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Preparation and characterization of tri-block poly(lactide)–poly(ethylene glycol)–poly(lactide) nanogels for controlled release of naltrexone

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ABSTRACT

Tri-block poly(lactide)–poly(ethylene glycol)–poly(lactide) (PLA–PEG–PLA) copolymers and related acrylated derivative were synthesized and used to prepare micelles and nanogels for controlled release of naltrexone. The resulting copolymers, micelles and nanogels were characterized by various techniques such as proton nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, gel permeation chromatography, fluorescence spectrometry, differential scanning calorimetry, photon correlation spectroscopy and scanning electron microscopy. The nanogels exhibited high encapsulation efficiency around 60% and excellent stability for long periods of time. The drug release profiles of micelles and nanogels were compared and it was found that the naltrexone loaded nanogels offered a steady and long-term release pattern for different periods of time up to 35 days, depending on the crosslinker concentration, compared to the micelles. The size of nanogels could be manipulated easily in the range of 128–200 nm by variations in polymer concentration used in the nanogels preparation step. From the results obtained it can be concluded that PLA–PEG–PLA nanogels can be considered as a promising carrier for drug delivery purpose.

1. Introduction

Naltrexone is an efficient narcotic antagonist with a remarkable safety profile, thereby widely used in the treatment of narcotic addiction as well as alcohol dependence. It is used mainly as an adjunct to prevent relapse in detoxified opioid-dependent patients. Naltrexone tightly binds to opioid receptors in the brain, thus blocking opioid effects and reinforcement from its use. In most cases, for naltrexone treatment to be effective, it is necessary to provide a sufficient level of the drug concentration for a long period of time. To achieve this goal, there are a number of issues associated with oral administration of naltrexone hydrochloride, known as REVIA™ tablet, including bioavailability and patient compliance which must be overcome. Consequently, considerable interests have been shown in recent years in the development of new drug delivery systems for naltrexone. In this context, time-controlled delivery systems can be regarded as a rational solution for the long term availability of the drug within the host blood circulation. Negishi et al. (1987) have reported a 30-days *in vitro* release period of the drug by covalently coupling of naltrexone to a biodegradable poly(α-amino acid) backbone. Salehi et al. (2009) used a thermosensitive poly(lactide-co-glycolide)-based delivery system for sustained release of naltrexone. However, most attention has been focused on the preparation of injectable polymeric micro and nanoparticles for this purpose (Yin et al., 2002). In April 2006, the U.S. Food and Drug Administration (FDA) approved an extended release injectable microparticles formulation of naltrexone (Vivitrol®) for the treatment of alcohol dependence. However, the idea of using nanoparticulate polymeric drug delivery systems for controlled release of naltrexone is relatively new and there are few studies in this context (Yin et al., 2002).

Block copolymers such as di-block poly(lactide)–poly(ethylene glycol) (PLA–PEG) and tri-block poly(lactide)–poly(ethylene glycol)–poly(lactide) (PLA–PEG–PLA) have been used extensively for drug delivery applications because of their superior biocompatibility, biodegradability and the ability to self assemble in aqueous media to form polymeric micelles with a core–shell structure (He et al., 2007; Panyam and Labhasetwar, 2003; Venkatraman et al., 2005). The drug delivery systems based on polymeric micelles suffer from instability and show very fast drug release behavior (Kim et al., 2010). Therefore, to develop systems with sustained drug release ability the block copolymer micelles need to be stabilized further. One of the preferred ways to reach this goal is to crosslink either the core or the shell of the micelles. In this regards, there are some studies in the literature which in that cases, the

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shell layers of polymeric micelles have been chemically linked to obtain various shell-cross-linked nanoparticles (Joralemon et al., 2005; Ma et al., 2001; Pochan et al., 2004). In another study, polyethylenimine (PEI) was cross-linked in aqueous solution to the micelles of bis-activated Pluronic block copolymer (Vinogradov et al., 2006). Furthermore, Lee et al. (2006) photo cross-linked the core of polymeric micelles of PLA–PEG–PLA end-modified by acrylate group. As a matter of fact, crosslinking the core of the micelle-like nanoparticles of PLA–PEG–PLA triblock copolymers, turns them into nanogels. Nanogels are defined as nanosized networks of chemically or physically cross-linked polymers that can swell in an appropriate solvent (Kabanov and Vinogradov, 2009; Vinogradov et al., 2002). Nanogels with the same core–shell structure as the micelles can easily incorporate both hydrophilic and hydrophobic drugs, with their small size being suitable for site-specified delivery by IV injection. In addition, nanogels due to their crosslinked structure exhibit high stability which makes them more advantageous in application as sustained drug delivery systems. This work sought to fabricate nanogels with crosslinked hydrophobic core and hydrophilic shell with the purpose of obtaining a biocompatible and biodegradable polymeric injectable controlled delivery system capable of timed-release of naltrexone for a long period of time.

In this contribution, the PLA–PEG–PLA with acrylated groups at the PLA termini was used to prepare nanogels and micelles. The nanoparticles were characterized *in vitro* with different techniques. The release profile and the stability of nanogels and micelles were also evaluated.

2. Materials and methods

2.1. Materials

L,D-Lactide [(3S)-cis-3,6-dimetyl-1,4-dioxane-2,5-dione], 98% purity, and stannous 2-ethyl-hexanoate ($SnOct_2$) were from Aldrich and purchased locally. Dihydroxy-terminated poly(ethylene glycol) (PEG), Mn = 2000, 10000 g mol^{-1} (degrees of polymerization 45 and 227, respectively), acryloyl chloride, ethylene glycol dimethacrylate (EGDMA), and 2,2′-azobisisobutyronitrile (AIBN) were all from Merck (Darmstadt, Germany), purchased locally and used as received.

2.2. Synthesis of PLA–PEG–PLA diacrylate copolymer

The PLA–PEG–PLA copolymer was synthesized by a ring-opening polymerization of D,L-lactide with PEG as initial molecule and $Sn(Oct)_2$ as catalyst (Du et al., 1995). Briefly, appropriate amounts of lactide, PEG, and $Sn(Oct)_2$ were heated to 120 °C to start polymerization. After 11 h, the resulting polymer was cooled to room temperature, dissolved in chloroform, and precipitated in diethyl ether. The copolymer was dried under vacuum at room temperature for 24 h before acrylation. The copolymer and triethylamine were dissolved in dry dichloromethane (DCM) at 0 °C. After stirring for 1 h, the mixed solution was treated drop-wise with acryloyl chloride. The reaction was kept under dry nitrogen and in an ice-bath for 12 h, and then was stirred for a further 12 h at room temperature. Afterwards, the precipitates were removed by simple paper filtration and the filtrate was added into iced diethyl ether to precipitate the acrylated copolymer. The obtained materials were further purified by precipitation in hexane from DCM, and finally were dried under vacuum for 24 h. The structure of polymer was determined by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) in $CDCl_3$ at 400 MHz (Bruker, Avance 400) and Fourier transform infrared spectroscopy (FT-IR) (Bruker, Tensor 27) and the average molecular weight and distribution of the PLA–PEG–PLA copolymers were determined by ^{1}H NMR and gel permeation chromatography (GPC) (Knaure, Germany). Differential scanning calorimetry (DSC) (Mettler Toledo, Star SW 9.30) was used for thermal analysis of synthesized copolymers. Samples were heated at a rate of 10 °C min^{-1} and the data were recorded from 0 to 200 °C.

2.3. Preparation polymeric micelles and nanogels

Polymeric micelles were prepared by nanoprecipitation method as described by Fessi et al. (1989) with THF used as the solvent. The procedures were as follows. PLA–PEG–PLA diacrylate copolymer (50 mg), naltrexone (5.0 wt.% based on copolymer), initiator (AIBN, 5.0 wt.% based on copolymer), and different contents of EGDMA were dissolved in 2 ml of tetrahydrofuran (THF). The mixture was then injected drop-wise through a syringe into the determined volume of distillated water under certain mixing rates and stirred magnetically at room temperature until complete evaporation of the organic solvent. Consequently, in aqueous environment, the amphiphilic copolymers self-associated with the evaporation of the organic solvent and the particles were formed.

For nanogels preparation, the suspension containing polymeric micelles was heated up to 70 °C under nitrogen for 24 h for crosslinking reaction. The resulting nanogel suspension was then lyophilized for 48 h to remove all the solvent, and preserved at −20 °C before further measurements.

2.4. Characterization of the micelles and nanogels

2.4.1. Particles morphology

The morphology of nanogels was determined using scanning electron microscopy (SEM) (LEO 1455 VP). The samples were prepared on aluminum stabs and coated with gold prior to examination by SEM.

2.4.2. Determination of particle size and zeta potential

The particle size distribution (mean diameters and polydispersity index) and zeta potential of prepared nanogels were determined by photon correlation spectroscopy (PCS) using a Malvern Nano/zetasizer (Malvern Instruments, UK). The zeta potential variation of nanogels after determined time incubation was used to investigate degradation of PLA blocks of nanogels. Dispersant viscosity was set as 0.8872 cP at 25 °C.

2.4.3. Critical micelle concentration (CMC)

The critical micelle concentration (CMC) of the copolymers was determined by fluorescence spectroscopy using pyrene as fluorescence probe. For fluorescence emission spectra, the excitation wavelength was set at 339 nm, and for excitation spectra, the detection wavelength was set at 390 nm.

2.4.4. Swelling ratio

The size distribution data of nanogels measured immediately after preparation and also after resuspending in deionized water were used for determination of swelling ratio according to the following equation (McAllister et al., 2002):

$$\text{swelling ratio} = \frac{(D_{\text{h aqueous solution}})^3}{(D_{\text{h emulsion solution}})^3}$$

To resuspend, in brief, the defined amount of freeze dried nanogels powder was added into deionized water and was sonicated for 30 min before size measurement.

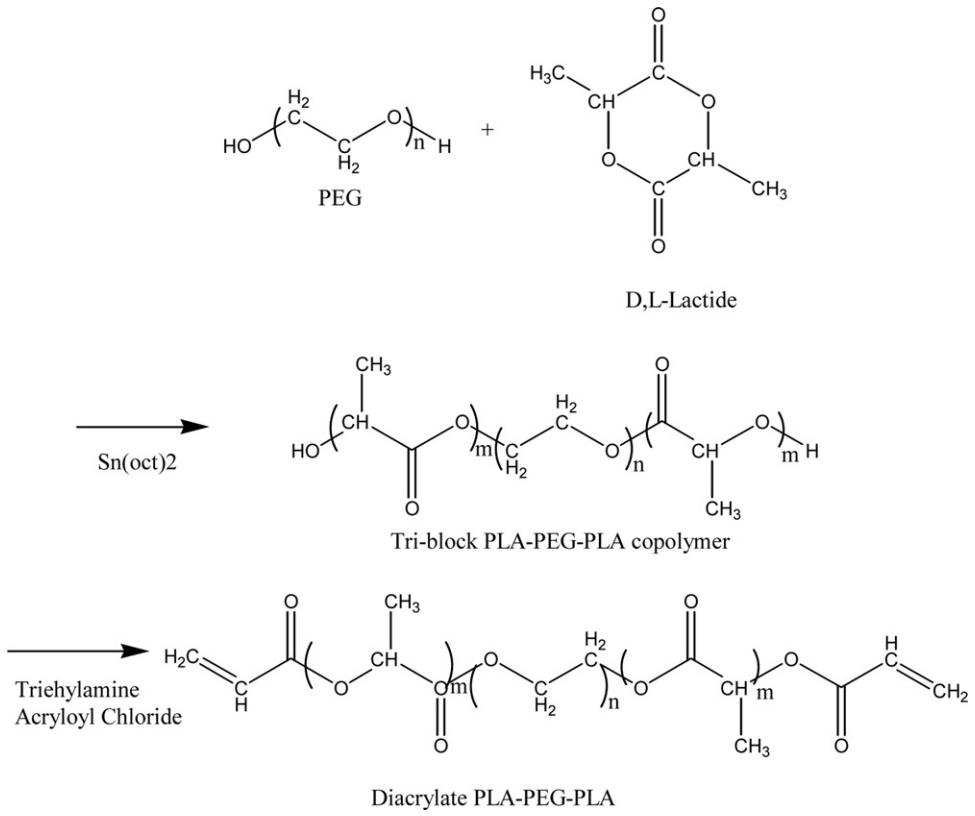

Fig. 1. Schematic synthesis route of PLA–PEG–PLA diacrylate copolymer.

2.4.5. Stability test

The stability of nanogels was investigated by following size variation of suspension of nanogels in aqueous medium. As detail, after preparation of nanogels suspension, the nanogels were incubated over determined time until size measurement.

2.5. Encapsulation ratio

The amount of naltrexone encapsulated in the nanogels was determined as difference between the total amount of naltrexone used and the amount that was not encapsulated (free naltrexone) (Liu et al., 2001). In order to determine the free naltrexone content, the solution containing naltrexone loaded nanogels and free naltrexone was dialyzed with dialysis membrane bag (cut off 2000) against deionized water over 48 h. The concentration of free naltrexone in the dialysis solution was measured by UV spectrophotometry (Biochrom, Biowave S2100) at $\lambda = 281$ nm. The naltrexone encapsulation ratio (ER) of the nanogels was calculated as follows:

$$\mathrm{ER} = \frac{\text{total amount drug} - \text{free amount drug}}{\text{total amount drug}} \times 100\%$$

2.6. Drug release

After separation of free naltrexone and naltrexone loaded in nanogels by dialysis, a 10-ml suspension of naltrexone-loaded nanogels (50 mg) was placed within the dialysis bag and incubated at 37 °C while immersed in 30 ml of phosphate-buffered saline (PBS, pH 7.4) for the course of the release study (Salehi et al., 2009). At scheduled time intervals, 3 ml of the dialysate was taken out and replaced by 3 ml fresh PBS. The concentration of free naltrexone in the medium was determined by UV spectrophotometer. All the release studies were carried out in triplicate. The results were presented in terms of cumulative release as a function of time:

$$\text{Cumulative amount released}\,(\%) = \left(\sum_{t=0}^{t=t} \frac{M_t}{M_0}\right) \times 100$$

where $\sum_{t=0}^{t=t} M_t/M_0$ is the cumulative amount of released naltrexone from the nanogels at time t, and M_0 is the total amount of naltrexone in the nanogel.

3. Results and discussion

3.1. Synthesis and characterization of PLA–PEG–PLA diacrylate copolymer

PLA–PEG–PLA tri-block copolymer was synthesized using the ring-opening polymerization of lactide in the presence of PEG, whose hydroxyl end groups initiate the ring opening (Fig. 1). The structure and composition of the synthesized PLA–PEG–PLA tri-block copolymer was determined by ^{1}H NMR spectroscopy in $CDCl_3$, as shown in Fig. 2(a). The presence of methine (CH) and methyl (CH_3) protons in PLA was observed around 5.15 ppm (tetralet split) (c) and 1.60 ppm (doublet split) (a), respectively. The methylene protons in (CH_2) group of PEG were around 3.63 ppm (triplet split) (b). Furthermore, the ratio of the peak area at 1.6 and 3.6 ppm was indicative of the number of each repeating units and the number-average molecular weight of the synthesized copolymer (Table 1).

Diacrylated PLA–PEG–PLA copolymers were synthesized by treatment of the PLA–PEG–PLA triblock copolymer with acryloyl chloride. In the ^{1}H NMR spectrum shown in Fig. 2(b), there are three different peak groups that represent acryl protons: –COCHC=C– at 6.14–6.21 ppm, –COCHC CH_2 (cis) at 5.86–5.91 ppm, and –COCHC

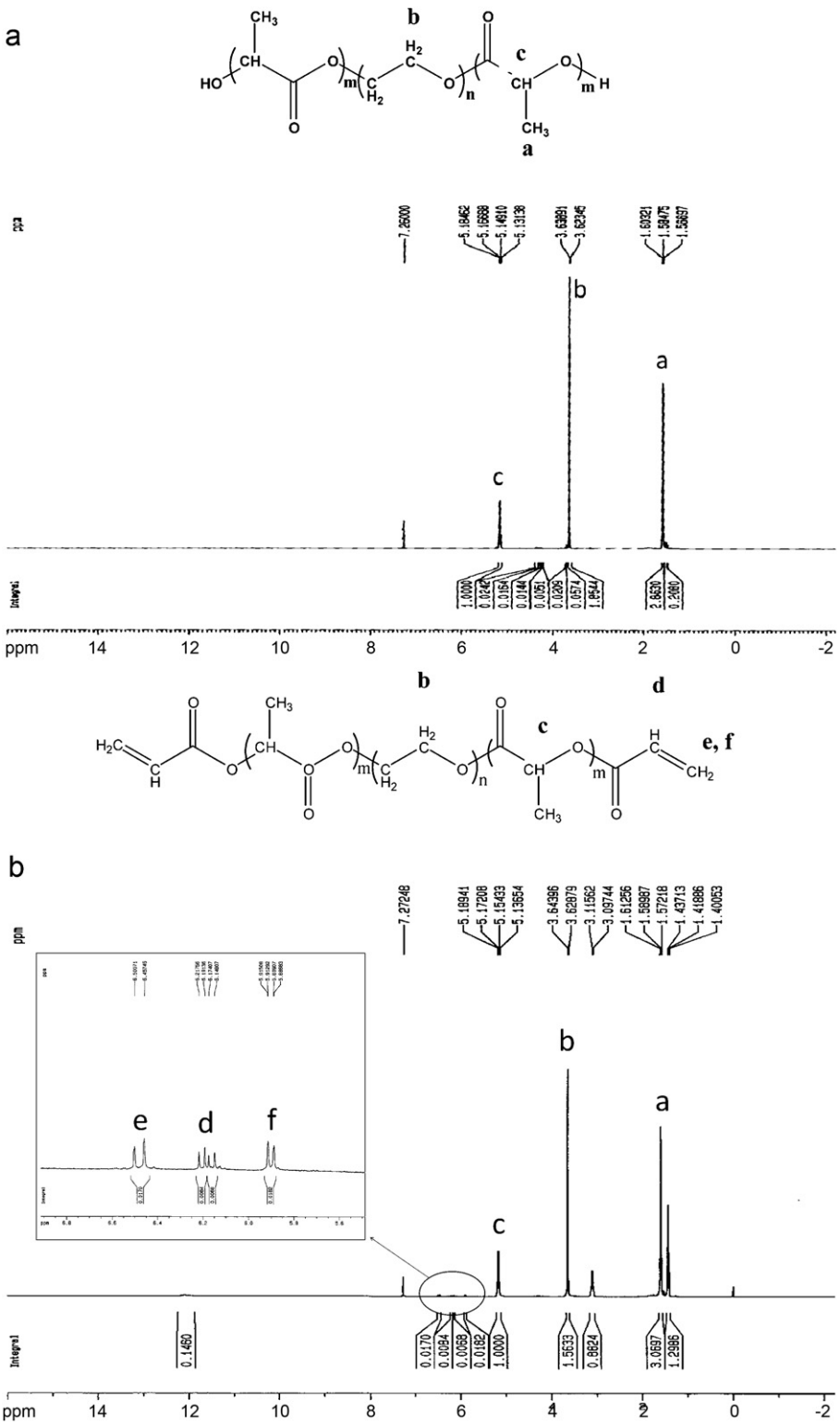

Fig. 2. ^{1}H NMR spectrum of (a) PLA_{48}–PEG_{45}–PLA_{48} tri-block copolymer and (b) PLA_{48}–PEG_{45}–PLA_{48} diacrylate copolymer in $CDCl_3$.

CH_2 (trans) at 6.45–6.50 ppm. From the ^{1}H NMR spectrum, the integration ratio of the acryl protons to the methylene protons (–CH_2–, 3.6 ppm) in the PEG segments reveals that both OH terminal groups of the PLA–PEG–PLA triblock copolymer have been derivatized successfully into acrylate groups.

FT-IR spectra of PEG, PLA–PEG–PLA copolymer and diacrylate PLA–PEG–PLA are shown in Fig. 3. For PEG homopolymer, the characteristic absorption band is an intense band due to the associated hydroxyl groups (3421 cm^{-1}). In the PLA–PEG–PLA copolymer spectrum, the sharp and intense band at 1757 cm^{-1} and at 1102 cm^{-1} confirm the presence of the carboxylic ester (C=O) and ether (C–O–C) groups indicating that formation of PLA–PEG–PLA copolymer has been taken place. The formation of PLA–PEG–PLA diacrylate copolymers is also confirmed by FT-IR spectroscopy as evidenced by the appearance of absorption band at 1638 cm^{-1} awardable to C=C in the vinyl groups.

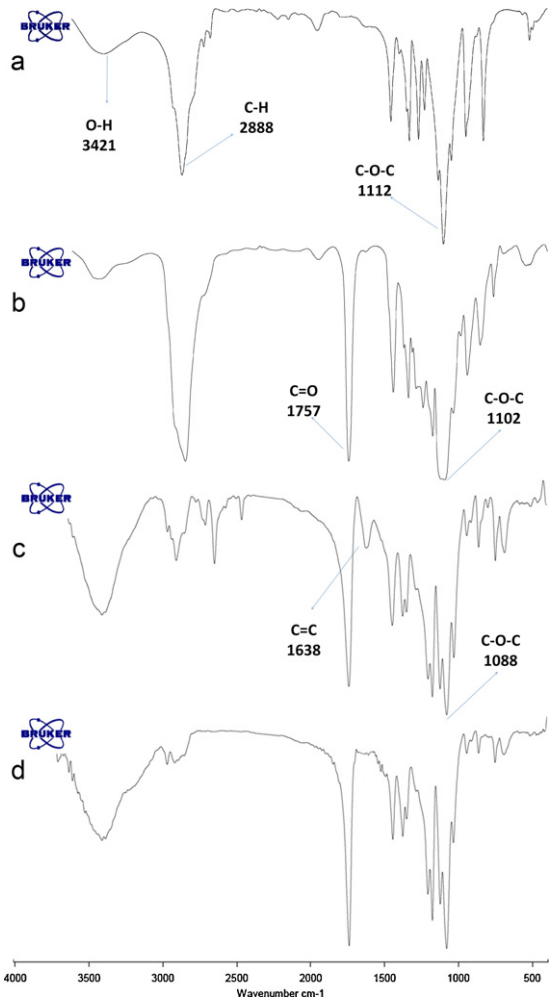

Fig. 3. FT-IR spectra of (a) PEG-2000, (b) PLA–PEG–PLA tri-block copolymers, (c) PLA–PEG–PLA diacrylate copolymers and (d) PLA–PEG–PLA nanogels.

DSC provides extensive information about physical state of copolymers, and detects phase transitions such as glass transition, (exothermic) crystallization and (endothermic) melting. Fig. 4 shows the DSC thermograms corresponding to PLA_{48}–PEG_{45}–PLA_{48} copolymers, PLA_{94}–PEG_{227}–PLA_{94} copolymers and diacylate PLA_{48}–PEG_{45}–PLA_{48} copolymers. The thermogram of PLA_{48}–PEG_{45}–PLA_{48} copolymers displayed an endothermic peak at 146.24 °C which are indicative for the melting of the crystalline PLA segment of copolymer and an exothermic peak around 95 °C which implies to the crystallization of the PLA segments. Of particular note was that in the thermogram of PLA_{94}–PEG_{227}–PLA_{94} copolymers, in addition to melting peak of PLA at 135.79 °C, an endothermic peak at 47.19 °C was observed which can be accounted to the melting of the PEG segments. The interpretion for this observation is that in the case of PLA_{48}–PEG_{45}–PLA_{48} copolymers with PLA blocks longer than the PEG ones, the PEG segments are in the amorphous state. Indeed, this finding is also consistent with results reported in the literature (Luo et al., 2002). Fig. 4 reveals that thermograms of PLA_{48}–PEG_{45}–PLA_{48} copolymers and diacylate PLA_{48}–PEG_{45}–PLA_{48} copolymers exhibited no significant differences except small shift at place of peaks which is due to alteration in termini groups of PLA.

Table 1
Composition and the average molecular weight of PLA–PEG–PLA.

Copolymer	LA/EO[a]		$\bar{M}_n$ [b]	$\bar{M}_w/\bar{M}_n$ [c]	DP_{PLA} [b]
	Initial feed	Final polymer			
PLA_{48}–PEG_{45}–PLA_{48}	2.44	2.12[b]	8920	1.17	48
PLA_{94}–PEG_{227}–PLA_{94}	2.44	0.824[b]	23540	1.22	94

LA/EO in product and DP_{PLA} are determined from the integration ratio of resonance due to PEG blocks at 3.62 ppm (–O–CH_2–CH_2–) and to the PLA blocks at 1.6 ppm (–CH_3) in the ^{1}H NMR. DP_{PEG} = 2000/44 = 45 and DP_{PEG} = 10000/44 = 227, $DP_{PLA} = DP_{PEG} \times (LA/EO)/2$.

[a] The molar ratio of repeating unit of LA (lactide) and EO (ethylene oxide).
[b] Determined by ^{1}H NMR measurement in $CDCl_3$.
[c] Determined by GPC measurement in THF.

3.2. Preparation of polymeric micelles

The PLA–PEG–PLA diacrylate copolymer micelles were prepared by solvent injection using THF as the solvent. It was clearly shown that amphiphilic block copolymers can form a micellar structure when exposed to an appropriate solvent (Letchford and Burt, 2007). The amphiphilic nature of the PLA–PEG–PLA diacrylate copolymers, with hydrophilic PEG and hydrophobic PLA blocks, provides an opportunity to form micelles in water. This behavior can be explained as a consequence of THF diffusion into the water as the polymer solution is injected, thus leading the PLA–PEG–PLA diacrylate copolymer to self-assemble into micelles structure because of its amphiphilic characteristics. In water, the hydrophilic PEG segments serve as hydrophilic shell and the hydrophobic PLA segments aggregate to become the micellar core.

Fluorescence techniques based on the selective partition of pyrene in hydrophobic phase against aqueous phase was used for determination of CMC of the PLA–PEG–PLA micellar systems as described by Dai et al. (2004). Fig. 5 illustrates the fluorescence excitation spectra of 6×10^{-7} mol l^{-1} pyrene in water in the presence of PLA–PEG–PLA of various concentrations. In spite of the constant pyrene concentration, obviously the fluorescence intensity extremely increased and the maximal peak position shifted from 335 to 337 nm as the polymer concentration increased. This phenomenon was attributed to the formation of the micelles in the system and entering pyrene molecules into the micelle phase which show much stronger fluorescence than in the water phase. Fig. 6 shows the intensity ratio (I_{337}/I_{335}) against logarithmic concentration of triblock PLA_{48}–PEG_{45}–PLA_{48}. It is clear that below a certain concentration, this ratio was approximately constant, and above this concentration, it increased with increasing copolymer concentration, indicating the formation of micelles. The CMC was taken as the intersection of the tangent of the curve in these two parts. The CMC of 1.28 mg l^{-1} for PLA_{48}–PEG_{45}–PLA_{48} micelles was calculated which was much lower than the CMC for low molecular surfactants and PEG–PCL (polycaprolactone) or PEG–PLA diblock copolymers (Nagasaki et al., 1998).

The structure of the PLA_{94}–PEG_{227}–PLA_{94} copolymer micelles was determined by ^{1}H NMR spectrometry in D_2O (Fig. 7). One surprising observation was that the signals at 1.6 and 5.15 ppm assigned to the PLA segments were disappeared as compared to copolymer spectrum in $CDCl_3$. These results may be attributed to the PLA block aggregation in the aqueous environment due to its hydrophobic character, which behaves as the solid core of the micelles. The surface orientation of the PEG segment was confirmed by the significantly intense signals of methylene protons of PEG at 3.7 ppm in D_2O. The results of ^{1}H NMR spectrometry in D_2O prove the PLA core and PEG shell structure of the micelles in aqueous media. Based on the structure of the tri-block copolymer, PLA–PEG–PLA, and also considering the results of the core–shell structure of the micelles, it is speculated that the nanostructure of the micelles to be a flower-like structure (Zhao et al., 2001).

DSC provides an attractive technique to study the influence of the nanoparticles preparation process on their properties.

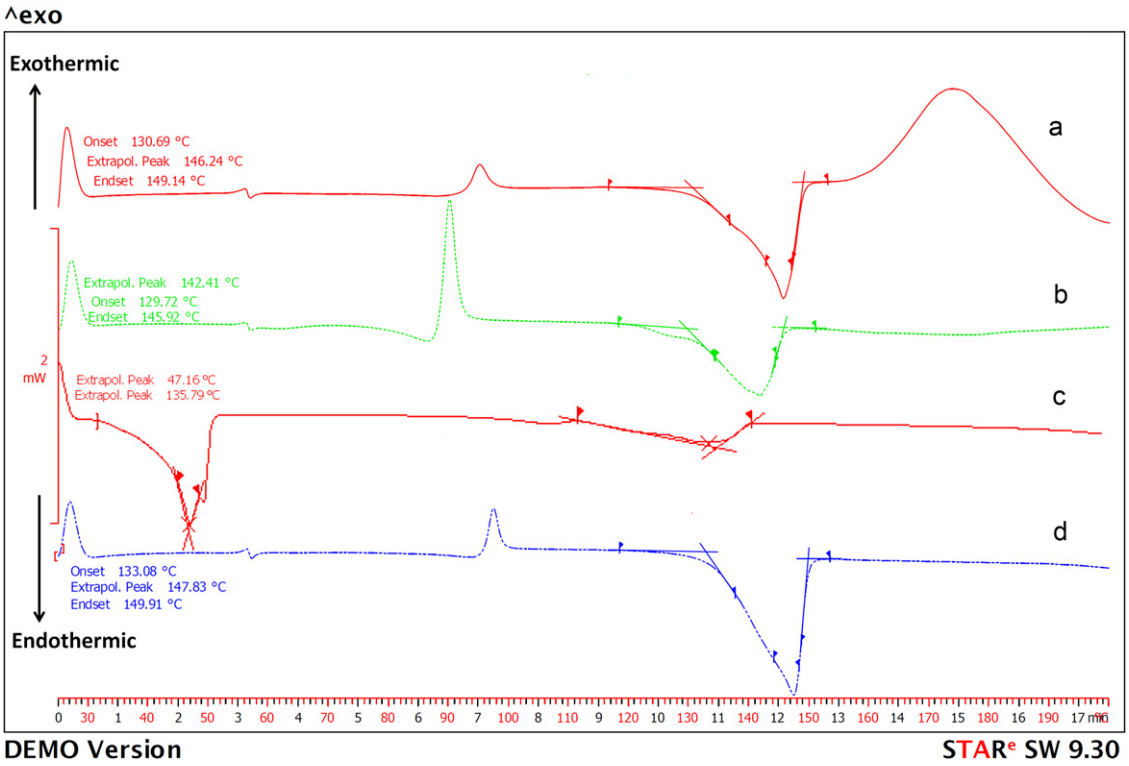

Fig. 4. DSC thermograms of (a) PLA_{48}–PEG_{45}–PLA_{48} copolymers, (b) PLA_{48}–PEG_{45}–PLA_{48} nanoparticles, (c) PLA_{94}–PEG_{227}–PLA_{94} copolymers and (d) PLA_{48}–PEG_{45}–PLA_{48} diacrylate copolymers.

Comparing thermograms of PLA_{48}–PEG_{45}–PLA_{48} copolymers and PLA_{48}–PEG_{45}–PLA_{48} nanoparticles, it was found that the intensity of crystallization peak of PLA segment in the thermogram of PLA_{48}–PEG_{45}–PLA_{48} nanoparticles increased (Fig. 4). This phenomenon seems to be due to the nanoprecipitation process which generally decreases the crystallinity of PLA blocks because the polymer network did not find an opportunity to be organized prior to the precipitation during the solvent diffusion into the outer phase (Izumikawa et al., 1991).

3.3. Preparation of nanogels

The nanogels were prepared by thermal crosslinking of the terminal vinyl group of PLA–PEG–PLA diacrylate copolymer in the micelles. FT-IR spectroscopy was used to confirm formation of nanogels. Disappearance of the C=C absorption at 1638 cm^{-1} indicates that the reaction of the vinyl groups was complete and crosslinking of the micellar core is accomplished successfully (Fig. 3(d)).

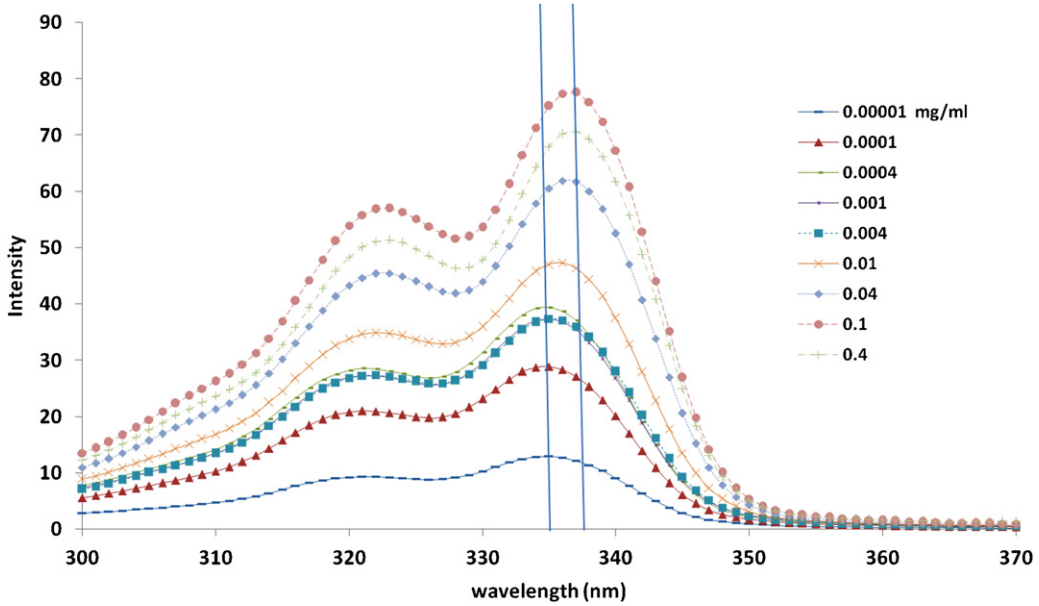

Fig. 5. Excitation spectra of pyrene in water for PLA_{48}–PEG_{45}–PLA_{48} at various concentrations at λ_{em} = 390 nm.

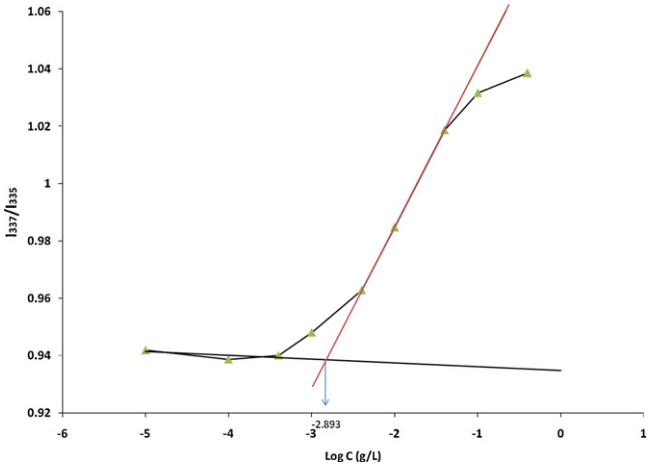

Fig. 6. Intensity ratio I_{337}/I_{335} of pyrene excitation spectra versus $\log C$ for PLA_{48}–PEG_{45}–PLA_{48}.

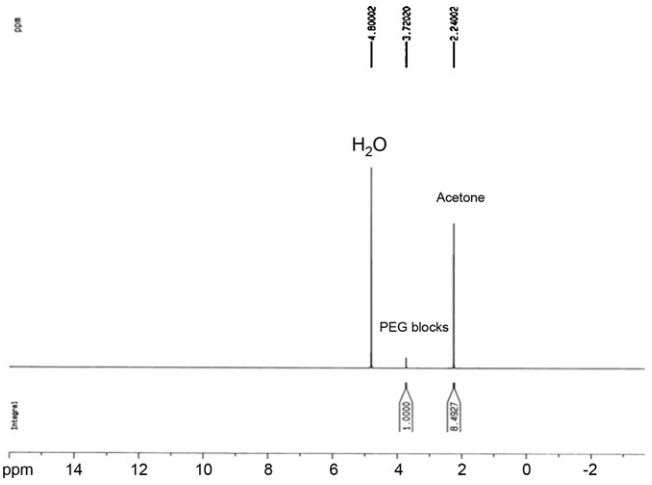

Fig. 7. ^{1}H NMR spectrum of PLA_{94}–PEG_{227}–PLA_{94} micelles in D_2O.

3.4. Characterization of micelles and nanogels

3.4.1. Nanogels morphology

The SEM analysis was used to obtain images of the nanogels and confirm their shape. As Fig. 8 illustrates the nanogels form spherical and discrete particles. The image also shows that the size of nanogels was around 200 nm.

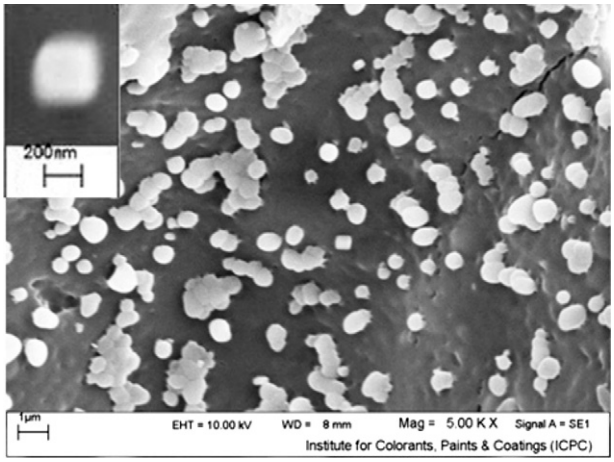

Fig. 8. The SEM image of PLA_{48}–PEG_{45}–PLA_{48} nanogels with 50 wt.% EGDMA.

Table 2
The effect of PLA_{48}–PEG_{45}–PLA_{48} copolymer concentration on final nanogels sizes.

Polymer concentration in THF (mg/ml)	Nanogels size (nm)	Polydispersity index (PdI)
12.5	197.1	0.280
5.00	150.8	0.159
3.33	128.2	0.262

3.4.2. Size of nanogels

The hydrodynamic diameters of particles which were determined by PCS, are reported in Tables 2 and 3. Since the particle size is a critical factor in the performance of nanogels, the effect of some preparation condition on the nanogels size was investigated. It was found that the final nanogels size is mainly affected by the micelles preparation step (Table 2). Since the nanoprecipitation method was used for micelles preparation, as expected, polymer concentration in solvent exhibited the most significant influence on nanoparticles size (Asadi et al., 2011). From the results shown in Table 2, it is clear that the size of nanogels increased as polymer concentration increased. This finding is important as it gives a chance to manipulate the nanogels size by changing polymer concentration while the other parameters remained constant (Table 2).

3.4.3. Swelling ratio

The swelling–deswelling is considered as a characteristic behavior of nanogels. The swelling ratio of nanogels was studied by following the size variations following the particle immersion in aqueous medium. The swelling ratios for PLA_{94}–PEG_{227}–PLA_{94} and PLA_{48}–PEG_{45}–PLA_{48} nanogels were calculated to be 4.50 and 2.15, respectively. The increase in swelling ratio when PEG content of copolymer increases indicates that the extent of swelling ratio depends on hydrophilic/hydrophobic ratio of copolymer. In other words, swelling of nanogels is due to the hydrophilic nature of PEG segments which become much more extended, as nanogels are exposed to the deionized water. Consequently, the size of the nanogels increases significantly according to the length of PEG segments in the copolymer structure. Furthermore, the size of nanogels was also affected by the surrounding environment. In this study, the nanogels were prepared by polymeric THF solution injection into the deionized water and nanogels were formed in the presence of small amount of THF mixed with deionized water. THF is a relatively good solvent for PLA segments and is a relatively poor solvent for PEG segments of the copolymers as compared to deionized water. From the discussion above, it is obvious that in THF solution, the PEG segments of nanogels do not expand much, but when freeze dried samples are resuspended in deionized water, in the absence of THF, the PEG segments become much more extended thus leading to swelling of nanogels.

3.4.4. Zeta potential

Zeta potential (ζ) can extremely influence the particles stability in suspension through the electrostatic repulsion between the particles. In addition, from the zeta potential, it is possible to obtain some valuable information about the dominated component on the particles surface (Dong and Feng, 2004). Both PLA_{48}–PEG_{45}–PLA_{48} micelles and nanogels exhibited zeta potential of −24.9. The same values of ζ potential for both nanoparticles can be attributed to their similar structure. Since the PLA or PLGA nanoparticles have a highly negative ζ potential values (around −50 mV) (Govender et al., 1999), increase in ζ potential value would be awarded as an indirect evidence for micelle like structure of nanogels where PEG is present in the surface of nanogels. This interpretation is further

Table 3
The composition of nanogels, the mean size of nanogels, polydispersity, encapsulation ratio of naltrexone, and the mean size of nanogels encapsulated with naltrexone.

Copolymer	EGDMA (wt.%)	Polymer (wt.%)	Mean size of nanogels (nm)	PdI without drug	Naltrexone ER (%)	Mean size of nanogels with naltrexone (nm)	PdI with drug
PLA_{48}–PEG_{45}–PLA_{48}	10	90	176.1	0.425	55.8	193.8	0.466
PLA_{48}–PEG_{45}–PLA_{48}	50	50	180	0.313	59.6	197.1	0.280
PLA_{94}–PEG_{227}–PLA_{94}	50	50	171.2	0.272	47.6	182.4	0.295

supported by increasing the ζ potential of nanogels with higher PEG content. The PLA_{94}–PEG_{227}–PLA_{94} nanogels showed the higher value of ζ potential (−13.3 mV) which is indicative of the higher coverage of nanogels surface by the PEG chains in this case. ζ potential values of PLA_{48}–PEG_{45}–PLA_{48} with 50 wt.% of EGDMA was also used to study degradation of nanogels. The results showed that during 180 days, the value of ζ potential changed from −24.9 mV to −12.3 mV. Since the surface negative ζ potential of nanogels is due to surface PLA blocks, the results can be accounted for degradation of PLA block on the surface of nanogels.

3.5. Drug encapsulation efficiency

Naltrexone encapsulation ratio of nanogels was calculated to be approximately 60% and 50% for PLA_{48}–PEG_{45}–PLA_{48} and PLA_{94}–PEG_{227}–PLA_{94}, respectively (Table 3). The results showed that naltrexone loading capacity in PLA–PEG–PLA nanogels was related to the PLA-to-PEG ratio where higher ratio of PLA/PEG block led to higher amount of drug loading. This could be largely due to hydrophobic nature of naltrexone which caused it to be encapsulated in the PLA core of the nanogels. The results are in accordance with the commonly accepted idea that for hydrophobic drugs, such as naltrexone, increasing the chain length of the PLA blocks causes the encapsulation efficiency to be increased, and conversely, the encapsulation efficiency of hydrophilic drugs increases by increasing the chain length of the PEG blocks (Liu et al., 2001). So it can be concluded that drug encapsulation ratio can be manipulated by the adjustment of the PLA–PEG–PLA triblock copolymer compositions used for nanogels preparation. The effect of crosslinker (EGDMA) concentration on the encapsulation ratio of naltrexone was also investigated and the results are shown in Table 3. It was found that there was no apparent relationship between the concentration of EGDMA and the encapsulation ratio of nanogels. It must be noted that, as shown in Table 3, the incorporation of naltrexone in nanogels slightly increased their size as compared to the naltrexone-free nanogels.

3.6. In vitro drug release

Fig. 9 illustrates the accumulative release curve of naltrexone from micelles and nanogels with different EGDMA contents in PBS solution. It can be observed from Fig. 9 that the release profile of the micelles (non-crosslinked particle) reaches over 90% in first 24 h of the test period. On the other hand, in the case of nanogels with 10 wt.% of EGDMA, about 96% naltrexone was released over 15 days, and for nanogels with 50 wt.% EGDMA only 19% of the naltrexone was released during the same period of time. A possible explanation for this behavior could be the instability of micelles that lead to rapid release of naltrexone from micelles compared to the nanogels. The drug release profiles of naltrexone from nanogels were approximately steady-linear throughout the time evaluated. The rate of drug release from the nanogels is theoretically affected by many factors such as rate of polymer degradation, diffusion of the drug through the nanogel particles, and extent of swelling of nanogels in aqueous media. In the case of PLA–PEG–PLA nanogels which used in this study, since they are composed of biodegradable polymer, as the polymer degrades the structure might become loose which, in turn, lead to drug release (Mellott et al., 2001). Additionally, increasing the size of pores due to the nanogels swelling in aqueous media can lead naltrexone to diffuse out of nanogels (Mellott et al., 2001). With respect to insignificant change of the nanogels size during drug release in the current study, naltrexone is speculated to be released from the nanogels mainly by diffusion.

The content of crosslinker has also showed significant impact on the release characteristics of naltrexone. A decrease of naltrexone release rate was observed as crosslinker content was increased, as shown in Fig. 9. Such findings can be attributed to a tightly packed core region of the nanogels, with increasing EGDMA levels lead-

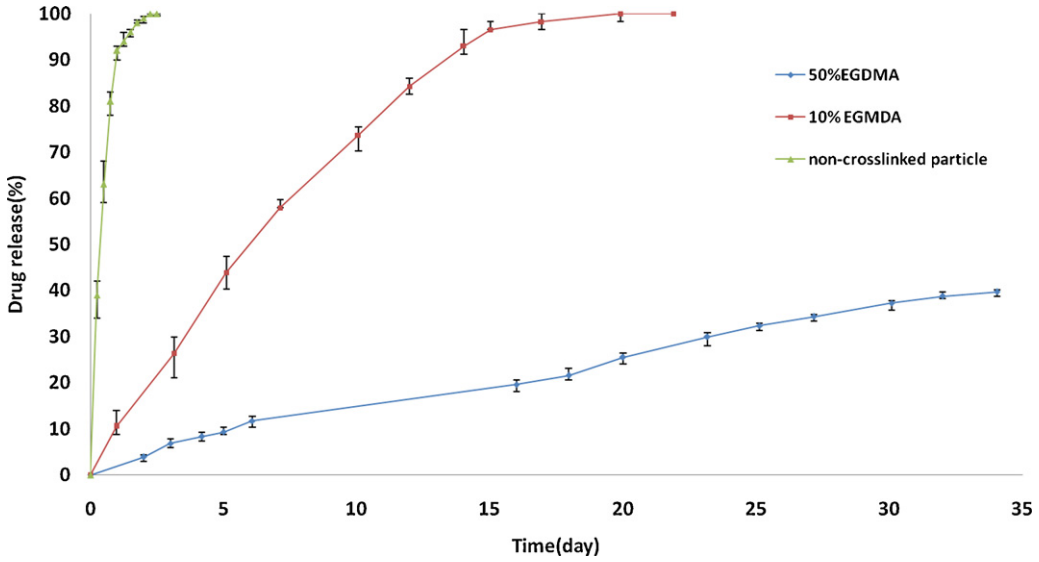

Fig. 9. The naltrexone release profiles of PLA_{48}–PEG_{45}–PLA_{48} nanogels with different EGDMA concentration.

Table 4
The stability of nanogels suspension with different crosslinker contents.

Nanogels	Mean size of nanogels after preparation (nm)	Mean size of nanogels after 40 day (nm)	Mean size of nanogels after 60 day (nm)
Nanogels with 10 wt.% EGDMA	193.8	206.5	–
Nanogels with 50 wt.% EGDMA	197.1	–	203.1

ing to slower drug release rate because of the formation of more crosslinks. So it is possible to adjust the rates of drug release by changing the content of crosslinker to attain predictable release rates form nanogels.

3.7. Physical stability of nanogels

In the clinical administration of nanoparticle dispersions, vessel occlusion resulting from the particle aggregation raises great concern. So it is highly valuable to evaluate the stability of nanoparticles in the biological milieu. The particle size stability was followed over a 60-days course. The variation of the size of nanogels with different crosslinker contents as a function of incubation time is shown in Table 4. As it can be seen the sizes of all nanogels were almost constant and there was no significant change in the size of nanogels within the time range investigated. This could be mainly due to core–shell like structure of nanogels, where hydrophilic PEG shell provided the steric stability by hydrophilic interaction in aqueous media.

4. Conclusion

The PLA–PEG–PLA triblock copolymers and related diacrylated derivative were synthesized and their structure and composition were confirmed by ^{1}H NMR, and FT-IR. The naltrexone-loaded micelles of PLA–PEG–PLA were fabricated by nanoprecipitation method and were turned to nanogels by thermal crosslinking. The formation of micelles and nanogels of PLA–PEG–PLA diacrylate was confirmed by various techniques such as fluorescence spectrometry, FT-IR, DSC, PCS, and SEM. Moreover, it was shown that by means of changing preparation condition, mainly polymer concentration, the size of nanogels is easily controlled in the range of 128–200 nm. The nanogels achieved high encapsulation efficiency around 60% and exhibited excellent stability for long periods of time without significant aggregation. Furthermore, the naltrexone-loaded nanogels were able to sustain the release of the naltrexone for different periods of time up to 35 days, depending on the crosslinker concentration. This feature makes the PLA–PEG–PLA nanogels a promising carrier for controlled release drug delivery systems.

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