Polymeric Micelles as Drug Delivery Systems: a Reactive Polymeric Micelle Carrying Aldehyde Groups

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ABSTRACT

Nanospheric particles as drug delivery systems are gaining increasing interest in the biomedical field. Nanospheres have been proven as efficient drug delivery systems for intravenous administration because of their comparatively long bloodstream circulation. A novel approach in the field of polymeric drug delivery systems was introduced by the formation of polymeric micelles and subsequently by functionalized polymeric micelles. Functionalized polymeric micelles are expected to find a wide application in the fields of drug delivery and diagnosis since the possibility of coupling to bioactive substances is provided. A large number of densely packed functional groups on the outer shell of the micelle allows an immobilization of biologically active substances at a high density. This is a great advantage for utilizing this particular type of nanosphere in the biomedical field. The possibilities of synthesizing heterobifunctional block copolymers will be demonstrated and the influence of the individual block length on the micelle properties will be discussed. Functionalized polymeric micelles were synthesized from poly(ethylene glycol) (PEG) and poly(lactide) (PLA), and combine the advantages given by the hydrophobic PLA core and the hydrophilic PEG corona. An established quantitative synthetic method for the formation of heterobifunctional PEG was advanced and applied to the block copolymerization. A heterobifunctional block copolymer was synthesized, terminated by an acetal group at the PEG end and a vinyl group was introduced at the PLA end in a one-pot synthesis. After the micellization the acetal groups on the micelle surface were converted into aldehyde groups by an acidic treatment. Aldehyde groups can react rapidly with primary amines forming Schiff bases, a potential future pathway for the conjugation of functionalized polymeric micelles with proteins. PLA was chosen as core-forming segment since it is a biodegradable, non-toxic polymer that is well established as implant material. Dynamic and static light scattering was applied to determine the micelle size and shape and to study the dependence of the micelle geometry on the block length of the copolymer. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: polymeric micelle; block copolymer; light scattering; site-specific drug delivery

INTRODUCTION

There has been a steadily increasing interest and demand for development of drug delivery systems that are not only highly efficient but also site-specific [1, 2]. Former drug delivery systems lacked...
performance in part due to rapid recognition by the reticuloendothelial system (RES) and subsequent kidney and/or hepatic elimination. Moreover, for drug delivery systems targeted at solid tumors, which are located outside the blood compartment, the vehicle is required to exhibit not just a sufficient half-life in the bloodstream to reach the tumor site, but also the capability of penetrating the vessel wall and docking at the tumor site. Recent developments led to the design of drug delivery vehicles with prolonged circulation in the vascular system. [3] Anticancer drugs cause severe side effects, leaving patients in chemotherapy under extreme distress. To overcome this problem, an ideal drug delivery system would be characterized by the following properties: (i) a drug compartment that conceals a drug while traveling through the blood stream, and (ii) releases the drug only after endocytosis into the targeted cells and lysosomal uptake inside these cells; and (iii) a long circulating delivery system that is blood-compatible but not subject to elimination from the bloodstream by scavenger cells. Considering these demands and requirements from a construction point of view, viruses would actually act as an ideal drug delivery system. A virus is constructed in such a way that the substance being transported, i.e. the viral gene, is concealed in a microcontainer (drug compartment); the virus carries pilot molecules for spatial recognition (site-specific delivery), it is compatible with the host and because of its stealth character and small size of about 20 nm it is not subject to RES recognition.

Based on this concept a drug delivery system was developed that mimics in part the characteristics of a virus [4-7]. The drug delivery system described here consisted of polymeric micelles that were formed from heterobifunctional block copolymers [8-10]. With a size of 30-50 nm in diameter, polymeric micelles range closely in size to that of viruses which is of advantage in order to prevent detection and elimination by the RES. Amphiphilic AB-block-copolymers were synthesized with poly(ethylene glycol) (PEG) as hydrophilic block and poly(lactide) (PLA) as hydrophobic block, both polymers approved by the Food and Drug Administration (FDA). Upon exposure to a selective solvent those amphiphilic block copolymers self-assembled in polymeric micelles with PEG as the shell-forming outer segment and PLA as the core-forming inner segment. The compatibility with the host system was guaranteed by the dense PEG shell, which has excellent blood compatibility and contributed to the stealth character of the system [11]. PEG chains attached to a surface or forming the corona of a nanospheric particle exhibit rapid chain motion in an aqueous medium and have a large excluded volume. The steric repulsion resulting from a loss of configurational entropy of the bound PEG chains upon the approach of a foreign particle and the low interfacial free energy of PEG in water contribute to the extraordinary physiologica properties of nanospheres covered with PEG [12-18]. Moreover, PEG grafted to surfaces of biomedical devices proved to increase their bio-compatibility and to reduce thrombogenicity [19-23]. The PLA segment formed the inner core of the polymeric micelle, providing a hydrophobic drug compartiment. In this microcontainer the substance being transported is well concealed and protected from early release. In order to prepare the drug delivery system for site specificity, that is, providing the ability of attaching a homing device, the outer shell of the polymeric micelle was built in such a way that it was covered with functional groups, which react readily with potential pilot molecules or target specific antibodies. Aldehyde groups were chosen for their instantaneous reaction with amine groups.

**EXPERIMENTAL**

**Materials and Methods**

Commercial tetrahydrofuran (THF), 2-methoxy-ethanol, 3,3-diethoxypropanol, ethylene oxide (EO) and lactide were purified conventionally [24]. Methacrylic anhydride was used without further purification. Potassium naphthalene was used as THF solution [25]. Gel Permeation Chromatography (GPC) measurements were carried out using a Shimadzu 6A Liquid Chromatograph equipped with a TSK gel column (G4000HXL, G3000HXL, G2500HXL) and an internal RI detector (RID-6A). THF containing 2% triethylamine was used as eluent at a flow rate of 1 ml/min. 1H-NMR (nuclear magnetic resonance) spectra were obtained using chloroform-d solutions with a Jeol Ex400 spectrometer at 400 MHz. High-performance liquid chromatography (HPLC) measurements were carried out using a Jasco 802-SC, 880-PV, 880-50, 851-AS chromatograph equipped with a Superox 6H10/30 column (Pharmacia Biotech) and a RI (Jasco 830-RI) and UV (Jasco 870-UV) detector at a flow rate of 0.5 ml/min and a pressure of 5 kg/cm² with water as eluent. A light-scattering spectrophotometer (DLS 700 Photol, Otsuka Electronics) equipped with a He-Ne laser was used at a wavelength of 488 nm for dynamic and static light-scattering measurements. The scattering angles ranged from 30° to 150° and the concentration varied from 0.1 to 10 g/l.

**Polymer Synthesis**

2-Methoxy-PEG-b-PLA, 2-diethoxy-PEG-b-PLA and 2-methoxy-PEG-b-ω-vinyl-PLA have been synthesized by a one-pot anionic ring-opening polymerization at room temperature under argon. One mmol (0.08 ml) initiator (2-methoxyethanol) and 1 mmol (3.5 ml) of potassium naphthalene were added to 25 ml of dry THF. After stirring for 10 min, 200 mmol (10 ml) of condensed EO were added via a cooled syringe to the formed potassium methoxyethanolate. The polymerization of EO proceeded for three days, resulting in a light brown, highly viscous solution. Supplementary THF was added to decrease the viscosity of the reaction mixture and

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potassium naphthalene (about 1.5ml) was added until the solution turned green to stabilize the living chain end. Some 20mmol (10.1ml) of a lactide solution in THF ($c = 1.98 \text{mol/l}$) were introduced, and the polymerization proceeded for 60min. Again, supplementary potassium naphthalene (about 1.5ml) was used to stabilize the living chain end and then a 10-fold excess of methacrylic anhydride (10mmol) was added. The reaction mixture turned immediately green and turbid. During the reaction time of 60h the mixture turned white, but remained turbid. The polymer was recovered by precipitation into 10-fold excess of cold isopropanol ($-15^\circ\text{C}$), stored for 2h in the freezer and centrifuged for 30min at 4200rpm. The polymer was then freeze-dried in benzene and obtained as a white powder with a yield of about 90%. 

Polymer Characterization

The molecular weight was determined by GPC at the end of the EO polymerization and at the end of the block copolymerization. The molecular weight for PLA was calculated from the difference. The chemical structure was determined by $^1\text{H}$-NMR spectroscopy using TMS (trimethylsilane) as an internal standard.

Micelle Formation

The procedure has been described detailed previously [10]. Briefly, 200mg of copolymer were dissolved in 40ml dimethyl acetamide and the polymer solution was transferred into a pre-swollen membrane (Spectra/Por molecular weight cut-off size 12,000–14,000) and dialyzed against water for 24h and subsequently lyophilized. The yield of the micelle formation was about 90%.

To convert an $\alpha$-diethoxy terminated micelle into a micelle with aldehyde groups at the end of the PEG chain, 50mg of micelle were solubilized by sonication for 40min in water and stirred for another 3h. The pH of the solution was adjusted by HCl to pH 2, kept for 14h and readjusted to pH 7 by NaOH. The solution was again dialyzed against water for 24h using a pre-swollen membrane to remove the salt and then frozen in liquid nitrogen and lyophilized, resulting in a yield of 85 to 90%.

Micelle Characterization

The procedure to confirm terminal aldehyde groups at the outer shell of the polymeric micelle has been described before [10]. A Schiff base was formed upon the reaction of benzyl hydrazide, which was employed as a UV probe, with aldehyde groups. The modified micelle was characterized by

\[ \text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-OH} \xrightarrow{\text{K-naphthalate}} \text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-OK} \]

[10 min, RT]

\[ \xrightarrow{\text{48 hr, RT}} \text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-O-(CH}_2\text{-CH}_2\text{-O)}_m\text{K} \]

\[ \xrightarrow{\text{1 hr, RT}} \text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-O-(CH}_2\text{-CH}_2\text{-O)}_m\text{-(CO-CH(CH}_3\text{-O)}_n\text{K} \]

\[ \text{Methacrylic anhydride} \xrightarrow{\text{72 hr, RT}} \text{CH}_3\text{-O-CH}_2\text{-CH}_2\text{-O-(CH}_2\text{-CH}_2\text{-O)}_m\text{-(CO-CH(CH}_3\text{-O)}_n\text{-CO-C(CH}_3\text{)CH}_2} \]

**SCHEME 1.**

HPLC using an UV detector. In addition, aldehyde groups were verified by \(^1\)H-NMR spectroscopy.

**RESULTS AND DISCUSSION**

**Polymer and Micelle Characterization**

As reported previously a potassium alkoxide initiator, which possesses an acetal or methoxy moiety, can initiate the polymerization of EO without any side reaction to form heterotelechelic PEG having an acetal or methoxy moiety at one end and a potassium alkoxide at the other end [8±10]. This polymerization technique is also applicable to a block copolymerization of EO with lactide. \(\alpha\)-Diethoxy-PEG-b-PLA and \(\alpha\)-methoxy-PEG-b-PLA were synthesized via a one-pot ring-opening polymerization of EO and lactide using 3,3-diethoxy propanol and 2-methoxyethanol, respectively, as initiators. After the polymerization of EO with the respective potassium alkoxide as initiator, lactide was added to the reaction mixture. The reaction was terminated either by introducing a vinyl group using methacrylic anhydride as a chain-terminating compound (Scheme 1) or by moisture after opening the reaction flask and exposing the reaction mixture to air. The molecular weight was controlled by the initiator to monomer ratio. If the PLA content is very high in comparison to short PEG chains, the resulting micelle will not be covered completely by a PEG corona, exposing the hydrophobic and hydrolyzable PLA core to the environment. This would probably cause detrimental effects upon exposing the polymeric micelle to tissue or blood. If however, the PEG chain length is very high compared with a small PLA segment, then the shape of the resulting self-assembling block copolymer will deviate from a sphere, since PEG chains dominate the spatial arrangement of the polymer chains. In the state of an ideal micelle the PEG chains are densely packed around the core.

The chemical composition and polymerization yields of the block copolymers (I, II, III) considered in the present study, are listed in Table 1. Table 2 shows the molecular weights of the individual blocks, as well as the total molecular weight of the block copolymer. As expected for an anionic polymerization, the molecular weight distribution \(n\) was close to 1.00. The molecular weight distribution after the EO polymerization ranged between 1.05 and 1.12, and the subsequent polymerization of lactide resulted in a slight increase of \(n\).

The polymer structure and functionalities at the \(\alpha\)- and \(\omega\)-ends of the block copolymers were determined by \(^1\)H-NMR. Referring to literature on acetal-terminated PEG [8] and methoxy-terminated PEG [26] functional end groups were assigned. Using the methyl proton of the acetal group at 1.2ppm, methylene proton of the PEG chain at 3.6ppm and methine proton of the PLA chain at 5.2ppm, the amount of acetal groups for polymer II was determined to be 46%. Using the methyl proton of the methoxy group at 3.3ppm in a similar manner, the methoxy contents for polymers I and III were determined to be 40% and 63%, respectively. These results indicated that besides potassium 3,3-diethoxypropoxide and 2-methoxyethoxide other species, most likely water traces, initiated the polymerization. The amount of vinyl groups in polymer III (52%) was determined by using the split signal of the methylene protons at 5.6 and 6.2ppm, respectively; Table 3. Figure 1 shows the \(^1\)H-NMR spectrum of polymer III with the signals assigned in the figure. When the polymerization was terminated by introducing moisture into the reaction mixture,

<table>
<thead>
<tr>
<th>Block copolymer Sample</th>
<th>Initiator PEG chain end</th>
<th>Terminal PLA chain end</th>
<th>Polymer yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CH(_3)O-(CH(_2))_2-OH</td>
<td>OH</td>
<td>71</td>
</tr>
<tr>
<td>II</td>
<td>(C(_2)H(_5)O)(_2)-CH-(CH(_2))_2-OH</td>
<td>OH</td>
<td>86</td>
</tr>
<tr>
<td>III</td>
<td>CH(_3)O-(CH(_2))_2-OH</td>
<td>C(CH(_3))(_3)-CH(_2)</td>
<td>90</td>
</tr>
</tbody>
</table>

**TABLE 1.** Chemical Composition and Polymer Yield of Functionalized PEG-b-PLA Copolymers

<table>
<thead>
<tr>
<th>Sample</th>
<th>PEG</th>
<th>PLA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(M_w)</td>
<td>(M_n)</td>
<td>(M_w)</td>
</tr>
<tr>
<td>I</td>
<td>4,700</td>
<td>4,500</td>
<td>2,700</td>
</tr>
<tr>
<td></td>
<td>(n = 1.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>6,400</td>
<td>5,700</td>
<td>3,900</td>
</tr>
<tr>
<td></td>
<td>(n = 1.12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>7,900</td>
<td>7,400</td>
<td>1,800</td>
</tr>
<tr>
<td></td>
<td>(n = 1.07)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

the amount of terminal hydroxyl groups was presumed to be 100% since there is no other possibility for the anion of the living chain end than to react with water. The actual amount was, however, not quantified. The amount of terminal groups at the PEG as well as the PLA chain end depended strongly on the amount of water present in the reaction mixture and traces of water suppressed the end group yield.

After polymeric micelles were formed by dialyzing a dimethyl acetamide solution of the respective block copolymer against water, terminal acetal groups were converted into aldehyde groups. The freeze-dried micelle was dissolved in water and the solution was acidified by hydrochloric acid. The extent of conversion of acetal into aldehyde groups, determined by 1H-NMR (\( \delta = 9.8 \text{ ppm} \)) was ca. 22% corresponding to a 48% conversion of the total amount of acetal groups. As indicated by 1H-NMR investigations the remaining acetal groups formed semiacetals. The hydroxyl groups of the semiacetal were characterized by the proton signal at 2.7 ppm (in CHCl₃). Ethanol, which formed during the acetal conversion, reacted with already formed aldehyde moieties, a reaction favored at acidic pH, resulting in the formation of semiacetals. The formation of the semiacetals had to be considered as a competition reaction to the aldehyde formation. Figure 2 shows the 1H-NMR spectra of polymer II before and after the aldehyde/semiacetal formation.

The procedure to verify the presence of surface aldehyde groups using benzyl hydrazide as a UV probe which reacted instantaneously with aldehyde groups, forming a Schiff base, was described previously [10]. By using the UV signal in HPLC analysis it was verified that benzyl hydrazide reacted with the polymeric micelle. The UV signal which was not present before the conversion, was then observed at the same retention time in the

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**TABLE 3. Yield of Terminal Functional Groups at the PEG and PLA Terminal of Block Copolymers**

<table>
<thead>
<tr>
<th>Sample</th>
<th>PEG terminal</th>
<th>Yield (%)</th>
<th>PLA terminal</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>( \text{CH}_3-O)</td>
<td>40</td>
<td>( -\text{OH} )</td>
<td>ND(^a)</td>
</tr>
<tr>
<td>II</td>
<td>( (\text{C}_2\text{H}_5-O)_2\text{-CH} )</td>
<td>46</td>
<td>( -\text{OH} )</td>
<td>ND(^a)</td>
</tr>
<tr>
<td>III</td>
<td>( \text{CH}_3-O)</td>
<td>63</td>
<td>( -\text{C(R}_3\text{H}_7)\text{-CH}_2 )</td>
<td>52</td>
</tr>
</tbody>
</table>

\(^a\) Not determined, presumably 100%, since the reaction was terminated by introducing moisture.

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\( \delta \) in ppm

**FIGURE 1.** 1H-NMR spectrum of block copolymer III, \( \alpha \)-methoxy-PEG\( -b\)-\( \omega \)-vinyl-PLA.
HPLC chromatogram as the RI signal derived from the polymeric micelle, indicating that the UV probe was attached to the micelle.

The polymeric micelles investigated in this study were considered for a long-term application under physiological conditions. Micelle stability is of great importance in this respect and in order to increase the stability, vinyl groups were introduced at the PLA terminal for subsequent crosslinking. Crosslinking reactions are under investigation and will be reported elsewhere.

**Dynamic and Static Light-scattering Studies**

Extensive studies were conducted to determine the shape and size of the polymeric micelles employing dynamic and static light scattering. Micelles formed from block copolymers II and III as well
as micelles formed from block copolymer II with subsequent conversion of acetal groups into aldehyde groups (II-CHO) were examined. The parameters characterizing the micelles are listed in Table 4.

Dynamic light scattering was employed to determine the micelle diameter and to obtain information about the geometric shape of the micelle. First, the micelle parameters were studied as to their dependence on the scattering angle and the sample concentration. Laplace inversion was used to analyze the data. The characteristic line width $\Gamma$ proved to be linear-dependent on the scattering angle for all concentrations investigated (0.1; 0.5; 1.0; 2.5; 5.0; 10.0 g/l), data not shown, indicating that according to $\Gamma = Dk^2$ internal motions were negligible, and the translational diffusion coefficient $D$ was determined, $k$ is the scattering vector. Based on this result the diffusion coefficient $D_0$ was determined by extrapolation to $c = 0$ to range between $5.0 \times 10^{-8}$ and $8.0 \times 10^{-8}$ cm$^2$/sec. Using the obtained $D_0$ values and applying them in the Stokes–Einstein equation led to the determination of the hydrodynamic radii of the polymeric micelles:

$$R_h = \frac{k_B \cdot T}{6\pi \cdot \eta \cdot D_0}$$

where $R_h$ = hydrodynamic radius; $k_B$ = Boltzmann constant; $T$ = absolute temperature; $\eta$ = solvent viscosity; and $D_0$ = diffusion coefficient at infinite dilution.

Typically the hydrodynamic radii are used to describe the micelle size. The conversion of acetal into aldehyde groups (micelle II and II-CHO) did not result in a considerable change of the micellar diameter (30 and 34 nm), indicating that the conversion reaction did not effect the micelle geometry. Micelle III was characterized by a larger diameter of 46 nm. Considering the $z$-average particle diameter, obtained by the cumulative analysis approach and determined at a sample concentration of 0.5 g/l, the same result was established. Micelle III had the largest $z$-average particle diameter of 88 nm compared with 58 nm for micelle II and 69 nm for the converted II-CHO micelle. The $z$-average diameters were in good agreement with the hydrodynamic radii. The polydispersity determined by the cumulant approach proved to be moderate for all samples, ranging between 0.10 and 0.24 with micelle III exhibiting the higher values.

Figure 3 shows the dependence of the scaled characteristic line width on the scattering vector, which corresponded to the scattering angle. The scaled characteristic line width ($\Gamma/k^2$) of a perfectly spherical particle is independent of the scattering angle [27]. The present data showed a negligible angular dependence of the scaled characteristic line width on the scattering vector for micelle II (slope 0.073) and micelle II-CHO (slope 0.088) and a stronger angular dependence for sample III (slope 0.190). These results indicated that the shape of polymeric micelles II and II-CHO was almost spherical. The shape of polymeric micelle III, however, deviated from a perfect sphere.

In order to obtain information regarding the molecular weight of the micelles, their radii of

### Table 4. Micelle Parameters Determined by Dynamic and Static Light Scattering

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Micelle III</th>
<th>Micelle II</th>
<th>Micelle II-CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_h$ (Stokes–Einstein) (nm)</td>
<td>46</td>
<td>30</td>
<td>34</td>
</tr>
<tr>
<td>Radius of gyration (s)(nm)</td>
<td>45</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>$\langle s \rangle / R_h$</td>
<td>0.97</td>
<td>0.83</td>
<td>ND</td>
</tr>
<tr>
<td>Slope ang. dependence</td>
<td>0.190</td>
<td>0.073</td>
<td>0.088</td>
</tr>
<tr>
<td>$\Gamma / k^2$ over k^2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle diameter 0.5 g/l (nm)</td>
<td>88</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>Diff. coeff. $D_0$; 90° (cm^2/sec)</td>
<td>$5.13 \times 10^{-8}$</td>
<td>$7.93 \times 10^{-8}$</td>
<td>$6.66 \times 10^{-8}$</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>1.13 $\times 10^4$</td>
<td>6.29 $\times 10^7$</td>
<td>ND</td>
</tr>
<tr>
<td>2nd virial coefficient $A_2$ (mol cm^3/g)</td>
<td>4.10 $\times 10^{-5}$</td>
<td>4.03 $\times 10^{-5}$</td>
<td>ND</td>
</tr>
<tr>
<td>Average polymer density (g/cm^3)</td>
<td>0.046</td>
<td>0.092</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined.
gyration and the second viral coefficient, static light scattering was performed for micelles II and III and data were analyzed by a Zimm plot. The molecular weight of micelle II was determined to be $6.29 \times 10^6$ g/mol, and considering the molecular weight of the block copolymer ($M_n = 14,200$), it was concluded that the polymeric micelle consisted on average of 450 macromolecules. The molecular weight of micelle III was determined to be $1.13 \times 10^6$ g/mol, indicating that micelle formation involved in average 930 macromolecules per micelle. Since in micelle III twice the number of macromolecules were involved in forming an individual polymeric micelle, the resulting micelle was larger in diameter as micelle II, as observed by dynamic light scattering for the $z$-average diameter and the hydrodynamic radius, respectively.

As observed for the hydrodynamic radius and $z$-average micelle diameter, the radius of gyration ($\langle s \rangle$) is larger for micelle III, 45 nm, compared with 25 nm for micelle II. Again, this corresponded to the fact that in micelle III about twice as many macromolecules were combined to form a micelle. The ratio of the radius of gyration to the hydrodynamic radius ($\langle s \rangle / R_h$) gave additional information regarding the micelle shape. A ratio of 0.77 indicates a hard sphere and a ratio of 1.30 characterizes a linear coil [28]. For micelle II ($\langle s \rangle / R_h$ was determined to be 0.83. This result shows that micelle II was of nearly spherical shape. Micelle III was characterized by an ($\langle s \rangle / R_h$ ratio of 0.97, demonstrating once again that micelle III deviated from a spherical shape, tending to a random coil, as the results of the investigation of the angular dependence of the scaled characteristic line width on the scattering vector already implied. The properties of micelle III were largely determined by the higher content of PEG (82%) in the micelle. Flexible PEG chains occupied a larger space compared with the densely packed PLA segments in the micelle core, which accounted in this sample for only 18% of the total molecular weight. The small PLA core of this micelle was not capable of arranging the PEG chains in a dense spherical corona surrounding the PLA core. The longer PEG chains tended to assemble in a state closer to a random coil.

Micelle II was characterized by an optimum ratio of the hydrophilic to hydrophobic segment of the amphiphilic block copolymer. It allowed the formation of a densely packed PEG corona surrounding a sufficiently large PLA core. These findings were confirmed by the average polymer density according to [29]:

$$\Phi = \frac{3M}{4\pi \cdot R_h^3 \cdot N_A}$$

where $\Phi$ = average polymer density; $M$ = molecular weight; $R_h$ = hydrodynamic radius; and $N_A$ = Avogadro number.

The density of micelle II ($\Phi = 0.093 \text{g/cm}^3$) was twice as high as the one for micelle III ($\Phi = 0.046 \text{g/cm}^3$). This result characterized micelle II as a hard nanosphere of densely packed PEG chains surrounding the PLA core. In contrast, micelle III showed a less densely packed structure which deviated from a spherical shape.

**CONCLUSION**

Heterobifunctional amphiphilic block copolymers consisting of PEG and PLA were synthesized having an acetal or methoxy terminal group at the PEG chain end and a hydroxyl or vinyl group at the PLA chain end. Evidence was presented which suggested that these block copolymers associated in water to form polymeric micelles. Acetal groups located at the outer shell of the polymeric micelle were partially transformed into aldehyde groups after micellization by a treatment with hydrochloric acid. The presence of aldehyde groups at the micelle surface was confirmed by HPLC after forming a Schiff base with a UV probe. The degree of aldehyde conversion was established by $^1$H-NMR spectroscopy. The micelle size and shape were determined by dynamic and static light scattering. The molecular weight ranged between $6 \times 10^6$ and $10 \times 10^6$ g/mol. It was concluded that between 450 and 930 macromolecules were involved in the micelle formation. The micelle size characterized by the hydrodynamic radius ranged between 25 and 45 nm, a size slightly larger than common viruses. However, nanospheres in this size range are considered suitable drug delivery systems which are not subject to immediate RES uptake. Measurements of the hydrodynamic radii, $z$-average diameter and radii of gyration were in good agreement. The angular dependence of the scaled characteristic line width and the ratio of the radius of gyration to the hydrodynamic radius gave information about the micelle shape. The ratio of the hydrophobic to the hydrophilic block copolymer segment determined the ratio of micelle core to micelle corona and therefore the geometric shape.

**ACKNOWLEDGMENT**

This research was in parts financially supported by the Japan Research Promotion Society for Cardiovascular Diseases.

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