Thermosensitive Nanoparticles Prepared from Poly(*N*-isopropylacrylamide-acrylamide-vinilpyrrolidone) and its Blend with Poly(lactide-*co*-glycolide) for Efficient Drug Delivery System

Roya Salehi,[1] Soodabeh Davaran,[2] Mohammad R. Rashidi,[3] Ali Akbar Entezami[1]

[1]*Laboratory of Polymer, Department of Organic Chemistry, Faculty of Chemistry, University of Taabriz, Tabriz, Iran*
[2]*Department of Biomedical, Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Science, Tabriz, Iran*
[3]*Department of Polymer Synthesis, Drug Applied Research Center, Tabriz University of Medical Science, Tabriz, Iran*

Received 12 November 2007; accepted 14 August 2008
DOI 10.1002/app.29199
Published online 6 November 2008 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Temperature-responsive polymers have become increasingly attractive as carrier for the injectable drug delivery systems. In the present work, we have studied the preparation of poly(*N*-isopropylacrylamide-acrylamide-vinilpyrrolidone) (NIPAAm-AAm-VP terpolymer) nanoparticulated terpolymer and its blend with poly(lactide-*co*-glycolide, PLGA; molar ratio of lactide/glycolid 1/3). Thermosensitive terpolymer, poly(NIPAAm-AAm-VP) was prepared by free-radical polymerization in aqueous solution. The nanoparticles of poly(NIPAAm-AAm-VP) and its blend with PLGA containing naltrexone were prepared using the evaporation and w/o emulsion-solvent evaporation methods, respectively. Nanoparticles prepared from terpolymer-PLGA blend at low polymer concentration (5%) shows larger particle size (>300 nm) and higher drug content%. Various types of nanoparticles showed a burst release of less than 10% after **24 h**. The results suggest that by regulating different variables, desired release profiles of naltrexone can be achieved using a blend of PLGA-poly(NIPAAm-AAm-VP) nanoparticulate system. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 1905–1910, 2009

Key words: thermosensitive; nanoparticles; poly(*N*-isopropylacrylamide); poly(lactide-*co*-glycolide); blend; naltrexone; drug delivery

INTRODUCTION

Over the past years, temperature and pH-sensitive hydrogels was designed and developed for the fabrication of "smart" or "intelligent" drug delivery systems (DDS).[1–8] These hydrogels (stimuli-sensitive hydrogels) change their structure and physical properties in response to their surrounding environment.[9–11] Example of this behavior includes change in swelling ratio and lower critical solution temperature (LCST) of the temperature- and pH-sensitive hydrogels.[12–14] Temperature-responsive polymers have become increasingly attractive as carrier for the injectable drug delivery systems.[15,16] One of the most widely investigated temperature-sensitive polymers are poly(*N*-isopropyl acrylamide) (PNIPAAm) and related copolymers.[17,18] Also numerous studies and promising results of the thermosensitive hydrogels and thermosensitive polymeric nanoparticles as drug carriers have been reported.[19,20]

Subject of the present article is to develop an improved method for preparation of thermosensitive biocompatible nanoparticles for the purpose of serving as drug delivery systems in particular for injectable systems. Preferably, the biocompatible polymer is a polymer which can be readily melted or used in a solvent deposition process for preparing nanoparticles. If the polymer can be readily melted this is quite convenient, because it must not be dissolved and can be directly mixed intimately with the biocompatible interacting agent. During the melting process, the polymer should not be decomposed or start to decompose. When the biocompatible polymer is to be dissolved, preferably organic solvents are used. In particular, the biocompatible polymer is aliphatic polyester, such as poly ε-caprolactone, polylactide or polyglycolide, or a copolymer thereof. Preferably, poly(D, L) lactide or poly(D, L lactide-*co*-glycolide) are used in particular in a 50 : 50% molar ratio. The stimuli-responsive nanoparticles described in present article are obtained using a thermosensitive PNIPAAm terpolymer blend with PLGA. These polymeric blends were obtained by mixing a biodegradable polymer able to form nanoparticles (poly lactide-*co*-glycolide, PLGA) and a thermosensitive

Correspondence to: A. A. Entezami (aaentezami@yahoo.com or aentezami@tabrizu.ac.ir).

sol-gel system (PNIPAAm terpolymer) for making the polymeric blend acceptable for a injectable drug delivery. The thermosensitive polymer used in present work is poly *N*-isopropylacrylamide-acrylamide-vinyl pyrrolidone terpolymer [P(NIPAAm-AAm-VP)] and the biodegradable polyester is poly lactide-*co*-glycolide (PLGA; LA: GA 3 : 1). The hydrophilic/lipophilic balance of the blend can be controlled by the comonomer molar ratio of terpolymer, the molecular weight of PLGA. This polymeric blend was used to prepare the nanoparticles containing naltrexone.[21]

Naltrexone is an opiate antagonist used mainly as an adjunct to prevent relapse in detoxified opioid-dependent patients. It is currently given orally as tablets or capsules in daily dose of 50 mg. naltrexone is orally active with a relatively short half-life and subject to extensive hepatic first-pass metabolism.[22] Slow release naltrexone formulations are provided for use in the treatment of alcoholics and heroin addicts and such other indications for which naltrexone has been found to be efficacious. In present work, we have studied the preparation of poly(*N*-isopropylacrylamide-acrylamide-vinylpyrrolidone) (PNIPAAm-AAm-VP) terpolymer and its blend with poly(lactide-*co*-glycolide) (PLGA) with molar ratio of lactide/glycolide 3/1. Naltreoxone loaded nanoparticles were prepared by solvent evaporation method.

EXPERIMENTAL

Materials and methods

Poly(DL-lactide-*co*-glycolyde, PLGA) with a weight average molecular weight of 75,000–120,000, whose copolymer ratio of DL-lactide to glycolide is 3 : 1 and naltrexone hydrochloride were purchased from Sigma (St Louis, MO). NIPAAm purchased from Fluka and was purified by recrystallization in hexane and dried under vacuum at 25°C. VP (Merck Chemical) was freed from stabilizer by twice vacuum distillation with continuous bubbling argon. AAm (Fluka) was purified by recrystallization in $CHCl_3$ and dried under vacuum at 25°C. Initiator ammonium persulfate (APS) (Aldrich Chemical) was purified by recrystallization in$EtOH/H_2O$ (2/1) solvent mixture and dried under vacuum at 40°C and the accelerator N, N, N′, N′-tetramethyl ethylene diamine (TEMED) (Fluka) was used as supplied.

The structure of copolymers were characterized by FT- IR (Shimadzu 8400),^{1}H NMR (Bruker AC 80).

Preparation of poly(Nipaam-AAm-VP) terpolymrs

Terpolymers of NIPAAm - AAm - VP were synthesized by free-radical polymerization method. Water soluble NIPAAm, AAm, and VP monomers were used in 86 : 9.5 : 4.5*M* ratios. The monomers were dissolved in 13 mL of distilled water. APS (0.3 mol% with respect to the monomers) was added. The mixture was magnetically stirred and degassed with argon for 30 min. Then 30 μL of TEMED was added as an accelerator. The polymerization was carried out at room temperature for 16 h with continuous argon bubbling. The obtained reaction mixture was purified by dialysis for 5 days. Then polymer solutions precipitated by heating the polymer solutions at temperature above LCST.

Preparation of polymer blend

PLGA (200 mg) and PNIPAAm-AAm-VP (200 mg) were dissolved in 5 mL of methylene chloride at room temperature for 15 min. The organic solvent was evaporated under nitrogen at room temperature and then at 40°C. This polymer blend could be used immediately or stored at 4°C.[21]

Preparation of naltrexone loaded poly(Nipaam-AAm-VP) nanoparticles

To prepare naltrexone-loaded nanoparticles, solvent evaporation method was employed. Appropriate amounts of polymer were added to 5 mL THF to provide concentration of 5, and 10% w/w. Then, appropriate amount of naltrexone were dissolved in the polymer solution to give theoretical drug loading of 5%. Then, the solution was slowly added into 80 mL of chilled deionized water with sonication (200 W) by using probe type ultrasonic generator. After formation of naltrexone-loaded polymeric nanoparticles, the organic solvent was removed by evaporation under reduced pressure. The nanoparticles suspension was concentrated by ultrafiltration with 50 kDa membrane (poly ether sulfon, purchased from Millipore). Finally, the concentrated nanoparticles solution was filtered and lyophilized. Particle morphology was studied with scanning electron microscopy (SEM). Particle size was determined with a laser diffraction particle size analyzer (Shimadzu SALD 2101).

Preparation of naltrexone loaded poly(Nipaam-AAm-VP)–Plga nanoparticles

Appropriate amounts of polymer blend were added to 10 mL methylene chloride to provide concentration of 5%, and 10% w/w; then appropriate amount of naltrexone were dissolved in the polymer solution to give theoretical drug loading of 5%. The solution was then added dropwise to aqueous phase solution containing 0.5% poly vinyl alcohol (PVA). The mixture was stirred by a high-speed hemogenizer

(Edmund Buhler HO 4AP) to form a stable emulsion. The organic solvent was evaporated and the suspension obtained was purified by ultrafiltration with 50 kDa membrane. Finally, the concentrated nanoparticles solution was filtered and lyophilized.

Drug loading and drug release studies

To measure drug loading efficiency, the naltrexone loaded nanoparticles were dissolved in phosphate buffer (pH 7.4). The drug content was determined using a high performance liquid chromatography (HPLC) method. The HPLC system consisted of a Waters 515 pump, automatic injector (7plus Waters autosampler), and Waters 2487 dual λ absorbance detector. Chromatographic separation was achieved using a Nucleosil (Phenomenex) ODS 5 μm (25 cm × 4.6 mm, i.d.) column and phosphate buffer (25 m*M*, pH 6.5)/acetonitrile (53/47) as the mobile phase at a flow rate of 1.0 mL/min. The UV detection was at 282 nm. The run time for the assay was 10 min and the retention time for naltrexone was 5.5 min. Naltrexone loading efficiency of the nanoparticles was defined as following:

Drug incorporation efficiency was expressed both as drug content (%w/w) and drug entrapment (%). The individual values for three determinations and their mean values are reported.

$$\text{Drug content (\%w/w)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of nanoparticles}}$$

$$\text{Drug entrapment (\%)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of drug used in formulation}}$$

Drug release studies

The in vitro release of naltrexone from nanoparticles was carried out at 37°C. A 5 mL suspension of the naltrexone containing nanoparticles (40 mg nanoparticles in 5 mL PBS) was taken in to a cellulose acetate dialysis membrane (Sigma, 2000 Da), and the dialysis membrane was allowed to float in a beaker containing 30 mL of phosphate buffered saline (PBS; pH = 7.4, *M* = 0.1). The beakers were placed in a shaker incubator maintained at 37°C. Three millilitre samples were removed from the external buffer solution and was replaced with fresh PBS. The naltrexone released in to the medium was analyzed using an HPLC assay as described above.

All release studies were carried out in triplicate. The results were presented in the terms of cumulative release as a function of time:

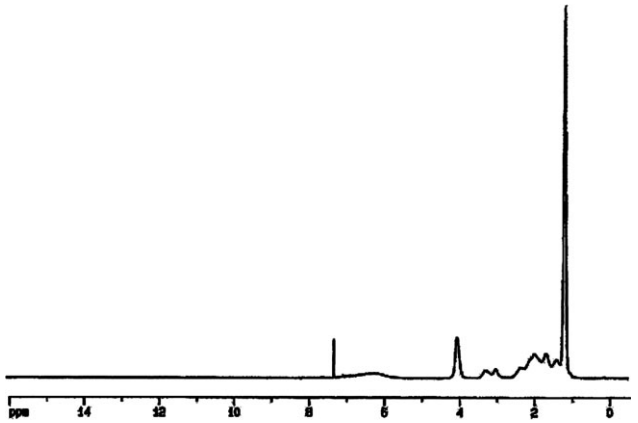

Figure 1 The ^{1}H NMR spectra of poly(NIPAAm-AAm-VP) in D_2O.

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

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

Glass transition determination

The glass transition temperature (T_g) of vacuum dried polymers were investigated by DSC (Mettler-Toledo model 822). Pans containing about 8 mg of vacuum dried gels heated from 40 to 200°C at 20°C min^{-1}, cooled back to 50°C at 10 °C min^{-1}, and heated again to 200°C at 20 °C min^{-1}. *Tg* (average of two measurements from the second heating segment) was considered at the mid-point temperature of the exothermic drift in the heating curves.[23]

RESULTS AND DISCUSSION

Characterization of copolymers

The structure and composition of terpolymer were established by ^{1}H NMR in $CDCl_3$ (Fig. 1).

Several characteristic peaks from NIPAAm, AAm and VP were overlapped. But the signals pertaining to NIPAAm are found in 1.15 ppm; $(CH_3)_2CH$, 3. 9 ppm; N—CH—$(CH_3)_2$. and the signals pertaining to VP are found in, 3.2 ppm (N—CH_2).

Figure 2; exhibit the FTIR spectra of the gel. As shown in the Figure 2, strong peaks in the range of 800–1000 cm^{-1} correspond to the stretching mode of vinyl double bonds disappeared in the spectrum of polymer indicating that polymerization has taken place. And intense peak at 3437 cm^{-1} can be attributed to the —NH_2 group of AAm. The C—H

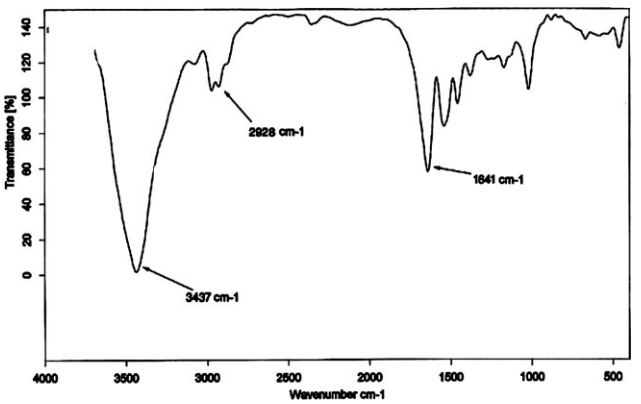

Figure 2 FTIR spectra of poly(NIPAAm-*co*-AAm-*co*-VP).

stretching vibration of the polymer backbone is manifested through strong peak at 2928 cm^{-1}. Peak at 1641 cm^{-1} corresponded to C=O stretching from all three monomer units.

Figure 3 shows the DSC thermograme of poly (Nipaam-AAm-VP) (3A) and its blend with Plga (3B).

As shown in the Figure 3, terpolymer has a single transition temperature (Tg) between the Tg values of corresponding homopolymers.[14] The blend shows two *Tg* values corresponding to terpolymer and PLGA components (52°C corresponds to PLGA and 140°C corresponds to terpolymer).

As mentioned above, to prepare thermosensitive nanoparticles, a specially designed PNIPAAm terpolymer was used. The monomers were chosen because (i) PNIPAAm hydrogel swollen in water is a typical temperature-sensitive hydrogel that exhibits a volume phase transition in response to temperature changes at around 33°C. (ii) AAm is a versatile hydrophilic comonomer, but its homopolymer does not show a volume phase transition temperature in water. Introduction of AAm component improves the mechanical strength of PNIPAAm hydrogels. (iii) Poly(1-vinyl-2-pyrrolidone) (PVP) is one of the hydrophilic monomers, which is nontoxic in nature and is of particular interest to research for its affinity to water.[23,24]

Different formulation parameters used for the preparation of nanoparticles. Their characterization for mean particle size and drug loading are described in Table I. Nanoparticles were prepared through a solvent evaporation method for poly(NIPAAm-AAm-VP) nanoparticles and a modified oil-in-water emulsion-solvent evaporation method for poly(NIPAAm-AAm-VP) - PLGA nanoparticles. In this process, the separation of nanoparticles in the liquid phase is achieved by filtration. The nanoparticles are separated from the original liquid phase to eliminate the organic solvent(s) and the nonencapsulated drug. Preferably, the filtration is carried out at 25°C with an ultrafiltratio membrane.

Different polymer concentration and polymer type were used in formulations to examine its effect on the properties of the particles formed. The composition of the polymer blend (weight ratio of the two polymers) had an effect on the average particle size. The process conditions were adjusted so that nanoparticles of a size of 200–350 nm (average size) were formed (PLGA: terpolymer 0 : 100 and 50 : 50). By increasing the PLGA contents of the blend above 50%, the particle size increased (above 500 nm). By decreasing the PLGA contents of the blend below 50% the encapsulation efficiency was decreased (below 30%). Selected formulations are given in Table I. Figure 4 shows SEM micrographs of thermosensitive nanoparticles. The SEM micrographs present the spherical morphology of particles; these particles were similar to the particles in which the mean diameters, measured by particle size analyzer (PSA), were in the range of 198–360 nm.

Figure 4 shows the typical SEM micrographs of the naltrexone loaded nanoparticles.

Nanoparticles prepared by 10% polymer concentration tend to have a large size (more than 240 nm for terpolymer and approximately 540 nm for its

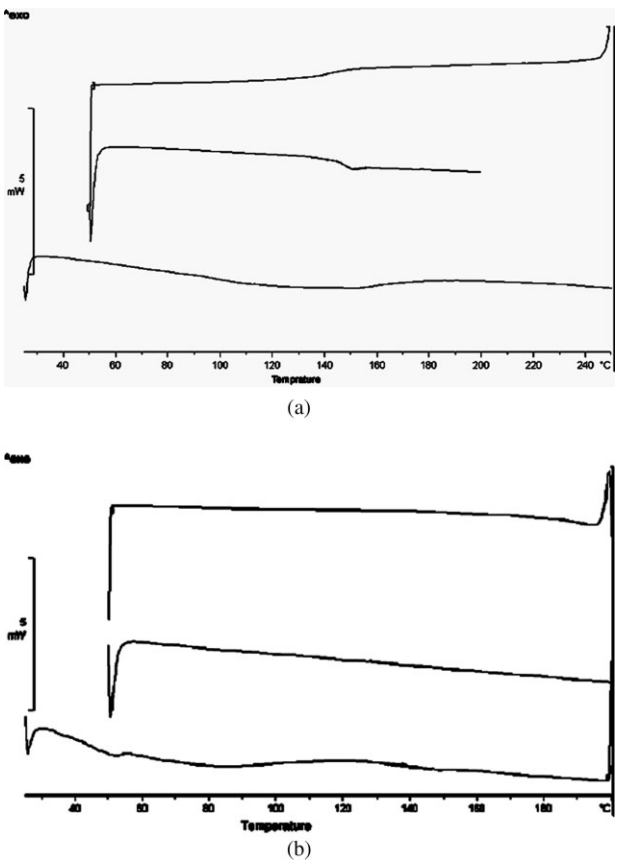

Figure 3 The DSC thermograme of poly(NIPAAm-AAm-VP) (3A) and its blend with PLGA (3B).

TABLE I
Formulation of Naltrexone Loaded Nanoparticles Prepared from Poly(NIPAAm-AAm–VP) and its PLGA Blend[a]

Polymer code	Polymer type	Polymer (%)	Drug content %	Particle size (nm)
PI	Poly(NIPAAm-VP-AAm)	10	34.5 ± 8.5	220 ± 23.7
PII	Poly(NIPAAm-VP-AAm)-PLGA	10	41.3 ± 11.2	520 ± 18.8
PIII	Poly(NIPAAm-VP-AAm)	5	53.2 ± 9.6	198 ± 21.2
PIV	Poly(NIPAAm-VP-AAm)-PLGA	5	64.7 ± 13.4	360 ± 14.65

[a] Theoretical drug loading was 5% for all formulations.

blend with PLGA). The nanoparticles prepared by 5% polymer concentration yielded the smaller particle size(less than 220 nm for terpolymer and approximately 375 nm for its blend with PLGA), highest encapsulation efficiency, and drug loading %. Nanoparticles prepared from terpolymer-PLGA blend had bigger size and higher encapsulation efficiency.

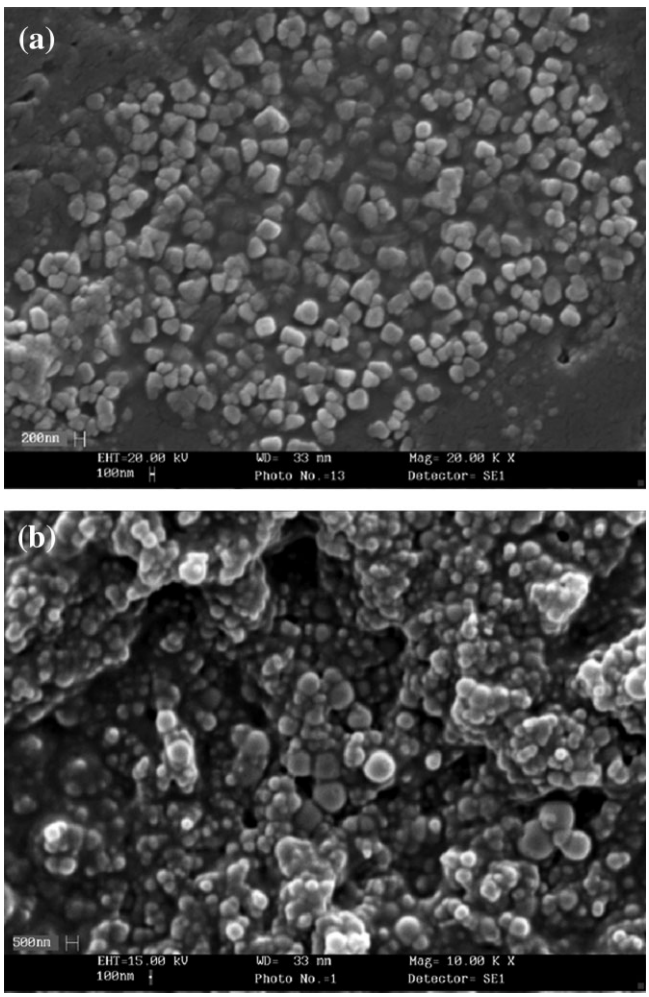

Figure 4 Scanning electron micrographs of nanoparticles prepared from poly(NIPAAm-AAm-VP); (a) and poly(NIPAAm-AAm-VP) -PLGA blend; (b) and with a theoretical drug loading of 5% and polymer content of 10%.

The drug release tests were performed at 37°C in buffer solution at (pH = 7.4). Figure 5 shows the release profiles of naltrexone loaded nanoparticles (PIII and PIV). As illustrated in Figure 5 the naltrexone loaded nanoparticles showed well-developed sustained drug release patterns. Various types of nanoparticles (PIII-PIV) showed a burst release of less than 10% after 24 h. At 37°C, the 33% of drug was released in 336 h from terpolymer (PIII) and 17% from terpolymer-PLGA blend (PIV). A typical release profile is shown in Figure 5, for selected formulations PIII and PIV. In this system, polymer degradation did not occur within a few days; accordingly, we can consider that the drug is released from the polymer blend through the diffusion mechanism *in vitro*. There are small initial releases(less than 10%) because of the small amount of drug embedded on the surface at first stage. And, the second stage is the release of drug encapsulated within the matrix of the nanoparticles.

It seems that the incorporation of hydrophobic PLGA component leads to a lower burst effect and slower release rate of naltrexone from terpolymer-PLGA blend. Therefore, the naltrexone loaded nanoparticles showed well developed sustained drug release in presence of PLGA component. Also, the

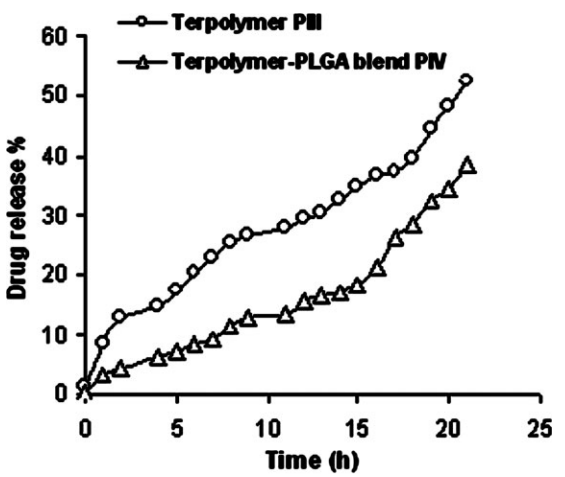

Figure 5 Release profile of naltrexone from naoparticles prepared from PIII and PIV at 37°C, pH 7.4.

drug release can be affected by particle size. In the case of smaller nanoparticles (PIII), greater surface area produces a higher number of drug molecules at the surface of nanopartcicles ready for faster release (also higher burst release in PIII in comparison with PIV). And, it is possible to conclude that the formation of hydrogel layer at 37°C might act as additional diffusion barrier for the drug release.[19,22,25]

CONCLUSIONS

Naltrexone was successfully encapsulated into the Poly(NIPAAm-AAm-VP) and its blend with PLGA. The yield of encapsulation of naltrexone in Poly(NIPAAm-AAm-VP) and PLGA (NIPAAm-AAm-VP) was 34–65%. Nanoparticle sizes were depended on the polymer concentration and type. From SEM observations, all copolymers resulted in almost spherical particles with a size range of 200–500 nm, which was similar to particle size analyzer measurements. These nanoparticles can be used as vehicles for the controlled delivery of naltrexone. Thermosensitive PLGA- based nanoparticles reported in present article could provide protection to the therapeutic agent from the fast degradation by physiological conditions, and release the drug in a controlled manner so that its concentration is maintained within therapeutic levels for longer periods of time. Preparation process was optimized to reduce the burst effect and provide *in vitro* sustained release. Therefore, we developed polymeric injectable nanoparticulated (matrix) naltrexone controlled delivery systems capable of sustaining availability of naltrexone for a long-period of time.

References

1. Hoffman, A. S. J Controlled Release 1987, 6, 297.
2. Kim, S. W.; Bae, Y. H.; Okano, T. Pharm Res 1992, 9, 283.
3. Bromberg, L. E.; Ron, E. S. Adv Drug Deliv Rev 1998, 31, 197.
4. Kost, J.; Langer, R. Adv Drug Deliv Rev 2001, 46, 125.
5. Bezemer, J. M.; Grijpma, D. W.; Dijkstra, P. J.; Blitterswijk, C. A.; Feijen, J. J Controlled Release 1999, 62, 393.
6. Hirosue, S.; Müller, B. G.; Mulligan, R. C.; Langer, R. J Controlled Release 2001, 70, 231.
7. Gruet, P.; Maincent, P.; Berthelot, X.; Kaltsatos, V. Adv Drug Deliv Rev 2001, 50, 245.
8. Zhang, X. Z.; Wu, D. Q.; Chu, C. C. Biomaterials 2004, 25, 3793.
9. Massoumi, B.; Entezami, A. J Bioact Compat Polym 2002, 17 1.
10. Massoumi, B.; Entezami, A. Eur polym J 2001, 37, 1015.
11. Davaran, S.; Entezami, A. J Controlled Release 1997, 47, 41.
12. Benrebouh, A.; Avoce, D.; Zhu, X. X. Polymer 2001, 42, 4031.
13. Avoce, D.; Liu, H. Y.; Zhu, X. X. Polymer 2003, 44, 1081.
14. Zhang, X. Z.; Lewis, P. J.; Chu, C. C. Biomaterials 2005, 26, 3299.
15. Inomata, H.; Wada, N.; Yagi, Y.; Goto, S.; Saito, S. Polymer 1995, 36, 875.
16. Grinberg, V. Y.; Dubovik, A. S.; Kuznetsov, D. V.; Grinberg, N. V.; Grosberg, A. Y.; Tanaka, T. Macromolecules 2000, 33, 8685.
17. Eeckman, F.; Möes, A. J.; Amighi, K. Eur Polym J 2004, 40, 837.
18. Li, X. W.; Liu, W.; Ye, G.; Zhang, B.; Zhu, D.; Yao, K.; Liu, Z.; Sheng, X. Biomaterials 2005, 26, 7002.
19. Choi, C. Y.; Chae, S. Y.; Nah, J. W. Polymer 2006, 47, 4571.
20. Huang, G.; Gao, J.; Hu, Z; St, J. V.; Ponder, B. C.; Moro, D. J Controlled Release 2004, 94, 303.
21. Grandfils, C.; Jerome, R.; Nihant, N.; Teyssie, P.; U.S. pat. 5, 962, 566.
22. Dinavaran, R.; Mogadam, S. H.; Mohammadyari-Fard, L.; Atyabi, F. AAPS Pharm Sci Tech 2003,4 Article 34.
23. Geever, L. M.; Devine, D. M.; Nugent, J. D.; Kenedy, J. E.; Lyons, J. G.; Higginbotham, C. L. Eur Polym J 2006, 42, 69.
24. Caykara, T.; Kiper, S.; Demirel, G. Eur Polym J 2006, 42, 348.
25. Yin, W.; Akala, E. O.; Taylor, R. E. Int J Pharm 2002, 244, 9.