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ORIGINAL CONTRIBUTION

Novel pentablock copolymer (PLA–PCL–PEG–PCL–PLA) -based nanoparticles for controlled drug delivery: effect of copolymer compositions on the crystallinity of copolymers and in vitro drug release profile from nanoparticles

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Abstract The purpose of this investigation was to design novel pentablock copolymers (polylactide–polycaprolactone–polyethylene glycol–polycaprolactone–polylactide) (PLA–PCL–PEG–PCL–PLA) to prepare nanoparticle formulations which provide continuous delivery of steroids over a longer duration with minimal burst effect. Another purpose was to evaluate the effect of poly(L-lactide) (PLLA) and poly(D,L-lactide) (PDLLA) incorporation on crystallinity of pentablock copolymers and in vitro release profile of triamcinolone acetonide (selected as model drug) from nanoparticles. PLA–PCL–PEG–PCL–PLA copolymers with different block ratio of PCL/PLA segment were synthesized. Release of triamcinolone acetonide from nanoparticles was significantly affected by crystallinity of the copolymers. Burst release of triamcinolone acetonide from nanoparticles was significantly minimized with incorporation of proper ratio of PDLLA in the existing triblock (PCL–PEG–PCL) copolymer. Moreover, pentablock copolymer-based nanoparticles exhibited continuous release of triamcinolone acetonide. Pentablock copolymer-based nanoparticles can be utilized to achieve continuous near–zero-order delivery of corticosteroids from nanoparticles without any burst effect.

Keywords Block copolymers · Nanotechnology · Corticosteroids · Drug delivery

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Introduction

Chronic posterior-segment diseases such as macular edema, diabetic retinopathy, age-related macular degeneration, and retinovascular diseases require sustained levels of corticosteroids [1]. Direct intravitreal injections provide therapeutic levels in ocular tissues and avoid systemic toxicity [2, 3]. However, high bolus dose and repeated intravitreal injections are required to maintain therapeutic concentrations. The common side effects associated with frequent intravitreal corticosteroid injections are development of endophthalmitis, blurred vision, increase in intraocular pressure, cataract formation, and increased risk of retinal detachment [4]. Moreover, a high dose of steroids may cause retinal toxicity [5]. To overcome these problems and to avoid direct tissue exposure of high concentration of steroids, encapsulation of the drug molecules in polymeric nanoparticles may be an ideal strategy. In this regard, biodegradable polymeric nanoparticles hold significant promise. Unlike implants, biodegradable polymeric nanoparticles do not require surgical removal and thus may avoid autoimmune responses and disorganization of ocular tissues. Biodegradable nanoparticles with appropriate size and narrow size distribution can provide adequate ocular bioavailability [6]. In the last decade, numerous investigators evaluated the significance of nanoparticles for ocular drug delivery [7–9]. Biodegradable polymers such as poly(DL-glycolide-co-lactide) (PLGA) [10], poly(lactide) (PLA) [11], and poly(caprolactone) (PCL) [12] are widely studied for the preparation of nanoparticles. Particularly, micelle-like nanoparticles having amphiphillic block with polyethylene glycol (PEG) such as PLA–PEG [13], PCL–PEG [14], PCL–PEG–PLA [15], PLGA–PEG [16], and PCL–PEG–PCL [17] have gained attention in controlled drug delivery. In these block copolymers,

PEG block forms the outer shell of nanoparticles whereas PCL or PLA, due their hydrophobic nature, forms nanoparticle core. PEG is well known for its non-antigenic and non-immunogenic nature [18, 19]. In addition, because of its hydrophilic nature, it facilitates the diffusion of water into nanoparticle matrix and provides diffusion-mediated drug release from nanoparticles. PCL is hydrophobic biodegradable polyester which enables high permeability for small molecules [20, 21]. Being hydrophobic in nature, it also provides good encapsulation efficiency to lipophilic drugs via hydrophobic interactions. However, it exhibits very slow degradation because of its hydrophobic and crystalline nature.

PCL- and PEG-based triblock copolymers (i.e., PCL–PEG–PCL or PEG–PCL–PEG) are widely explored for drug delivery. In attempt to sustain the drug release profile, investigators generally increase the molecular weight of PCL block. However, high-molecular-weight PCL block increases the overall crystallinity and hydrophobicity of the polymer. Nanoparticles prepared from such triblock polymers have limitations of initial burst release due to higher crystallinity of PCL block [17, 22]. In addition, lipophilic drugs often get trapped in the hydrophobic core of the nanoparticles and achieve no or limited release in the later time intervals. Therefore, there is an unmet need of optimized block copolymers that can provide continuous delivery of corticosteroids for longer duration with minimal burst release. Literature suggests that crystallinity of PCL can be modulated by conjugating with PLA segment [23, 24]. In vitro drug release profile can be optimized by adjusting the block length ratio of PCL to PLA and may be further optimized by changing the molecular weight of each polymeric block. Considering these facts, we have developed novel pentablock copolymers (PLA–PCL–PEG–PCL–PLA) to achieve sustained delivery of steroids from nanoparticles. These pentablock polymers have unique block arrangement, block ratio, and molecular weight, which can influence the drug release profile of highly hydrophobic molecules. There is no report published till now which suggest how pentablock copolymers can be explored to optimize the release profile of a steroid molecule from nanoparticle formulation by changing crystallinity.

Triamcinolone acetonide was selected as a model corticosteroid to evaluate the potential of PLA–PCL–PEG–PCL–PLA-based nanoparticles as carrier for hydrophobic steroids. Triamcinolone acetonide is a water-insoluble synthetic corticosteroid with longer intravitreal half-life compared with other steroids [25]. It is very effective in the treatment of macular edema and is also being used off-label for the treatment of many sight-threatening ocular disorders such as age-related macular degeneration and vitreoretinopathy [1, 26]. Marketed formulations of triamcinolone acetonide are available in the form of injectable suspension and require repeated administrations to maintain therapeutic levels at the target sites [1, 26]. Therefore, there is a need for sustained-release drug delivery system for lipophilic drugs such as triamcinolone acetonide, which can continuously provide therapeutic levels in ocular tissues over longer periods and avoid repeated administrations [27–28].

We believe that novel pentablock copolymers can be utilized for the sustained delivery of corticosteroids. We conducted proof-of-concept studies to support our hypothesis. Different compositions of pentablock copolymers were synthesized and characterized for nanoparticle preparation. Molecular weight and molecular weight distribution of copolymer were determined by ^{1}H nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). The effect of copolymer compositions on crystallinity of polymers were evaluated by X-ray diffraction analysis (XRD) and differential scanning calorimetry (DSC) analysis. Nanoparticles were characterized for drug entrapment efficiency, size, and surface charge. Moreover, effect of copolymer compositions on triamcinolone release profile was determined.

Materials and methods

Material

Triamcinolone acetonide, PEG (Mw, 2 kDa), ε-caprolactone, stannous octoate, poly(vinyl alcohol) (PVA) were purchased from Sigma Aldrich company (St. Louis, MO, USA). D,L-lactide and L-lactide were purchased from Acros Organics (Morris Plains, NJ, USA). All other chemicals purchased were of analytical grade and used as received.

Synthesis of pentablock copolymers

Pentablock copolymers were synthesized in two steps. In the first step, triblock copolymer PCL–PEG–PCL was synthesized by ring opening polymerization of ε-caprolactone [17]. PEG of Mw=2,000 was selected as initiator, and stannous octoate was added as catalyst. After purification, a predetermined amount of PCL–PEG–PCL was used as initiator for the synthesis of D,L-lactide- or L-lactide-containing pentablock copolymers (PLA–PCL–PEG–PCL–PLA).

Briefly, for synthesis of triblock copolymer, PEG (Mw=2,000) was dried under vacuum for 3 h before copolymerization. Calculated amounts of PEG (0.001 mol), ε-caprolactone (0.001 mol), and stannous octoate (0.5 wt%) were added in the round-bottom flask and degassed for 30 min. Then the flask was purged with nitrogen, and the reaction was performed for 24 h at 130 °C. The resulting crude product was dissolved in methylene chloride and precipitated with cold petroleum ether to remove un-reacted monomers. The precipitated polymer was filtered and vacuum-dried for 24 h.

For the synthesis of pentablock copolymer, purified PCL–PEG–PCL was utilized as initiator and copolymerized

with respective amount of L-lactide. Calculated amount of triblock copolymer and L-lactide (0.001 mol) monomer were added in the round-bottom flask, and stannous octoate (0.5 wt %) was added as a catalyst. Then, the flask was purged with nitrogen, and a reaction was performed for 24 h at 130 °C. The final product was again purified by dissolving in methylene chloride followed by precipitation with cold petroleum ether, and the precipitate was vacuum-dried for 24 h. A similar procedure was followed for the synthesis of different compositions of pentablock copolymer.

Characterization of copolymers

NMR

^{1}H NMR spectroscopy was performed to characterize copolymer compositions. Spectra were recorded with a Varian-400 NMR instrument by dissolving polymeric material in deuterated chloroform ($CDCl_3$).

Gel permeation chromatography (GPC) analysis

GPC analysis was performed with the Shimadzu refractive index detector to determine molecular weight and its distribution. The 1.5-mg polymeric material was dissolved in 1.5 ml tetrahydrofuran (THF). Polystyrene standards of different molecular weights were utilized as reference. THF was used as eluting solvent at a flow rate of 1 ml/min, and Styragel HR-3 column, maintained at 35 °C, was utilized for separation.

Fourier-transform infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) spectra were recorded with a Nicolet-100 infrared spectrophotometer at a resolution of 8 cm^{-1}. Polymer was dissolved in methylene chloride, and film was casted on KBr plates.

X-ray diffraction analysis of copolymers

XRD analysis was performed for triblock and pentablock copolymers, by MiniFlex automated X-ray diffractometer with Ni-filtered Cu-Kα radiation (30 kV and 15 mA) at room temperature at the scanning rate of 5°/min.

Differential scanning calorimetry (DSC) analysis of copolymers

DSC analysis was performed for tri- and pentablock copolymers by Perkin Elmer, Diamond DSC thermal analyzer. Approximately 5 mg sample mass of each copolymer was taken. DSC heating and cooling run were performed at the rate of 5 °C/min. Samples were heated till 100 °C and kept for 2 min at that temperature. Then samples were cooled down to 10 °C and followed by a second heating till 100 °C with the same rate.

Preparation of drug-loaded nanoparticles

Triamcinolone acetonide-loaded nanoparticles were prepared as per a previous reported method with minor modifications [14]. Concisely, single oil/water (o/w) emulsion solvent evaporation method was used to prepare triblock and pentablock nanoparticles. Single o/w emulsion solvent evaporation method is mostly utilized for encapsulation of hydrophobic drugs [29]. Copolymer (100 mg) and drug (10 mg) were dissolved in 1 ml methylene chloride at room temperature. The organic phase containing drug and copolymer was emulsified in an aqueous solution of 2 wt% PVA (surfactant) by sonication for 5 min at 65 W to obtain o/w emulsion. The organic solvent was evaporated by continuous stirring for 2 h at room temperature to generate nanoparticles. The resulting nanoparticle suspension was then centrifuged at 21,000 rpm, 4 °C for 1 h. The prepared nanoparticles were washed three times with distilled water to remove un-entrapped drug and excess PVA. Nanoparticle suspensions were lyophilized with 5 wt% mannitol (act as lyoprotectant) for 24 h and stored at 4 °C for further studies.

Characterization of nanoparticles

Size and zeta potential determination of nanoparticles

The mean diameter and size distribution of nanoparticles were determined by dynamic light scattering with a 90 Plus Particle Size Analyzer (Brookhaven Instrument Corporation). Nanoparticle suspension was approximately diluted with distilled water, and analysis was performed for 3 min at 15 °C and 90° scattering angle. All measurements were made in triplicate, and average particle size and size distribution data were reported±SD. Zeta potential of nanoparticles was determined by Nano-ZS Malvern Zeta sizer. All analyses were performed at 25 °C. Values were reported as mean value±SD of three test runs.

Drug loading and entrapment efficiency

Drug-loaded freeze-dried nanoparticles were employed for the determination of drug loading and entrapment efficiency. Briefly, 5 mg of nanoparticles were dissolved in 0.5 ml of dimethyl sulfoxide and diluted approximately with deionized water. Total amount of drug in solution was analyzed by high-performance liquid chromatography (HPLC) instrument at a wavelength of 240 nm. The mobile phase containing acetonitrile/water (40/60) mixture was used as eluent solvent at the rate of 1 mL/min, and C18 reverse phase column was utilized for separation [30]. Drug loading and

entrapment efficiency of nanoparticles were determined by the Eqs. 1 and 2:

$$\text{Drug loading} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of polymer} + \text{drug}} \times 100 \quad (1)$$

$$\text{Entrapment Efficiency} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of of feeding drug}} \times 100 \quad (2)$$

In vitro release studies of triamcinolone acetonide-loaded nanoparticles

Drug-loaded nanoparticles (corresponding to 0.5 mg triamcinolone acetonide) were suspended in 500 μl 10 mM phosphate buffer saline (PBS) of pH 7.4, and this nanosuspension was placed in a dialysis bag with molecular weight cutoff of 6,500 Da. (Sigma Aldrich, MO, USA). The dialysis bag was immersed in tube containing 10 ml of 10 mM PBS (pH 7.4) at 37 °C with continuous shaking (60 rpm). At pre-determined time intervals, samples were withdrawn, and the entire release medium was replaced with fresh buffer to maintain sink conditions. The amount of triamcinolone acetonide released at each time point was determined by HPLC analysis as mentioned above. The experiments were repeated three times, and mean value±SD was expressed as cumulative percent drug released with time.

Drug release kinetics

Drug release parameters were computed by two different methods utilizing Higuchi and Korsmeyer equations. Drug release mechanism was evaluated by model equations as described below.

Higuchi equation:

$$M_t = K_H t^{1/2} \quad (3)$$

K_H indicates the Higuchi release rate constant obtained by plotting cumulative percent drug released against the square root of time.

Korsmeyer–Peppas equation:

$$Mt/M\infty = kt^n \quad (4)$$

Mt and $M\infty$ denote the cumulative amount of drug released at time t and at the equilibrium, respectively. The constant k represents the kinetic constant and is obtained by plotting logarithmic values of cumulative percent drug release versus logarithmic values of time. The release exponent n represents the drug release mechanism. Values of $n<0.5$ indicate Fickian (ideal) diffusion mechanism, and values of $0.5<n<1.0$ suggest non-Fickian diffusion. When value of n is greater than 1.0, it represents case II transport or zero-order release kinetics [21].

Statistical analysis

All release studies were performed in triplicate. The results were reported as mean±standard deviation. Statistical analysis of the effect of copolymer compositions on triamcinolone acetonide release profile from nanoparticles were compared by one-way ANOVA. Statistical package for social science (SPSS) version 11 was applied to compare mean of each group. A level of $P<0.05$ was considered statistically significant in all cases.

Results and discussion

Polymer synthesis and characterization

Pentablock copolymers were synthesized by sequential ring opening polymerization of ε-caprolactone and L-lactide or

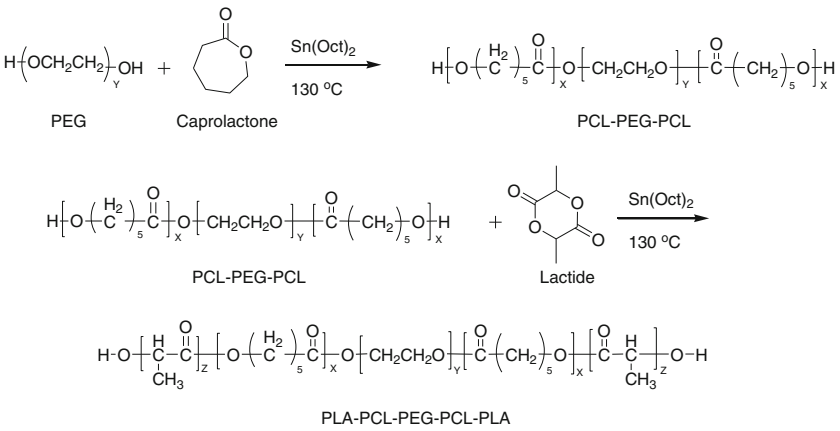

Fig. 1 Synthetic scheme of PLA–PCL–PEG–PCL–PLA

Table 1 Characterization of copolymers

Code	Copolymers	PEG/PCL/PLA ratio	Mn[a]	Mn[b]	Mw[b]	PDI[b]
P-1	PCL–PEG–PCL	1/2.5/0	7,105	4,083	6,080	1.63
P-2	PCL–PEG–PCL	1/5/0	12,450	8,975	11,630	1.50
P-3	PLLA–PCL–PEG–PCL–PLLA	1/5/2.5	19,072	10,964	16,260	1.48
P-4	PLLA–PCL–PEG–PCL–PLLA	1/2.5/2.5	11,062	6,186	9,526	1.54
P-5	PDLLA–PCL–PEG–PCL–PDLLA	1/5/2.5	18,803	11,739	16,246	1.38
P-6	PDLLA–PCL–PEG–PCL–PDLLA	1/2.5/2.5	11,665	6,165	10,160	1.64

[a]Values calculated from ^{1}H NMR spectra

[b]Values obtained by GPC analysis

D,L-lactide. In the first step, triblock copolymers with different ratios of PEG/PCL were synthesized by ring opening polymerization of ε-caprolactone in the presence of PEG and a small amount of stannous octoate [17]. In the second step, these triblock copolymers served as initiators for the synthesis of pentablock (PLA–PCL–PEG–PCL–PLA) copolymers by ring opening polymerization of D,L-lactide or L-lactide (Fig. 1). Different compositions of pentablock copolymers were synthesized by changing the molar ratios of PEG, ε-caprolactone, and L-lactide or D,L-lactide, as listed in Table 1. We synthesized a series of copolymers with different molecular weights by changing the hydrophobic segment block length of amphiphillic block copolymers. We attempted to modulate the crystallinity of copolymers by changing the PCL/PLA block length ratio. In this study, we also wanted to evaluate the effect of hydrophobic segment of copolymer on the release profile of a hydrophobic drug.

PEG of molecular weight 2,000 with two hydroxyl terminals was utilized for the synthesis of tri- and pentablock copolymers. Polymers were characterized for molecular weight and molecular weight distribution by ^{1}H NMR and GPC analysis. Figure 2 shows the ^{1}H NMR spectrum of PLLA–PCL–PEG–PCL–PLLA in $CDCl_3$. Typical signals of PEG, PCL, and PLA components were utilized to calculate the molecular weight of copolymers. Signal at 3.65 ppm (−CH2CH2–) was assigned to PEG block. Signals at 1.28, 1.6, 2.3, and 4.09 ppm were assigned to different methylene protons (−CH2–) of PCL blocks, and signals at 1.4 (−CH3) and 5.19 ppm (−CH) were assigned to PLA block. The molar ratio of PEG/PCL/PLA was determined by integrating peak intensities of methylene protons from PEG block at 3.65 ppm, PCL block at 4.09 ppm, and to PLA block at 5.10 ppm. The number average molecular weight (Mn[a]) of copolymers was calculated as per the previously reported equations [31]. The Mn[b] and Mw (weight average molecular weight) values obtained by GPC analysis are summarized in Table 1. The Mn values of copolymers determined by GPC analysis were lower than the Mn values calculated from ^{1}H NMR analysis. This

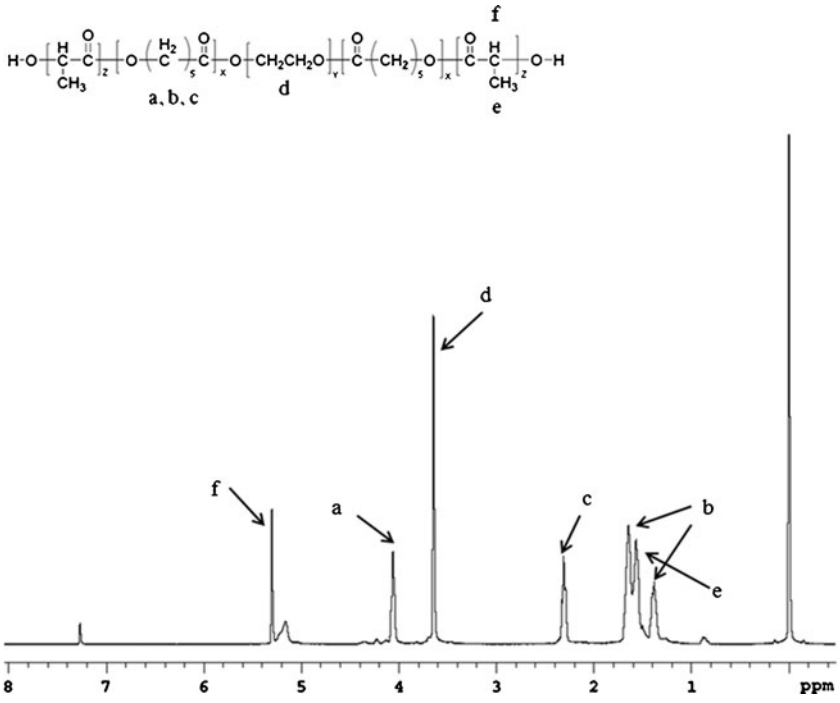

Fig. 2 ^{1}H NMR spectra of PLA–PCL–PEG–PCL–PLA copolymer in $CDCl_3$

result was attributed to the change in hydrodynamic volume of block copolymers as compared with parent homopolymers [31].

Figure 3 depicts FT-IR spectra of P-2, P-3, and P-5 block copolymers. On the spectrum of PCL–PEG–PCL (P-2) (Fig. 3a), band for C=O stretching appeared at 1,732 cm^{-1} and bands for C–H stretching appeared at 2,941 and 2,860 cm^{-1} for PCL block. Absorption band at 1,140 cm^{-1} appeared because of C–O–C stretching vibrations of the repeated OCH2CH2 units of PEG, and band at 1,279 cm^{-1} was attributed to the –COO– stretching vibrations [14]. For copolymers P-3 (Fig. 3b) and P-5 (Fig. 3c), another C=O stretching band was observed at 1,757 cm^{-1}, which can be attributed to PLA block.

To modulate the crystallinity of PCL-based triblock copolymers (i.e., PCL–PEG–PCL), PLA block was introduced in the pentablock copolymers [24]. Earlier reports suggest that, by changing the hydrophobic/hydrophilic constituents, crystallinity and degradation of the copolymers can be modulated [22, 32–35]. However, not much information is available in the literature suggesting the effect of copolymer crystallinity on drug release profile from nanoparticles. Therefore, we synthesized four compositions of pentablock copolymers with different ratios of PCL/PLA in the hydrophobic segments. We also evaluated the effect of different isomeric forms of PLA block on the crystallinity of copolymers. Poly(L-lactide) (PLLA) is a semicrystalline polymer where as poly(D,L-lactide) (PDLLA) has an amorphous structure [36]. Both polymers may act differently in reducing the crystallinity of PCL block, which eventually may affect drug release profile. The X-ray diffractograms were obtained to evaluate the crystallinity of different triblock and pentablock copolymers (Fig. 4). For triblock copolymer (P-1 and P-2), diffraction pattern exhibited two characteristic crystalline peaks of PCL block at $2\theta=21.5°$ and 23.8°. Poly(L-lactide)-containing pentablock copolymer, P-4, showed less intense peaks for PCL and two another crystalline peaks at $2\theta=16.5°$ and 19.05° for PLA block. These peaks suggest that incorporation of PLLA in the copolymers has reduced the crystallinity of PCL, and because of its semicrystalline nature, PLLA exhibited its own crystalline peaks. However, P-6 represented a wide amorphous peak, indicating amorphous state of PDLLA-containing pentablock copolymer. This result suggested that PDLLA exhibited significant effect on reducing crystallinity of PCL in comparison to PLLA. However, there is no significant effect on crystalline peak of PCL in the case of P-3 and P-5 copolymers compared with P-2 polymer having same molecular weight of PCL. The PCL/PLA block length ratio in the case of P-3 and P-5 copolymers was higher as compared with P-4 and P-6, respectively. The ratio of PCL/PLA in the case of P-4 and P-6 is 1/1 whereas in the case of P-3 and P-5 the ratio is 2/1. Therefore, effect of PLA in reducing the PCL crystallinity was not as intense for P-3 and P-5 copolymers as observed in the case of P-4 and P-6 copolymers. From the XRD results, it can be concluded that a proper ratio of PCL/PLA in the copolymer compositions can significantly alter the crystallinity of PCL.

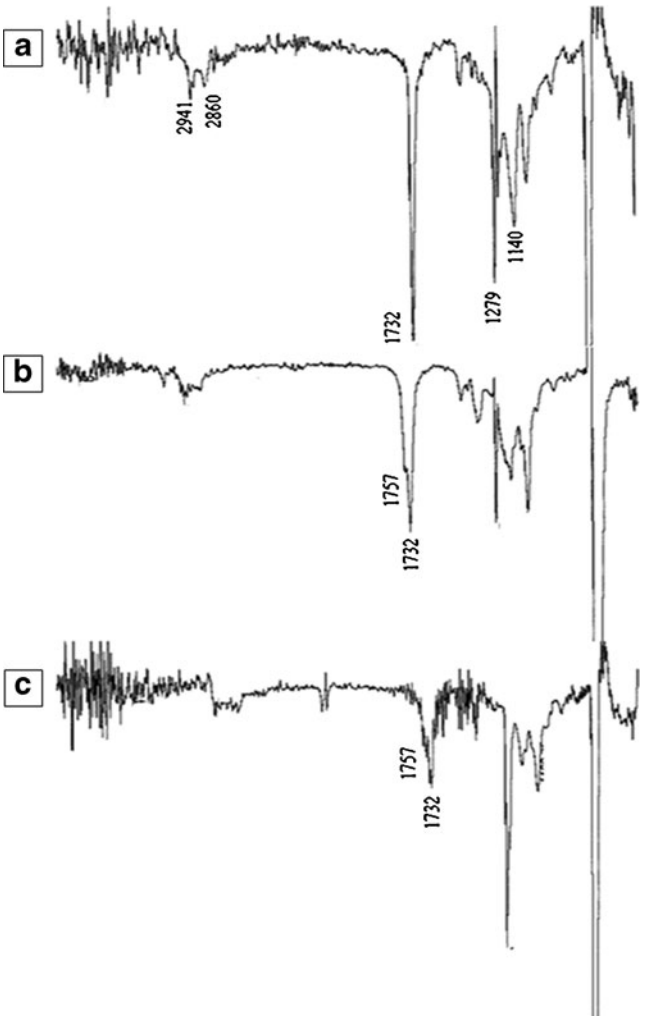

Fig. 3 FT-IR spectra of copolymers **a** P-2, **b** P-3, **c** P-5

Figure 5a shows DSC results of first heating scan, and Fig. 5b shows the DSC scan of second heating cycle of all prepared copolymers. The endotherms located in between 30 °C and 60 °C correspond to the melting of PCL component in the copolymers [31]. The melting points of PCL in pentablock copolymers were clearly decreased with incorporation of PLLA or PDLLA in the polymer, suggesting the reduction in crystallinity of PCL block in different pentablock copolymers. For instance, in the case of P-1, PCL melting point was 50.3 °C whereas, for P-4 it, was 45.8 °C and, for P-6, it was 44.8 °C. This finding suggests that PLA block has reduced the crystallization ability of PCL block by limiting the chain mobility. Melting point of PDLLA-

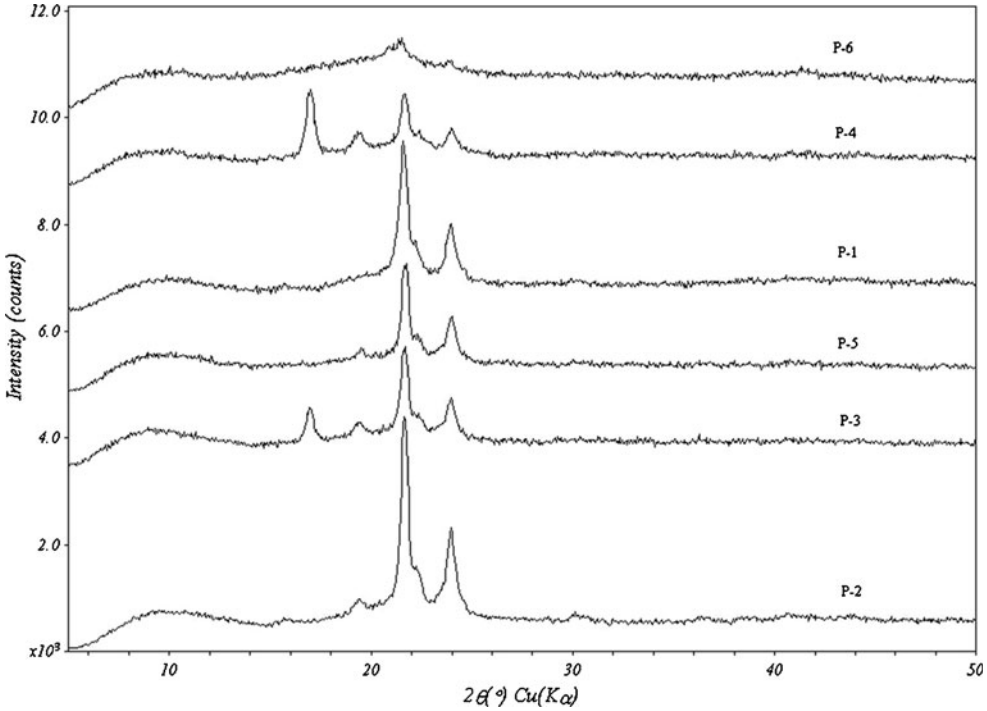

Fig. 4 X-ray diffraction diagrams of polymer temperatures (°C)

containing copolymer (P-6) is further lowered than PLLA-containing copolymer (P-4). These results describe that PDLLA has more pronounced effect in reducing the crystallinity of PCL block compared with PLLA. These results were in agreement with our XRD results. PDLLA contains random mixture of L-lactide and D-lactide and interrupts the crystallization of PCL more significantly than PLLA. Also, second heating scan shows similar decrease in PCL melting

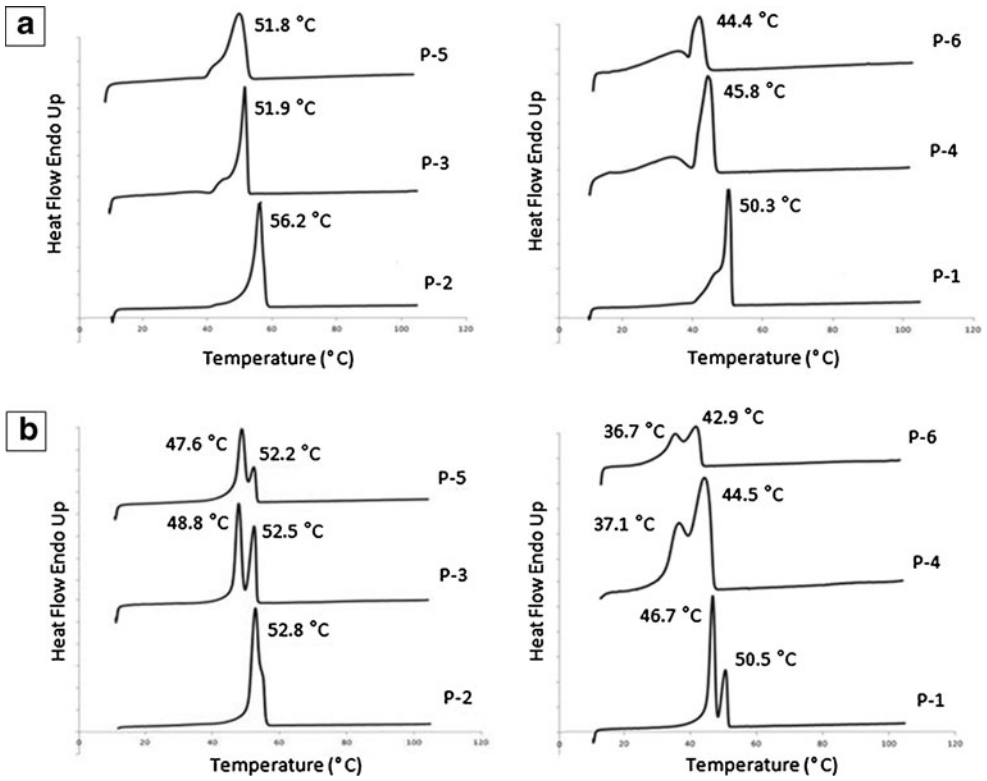

Fig. 5 DSC thermograms of polymers **a** first heating, **b** second heating

points of pentablock copolymer in comparison to triblock copolymers. It is interesting to observe that second heating cycle shows two melting peaks for PCL. The second melting peaks were attributed to the imperfect crystallization of PCL chains during cooling and second heating process [37]. Our DSC results further confirmed that crystallization ability of PCL in copolymer compositions was altered by incorporation of PLLA or PDLLA. Hydrolytic degradation of polymers depends on the crystallinity. Amorphous polymers degrade at a faster rate than the crystalline polymers [36]. Earlier reports suggest that the higher the PLA content in PCL–PLA block copolymers or PCL/PLA blends, the lower the crystallinity of PCL [37, 38]. Also, degradation rate of block copolymer was faster in comparison to PCL and PLA homopolymers [39]. In this study, two feed ratios of PCL/PLA, i.e., 2/1 and 1/1, were selected for the synthesis of PDLLA- and PLLA-containing pentablock copolymers in which PLA content was more than 50 % in each prepared copolymer. We attempted to modify the crystallinity of triblock copolymers by incorporating PLA segment. We observed that more reduction in the crystallinity of PCL upon polymerization with D,L-lactide in comparison the L-lactide. It would be more interesting to observe the effect of crystallinity on drug release profile. Therefore, we utilized all synthesized copolymers for preparation of triamcinolone acetonide-loaded nanoparticles. Crystallinity of PCL has also affected release rate of triamcinolone acetonide from nanoparticles that is discussed in another section.

Preparation and characterization of nanoparticles

Particle size and zeta potential

Table 2 summarizes the properties of each triblock and pentablock copolymers nanoparticles prepared by single o/w emulsion solvent evaporation method. There was not much difference on size of nanoparticles prepared from different copolymer compositions. Nanoparticles were in the size range of 229–310 nm and with polydispersity in the range of 0.237–0.322. Figure 6 shows the unimodel particle size distribution for nanoparticles prepared from P-4 polymer. Zeta potential of prepared nanoparticles sample was also listed in Table 2. It appears that nanoparticles have very high negative zeta potential, indicating stable nature of the particles in water due to repulsion [40].

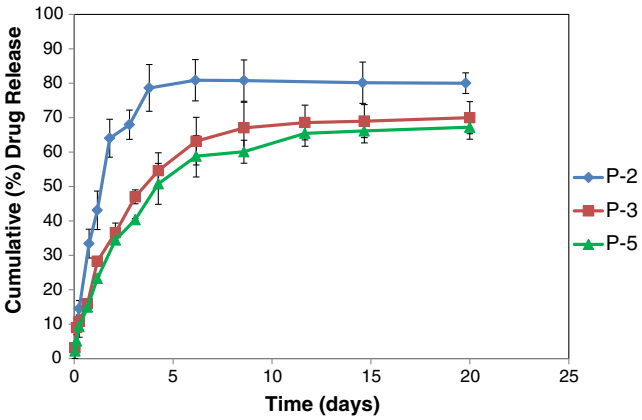

Fig. 6 Release of triamcinolone acetonide from P-2, P-3, and P-5 copolymer nanoparticles in PBS buffer (pH 7.4) at 37 °C. The values are represented as mean±standard deviation of $n=3$

Drug loading content and entrapment efficiency

Drug loading capacity and entrapment efficiency of nanoparticles mainly depended on copolymer compositions. Since triamcinolone acetonide has very low aqueous solubility, it precipitates in water during fabrication of nanoparticles that resulted in higher drug loading in all nanoparticle batches. As shown in Table 2, molecular weight of hydrophobic segment of the copolymer affected drug loading and percentage entrapment efficiency of nanoparticles. Higher drug loading in P-3 and P-5 in comparison to P-4 and P-6, respectively, could be attributed to longer chain length of PCL segment that provides more hydrophobicity to the polymers. These results suggest that nanoparticles drug loading can be increased by increasing the molecular weight of hydrophobic segment of copolymers. However, triamcinolone acetonide is very hydrophobic in nature, and higher-molecular-weight PCL segment would not provide ideal drug release rate. Therefore, we believe that P-4 and P-6 are better polymeric compositions for delivery of hydrophobic corticosteroids such as triamcinolone acetonide, which provided near–zero-order drug release profile (discussed in detail in the next section).

Table 2 Characterization of nanoparticles

Polymer	Size (nm)	PDI	EE	Drug loading	Zeta potential
P-2	229±2	0.294	74.4±2.4 %	6.7±1.2 %	−26.4±4.5
P-3	279±2	0.257	76.8±3.5 %	6.5±0.6 %	−34.2±6.0
P-4	272±12	0.237	63.1±2.7 %	5.7±0.3 %	−29.2±3.9
P-5	310±14	0.286	80.6±2.9 %	7.7±0.2 %	−29.9±4.8
P-6	281±8	0.322	68.3±2.5 %	6.2±0.3 %	−30.3±4.8

In vitro drug release study

Figures 6 and 7 show the comparison of triamcinolone acetonide release profile from triblock (P-2) and different pentablock copolymer-based nanoparticles. Pentablock copolymers with different block ratios of PCL/PLA were used to prepare nanoparticles. In vitro studies revealed that drug release from nanoparticles depended on hydrophobic segment block length and crystallinity of the copolymers. Pentablock copolymer-based nanoparticles exhibited lower burst release in the initial time points in comparison to triblock nanoparticles. Triblock-based, P-2, nanoparticles exhibited high burst release and observed almost 64 % drug release in first 2 days, whereas pentablock copolymer, P-3, exhibited almost 36 %, and P-5 exhibited 34 % drug release for the same duration. Similar results were observed by other investigators and can be attributed to the assumption that pentablock copolymer-based nanoparticles have very less surface-adsorbed drug in comparison to triblock copolymer-based nanoparticles [19]. Figure 6 also suggests that, in comparison to triblock copolymer (P-2), drug release rate from both pentablock copolymers (P-3 and P-5) was much slower. Almost 90 % drug got released in 20 days in the case of P-2 copolymer, whereas only 70 % drug got released in almost 20 days in the case of P-3 and P-5 polymers. This observation may be explained by the fact that pentablock copolymers P-3 and P-5 have high molecular weight compared with P-2 copolymer. Although all three copolymers have same PCL block length, P-3 and P-5 have additional PLA segment in their composition, which made their structure more hydrophobic in comparison to triblock copolymers. However, these two polymeric compositions (i.e., P-3 and P-5) did not resolve the problem of no release or very slow drug release

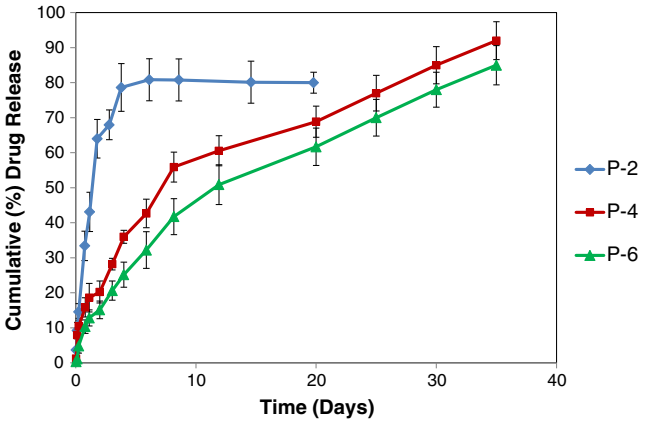

Fig. 7 Release of triamcinolone acetonide from P-2, P-4, and P-6 copolymer nanoparticles in PBS buffer (pH 7.4) at 37 °C. The values are represented as mean±standard deviation of $n=3$

phase in the later time points. As mentioned earlier, lipophilic drugs often get trapped in the hydrophobic core of the nanoparticles and achieve no or limited drug release in the later time intervals [41]. Because of the high molecular weight of PCL block, triamcinolone acetonide observed slow release rate in the later time points in all three copolymers (i.e., P-2, P-3, and P-5), which is not ideal for continuous delivery of drug at the target site. Polymer crystallinity and hydrophobicity play an important role in drug release rate from nanoparticles. Therefore, we tried to optimize the drug release rate by changing the molecular state of the copolymers. For copolymers P-4 and P-6, we decreased the block length of PCL in comparison to P-3 and P-5 (Table 1). The overall block ratio of PCL/PLA was changed from 2/1 to 1/1 in P-4 and P-6 as compared with P-3 and P-5, respectively. Our XRD results also suggested that incorporation of PLLA has reduced the crystallinity of PCL block in P-4 whereas, in the case of P-6, PCL was present in amorphous state due to conjugation of PDLLA. Drug diffusion from amorphous structure takes place at slower rate compared with the pores of crystalline copolymers [42]. Therefore, we observed slow release of triamcinolone acetonide from and P-4 and P-6 (Fig. 7) in comparison to other copolymers. P-4 and P-6 copolymer-based nanoparticles exhibited continuous release of triamcinolone acetonide for 35 days without any slow release phase because of low crystallinity and hydrophobicity of copolymers. Moreover, drug release from P-6 was much slower in comparison to P-4 because of amorphous nature of P-6 copolymer. Initial burst release profile of triamcinolone acetonide was also significantly decreased in P-6 nanoparticles with only 15 % drug released in 2 days in comparison to P-2, which exhibited 64 % drug release from nanoparticles. The overall release rate of triamcinolone acetonide also decreased in the case of P-6 nanoparticles in comparison to all other nanoparticle formulations. Therefore, we were successful in modulating the release profile of hydrophobic drug from pentablock copolymer-based nanoparticles by

Table 3 Kinetic parameters for drug release

Polymer	Higuchi		Korsmeyer–Peppas		
	r^2	k_H (day$^{-1/2}$)	r^2	K_{KP} (day^{-n})	n
P-2	0.711	18.79	0.998	40.70	0.744
P-3	0.891	17.43	0.983	23.57	0.583
P-4	0.974	15.71	0.920	15.97	0.706
P-5	0.913	17.05	0.989	19.27	0.634
P-6	0.996	14.86	0.952	8.22	0.847

changing the copolymer compositions. Also, optimized pentablock copolymer-based, P-6, nanoparticles exhibited continuous release of triamcinolone acetonide without producing any significant burst effect. Therefore, pentablock copolymers are very advantageous to prepare nanoparticle formulations in comparison to existing triblock and other PLGA-based polymers which show very high burst release [43].

Drug release kinetics

Drug release from nanoparticles generally follows diffusion/degradation or a combination of diffusion and degradation-mediated release phenomena [44]. Analysis of drug release kinetics, as shown in Table 3, correlated well with Korsmeyer model. This observation suggested that drug release primarily depended on diffusion from the nanoparticles matrix rather than erosion process of the copolymer. In addition, analysis of first 60 % release data according to Korsmeyer model suggested that release rate of triamcinolone acetonide was slowest from the nanoparticles prepared from P-6 pentablock copolymer. A diffusion exponent value in between $0.5<n<1.0$ suggested anomalous diffusion mechanism from all nanoparticles matrixes.

Conclusion

PLA–PCL–PEG–PCL–PLA pentablock copolymers with different block ratios of PEG, PCL, and PLA were synthesized. Pentablock copolymer crystallinity can be modulated by changing the PCL/PLA ratio as well as copolymer composition. Triamcinolone acetonide nanoparticles were successfully prepared from pentablock copolymers. The copolymer compositions and crystallinity can influence the drug release kinetics from nanoparticles. Copolymer crystallinity had significantly affected the drug release rate from nanoparticles. Nanoparticles prepared from pentablock copolymers had minimized the limitations of existing triblock polymer nanoparticles such as burst release effect and provided sustained release for longer duration. Novel pentablock copolymers are excellent biomaterials that could serve as a vehicle for ocular drug delivery as well as for other disorders where sustained levels of corticosteroids are required.

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