ORIGINAL PAPER

Preparation and analysis of a sustained drug delivery system by PLGA–PEG–PLGA triblock copolymers

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Abstract   Traditional drug delivery systems that are based on multiple dosing are usually accompanied by many shortcomings, including unwanted fluctuations in the plasma concentration of the drug and poor patient compliance. In this study, we aimed to synthesize a polymeric drug delivery system based on a triblock copolymer of PLGA–PEG1000–PLGA and investigate its application as a controlled drug delivery system. Naltrexone hydrochloride and vitamin B12 were used as model drugs here. The copolymer was successfully synthesized by the ring-opening method. A phase transition analysis indicated that the copolymer is in gel at body temperature. The release profiles from the formulations showed a higher initial release followed by a slower pattern for up to 4 weeks. More than 50 % of the vitamin B12 and 60 % of the naltrexone hydrochloride were released during this period.

Keywords   PLGA–PEG–PLGA · Triblock copolymer · Naltrexone hydrochloride · Vitamin B12 · Controlled release · Hydrogel · In situ-forming system

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Introduction

Over the past few decades, new, biodegradable, injectable, and in situ-forming drug delivery systems have received considerable attention because of their unique properties, such as their ease of administration, localized drug delivery, and controlled drug release for a long period of time [1, 2].

Furthermore, depending on the type of polymer, some of the novel drug delivery systems can release drugs in response to the environment, as occurs in smart systems, such as thermo responsive and pH sensitive hydrogels [3–5].

One of the in situ-gelling and temperature-sensitive block copolymer hydrogels that are available for drug delivery is PLGA–PEG–PLGA triblock copolymer that was made from poly (lactic-*co*-glycolic acid) as the hydrophobic segment and polyethylene glycol as the hydrophilic segment. This copolymer is very attractive because of its biodegradability, biocompatibility, and ease of formulation and application. An organic solvent is not required for the synthesis of this polymer, and it is appropriate for delivery of both hydrophilic and hydrophobic molecules [6, 7].

PLGA–PEG–PLGA hydrogels with different lactide-to-glycolide ratios have been used as delivery systems for various drugs, such as growth hormone [8], testosterone [9], insulin [10], 5-fluorouracil [7], and calcitonin [11]. Furthermore, PLGA–PEG–PLGA nano micelles have been used as nano carriers for targeted drug delivery [12, 13].

In this study, we investigated the release of naltrexone hydrochloride and vitamin B_{12} from PLGA–PEG (1000)–PLGA, with a lactide-to-glycolide ratio of 3:1.

Naltrexone hydrochloride is a specific opioid antagonist and is used to maintain abstinence after withdrawal in detoxified opioid- and alcohol-dependent patients [14, 15]. Because of the poor compliance of the addicted patients that regularly use naltrexone hydrochloride, the development of novel extended-release systems that can provide an effective drug concentration in the blood for a long time after just one injection is desirable [16–18].

Here, we also determined the effect of size and molecular weight (M_w) of the drug molecules on their release profile. Naltrexone hydrochloride ($C_{20}H_{23}NO_4$, HCl) and vitamin B_{12} ($C_{63}H_{88}CoN_{14}O_{14}P$) are both hydrophilic molecules with M_ws of 377.9 and 1355.4 g/mol, respectively.

Materials and methods

Materials

Glycolide A, D,L-lactide, stannous 2-ethylhexanoate, and 1,6-diphenyle-1,3,5-hexatriene (DPH) were purchased from Sigma Aldrich, USA. Iodine was purchased from the Kian Kave Pharmaceutical Co., Iran. B12 was kindly donated by the Iran Hormone Pharmaceutical Co., and naltrexone hydrochloride was purchased from the Razak Pharmaceutical Co., Iran.

Copolymer synthesis

PLGA–PEG–PLGA with a lactide (LA)-to-glycolide (GA) ratio of 3:1 was synthesized using a ring-opening method with slight modifications, as described previously by Zentner et al. [6]. First, 60 g of PEG 1000 were heated at 150 °C and stirred (250 rpm) in a stainless steel reactor under a vacuum (5 mmHg) for 3 h. Next, 113.46 g of D,L-lactide and 30.48 g of glycolide were added, and the mixture was heated and stirred at 150 °C under a vacuum for 30 min. As a catalyst, 0.04 g of stannous 2-ethylhexanoate was added, and the heating and stirring was continued at 160 ± 5 °C under a vacuum for 8 h [19].

Copolymer purification

The product was first dispersed in hot water (70–80 °C), and the temperature of the dispersion was subsequently reduced to 4 ± 1 °C to completely dissolve the copolymer in water. By heating the solution to 80 °C, the triblock copolymer was precipitated, and all of the impurities remained in the solution. This process was repeated three times. Finally, the purified copolymer was freeze-dried, and the product was kept at −20 ± 1 °C [6].

Copolymer characterization

The structure of the copolymer was confirmed by ^{1}H NMR. The molecular number (M_n) of the triblock copolymer was estimated by integrating the ^{1}H NMR signals pertaining to each monomer according to the method established by Jeong et al. [20] according to formula (1). In this formula, PEG, LA, and GA show the number of each monomer in copolymer structure. Aa, Ae, Ad, and Ac are related to the integrations of the CH of lactide, the CH_2 of the PEG that binds to the PLGA, the CH_2 of PEG, and the CH_2 of glycolide, respectively. The NMR spectra were recorded by an NMR instrument (Bruker AC-80) at 300 MHz at room temperature, using $CDCl_3$ as a solvent.

The molecular weight (M_w) and polydispersity of the copolymer were determined by gel-permeation chromatography (GPC) using a GPC-Addon apparatus and a RID-A refractive index signal detector coupled to Plgel$^®$ columns. The eluent was tetrahydrofuran with a flow rate of 1 mL/min. Polystyrene standards were used as a calibration agent [7, 19].

$$M_n = (44\text{PEG}) + (72 \times \text{LA}) + (58 \times \text{GA}) + 17 + 1 \tag{1}$$

$$\text{LA}: \frac{\text{Aa}}{\text{Ae}} = \frac{\text{LA}}{4}$$

$$\text{GA}: \frac{\text{Ac}}{\text{Ae}} = \frac{2\text{GA}}{4}$$

$$\text{PEG}: \frac{\text{Ae}}{\text{Ad}} = \frac{4}{4(x-2)+4}$$

The sol–gel transition temperature was determined by a refrigerated bath circulator instrument (WISD P-22, South Korea). PLGA–PEG–PLGA copolymers were dissolved in phosphate-buffered solution (PBS, pH 7.4) to make the concentrations of 17, 23, and 28 wt%. The temperature was then increased by 0.5 °C/min, from 0 °C to the temperature at which the magnet inside the copolymer solution stopped stirring and the gel formed.

Drug loading

A PLGA–PEG–PLGA solution (100 mg/mL) was prepared by dissolving the copolymer in chloroform. Naltrexone hydrochloride and vitamin B12 were separately dissolved in 70 % ethanol to make 1- and 5-mg/mL drug solutions. One milliliter of the copolymer solution was mixed with 1 mL of the drug solution and dried under a 5-mmHg vacuum at 30 °C. Finally, all of the samples were freeze-dried to remove all of the residual solvents. The blank was 100 mg of the copolymer without the drug.

To determine the amount of the drug that was loaded in the hydrogel, 50 mg of each sample was dissolved in 10 mL of cold distilled water (4 °C), and the drug content was measured by high-performance liquid chromatography (HPLC) (SPD-10Avp, Shimadzu). The naltrexone hydrochloride analysis was performed at 281.4 nm on a C18 column (5-m particle, 150×4.6 mm² i.d., DiamonsilTM) at 50 °C. The mobile phase was an isocratic mixture of 0.5 % (v/v) glacial acetic acid and 14 % (v/v) acetonitrile in deionized water. The injection volume was 50 μL, and the flow rate was 0.5 mL/min. For vitamin B12, the measurement was performed at 305 nm. The mobile phase was 30 % (v/v) methanol in deionized water. The temperature was 30 °C, and the flow rate was 0.8 mL/min.

In vitro drug release

Four milliliters of PBS were added to the each vial containing 100 mg of drug/copolymer freeze-dried powder. The vials were kept in a Reciprocal water bath (N-BIOTEK NB -304, South Korea) (20 ± 2 rpm) at 37 ± 0.1 °C. Every 24 h, a 1-mL aliquot was withdrawn from each sample and substituted with 1 mL of fresh PBS. The amount of drug was determined by reverse-phase HPLC, as described in "Drug loading" section.

Data analysis

The results were reported as means $\pm$ SDs ($n = 4$). Statistical comparisons were performed using paired t tests and one-way ANOVA. A significance level of $P < 0.05$ denoted significance in all cases.

Results and discussion

Characterization of triblock copolymers

The triblock copolymer was effectively synthesized by the ring-opening polymerization.

A typical ^{1}H NMR spectrum of the PLGA–PEG–PLGA copolymer containing PEG 1000 with a LA/GA ratio of 3:1 is presented in Fig. 1. This figure is very similar to the previously reported spectrum and confirms the synthesis of the triblock copolymer [7]. The signals appearing at 5.2 (a), 4.8 (c), 4.3 (e), 3.5 (d), 2.6 (f), and 1.5 (b) ppm correspond to the CH of LA, the CH_2 of GA, the CH_2 of PEG that binds to PLGA, the CH_2 of PEG, and the OH and CH_3 of LA, respectively. The LA/GA ratio determined by ^{1}H NMR matched the initial ratios of the components used in the polymerization. The results of ^{1}H NMR and GPC analyses are shown in Table 1.

A typical GPC chromatogram seems to show a symmetric peak and indicates the low polydispersity of the triblock copolymer (Fig. 2).

The sol–gel transition temperature of the PLGA–PEG–PLGA solutions with 17, 23, and 28 wt% were found to be 19 ± 0.5, 18 ± 0.5, and 16 ± 0.5 °C, respectively. A typical phase diagram that illustrates the phase transition behavior of the aqueous solutions of the triblock copolymer is shown in Fig. 3. As the diagram indicates, by increasing the copolymer concentration, the gelation temperature decreases because of an increase in the number of polymer–polymer
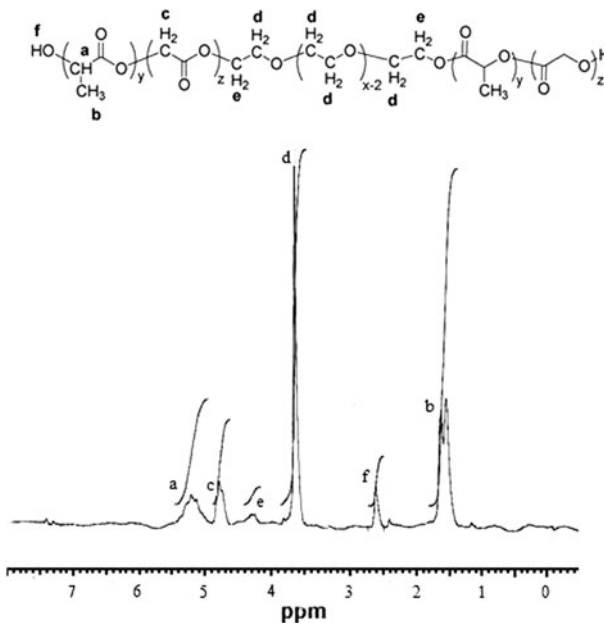

Fig. 1 The ^{1}H NMR spectrum and chemical structure of the PLGA–PEG–PLGA copolymer

Table 1 Copolymer composition determined by GPC and ^{1}H NMR

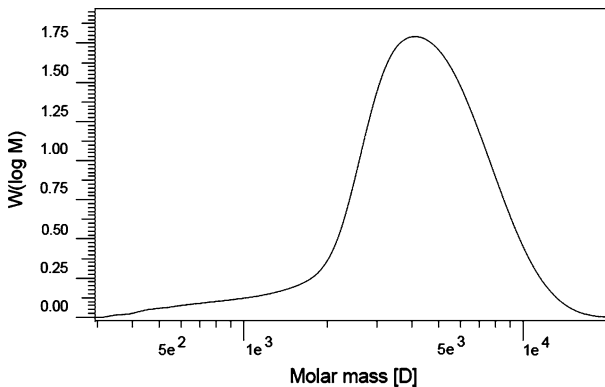| Copolymer	NMR		GPC		
	M_n^{a}	LA/GA[b]	M_n^{c}	M_w^{d}	M_w/M_n^{e}
PLGA–PEG–PLGA	1358–1000–1358	3.09	3290.3	4743.7	1.44

[a] Number-average molecular number determined by ^{1}HNMR

[b] LA/GA ratio determined by ^{1}HNMR

[c] Number-average molecular weight determined by GPC

[d] Weight-average molecular weight determined by GPC

[e] Polydispersity determined by GPC

Fig. 2 A GPC chromatogram of the PLGA–PEG–PLGA copolymer

interactions at higher concentrations. The gelation of the copolymer solution over the phase-transition temperature is due to the hydrophobic interaction between the PLGA segments and the weakness of the hydrogen bonding. At lower temperatures, because of hydrogen bonding between the hydrophilic PEG segments and water molecules, monomers, individual micelles, and grouped micelles persist in the aqueous environment, and the samples remain sol [7].

Drug-loading efficiency

The amount of drug loaded in the hydrogel was determined by HPLC. Approximately 85 % of each drug was loaded in the hydrogel by the method discussed in "Drug loading" section.

In vitro drug release

The release profiles of the drugs are shown in Figs. 4, 5, and 6. The drug release from the formulations showed a higher initial release followed by a slower pattern for up to 4 weeks. More than 50 % of the vitamin B12 and 60 % of the naltrexone hydrochloride were released during this period.
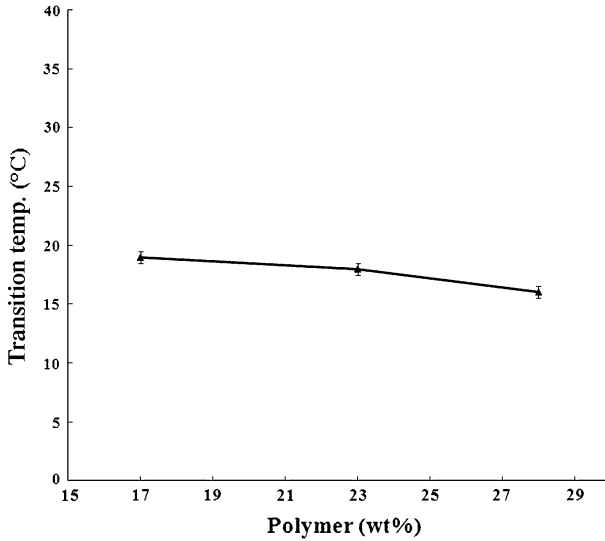
Fig. 3 The phase diagram of the PLGA–PEG–PLGA copolymers in aqueous solutions with different polymer concentrations
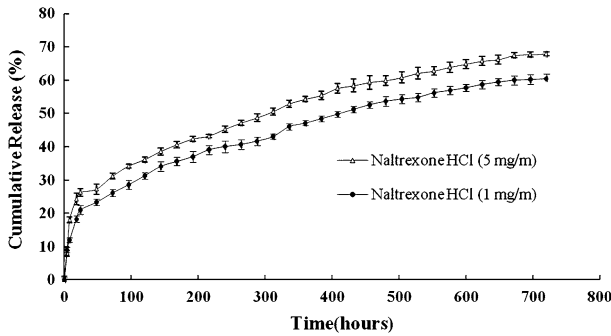

Fig. 4 The naltrexone hydrochloride release profile from the hydrogels with different drug concentrations

Naltrexone hydrochloride which is a small molecule released from the hydrogel via diffusion, as indicated in Table 2 (Higuchi modeling). By increasing the drug concentration, the release rate increased significantly ($P < 0.05$), as is expected from the Higuchi equation (2). Where Q is mass flux, C is initial drug concentration, D is diffusion constant, and t is time.

$$Q = 2c * \sqrt{\frac{Dt}{\pi}} \tag{2}$$

Our data indicated that the hydrogel released vitamin B_{12} according to the Higuchi model, especially in the first few hours of release when the concentration gradient of the drug was much higher, and the main release mechanism was
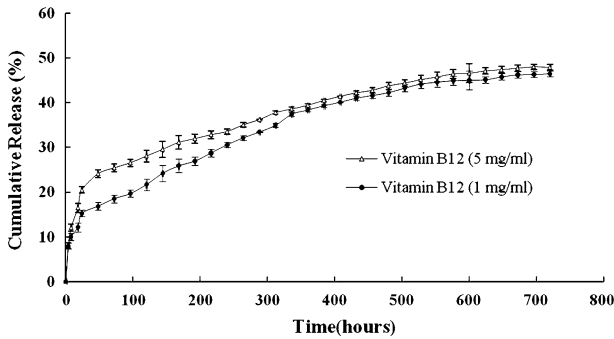
Fig. 5 The vitamin B_{12} release profile from the hydrogels with different drug concentrations
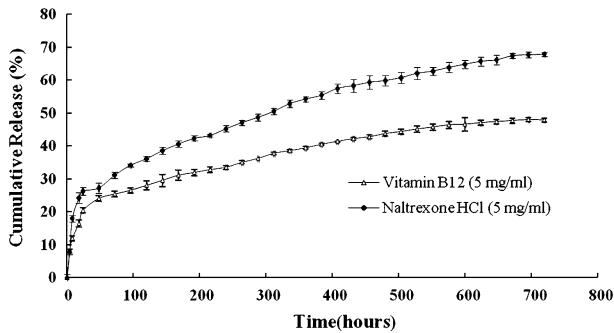

Fig. 6 A comparison of the release of naltrexone hydrochloride and B12 (drug concentration: 5 wt%)

Table 2 The kinetic profiles of drug release from the PLGA–PEG–PLGA hydrogel

Drug	Drug concentration (w/v%) (mg/mL)	Zero-order		Higuchi	
		Slope	R^2	Slope	R^2
Naltrexone hydrochloride	1	1.3707	0.9606	8.9513	0.9872
	5	1.462	0.962	9.5156	0.982
B_{12}	1	1.1487	0.9347	7.5601	0.9755
	5	0.9589	0.9452	6.3335	0.9935

diffusion. Figure 5 shows that, during the first few hours, the release rate increased significantly ($P < 0.05$) when the drug concentration increased. This result confirms our hypothesis for the release mechanism during these hours. As drug release continued, the rates of release from each of the samples with different concentrations became very similar, and polymer degradation became the main mechanism of release. Vitamin B_{12} is a large molecule that cannot easily diffuse through the small pores of a tortuous hydrogel. Therefore, when the drugs in and near the surface of the hydrogel were released, the main mechanism of drug release changed to polymer degradation. Thus, the fact that the naltrexone hydrochloride release rate

was significantly ($P < 0.05$) much faster than the B_{12} release rate is predictable because of the smaller size and lower M_w of naltrexone hydrochloride, as demonstrated in Fig. 6.

The burst release of drug is thought to be due to the surface location of the drug. The lower burst release and release slope of vitamin B_{12} (with its higher M_w) show that diffusion of vitamin B_{12} was very slow.

Conclusion

Biodegradable PLGA–PEG–PLGA triblock copolymer with PEG 1000 and LA/GA = 3 was synthesized by the ring-opening method and used for drug controlled release. This study confirmed that the drug release profile depended on the size and M_w of the drug molecules. Although the drug concentration had a critical role in controlling drug release from the hydrogel, it was not an important factor when the main mechanism of release was polymer degradation and the zero-order model had the best fit. PLGA–PEG1000–PLGA (LA/GA: 3/1) is suitable as a long-acting, controlled-release delivery system for naltrexone hydrochloride and vitamin B_{12}. As the sol–gel transition temperature of the copolymer and drug complexes was below body temperature, an injectable aqueous solution is simple to prepare and forms an implant upon injection. It can form a gel in the body and release the drug for a long period after just one injection. Therefore, this system is suitable for addicted patients with poor compliance.

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