low crystallinity and thermal degradation temperature. Static water contact angle testing suggests that graft copolymer has superior hydrophilicity compared with PLLA, which can be very useful for biomedical applications. 5-Fluorouracil loaded copolymer microspheres were prepared by phase separation method. The size and distribution of microspheres were measured by a Laser particle analyzer. The microspheres with LLA:CS feeding molar rotio (15:1) have a mean diameter of 332 nm with a narrow unimodal distribution. The spherical microspheres were observed by transmission electron microscopy (TEM). The microspheres shows good releasing property from drug release *in vitro*, and the drug release rate decreases as the increase of microspheres size.

Keywords: chitosan; poly(L-lactide); graft copolymer; hydrophilicity; microspheres

1. Introduction

Currently, the biodegradable polymer, which is an important material for biomedical applications has been widely and intensively investigated (1). Most natural biodegradable polymers like chitosan and its derivatives are low in mechanical strength but exhibit excellent cell adhesion, while synthetic biodegradable polymers such as polylactide and its copolymers have high initial strengths but show poor hydrophilicity (2–3). Development of new biomaterials by combining the advantage of natural and synthetic polymers is one of the challenging tasks for materials science today.

Chitosan (CS) containing 2-acetamido-2-deoxyb-β-D-glucopyranose and 2-amino-2-deoxy-β-D-glucopyranose groups is a natural biodegradable polysaccharide derived by partial deacetylation of chitin. This polymer displays interesting properties such as being non-carcinogenic, non-immunogenic and non-toxic, besides being biocompatibile and enzymatically biodegradable (4–6). Much attention has been paid to its prospective applications such as biomedicine, wastewater treatment and functional membranes (7). However, chitosan is soluble only in some acid aqueous solutions because of its rigid structure, which greatly limits its further applications (8). Different from chitosan, poly(L-lactide) (PLLA) and other poly(α-hydroxy-acid)s are the most widely used synthetic polyesters for biomedical application (9–10), such as absorbable implants, tissue engineering and drug delivery. The advantage of these polymers is that they are biocompatible and their degradation rates can be controlled and thus tailored to aspecific requirement for each application (11–12). However, some drawbacks are associated with the use of poly(L-lactide) (13): (i) hydrophobicity, (ii) generation of acidic species, resulting in decrease in local pH which potentially may cause tissue inflammation surrounding implant and initiate enzyme hydrolysis, (iii) self-acceleration of degradation. A decrease in local pH (acidic environment) catalyzes the hydrolytic degradation of ester bonds of the polymer. Because of these drawbacks, some natural biodegradable polymers have been combined with poly(L-lactide) to produce a new polymer hybrid applicable for a variety of purposes.

Recently, modification of chitosan by polylactide for balance of hydrophobicity and hydrophilicity has been explored as an interesting method to develop novel hybrid materials which combines the advantages of natural and synthetic polymers (14–15). Among various methods, graft

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copolymerization is the most attractive because it is a useful technique for modifying the chemical and physical properties of chitosan. Chitosan bears two types of reactive groups that can be grafted. First, the free amino groups on deacetylated units and secondly, the hydroxyl groups on the C_3 and C_6 carbons on acetylated or deacetylated units, they offer possibilities of modifications and graft reactions (16–18). For instance, Yao (19) has reported poly(chitosan-g-L-lactic acid) was prepared by grafting L-lactic acid onto the amino groups in chitosan without a catalyst. Liu (20) has synthesized a brush-like copolymer of polylactide grafted onto chitosan and studied its properties. Yang (21) has prepared a TMS-chitosan-g-PLA copolymer using macromolecular initiator sodium of trimethylsilyl-chitosan.

Very little has been reported about the synthesis of chitosan-g-poly(L-lactide) graft compolymer and its application for drug delivery vehicles. In this paper, a series of novel chitosan-g-poly(L-lactide) graft copolymers with different grafting percentage were obtained using triethylamine as the catalyst in dimethyl sulfoxide solution. The chemical structure and physical properties were studied. And the hydrophilicity of graft copolymers was analyzed. 5-Fluorouracil (5-Fu) loaded copolymer microspheres were prepared by phase separation method. The sizes and morphologies of microspheres were measured. The drug release *in vitro* of microspheres was observed. All results show that the biodegradable graft copolymers could be potential materials for biomedical applications.

2. Experimental

2.1 Materials

L-lactide from Shanghai TJL Bio-materials Co. was used after further purification. Chitosan was purchased from Shanghai Chemical Industry (Shanghai, China). Low molecular weight chitosan was prepared by oxidation of chitosan with hydrogen peroxide, and then dried at vacuum. The degree of degradation (93%) and viscosity average molecular weight (6279) were determined by elemental analysis and $[\eta] = 1.81 \times M_v^{0.93}$, respectively. Dimethyl sulfoxide and ethyl acetate were distilled under vacuum from calcium hydride and stored over molecular sieves. All other reagents and solvents used in the study were analytical grade and used as delivered.

2.2 Synthesis of Chitosan-g-polylactide Copolymers

The polymerizations were carried out for 12 h under magnetic stirring in dimethyl sulfoxide at 80°C. After being dissolved for 24 h, 0.005 mol low molecular weight chitosan was degassed for 1 h in vacuum below 1 mm Hg, and added into the reactor. A mixture of chitosan and L-lactide (in the molar ratio of 8:1∼20:1 (LLA/CS)) was suspended in a 30 ml dimethyl sulfoxide solution with magnetic stirring at room temperature under a vacuum below 1 mm Hg for 1 h. Then, 0.005 mol triethylamine was added dropwise via a syringe through a rubber septum under vigorous stirring at 80°C in nitrogen atmosphere. After a further 14 h continuous stirring, the mixture became a clear solution. When the reactor was cooled to room temperature, the product was precipitated in ethyl acetate and then extracted with chloroform in a Soxhlet's apparatus for 168 h. Dried at 30°C for 48 h under vacuum, the graft copolymers was obtained.

2.3 Characterization of Chitosan-g-polylactide Copolymers

IR spectra were obtained with a Fourier-transform infrared (FTIR) spectrometer (Nicolet, Magna-550) using KBr pellets.

^{1}H-NMR was peformed on a Bruker, DMX-500 spectrometer, ^{1}H-NMR chitosan was dissolved in the mixed solvent of D_2O and $(CD_3)_2SO$. The chitosan graft copolymer was dissolved in $(CD_3)_2SO$.

Analysis of the N of graft copolymers was carried out by Eario EL elemental analysis instrument.

X-ray diffraction spectrometric image was recorded on a Rigaku Dmax-rC diffractometer, in which the high-intensity monochromatic Ni-Filtered CuKα radiation was generated at 40 kV and 30 mA.

Thermogravimetry (TG) analysis was made with PE, Pyrolysis-1 equipment. The temperature range was 30–200°C and the heating rate was 10°C/min.

Static water contact angles were measured at room temperature with an image analyzing system using the sessile drop technique. Double-distilled water was used in the measurement. Each determination was obtained by averaging the results of at least eight droplets placed at different positions on the sample surface.

2.4 Preparation of Chitosan-g-polylactide Copolymer Microspheres

The phase separation method was used for the preparation of chitosan-g-polylactide copolymer microspheres. 0.02 g drug and 0.1 g copolymer were placed into a 10 ml organic solvent to form a solution that was then put into a 50 KHz ultrasonic device (Branson, America) to make good dispersion. The well-dispersed solution was then dropped into 10 ml distilled water solution of Tween-80 of 1 mg/ml concentration under magnetic stirring to form microspheres. The microspheres transferred into dialysis bags (cut-off molecular weight, 8000) were dialyzed against 1 l distilled water for 2 days to remove the organic solvent completely, and then the microspheres were prepared.

2.5 Laser Particle Size Analyzer

The particle size and size distribution of the copolymer microspheres were determined by a Laser particle analyzer (LS230, Culter, American) at 25°C.

2.6 Transmission Electron Microscope (TEM)

The morphology of microspheres was examined using the H-600 TEM (Hitachi, Tokyo, Japan) at a 75 kV electron beam accelerating voltage. Samples were loaded onto a copper halftone, and dried at room temperature after they were dyed by 1% (wt) phosphotungstic acid solution.

2.7 Release In Vitro

5-Fu loaded microspheres were dialyzed in a phosphate buffer saline solution (PBS, pH7.4) with dialysis bags (cut-off molecular weight, 8000), and then continuously stirred by a dissolution tester at 37°C. At specific time intervals, samples were removed from the release medium, 5-Fu concentration was measured by spectrophotometry at 265 nm. The release curve was recorded as the accumulated concentration of drug vs. time.

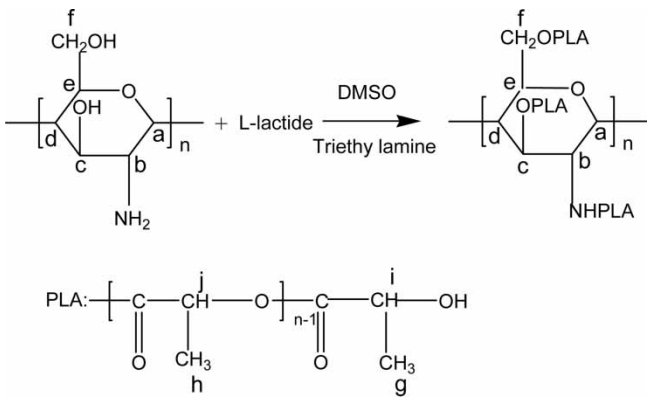
3. Results and Discussion

3.1 Synthesis and Characterization of Chitosan-g-poly (L-lactide) Copolymers

As shown in Scheme 1, the chitosan–polylactide grafting copolymers were prepared by reacting L-lactide (LLA) on chitosan.

The copolymerization of L-lactide onto chitosan in various molar ratio was carried out easily under the designated conditions. The results are shown in Table 1.

Grafting percentage and the amount of L-lactide introduced to a chitosan increase with the feeding molar ratio of L-lactide to a structural unit of chitosan is shown in Table 1. When the feeding molar ratio of L-lactide to chitosan increases from 8:1 to 20:1, the grafting percentage rises from 211% to 278%. This indicates that the higher the concentration of the L-lactide in dimethyl sulfoxide, the more the opportunity for the L-lactide to react with chitosan reactive centers. Grafting percentage of the copolymer could approach 211%, when the molar ratio of L-lactide/aminoglucoside units is 8:1.



Sch. 1. Graft copolymeration of L-lactide onto chitosan.

Table 1. Grafting percentage of Chitosan-g-poly(L-lactide) copolymers

Polymers	Molar ratio (L-lactidee:chitosan)	Grafting percentage (%)[a]
CS-g-PLLAA	8:1	211
CS-g-PLLAA	11:1	217
CS-g-PLLAA	12.5:1	228
CS-g-PLLAA	15:1	240
CS-g-PLLAA	20:1	278

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

[a]Structure changes of chitosan and its graft copolymers were confirmed by FTIR spectra (Fig. 1). Compared to the FTIR spectrum of chitosan, the copolymers have a new absorption peak appearing around 1744 cm^{-1}, corresponding to the carbonyl group of the branched polylactide. The methyl asymmetric deformation of polylactide appears at 1451 cm^{-1}. The 1189 and 1263 cm^{-1} doublets observed in the copolymer are assigned to the symmetric C-O-CC stretching modes of the ester group. There are two other peaks at 1128 and 1043 cm^{-1} attributed to methyl rocking and C-CH_3 stretching vibration respectively. The increase of amide I peak (1668 cm^{-1}) indicates an increase of the amidation by reacting chitosan with L-lactide (15), that demonstrates the formation of amide group between chitosan and lactide. This evidence suggests that the lactide can indeed react with chitosan with triethylamine as catalyst. Increasing of the feeding molar ratio of L-lactide to chitosan makes the absorption at 1189 cm^{-1} rise, which means that more L-lactide has been grafted to chitosan. The result is correlated well with the result of the gravimentric method (Table 1). The amount of grafted poly(L-lactide) increases with an increase in L-lactide content in the feeding molar ratio.

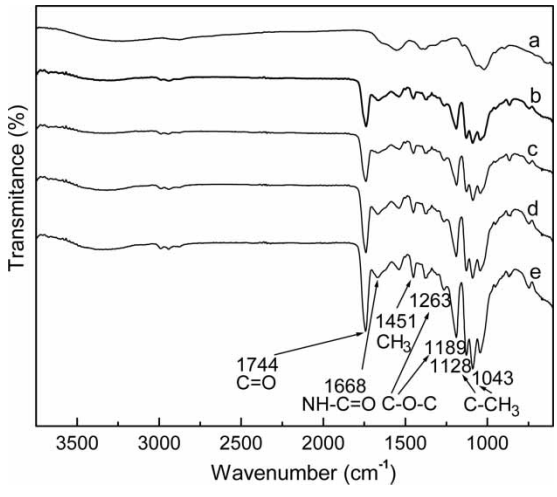


Fig. 1. FTIR spectra of chitosan and graft copolymer: (a) CS, (b) 8:1, (c) 12.5:1, (d) 15:1, and (e) 20:1.

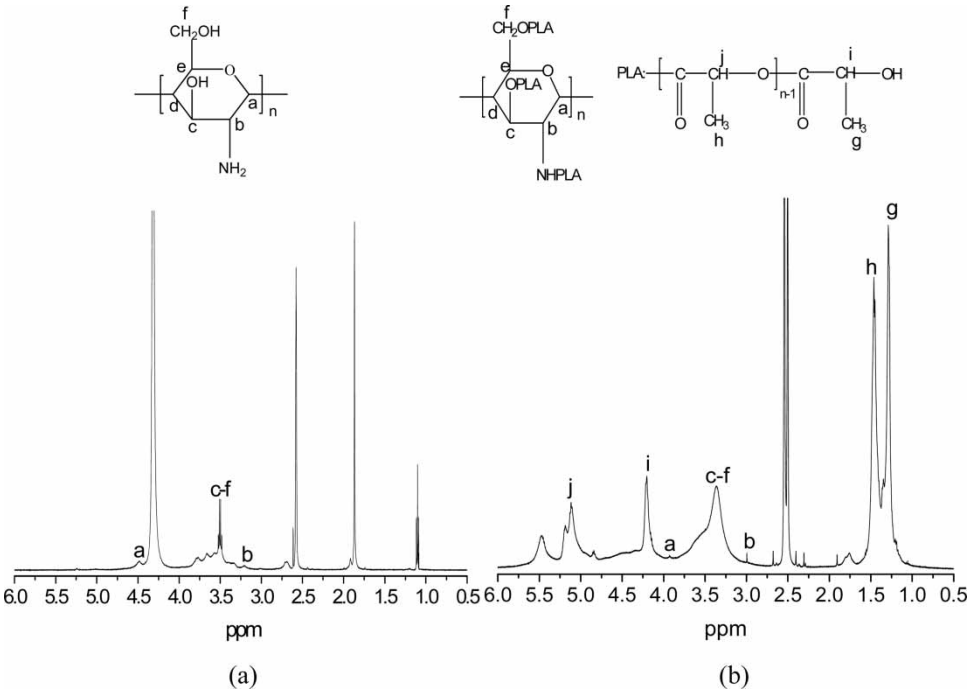


Fig. 2. [1]H-NMRR spectra of chitosan and graft copolymer: (a) CS and (b) CS-g-PLLAA.

The [1]H-NMR spectra of the chitosan and graft copolymer of 8:1 are compared in Figure 2. Chitosan shows a singlet at 3.22 (H-b) and mutiplets at 3.3–3.7 ppm (H-c, H-d, H-e, H-F) and a small singlet at 4.4 ppm (H-a) corresponding to the ring methenyl protons. The singlet at 1.87 ppm is due to the survival of the N-acetylglucosamine units of chitin. Compared with chitosan, the [1]H-NMR spectra of the graft copolymer shows that the signals at 4.2 and 5.1 ppm are assigned to the terminal methenyl protons of the grafted poly(L-lactide) 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 (20). The signals at 1.3 and 1.4 ppm are attributed to the methyl protons of the poly(L-lactide) moiety located at the terminal groups and the backbones (17, 18). All these results prove that the chitosan derivatives contain poly(L-lactide) side chains.

3.2 Physical Properties of Chitosan-g-poly(L-lactide) Copolymers

X-ray diffraction profiles of chitosan and its graft copolymer are shown in Figure 3. Chitosan has an orthorhombic unit cell with $a = 0.824$ nm, $b = 1.039$ nm and $c = 1.648$ nm. Chitosan has two peaks at around $2\theta = 10^\circ$ and 20°. The peak at around $2\theta = 10^\circ$ is assigned to (010) and (100). The strongest peak appears at around $2\theta = 20^\circ$, which corresponds to (020) and (200). Poly(L-lactide) crystallized in a

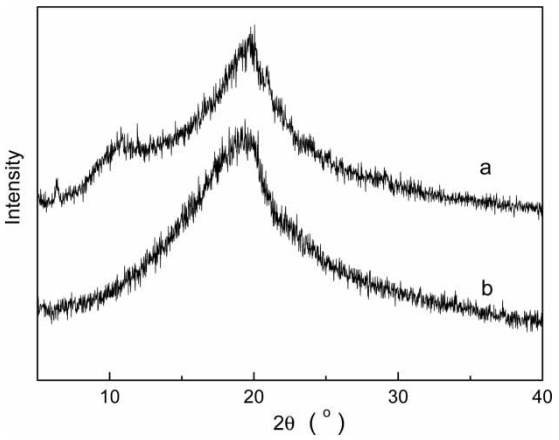


Fig. 3. XRD profiles of chitosan and graft copolymer: (a) CS and (b) CS-g-PLLAA.

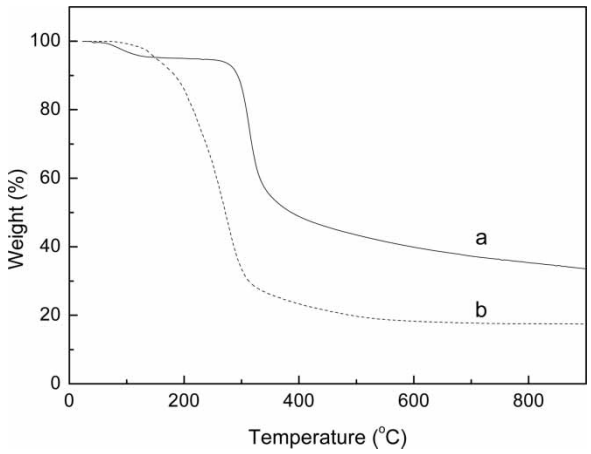


Fig. 4. TG Thermograms of chitosan and graft copolymer: (a) chitosan and (b) CS-g-PLLAA.

Table 2. Static water contact angles of polymers

Polymers	Grafting percentage (%)	θ (°)
CS-g-PLLAA	217	19
CS-g-PLLAA	228	22
CS-g-PLLAA	240	27
CS-g-PLLAA	278	41
PLLA	—	86

pseudo-orthorhombic unit cell (dimensions a = 1.07 nm, b = 0.595 nm and c = 2.78 nm) which contain two 10^3 helices (α-Form), the main peak in X-ray diffraction profile appears at 2θ value of 15, 17 and 19 (22). Compared with chitosan, the graft compolymer of 8:1 decreases the intensity at both peaks. When the feeding molar ratio reaches LLA/CS = 8:1, the graft copolymer shows only one broad peak at around $2\theta = 18°$. It suggests that when L-lactide is grafted onto chitosan, the ability of forming hydrogen bond of chitosan is decreased, and the graft copolymer becomes almost amorphous. Since the grafting by PLLA takes place at random along the chitosan chain, it gives rise to a random copolymer (19). This will efficiently destroy the regularity of the packing of the original chitosan chains, which results in the formation of almost amorphous copolymer.

TG curves for chitosan and grafted copolymer of 8:1 are shown in Figure 4. Compared to chitosan, graft copolymer has lower thermal degradation temperatures at 164°C. A fast process of weight loss appears in the TG curves for the graft copolymer in the thermal degradation ranges. These results show some decrease of the thermal stability for chitosan-polylactide graft copolymer relative to the original chitosan. Introduction of substituents into polysaccharide structures would disrupt the crystalline structure of chitosan, especially by the loss of the hydrogen bonding.

Static water contact angles of polymers are shown in Table 2 and Figure 5. Smaller contact angle between the water and material surface suggests superior hydrophilicity. It is found that hydrophilicity of graft copolymers decreases with the increase of grafting percentage. Because PLLA is hydrophobic, while CS is hydrophilic. Increase of the grafting percentage means an increase of the content of PLLA, which results in hydrophilicity declining. The results suggest that grafting PLLA onto CS can improve the hydrophilicity of PLLA.

3.3 Size and Morphology of Chitosan-g-poly(L-lactide) Copolymers Microspheres

The polymeric microspheres (LLA:CS molor ratio = 15:1) in water has a mean diameter of 332 nm. The size distribution of polymeric microspheres is shown in Figure 6. It can be seen from Figure 6 that the microspheres possess a narrow unimodal distribution (polydispersity = 80 nm).

The morphology of the copolymer microspheres was investigated by the transmission electron microscopy (TEM) technique. Figure 7 shows the TEM image of copolymer microspheres. It can be confirmed that copolymer microspheres are spherical in shape. The spherical shape of microspheres is attributed to the amphiphilic property of copolymers.

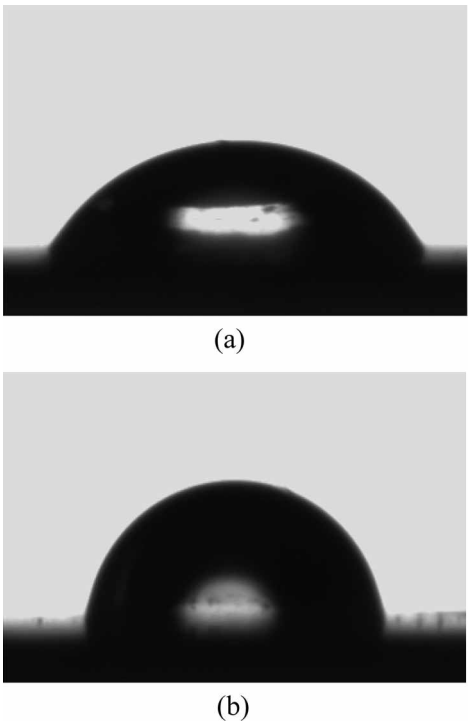


Fig. 5. Static water contact angles photos of graft copolymer and PLLA: (a) CS-g-PLLAA V and (b) PLLA.

3.4 Drug Release In Vitro of Chitosan-g-poly(L-lactide) Copolymers Microspheres

Drug release in vitro of microspheres is important for developing the successful formulations. The release rates of microspheres depend upon: (i) desorption of the surface-bound/adsorbed drug; (ii) diffusion through the microspheres

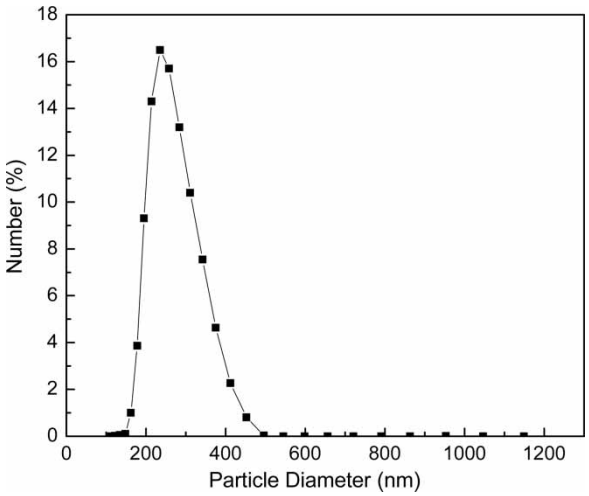


Fig. 6. Size distribution of microspheres.

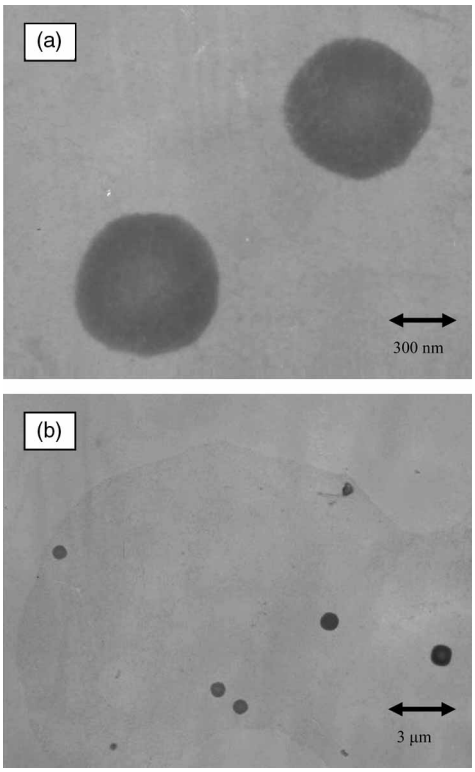


Fig. 7. TEM morphology of 5-Fu loaded copolymer microspheres.

matrix; (iii) diffusion through the polymer wall; (iv) microspheres matrix erosion; and (v) a combined erosion/diffusion process. Thus, diffusion and biodegradation dominates the process of drug release.

Figure 8 displays the drug release curve *in vitro* of 5-Fu loaded copolymer microspheres. The microspheres possess a slower, balanced release for up to 63 h after an initial burst release. As shown in Figure 8, the initial burst release is attributed to the fraction of the drug which is adsorbed or weakly bound to the large surface area of the microspheres instead of that incorporated in microspheres. Following the dilution of the dissolution medium under perfect sink conditions the drug partition shows an increase due to the immediate release phase. Later, an exponential delayed release rate is observed probably due to the drug diffusion from the matrix. Release in the matrix type of microspheres follows the first-order kinetics (23).

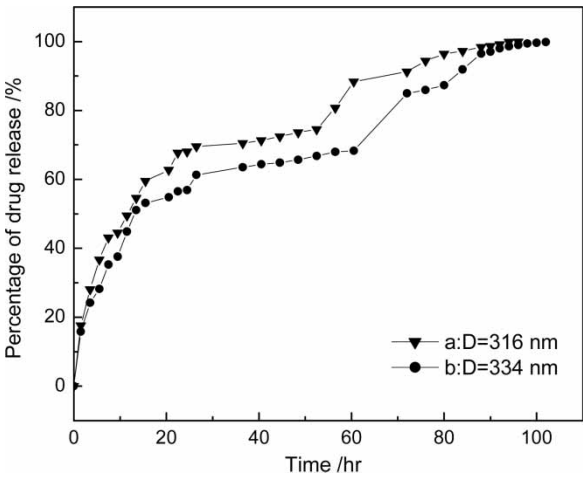


Fig. 8. Drug release curve *in vitro* of 5-Fu loaded copolymer microspheres.

The rate and accumulated amount of 5-Fu released decrease as the sizes of microspheres increase. In 23 h, 55% of 5-Fu is released from the microspheres with mean diameter 334 nm, while 70% from the microspheres with mean diameter 316 nm. With a larger size, the microspheres have smaller specific surface area, thus causing slower release in the release medium.

4. Conclusions

A novel biodegradable graft compolymer with L-lactide grafted onto chitosan was synthesized, and the graft copolymerization could be carried out well in dimethyl sulfoxide at 80°C. The structure of graft copolymers are confirmed by FTIR and ^{1}H-NMR. Grafting percentage increases with the molar feeding ratio of l-lactide to chitosan and the highest grafting percentage is 278% at the molar feeding ratio of 20:1. The measurements of XRD and TG show that graft copolymers exhibit low crystallinity and thermal degradation temperature. Static water contact angle testing suggests that hydrophilicity of graft copolymers descends with the increase of grafting percentage, which is much better than PLLA. 5-Fu loaded copolymer microspheres were prepared by phase separation method. The microspheres with LLA:CS feeding molar rotio (15:1) have a mean diameter of 332 nm with a narrow unimodal distribution (polydispersity = 80 nm). The spherical microspheres are observed by transmission electron microscopy (TEM). The microspheres show good drug releasing property *in vitro*, and the drug release rate decreases as the increase of microspheres size.

Biodegradable and amphiphilic properties of the graft copolymers can be very useful for biomedical applications, especially for drug delivery systems.

5. Acknowledgments

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6. References

1. Hurrell, S. and Cameron, R.E. (2002) *Biomaterials*, **2(3)**, 2401–2409.
2. Carsten, G. and Holger, F. (2006) *Macomoecules*, **39**, 1719–1723.

3. Cai, Q. and Bei, J. (2000) *Biomater. Sci. Polym. Ed.*, **11**, 273–288.
4. Jue, Y.L. and Sung, H.N. (2002) *Control. Release*, **78**, 187–197.
5. Chourasia, M.K. and Jain, S.K. (2004) *Drug. Deliv.*, **11(2)**, 129–148.
6. Maureen, D.B., Andress, G.S. and Ijeoma, F.U. (2001) *Int. J. Pharm.*, **229**, 1–21.
7. Marc, L., Nicolas, T.K. and Michael, D.B. (2006) *Biomaterials*, **27**, 4815–4824.
8. Aksungur, P., Sungur, A. and Unal, S. (2004) *J. Control. Release*, **98(2)**, 269–279.
9. Breitenbach, A., Li, Y.X. and Kissel, T. (2000) *J. Control. Release*, **64(1)**, 167–178.
10. Kissel, T., Li, Y. and Unger, F. (2002) *Adv. Drug. Deliver. Rev.*, **54(1)**, 99–134.
11. Kumar, N., Ravikumar, M.N.V. and Domb, A.J. (2001) *Adv. Drug. Deliver. Rev.*, **53(1)**, 22–44.
12. Vila, A., Sánchez, A., Tobío, M., Calvo, P. and Alonso, M.J. (2002) *J. Control. Release*, **78**, 15–24.
13. Mihaela, Z., Carsten, G. and Heidi, K. (2006) *Polymer*, **47**, 3740–3746.
14. Jayakumar, R., Prabaharan, M. and Reis, R.L. (2005) *Carbohyd. Polym.*, **62**, 142–158.
15. Wu, Y., Zheng, Y.L. and Yang, W.L. (2005) *Carbohyd. Polym.*, **59**, 165–171.
16. Ji, Y.K., Chang, S.H. and Nam, J.J. (2002) *Polym. Int.*, **51**, 1123–1128.
17. Qu, X., Wirsén, A. and Albertsson, A.C. (2000) *Polymer*, **4(12)**, 4589–4598.
18. Qu, X., Wirsén, A. and Albertsson, A.C. (2000) *Polymer*, **4(13)**, 4841–4847.
19. Yao, F.L., Chen, W. and Wang, H. (2003) *Polymer*, **44**, 6435–6441.
20. Liu, Y., Tian, F. and Hu, K.A. (2004) *Carbohyd. Res.*, **339**, 845–851.
21. Yang, H., Zhou, S.B. and Deng, X.M. (2005) *Chinese. Chem. Lett.*, **16(1)**, 123–126.
22. Tsuji, H. and Muramatsu, H. (2001) *J. Appl. Polym. Sci.*, **81(9)**, 2151–2160.
23. Soppimath, K.S., Aminabhavi, T.M. and Kulkarni, A.R. (2001) *J. Control Release*, **70**, 1–20.