Stereocomplexes of Triblock Poly(lactide- PEG_{2000}-lactide) as Carrier of Drugs

Ashgan Bishara,[1] *Hans R. Kricheldorf,*[2] *Abraham J. Domb**[1]

[1] Department of Medicinal Chemistry and Nature Products, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem, Jerusalem, 91120 Israel
[2] Institute of Technical and Macromolecular Chemistry, Hamburg University, Germany

Summary: Triblock copolymers of poly(lactide)-poly(ethylene-glycol)-poly(lactide) (PLA-PEG_{2000}-PLA) were synthesized by ring-opening polymerization of lactide and PEG_{2000} diol as co-catalyst. Stereocomplexes with particle sizes ranging from nanometers to microns were obtained by mixing acetonitrile solutions of pairs of enantiomeric homopoly(lactide) and the triblock copolymers. The stereocomplexes exhibited higher crystalline melting temperatures than the optically pure polymers. The ratio of PLA terminals in the copolymers had a significant effect on their stereocomplex degradation and drug release. These stereocomplexes were used for the encapsulation of dexamethasone for controlled release applications. Dexamethasone phosphate loading capacity, in vitro release, degradation and stability of polymers and formulation were investigated for one month. An increase in the dexamethsone phosphate content in the stereocomplex or a decrease in the PLA ratio in the copolymer resulted in a faster release of drug and polymer degradation.

Keywords: controlled release; degradation; dexamethasone phosphate; poly(ethylene glycol); polylactide; stereocomplex

Introduction

The formation of stereocomplexes by blending entiomeric poly(L-lactide) [L-PLA] and poly(D-lactide) has been extensively studied during the past decade[1,2]. A stereocomplex racemic crystallite having a 3/1 helical structure is different from a homopolymer crystallite with a 10/3 helical structure as found in individual L-PLA or D-PLA[3-5]. The crystallization between the two enatiomeric polymers results in a much high melting point and higher mechanical strength, and improved thermal and hydrolytic stability[6-8].

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Block copolymers composed of PLA and poly (ethylene glycol) possess more water-absorbing capacity, due to the inclusion of hydrophilic PEG segments within the relatively hydrophobic PLA chain[9-11]. PLA-PEG copolymers have been used for the preparation of polymeric micelles and biodegradable nanoparticles with the PEG chains on the particle surface[12-14]. These particles possess a long blood circulation time, due to the surface PEG chains that prevent adsorption by the RES system. PLA-peptide stereocomplexes for the delivery of leuprolide and insulin[15,16] have also recently been reported. The influence of polylactide chain lengths on the thermal behavior of their resultant stereocomplexed was also investigated[17].

In this work, stereocomplexes between PLA-PEG_{2000}-PLA and PLA were formed under various conditions, yielding nano- and microparticles. Stereocomplexes of a pair of enatiomeric PLA homopolymer with the PLA-PEG_{2000}-PLA triblock copolymer were prepared and used for the delivery of dexamethasone, a corticosteroid anti-inflammatory drug. The in vitro release of dexamethasone and the hydrolytic degradation of stereocomplexes were studied.

Experimental Methods

Materials

Lactide for the synthesis of isotactic and atactic PLA were obtained from Purac BV, Gorinchem, Holland. Dexamethasone phosphate was a gift from Taro Pharmaceutical Industries Ltd, Haifa, Israel. All other materials and agents were purchased from Sigma-Aldrich (Rehovot, Israel), and HPLC grade solvents were purchased from BioLab (Jerusalem, Israel).

Instrumentation

Thermal analysis was determined on a Metler TA 4000-DSC differential scanning calorimeter (DSC), calibrated with indium standards, using a heating rate of 10 ^{0}C/min. The average sample weight was 10 mg. Molecular weights of polymers and their co-polymers were estimated on a gel permeation chromatography (GPC) system consisting of Spectra Physics (Darmstadt, Germany) P 1000 pump with Waters 2410 refractive

index (RI) detector, and a Rhodyne (Caotati, CA) injection valve with 20-Ml loop (Waters Ma). Samples were eluted with $CHCl_3$ through a linear Ultratyrogel column (Waters, MA; 500 Å pore size) at a flow rate of 1ml/min. The molecular weights were determined relative to polystyrene standards (Polyscience, Warrington, PA), with a molecular weight range of 400-10,000, using a Breeze computer program. Particle size was determined by a Coulter N4 SD Sub-Micron Particle Size Analyzer, Coulter Electronics, USA. Scanning electron microscopy was performed on a SEM 505, Philips, Holland, (30 KV) after dried stereocomplex nanoparticles were fixed on a stub and gold coated using Polarone E5100. Infrared (IR) spectroscopy (Perkin-Elmer System 2000 FT-IR) was performed on polymers and stereocomplexes. The dried samples were powdered, ground with dried KBr powder, and pressed into thin pellets. Each sample was recorded by 16 scans between 4000-400 cm^{-1}.

Lactic acid was determined by High Performing Liquid Chromatography (HPLC) using a Lichrospher-60 RP column (125 x4 mm ID, 5μm) at room temperature, phosphoric acid solution (PH 2.5) as mobile phase with a flow rate of 1 ml/min and UV detection at 210 nm. A typical retention time of lactic acid was 4.4 min. For dexamethasone phosphate, a C-18 column was used with 55:45 acetonitrile:DDW as mobile phase, at a rate of 1 ml/min, and a UV detector at 254 nm.

Polymer Synthesis

Polylactic Acid (Homopolymers)

Poly (L-lactic acid) (PLLA) and poly (D-lactic acid) (PDLA) were prepared by the ring-opening polymerization of L-lactide and D-lactide using stannous 1-ethylhexanoate (Sn(Oct)2) as catalyst and an alcohol as co-catalyst. In a typical reaction, lactide (10 g, 70 mmole) and benzyl alcohol (1.5 g, 14 mmole) were dissolved in dry toluene (200 ml) and refluxed in a Dean-Stark apparatus for 3 hours. After azeotropic removal of the water, 140 mg (0.35 mmol, 0.05 mol % per lactide) of $Sn(Oct)_2$ was added and the mixture was stirred for 3 hours. After removal of the toluene, the reaction continued at 130 ^{0}C for 2 hours. The crude melt was dissolved in chloroform and precipitated into an ether/petroleum ether mixture and a white powder was obtained.

Triblock Copolymers (PLA-PEG-PLA)

Under an atmosphere of dry nitrogen L-lactide (60 mmol) and dry PEG-2000 (1.2 mmol) were weighed into a 50-mL glass Erlenmeyer flask. The reaction vessel was closed with a glass stopper and steel spring, and immersed into an oil bath preheated to 100°C. After homogenization by stirring with a magnetic bar, the initiator $SnOct_2$ was introduced in the form of a 1 M solution in dry chlorobenzene (0.12 mL). After 2 h at 100°C the ^1H NMR spectrum indicated ≥ 97% conversion. The cool reaction mixture was dissolved in CH_2Cl_2 and poured into cold diethyl ether. The precipitated polymer was isolated by filtration and dried at 20°C under vacuum. An analogous polymerization was conducted with 2.4 mmol of PEG-2000.

Stereocomplex Preparation

Stereocomplexes

Stereocomplexes were prepared by mixing solutions of the enantiomeric PLA polymers and copolymers in acetonitrile. In a typical experiment, D-PLA (10mg, 0.1 %w/v) having a number average molecular weight of 4000 Da and (PLA-PEG-PLA) (10mg, 0.1% w/v), with a number average molecular weight of between 6000 and 8000 Da, were dissolved separately in acetonitrile (10 ml). The solutions were then mixed and warmed to 60°C until a white milky dispersion was obtained. Upon evaporation of the solvent a white powder was obtained. A similar procedure was applied when concentrations of 0.2, 0.5 and 1% w/v were used.

Preparation of Dexamethasone Phosphate Formulations.

Dexamethsone was incorporated into stereocomplexes using the same procedure as above, followed by the addition of dexamethasone phosphate (2 mg) in 1 ml acetonitrile to the polymer solution containing 10 mg of each enatiomeric polymer in 10 ml acetonitrile. The obtained white precipitate was separated by centrifugation. The precipitate was characterized for thermal properties, particle size and morphology, in addition to drug content and release profile.

Determination of Particle Size, Morphology and Drug Content

Particle size of stereocomplexes was determined with a particle size analyzer (Coulter N4), in which the polymer sample was suspended with agitation. SEM analysis was conducted on dry powders fixed on a stub and gold coated using Polarone E5100. The drug content in the solutions was determined by HPLC, with UV detection of 254 nm.

In-Vitro Hydrolytic Degradation of Polymers and Stereocomplexes

The hydrolysis of these stereocomplexes was evaluated by placing a polymer sample (20 mg) in 20 ml of 0.1M phosphate buffer, pH=7.4 at 37°C with constant shaking (100 rpm). At each time point, a polymer sample was taken out of the buffer, washed in distilled water, and dried under vacuum at room temperature overnight. The hydrolysis was monitored by (a) the appearance of a carboxylic acid peak, by IR spectroscopy, (b) lactic acid release to the degradation solution, by HPLC, (c) weight loss of polymer mass, and (d) change in polymer molecular weight, as determined by GPC.

In-Vitro Drug Release

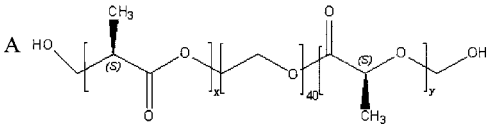
Drug release studies were conducted by placing 20 mg of the polymer formulation in 50 ml of phosphate buffer (0.1M, pH 7.4) at 37°C, with constant shaking (100 rpm). At each time point, a sample was taken for drug analysis. The solution that was removed was replaced with fresh buffer. Dexamethasone concentration in solution was determined by HPLC. All experiments were done in duplicate.

Results and Discussion

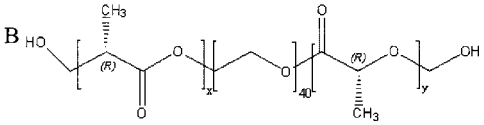
PLA Homopolymer and PLA-PEG_{2000}-PLA Copolymer

PLA copolymers were prepared by the ring-opening polymerization of chiral lactides. The structures of the polymers are shown in Scheme 1. The synthesized polymers were soluble in dichloromethane, chloroform, DMSO and acetonitrile. The chemical structures of the polymers were confirmed by ^{1}H-NMR: δ=5.15 (1H, q, CH); δ=1.60 (3H, d, CH_3), PEG-block: δ=3.61 (4H, m, CH_2-O). At δ=7-7.4 (m. alkyl) the aromatic peaks were

detected in those polymers synthesized with benzyl-alcohol as co-catalyst. The molecular weights were in the range of 4000 Da, as estimated by GPC.



PLLAx-PEG2000-PLLAy



PDLAx-PEG2000-PDLAy

Scheme 1. Structure of triblock copolymers.

The molecular weight and melting temperature of PLA-PEG-PLA triblock copolymers are given in Table 1. The molecular weights of the polymers are limited by the ratio of lactide and PEG_{2000}, where the PLA blocks grow on each side of the PEG. All triblock copolymers melted between 75 and 145°C. The enthalpy increased with the increase in the content of the PLA blocks ratio.

Table 1. Molecular weights and melting tegmperatures of triblock copolymers.

	polymer	Length	M_n (Da)*	M_w/M_n*	T_m (^{0}C)	ΔH (J/g)
1.	$PLLA_x$-PEG2000-$PLLA_y$	X+Y=25	6,600	1.03	120.8	20.40
2.	$PLLA_x$-PEG2000-$PLLA_y$	X+Y=50	8,800	1.04	145.4	28.10
3.	$PDLA_x$-PEG2000-$PDLA_y$	X+Y=25	6,260	1.03	119.0	9.09
4.	$PDLA_x$-PEG2000-$PDLA_y$	X+Y=50	7,750	1.06	142.6	35.00

*Polymers were synthesized by ring-opening polymerization using PEG_{2000} diol as co-catalyst. M_w and thermal properties were determined by GPC and DSC, respectively

Preparation and Characterization of Stereo Complex Formulations

Stereocomplexes were prepared by spontaneous formation and precipitation from solution. The stereocomplexes had different physical properties compared to the enantiomeric polymers. The stereocomplexes are powdery crystalline materials, insoluble in common organic solvents including: chloroform, dichloromethane, dioxane and dimethyl sulfoxide. The stereocomplexes melted at temperatures that are at least 40°C above the melting point of the corresponding enantiomers (see Table 1 and Table 2). It could be seen that the stereocomplexes I and V melting at 186 and 214°C, respectively, while the enatiomeric triblock copolymers melted at 110 and 142°C, respectively. A shift in the melting peak to a higher melting temperature was observed with enantiomeric peaks, which indicated complete complexation. Also, dexamethasone did not affect the stereocomplex thermal properties (Table 3).

Table 2. Stereocomplex composition and characteristics.

Stereocomplex No.	Polymer I	Polymer II	T_m (^{0}C)[a)]	particle size (nm)±S.D[b)]
I	$PLLA_x$-PEG2000-$PLLA_y$, x+y=25	D-PLA	186.6	498±22
II	$PDLA_x$-PEG2000-$PDLA_y$, x+y=25	L-PLA	187.5	563±17
V	$PLLA_x$-PEG2000-$PLLA_y$, x+y=50	D-PLA	214.4	1040±59
VI	$PDLA_x$-PEG2000-$PDLA_y$, x+y=50	L-PLA	209.7	1055±66

[a)] Thermal properties were determined by DSC.
[b)] Particle size was determined with a particle size analyzer, from suspension in acetonitrile.

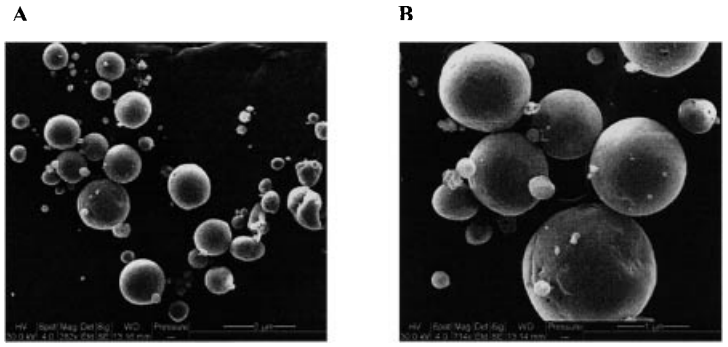


Figure 1. Scanning electron micrographs of nanoparticle-stereocomplexes: A) stereocomplex-I (Table 2), B) stereocomplex-I 10% w/w dexamethasone phosphate (Table 3).

The surface of the particles was analyzed by scanning electron micrographs (SEM) (Figure 1). It was seen that the nanoparticles prepared from triblock copolymers had a relatively smooth surface, probably due to the PEG segments. The particle size as determined by a particle size analyzer is given in Table 2. As shown in Figure 2, the particle size increased with increasing the concentration of polymer solution. For example, complex III formed particle size of 0.76 μm in 0.1% w/v but 2.3 μm in 0.5% w/v. When increasing the PLA content in the copolymer it also increased the stereocmplex particle size, (complex III and complex VII, Table 2).

When dexamethasone was incorporated in the stereocomplex, then after spontaneous precipitation from a 0.1% w/v polymer solution, micron size particle were obtained with a high encapsulation yield (>80%, Table 3).

Table 3. Characteristics of stereocomplex-formulations containing dexamethasone phosphate.

Stereocomplex	Particle Size Mean (nm)±S.D. Dexamethasone (10wt%) 0.1%w/v	Drug Loading (%)*	T_m (^{0}C)	ΔH (J/g)
I	822±45	84.5	189.5	29.5
V	1102±222	77.9	214.9	37.2

*Stereocomplexes loaded with dexamethasone phosphate (10% w/w) prepared from solution.

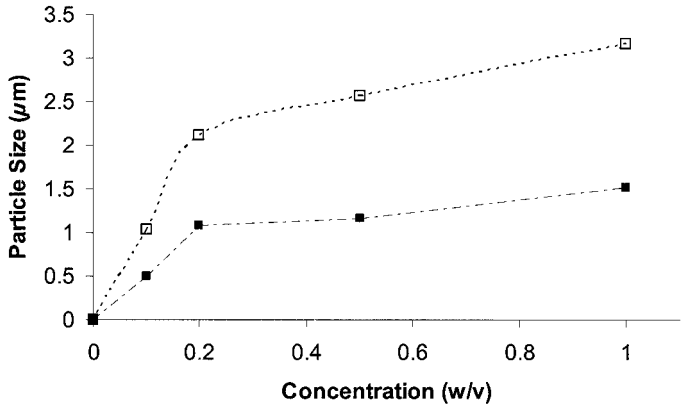


Figure 2. Particle size analysis of stereocomplexes with different concentrations ;(■) stereocomplex I and (□) stereocomplex V, (see Table 3).

In vitro hydrolysis of copolymers and stereocomplexes

The hydrolysis of the PLA-PEG_{2000}-PLA triblock copolymers and their stereocomplexes was studied in vitro under physiological conditions (phosphate buffer pH 7.4 at 37°C), as followed by weight loss, hydrolysis of ester bonds by IR, lactic acid release by HPLC, and change in polymer molecular weight during hydrolysis.

Mass loss during a specified sampling period was determined by measuring the weight loss (%) using the following equation:

Remaining mass (%) = [(initial weight-loss weight)/initial weight] ×100

Weight loss of PLA-PEG_{2000}-PLA and their stereocomplexes with different block lengths are shown in Figure 3. The higher the PLA content in the copolymer, the lower was the rate of weight loss obtained. Copolymer 1 lost about 35% of its weight in 40 days, whereas copolymer 2 lost less than 25% of its weight during the same period. With regard to their stereocomplexes, complex I lost about 45% of its weight in 40 days, resulting in a reduction in the overall crystallinity of stereocomplexes compared to polymer sample.

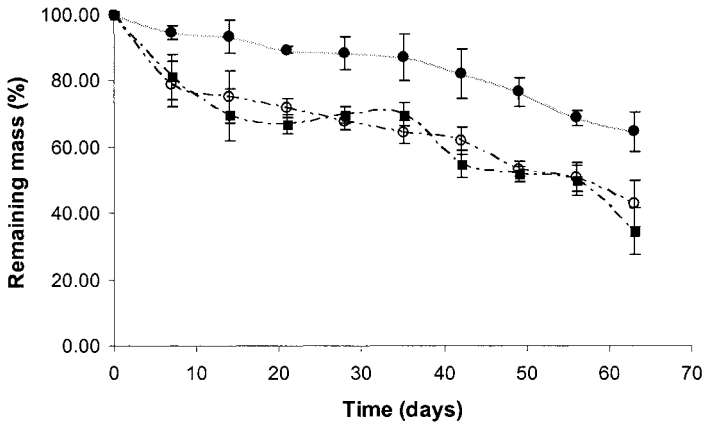


Figure 3. Hydrolysis of copolymers monitored by weight loss. Hydrolysis was conducted in phosphate buffer pH 7.4 at 37°C. Polymer 1 (○), polymer 2 (●) and stereocomplex I (■) (see Table 3).

The disappearance of the ester bonds in the copolymers and stereocomplexes as a function of time is shown in Figure 4. The ester peaks at 1748 cm^{-1} diminished with time while the acid peak at 1700 cm^{-1} intensified. The ester bond (%) was therefore determined by using the following equation:

$$\textbf{Ester bond (\%)} = \left(\frac{\textbf{Length of acid peak (cm)}}{\textbf{(Length of acid peak (cm) + length of ester peak (cm))}} \right)$$

Typical IR spectra for the original copolymer and after 30 days of degradation are shown in Figure 5. The stereocomplex containing dexamethasone (10% wt) degraded much faster, probably due to the water solubility of dexamethasone phosphate which increased the surface area of the stereocomplex.

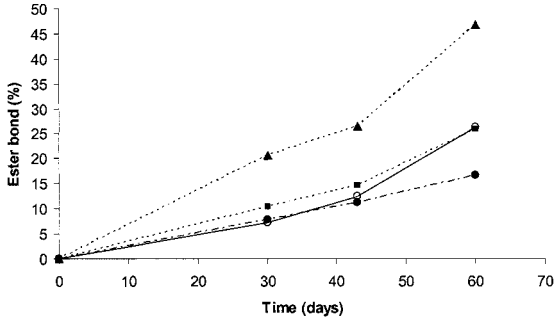


Figure 4. Hydrolysis of copolymers monitored by ester bond degradation. Hydrolysis was conducted in phosphate buffer pH 7.4 at 37°C. Polymer 1(○), polymer 2 (●), stereocomplex I (■) and stereocomplex I loaded with 10% w/w dexamethasone phosphate (▲).

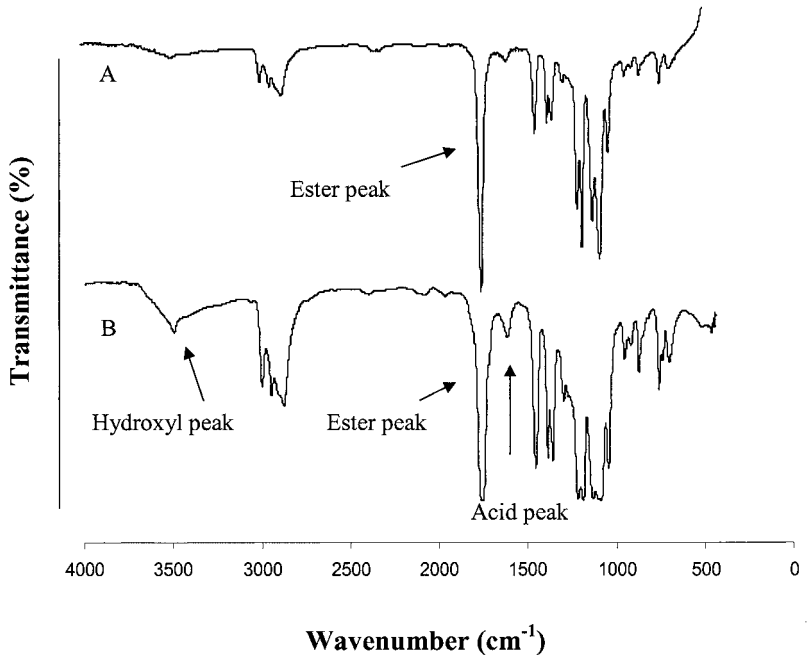


Figure 5. IR-spectra; copolymer 1 (A) and copolymer after 30 days degradation (B).

The number average molecular weight of the degraded enantiomeric polymer samples was monitored by GPC (Figure 6). Stereocomplexes were not monitored due to their insolubility in chloroform. A gradual degradation phase was observed during the first month, followed by a slow degradation phase which kept the number average molecular weight of the sample at 3000 Da for the following month (Figure 6).

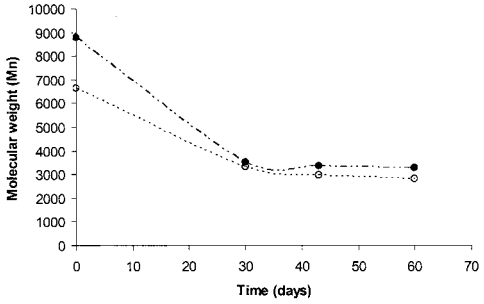


Figure 6. Molecular weight decrease during hydrolytic degradation. Average molecular weight determined by GPC; Polymer 1(○), polymer 2 (●).

Lactic acid release from the copolymers and stereocomplexes was monitored by HPLC chromatography (Figure 7). Stereocomplexes with small particle size and large surface area degraded faster. The dexamethasone-containing stereocomplex degraded faster, probably due to the increase in surface area. The enantiomeric polymers with a large particle size and low surface area degraded slower.

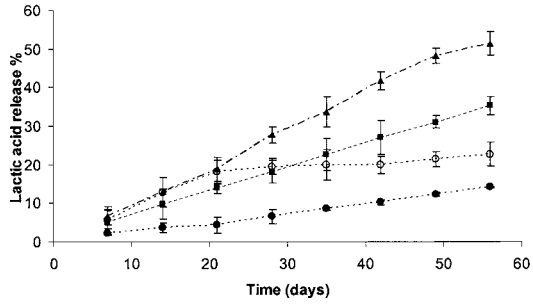


Figure 7. Lactic acid release from copolymers and stereocomplexes during hydrolysis. Lactic acid release was determined by HPLC. Polymer 1 (○), polymer 2 (●), stereocomplex I (■), and stereocomplex I containing 10 % w/w dexamethasone phosphate (▲).

The stereocomplexes had a faster rate of degradation, as shown by IR and lactic acid release, than their copolymers. This might be caused by the presence of more amorphous regions in the former, and degradation due to greater water accessibility[18]. This caused cleavage sites in the polymer chains of the stereocomplexes from those triblocks copolymers, resulting in significantly faster degradation.

In-Vitro Drug Release From the Stereocomplex-Formulation

In vitro release of dexamethasone from the stereocomplexes I and V was analyzed by HPLC (Figure 8). The encapsulation yield of drug was 77 and 84% (Table 3). Dexamethasone phosphate, a water soluble drug, was released over a period of 30 days from stereocomplex particles. PLA-PEG_{2000}-PLA with higher % PLA content (complex V) released the drug at a slower rate (Figure 8).

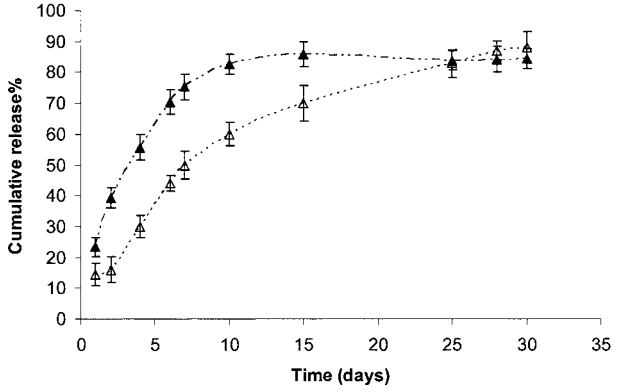


Figure 8. In vitro release of dexamethasone from stereocomplexes I (▲) and V (Δ). (See Table 3). Dexamethasone release was conducted in phosphate buffer pH 7.4 at 37° and the concentration of the drug was determined by HPLC.

In summary, PLA and the triblock copolymer PLA-PEG_{2000}-PLA spontaneously formed stereocomplexes from solution, and exhibited higher melting temperatures than those of the optically pure polymers. The resultant stereocomplexes were used to encapsulate dexamethasone phosphate in high yield. Dexamethasone phosphate was constantly released for a few weeks whereas the polymers were degraded for over two months. The incorporation of water soluble dexamethasone phosphate enhanced the degradation of the stereocomplex.

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