POLYMERS FOR ADVANCED TECHNOLOGIES
Polym. Adv. Technol. **10**, 647–654 (1999)

Core-stabilized Polymeric Micelle as Potential Drug Carrier: Increased Solubilization of Taxol

Ji-Heung Kim†, Kazunori Emoto[1], Michihiro Iijima[1], Yukio Nagasaki[1], Takao Aoyagi[2], Teruo Okano[2], Yasuhisa Sakurai[2] and Kazunori Kataoka[3]*

[1]*Department of Material Science and Technology, Science University of Tokyo, Noda, Chiba 278-8510, Japan*
[2]*Institute of Biomedical Engineering, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ku, Tokyo 162-8666, Japan*
[3]*Department of Materials Science, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan*

ABSTRACT

Poly(ethylene glycol-b-lactide) possessing a methoxy group at the poly(ethylene glycol) (PEG) chain end and a polymerizable methacryloyl group at the poly(lactic acid) (PLA) chain end (MeO–PEG/PLA–methacryloyl) was prepared by an anionic ring-opening polymerization of ethylene oxide and DL-lactide in tandem manner initiated with a potassium 2-methoxyethanolate, followed by end-capping with an excess of methacrylic anhydride. The molecular weight of the obtained polymer was controlled by the initial monomer/initiator ratio, which was confirmed by the combination of gel permeation chromatography and nuclear magnetic resonance analyses. The functionality of the methacryloyl–PLA end was almost quantitative. The MeO–PEG/PLA–methacryloyl (38/35; these numbers in parentheses denote the molecular weights of PEG and PLA segments divided by 100, respectively) formed a core–shell type spherical micelle in aqueous media obtained by a dialysis technique, the cumulant diameter of which was ca. 30 nm with very low polydispersity factor.

The methacryloyl group adjacent to the PLA was polymerized in the PLA core of the micelle. The polymerization proceeded thermally with radical initiator and photochemically with photo-initiator to produce core-polymerized nanoparticles, which was found by spectroscopic and light-scattering techniques. Taxol-incorporated micelles were prepared to entrap Taxol into MeO–PEG/PLA–methyacryloyl block copolymer micelles by the oil/water emulsion method. Copyright © 1999 John Wiley & Sons, Ltd.

KEYWORDS: polymeric micelle; stable nanoparticle; drug delivery system; PEG/PLA block copolymer; micelle stability; taxol

INTRODUCTION

Polymeric micelles have been studied as a novel type of drug carrier system for the last decade [1–4]. Block copolymers composed of hydrophilic and hydrophobic segments can form a micellar structure with a hydrophobic inner core and a hydrophilic outer shell in aqueous media as a result of its amphiphilic nature. Because hydrophobic interac-

* Correspondence to: K. Kataoka, Department of Materials Science, Graduate School of Engineering, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan.
†Present address: Department of Chemical Engineering, SungKyun Kwan University, Suwon, Kyonggi 440-746, Korea.
Contract grant sponsor: Research Project on Biointegrated Materials, Science and Technology Agency of Japan.

CCC 1042–7147/99/110647–08 $17.50

Received 12 February 1999
Accepted 10 June 1999

tions are utilized effectively for the formation of micellar structures, drug carrier systems for hydrophobic drugs may be constructed using polymeric micelles. Biodegradable block copolymers consisting of poly(ethylene glycol) (PEG) and poly(lactic acid) (PLA) exhibit good potential for formulating a drug delivery system. PLAs are well-known biodegradable polymers which have been utilized in various biomedical applications because of their excellent biocompatibility and degradability. Also, PEG is one of the few water-soluble polymers that has been widely used to improve the biocompatibilities of blood-contacting biomaterials. A number of laboratories have already studied the synthesis of PEG/PLA block copolymers with various block ratios, molecular masses and structures using different synthetic methods [5–9]. By adjusting the hydrophobic and hydrophilic segments (their structure and sizes), micelles in aqueous media are formed. Recently, the association behavior of these PEG/PLA block copolymer micelles and evaluation as a drug carrier were studied by several groups [10–13].

It is generally known that polymeric micelles possess a very low critical micelle concentration (CMC) value and exhibit a very slow rate of micelle dissociation compared with low-molecular surfactants, suggesting apparent stability for the application in current drug delivery systems [14, 15]. However, the physical coagulation force, i.e. intermolecular hydrophobic or van der Waals interaction, as the main driving force to form the core–shell micelle structure may not be strong enough to maintain its stable form under various physiological conditions and on ultimate dilution *in vivo*. The stability of drug carrier micelles will have an extremely important influence on the drug-carrying capacity and their sustained release characterictics. Thus, chemical stabilization of the hydrophobic core in polymeric micelles was proposed and examined in this work.

For this study, an MeO–PEG/PLA block copolymer with a polymerizable methacryloyl end was prepared by anionic ring-opening polymerization and subsequent end-capping with methacrylic anhydride (MeO–PEG/PLA–methacryloyl). A stable core–shell type spherical micelle was obtained by the dialysis method, and the resulting aqueous micelle solution was core-polymerized both chemically and photochemically. The characterization of the copolymer and its micelle, before and after core-polymerization, by spectroscopic and light-scattering techniques are discussed. Also, paclitaxel (Taxol) was incorporated into these copolymer micelles to improve its solubility in aqueous media.

EXPERIMENTAL

Chemicals

Ethylene oxide (3M) was dried and distilled over CaH_2. 2-Methoxyethanol (Wako) and methacrylic anhydride (Aldrich) were distilled before use. Naphthalene (Wako, 98%) and potassium piller (Wako, 98%) were used as received. DL-Lactide (Tokyo Kasei) was purified by recrystallization from ethyl acetate and sublimation. Tetrahydrofuran (THF) was stirred over $LiAlH_4$ and was fractionally distilled before use [16]. Potassium naphthalene was used as a THF solution [17], the concentration of which was determined by titration.

Instrument

^{1}H-NMR (nuclear magnetic resonance) spectra were measured with a Jeol EX-400 spectrometer using $CDCl_3$ with tetramethylsilane (TMS) as an internal reference. Gel permeation chromatography (GPC) measurements in organic solvent were carried out using a Tosoh GPC model HLC-8020 equipped with a Shodex gel permeation column (KD-806M $\times$ 2). *N, N*-Dimethylformamide (DMF) containing 10 mmol/l of lithium bromide was used as the eluent at a flow rate of 1 ml/min. GPC measurements in aqueous system were carried out using a Jasco high-performance liquid chromatography (HPLC) system equipped with a Shodex gel permeation column (GF-7MHQ) and an internal reflective index (RI) detector (RI-930). Water containing 0.1 wt% sodium azide was used as the eluent at a flow rate of 0.5 ml/min at 25 °C. Thermal analysis (differential scanning calorimetry, DSC, and thermogravimetric analysis, TGA) was conducted on a Mettler DSC 30 with a low-temperature cell and a Mettler TG 50 thermobalance system under an argon flow at a heating rate of 10 °C/min. The first scan was measured using freeze-dried samples, and the second scan was measured after the first run to 150 °C and subsequent cooling to −100 °C.

Dynamic and static light scattering (DLS and SLS) measurements on the micelle solution were carried out using a Photal dynamic laser light-scattering spectrometer DLS-7000 (Otsuka Electromics Co. Ltd, Tokyo, Japan) using an argon laser at 488 nm and a He–Ne laser at 682 nm, respectively. The sample was filtered using Millipore filters of pore size 0.45 μm, if not specially mentioned. The refractive index increments of the polymeric micelles were obtained at 25 °C using a double beam differential refractometer (DRM-1020, Otsuka Electronics, Japan). All light-scattering experiments were done at 25 °C.

Synthesis of MeO–PEG/PLA–Methacryloyl Block Copolymer

A typical procedure was as follows. A 200 ml flask was equipped with a three-way stopcock and dried by applying a vacuum and an argon purge repeatedly. To the above flask under an argon atmosphere were added 30 ml of fractionally distilled THF and 0.08 ml (1 mmol) of 2-methoxyethanol, and subsequently 3.51 ml of 0.285 M solution (1 mmol) of potassium naphthalenide in THF wad added to generate 2-methoxyethanolate as an

active initiator for this polymerization. The dark green color of potassium naphthalenide disappeared immediately upon initial mixing, and a quantitative addition was assured by the color change at the final stage. To the above initiator solution was carefully added 6.0 ml (120 mmol) of ethylene oxide (EO) monomer with a chilled syringe, and the mixture was stirred at room temperature for two days. Several drops of solution were sampled for GPC measurement after the reaction. After the EO polymerization for two days, a light yellow and slightly viscous solution resulted. To this solution was added 43.6 ml of 1.1 M solution (48 mmol) of DL-lactide in THF, and the reaction mixture was stirred for 2 hr at room temperature before addition of 3.1 ml (20 mmol) of methacrylic anhydride to terminate the reactions and introduce the methacryloyl moiety to the living polymer chain end (see Scheme 1). The mixture was stirred for another two days to complete the reaction, then the final viscous solution was precipitated into a 30-fold excess of 2-propanol which was pre-chilled in a freezer at −15 °C. The white precipitate was centrifuged to collect white powder, and the solvent was evaporated under vacuum. The product was freeze-dried from benzene to provide 10.5 g of the desired polymer (yield *ca*. 85%).

Preparation of Block Copolymer Micelle

For the preparation of micelles, a dialysis method was employed [18], viz. briefly, 100 mg of the copolymer was dissolved in 20 ml of dimethyl acetamide, and the polymer solution was transferred into a pre-swollen membrane (Spectra/Por molecular weight cut-off size 12,000–14,000 Spectrum Medical Industries Inc., Houston, TX), dialyzed against water (1 l) for 24 hr.

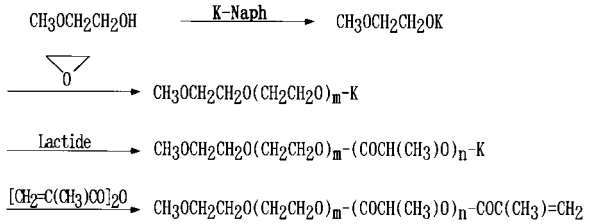
The water was exchanged three times, after 2, 5 and 8 hr and the solution was subsequently lyophilized. The yield of the formed micelle was about 90%.

Core-Polymerization and/or *in situ* Crosslinking of Dialyzed Micelle

For the core stabilization, the polymerization of the methacryloyl end-group of block copolymer in its aqueous micelle form was carried out:

(1) *Chemical polymerization*. Azobis-2,4-dimethylvaleronitrile (1 wt% of polymer, V-65, Wako, $t_{1/2} = 51$ °C) as a suitable free-radical initiator was incorporated into the micelle core by mixing into 100 mg of block copolymer solution in DMAc and subsequently dialyzing against water. The resulting micelle solution containing V-65 was transferred into a 100 ml flask and was purged with argon for 30 min to remove oxygen. The polymerization reaction was carried out in an oil bath at 60 °C for 20 hr. After the reaction, the micelle solution was directly used for several measurements or freeze-dried to give a powdery product.

(2) *Photo-polymerization.;* MeO-PEG/PLA-methacryloyl was dialyzed with 1–3 wt% of 2,2-dimethoxy-1,2-diphenylethane-1-one (BDMK) as a typical photoinitiator, against water. The dialyzed micelle solution was transferred into a round cylindrical flask and was purged with argon for 1 hr before starting photolysis. The solution was irradiated with a high-pressure mercury lamp (500 W, Ushio Inc.) with an IR filter (HA-30 type, Kenko). The light intensity was about 13–14 mW/cm^2. The photopolymerization was carried out for total of 4 hr. The aliquot of the solution was intermittently sampled for NMR analysis.



SCHEME 1

For further stabilization of the micelle core, chemical crosslinking of the core was attempted by introducing additional dimethacrylate compound into the above polymerization system. Into the aqueous micellar solution in a two-necked flask were added dimethacrylate compound (e.g. ethylene glycol dimethacrylate) and subsequently an azo initiator (V65) in methylene chloride using a microsyringe. After stirring for 1 hr, the solution was degassed by bubbling argon to remove the remaining organic solvent and dissolved oxygen and then stirred for 20 hr in an oil bath preheated to 60 °C.

Scanning Probe Microscopy of Micelle in Aqueous Solution

The scanning probe microscopy (SPM) image of stabilized micelles on a piece of Si wafer was observed with Bioscope (Olympus Co., Tokyo, Japan) equipped with Digital Instruments (Santa Barbara, CA) controller. The wafer cleaned in the Piranah method [19] was immobilized on the Petri dish, and exposed to micelle solution ($\sim 2 \times 10^{-3}$ mg/ml) for 1 min. The micelles attached to the surface via hydrogen bonding of PEG with the silanol group of the wafer [20, 21]. The SPM image was observed by tapping mode in 0.01 M NaCl solution. The area of $1 \times 1\,\mu$m was scanned with pyramidal cantilever with spring constant of 0.09 N/m and resonance frequency of *ca*. 5 kHz.

Peclitaxel (Taxol) Loading

Taxol-containing micelles were prepared by the oil/water (O/W) emulsion method. To the micelle solution in PBS (10 ml; 2 mg polymer/ml), a methylene chloride solution of Taxol (2 mg; 1 mg/

ml) was added dropwise using a microsyringe with vigorous stirring at room temperature. Alternatively, methylene chloride solution (2 ml) containing the block copolymer (20 mg) and Taxol (2 mg) was added to water (10 ml) with vigorous stirring to obtain a microemulsion. The solution in a vial with a porous cover was kept in a cold room with vigorous stirring overnight to remove the organic solvent completely. The solution was then centrifuged (3000 rpm, 30 min at 5 °C) to remove the unincorporated Taxol. The amount of Taxol entrapped in the micelle was determined by HPLC.

Core-polymerized micelle as well as core-crosslinked micelle were utilized in the same way as described above.

RESULTS AND DISCUSSION

Synthesis of MeO–PEG/PLA Block Copolymer With Methacryloyl End (MeO–PEG/PLA–Methacryloyl)

The MeO–PEG/PLA block copolymer was prepared by anionic ring-opening polymerization of EO and DL-lactide monomers by sequential addition in THF using potassium methoxyethanolate as the reactive initiator which was prepared *in situ* from 2-methoxyethanol and potassium naphthalenide. At the final stage of polymerization, the block copolymer with a living alkoxide end was terminated by adding an excess amount of methacrylic anhydride to introduce a polymerizable methacryloyl group into each polymer chain end (Scheme 1). Figure 1 shows the GPC chromatograms of an MeO–PEG sample after EO polymerization and the MeO–PEG/PLA–methacryloyl block copolymer in DMF. The number average molecular weight, M_n, and the polydispersity (M_w/M_n) of PEG were 3800 and 1.07, respectively, while those of the block copolymer were 6400 and 1.12, respectively. Both peaks appeared with quite narrow molecular mass distributions without any shoulder.

More precise estimation of molecular mass could be obtained from ^{1}H-NMR shown in Fig. 2 (a). Two vinyl protons of the methacryloyl end appeared at 5.65 and 6.21 ppm, and the methine proton of PLA and the methylene proton of the PEG block appeared at 5.20 and 3.65 ppm, respectively. Also the methyl protons of the methoxy end group and the α-methyl protons of the methacryloyl end group appeared at 3.4 and 2.0 ppm, respectively. The M_n values of each block segment, which were obtained by calculation based on the M_n from GPC and the integration ratio of backbone protons, were 3800 and 3500 for the PEG and PLA blocks, respectively. The integration ratio of methoxy methyl v. one vinyl proton was almost 3, suggesting a quantitative introduction of the methacryloyl group onto each polymer chain.

Figure 3 shows TGA and DSC traces of the block copolymer. The TGA thermogram under argon showed a two-step decomposition curve with the first onset at 310 °C and a second one at 385 °C, respectively, and the ratio between the first mass loss corresponding to the PLA block and the subsequent loss of PEG was almost 50:50, matching the NMR results. The DSC thermogram of the first scan showed only the PEO melting endotherm at 51.5 °C ($\Delta H \sim 95$ J/g) with a shoulder at around 35 °C, which seemed to come from the glass transition of the PLA block. The second scan after the first run to 150 °C showed a crystallization exotherm of PEG starting at −15 °C with a peak temperature at −4.2 °C and melting peak at 50 °C ($\Delta H \sim 90$ J/g).

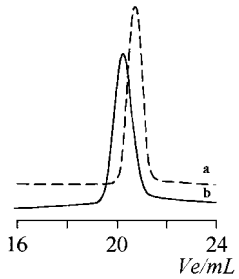


FIGURE 1. GPC chromatograms of (a) MeO-PEG and (b) MeO-PEG/PLA–Methacryloyl.

Preparation and Core-polymerization of Block Copolymer Micelles

The preparation of micelles was achieved by dialysis against water of a block copolymer solution in DMAc. From the ^{1}H-NMR analysis of the freeze-dried polymer after the dialysis, no signal based on the DMAc could be observed, indicating complete solvent exchange to water. The size and shape of the resulting polymeric micelles were estimated by the dynamic light scattering (DLS) measurement. Figure 4 shows a representative gamma-averaged distribution of the obtained micelles. A unimodal distribution was observed, and the average diameter and the distribution factor (μ/Γ^2), which were determined by a cumulant method, were 30 nm and 0.15, respectively. The prepared micelles were found to be very stable over the temperature range of 20–50 °C, showing no appreciable change in its size and distributions from the DLS measurements in this range.

It is generally known that polymeric micelles possess static stability with a very low CMC and dynamic stability that can be expressed in terms of the equilibrium between micelle and unimer, compared with low molecular mass surfactants. There are numerous requirements on the creation of drug carrier depend on disease, medicine and medical treatments. To meet these demands, versatile types of drug carrier must be created. For some of these demands, however, the physical coagulation force, i.e. intermolecular hydrophobic or van der Waals interaction, as the main driving force to form the core–shell micelle structure may not be strong enough to maintain its integrity under various physiological conditions and on ultimate dilution. Thus, the chemical stabilization of the

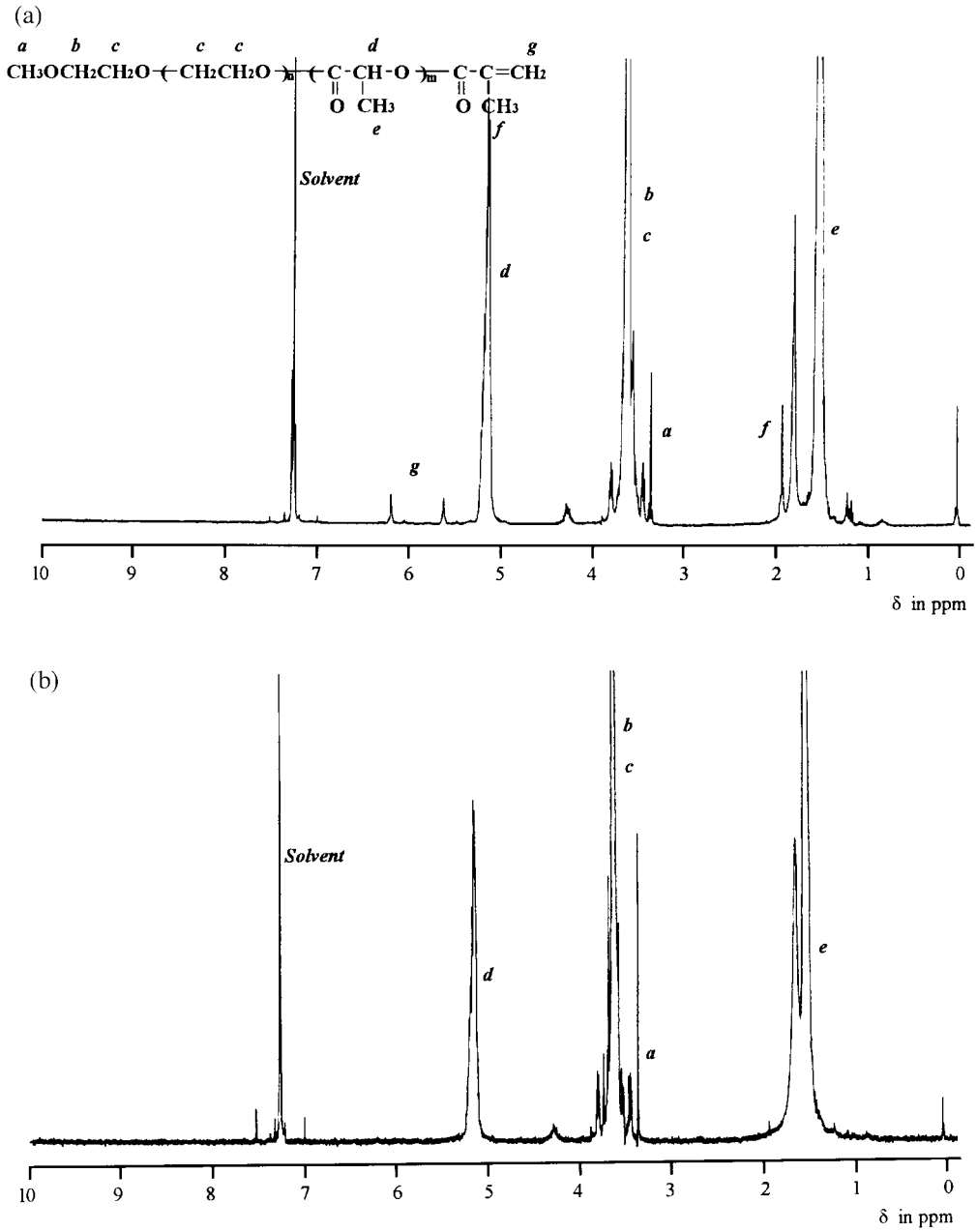


FIGURE 2. ^{1}H-NMR spectra (a) before and (b) after polymerization of MeO-PEG/PLA–Methacryloyl.

hydrophobic core in the polymeric micelle was proposed and examined here.

As was described earlier, the prepared block copolymer contains a polymerizable methacryloyl group at the end of the PLA block chain, which should distribute within the micelle core. Both chemical and photochemical methods were employed to accomplish core-polymerization. The first method was the thermally-initiated polymerization of the terminal methacrylate macromonomer in the presence of an azo-type free radical initiator. The second method was photo(UV)-induced polymerization in the presence of a suitable photosensitizer, such as benzoin methyl ether (BME) or BDMK. The photochemical method may have an advantage, because the process can be executed at low temperature without supplying heat to the system. An aqueous micelle solution containing an azo initiator was prepared by dialysis, and the solution was heated at 60 °C for 2 hr. Before the core polymerization, a freeze-dried sample of the solution was analyzed by ^{1}H-NMR to confirm there was initiator in the core. Though the remarkable initiator signal was not observed in the NMR analysis, the polymerization proceeded effectively (see below). The fact indicates that most of the initiator was eliminated by the dialysis, but the small amount of the initiator remained in the core and worked effectively. Actually, with increasing initial initiator concentration in the case of the photoinitiator, the rate of the polymerization and maximum conversion of the polymerization increased, which indicated that a part of the initiator was remaining in the core and worked

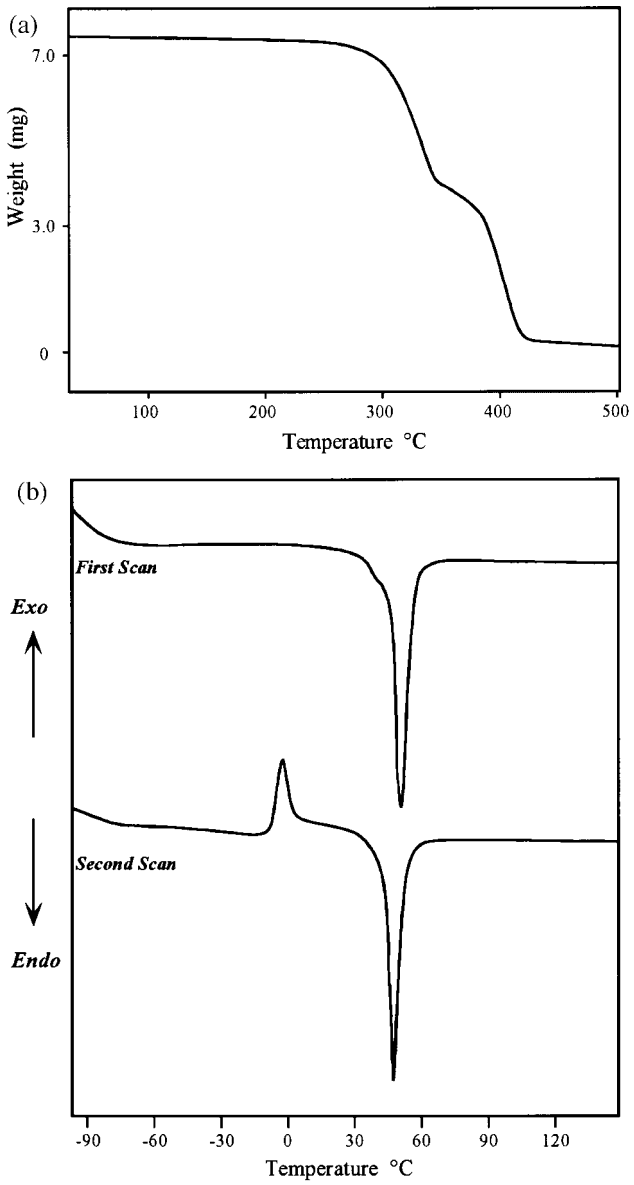


FIGURE 3. (a) DSC and (b) TGA thermograms.

effectively (see below). Figure 2 (b) shows the ^{1}H-NMR spectrum of a freeze-dried sample after core-polymerization. The original vinyl protons at 5.65 and 6.21 ppm and also the α-methyl protons at 2.0 ppm disappeared completely, indicating consumption of all of the methacryloyl moiety in the radical process. Photopolymerization was also tried at room temperature using the micelle solution dialyzed from a block copolymer solution containing BDMK or BME as a typical photosensitizer. Conversion of the vinyl moiety was estimated by a change in the intensity ratio of the vinyl proton v. methoxy methyl protons from ^{1}H-NMR. Figure 5 shows the increase in the conversion as a function of exposure time. The initial fast increase leveled off after 2 hr stirring, and about 75–80% conversion could be obtained in 4 hr. When the amount of photosensitizer was increased, up to 95% conversion of the vinyl group in 4 hr was obtained.

The DLS measurement of the micelle solutions

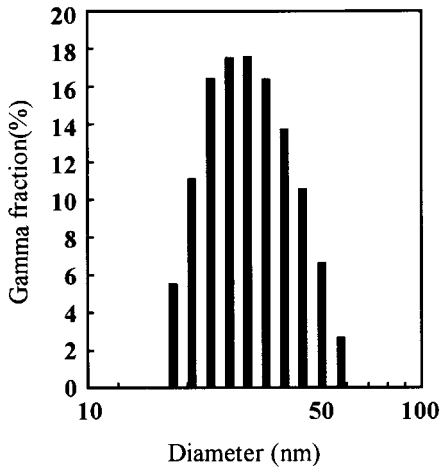


FIGURE 4. DLS histograms of dialyzed micelle.

after polymerization did not exhibit any noticeable changes in its size and distribution. The micelle solution seemed to remain stable during the polymerization reaction. The cumulant diamater was 32.8 nm and the μ/Γ^2 value was 0.144 by the DLS analysis. Thermal analysis by DSC of a polymerized micelle sample showed that the crystallinity of PEG decreased by about 15% ($\Delta H \sim 71$ J/g) from the quenched sample compared with that of a sample before polymerization and the crystallization seemed to be slower than the unpolymerized sample. The decrease in the crystallinity and crystallization rate is probably due to the restriction in chain mobility and the packing of PEG chains caused by the chemically linked PLA core domain.

The crosslinking of the core in a polymeric micelle was carried out using ethylene glycol bismethacrylate as a crosslinking agent. The size and distribution of the crosslinked micelle analyzed by GPC and DLS showed a tendency similar to that of a polymerized micelle (cumulant diameter = 34 nm; $\mu/\Gamma^2 = 0.114$). The difference between

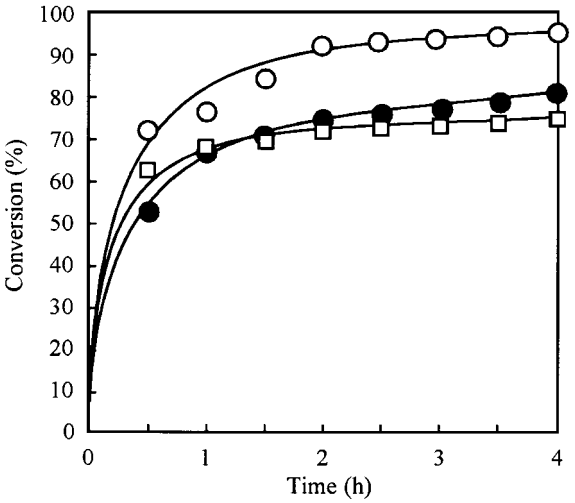


FIGURE 5. Conversion of vinyl group as a function of exposure time: ○ DMBK 3 wt%; ● DMBK 1 wt%; □ BME 1 wt%.

polymerized and crosslinked micelles appeared in their stability which is shown in the following section.

Stability of Polymerized and Cross linked Block Copolymer Micelle

The stability of the micelle after polymerization was studied by light-scattering analysis, and compared with micelle before polymerization. To evaluate the stability of micelles before and after the core-polymerization reaction, SDS treatment of a micelle solution was carried out, viz. to the micelle solution thus obtained was added a half volume of sodium dodecylsulfonate (SDS) solution (20 g/l). The change in the micelle solution after mixing as a function of time was observed by dynamic and static light scattering. The DLS measurement of the SDS-treated micelle solution before polymerization exhibited a rapid decrease in its scattering photon counting number within 2 hr (below *ca*. 20% of solution just after mixing with SDS), and it remained at a very low level, suggesting the disappearance of most of the micelles in solution. In contrast, the micelle solution after polymerization showed only an initial small drop in its photon counting which remained at high level afterward. As time elapsed, only a very slow decrease was observed. Even after one month, the solution exhibited about 70% photon counting, and a narrower size distribution with a somewhat increased size was observed. Static light-scattering measurements supplemented these results again. Figure 6 shows the decrease in light scattering intensity (I_s) due to SLS for the different micelle solutions which were treated with SDS. The relative intensity was normalized based on the light-scattering intensity of the first measurement of each solution just after mixing. Compared with the micelle solution before polymerization, the scattering intensity remained high for the samples after

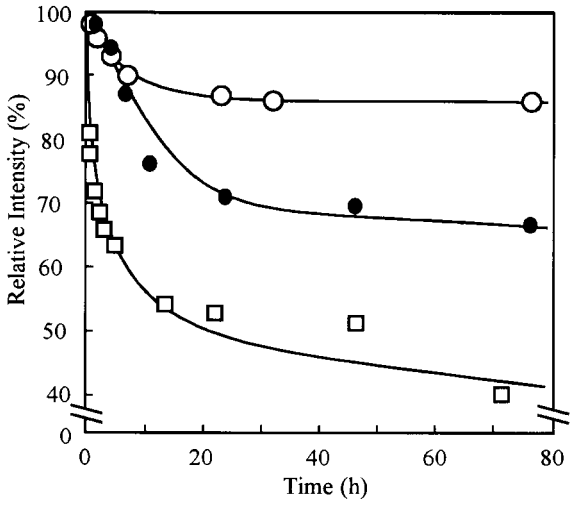


FIGURE 6. Stability of micelles against SDS: ○ crosslinked micelle; ● polymerized micelle; □ just dialyzed micelle.

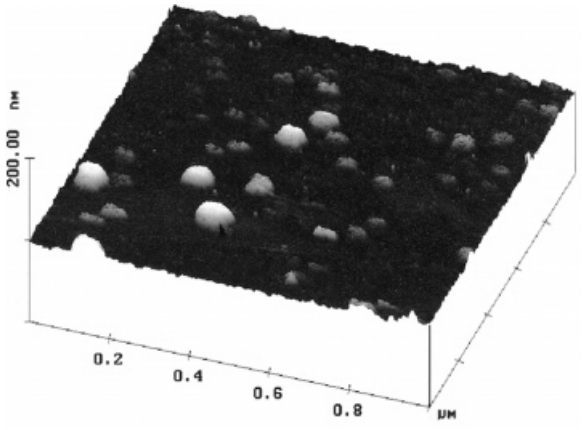


FIGURE 7. Scanning force image of polymerized micelle adsorbed on Si wafer.

polymerization, and even higher for the crosslinked micelle. These results strongly indicate the effectiveness of the stabilization of micelles by core-polymerization and/or crosslinking.

The image of micelle on a Si wafer in aqueous medium was observed by SPM. As in Fig. 7, the spherical structure of micelles of about 30–50 nm was observed, although the solution below CMC ($\sim 3 \times 10^{-3}$ mg/ml) was used. The size is slightly larger than the DLS measurement probably because of the nature of the cantilever and the flattening of micelle on the surface. We previously showed the images of surface-attached micelles from 1 mg/ml solution [22]. Whereas the micelle structure was maintained for the core-polymerized micelle, the nonpolymerized micelle disrupted and the surface got smoother than the original one, although the force-distance indicated a presence of soft polymer. This image also suggests the high structural stability of the core-polymerized micelle even below the CMC.

Taxol Loading

Taxol is anticipated to be one of the next anticancer drugs with extremely high efficiency. Its solubility, however, is one of the problems preventing its practical use. For example, the solubility of taxol in water is only 0.5 μg/ml. Burt and coworkers reported that a PEG/PLA block copolymer increased the solubility of taxol in aqueous media when the concentration of the copolymer was more than the CMC [23]. Actually, 20 μg/ml of Taxol was solubilized in aqueous media with a polymer concentration above the CMC (copolymer (MW = 4000) concentration was 45 μM), though a certain amount of Taxol leaked out during several hours. Because the core-polymerized micelle is stable enough even in the presence of SDS as stated above, the micelle can be anticipated to sustain a drug-retaining spherical shape in nano-size even in the bloodstream, which is a non equilibrated open system. The question is whether enough of a high molecular mass drug such as Taxol can be solubilized in the core of the micelle even after

the core polymerization. Thus, a drug loading test with the stabilized polymeric micelle were carried out. The incorporation of Taxol into the stabilized polymeric micelle was carried out via a general O/W emulsion method. Typically, a methylene chloride solution of Taxol was loaded into the micelle solution in PBS (10 mM, 1.5 wt% NaCl, pH 7.4) by dropwise addition using a microsyringe with vigorous stirring at room temperature. The loading of Taxol in the polymerized micelle attained 3–6 wt% using the above method. (3–6 mg/100 mg micelle/10 ml, which is 30–60 μg/ml; the polymer (M_w = 7000) concentration was 140 μM). Therefore, the solubilization of Taxol in core-polymerized micelles was at the same level as that of the physically aggregated micelles published previously.

The cumulant diameter and μ/Γ^2 values of the Taxol-loaded micelles were observed to be *ca.* 45–47 nm and 0.18–0.20, respectively, indicating the increased size and distribution of the micelles after drug incorporation. The results of *in vitro* and *in vivo* release experiments with Taxol from the stabilized micelles will be published elsewhere.

In conclusion, the MeO–PEG/PLA block copolymer with a polymerizable methacryloyl end was prepared by anionic ring-opening polymerization followed by end-capping with methacrylic anhydride. A stable core–shell type, spherical micelle with a number-averaged diameter of *ca.* 30 nm was obtained by a dialysis method, and the resulting aqueous micelle solution was core-polymerized chemically and photochemically to produce more stable nanoparticles, which was evidenced by spectroscopic and light-scattering techniques. Taxol-incorporated micelles were prepared to entrap Taxol into MeO–PEG/PLA–methacryloyl block copolymer micelles by the O/W emulsion method.

ACKNOWLEDGMENT

The authors express their sincere appreciation to the Research Project on Biointegrated Materials, Science and Technology Agency of Japan, for financial support of a part of this study.

REFERENCES

1. Kataoka K, Kwon GS, Yokoyama M, Okano T, Sakurai Y. Micelles based on AB block copolymers of poly(ethylene oxide) and poly(β-benzyl L-aspartate). *J. Controlled Release* 1993; **24**: 119–132.
2. Kwon GS, Naito M, Yokoyama M, Okano T, Sakurai Y, Kataoka K. Micelles based on AB block copolymers of poly(ethylene oxide) and poly(β-benzyl L-aspartate). *Langmuir* 1993; **9**: 945–949.
3. Yokoyama M, Kwon GS, Okano T, Sakurai Y, Sato T, Kataoka K. Preparation of micelle-forming polymer-drug conjugates. *Bioconjugate Chem.* 1992; **3**: 295–301.
4. Yokoyama M. Block copolymers as drug carriers. *Crit. Rev. Ther. Drug Carrier Systems* 1992; **9**: 213–248.
5. Jeong B, Bae YH, Lee DS, Kim SW. Biodegradable block copolymers as injectable drug-delivery systems. *Nature* 1997; **388**: 860–862.
6. Zhu KJ, Xianzhou L, Shilin Y. Preparation, characterization and properties of polylactide (PLA)-poly(ethylene glycol) (PEG) copolymers: a potential drug carrier. *J. Appl. Polym. Sci.* 1990; **39**: 1–9.
7. Tanodekaew S, Pannu R, Heatley F, Attwood D, Booth C. Association and surface properties of diblock copolymers of ethylene oxide and DL-lactide in aqueous solution. *Macromol. Chem. Phys.* 1997; **198**: 927–944.
8. Ramasmamy M, Zhang X, Burt HM, Wasan KM. The human plasma distribution of free paclitaxel and paclitaxel associated with diblock copolymers. *J. Pharm. Sci.* 1997; **86**: 460–464.
9. Peracchia MT, Gref R, Minamitake Y, Domb A, Lotan N, Langer R. EG-coated nanospheres from amphiphilic diblock and multiblock copolymers: investigation of their drug encapsulation and release characteristics. *J. Controlled Release* 1997; **46**: 223–231.
10. Hagan SA, Coombes GA, Garnett MC, Dunn SE, Davies MC, Illum L, Harding SS, Purkiss S, Gellert PR. Polylactide-poly(ethylene glycol) copolymers as drug delivery systems. Characterization of water dispersible micelle-forming systems. *Langmuir* 1996; **12**: 2153–2161.
11. Bazil D, Prudhomme C, Bassoullet MT, Marlard M, Spenlehauer G, Veillard M. Stealth Me.PEG-PLA nanoparticles avoid uptake by the mononuclear phagocytes system. *J. Pharm. Sci.* 1995; **84**: 493–498.
12. Piskin E, Kaitian X, Denkbas EB, Kucukyavuz Z. Novel PDLLA/PEG copolymer micelles as drug carriers: loading and release. *J. Biomater., Sci., Polym. Edn*. 1995; **7**(4): 359–373.
13. Zhang X, Jackson JK, Burt HM. Development of amphiphilic diblock copolymers as micellar carriers of taxol. *Int. J. Pharmaceutics* 1996; **132**: 195–206.
14. Yokoyama M, Okano T, Sakurai Y, Ekimoto H, Shibazaki C, Kataoka K. Toxicity and antitumor activity against solid tumors of micelle-forming polymeric anticancer drug and its extremely long circulation in blood. *Cancer Res.* 1991; **51**: 3229–3236.
15. Munk P, Ramireddy C, Tian M, Webber SE, Prochazka K, Tuzar Z. Block copolymer micelles in aqueous media. *Makromol. Chem. Macromol. Symp*. 1992; **58**: 195–199.
16. Perrin DD, Armarego WLF, Perrin DR. Purification of Laboratory Chemicals. Pergamon Press: Oxford, 1980.
17. Coates CE, Green MLH, Wade K. *Organometallic Compounds. Vol. 1: Three Main Group Elements.* Methuen: London, 1956.
18. Scholz C, Iijima M, Nagasaki Y, Kataoka K. A novel reactive polymeric micelle with aldehyde groups on its surface. *Macromolecules* 1995; **28**: 7295–7297.
19. Bearinger JP, Castner DG, Golledge SL, Rezania A, Hubchak S, Healy KE. P(AAm-co-EG) interpenetrating polymer networks grafted to oxide surfaces: surface characterization, protein adsorption, and cell detachment studies, *Langmuir* 1997; **13**: 5175–5183.
20. Preisler J, Yeung ES. Characterization of nonbonded poly(ethylene oxide) coating for capillary electrophoresis via continuous monitoring of electroosmotic flow. *Anal. Chem.* 1996; **68**: 2885–2889.
21. Emoto K. PhD Thesis. Chemical modification of surfaces with hydrophilic polymers. University of Alabama: Huntsville, AL. 1997.
22. Emoto K, Nagasaki Y, Kataoka K. Coating of surface with stabilized reactive micelles from poly(ethylene glycol)-poly(DL-lactic acid) block copolymer. *Langmuir* 1999; **15**: 5212–5218.
23. Zhang X, Jackson JK, Burt HM. Development of amphiphilic diblock copolymers as micellar carriers of taxol. *Int. J. Pharm.* 1996; **132**: 195–206.

