Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 322 (2006) 154–160
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Adriamycin release from self-assembling nanospheres of poly(DL-lactide-co-glycolide)-grafted pullulan

Young-Il Jeong [a,1], Hee-Sam Na [a], Jong-Suk Oh [a], Ki-Choon Choi [b], Chae-Eun Song [c], Hyun-Chul Lee [a,*]

[a] *Department of Microbiology, Chonnam National University Medical School, 5, Hak-1-dong, Dong-Gu, Gwang-ju 501-746, Republic of Korea*
[b] *Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine, Korea University, Seoul, Republic of Korea*
[c] *Korea Institute of Natural Science Inc., Naju, Jeonnam 520-330, Korea*

Received 5 December 2005; accepted 9 May 2006
Available online 16 May 2006

Abstract

Poly(DL-lactide-co-glycolide)-graft pullulan (PuLG) was synthesized to produce a hydrophobically modified polysaccharide. Specific pullulan and poly(DL-lactide-co-glycolide) (PLGA) (abbreviated as PuLG) appeared in the peaks of the PuLG spectra on ^{1}H NMR spectroscopy, suggesting that PLGA was successively grafted to the pullulan backbone. PuLG nanospheres have a round shape with a particle size of about 75–150 nm. From the fluorescence excitation spectra in a fluorescence probe study, the critical association concentration (CAC) values were determined to be 0.017 g/l for PuLG-1, 0.0054 g/l for PuLG-2, and 0.0047 g/l for PuLG-3. The drug contents of the PuLG nanospheres were approximately 20–30% (w/w). As the drug contents of PuLG nanospheres increased, the drug release rate from nanospheres decreased. The drug release rate from PuLG nanospheres was delayed as the molecular weight of PuLG increased. PuLG copolymer with higher graft ratio of PLGA showed slower degradation rate rather than that with lower graft ratio. Since degradation rate of PuLG was taken over 1 month, drug release was governed by diffusion mechanism rather than degradation mechanism.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Self-assembly; Pullulan; PLGA; Controlled release; Graft copolymer

1. Introduction

Because of their desirable properties, nanoparticles and colloidal carriers occupy a unique position in drug delivery technology (Majeti and Kumar, 2000). Nanoparticles are suitable devices for parenteral injection because their sizes are below 1000 nm (Kreuter, 1991), which minimize irritation at injection sites. Targeting the desired site of action would not only increase the therapeutic efficiency of a drug, but also allow a reduction in the amount of drug administered, thus minimizing side effects. Various nanosized carriers, such as liposomes (Estey et al., 1996), polymeric micelles (Kataoka et al., 1993; La et al., 1996), and core-shell type nanoparticles (Gref et al., 1994; Jeong et al., 1998) have been reported.

Polymeric micelles (Kataoka et al., 1993; Lehn, 1993), core-shell type nanoparticles (Gref et al., 1994; Jeong et al., 1999), and hydrophobized polysaccharides (Akiyoshi et al., 1993) have received considerable attention due to their self-assembling characteristics in aqueous solution. Self-assembled nanoparticles are generally composed of a hydrophobic core and a hydrophilic shell. Thus, they are superior drug carriers and/or drug-targeting vehicles. These properties of self-assembled nanoparticles offer a unique biodistribution of drugs and target solid tumors (Yokoyama et al., 1990). Yokoyama et al. reported that adriamycin-conjugated block copolymeric micelles were effective in treating solid tumors with prolonged blood circulation (Yokoyama et al., 1990, 1991). Jung et al. (2004) reported previously poly(ethylene glycol) (PEG)-grafted pullulan acetate (PA) as an amphiphilic macromolecule for drug carriers.

A variety of macromolecular drug carrier systems have been developed in animal and clinical experiments. Among these systems, polysaccharides appear to be one of the most promising carriers for delivery of both drugs and enzymes (Poznansky and

* Corresponding author. Tel.: +82 62 220 4133; fax: +82 62 228 7294.
E-mail address: hclee@jnu.ac.kr (H.-C. Lee).
[1] Present address: Department of Polymer Science and Engineering, Sunchon National University, Sunchon, Jeonnam 540-742, Korea.

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.05.020

Cleland, 1980; Molteni, 1979; Schacht et al., 1985; Kaneo et al., 1989). Pullulan, a nonionic polysaccharide, has several advantages as a macromolecular drug carrier. It is highly water-soluble and non-toxic, has multiple hydroxyl groups that can readily be chemically modified, lacks immunogenicity, and is useful as a plasma expander (Yuen, 1974; Jeanes, 1977).

In this study, we have synthesized amphiphilic macromolecules composed of pullulan and poly(DL-lactide-co-glycolide) (PLGA) (abbreviated as PuLG) to give amphiphilicity and biodegradability as novel drug carriers. Due to its biodegradability, PLGA is commonly used for the controlled release of drugs. Furthermore, PLGA is approved for use in the human body because of its good biodegradability and biocompatibility. Pullulan is water-soluble and has been used extensively as an additive in the food industry. We expected that self-assembling nanospheres of PuLG can be formed at aqueous environment. PLGA should compose the hydrophobic domain as a drug incorporation site, and its biodegradation results in the controlled release of a drug. Pullulan should compose the hydrophilic domain. Introduction of PLGA into a water-soluble polysaccharide, pullulan, successfully induced amphiphilicity and provided unique physicochemical properties.

2. Materials and methods

2.1. Materials

Pullulan with an average molecular weight of 50,000–100,000 (g/mol) and poly(DL-lactide-co-glycolide) (PLGA 5005) were purchased from the Wako Chem. Co., Japan. Adriamycin (ADR) was purchased from Sigma Chem. Co. (St. Louis, USA). *N*,*N*′-dicyclohexyl carbodiimide (DCC) and dimethylaminopyridine (DMAP) were purchased from the Aldrich Chemical Company (Milwaukee, USA). The dialysis membranes with a molecular weight cut-off (MWCO) of 12,000 g/mol were purchased from Spectra/Pro™ membranes. Dimethyl sulfoxide (DMSO), *N*,*N*-dimethylacetamide (DMAc), and dichloromethane (DCM) were of HPLC grade and used without further purification.

2.2. Synthesis of PuLG graft copolymer

Pullulan (1 g) was dissolved in DMSO (15 ml) for 3 h. Various amounts of PLGA were dissolved in DMSO (5 ml) with a 1.3 equiv. amount of DCC and DMAP. PLGA solution was added into pullulan solution and then stirred for 1 day in a nitrogen atmosphere. The resultant solution was filtered to remove byproducts and dialyzed against deionized water using a dialysis tube (molecular weight cut-off (MWCO) 12,000 g/mol, Sigma Chem. Co.) for 3 days, followed by lyophilization for 3 days. The resultant solid was then precipitated into DCM to remove unreacted PLGA. Filtration followed. This procedure was repeated three times. After filtration, the final product was dried in a vacuum oven for 3 days. Synthesized PuLG graft copolymer was analyzed and characterized.

2.3. Gel permeation chromatography (GPC)

The molecular weights of PuLG copolymers were estimated with GPC (Viscotek, Houston, TX). The GPC system is composed of a degasser (VE 7510, Viscotek), a pump (VE 1121, Viscotek), a Waters 712 WISP injector, a Waters 410 differential refractometer mounted in parallel with a Viscotek T60A detector, and a Waters TCM heating column system. Polystyrene standards (Polymer Laboratories, Shropshire, UK) were used to estimate molecular weights from universal calibration. For estimation of the molecular weight of PLGA 5005, THF was used at a flow rate of 1 ml/min (column temperature: 40 °C). For the analysis of PuLG, the mobile phase was DMAc containing 0.4% LiBr at a flow rate of 0.5 ml/min. The columns (GMHHR N) were heated at 60 °C.

2.4. ^{1}H nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR spectra were recorded with a 300 MHz NMR spectrometer (FT NMR, Bruker AC-300F, 300 MHz). PLGA and PuLG copolymers were analyzed in DMSO-d_6 (99.9 at.% deuterium (D), Sigma Co., USA).

2.5. Preparation of PuLG nanospheres

PuLG nanospheres were prepared by the dialysis method (Jeong et al., 1999). PuLG (40 mg) was dissolved in DMSO (5 ml). The solutions formed were dialyzed using a dialysis tube (molecular weight cut-off (MWCO) 12,000 g/mol, Sigma Chem. Co.) against deionized water. The deionized water was exchanged every 1 h for the first 3 h and every 3 h for an additional 21 h; the dialyzed solution was then analyzed or freeze-dried.

To prepare drug-encapsulated nanospheres, PuLG (40 mg) was dissolved in DMSO (4 ml). ADR (10–20 mg) was dissolved in DMSO (1 ml) with an equivalent molar amount of triethylamine (TEA). The each solution was mixed and stirred for 3 h. The resultant solution was dialyzed using a dialysis tube (molecular weight cut-off (MWCO) 12,000 g/mol, Sigma Chem. Co.) against 1 l of acetate buffer (pH 5.5, 0.1 M) for 2 h and dialyzed against deionized water for 9 h with water exchange every 1 h. The dialyzed solution was analyzed or freeze-dried.

2.6. Determination of drug contents and loading efficiency

After dialysis, the volume of the aqueous solution of ADR-encapsulated nanospheres was adjusted to 20 ml with deionized water (i.e. 40 mg of PuLG polymer/20 ml of water). The adjusted solution (0.2 ml) was mixed with DMSO (9.8 ml). The resulting solution was measured at 479 nm using a UV spectrophotometer (UV-1201, Shimadzu Co. Ltd., Japan). PuLG nanospheres without any drug were used as a blank test:

$$\text{Drug contents} = \frac{\text{Amount of ADR in the nanospheres}}{\text{Weight of nanospheres}} \times 100$$

$$\text{Loading efficiency} = \frac{\text{Residue amount of ADR in the nanospheres}}{\text{Feeding amount of adriamycin}} \times 100$$

2.7. Photon correlation spectroscopy (PCS)

Particle sizes were measured with a Zetasizer 3000 (Malvern Instruments, UK) with a He–He laser beam at a wavelength of 633 nm at 25 °C (scattering angle of 90°). The nanospheric content in the sample suspension was 1 g/l and was measured without filtering.

2.8. Transmission electron microscope (TEM)

A drop of nanospheres suspension containing 0.03% (w/v) of phosphotungstic acid was placed on a TEM copper grid coated with carbon film and dried at room temperature. Observation was performed at 80 kV with JEM-2000 FX II (Jeol, Japan).

2.9. Fluorescence spectroscopy

Fluorescence spectroscopy (Shimadzu RF-5301 PC spectrofluorophotometer, Shimadzu Co. Ltd., Japan) was performed to prove the potential for self-assembly formation of PuLG. PuLG nanoparticle suspensions were prepared without any drugs as follows: various PuLG (40 mg) were dissolved in DMSO (7 ml) and dialyzed against distilled water for up to 2 days in the same method as described above. The resultant suspension was adjusted to various nanospheric concentrations.

The critical association concentration (CAC) of the PuLG was estimated using pyrene as a hydrophobic probe (Wilhelm et al., 1991; Kwon et al., 1993). To prepare sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 ml vials, and the acetone was evaporated. The final concentration of pyrene was 6.0×10^{-7} M. To each vial, 10 ml of various concentrations of the nanosphere suspensions was added, and the solutions were heated for 3 h at 65 °C. Equilibration of the pyrene and the PuLG nanospheres was achieved by allowing the solutions to cool overnight at room temperature. The fluorescence excitation spectra were measured at an emission wavelength of 390 nm. Excitation and emission bandwidths were 1.5 and 1.5 nm, respectively.

2.10. In vitro drug release studies

The release experiment was carried out in vitro as follows: volumes of an aqueous solution of ADR-encapsulated nanospheres were adjusted to 20 ml with deionized water (i.e. 40 mg of PuLG polymer/20 ml of water). ADR-encapsulated PuLG nanospheres (5 ml) were introduced into a dialysis tube (molecular weight cut-off: 12,000 g/mol, Sigma Co. USA), and this dialysis tube was introduced into a bottle with 95 ml of PBS (0.1 M, pH 7.4). The media were stirred at 100 rpm and 37 °C. At specific time intervals, the whole medium was replaced with fresh medium and the amount of drug released into the medium was measured using a UV spectrophotometer at 479 nm (Jeong et al., 1999).

2.11. Degradation of PuLG nanoparticle in vitro

For degradation test, 100 mg of PuLG copolymer was dissolved in 12 ml of DMSO. Nanospheres were prepared by dialysis (dialysis tube: 12,000 g/mol, Sigma Chem. Co.) against deionized water for 2 days. The deionized water was exchanged every 1 h for the first 3 h and every 6 h for an additional 2 days. After that, the dialyzed solution was taken and the volume of dialyzed solution was adjusted to 40 ml (i.e. 100 mg of polymer/40 ml of deionized water. Thus, concentration of polymer: 2.5 mg/ml). About 8 ml of this solution was introduced into dialysis tube (12,000 g/mol) and this dialysis tube was introduced into bottle with 92 ml of PBS (pH 7.4, 0.1 M). PBS medium was exchanged every day. At specific time intervals, dialysis tube was taken and dialyzed against deionized water to remove trace elements. Then, PuLG nanosphere solution was lyophilized for 2 days. Lyophilized nanosphere solution was analyzed MW by GPC or stored at −20 °C untill use it.

3. Results and discussion

Pullulan consists of α-1,4 and α-1,6 glycosidic linkages and is a water-soluble, neutral, linear polysaccharide. Due to its biocompatibility and biodegradability, pullulan was extensively used in drug delivery carriers (Akiyoshi et al., 1998). However, pullulan itself cannot be used as a drug carrier due to its water solubility and, therefore, it first needs to be rendered hydrophobic (Jung et al., 2004). Since PLGA is well known for its biodegradable and biocompatible copolymers, PLGA was grafted to pullulan to make PLGA-grafted pullulan (PuLG). PuLG graft copolymer was not water-soluble, but was soluble in DMSO. It was expected that core-shell type nanospheres of PuLG may be formed due to their amphiphilic characteristics. The pullalan backbone may act as an outershell and PLGA may form the hydrophobic innercore of the nanospheres. The synthesis scheme is shown in Fig. 1. ^{1}H NMR results of PLGA, pullulan, and PuLG copolymer are shown in Fig. 2. PLGA and pullulan each have their own specific peaks, as shown in Fig. 2(a) and (b), respectively. The individual peaks of PLGA and pullulan were shown in NMR spectra of PuLG copolymer. As shown in Fig. 2(c), specific peaks of PLGA were shown at about 1.5 ppm, 3.4 ppm, and 4.8–5.3 ppm. Specific peaks of pullulan appeared at 3.6–3.8, 4.4–4.8, and 5.3–5.8 ppm (arrows). The characteristics of PuLG copolymer are summarized in Table 1.

To create the nanospheres, PuLG copolymer was dissolved in DMSO and the nanospheres were prepared by the dialysis method. To characterize the nanospheres of the PuLG copolymer, the nanospheres of PuLG were observed by TEM and the particle sizes were measured using photon correlation spectroscopy. The TEM photograph of PuLG-2 nanospheres is shown in Fig. 3. Nanospheres of PuLG-2 were roundly shaped and their size ranged from about 75 to 150 nm (Fig. 3). Theses results verified that PuLG copolymer can form nanospheres in the aqueous environment. Additionally, nanospheres or poly-

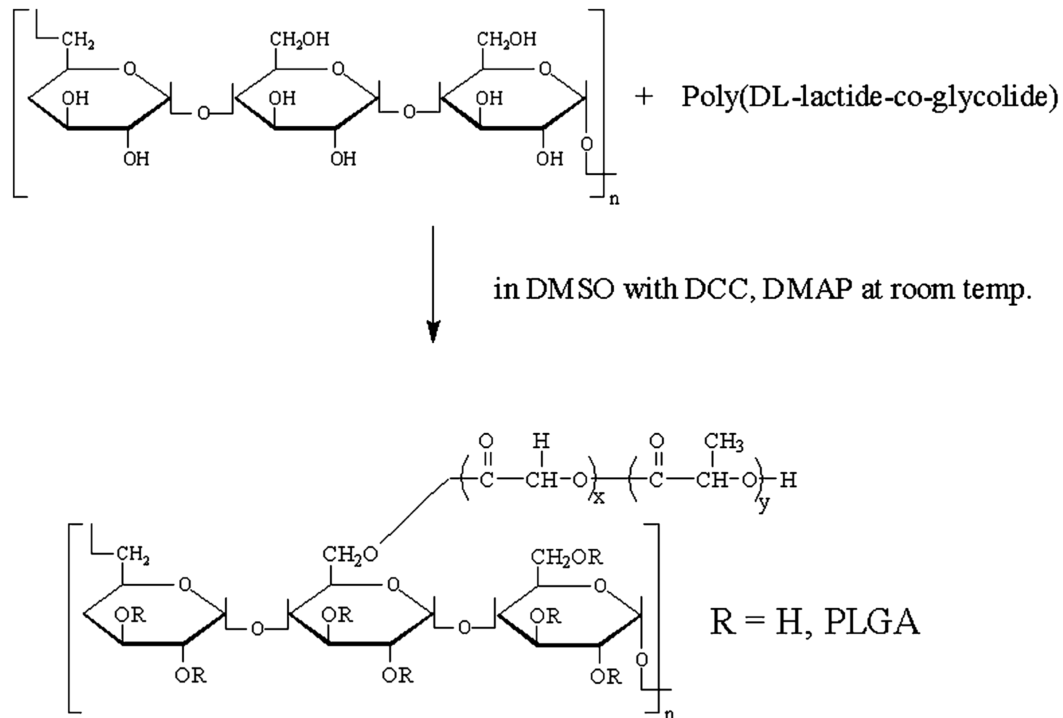

Fig. 1. Synthesis scheme of PuLG graft copolymer.

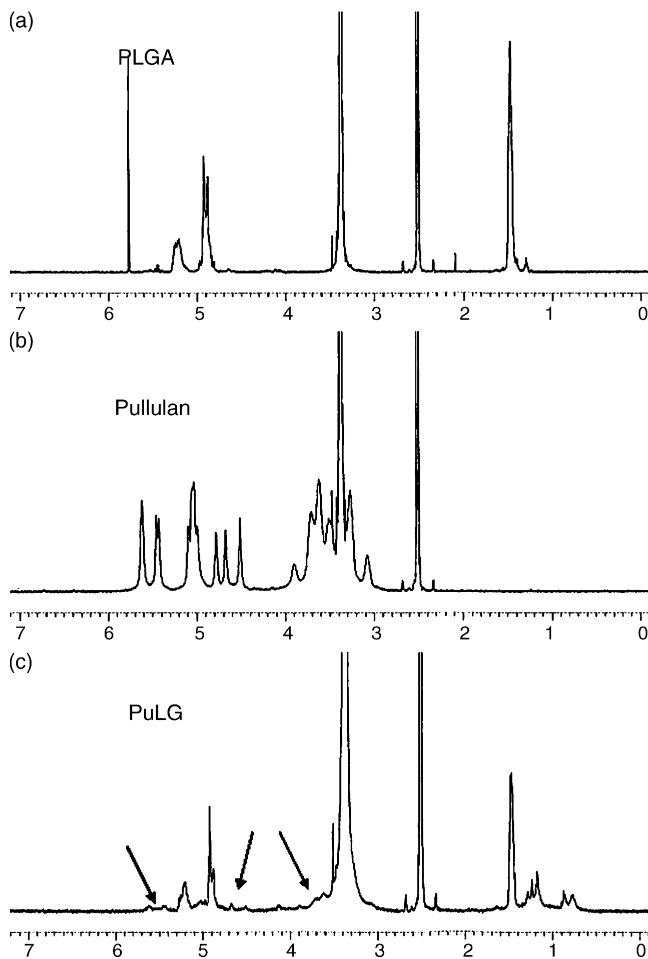

Fig. 2. ^{1}H NMR of (a) PLGA, (b) pullulan, and (c) PuLG, respectively.

meric micelles with a particle size less than 200 nm were reported as suitable drug-targeting carriers to solid tumors and specific disease sites (Gref et al., 1994; Yokoyama et al., 1990).

Generally, block and graft copolymers have self-assembling behavior and amphiphilic characteristics in an aqueous environment (Gref et al., 1994; Jeong et al., 1998; Jung et al., 2004; Kwon et al., 1993). It was thought that the PuLG copolymer would also show amphiphilic characteristics in water and would form self-aggregating nanospheres. To characterize the self-assembling behavior of the PuLG copolymer in aqueous media, the fluorescence probe technique was employed, using pyrene as the hydrophobic probe. The fluorescence excitation spectra of pyrene at various concentrations of PuLG copolymer are shown in Fig. 4. The fluorescence intensity of pyrene increased with increasing concentrations of PuLG copolymer, which strongly

Table 1
Characterization of PuLG graft copolymer

	MW of PuLG[a]		Polydispersity	d.s.[b]	CAC[c] (g/l)
	M_w	M_n			
PuLG-1	83900	76900	1.09	2.0	0.017
PuLG-2	102800	96200	1.07	4.3	0.0054
PuLG-3	121000	109000	1.11	6.5	0.0047

[a] The molecular weight (MW) of PuLG was measured by GPC. M_w, M_n, and polydispersity of pullulan from GPC results were 67,500, 62,400, and 1.081 ± 0.019, respectively. M_w, M_n, and polydispersity of PLGA 5005 were 8200, 5800, and 1.41, respectively.

[b] The degree of substitution (d.s.) of PLGA chains grafted per pullulan was estimated by subtracting the determined M_w of pullulan (67,500) from the M_w of PuLG copolymers and dividing by M_w of polyester grafts (8200).

[c] CAC was estimated from fluorescence spectroscopy measurements as explained in Section 2.

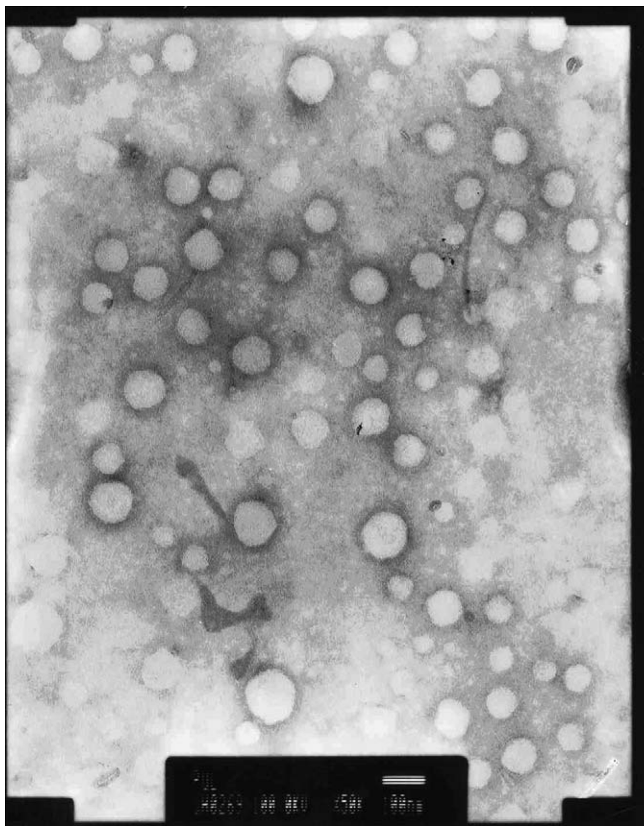

Fig. 3. TEM photograph of ADR-encapsulated PuLG-2 nanoparticles (bar = 100 nm). Particle size was 92.1 ± 15.1 at Table 2.

suggests the self-assembly of the PuLG copolymer in water. Additionally, a red shift was observed in the excitation spectra with increasing PuLG copolymer concentration, which suggests that pyrene is preferentially solubilized into the core part of the core-shell type nanospheres. The intensity ratios of I_{338}/I_{335} versus log c of PuLG copolymer for the pyrene excitation spectra are shown in Fig. 5. A flat region and a sigmoid change in the crossover region were observed at extremely low concentrations. This result indicates that the signal change in the crossover region could be related to the critical association concentration (CAC) value of PuLG copolymer. The CAC values were determined from the fluorescence excitation spectra and were found to be 0.017 g/l for PuLG-1, 0.0054 g/l for PuLG-2, and 0.0047 g/l for PuLG-3. The hydrophilic domain, pullulan, formed a flexible shell of self-assembling nanospheres in the aqueous environment; PLGA, the hydrophobic domain, formed the rigid inner core. Consequently, nanospheres were formed, due to the self-aggregating properties of the PuLG copolymer in the aqueous environment.

ADR was encapsulated into PuLG nanospheres and the results are summarized in Table 2. As shown in Table 2, drug contents into PuLG nanospheres and particle size increased as the

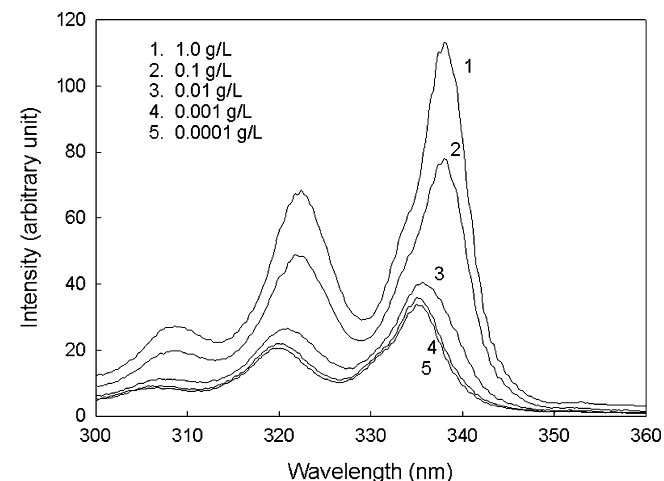

Fig. 4. Fluorescence excitation of pyrene (6.0×10^{-7} M) vs. the concentration of PuLG nanoparticles in distilled water (λ_{em} = 390 nm).

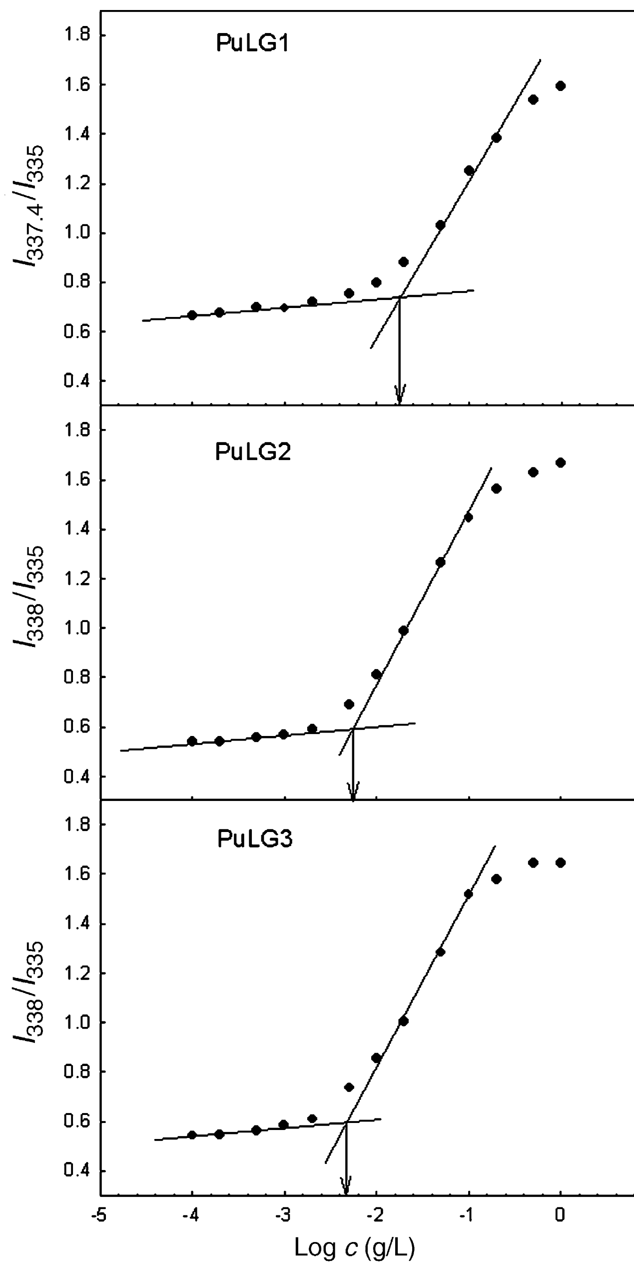

Fig. 5. Plots of the intensity ratios, I_{338}/I_{335}, from the pyrene excitation spectra vs. log c of the PuLG nanoparticles in distilled water. Each point was derived from Fig. 3. The arrows indicate the signal changes in the crossover region.

Table 2
Characterization of adriamycin-encapsulated PuLG nanoparticles

	PuLG/ADR weight ratio (mg/mg)	Drug contents (%, w/w)	Loading efficiency (%, w/w)	Particle size (nm)
PuLG-1	40/10	4.9	20.7	76.8 ± 12.4
PuLG-2	40/10	5.9	25.1	92.1 ± 15.1
	40/20	9.1	20.1	121.8 ± 23.7
PuLG-3	40/10	7.2	31.2	147.0 ± 30.4

molecular weight of PuLG copolymer increased. An increased drug-feeding ratio induced an increase in the drug content and the particle size, but decreased loading efficiency. The particle size of ADR-encapsulated PuLG nanospheres was below 150 nm, which suggests that PuLG nanospheres may suitable for intravenous injection.

The release of ADR from PuLG nanospheres is shown in Fig. 6. As shown in Fig. 6a, as the molecular weight of PuLG increased, the ADR release rate decreased. Higher contents of the PLGA domain may have increased the hydrophobic interaction between ADR and the PLGA domain. As shown in Fig. 6b, the drug release rates were slower as the drug contents increased. It was well known that hydrophobic drugs may be crystallized in the inner core of the self-assembling nanospheres, and a phase separation occurs at higher levels of drug-loading contents, which leads to slow dissolving into the medium. Therefore, ADR release from the PuLG nanospheres with higher drug contents and higher molecular weights of PuLG showed slower releases than nanoparticles with lower drug contents and molecular weights. On the other hand, at low drug contents, ADR might be somewhat present as a molecular dispersion inside the nanoparticles (Gref et al., 1994). ADR The release of ADR from PuLG nanospheres continued over 10 days. The release pattern of PuLG nanospheres showed an initial burst for 1 day, followed by a pseudo zero-order pattern. Initial burst of ADR from nanospheres was also reported previously (Jeong et al., 1999). It suggested that some part of drug was absorbed onto the surface of nanoparticles or loosely encapsulated in the hydrophilic domain of pullulan and these part of drug may release fast at initial stage.

For degradation test of PuLG nanospheres, aqueous solution of PuLG nanospheres was incubated at PBS and remained nanoparticles in the dialysis tube was used to analysis of decreased MW as an indication of degradation of polymers. Since pullulan does not degraded at normal physiological solution in vitro, PLGA is only degradable at PBS and pullulan is remained in the dialysis tube. Thus, degradation of copolymer can be estimated by analysis of MW of remained pullulan or PuLG copolymer. As shown in Fig. 7, nanospheres of PuLG copolymer was slowly degraded over 1 month. As expected,

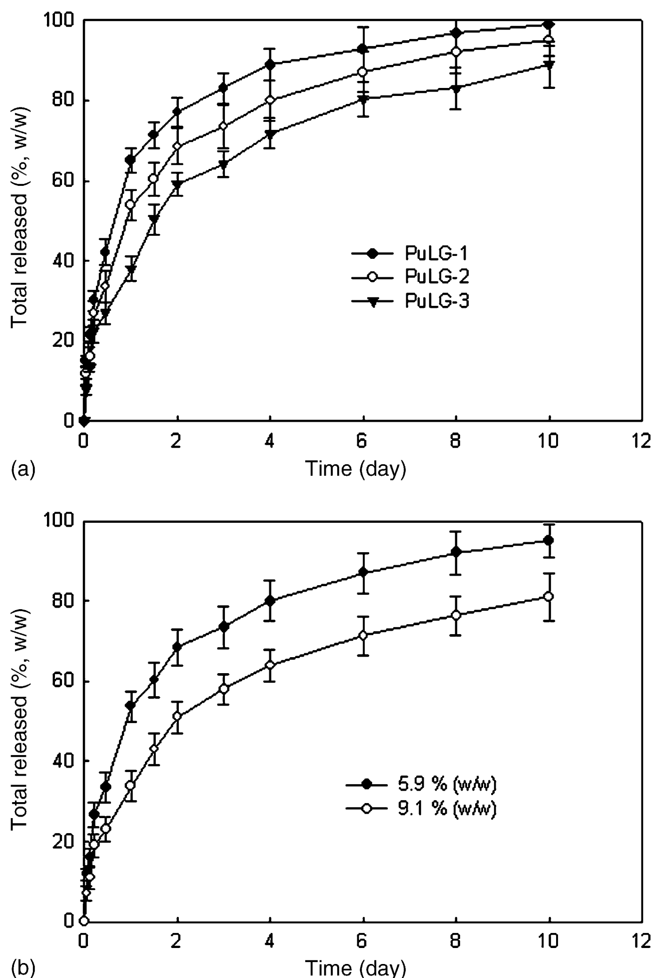

Fig. 6. ADR release from PuLG nanoparticles. The effect of a series of PuLG graft copolymer (a) and drug contents of nanoparticles (PuLG 2) (b).

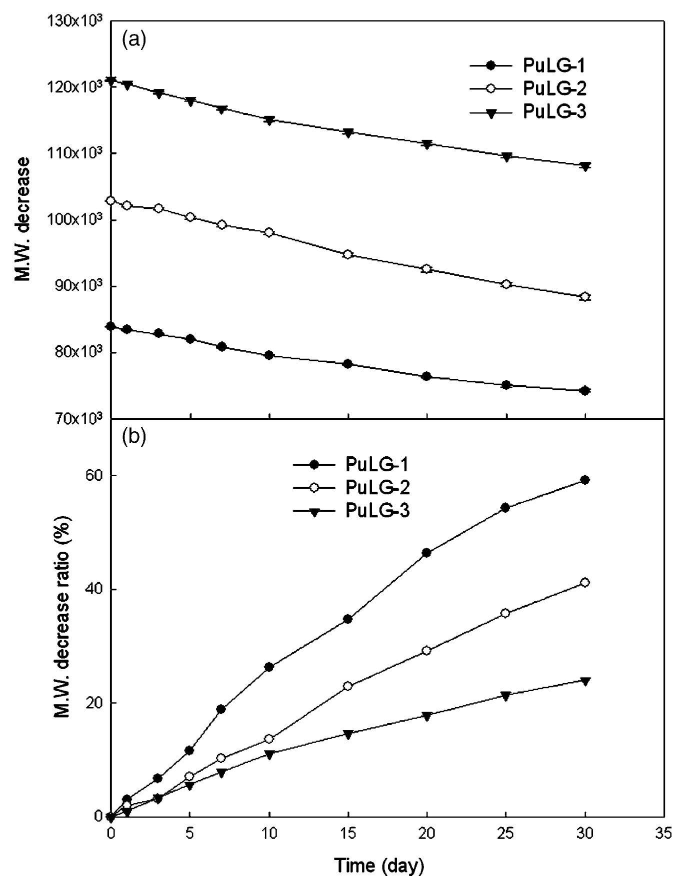

Fig. 7. Time course of degration of PuLG nanoparticles.

degradation rate of PuLG copolymer was slower in higher graft ratio of PLGA than lower graft ratio. These results indicated that PuLG copolymer with lower graft ratio of PLGA might more ease to swell at aqueous environment and then PLGA can degrade easily.

From the results of Figs. 6 and 7, we suggested that ADR release was dominantly governed by diffusion mechanism rather than degradation of PuLG copolymer. Degradation of PuLG copolymer was slowly degraded over 1 month and higher graft ratio induced slower degradation profiles, i.e. degradation of PuLG-1, -2, and -3 was below 25%, 45%, and 60%, respectively. However, drug release from nanospheres was higher than 80% at all formulation variables, indicating that ADR release rate was dominantly controlled by diffusion mechanism rather than degradation mechanism.

4. Conclusion

PLGA was grafted to pullulan to produce a hydrophobically modified polysaccharide and self-assembling nanospheres. The synthesized PuLG copolymer was characterized by ^{1}H NMR spectroscopy. From the results of the ^{1}H NMR spectra, specific peaks of pullulan and PLGA were shown in the NMR results of PuLG, suggesting the PLGA was successfully grafted to the pullulan backbone. From TEM, it was observed that PuLG nanospheres are roundly shaped, and their particle size is approximately 75–150 nm. In the fluorescence probe study, the CAC values were determined from the fluorescence excitation spectra, and were found to be 0.017 g/l for PuLG-1, 0.0054 g/l for PuLG-2, and 0.0047 g/l for PuLG-3. The drug content of the PuLG nanospheres was about 20–30% (w/w). As the drug contents of the PuLG nanospheres increased, the drug release rate from the nanospheres decreased. The drug release rate from PuLG nanospheres was delayed as the molecular weight of PuLG increased. PuLG copolymer with higher graft ratio of PLGA showed slower degradation rate rather than that with lower graft ratio. Since degradation rate of PuLG was taken over 1 month, drug release was governed by diffusion mechanism rather than degradation mechanism.

Acknowledgement

This study was financially supported by research fund of Chonnam National University in 2003.

References

Akiyoshi, K., Deguchi, S., Moriguchi, N., Yamaguchi, S., Sunamoto, J., 1993. Self-aggregates of hydrophobized polysaccharides in water. Formation and characteristics of nanoparticles. Macromolecules 26, 3062–3068.

Akiyoshi, K., Kobayashi, S., Shichibe, S., Mix, D., Baudys, M., Kim, S.W., Sunamoto, J., 1998. Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. J. Control. Release 54, 313–320.

Estey, E., Thall, P.F., Mehta, K., Rosenblum, M., Brewer Jr., T., Simmons, V., Cabanillas, F., Kurzrock, R., Lopez-Berestein, G., 1996. Alterations in tretinoin pharmacokinetics following administration of liposomal all-trans retinoic acid. Blood 87, 3650–3654.

Gref, R., Minamitake, Y., Peracchia, M.T., Trubetskoy, V., Torchilin, V., Langer, R., 1994. Biodegradable long-circulating polymeric nanospheres. Science 263, 1600–1603.

Jeanes, A., 1977. Dextrans and pullulans: industrially significant. ACS Symp. Ser. 45, 284–298.

Jeong, Y.I., Nah, J.W., Lee, H.C., Kim, S.H., Cho, C.S., 1999. Adriamycin release from flower type polymeric micelle based on star-block copolymer composed of poly(gamma-benzyl L-glutamate) as the hydrophobic part and poly(ethylene oxide) as the hydrophilic part. Int. J. Pharm. 188, 49–58.

Jung, S.W., Jeong, Y.I., Kim, Y.H., Kim, S.H., 2004. Self-assembled polymeric nanoparticles of poly(ethylene glycol) grafted pullulan acetate as a novel drug carrier. Arch. Pharm. Res. 27, 562–569.

Kaneo, Y., Fujihara, Y., Tanaka, T., Kozawa, Y., Mori, H., Iguchi, S., 1989. Intrahepatic delivery of glutathione by conjugation to dextran. Pharm. Res. 6, 1025–1031.

Kataoka, K., Kwon, G.S., Yokohama, M., Okano, T., Sakurai, Y., 1993. Block copolymer micelles as vehicles for drug delivery. J. Control. Release 24, 119–132.

Kreuter, J., 1991. Nanoparticle-based drug delivery system. J. Control. Release 16, 169–176.

Kwon, G., Naito, M., Yokoyama, M., Okano, T., Sakurai, Y., Kataoka, K., 1993. Micelles based on AB block copolymers of poly(ethylene oxide) and poly(β-benzyl L-asparate). Langmuir 9, 945–949.

La, S.B., Okano, T., Kataoka, K., 1996. Preparation and characterization of the micelle-forming polymeric drug indomethacin-incorporated poly(ethylene oxide)-poly(β-benzyl L-aspartate) block copolymer micelles. J. Pharm. Sci. 85, 85–90.

Lehn, J.M., 1993. Supramolecular chemistry. Science 260, 1762–1763.

Majeti, N.V., Kumar, Ravi, 2000. Nano and microparticles as controlled drug delivery devices. J. Pharm. Pharmaceut. Sci. 3, 234–258.

Molteni, L., 1979. Dextrans as drug carriers. In: Gregoriadis, G. (Ed.), Drug Carriers in Biology and Medicine. Academic Press, London, pp. 107–125.

Poznansky, M.J., Cleland, L.G., 1980. Biological macromolecules as carriers of drugs and enzymes. In: Juliano, R.L. (Ed.), Drug Delivery Systems. Oxford University Press, New York.

Schacht, E., Ruys, L., Vermeersch, J., Remon, J.P., Duncan, R., 1985. Use of polysaccharides as drug carriers: dextran and inulin derivatives of procainamide. In: Tirrell, D.A., Donaruma, G., Turek, A.B. (Eds.), Macromolecules as Drugs and as Carriers for Biologically Active Materials. The New York Academy of Science, New York, pp. 199–212.

Wilhelm, M., Zaho, C.L., Wang, Y., Xu, R., Winnik, M.A., Mura, J.L., Riess, G., Croucher, M.D., 1991. Poly(styrene-ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. Macromolecules 24, 1033–1040.

Yokoyama, M., Miyauchi, M., Yamada, N., Okano, T., Sakurai, Y., Kataoka, K., 1990. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-conjugated poly(ethylene glycol)-poly(aspartic acid) block copolymer. Cancer Res. 50, 1693–1700.

Yokoyama, M., Okano, T., Sakurai, Y., Ekimoto, H., Shibazaki, C., Kataoka, K., 1991. Toxicity and antitumor activity against solid tumors of micelle-forming polymeric anticancer drug and its extremely long circulation in blood. Cancer Res. 51, 3229–3236.

Yuen, S., 1974. Pullulan and its applications. Process Biochem. 9, 7–9.