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Research Article

Preparation and Investigation of Sustained Drug Delivery Systems Using an Injectable, Thermosensitive, *In Situ* Forming Hydrogel Composed of PLGA–PEG–PLGA

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Abstract. *In situ* gelling systems are very attractive for pharmaceutical applications due to their biodegradability and simple manufacturing processes. The synthesis and characterization of thermosensitive poly(D,L-lactic-co-glycolic acid) (PLGA)–polyethylene glycol (PEG)–PLGA triblock copolymers as *in situ* gelling matrices were investigated in this study as a drug delivery system. Ring-opening polymerization using microwave irradiation was utilized as a novel technique, and the results were compared with those using a conventional method of polymerization. The phase transition temperature and the critical micelle concentration (CMC) of the copolymer solutions were determined by differential scanning calorimetry and spectrophotometry, respectively. The size of the micelles was determined with a light scattering method. *In vitro* drug release studies were carried out using naltrexone hydrochloride and vitamin B12 as model drugs. The rate and yield of the copolymerization process *via* microwave irradiation were higher than those of the conventional method. The copolymer structure and concentration played critical roles in controlling the sol–gel transition temperature, the CMC, and the size of the nanomicelles in the copolymer solutions. The rate of drug release could be modulated by the molecular weight of the drugs, the concentration of the copolymers, and their structures in the formulations. The amount of release *versus* time followed zero-order release kinetics for vitamin B12 over 25 days, in contrast to the Higuchi modeling for naltrexone hydrochloride over a period of 17 days. In conclusion, PLGA–PEG1500–PLGA with a lactide-to-glycolide ratio of 5:1 is an ideal system for the long-acting, controlled release of naltrexone hydrochloride and vitamin B12.

KEY WORDS: hydrogel; naltrexone; PLGA–PEG–PLGA; thermosensitive; triblock copolymer; vitamin B12.

INTRODUCTION

Naltrexone hydrochloride is a specific opioid antagonist that is used to maintain abstinence after withdrawal in detoxified opioid-dependent patients (1–3). Naltrexone was the first drug to receive FDA approval to treat alcohol dependence (4–7). Because of the extensive first-pass metabolism in the liver, only 5–20 % of the oral dosage of this drug reaches the systemic circulation unchanged (4,8). In addition, there are certain side effects associated with its oral administration, such as abdominal pain, nausea, and vomiting (9). The major problem with naltrexone usage is the motivation and poor compliance of addicted patients (10–12). Therefore, developing a controlled-release parenteral formulation of which a single injection may release the drug over a week, month, or even longer is especially desirable. Early trials have suggested that the sustained release of naltrexone may be appropriate for the management of either alcohol or opioid dependence (5,13,14). There are several systems for the subcutaneous implantation of naltrexone hydrochloride (9,15–17), but this route of administration requires intricate technology, and it is too expensive (8).

In the past few years, a number of smart hydrogels have been reported for various biomedical applications, including drug delivery (18–21), gene delivery (22–24), cell encapsulation (25,26), and tissue engineering (27). Injectable *in situ* forming gels are one type of stimuli-sensitive polymers. These gels are fluid at room temperature, but in the body, they quickly convert to a very high viscous gel (28). Injectable gel-forming matrices have several advantages over other implantable systems that convert into the final form before

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placement in the body. For example, injectable materials do not require surgery for placement or withdrawal, and various therapeutic agents can be easily loaded into these systems (29).

One of the polymers that exhibits thermoresponsive properties is a triblock copolymer (ABA-type) composed of poly(D,L-lactic-co-glycolic acid) (PLGA) (A-block) and polyethylene glycol (PEG) (B-block). Some advantages of this copolymer (PLGA–PEG–PLGA) are that it does not require any organic solvent for its synthesis and purification, it has no systemic toxicity, it can deliver both hydrophobic and hydrophilic drugs, it is biodegradable and biocompatible, and it can stabilize and solubilize peptide and protein drugs, such as insulin (30), porcine growth hormone (31), testosterone (32), 5-fluorouracil (33), calcitonin (34), granulocyte colony-stimulating factor, and recombinant hepatitis B surface antigen. Gel formulations of this copolymer have been shown to provide a unique controlled release of paclitaxel (Oncogel®) in tumors for approximately 50 days with a minimal distribution in other organs (35). PLGA–PEG–PLGA is also used as a biosynthetic bandage for corneal wound repair (36).

In a study done by Salehi and coworkers, naltrexone hydrochloride was loaded into triblock copolymer solutions of PLGA–PEG1000 with copolymer concentrations of 36.9, 65, and 70 %. Incorporated naltrexone was released during a 14-day period. However, very high concentrations of the copolymer were used in this work, which is not economical, and there was an undesirable burst effect after injection (37).

The phase transition temperature of the hydrogel and the rate of its degradation and drug release can be regulated by the molecular weight of the triblock copolymer, the PEG content, the D,L-lactide (LA)-to-glycolide (GA) ratio (33,38,39) and the additives used in the formulation (31). Although significant efforts have been undertaken for the synthesis of PLGA–PEG–PLGA copolymers during recent years, ring-opening polymerization using microwave irradiation has not yet been reported. In this study, ring-opening polymerization using microwave irradiation was compared with a classical method in a stainless steel reactor.

We also investigated the suitability of PLGA–PEG1500–PLGA with a lactide/glycolide molar ratio of 5:1 as a drug delivery system to control the release of naltrexone hydrochloride and vitamin B12 at different concentrations of the copolymer and drug. The release of naltrexone hydrochloride and vitamin B12 (both water soluble with different molecular weights) from the system was investigated to determine the influence of the size of the drug on the rate and pattern of release.

MATERIALS AND METHODS

Materials

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

Synthesis and Purification of PLGA–PEG–PLGA Triblock Copolymers

Synthesis of PLGA–PEG–PLGA Triblock Copolymers Using the Classic Method

Triblock copolymers were synthesized using a ring-opening method (37) with minor modifications (36). PEG1500 (30 %, *w*/*w*) was loaded into a stainless steel reactor and stirred at 150 °C under vacuum for 2 h. Next, D,L-lactide and glycolide were loaded into the reactor at a molar ratio of either 3:1 or 5:1. The system was heated at 150 °C under vacuum for approximately 30 min, and then the catalyst stannous 2-ethylhexanoate (0.06 g) was added to the mixture, which was stirred at 250 rpm. Heating continued at 155 °C during this time. After the reaction was complete, the copolymers were dissolved in cold water (4 °C) to remove water-soluble impurities, and then they were heated to 80 °C to precipitate the polymer. The purification process was repeated three times, and then purified copolymers were dried by lyophilization.

Synthesis of PLGA–PEG–PLGA Triblock Copolymers Using Microwave Irradiation

D,L-Lactide, glycolide, PEG1500, and stannous 2-ethylhexanoate in the same amounts as described above were loaded into a flask. After sealing the system at 150 °C, the mixture was stirred at 250 rpm. Irradiation was performed at 800 W with a Milestone MicroSYNTH for approximately 5 min. Synthesized copolymers using the different methods and different ratios of LA/GA are shown in Table I.

Characterization of PLGA–PEG–PLGA Triblock Copolymers

Gel Permeation Chromatography

The molecular weights of the PLGA–PEG–PLGA copolymers and their size distributions were determined using an Agilent GPC Addon apparatus and a RID-A refractive index signal detector coupled to Plgel® columns. Tetrahydrofuran was used as an eluent with a flow rate of 1 ml/min. Polystyrene standards were used for calibration.

^1H Nuclear Magnetic Resonance

^{1}H Nuclear magnetic resonance (^{1}H NMR) spectra were collected to confirm the structure of the copolymers, to determine the LA/GA ratio, and to determine the number

Table I. Copolymers Synthesized by Different Methods and Using Different Ratios of LA/GL

	Method	LA/GA ratio
P1	Microwave irradiation	5:1
P2	Microwave irradiation	3:1
P3	Conventional method	3:1
P4	Conventional method	5:1

LA D,L-lactide, *GA* glycolide

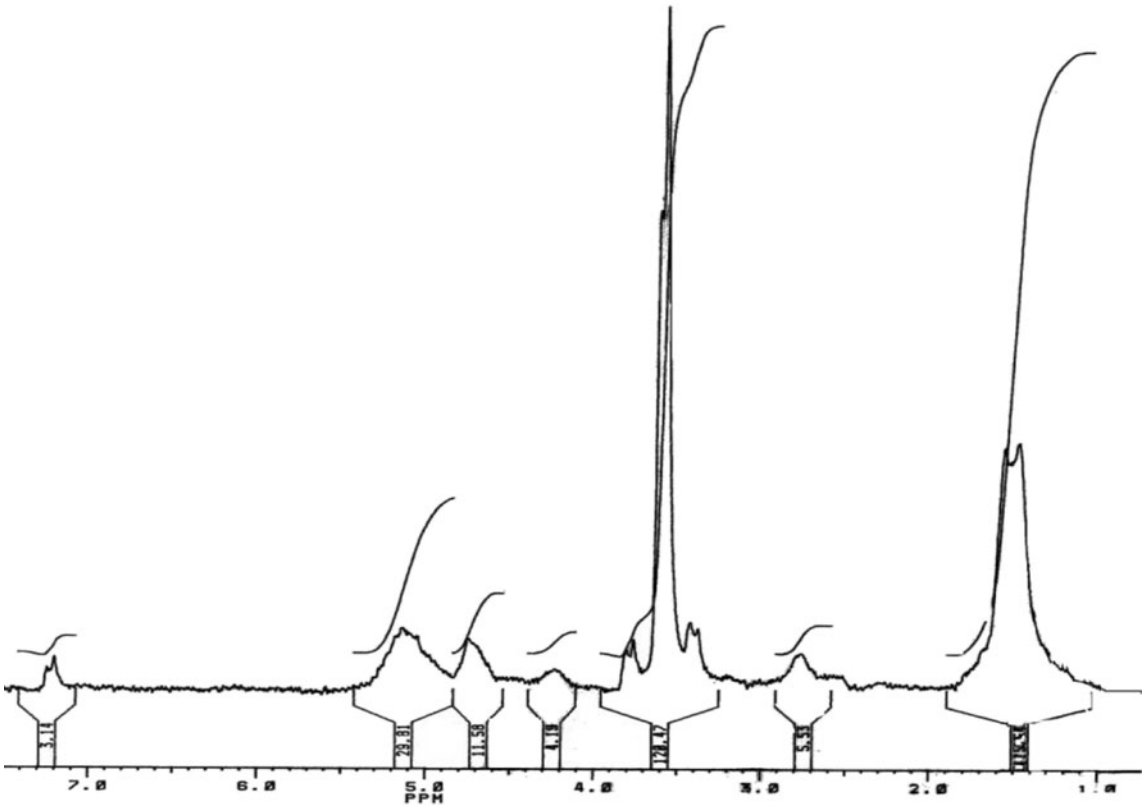

Fig. 1. ^{1}H NMR spectrum of PLGA–PEG–PLGA (5:1) copolymer (P1)

average molecular weight (M_n) of the PLGA–PEG–PLGA copolymers. ^{1}H NMR was performed in $CDCl_3$ using an NMR instrument (Bruker AC-80) at 300 MHz at room temperature. The Mn and the LA/GA ratio were determined by integration of the signals pertaining to each monomer, such as the peaks from the CH and CH_3 groups of lactide and from the CH_2 groups of ethylene glycol and glycolide (40).

FTIR Study

The structure of PLGA–PEG–PLGA was confirmed by Fourier transform infrared (FTIR) spectroscopy. The copolymer samples were dissolved in chloroform and were cast on KBr plates before FTIR analysis.

Measurement of the Phase Transition Temperature

Solutions containing 10, 15, 20, 25, and 30 % (*w*/*v*) PLGA–PEG–PLGA copolymers with LA/GA ratios of 3:1 and 5:1 were prepared in distilled water. Vials containing 1.5 ml of each copolymer solution were transferred to a water bath, and the temperature was increased at a rate of 0.5 °C/min from 4 to 60 °C. The sol–gel transition was determined by inverting the vial horizontally after keeping the sample at a constant temperature for 2 min to allow the establishment of equilibrium. The contents of the vials do not flow after inverting once the hydrogel is formed. This experiment was also performed after drug loading (0.1 and 0.5 % (*w*/*v*) of naltrexone hydrochloride and vitamin B12) of 15 and 25 % (*w*/*v*) copolymer solutions.

The sol–gel transition temperature was also determined by differential scanning calorimetry (DSC) (Mettle-Toledo, USA) to confirm the results of the inverted test tube. Aliquots of 10 μl of different concentrations of copolymer solutions were inserted into the pan and heated from 4 to 55 °C at a rate of 3 °C/min. Distilled water was used as a blank.

Critical Micelle Concentration Determination

Micelle formation was studied using our modified dye solubilization method in which iodine was used instead of DPH. One milliliter of 0, 0.001, 0.01, 0.05, 0.1, 0.25, 0.5, 1, or 2.5 wt.% copolymer solution was prepared in distilled water. Then the same amount of iodine powder was added into each vial to make a saturated solution of iodine, and the vials were incubated at room temperature (25±1 °C) for 24 h. All samples were then centrifuged at 200 rpm for 5 min. UV–vis spectra were recorded from 200 to 800 nm (UV-160A Shimadzu), and the absorbance

Table II. Copolymer Composition Determined by GPC and ^{1}H NMR

	M_n[a]	LA/GA[b]	M_n[c]	M_w[d]	M_w/M_n[e]
P3	5,264	3.15	2,940	4,270	1.45
P4	5,767	4.76	3,290	6,180	1.25

LA D,L-lactide, *GA* glycolide
[a] Number average molecular weight determined by ^{1}H NMR
[b] LA/GA ratio determined by ^{1}H NMR
[c] Number average molecular weight determined by GPC
[d] Weight average molecular weight determined by GPC
[e] Polydispersity determined by GPC

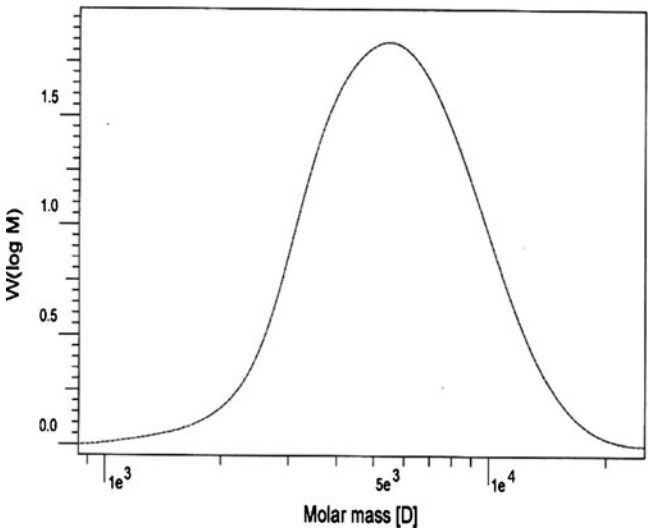

Fig. 2. GPC chromatogram of P3

of each sample was determined at 411 and 255 nm. An abrupt increase in the absorbance measurement indicated the formation of micelles. For comparison, the determination of the critical micelle concentration (CMC) was also done using the dye solubilization method with DPH (40).

Size Determination of Micelles in PLGA–PEG–PLGA Solutions

Solutions of 0.025, 2.5, and 25 wt.% of PLGA–PEG–PLGA triblock copolymer with LA/GA ratios of 5:1 were prepared in distilled water. The size of the micelles was determined by laser light scattering (Malvern Zetasizer ZS; Malvern, UK) at 25 °C in distilled water.

In Vitro Drug Release

The PLGA–PEG–PLGA (LA/GA 5:1) triblock copolymers were dissolved in phosphate-buffered saline (PBS; pH 7.4) at room temperature to make 20 and 25 wt.% solutions. Naltrexone hydrochloride and vitamin B12 were dissolved to prepare 0.1 and 0.5 % (*w*/*v*) drug-loaded copolymer solutions. The syringability of the systems was examined by passing each formulation through a 25-gauge needle at room temperature.

Then, 1 ml of each formulation was placed in a vial and incubated at 37±0.1 °C for 5 min until gelled, and 4 ml of PBS release medium was added. Copolymer hydrogels without drug were prepared as blanks. All the samples were incubated at 37± 0.1 °C and were shaken 20 rpm in a reciprocal water bath (N-BIOTEK NB-304, South Korea). Release medium samples (1 ml) were withdrawn and replaced each time to maintain sink conditions. UV–vis detection at 361 nm (UV-160A Shimadzu) was used for analysis of vitamin B12. The amount of naltrexone hydrochloride released was assayed by a reversed-phase HPLC method. Chromatographic determination of the components was performed on a Young Lin (South Korea) Acme 9000 system, which consisted of an SP930D solvent delivery module, an SDV50A solvent mixing vacuum degasser, a CTS30 column oven, a UV730 dual wavelength UV/VIS detector, and an ODSA C18 (4.6×250 mm, 5 μm) column. The UV detection wavelength was set at 281 nm. The data analysis was accomplished using Autochro-3000 software. The injection volume was 20 μl, the flow rate was 0.5 ml min^{-1}, and the column temperature was fixed at 50 °C. An isocratic method was used, and the mobile phase composition was acetonitrile/acetic acid 0.5 % (14:76, *v*/*v*).

Statistics

The results were reported as means±SDs (n=4). Statistical analysis was performed using a paired t test and one-way ANOVA. A significance level of $P<0.05$ denoted significance in all cases

RESULTS AND DISCUSSION

Characterization of PLGA–PEG–PLGA Triblock Copolymers

Triblock copolymers were synthesized effectively by ring-opening polymerization using microwave irradiation and a conventional method that made use of a stainless steel reactor. By microwave method, the reaction took relatively short times

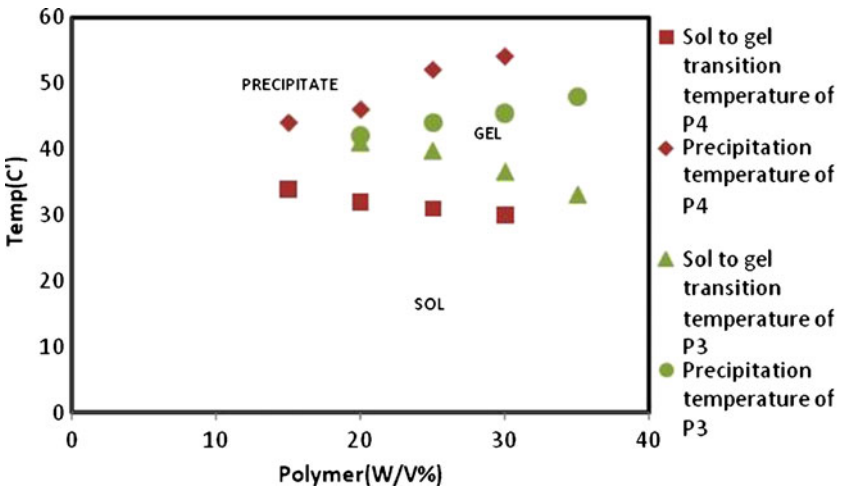

Fig. 3. Phase diagram of aqueous solutions of PLGA–PEG–PLGA copolymers

Table III. Sol-to-Gel Transition and Precipitation Temperatures of Two Different PLGA–PEG–PLGA Triblock Copolymers (LA/GA 5:1) with Different PEG/PLGA Ratios

	PEG/PLGA 0.45 (P1)		PEG/PLGA 0.35 (P4)	
Triblock copolymer concentration (%, *w*/*v*)	Sol-to-gel transition temperature (°C)	Precipitation temperature (°C)	Sol-to-gel transition temperature (°C)	Precipitation temperature (°C)
30	34.5	48.2	30	54
25	37	43.3	31.3	52
20	40	43	32.5	46.1
15	–	42.6	34.4	44.6
10	–	42	37	43

PEG polyethylene glycol, *PLGA* poly(D,L-lactic-co-glycolic acid)

and also the side reaction was limited, and consequently, greater yields were usually obtained. The result indicated that prolonged microwave irradiation did not significantly change the M_w of the copolymer.

A typical spectrum of the PLGA–PEG–PLGA copolymer (P1) is shown in Fig. 1. This figure is very similar to the previously reported spectrum (39). The complicated split in these peaks is due to the random copolymerization of the glycolide and lactide. The characteristic signals appearing at 5.2, 4.8, 4.3, 3.5, 2.6, and 1.5 ppm are from the CH of LA, the CH_2 of GA, a CH_2 of PEG, another CH_2 of PEG, and the OH and CH_3 of LA, respectively. The LA-to-GA ratio determined by ^{1}H NMR matched very well with the initial ratios of the monomers that were used in the polymerization process. The number average molecular weights (M_n) of P1 and P2 were determined to be 4,684 and 4,495, respectively, according to the ^{1}H NMR spectrum.

Table II presents the results from ^{1}H NMR and gel permeation chromatography (GPC) analysis of P3 and P4. As indicated in Table II, the molecular weight increased as the LA/GA ratio increased. The polydispersity of the copolymers was found to be approximately 1.45 and 1.25 for P3 and P4, respectively.

Figure 2 shows a typical GPC chromatogram of P3, which is a nearly symmetric peak. The unimodal GPC trace with the low polydispersity of the triblock copolymers suggested that the purity was sufficient to study the physical properties of the copolymers. IR spectra of PLGA–PEG–PLGA clearly illustrated the presence of both PEG and PLGA blocks.

A typical phase diagram demonstrating the phase transition behaviors of aqueous solutions of P3 and P4 is shown in Fig. 3. An increase in the copolymer concentration from 15 to 35 % (*w*/*v*) and an increase in the molar ratio of LA/GA from 3:1 (P3) to 5:1 (P4) caused a decrease in the phase transition temperature. At all concentrations, there was a significant difference ($P<0.05$) between the sol–gel transition temperatures of P3 and P4. The critical gel concentration gradually decreased from 20 % to lower than 15 % (*w*/*v*) by changing the LA/GA ratio from 3:1 (P3) to 5:1 (P4).

PLGA–PEG–PLGA triblock copolymers are thermosensitive copolymers composed of hydrophobic PLGA and hydrophilic PEG blocks. Lactide is a hydrophobic monomer that is used to produce PLGA. According to the iceberg theory, when hydrophobic molecules are surrounded by water molecules, the entropy of the system decreases. Therefore, hydrophobic molecules aggregate to decrease their exposed surface area in an aqueous environment. In this system, the triblock copolymers form micelles to decrease the exposed surface area of hydrophobic PLGA segments to water. Before increasing the temperature, because of hydrogen bonding between hydrophilic PEG segments and water molecules, the system is a sol. In this condition, monomers, individual micelles, and grouped micelles all exist in the aqueous environment. As the temperature increases, the hydrogen bonds become weaker, and hydrophobic bonds between the PLGA segments begin to form a hydrogel network by forming bridges between the micelles. After a specific temperature, which may be different for different systems depending on chemical structure, molecular weight, and the concentrations of triblock copolymers, phase separation occurs, and the copolymers are phased out.

According to the results, with increasing copolymer concentration, the sol–gel transition occurred at lower temperatures due to an increase in the number of polymer–polymer interactions at the higher concentrations. Higher LA/GA ratios also increased the hydrophobic interactions between PLGA segments and caused a decrease in the sol–gel transition temperature and precipitation at higher temperatures (Fig. 3).

Table III shows the sol-to-gel transition and precipitation temperatures of two different PLGA–PEG–PLGA triblock

Table IV. Effect of Drug Loading on the Sol–Gel Transition Temperature of P4

		Sol-to-gel transition temperature (°C) after drug loading			
		B12 (%, *w*/*v*)		Naltrexone (%, *w*/*v*)	
Triblock copolymer concentration (%, *w*/*v*)	Sol-to-gel transition temperature (°C) before drug loading	0.1 %	0.5 %	0.1 %	0.5 %
25	31.2	30	29	31	31
15	34.4	33	32	34	33.5

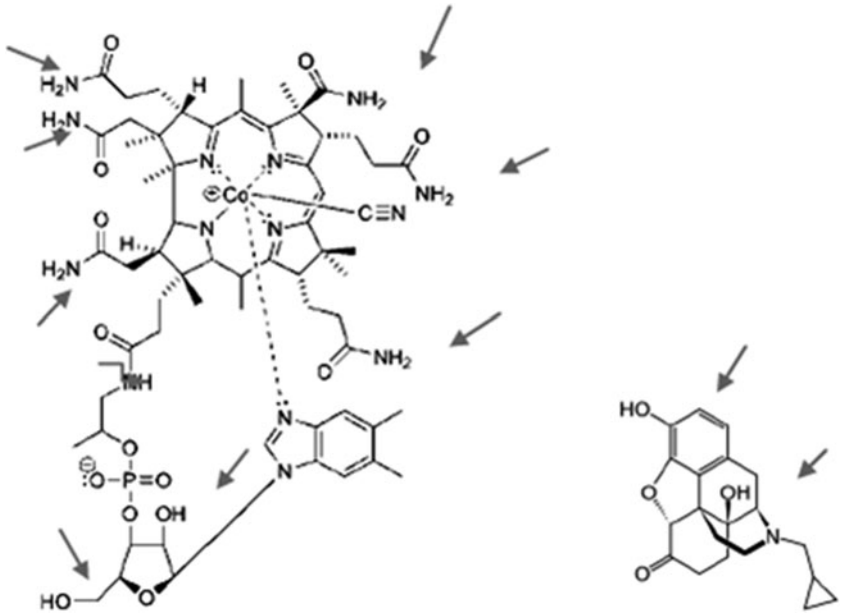

Fig. 4. The chemical structure of vitamin B12 (*left*) and naltrexone (*right*)

copolymers (P1 and P4) with different PEG-to-PLGA ratios due to the polymerization method used. By increasing the PEG/PLGA ratio, the sol–gel transition temperature increased, and the precipitation temperature decreased. By increasing the proportion of the hydrophilic segment (PEG) of the triblock copolymers, the amount of hydrogen bonding between the water molecules and PEG increases. As a result, the breaking of these bonds and the subsequent formation of hydrophobic bonds between PLGA segments occurred at higher temperatures.

Solutions of 20 and 25 % (*w*/*v*) copolymers with LA/GA ratios of 5:1 seemed to be the most ideal *in situ* gelling matrices for drug delivery systems according to the sol–gel transition temperatures. These solutions were fluid at room temperature and turned into a gel network at body temperature; thus, these solutions would not need to be cooled or warmed before injection. Gelation began from a solution of 15 % (*w*/*v*) copolymers. The phase separation temperatures of all copolymers were above 42 °C, indicating that none of these copolymer solutions exhibited phase separation at normal body temperature.

Table IV shows the influence of drug loading on the sol–gel transition temperatures of copolymer solutions. These temperatures decreased after loading the copolymer solutions with vitamin B12. In contrast, the gelation temperatures did not change significantly after loading the copolymer solutions with naltrexone hydrochloride.

As shown in Fig. 4, in contrast to naltrexone hydrochloride, vitamin B12 is a large molecule that can form many hydrogen bonds with water molecules in aqueous solutions. The migration of water toward the drug lowers the activity of water and favors hydrophobic interactions between the PLGA segments of the copolymers. This behavior may shift the gelation temperatures of the copolymer solutions to lower temperatures. The data did not show a significant influence of naltrexone on the sol–gel transition temperatures, likely because fewer hydrogen bonds form between naltrexone and water than between vitamin B12 and water. The arrows in

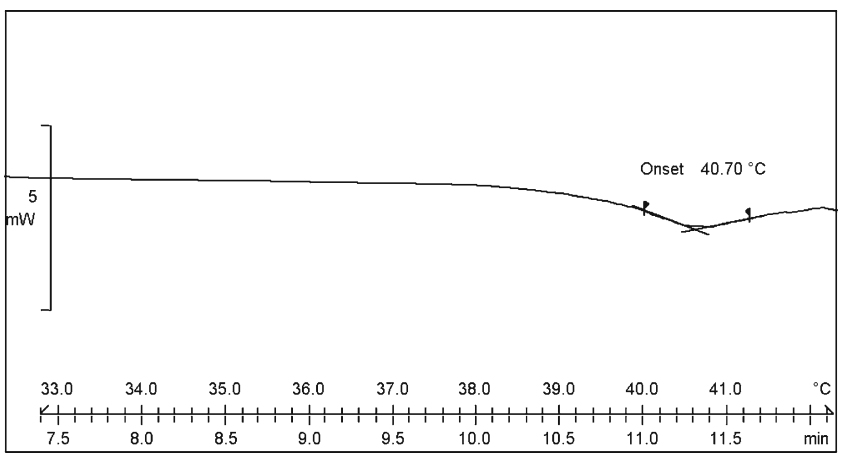

Fig. 5. DSC chromatogram of a P3 solution (25 %) of PLGA–PEG–PLGA

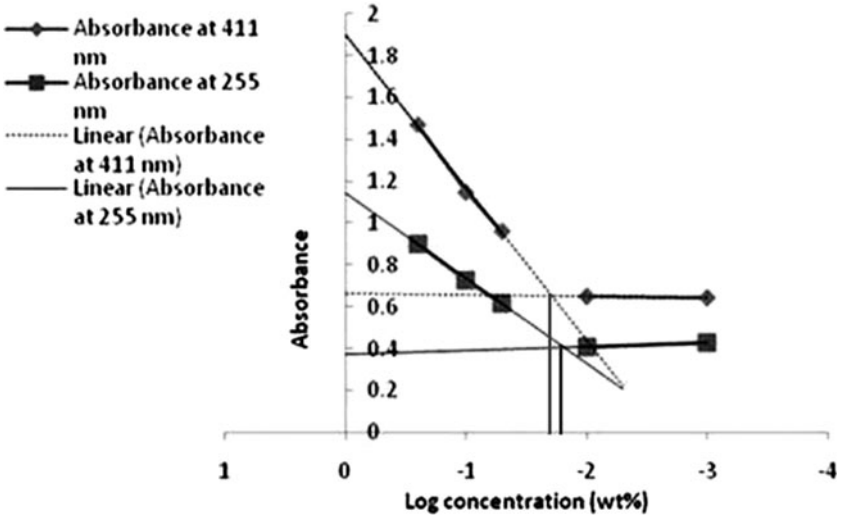

Fig. 6. Absorbance diagram of iodine copolymer solutions at different concentrations of PLGA–PEG–PLGA

Fig. 4 show the sections of the molecules that participate in hydrogen bonding.

Another mechanism that may favor gelation is the ability of vitamin B12 to act as a cross-linker between the hydrophilic segments of copolymers through hydrogen bonding. The DSC results of P3 (25 %, *w*/*v*) are shown in Fig. 5. An endothermic peak was observed between 39 and 41.8 °C. The onset of the peak (40.7 °C) was determined by the intersection point of the tangents of the baselines and the downward slope of the peak. The sol-to-gel transition temperature that was obtained by

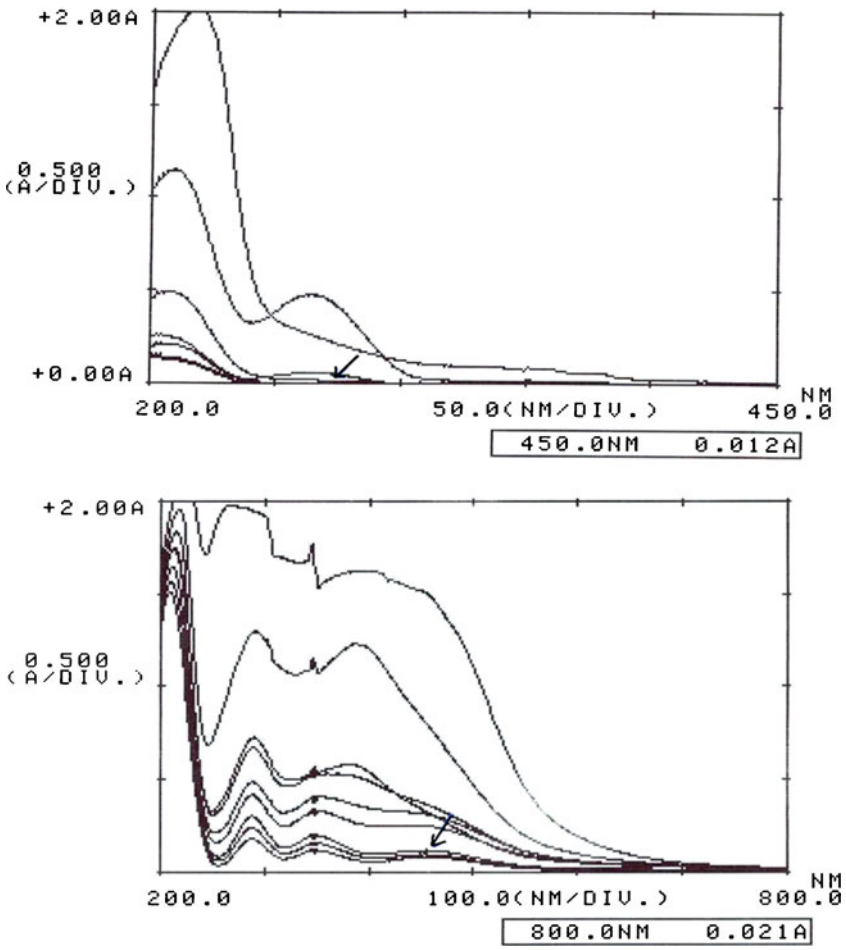

Fig. 7. Absorbance diagram of DPH (*first picture*) and iodine (*second*) solutions in the presence of P4. The *arrows* show the abrupt increase in absorbance, indicating the CMC

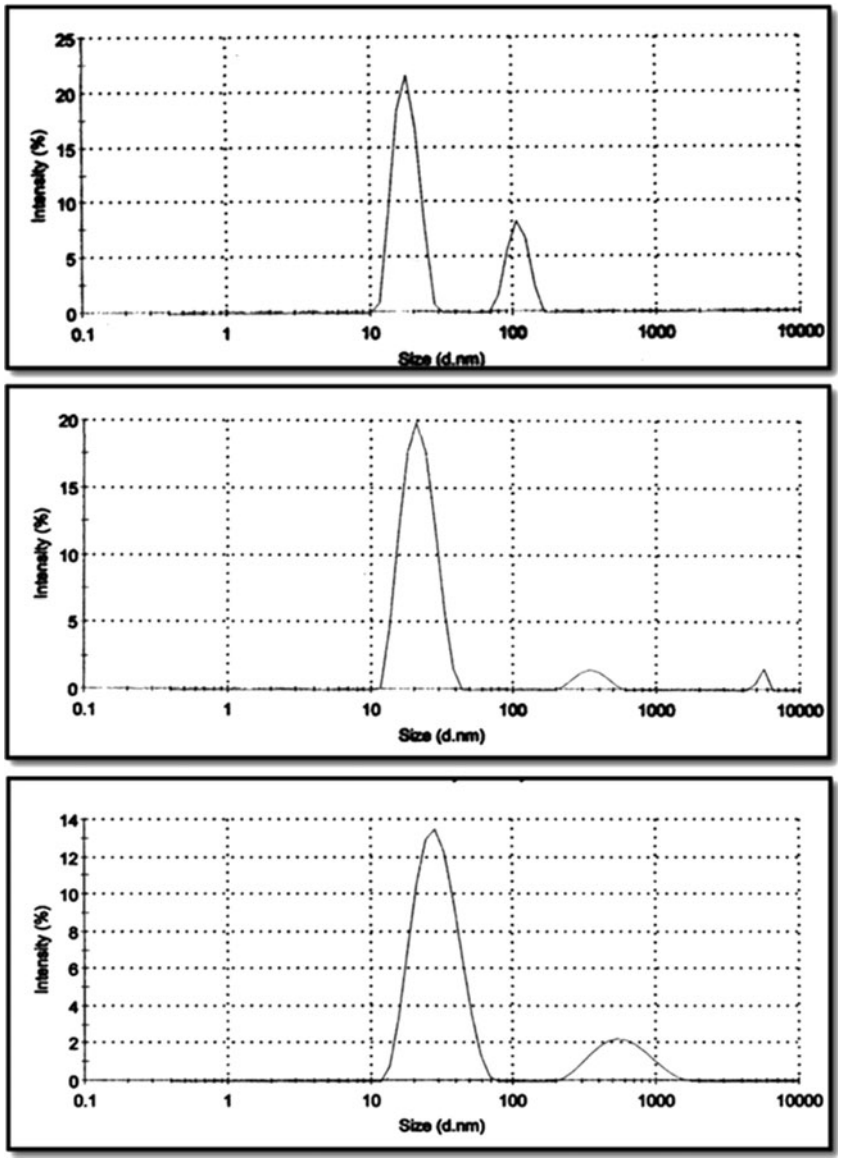

Fig. 8. Size distribution of P4 solutions of different concentrations (P4 concentrations **a**–**c** were 0.025, 2.5, and 25 wt.%, respectively)

DSC was in good agreement with that determined by the inverted test tube method described above.

As discussed above, these copolymers associate as micelles in water due to the presence of both hydrophobic and hydrophilic segments in their structures. The formation of PLGA–PEG–PLGA micelles was confirmed by a solubilization method, shown in Figs. 6 and 7. In a hydrophobic environment, iodine has a higher absorbance (at 411 and 255 nm)

Table. V. Size Distribution of P4 Solutions of Different Concentrations

Copolymer concentration (wt.%)	*Z*-average (nm)	PDI
0.025	18.27	0.313
2.5	26.68	0.273
25	32.16	0.218

PDI polydispersity index

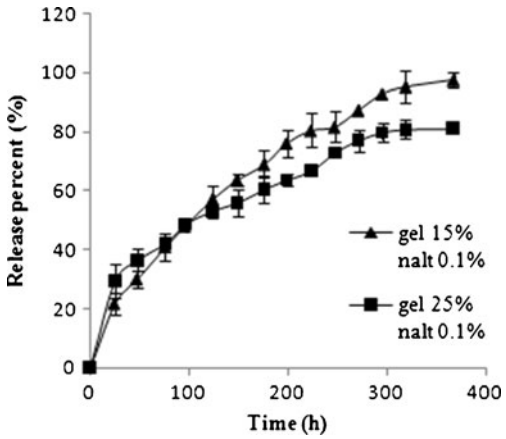

Fig. 9. Effect of the copolymer concentration on naltrexone hydrochloride (0.1 %, *w*/*v*) release

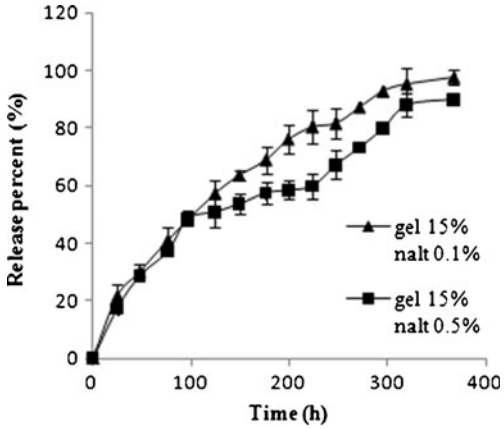

Fig. 10. Effect of the drug concentration on naltrexone hydrochloride release

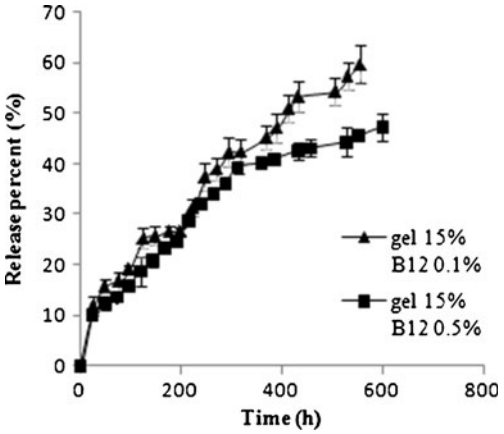

Fig. 12. Effect of the drug concentration on B12 release

than it does in an aqueous solution due to its higher solubility in hydrophobic media. The addition of iodine to solutions of PLGA–PEG–PLGA caused preferential partitioning of iodine molecules into the hydrophobic core of micelles. This partitioning caused the absorbance of iodine to increase. As the concentration of the copolymer increased, the number of micelles increased, and as a result, the absorbance of iodine also increased. As shown in Fig. 6, the absorbance of iodine was plotted against the logarithm of different concentrations of P4. The abrupt increase in absorbance reflected the micelle formation. According to this technique, the CMC (i.e., the concentration at which micelles are formed) of PLGA–PEG–PLGA with an LA/GA ratio of 5:1 (P4) was approximately 0.017 wt.%. Such a low CMC confirmed the stability of the micelles against the possible dilution of the drug delivery system in the body. Because iodine was used to determine the CMC for the first time, a dye solubilization method using DPH was also performed to validate the results (Fig. 7). The results of both methods were in good accordance.

The size distribution of the micelles is shown in Fig. 8 and Table V. When increasing the concentration of copolymer (P4), the sizes of the micelles increased. Below the sol–gel transition temperature, the two distinct peaks were assigned to individual micelles of approximately 20 nm in diameter and grouped micelles (>100 nm) (group 1) and bridged structures of micelles (>1,000 nm) (group 2).

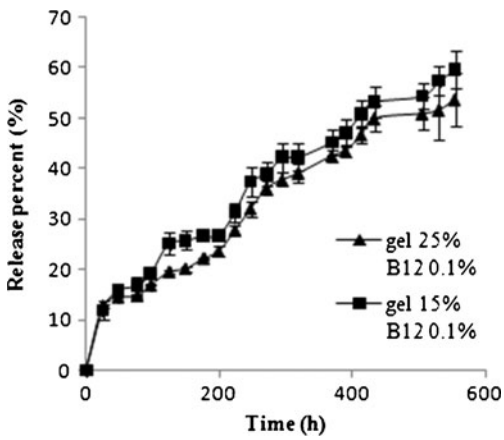

Fig. 11. Effect of the copolymer (P4) concentration on B12 release

All formulations easily passed through the 25-gauge needle at room temperature. Drug release from hydrogel occurs by two principal mechanisms: drug diffusion from the hydrogel during the initial release phase and drug release by the erosion of the hydrogel matrix during the later release phase (33).

In Vitro Release Study

The *in vitro* release of naltrexone hydrochloride and vitamin B12 from the triblock copolymer (P4) hydrogels is shown in Figs. 9, 10, 11, 12 and 13. The amount of drug released during the first day was treated as the burst release. We observed burst releases of less than 10 and 20 % for vitamin B12 and naltrexone, respectively.

The burst release is considered to be the result of the drug located near the surface. The lower burst release and release slop of vitamin B12 relative to naltrexone hydrochloride showed that the sol–gel transition at the surface and bulk erosion predominantly controlled vitamin B12 release from this system, rather than diffusion (Fig. 13).

In Table V, the drug release data were fit according to zero-order and Higuchi kinetic models of drug release to evaluate whether the drug release was through bulk erosion (zero or first order) or diffusion (Higuchi). As shown in Table II, all of the B12 formulations exhibited zero-order

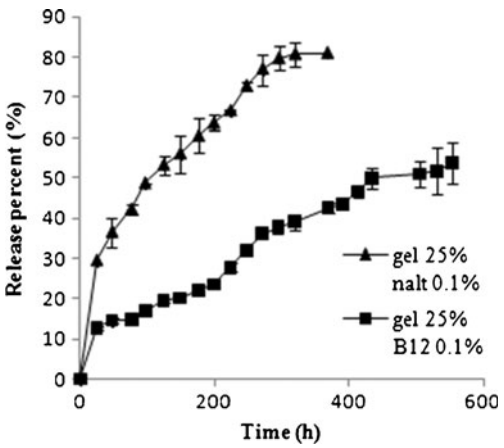

Fig. 13. Release of naltrexone hydrochloride and B12 from the hydrogel prepared with 25 wt.% (P4) copolymer

Table VI. Kinetic Profile of Naltrexone Release from P4

Drug	Drug concentration (%, *w*/*v*)	PLGA–PEG–PLGA concentration (%, *w*/*v*)	Zero order		Higuchi	
			Slope	R^2	Slope	R^2
Naltrexone	0.1	15	0.2512	0.9316	5.5003	0.9933
		25	0.1882	0.8779	4.3456	0.9915
	0.5	15	0.2163	0.932	4.6962	0.9767
		25	0.192	0.9297	4.1730	0.9766
Vitamin B12	0.1	15	0.0903	0.9788	2.5987	0.9762
		25	0.0852	0.9749	2.4071	0.9578
	0.5	15	0.068	0.9252	2.092	0.9757
		25	0.074	0.9576	2.0545	0.9600

PLGA poly(D,L-lactic-co-glycolic acid), *PEG* polyethylene glycol

release profiles, except for the formulation with 0.5 % drug and 15 % copolymer.

Vitamin B12 is a large molecule with many hydrophilic groups, such as NH_2 and OH (Fig. 4). The large molecules do not diffuse easily through the small pores of the PLGA–PEG–PLGA hydrogel. In addition, vitamin B12 may remain in the hydrogel because of the hydrogen bonding between the drug and the hydrophilic domain of the copolymer; therefore, this drug mainly is released by copolymer degradation. However, high drug concentration gradients and increased porosity among the hydrogel chains with lower concentrations of copolymer can allow B12 to diffuse out of this system, as we observed for the formulation with 15 % copolymer and 0.5 % drug.

Naltrexone release was mainly controlled by a diffusion mechanism (Higuchi model) (Table VI). Naltrexone was released from these formulations for more than 15 days. Naltrexone hydrochloride is a small water-soluble salt, and because of the large hydrophobic section (lactide) of PLGA–PEG–PLGA (LA/GA 5:1), the naltrexone hydrochloride diffusion rate through the system was greater than the hydrogel bulk erosion rate. Drug molecules that were distributed in the hydrophilic domain (PEG1500) were released by diffusing through the hydrophilic channels of the hydrogel.

We found that with increasing drug concentrations, the release rate decreased. Increasing hydrophilic drug concentrations causes a surface gel-to-sol transition. Additionally, the copolymer erosion rate decreases because of a salting-out effect and a decrease in water activity because of the drugs. Also, by increasing the drug concentration, the copolymer hydrogel viscosity increases, and it can also cause a slower release by diffusion (34).

Increasing the PLGA–PEG–PLGA concentration in the formulations significantly ($P<0.05$) reduced the release rate of naltrexone in the *in situ* forming system. Increasing the copolymer concentration induced more cross-links between the copolymer molecules, lowered the hydrogel network porosity, increased the tortuosity and viscosity of the hydrogel, and finally lowered the drug diffusion rate. The copolymer concentration did not have a significant effect on the release profile of vitamin B12. The copolymer degradation kinetic profile was zero order, and it did not depend on the initial concentration of the copolymer. We found that the drug molecules with high M_w, such as B12, released more slowly than the smaller drug molecules, such as naltrexone hydrochloride, from the PLGA–PEG–PLGA hydrogel.

CONCLUSION

Biodegradable, thermogelling PLGA–PEG–PLGA copolymers with different compositions were synthesized for use as controlled-release systems using two different methods, a conventional method with a reactor and a method using microwave irradiation. The synthesis of triblock copolymers using microwave irradiation was much faster than that using conventional heating. We found that the copolymer structure could control the phase transition temperature. The copolymer concentration played a critical role in controlling the sol–gel transition temperature, the CMC, and the size of the micelles in the copolymer solution. This study confirmed that the drug release rates and the release mechanisms can be changed by the size and M_w of the drug-loaded molecules and copolymer concentrations in formulations. PLGA–PEG1500–PLGA (LA/GA 5:1) is a suitable system for the long-acting, controlled-release delivery of naltrexone and peptide drugs.

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