Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

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To cite this Article Chang, Jun , Du, Jie and Zheng, Yubin(2007) 'Synthesis and Characterization of Novel PGA and PLA Prodrugs with Sulfadiazine and 5-Fluorouracil Terminal Groups', Journal of Macromolecular Science, Part A, 44: 8, 887 — 892
To link to this Article: DOI: 10.1080/10601320701407870
URL: http://dx.doi.org/10.1080/10601320701407870

Journal of Macromolecular Science®, Part A: Pure and Applied Chemistry (2007) **44**, 887–892

ISSN: 1060-1325 print/1520-5738 online
DOI: 10.1080/10601320701407870

Synthesis and Characterization of Novel PGA and PLA Prodrugs with Sulfadiazine and 5-Fluorouracil Terminal Groups

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Received November, 2006, Accepted January, 2007

PGA and PLA prodrugs containing sulfadiazine and 5-fluorouracil terminal groups were prepared in three steps: (1) Chloride-terminated polymeric carriers were synthesized by polymerization of chloroacetic acid and L-lactic acid (PLA); and chloride-terminated PLA was prepared by reaction of PLA and α-chloroacetyl isocyanate; (2) The obtained polymeric carriers reacted with 5-FU to get polymer-5-FU conjugates in the presence of dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP); (3) The conjugates reacted with potassium sulfadiazine using tetrabutyl ammonium chloride as phase transfer catalyst. Structures of the synthesized prodrugs were confirmed by IR and ^{1}H-NMR. The molecular weights of prodrugs were determined by GPC. The UV measurement showed that 5-FU could be fully released from the prodrugs in acidic solution *in vitro*. The drug content measured by UV spectrometer is 66.2 in maximum.

Keywords: sulfadiazine; 5-fluorouracil; drug carriers; drug release

1 Introduction

5-Fluorouracil (5-FU) is an anti-metabolite with a broad spectrum of activity against solid tumor. However, it showed severe toxic side effects and delivery problems on the living body. Thus, how to reduce or remove the toxicity of 5-FU is a very important subject for pharmaco-chemists. Many investigations have reported that it is possible to reduce the toxic side effects and prolong the duration of activity using polymer as drug carrier (1–2). So far, a number of drug delivery systems utilizing polymeric drug carriers have been successfully developed to increase the therapeutic efficiency of the anti-tumor drugs.

The target-directing anti-tumor drugs are recognized as the most effective way to kill malignant tissue selectively and minimize the unfavorable side effects (3–4). A great deal of work has been done to prepare target-directing prodrugs (5–7). In the 1950's, Stevens (8) found that sulfadiazine (SF), an antibiotic, could be concentrated selectively in Yoshida Sarcoma growing in rats. Recently, polyethylene oxide (PEO) with sulfadiazine and diethylenetriaminepentaacetic acid (DTPA) end groups was synthesized (9). After complexation with ^{153}Sm and ^{99}Tc, it could be concentrated selectively in the tumor tissue. Currently, a great deal of interest has been concentrated in utilizing poly(glycolic acid) (PGA) and poly(lactic acid) (PLA) to regulate the delivery of pharmaceuticals. Pan et al. (10) used poly(lactic-co-glycolic acid) nanoparticles as a carrier for loading insulin and it was proved that these nanoparticles might be used as a new oral carrier for protein drug delivery system. A paclitaxel/MPEG-PLA block copolymer conjugate was prepared by Zhang et al., (11) and the results showed that the conjugate exhibited the same anti-tumor activity as the pure paclitaxel.

In this article, we prepared a series of novel PGA and PLA prodrugs with SF and 1,3-dihydroxymethyl-5-FU terminal groups. Owing to the biodegradability of the prodrugs, 5-FU can be released not only by the hydrolysis of the chemical bonds directly formed by 5-FU and polymeric carriers, but also by the biodegradation of polymeric carriers. Thus, the release kinetics of 5-FU may be adjusted to certain extent, in comparison with the cleavage of a single chemical bond of a prodrug (12).

Furthermore, the SF terminal group can enhance the targeting and selectivity of the prodrugs, and hence, minimize the undesirable side effects. In this work, the effects of drug carrier contents on thermal properties and hydrolytic degradation were examined, respectively.

2 Experimental

2.1 Materials

Sulfadiazine was supplied by Northeast Pharmaceutic Manufacturing Co. (Shenyang, China), recrystallized twice from

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DMSO/ethanol (1/4 v/v) before use; 5-Fluorouracil was purchased from Nantong Pharmaceutic Manufacturing Co. (Nantong, China), recrystallized from water and dried in vacuum. L-Lactic acid (LA) and chloroacetic acid (Shenyang Lianbang reagent Co., China) were dehydrated before use. Dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) were purchased from Yanchang Biotechnology company (Shanghai, China) and used as received. Stannous octoate, tetrabutyl ammonium chloride (TAC) and triethylamine (TEA) were purchased from Shanghai Chemical Regent Company (Shanghai, China), and TAC was used as phase transfer catalyst (PTC). All the solvents were purified by conventional methods. 1,3-Dihydroxymethyl-5-fluorouracil, potassium sulfadiazine (SF-K) and α-chloroacetyl isocyanate were synthesized according to the literatures (13–15).

2.2 Measurements

IR spectra were recorded on a Nicolet 220DXB IR spectrometer. ^{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 THF as the eluent. The UV spectra were obtained on a 756MC spectrophotometer. DSC measurements were performed by a Perkin-Elmer TAC/DX in nitrogen atmosphere with the heating rate of 10°C/min. The inherent viscosities were measured with an Ubbelohde viscometer at 25°C in chloroform. MS spectra were measured with HP1100LC/MSD mass spectrometer. XRD measurement was performed by Japan Shimadzu XD32A X-ray diffractometer.

2.3 Polymer Synthesis

2.3.1 Synthesis of the Chlorinated PGA

Chloroacetic acid (0.21 mol, 20 g) and THF (20 mL) was added into a three-necked flask equipped with mechanical stirrer and a condenser, the solution was stirred at reflux temperature. Then, TEA (0.19 mol, 19.2 g) in THF (10 mL) was added within 1 h, and the temperature was maintained at 80°C for 6 h. The solvent was evaporated under reduced pressure and the residue was washed three times with ethanol, and then dried at room temperature in vacuum. White solid (**a1**) was obtained in the yield of 85.6%. MS (m/z):847. Using the same method, when the solvent was about to evaporate off, the temperature was raised to 200°C and maintained for 2 h. Then, chlorinated PGA with higher molecular weight (**a2**) was obtained in the yield of 42.6%. MS (m/z):1435. ^{1}H-NMR (δ, ppm): 4.92 (m, 2H,-CH_2COO-); IR (KBr, cm^{-1}): 2976, 2939 (-CH_2-), 1758 (C═O), 1215, 1095 (C-O-C), 672 (C-Cl).

2.3.2 Synthesis of Chlorinated PLA

PLA with different molecular weights (M_w = 1860, 4540 and 7880) were synthesized using stannous octoate as catalyst according to the literatures (16, 17).

^{1}H-NMR (δ, ppm): 5.21 (m, 1H, -CH(CH_3)COO-), 1.53 (m, 3H, -CH_3); IR (KBr, cm^{-1}): 2994, 2944(-CH_3), 1755(C═O), 1186, 1087 (C-O).

PLA (M_w = 1860) (2 mmol, 4 g) and DMF (20 mL) was added into a three-necked flask with magnetic stirrer, α-chloroacetyl isocyanate (7.32 mmol, 0.6 mL) was added dropwise and stirred for 12 h at room temperature. The synthesized product was dissolved in methylene chloride, and precipitated in methanol. The resultant precipitate (**d1**) was filtered and dried at room temperature in vacuum in the yield of 89.2%.

^{1}H-NMR (δ, ppm): 7.67 (m, 1H, -CONHCOO-), 5.21 (m, 1H, -CH(CH_3)COO-), 1.53 (m, 3H, -CH_3), IR (KBr, cm^{-1}): 2994–2944 (-CH_3), 1755 (C═O), 1688 (-NHCOO-), 1186, 1087 (C-O), 682 (C-Cl).

The other chlorinated PLA (**d2, d3**) were respectively prepared by the same reaction of PLA (M_w = 4540, 7880) and α-chloroacetyl isocyanate.

2.3.3 Synthesis of the Polymer-5-fluorouracil Conjugate

d1 (2 g) 1,3-dihydroxymethyl-5-fluorouracil (11 mmol, 2.1 g) and DMAP (0.06 g, 1 wt%) were dissolved in DMF (25 mL). The solution was cooled to 0–5°C and DCC (3.88 mmol, 0.8 g) in DMF (5 mL) 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. The product (**e1**) was obtained in the yield of 81.4%.

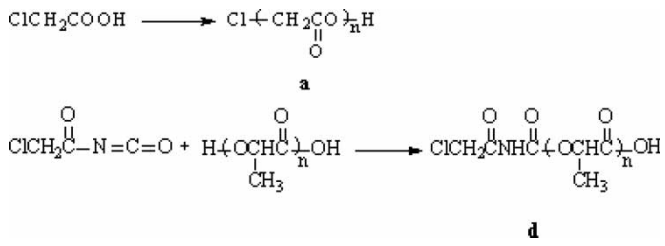
^{1}H-NMR (δ, ppm): 7.75 (-CH═C-for 5-FU), 7.67 (m,1H, -CONHCOO-), 5.21 (m, 1H, -CH(CH_3)COO-), 4.68 (m, 4H, CH_2-FU), 1.53 (m, 3H, -CH_3). IR (KBr, cm^{-1}): 3083 (C-H for 5-FU), 2994, 2944 (-CH_2- and -CH_3), 1755 (C═O), 1723, 1673 (-COO- for 5-FU), 1688 (-NHCOO-), 1186, 1087 (C-O), 682 (C-Cl).

The Cl-PGA-FU (**b1**, **b2**) and other Cl-PLA-FU (**e2**, **e3**) were prepared by the same method.

2.3.4 Synthesis of the Polymers with 5-FU and Sulfadiazine Terminal Groups

e1 (1 g) dissolved in methylene chloride (10 mL) was placed into a 100 mL flask equipped with a magnetic stirrer, SF-K (2 mmol, 0.576 g) in distilled water (5 mL) was then added. The mixture was reacted in the presence of TAC (0.01 g, 0.5 wt%, as phase transfer catalyst) 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 (**f1**) was obtained in the yield of 68.2%.

The SF-PGA-FU (**c1**, **c2**) and other SF-PLA-FU (**f2**, **f3**) were prepared by the same method.



Sch. 1. Synthesis of chlorinated PGA and chlorinated PLA.

2.3.5 In vitro Hydrolytic Degradation and Drug Contents Measurement

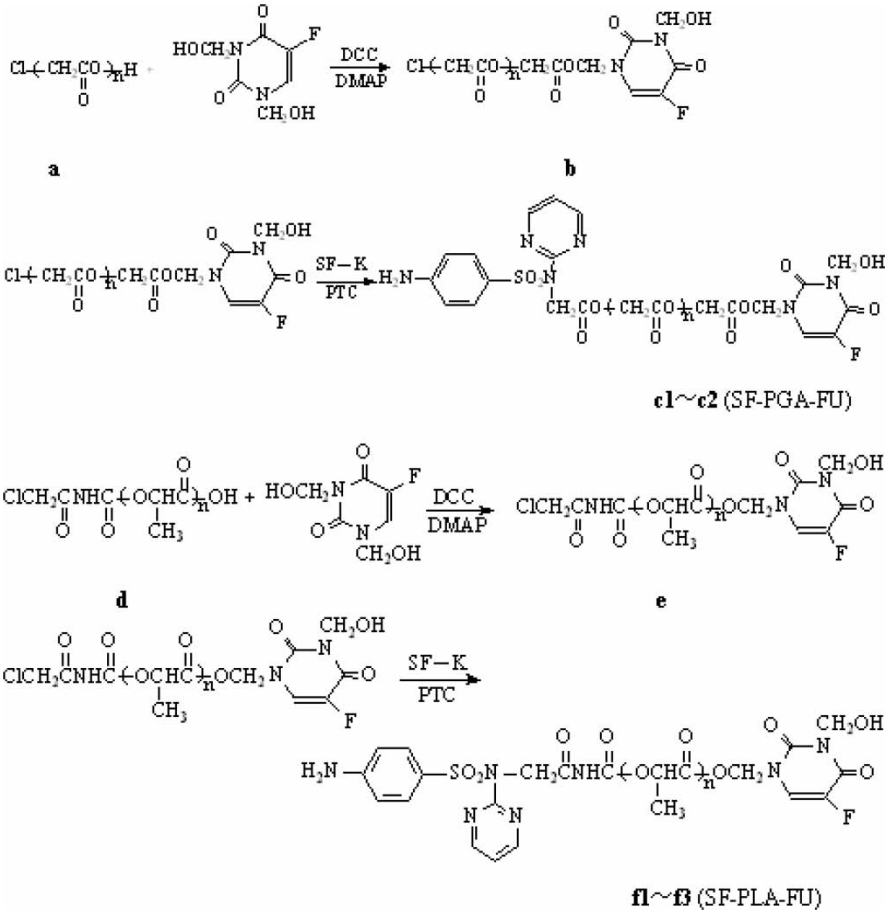
The weight loss of prodrugs was determined as follows: 25 mg of prodrugs were pressed to a tablet, the samples were then immersed in a glass test tube containing 10 mL of pH 7.2 phosphate buffer solution (PBS) at 37°C. At every fixed period of time, the samples were taken out to be washed with distilled water thoroughly, dried overnight in vacuum, and then weighed. The degree of degradation was estimated from the weight loss according to the following equation: Weight loss (%) $= 100(W_0 - W_t)/W_0$, where W_0 and W_t are the dry sample weight before and after the degradation. The drug release rate 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 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.

3 Results and Discussion

3.1 Synthesis of Objective Polymer

In our research, to synthesize the prodrugs with PGA or PLA as polymeric carriers containing 5-FU terminal groups, the hydroxyl terminated PGA or PLA should be functionalized with an active group which can react with sulfadiazine. Thus, Cl group was chosen and α-chloroacetyl isocyanate was employed to ensure high reaction activity with 5-FU and to simplify subsequent purification. The synthesis routes of chlorinated PGA and chlorinated PLA are shown in Scheme 1.



Sch. 2. Synthesis of SF- PGA-FU and SF- PLA-FU.

The resultant polymeric prodrugs were composed of degradable aliphatic polyester units as drug carrier, sulfadiazine unit as target-directing terminal group, and 5-fluorouracil unit as drug-active terminal group. Using polyethylene oxide (PEO) as carrier, Chen et al. (18) have prepared SF-PEO-FU by ring-opening polymerization. Compared with this drug delivery system, our research has two obvious advantages. Firstly, the released rate of 5-FU could be adjusted by changing the molecular weights of PGA and PLA. Secondly, the polymeric carriers couldn't accumulate in the body because of their biodegradability. It is generally considered that PLA lacks the functional groups which can react with SF-K. Therefore, a series of polymeric prodrugs were synthesized by a two-step reaction of chlorinated PLA, 1,3-dihydroxymethyl-5-fluorouracil and SF-K, as described in detail in Scheme 2. The structures of objective polymers were confirmed by IR and ^{1}H-NMR, as shown in Figures 1 and 2. Concretely, the IR absorption peaks, included 3418, 3356 (amine group conjugate with phenyl ring), 3083 (C-H for 5-FU), 3042, 1595, 1581 (phenyl and pyrimidinyl rings), 2991, 2963, 2944 (-CH_3 and -CH-for polylactic acid), 3052, 1688 (-NHCOO-), 1747 (-COO-for PGA and PLA), 1723, 1673 (-COO-for 5-FU), 1264 (C-F for 5-FU), 1092, 1053 cm^{-1} (-COO-CH_2-FU); ^{1}H-NMR (δ, ppm) peaks: 5.21(-CH-for PLA), 4.92 (-CH_2CO- for PGA), 4.54 (-NH_2 conjugated with benzene ring), 6.5–6.7 (phenyl ring) and 7.5–8.5 (pyrimidinyl ring) and 1.45 (-CH_3 for PLA).

From GPC curves of synthesized polymeric prodrugs presented in Figure 3, we could find a single sharp peak, which confirmed that no chain cleavage of whole polymers occurred. The molecular weights of polymeric prodrugs determined by GPC and ^{1}H-NMR are listed in Table 1. Polymeric prodrugs with different molecular weights were synthesized by altering the reaction condition and changing the reaction time. For prodrugs **f**, with the increased molecular weight, the hydrophilicity of the polymer decreased. Therefore, the molecular weight of polymer **g** should be controlled below 10000 in order to maintain its hydrophilicity. The average molecular weights of the polymers were also calculated using ^{1}H-NMR spectrum by comparing the integrated

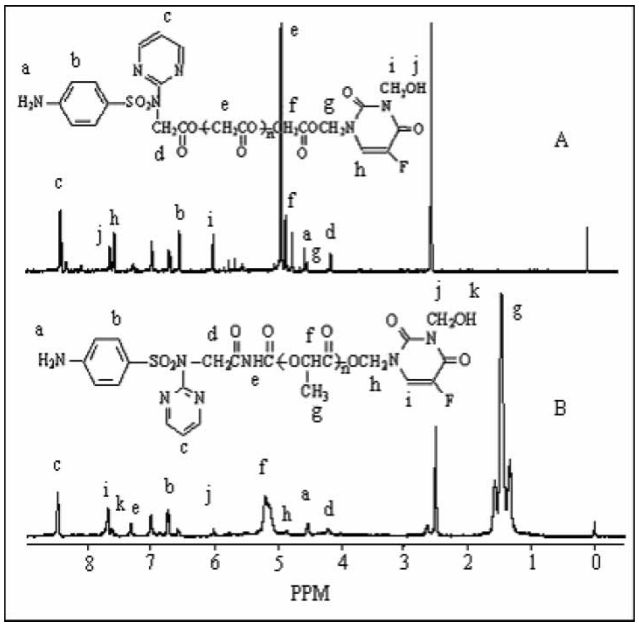


Fig. 2. ^{1}H-NMR spectrum of SF-PGA-FU (A) and SF-PLA-FU (B).

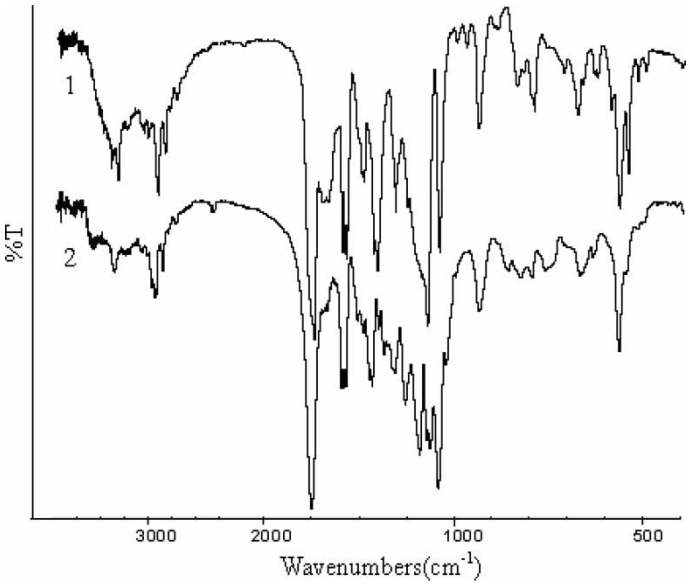


Fig. 1. IR spectrum of SF-PGA-FU (1) and SF-PLA-FU (2).

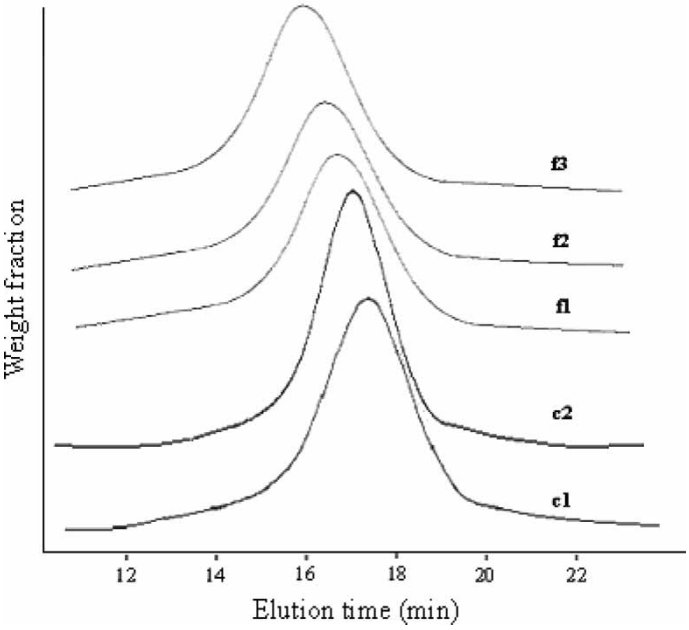


Fig. 3. GPC measurement of polymers SF-PGA-FU and SF-PLA-FU.

Table 1. Molecular weights of synthesized copolymers

Polymers	Yield (%)	$[\eta]^a$ (dL · g^{-1})	Elution time^b (min)	$M_w{}^b$	$M_w/M_n{}^b$	M_n by ^{1}H-NMR
c1	63.8	0.175	17.6	1345	1.25	1310
c2	42.5	0.178	17.4	2130	1.13	1947
f1	76.5	0.168	16.7	2320	1.42	2140
f2	58.1	0.232	16.4	4950	1.36	4720
f3	32.1	0.346	15.8	8310	1.33	7980

[a]Inherent viscosities measured in chloroform.
[b]Measured by GPC.

areas of the peak at 4.92 ppm (-CH_2- in PGA) or 5.21 ppm (-CH- in PLA) with that of the peak at 7.72 ppm (-CH═C-in 5-FU). The molecular weights thus determined were consistent with the results measured by GPC.

3.2 Thermal Properties

DSC measurements were performed for polymeric prodrugs samples in order to investigate their thermal properties. The determined data are listed in detailed in Table 2 and the representative DSC curves are shown in Figure 4. It was found that T_g (112°C) and T_m (126°C) of **c1** was much lower than T_g (153°C) and T_m (203°C) of **a1**. The results indicated that introducing 1,3-dihydroxymethyl-5-fluorouracil and sulfadiazine terminal groups destroyed the symmetric structure of chlorinated PGA and declined its crystallinity. It was also found that PLA and SF-PLA-FU were both crystalline. T_g (67°C) and T_m (127°C) of **f1** were increased compared with that of PLA. The probable reason was that when 1,3-dihydroxymethyl-5-fluorouracil and sulfadiazine terminal groups were introduced, the chain rotation became more difficult, T_g and T_m and thus increased.

3.3 Crystallinity and Solubility

The crystallinity behaviors of polymeric carriers and prodrugs were carried out by X-ray diffraction (XRD) measurements, and the comparison of XRD patterns was shown in Figure 5. The sharp diffraction peaks were indicative of the presence of crystallites in the polyesters. The crystallinity of the prodrugs was dependent on the polymeric carriers (PGA or PLA). It was also found that the crystallinity of prodrugs declined compared with PGA and PLA. The probable reason was that introducing 5-FU and SF-K could destroy the symmetric structure of PGA and PLA, and thus decreased the degree of relative crystallinity. The solubility measurements were carried out in chloroform at 25°C. Compared with chlorinated PLA (**d1**–**d3**), the solubility of prodrugs **f1**–**f3** was enhanced. However, the solubility of prodrugs **c1**–**c2** was comparatively poor, which might be due to the introduced rigid and symmetric structure of PGA. For **f1**–**f3**, introducing the lateral methyl group of LA into the polymer backbones could reduce the energy of internal rotation of the polymer chains, thus declined the crystallinity and increased the solubility of the products.

Table 2. Hydrolysis and thermoanalysis results of synthesized copolymers.

Polymers	Tg^a (°C)	Tm^a (°C)	Drug content^b mol (%)	Weight loss^c (%)
c1	112.3	126.1	66.2	54.2
c2	126.5	138.4	61.4	47.6
f1	67.4	127.6	56.2	67.5
f2	74.7	137.4	41.3	45.6
f3	84.2	148.3	23.2	34.1

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

3.4 In vitro Hydrolytic Degradation and Drug Contents Measurement

The UV measurements of SF, 5-FU and prodrugs are illustrated in Figure 6. The prodrugs had the absorption at 240 nm and 25 6 nm, compared with the absorption at

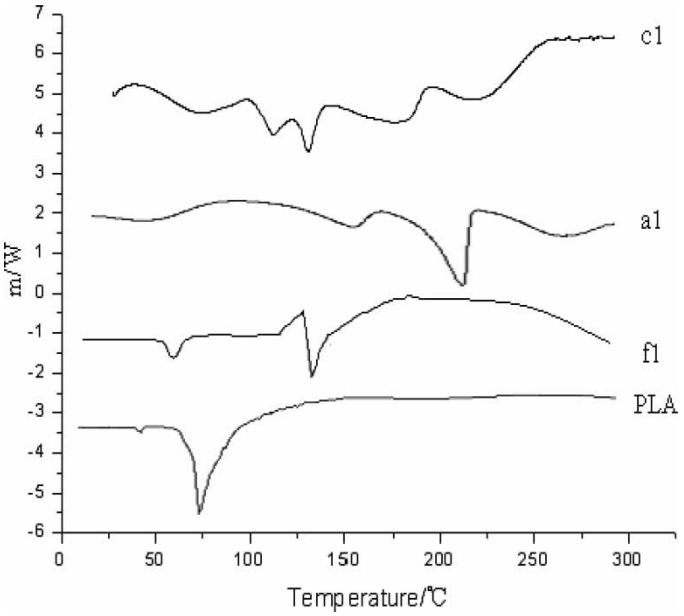


Fig. 4. DSC measurement of a1, c1, PLA and f1.

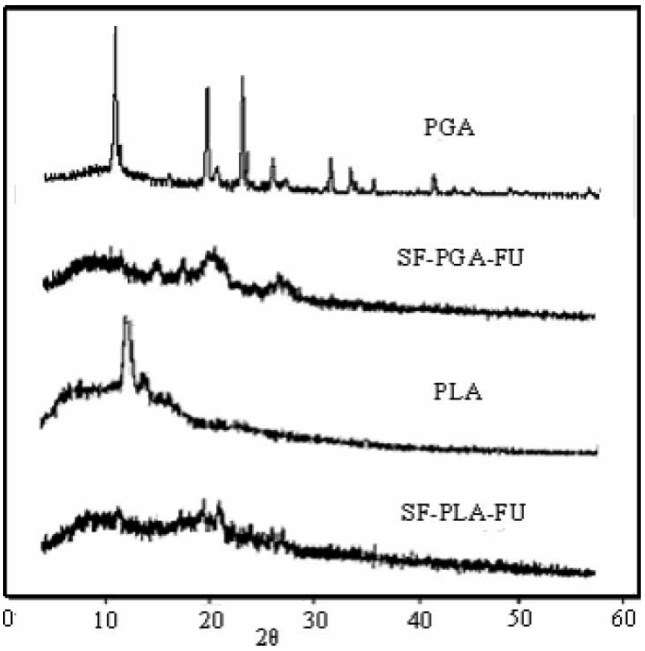


Fig. 5. X-ray diffraction of polymeric carriers and prodrugs.

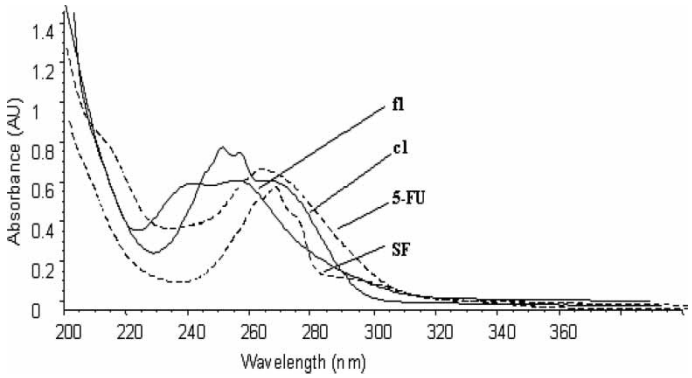


Fig. 6. UV spectra of SF, 5-FU, c1 and f1 (concentration: 1×10^{-4} mol/L, solvent: 1.0 N hydrochloric acid).

265 nm and 270 nm of 5-FU and SF, respectively. From the *in vitro* hydrolysis degradation test, it was found that 5-FU was released from the prodrugs, and the release rate strongly depended on pH values of the dissolution medium, the molecular weight and composition of the polymer employed. 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 drug contents and the percent of weight loss after 10 days' degradation are listed in Table 2. It was found that the hydrolytic degradation rate was decreased with the increased molecular weight of polymeric carriers. The results indicated that the degradation in PBS was 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. Consequently, the molecular weight and surrounding medium properties might influence the hydrolytic degradation in our research.

4 Conclusions

A series of target-directing biodegradable polymeric antitumor prodrugs with sulfadiazine and 5-fluorouracil terminal groups were prepared by the functionalization of the hydroxyl terminal group of PGA or PLA. The results indicated that 5-fluorouracil could be fully released in acidic solution. The drug release rate could be adjusted by changing the molecular weights of drug carriers. Further investigations on drug release behavior are in progress and will be reported later.

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