The Reactive Polymeric Micelle Based on An Aldehyde-Ended Poly(ethylene glycol)/Poly(lactide) Block Copolymer

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ABSTRACT: Formation of amphiphilic poly(ethylene glycol)-*b*-polylactide (PEG/PLA) block copolymers was accomplished by using potassium alkoxides to initiate the anionic polymerization of ethylene oxide, with the living chain end initiating the polymerization of lactide. By using potassium 3,3-diethoxypropoxide as an initiator, block copolymers with an acetal moiety at the PEG chain end, which was later converted into an aldehyde group, were obtained. The amphiphilic block copolymers formed micelles in aqueous milieu. The conversion of acetal end groups to aldehyde groups was carried out by an acid treatment using 0.01 mol L^{-1} hydrochloric acid. The extent of the conversion attained was >90%, without any side reaction such as aldol condensation. The micellar structure may play an important role in preventing a possible aldol condensation between the neighboring two aldehyde groups at the PEG chain end. From dynamic light scattering measurements, no angular dependence of the scaled characteristic line width was observed in the case of the acetal-PEG/PLA(52/56) micelle, suggesting the spherical structure. The diameter and polydispersity factor of the polymeric micelle were influenced by the molecular weights and the composition of two components of the block copolymer. The block copolymer with the molecular weight of 5200 for PEG and 5600 for PLA was a most suitable balance for micelle formation with narrow distribution. Actually, the diameter and polydispersity factor (μ/Γ^2) of acetal-PEG/PLA(52/56), determined by a cumulative method, were 33 nm and 0.03, respectively. No change in the micelle size and shape was observed before and after the conversion of the acetal end groups to aldehyde groups on the micelle. The critical micelle concentrations (cmc) of the polymeric micelle was 2−4 mg L^{-1}, as determined by fluorescence spectroscopy using pyrene. This functionalized micelle, in particular the one carrying terminal aldehyde groups, is expected to have a wide utility not only in biomedical applications (e.g., drug delivery, diagnosis, and surface modification through the coupling of bioactive substances), but also for the construction of the supramolecular architecture.

Introduction

A polymeric particle of nano dimension in diameter has become attractive in the field of nano-fabrication chemistry for construction of a supramolecular structure.[1] A dendrimer is well known to provide such a nanoparticle of a few nanometers in diameter.[2] In general a dendrimer is prepared by successive 1:2 consecutive reactions to form a dendritic structure. Thus, the surface of the dendrimer possesses many reactive groups, which is one of the reasons for utilization of the dendrimer as a starting material for nano-fabrication chemistry. However, it is not easy to complete the consecutive reactions for the preparation of the dendrimer. Actually, several defects often appear, especially in the cases higher than the 4th generation. These defects sometimes induce a serious problem for nano-supramolecular fabrication and also for applications of the supramolecules, such as in a drug delivery system.

Amphiphilic AB block copolymers form micellar structures in selective solvents.[3] Although these nanospheric particles are formed by intermolecular interactions of one of the block segments, which is insoluble in the selective solvents, it is fairly stable compared with low molecular weight (lipid) micelles. Such polymeric micelles tend to form a spherical structure with a size of a few tens to a few hundreds of nanometers in diameter. However, most of polymeric micelles prepared so far possess no reactive group on the surface.[4] If reactive groups can be introduced on the surface, the reactive polymeric micelles can be utilized as a starting material for nano-fabrication chemistry.

Recently, we reported a facile and quantitative synthetic method for the formation of heterobifunctional poly(ethylene glycol) (PEG).[5] When one of the functional end-groups in the heterobifunctional PEG selectively inititates the polymerization of a hydrophobic monomer, a new heterobifunctional AB block copolymer can be created, retaining the other functional group at the PEG chain end. In our previous communication,[6] lactide (LA) was chosen as the hydrophobic segment, because (i) the ring opening reaction of LA can be initiated by the living chain end of potassium alkolate at the PEG end without any side reaction,[7] (ii) polylactides (PLAs) are biodegradable and nontoxic polymers that are widely utilized as implant materials,[8] and (iii) nanoparticles, consisting of block copolymers of α-methoxy-PEG and PLA, are suited for drug delivery.[9]

This paper deals with a reactive PEG/PLA polymeric micelle in detail; *viz*., synthesis of acetal-PEG/PLA block copolymers, preparation of the polymeric micelle with acetal groups on their surface, and conversion of the acetal groups into aldehyde groups to prepare stable polymeric micelles with aldehyde groups on their surface.

Experiments

Materials and Methods. Commercial tetrahydrofuran (THF), 3,3-diethoxypropanol, ethylene oxide (EO),

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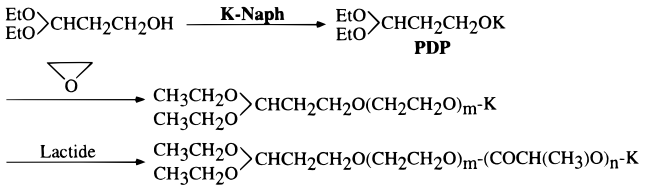
and *dl*-lactide (LA) were purified conventionally.[10] Potassium naphthalene was used as a THF solution,[11] the concentration of which was determined by titration. Gel permeation chromatography (GPC) measurements were carried out using a Shimadzu 6A liquid chromatograph equipped with a TSK gel columns ($G4000_{HXL}$ + $G3000_{HXL}$ + $G2500_{HXL}$) and an internal refractive index (RI) detector (RID-6A). THF containing 2% triethylamine was used as eluent at a flow rate of 1 mL min^{-1}. The ^1H NMR spectra were obtained using chloroform-*d* solutions with a JEOL EX400 spectrometer at 400 MHz. Fluorescence measurements were carried out using a 770F JASCO fluorometer at an excitation wavelength of 333 nm and emission wavelengths of 339 and 390 nm. A light scattering spectrophotometer (DLS-7,000 Photal, Otsuka Electronics) equipped with a 75 mW Ar-laser that produces vertically polarized incident beams at λ_0 = 488 nm was used in the present study for dynamic and static light scattering measurements. The vertically scattered beam was collected over an angular range of 30 to 150° for photon correlation spectroscopy measurements. The concentration varied from 5×10^{-4} to 2 g L^{-1}.

Polymer Synthesis. One of the representative procedures for preparation of α-acetal-PEG/PLA block copolymers is described. α-Acetal-PEG/PLA block copolymers were synthesized by a one-pot anionic ring opening polymerization of EO followed by LA, that was initiated with potassium 3,3-diethoxypropanolate (PDP) as an initiator at room temperature under argon. One millimole (0.16 mL) 3,3-diethoxypropanol and 1 mmol (3.5 mL) potassium naphthalene were added to 30 mL of dry THF to form PDP. After stirring for 10 min, 120 mmol (6 mL) of condensed EO was added via a cooled syringe to the formed PDP solution. The polymerization of the EO proceeded for 2 days and resulted in a light brown, highly viscous solution. Supplementary THF was added to decrease the viscosity of the reaction mixture, and potassium naphthalene (≈1.5 mL) was added, until the solution turned green, to stabilize the living chain end. Twenty millimole (10.1 mL) of an LA solution in THF (1.98 mol L^{-1}) was introduced, and the polymerization proceeded for 90 min. The polymer was recovered by precipitation into a 20-fold excess of cold isopropyl alcohol (−15 °C), stored for 2 h in the freezer, and centrifuged for 30 min at 6000 rpm. The polymer was then freeze-dried in benzene and the yield of the obtained polymer was ≈90%.

Polymerization Characterization. The molecular weight of the PEG segment was determined by GPC at the end of the EO polymerization. The molecular weight of the PLA segment was determined with an ^1H NMR spectrum by examining the ratio of methine protons in the PLA segment and methylene protons in PEG segment based on the number-average molecular weight (M_n) of PEG determined from the GPC result. The extent of conversion of the acetal to aldehyde groups at the end of the polymer chain was estimated by ^1H NMR spectroscopy after the acid treatment and purification (described later).

Micelle Preparation. The procedure has been detailed previously.[6] Briefly, 100 mg of the copolymer was dissolved 20 mL of dimethyl acetamide, and the polymer solution was transferred into a pre-swollen membrane (Spectra/Por, molecular weight cutoff size, 12 000−14 000), dialyzed against water for 24 h and subsequently lyophilized. The yield of the micelle formation was ≈90%.

Scheme 1



To convert the α-diethoxy-terminated micelle into a micelle with aldehyde groups at the end of the PEG chain, the polymeric micelle solution was adjusted to pH 2 without lyophilization, kept for 1 h, and readjusted to pH 7 with NaOH. The solution was again dialyzed against water for 24 h using a pre-swollen membrane to remove the salt. The aldehyde micelle thus obtained was analyzed directly by dynamic light scattering (DLS) or frozen in liquid nitrogen and lyophilized, resulting in a yield of 85 to 90%.

Micelle Characterization. The size and shape of the polymeric micelle were characterized by DLS measurements.[12] The critical association concentration (cac) was determined using pyrene as a fluorescence probe.[13] Pyrene, used as a hydrophobic fluorescence probe, parititioned preferably in the micelle core, causing changes in the photophysical properties of the nanoparticle under investigation.[14] A saturated aqueous solution of pyrene (6×10 L^{-7} mol L^{-1}) was used for the measurements. The micelle concentration varied from 5×10^{-4} to 1.0 g L^{-1}.

Results and Discussion

Synthesis of Acetal-PEG/PLA BLock Copolymers. An initiation method was used for introduction of a functional group at the PEG end. Potassium alkoxide with a functional group was used for the polymerization of EO to obtain the α-functionalized PEG. To introduce an aldehyde group at the α-terminal, an acetal group was employed as a protective group (Scheme 1).

When PDP is used as an initiator for EO polymerization, PEG with an acetal moiety at the α-terminus is obtained quantitatively. Because potassium alkoxide has the ability to initiate LA polymerization, a PEG/PLA block copolymer with a functional group at the PEG chain end can be prepared. The molecular weight of each segment can be controlled by the initial monomer/initiator ratio. Figure 1 shows the stepwise block polymerization profile monitored by GPC. After the EO polymerization initiated with PDP, the M_n and the molecular weight distribution (MWD) were 5200 and 1.04, respectively. The MW of the obtained PEG was in good accordance with the initial monomer/initiator ratio ($[EO]_0/[PDP]_0$ = 120/1). After the block copolymerization of LA, the M_n and the MWD determined by the GPC data were 9100 and 1.07, respectively. The MW of the PLA segment calculated by subtraction of the MW of the PEG segment from the total MW was only 3900, which was lower than the value expected by the initial monomer/prepolymer ratio (=57/1). In the case of the GPC analysis of acetal-PEG/PLA block copolymers using THF as a carrier, the block copolymer tends to appear rather on the lower MW, side probably due to the adsorption of the block copolymer on the gel.

The segment length of the PLA in acetal-PEG/PLA block copolymers was estimated from the ^1H NMR

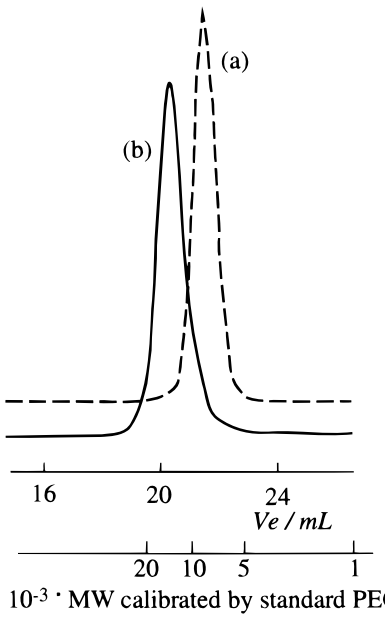


Figure 1. Gel permeation chromatograms of (a) acetal-PEG and (b) acetal-PEG/PLA.

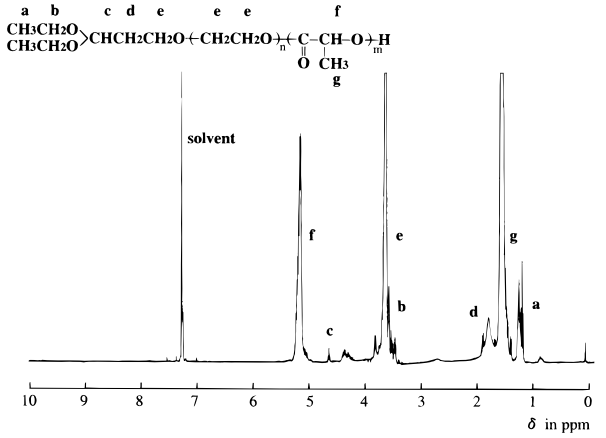


Figure 2. ^{1}H NMR spectrum of the acetal-PEG/PLA (the same sample as in Figure 1).

spectrum. Using PLA and acetal-ended PEG as reference compounds, the assignments of the spectrum were carried out and are described in Figure 2. The ratio of methine in the LA unit appearing at 5.2 ppm versus methylene in the EO unit appearing at 3.6 ppm can be used to determine the ratio of LA versus EO units in the block copolymer. Assuming the MW of PEG determined by GPC, the MW of the PLA unit can be calculated by the ratio as 5600, which agreed with the initial molar ratio of initiator versus LA. Thus, we employed the MW data for the PEG segment from GPC analysis and for the PLA segment from ^{1}H NMR analysis.

The ^{1}H NMR spectrum of the acetal-PEG/PLA block copolymer provides additional information on the end group of the polymer.[5c] As evident in Figure 2, the triad signal appearing at 4.6 ppm can be attributed to the acetal methine protons at the PEG end. By comparison of the acetal protons with methylene protons in the PEG segment and with methine protons in the PLA segment, each block copolymer should possess an acetal end group almost quantitatively.

(ii) Preparation of Acetal-PEG/PLA Micelle. It is known that amphiphilic block copolymers with a suitable hydrophilic/hydrophobic balance form micelle structures when exposed to a selective solvent. For the preparation of polymeric micelles of acetal-PEG/PLA block copolymers, water was employed which is a good solvent for PEG and a poor solvent for PLA. It is very important, however, to choose preparation conditions so that the polymeric micelle will have a desirable size and shape. Very large aggregates with a wide polydispersity factor are often observed. For example, it is reported that 80−110 nm size micelles were prepared by direct dissolution of PEG/PLA(40/80) and PEG/PLA(40/26) block copolymers into water.[15] When the block copolymers with a rather longer hydrophilic segment [PEG/PLA(20/9)−PEG/PLA(50/11)] were exposed to water, however, micelles of 15−25 nm in diameter could be prepared, but the polydispersity factors were fairly large.[16] Bazile et al.[17] reported the preparation of PEG/PLA nanoparticles by a solvent diffusion method; that is, an acetone solution of the block copolymer was exposed to water. In this case, micelles of 50−110 nm in diameter were prepared from PEG/PLA with fairly large hydrophobic segments [PEG/PLA(20/100)−PEG/PLA(50/350)].[17]

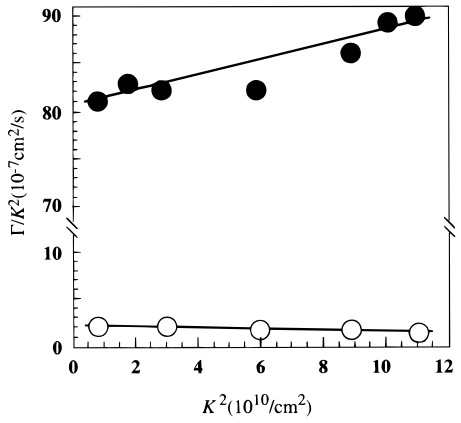


Figure 3. Plots of the K^2-scaled average characteristic line width Γ (Γ/K^2) versus K^2 for the acetal-PEG/PLA(52/56) (open circle) and acetal-PEG-PLA(91/51) (closed circle). (Concentration, 1.0 g L^{-1}; temperature = 25 °C.)

In the present study, a dialysis method was employed to prepare the polymeric micelle; that is, after the block polymer was dissolved in a good solvent for both segments, such as dimethylacetamide (DMAc), the solution was dialyzed against water.[18] The size and the shape of the obtained polymeric micelle were estimated by DLS measurement.

Before the estimation of the size of the prepared micelles, the angular dependency of the sample solutions was analyzed by the DLS. Figure 3 shows the dependence of the scaled characteristic line width on the scattering vector, which corresponds to the scattering angle. The scaled characteristic line width (Γ/K^2) of a perfectly spherical particle is independent of the scattering vector.[19]

The present data shown in Figure 3 exhibit a negligible angular dependence of the scaled characteristic line width on the scattering vector for the acetal-PEG/PLA(52/56) micelle, which is in sharp contrast to the fairly large angular dependency of acetal-PEG/PLA(91/51) (slope = 0.088). From these results it is concluded that the acetal-PEG/PLA(52/56) micelle is spherical in nature. The fairly large angular dependency of acetal-PEG/PLA(91/51) is probably due to the secondary aggregations of the micelles of acetal-PEG/PLA(91/51).

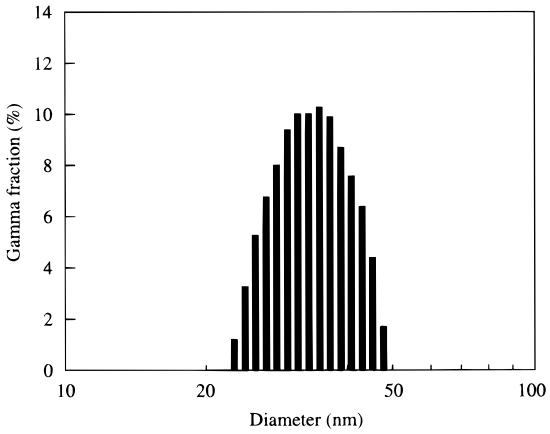


Figure 4. Gamma distribution of the acetal-PEG/PLA(52/56) micelle analyzed by DLS.

Representative data for the γ-distribution of the obtained polymeric micelle are shown in Figure 4. The acetal-PEG/PLA micelles thus obtained possess unimodal distribution in histogram analysis. The micelle size and polydispersity factor (PDF; μ/Γ^2) determined by a cumulant method are summarized in Table 1. The polymeric micelle thus obtained by the dialysis method shows fairly low PDF. Especially, the PDF of the micelle prepared from acetal-PEG/PLA(52/56) was 0.03. In addition, the size of the micelle for acetal-PEG/PLA-(52/56) was very small (30 nm). Thus, a unimodal spherical micelle with a small diameter can be prepared from acetal-PEG/PLA with a MW of both segments of $\approx$5000.

The critical micelle concentration (cmc) is a measure describing the physical properties of the micelle and refers to the micelle stability.[20] The term is actually derived from low molecular weight micelles, known for instance as detergents, but it is also an appropriate measure characterizing the stability of polymeric micelles. Pyrene was used as fluorescence probe, which partitioned preferably into the hydrophobic microdomain (i.e., the PLA-core of the micelle) and caused changes in the photophysical.[21] The total fluorescence increased with increasing concentrations of acetal-PEG/PLA polymer (data not shown). The total fluorescence intensity of the pyrene monomer emission versus the logarithm of the block copolymer concentration is shown in Figure 5a, indicating a cmc of 3 mg L^{-1} for acetal-PEG/PLA(52/56). With increasing concentration of the acetal-PEG/PLA polymer, a red shift was observed in the excitation spectrum; that is, the (0,0) band of pyrene shifted from 334 nm (in water) to 339 nm upon the addition of the block copolymer. Plotting the ratio of the intensity of the signal at 339 nm to that of the signal at 334 nm versus the logarithm of the block copolymer concentration (Figure 5b) resulted in a cmc of 1 mg L^{-1}. Moreover, the vibrational structure of the pyrene monomer emission spectrum changed upon the formation of a polymeric micelle, due to changes in the local polarity, the Ham effect.[21] Plotting the **I/III** band intensity ratio of the pyrene monomer versus the dependence of the logarithm of the acetal-PEG/PLA concentration indicated a cmc of 3 mg L^{-1} (Figure 5c). The mean value of the cmc based on the changes in the total fluorescence, the red shift of the pyrene excitation spectrum and the change in the **I/III** band intensity ratio of pyrene, was $<$2 mg L^{-1}. On the other hand the mean value of acetal-PEG/PLA(91/51) was 12 mg L^{-1}, indicating the difference in stability of acetal-PEG/PLA caused by the PEG segment length. These phenomena agreed well with the previously reported data that a PEG/PLA block copolymer with a rather longer PEG segment length relative to PLA tends to increase the cmc value in aqueous media [i.e., 350 mg L^{-1} for PEG/PLA(18/9)[22]; 35 mg L^{-1} for PEG/PLA(20/9)[13]]. The low coagulation force of the PLA unit may be one of the reasons for the rather lower stability of the PEG/PLA micelle with long PEG chains. The very low cmc for acetal-PEG/PLA(52/56) indicates that PEG/PLA block copolymers with the same segment length form very stable micelles in aqueous media as do PEG/polystyrene polymeric micelles in water (e.g., the cmc of PEG/polystyrene was 1$-$4 mg L^{-1} [23]).

(iii) Conversion of the Acetal to Aldehyde. The conversion of the acetal end group into an aldehyde end group was conducted after the micelle formation. After the acetal-PEG/PLA(52/56) micelle was prepared by the dialysis technique, the solution was adjusted to pH 2 with hydrochloric acid. After a predicted period of time, the reaction was quenched by neutralization with $NaOH_{aq}$, and the polymeric micelle was purified by dialysis. The conversion reaction of acetal into aldehyde was monitored by the ^{1}H NMR of the polymer after freeze-drying with water. The ^{1}H NMR spectrum of PEG/PLA-(52/56) after the hydrolysis reaction is shown in Figure 6. As can be seen in the figure, the end-aldehyde proton appears at 9.8 ppm, and the acetal methine proton disappears around 4.6 ppm. The extent of the conversion of the acetal group to the aldehyde group was determined by the ^{1}H NMR spectrum. More than 80% of the acetal was converted to aldehyde by the 4-h reaction. With a longer reaction period, however, the conversion tends to decrease, especially at $>$10 h. Some side reactions may take place with further acid treatment.

An aldehyde group possessing α-hydrogen(s) is generally known to undergo an aldol condensation reaction under acid conditions. Because the produced aldehyde group at the end of PEG/PLA chain end possesses a methylene group at the α-position, aldol condensation might take place. In the present case, the MW of the polymer should increase if the aldol reaction took place. As shown in Figure 7, no change in the MW and the MWD of the aldehyde-PEG/PLA(52/56) was observed before or after the acid treatment. The reactivity of the two chain ends might be different from low MW compounds; that is, the negative entropy factor may prevent the reaction between the two free ends because the mobility of the other chain end is restricted by the hydrophobic core formation.

To obtain further information on the shape and size of the aldehyde micelle in aqueous media, ^{1}H NMR and DLS measurements were carried out. The ^{1}H NMR spectrum of the aldehyde-PEG/PLA(52/56) micelle in D_2O is shown in Figure 8. The proton signal based on the aldehyde appears at 9.5 ppm along with large oxymethylene protons based on the PEG chain. The protons signals based on PLA units almost disappeared, indicating that the PLA segments form a solid core that causes a broadening effect due to the restricted mobility in NMR spectroscopy. Therefore, it is reasonable to consider that the aldehyde-PEG/PLA micelle possesses a core-shell structure with aldehyde groups on its surface.

The size and the shape of the aldehyde-PEG/PLA micelle after the acid treatment was investigated by

Table 1. Data of Acetal-PEG-PLA Block Copolymers and Their Micelles

	PEG		PLA				
polymer code	unit	M_n	unit	M_n	MWD	d (nm)	μ/Γ^2
PEG/PLA(52/56)	118	5200	39	5600	1.11	33	0.03
PEG/PLA(91/51)	207	9100	35	5100	1.10	30	0.13
PEG/PLA(77/18)[a]	175	7700	13	1800		172	0.14

[a] *l*-Lactide was used.

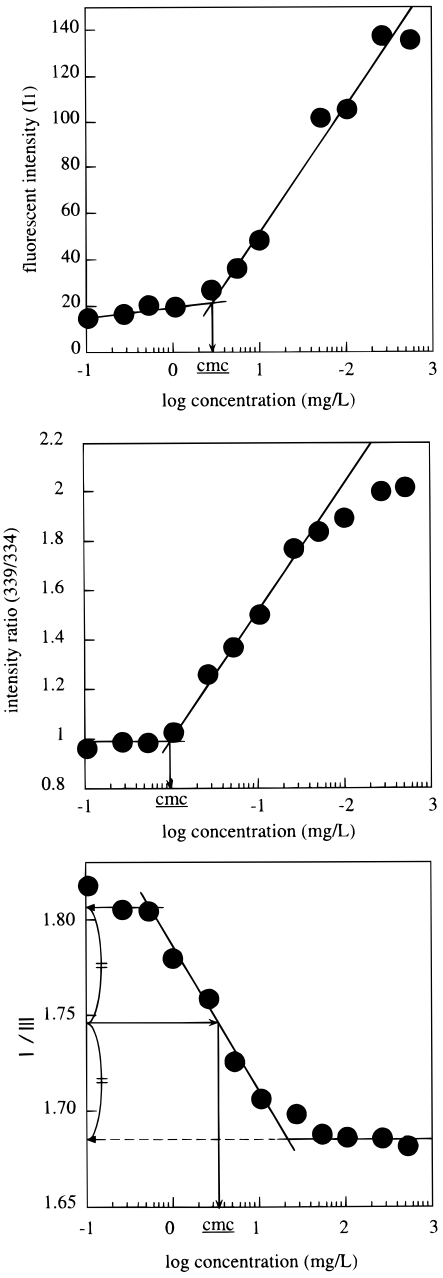


Figure 5. (a) Total fluorescence of pyrene emission, (b) intensity ratio (339/334) of pyrene, and (c) **I/III** band intensity ratio of pyrene as a function of acetal-PEG/PLA(52/56) concentration.

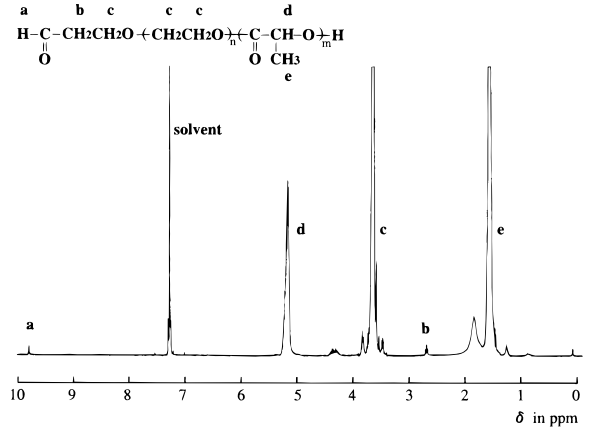


Figure 6. ^{1}H NMR spectrum of aldehyde-PEG/PLA(52/56) in $CDCl_3$ (the sample shown in Figure 2 was converted by acid hydrolysis).

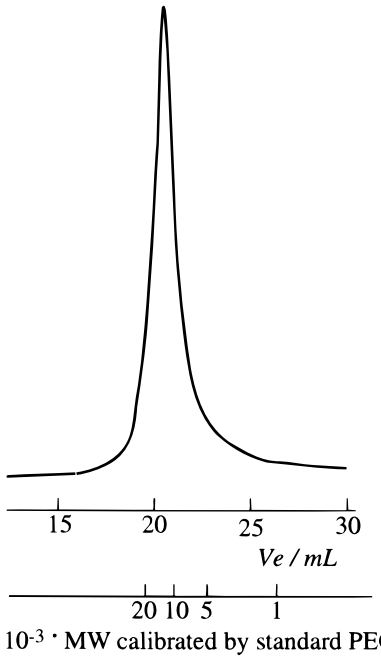


Figure 7. Gel permeation chromatogram of aldehyde-PEG/PLA(52/56).

DLS analysis.

The angular dependency of the Aldehyde-PEG/PLA-(52/56) from the DLS measurements was estimated. The dependence of the scaled characteristic line width on the scattering vector, which corresponds to the scattering angle, is shown in Figure 9. Even after the conversion of the acetal to aldehyde, no angular dependence of the scaled characteristic line width on the scattering vector was observed, indicating the spherical structure of the aldehyde micelle. The gamma distribution of the aldehyde-PEG/PLA(52/56) (meaning after acid treatment of the acetal-micelle) also shows unimodal distribution; the cumulant diameter and μ/Γ^2 were 31.6 nm and 0.04, respectively, indicating no change in sizes before or after the conversion of acetal to aldehyde.

Figure 10 is a plot of the scaled characteristic line width versus aldehyde-PEG/PLA(52/56) concentration. No change in Γ/K^2, which is proportional to size, was observed in the concentration range from 0 to 1.5 g L^{-1}, even though the micelle possesses reactive aldehyde groups on the surface. From the diffusion constant D_0, which can be obtained from Γ/K^2 extrapolated to a

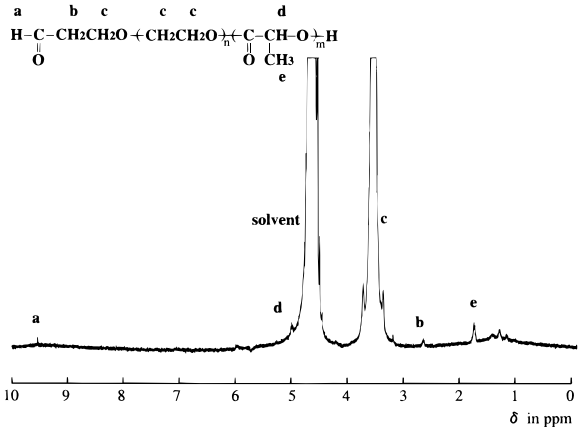


Figure 8. ^{1}H NMR spectrum of aldehyde-PEG/PLA(52/56) analyzed in D_2O (the same sample as in Figure 6).

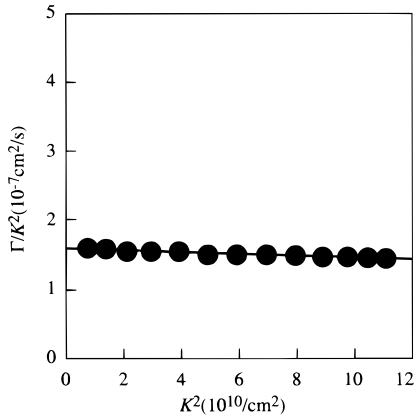


Figure 9. Plots of the K^2-scaled average characteristic line width Γ, (Γ/K^2) versus K^2 for aldehyde-PEG/PLA(52/56) at a concentration of 1.0 g L^{-1} and temperature of 25 °C.

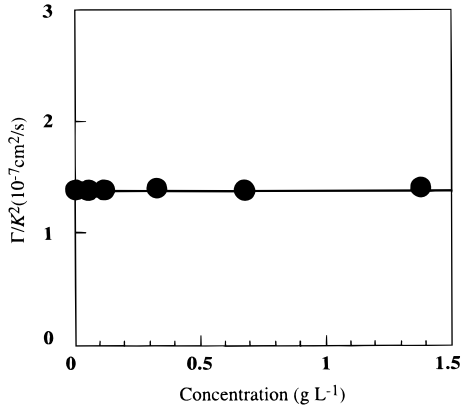


Figure 10. Change in Γ/K^2 as a function of the aldehyde-PEG/PLA(52/56) concentration. (Temperature, 25 °C).

concentration of 0, the Stokes diameter was calculated to be 35.8 nm, which is in good agreement with the diameter determined by histogram analysis. This result means that the reactive polymeric micelle consisting of aldehyde-PEG/PLA(52/56) shows almost no secondary aggregation in the wide concentration range.

On the basis of all these results it is concluded that acetal-PEG/PLA(52/56) shows spherical core-shell type polymeric micelles in aqueous media. The conversion of the acetal to aldehyde proceeds smoothly to form the aldehyde micelle, which has a spherical structure. This functionalized micelle (in particular, the one carrying terminal aldehyde groups) is not only expected to have wide utility in biomedical application (including drug delivery, diagnosis, and surface modification through the coupling of bioactive substances), but also to be of good interest as a novel supramolecular architecture.

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