Synthesis and Characterization of Novel Biodegradable Polymeric Prodrugs Containing 5-Fluorouracil and 4-Amino-*N*-(2-pyrimidinyl) Benzene Sulfonamide Terminal Groups

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ABSTRACT: To develop novel biodegradable polymeric prodrugs with target-directing and drug-active functional groups, a series of polymeric antitumor prodrugs containing sulfadiazine and 5-fluorouracil terminal groups were prepared via the two-step reaction of chlorinated poly(lactic acid) or chlorinated poly(lactic acid-*co*-glycolic acid) with potassium sulfadiazine (SF-K) and 1,3-dihydroxymethyl-5-fluorouracil. The synthesized polymers were characterized by means of infrared spectroscopy, proton nuclear magnetic resonance spectroscopy, gel permeation chromatography, viscosity measurements, differential scanning calorimetry, and ultraviolet (UV) spectroscopy. The GA/LA value was varied, so that the effects of the comonomer content on the solubility, thermal properties, and degradable behaviors were examined respectively. It was found that introducing the GA units could increase the melting temperature (T_m), the hydrolytic degradation, and the hydrophilicity, while it decreased the glass transition temperature (T_g). The drug content of 5-FU measured by UV spectra is 56.3 in maximum. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 105: 2339–2345, 2007

Key words: polycondensation; biodegradable; drug delivery systems

INTRODUCTION

5-Fluorouracil (5-FU), an antimetabolite, has been widely used for a long time as an antitumor agent. However, it showed severe toxic side effects and delivery problems on living body. Thus, reducing or removing the toxicity of this antitumor drug is a very important subject for pharmacochemists. Much attention has been focused on the attachment of drug to polymeric backbone. This is because the drug released from the polymer increases the duration of drug activity due to slow release or of target-directing function of polymeric drug in the body.

The targeting antitumor drugs are recognized as the most effective way to kill malignant tissue selectively and to minimize the unfavorable side effects.[1,2] It was as early as 1950s, Stevens found that sulfadiazine (SF), an antibiotic, and its homologues could be concentrated selectively in growing Yoshida Sarcoma rats.[3] Abel attempted to exploit its ability to concentrate in tumor cell by designing SF-mustard (Scheme 1), but it lost the ability to be taken up by those tumor cells which concentrate SF.[4] Recently, poly(ethylene oxide) with SF and diethylenetriaminepentaacetic acid terminal groups were synthesized by Huang.[5] After complexation with ^{153}Sm and ^{99}Tc, it could be concentrated selectively in the tumor tissue, the ratio of concentration of the polymeric drug in tumor tissue to that in ordinary organ was about 2.3 : 1 and 10 : 1.[6] It would be of considerable interest to develop polymeric drugs with target-directing function and durative drug action. Thus, our current research is focused on the synthesis and characterization of poly(lactic acid) (PLA) or poly(lactic acid-*co*-glycolic acid) (PLGA) with SF and 1,3-dihydroxymethyl-5-FU at two ends. Owing to the biodegradability of PLA and PLGA, the release of 5-FU will be caused not only by the hydrolysis of the ester linkage directly formed by 5-FU and polymeric carriers, but also by the biodegradation of polymeric carriers. Therefore, the release kinetics of 5-FU may be adjusted to a certain extent.

EXPERIMENTAL

Materials

Sulfadiazine was purchased from Northeast Pharmaceutic Manufacturing (Shenyang, China), recrystallized twice from DMSO/ethanol (1/4 v/v) before use; 5-fluorouracil was purchased from Nantong Pharmaceutic Manufacturing Co. (Nantong, China),

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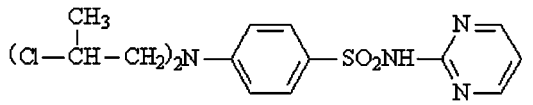


Scheme 1 The structure of sulfadiazine-mustard.

recrystallized from water, and dried in vacuum. L-Lactic acid (LA) and glycolic acid (GA) (Shenyang Lianbang Reagent, China) was dehydrated before use. Dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) were purchased from Yanchang Biotechnology (Shanghai, China) and used as received. Stannous octoate and tetrabutyl ammonium chloride (TAC) were purchased from Shanghai Chemical Reagent (Shanghai, China), and TAC was used as phase transfer catalyst (PTC). All the solvents were purified by conventional methods. 1,3-Dihydroxymethyl-5-FU, potassium sulfadiazine (SF-K), and α-chloroacetyl isocyanate were synthesized as per literature.[7–10]

Synthesis of chlorinated PLA and chlorinated PLGA

Using stannous octoate as catalyst, PLA, PLGA (75 : 25), PLGA (50 : 50), and PLGA (25 : 75) were prepared as per literature.[11,12] The molecular weights of polymeric carriers were 3160, 4650, 3760, and 2780, respectively.

Four grams (1.1 mmol) of PLGA (50 : 50) and 20 mL of DMF was added in a three-necked flask with magnetic stirrer, to which 0.4 mL (4.88 mmol) of α-chloroacetyl isocyanate was added and stirred for 12 h at room temperature. The synthesized product was dissolved in methylene chloride and precipitated in methanol. The resultant precipitate was filtered and dried at room temperature in vacuum with the yield of 89.2%. ^{1}H NMR (δ, ppm): 7.65 (m, 1H, —CO NHCOO—), 5.21 (m, 1H, $CH(CH_3)COO$—), 1.53 (m, 3H, —CH_3). IR (KBr, cm^{-1}): 2994, 2944 (—CH_2— and —CH_3), 1755 (C=O), 1688 (—NHCOO—), 1186, 1087 (C—O), 682 (C—Cl). Chlorinated PLA and other chlorinated PLGA were prepared by the same method.

Synthesis of polymers with 5-FU terminal group

Two grams (11 mmol) of 1,3-dihydroxymethyl-5-fluorouracil, 0.04 g (1 wt %) DMAP, and 2 g of chlorinated PLGA (50:50) were dissolved in 25 mL of DMF. The solution was cooled to 0–5°C, and 0.8 g (3.88 mmol) of DCC in 5 mL of DMF was added dropwise. After 24 h of stirring at room temperature, the precipitate was filtered; washed with ether, acetone, and distilled water; and dried in vacuum at room temperature with the yield of 81.4%. ^{1}H NMR (δ, ppm): 7.75 (—CH=C— for 5-FU), 7.67 (m, 1H, —CONHCOO—), 5.21 (m, 1H, —$CH(CH_3)COO$—), 4.68 (m, 4H, CH_2—FU), 1.53 (m, 3H, —CH_3). IR (KBr, cm^{-1}): 3083 (C—H for 5-FU), 2994, 2944 (—CH_2— and —CH_3), 1755 (C=O), 1723, 1673 (—COO— for 5-FU), 1688 (—NHCOO—), 1186, 1087 (C—O), 682 (C—Cl). The other polymers with 5-FU terminal group were prepared by the same method.

Synthesis of the objective polymeric prodrugs

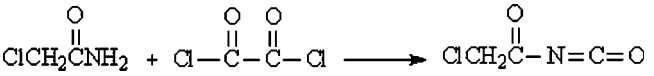
Two grams of PLGA (50:50)-5-FU conjugate in 10 mL of methylene chloride was placed in a 100 mL flask equipped with a magnetic stirrer, 0.576 g (2 mmol) of SF-K in 5 mL of distilled water was then added. The mixture was reacted in the presence of TAC (0.01g, 0.5 wt %, as PTC) at room temperature for 12 h, and the oil layer was extracted and poured into excessive ether. The whole precipitate was filtered, purified by reprecipitation from methylene chloride/ether, and then dried in vacuum at room temperature. Light yellow solid product was obtained with the yield of 68.2%. The other polymeric prodrugs were prepared by the same method.

Measurements and instruments

Infrared (IR) spectra were recorded on a Nicolet220DXB IR spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on a Bruker Unity 400 NMR spectrometer at room temperature, with DMSO-d_6 as solvent and TMS as internal reference. The gel permeation chromatography (GPC) measurements were conducted at 25°C with a Waters 410 GPC instrument equipped with microcomputer, using the monodistrbuted polystyrene as the standard sample and tetrahydrofuran as the eluent. The ultraviolet (UV) spectra were obtained on a 756MC spectrophotometer. Differential scanning calorimetry (DSC) measurements were performed by a Perkin-Elmer TAC/DX in nitrogen atmosphere, at the heating rate of 10°C/min. The inherent viscosities were measured with an Ubbelohde viscometer at 25°C in chloroform.

In vitro hydrolytic degradation and drug contents measurement

The weight loss of prodrugs was determined as follows: 25 mg prodrugs were pressed to tablet. Then the samples were immersed in a glass test tube containing 10 mL phosphate buffer solution (PBS; pH 7.2) that was kept in a thermostated bath at 37°C. At every fixed period of time, the samples were taken out and washed with distilled water thoroughly, dried overnight in vacuum, and then weighed. The degree of degradation was estimated from the weight loss according to the following equation:



Scheme 2 Synthesis of α-chloroacetyl isocyanate.

weight loss (%) = $100(W_0 - W_t)/W_0$, where W_0 and W_t are the dry sample weight before and after degradation. The weight loss averaged for two specimens was employed, and the degradation rate of prodrugs was simply determined by the percent of weight loss after 10 days.

It was proved that the synthesized polymer could be fully hydrolyzed in acidic solution. Therefore, the drug contents were determined by releasing it in 0.1 mol/L HCl buffer solution at 37°C. The extent of hydrolysis was estimated from the amount of released 5-FU measured by UV spectra. When the absorbance was invariable, it was proved that the drug was completely released. Then compared with 5-FU standard curve, drug contents of the prodrugs were thus calculated.

RESULTS AND DISCUSSION

Synthesis of α-chloroacetyl isocyanate

Literature is available on preparation of target-directing prodrugs.[13–15] In our research, to synthesize the prodrugs with PLA or PLGA as polymeric carriers containing 5-FU and SF terminal groups, the hydroxyl-terminated PLA or PLGA should be functionalized with an active group that can react with SF. Thus, Cl group was chosen as an active group and α-chloroacetyl isocyanate was employed in this paper to ensure high reaction activity with hydroxyl and to simplify subsequent purification. To synthesize α-chloroacetyl isocyanate with a high yield, HCl should be taken out of the system smoothly. In this reaction, control of reaction temperature was very important and the optimum temperature was 82°C, and any change in this temperature would cause low yield. The optimum reaction condition was as follows: the molar ratio of chloroacetamide and oxalyl chloride is 1.2 : 1, reaction temperature 82°C, and reaction time 16 h. Scheme 2 is the synthesis route of α-chloroacetyl isocyanate.

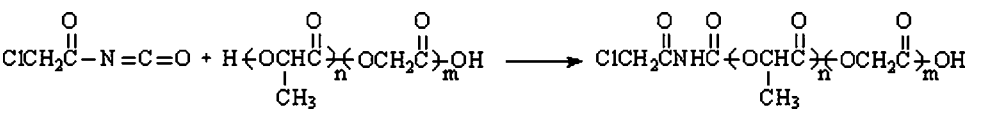
Synthesis of chlorinated PLA and chlorinated PLGA

With the increased reaction time and vigorous stir, the NCO group of α-chloroacetyl isocyanate could contact with hydroxyl groups of PLA or PLGA, and the coupling reaction between isocyanate and hydroxyl should occur according to Scheme 3. During the reaction, the temperature should be controlled below 40°C, because PLA or PLGA tend to decompose into oligomers, and thus decrease the molecular weight in higher temperature. The ^{1}H NMR and IR spectra indicated that α-chloroacetyl isocyanate reacted with hydroxyl, and Cl-terminated carbamate was formed as expected. The urethane bond of the carbamate, through which the SF unit linked to the polymer backbone, was very stable in physiological conditions.[16]

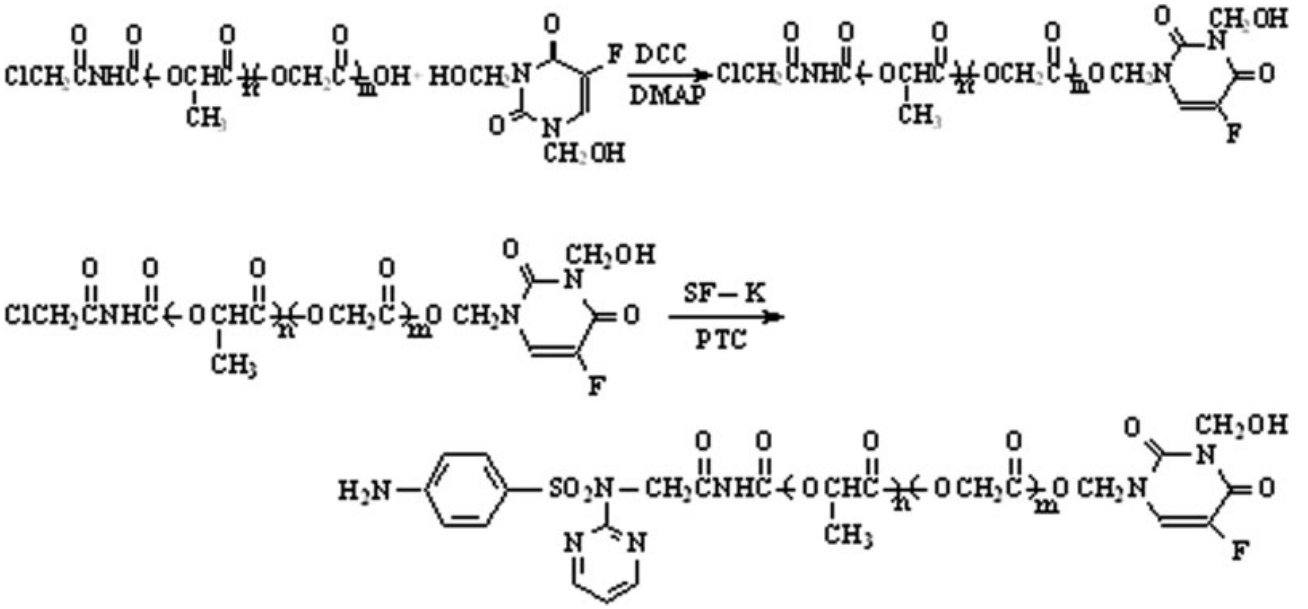
It is reported that the hydroxyl and carboxyl groups of PLA and PLGA could react with isocyanate group to form carbamate and amide, respectively. But the reaction of forming carbamate was a dominant reaction, and the formation of amide could be hold back by controlling the reaction temperature and the content of α-chloroacetyl isocyanate. It was observed that when α-chloroacetyl isocyanate was added into the system, some gas would give out, the temperature of system simultaneously increased 3–4°C, which proved that it was an exothermic reaction. Therefore, α-chloroacetyl isocyanate should be added into the system dropwise to control the system temperature. Scheme 3 is the synthesis route of chlorinated PLA or chlorinated PLGA.

Synthesis of objective polymeric prodrugs

The resultant polymeric prodrugs were composed of degradable aliphatic polyester units as drug carrier, sulfonamide unit as target-directing terminal group, and 5-FU unit as drug-active terminal group. It is generally considered that PLA or PLGA lacks the functional groups that can react with SF-K. Therefore, a series of polymeric prodrugs were synthesized by two-step reaction of chlorinated PLA or chlorinated PLGA with 1,3-dihydroxymethyl-5-FU and SF-K, as detailedly described in Scheme 4. All the objective polymers were structurally confirmed by IR and ^{1}H NMR, as shown in Figures 1 and 2. Concretely, the IR (KBr, cm^{-1}) absorption peaks included 3418, 3356 (amine group conjugate with phenyl ring), 3052, 1595, 1581 (phenyl and pyrimidinyl rings), 2991, 2963, 2944 (—CH_3 and —CH— for PLA), 1754 (—COO— for PLA or PLGA), 1721, 1669 (—COO— for 5-FU), 1688 (—NHCOO—), 1270 (C—F for 5-FU), and



Scheme 3 Synthesis of chlorinated PLA and chlorinated PLGA.



Scheme 4 Synthesis of objective polymer (a: $m = 0$,SF-PLA-FU; b, c, d: $m \neq 0$, SF-PLGA-FU).

1092, 1053 (—COO—CH_2—FU); ^{1}H NMR (δ, ppm) peaks: 7.5–8.5 (pyrimidinyl ring), 7.75 (—CH=C— for 5-FU), 6.5–6.7 (phenyl ring), 5.21 (—CH— for PLA or PLGA), 4.92 (—CH_2— for PLGA), 4.54 (—NH_2 conjugated with benzene ring), and 1.45 (—CH_3 for PLA.).

The GPC curves of PLGA (50 : 50), Cl-PLGA (50 : 50)-FU, and SF-PLGA (50 : 50)-FU revealed a single and sharp peak (Fig. 3), which confirmed that no chain cleavage of whole polymers occurred. For copolymer SF-PLGA (25 : 75)-FU, increased GA content caused an increase in crystallinity and a decrease in solubility. The inherent viscosity of SF-PLGA (25 : 75)-FU could not be obtained, because it was partly soluble in chloroform. Molecular weights and composition of the polymeric prodrugs are listed in Table I.

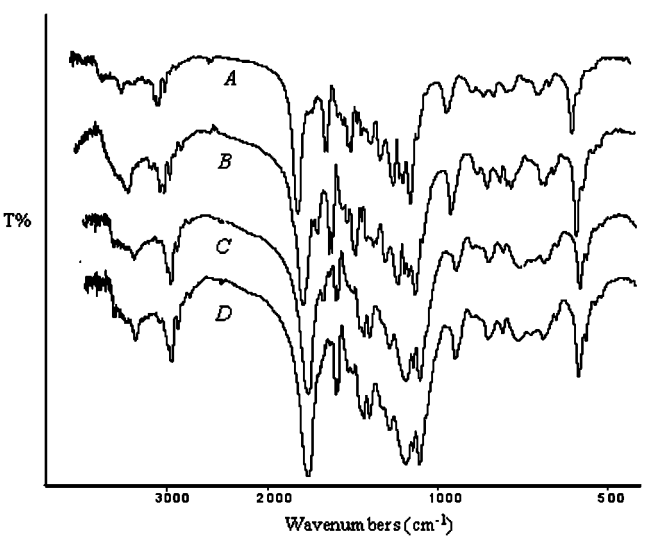


Figure 1 IR spectra of SF-PLA-FU (A), SF-PLGA (75 : 25)-FU (B), SF-PLGA (50 : 50)-FU (C), and SF-PLGA (25 : 75)-FU (D).

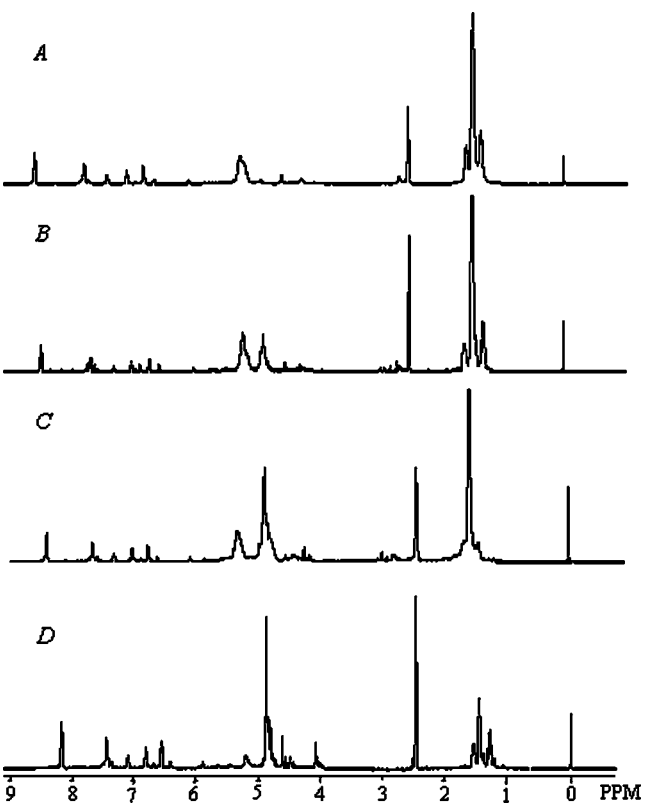


Figure 2 ^{1}H NMR spectra of SF-PLA-FU (A), SF-PLGA (75 : 25)-FU (B), SF-PLGA (50 : 50)-FU (C), and SF-PLGA (25 : 75)-FU (D).

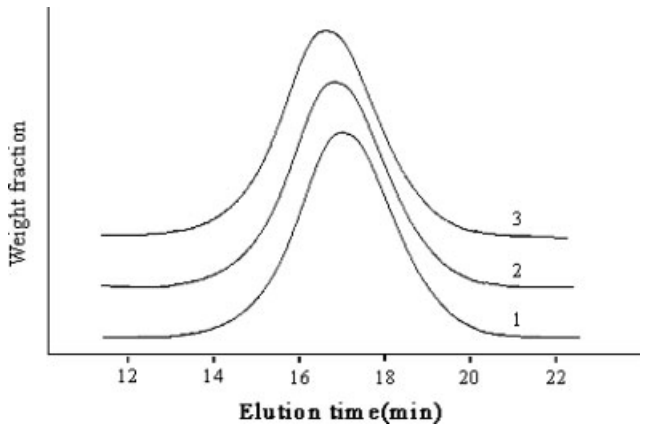


Figure 3 GPC traces of PLGA (50 : 50) (1), Cl-PLGA (50 : 50)-FU (2), and SF-PLGA (50 : 50)-FU (3).

The amounts of comonomer incorporated into the copolymers could be calculated by comparing the integrated areas of the absorption peaks (δ 5.21 ppm) of the —CH of the LA with the absorption peaks (δ 4.92 ppm) of the $—CH_2$ of the GA. The molar ratios of comonomers thus determined are given in Table I. The results showed that the contents of GA in the copolymers are higher than the contents of GA in the feed. It has been reported[17] that, in 200°C, the reactivity ratios of GA and LA are 2.8 and 0.2, respectively. So we concluded that GA also had a higher value of reactivity ratio than LA in the temperature (170°C) for preparation of PLA and PLGA in our research, and it had priority in melt polymerization over LA.

Thermal properties of polymeric prodrugs

DSC measurements were performed for polymeric prodrugs samples to investigate their thermal properties. The second heating curves for melt-quenched samples were chosen to remove previous thermal history and to make the T_g more clear and obvious. The determined data are listed in detail in Table II, and the representative DSC curves are shown in Figure 4. Consequent to the DSC studies, we have compared the T_g and T_m of the copolymer with different comonomer composition in the feed. It was observed that, for SF-PLA-FU, the value of T_g was 72°C and this was higher than the value of T_g of SF-PLGA (75 : 25)-FU and SF-PLGA (50 : 50)-FU, whose values were 61°C and 39°C respectively. This was due to the lower flexibility of the copolymer backbones caused by the introduction of the lateral methyl group of LA. As the contents of LA decreased, the polymer chain became more flexible, and T_g of the copolymers thus decreased. Compared with T_m of SF-PLA-FU and SF-PLGA (75 : 25)-FU, T_m of SF-PLGA (50 : 50)-FU increased. Probable reason was that T_m was affected by regularity of copolymer backbones. As the contents of GA increased, the main chain structure became more regular and easier to crystallize, and T_m of the copolymers thus increased. For SF-PLGA (25 : 75)-FU, the crystallinity was higher than that of the other polymeric prodrugs, and the contribution of crystallizability exceeds the contribution of flexibility, and T_g and T_m thus increased remarkably.

In vitro hydrolytic degradation and drug contents measurement

The UV spectra of SF, 5-FU, and SF-PLGA (50 : 50)-FU are illustrated in Figure 5. It was found that the prodrug had the absorption at 240 and 256 nm, compared with the absorption at 265 and 270 nm of 5-FU and SF, respectively. From the *in vitro* hydrolysis degradation test, we found that 5-FU was released from the prodrugs, and the release rate was strongly dependent on pH of the dissolution medium, the molecular weight, and composition of the polymer employed.[18] The result showed that the degradation in PBS likely occurred by simple hydrolytic cleavage of ester linkage. Low-molecular-weight polymers, accompanied with elevated amounts of —COOH and —OH end group, could lead to enhanced hydrophilicity and increased degradation rate. Fur-

TABLE I
Composition and Molecular Weights of Prodrugs

No.	Polymers	LA/GA[a] (mol %)	LA/GA[b] (mol %)	Solubility[c]	Yield	$[\eta]$[d] (dL/g)	Elution time[e] (min)	M_w[e]	M_w/M_n[e]
A	SF-PLA-FU	100/0	100/0	+	61.7	0.158	17.3	3660	1.31
B	SF-PLGA(75 : 25)-FU	75/25	68.8/31.2	+	66.5	0.164	16.7	4980	1.42
C	SF-PLGA(50 : 50)-FU	50/50	46.9/53.1	+	48.1	0.178	16.4	4120	1.26
D	SF-PLGA(25 : 75)-FU	25/75	20.2/79.8	±	42.1	–	16.8	3180	1.24

[a] Feed molar ratios.
[b] Molar ratios of two components estimated from ^1H NMR.
[c] Solubility in chloroform at 25°C (+ soluble; ± partly soluble).
[d] Inherent viscosities measured in chloroform.
[e] Measured by GPC.

TABLE II
Hydrolysis and Thermoanalysis Results of Prodrugs

Polymer's no.	Drug content[a] (mol %)	Weight loss[b] (%)	T_g[c] (°C)	T_m[c] (°C)
A	56.3	51.2	72	128
B	52.2	42.5	61	113
C	41.3	57.6	39	131
D	43.2	46.1	81	139

[a] Measured by UV spectra.
[b] The percent of weight loss after 10 days (degradation in pH 7.2 PBS at 37°C).
[c] Measured by DSC.

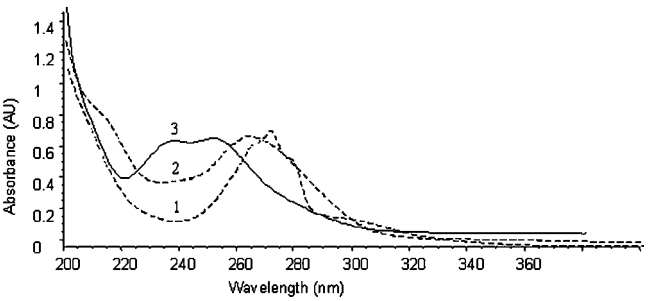


Figure 5 UV spectra of SF (1), 5-FU (2), and SF-PLGA (50:50)-FU (3). (Concentration: 1×10^{-4} mol/L, solvent: 1.0*N* hydrochloric acid.)

thermore, GA had a higher hydrophilicity compared with LA. Therefore, when more GA was introduced into the polymer backbones, the packing density of the copolymer chains decreased and led to an increase in hydrophilicity. Thus, the degradation rate and hydrophilicity of copolymers could be adjusted by varying the ratio of LA and GA in the polymer chains.

The 5-FU terminal group would be released during polymer degradation, and the content of 5-FU introduced into the polymer backbones could be determined using UV spectra by measuring the amount of released 5-FU after full hydrolysis. The contents of 5-FU determined by UV are listed in Table II. The *in vitro* degradation of prodrugs immersed in PBS (pH 7.2) at 37°C against degradation time are shown in Figure 6, and the percent of weight loss after 10 days' degradation are listed in Table II. It was found that SF-PLGA (50 : 50)-FU degraded much faster than the other polymer samples, which was due to the fact that the more the number of GA units introduced into the polymer, the more easily the chain scission could occur during hydrolytic degradation.[19] Therefore, the hydrolytic degradation could be enhanced with the increased contents of GA, attributed to the increased hydrophilicity. For SF-PLGA (25 : 75)-FU, the lower solubility and hydrolytic degradability might be due to the formation of substantive poly(glycolic acid) with rigid and symmetric structure. Consequently, the crystallization tendency was remarkably increased, and the solubility significantly declined compared with SF-PLGA (50 : 50)-FU.

CONCLUSION

A series of target-directing biodegradable polymeric antitumor prodrugs with SF and 5-FU terminal groups were prepared by the two-step reaction of

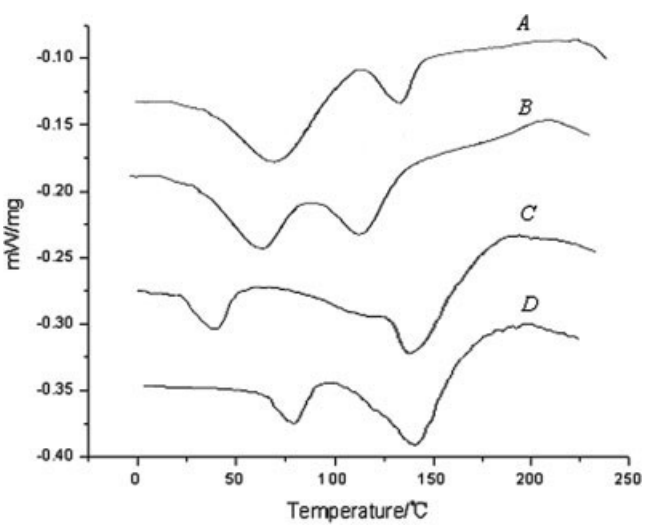


Figure 4 DSC measurement of SF-PLA-FU (A), SF-PLGA (75 : 25)-FU (B), SF-PLGA (50 : 50)-FU (C), and SF-PLGA (25 : 75)-FU (D).

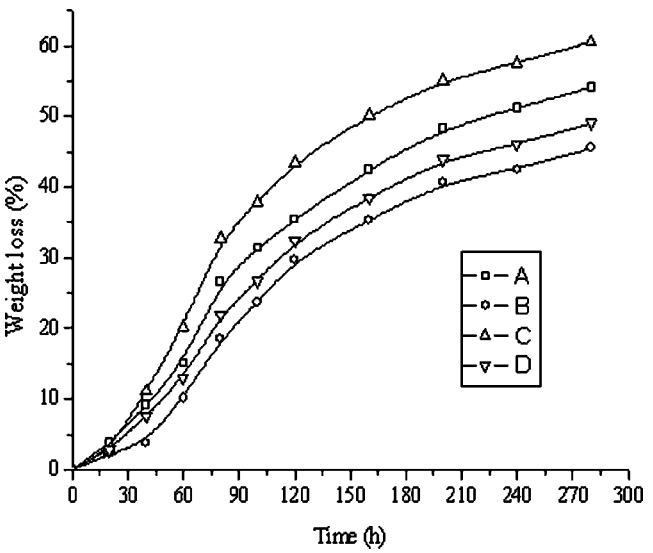


Figure 6 Weight loss of SF-PLA-FU (A), SF-PLGA (75 : 25)-FU (B), SF-PLGA (50 : 50)-FU (C), and SF-PLGA (25 : 75)-FU (D) in PBS at 37°C.

chlorinated PLA or chlorinated PLGA with 1,3-dihydroxymethyl-5-FU and SF-K. The structures of synthesized copolymers were confirmed by IR and ^{1}H NMR spectra. The effects of copolymer composition on solubility, thermal properties, and hydrolytic degradation were investigated respectively. The results indicated that increased contents of GA could increase the melting temperature, the hydrolytic degradation, and the hydrophilicity, while it decreased the glass transition temperature. When the contents of GA increased to 75 mol % (SF-PLGA (25 : 75)-FU), the crystallization tendency remarkably increased; thus the solubility and hydrolytic degradability declined, and the glass transition temperature enhanced, when compared with SF-PLGA (50:50)-FU. Further investigations on drug release behavior are in progress and will be reported later.

References

1. Kim, T. H.; Park, I. K.; Nah, J. W.; Cho, Y. J.; Cho, C. S. Biomaterials 2004, 25, 3783.
2. Liu, Z.; Rimmer, S. J Control Release 2002, 81, 91.
3. Stevens, C. D.; Quinlin, P. M.; Meinken, M. A. Science 1950, 112, 561.
4. Abel, G.; Connors, T. A.; Ross, W. C. J. Eur J Cancer 1973, 9, 49.
5. Huang, J. L.; Wang, H. Y.; Zhou, C. L. J Appl Polym Sci 1995, 58, 11.
6. Huang, J. L.; Chen, S.; Tan, L. S.; Wang, H. Y. Sci China (Ser B) 1998, 28, 85.
7. Fan, C. L.; Mai, C. H.; Yu, H.; Xiang, J. N.; Ding, Q. Z.; Zhuo, R. X. J Wuhan U 1984, 2, 115.
8. Fan, C. L.; Li, B.; Zhuo, R. X. Chem J Chinese U 1996, 11, 1788.
9. Huang, J. L.; Wang, H. Y. Sci China Ser B 1996, 26, 214.
10. Speziale, A. J.; Smith, L. R. J Org Chem 1963, 28, 1805.
11. Takahashi, T.; Taniguchi, I.; Kimura, Y. Polymer 2000, 41, 8725.
12. Moon, S. I.; Lee, C. W.; Miyamoto, M.; Kimura, Y. J Polym Sci Part A: Polym Chem 2000, 38, 1673.
13. Cai, T. B.; Tang, X.; Nagorski, J.; Brauschweiger, P. G.; Wang, P. G. Bioorg Med Chem 2003, 11, 4971.
14. Chen, S.; Huang, Z. H.; Huang, J. L. Eur Polym Mater 2000, 36, 1703.
15. Jia, Z. F.; Zhang, H. T.; Huang, J. L. Bioorg Med Chem Lett 2003, 13, 2531.
16. Milton, H. J Polym Prepr 1997, 38, 520.
17. Gilding, D. K.; Reed, A. M. Polymer 1979, 20, 1459.
18. Zhang, X. F.; Li, Y. X.; Chen, X. S.; Wang, X. H.; Xu, X. Y.; Jing, X. B. Biomaterials 2005, 26, 2121.
19. Fu, Y. J.; Shyu, S. S.; Su, F. H.; Yu, P. C. Colloids Surf B 2002, 25, 269.