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Functionalized micelles from new ABC polyglycidol-poly(ethylene oxide)-poly(D,L-lactide) terpolymers

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Abstract

New ABC type terpolymers of poly(ethoxyethyl glycidyl ether)/poly(ethylene oxide)/poly(D,L-lactide) were obtained by multi-mode anionic polymerization. After successive deprotection of the ethoxyethyl groups from the first block, highly hydroxyl functionalized copolymers of polyglycidol/poly(ethylene oxide)/poly(D,L-lactide) were obtained. These copolymers form elongated ellipsoidal micelles by direct dissolution in water. The micelles consist of a poly(D,L-lactide) core and stabilizing shell of polyglycidol/poly(ethylene oxide). The hydroxyl groups of polyglycidol blocks situated at the micelle surface provide high functionality, which could be engaged in further chemical modification resulting in a potential drug targeting agents. The micellization process of the copolymers in aqueous media was studied by hydrophobic dye solubilization, static and dynamic light scattering, and transmission electron microscopy.

Keywords: Functionalized micelles; Poly(D,L-lactide)

1. Introduction

Di- and triblock copolymers of poly(ethylene oxide) (PEO) and poly(D,L-lactide) (PL) are promising and extensively studied materials for modern drug delivery systems due to the biocompatibility of PEO as well as the biocompatibility and biodegradability of PL [1–3]. The micellization of these block copolymers leads to polymeric nanoparticles consisting of hydrophobic PL cores and hydrophilic PEO shells [4,5]. The biodegradable PL core can serve as a microenvironment which can entrap the hydrophobic drug after its solubilization. The PEO shell provides a masking shield against the biological milieu thus reducing the undesired interactions between the drug-carrying micelle and the host reticuloendothelial system. In order to confer drug-targeting capabilities to such systems, Kataoka et al. have developed acetal end-functionalized Ac-PEO-PL block copolymers [6–10]. After deprotection of the acetal group an aldehyde function was formed with the aid of which different micelle surface modulators, such as peptides [7] and carbohydrates [9] could be attached. It was also shown [7] that the end-functions had almost no influence on the micellization of these copolymers and thus micelles with predicted properties with regard to the degrees of polymerization of either the PEO or the PL blocks could be obtained.

Polyglycidol (PG) is a very hydrophilic and highly hydroxyl-functional polymer [11–13], which we have already applied in a number of new amphiphilic block copolymers [14]. Linear PG is obtained by anionic polymerization of ethoxyethyl glycidyl ether (EEGE) followed by cleavage of the ethoxyethyl groups [15–17]. Recently and independently from our study Gadzinowski et al. [18] have synthesized copolymers of PEO-PG-PL as to obtain micelles, whose PL core surface is linked with short PG blocks, i.e. core functionalization was achieved.

The goal of the present work is to synthesize new functionalized amphiphilic block copolymers of PG-PEO-PL and to further investigate their self-assembly in aqueous media. Shell-functionalized micelles of PG-PEO-PL were characterized by hydrophobic dye solubilization, static and

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dynamic light scattering, and transmission electron microscopy.

2. Experimental

2.1. Copolymer synthesis

2.1.1. Materials

All solvents were purified by standard methods. 1-Methoxy-2-ethanol was purified by vacuum distillation. Ca (Aldrich) and $CsOH \cdot H_2O$ 99.5% (Acros Organics) were used as received. EEGE was synthesized according to procedure, described elsewhere [19] and purified by vacuum distillation. Fractions of purity exceeding 99.0% (GC) were used for polymerizations. Ethylene oxide (EO) (Clariant) was used as received. D,L-Lactide (L) was purchased from Polysciences and purified by recrystallization from toluene.

2.1.2. Synthesis of PEEGE-PEO-PL

To $CsOH \cdot H_2O$ (9 mmol), magnetically stirred in a reaction vessel equipped with argon and vacuum line, an equimolar amount of 1-Methoxy-2-ethanol was added at 90 °C. After stirring for 1 h, the system was switched to the vacuum line for two more hours. After flushing the vessel with dry argon another 9 mmol of 1-methoxy-2-ethanol were added. The polymerization of the PEEGE block was started by adding 4, 7, or 16 fold molar excess of the EEGE monomer to the fresh initiator mixture at 60 °C. The formation of the first block was completed in 24 h. After removing a small sample for analysis, the reaction temperature was raised to 90 °C and EO was bubbled through the system for 2, 3, or 5 h depending on the desired length of the PEO block. The PEEGE-PEO-OH diblock copolymers were carefully purified and analyzed. The polymerization of L initiated by PEEGE-PEO-O-$CaNH_2$ was performed in THF at 40 °C by the procedure described by Piao et al. [20].

2.1.3. Copolymer purification

The copolymers were dissolved in methylene chloride and filtered through Hylfo Super Cel® (diatomaceous earth). After precipitation from freshly distilled dry diethyl ether the products were extensively dried under reduced pressure at 40 °C.

2.1.4. Preparation of PG-PEO-PL by cleavage of the ethoxyethyl protective groups

The described procedure below is similar to a recent method for deprotection of tetrahydropyranyl ethers [21]. Given amount of PEEGE-PEO-PL was dissolved in MeOH. Then $AlCl_3 \cdot 6H_2O$ was added and the reaction was kept for 0.5 h at room temperature. The [–EEGE–]/$AlCl_3$/MeOH molar ratio was 100:1:800. The reaction product was filtered through diatomaceous earth and the solvents were evaporated under reduced pressure.

2.1.5. Preparation of PG-PEO-PL micellar dispersions

Stock solutions were prepared directly by dissolving 0.2 g of copolymer in 19.8 g of bi-distilled water to produce 10 g/l solution, which was placed at 80 °C for 30 min. After cooling down, desired concentrations ranging from 10 to 0.01 g/l were prepared by dilution. Both the stock solution and the bi-distilled water were filtered through Gelman 0.45 μm PVDF membranes.

2.2. Methods

2.2.1. NMR

The ^1H NMR spectra were recorded at 250 MHz on a Bruker WM 250, using $CDCl_3$ as a solvent.

2.2.2. Size exclusion chromatography (SEC)

SEC experiments were performed on a chromatography line consisting of an M 510 pump, a U6K universal injector, three Styragel columns with nominal pore sizes of 100, 500 Å, Linear, a differential refractive index detector M 410, and a tunable absorbance detector M 490 (all Millipore Co., Waters Chromatography Division). THF was used as an eluent at 45 °C and a flow rate of 1 ml min^{-1}. Molecular weight characteristics of the copolymers were calculated using a 'universal calibration' curve constructed with monodisperse polystyrene standards. Toluene was used as an internal standard.

2.2.3. Hydrophobic dye solubilization

Aqueous solutions (2 ml) of a triblock copolymer in the concentration range from 0.01 to 10 g/l were prepared as described above. 20 μl of a 0.4 mM solution of 1,6-diphenyl-1,3,5-hexatriene (DPH) in methanol were added to each of the copolymer solutions. Solutions were incubated in the dark for 16 h at 25 °C. The absorbance in the range of $\lambda = 300$–500 nm was followed at 25 °C on a Specord UV–VIS spectrometer. The main absorption peak characteristic of DPH solubilized in a hydrophobic domain was observed at 356 nm [22].

2.2.4. Dynamic light scattering

Dynamic light scattering (DLS) measurements were performed on a Malvern Autosizer 4700 instrument with vertically polarized incident light of wavelength $\lambda = 488$ nm supplied by an argon-ion laser operated at 500 mW or less, and a Malvern 7032 correlator. Measurements of scattered light from the 2 g/l polymer aqueous solutions were made at angle 90° to the incident beam at 25 °C. The correlation functions from DLS were analyzed by the constrained regularized CONTIN method [23] to obtain distributions of decay rates (Γ). The decay rates gave distributions of apparent diffusion coefficient ($D_{app} = \Gamma / q^2$, where q is the magnitude of the scattering vector) and the apparent hydrodynamic radii were obtained by the Stokes–Einstein equation

$$R_h = kT/(6\pi\eta D_{app}) \quad (1)$$

where k is the Boltzmann constant and η is the viscosity of water at temperature T.

2.2.5. Static light scattering

Static light scattering (SLS) measurements were carried out on a multi-angle laser light scattering detector (MALLS) DAWN DSP laser photometer (Wyatt Technology Co.) equipped with an argon laser emitting at a wavelength of 633 nm. Analyses were performed in a microbatch mode at 25 °C. The specific refractive index increment was measured at 25 °C on an Optilab 903 interferometric refractometer and determined with the Wyatt dn/dc software. The data analysis was performed by ASTRA (Wyatt Corp.) software using the Rayleigh–Gans–Debye equation, valid for small interacting particles in the form [24]:

$$\frac{Kc}{R_\theta} = \frac{1}{\bar{M}_w^{app}} + 2A_2c \quad (2)$$

where $K \equiv 4\pi^2 n_0^2 (dn/dc)^2/N_A\lambda^4$ is an optical parameter with n_0 being the refractive index of toluene, N_A is the Avogadro's constant, λ is the laser wavelength (633 nm); $\bar{M}_w^{app}$ is the apparent weight average molecular weight, A_2 is the second virial coefficient, R_θ is the Rayleigh ratio of the polymer solution at a given angle.

2.2.6. Transmission electron microscopy (TEM)

Samples for TEM were prepared by adding a drop of aqueous micellar dispersion to the grid followed by slow evaporation of the solvent. Measurements were performed on JEM 200 CX apparatus.

3. Results and discussion

3.1. Synthesis of PEEGE-PEO-PL block copolymers

The synthetic route to PEEGE-PEO-PL is represented in Schemes 1 and 2. The sequential polymerization of EEGE and EO resulting in a PEEGE-PEO precursor was performed by means of a cesium initiating system due to its high activity and efficiency towards a number of oxirane monomers [25]. However, it is well-known that PL is not stable in strong basic media [26] as in the case of cesium initiating systems, which we have observed as well. Therefore the formation of the PL block was initiated by calcium alkoxide initiating system (Scheme 2), which was found to be very efficient for the polymerization of cyclic esters [20,27]. In general calcium-based initiators are favored for the ring-opening polymerization of lactides as they show: (i) very high efficiency at temperature as low as 40 °C; (ii) low toxicity, which is very important for materials, intended for use in drug delivery.

3.2. Characterization of the PEEGE-PEO-PL copolymers

The molecular weight characteristics of the pre-polymers and the triblock copolymers are summarized in Table 1. SEC measurements result in monomodal and relatively narrow molecular weight distributions for both the PEEGE and PEEGE-PEO pre-polymers, and the triblock PEEGE-PEO-PL copolymers as well (Fig. 1).

The apparent number average molecular weights obtained by SEC for the final products have not been taken into account, since their values seemed to be lower than expected, most probably due to the complex composition of the copolymers. Therefore, $\bar{M}_n$ of the products was calculated by NMR, taking into account the degree of polymerization of the PEEGE first block obtained by the relationship:

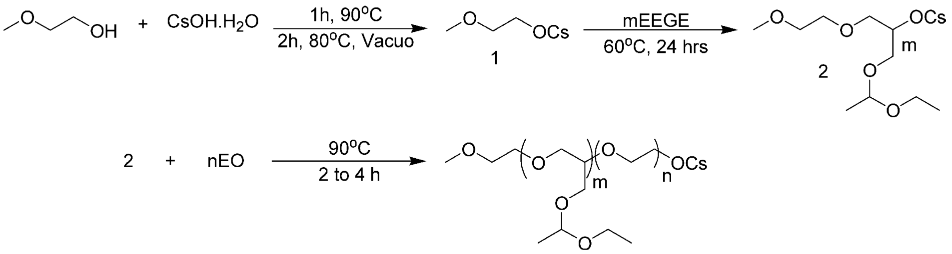
$$\overline{\mathrm{DP}}_{\mathrm{PEEGE}} = 3\frac{I_{[\mathrm{O{-}\underline{CH}{-}O}]}}{I_{[\mathrm{\underline{CH_3}{-}O{-}}]}} \quad (3)$$

where $I_{[\mathrm{\underline{CH_3}{-}O{-}}]}$ is the area of the peak related to the methoxy-protons at 3.4 ppm belonging to the starter alcohol (1-methoxy-2-ethanol), and $I_{[\mathrm{O{-}\underline{CH}{-}O}]}$ is the area of the peak related to the acetal proton of the EEGE units at 4.6 ppm (Fig. 2(a)).

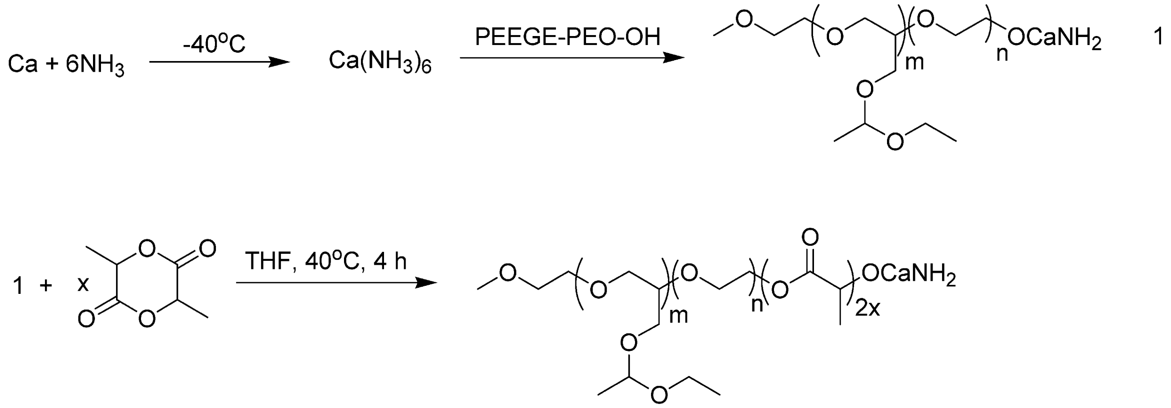
Provided that the DP of PEEGE is known and the area $I_{[\mathrm{O{-}\underline{CH}{-}O}]}$ is equal to 1, the DP of the PEO block within the PEEGE-PEO diblock copolymer was calculated by the following expression:

$$\overline{\mathrm{DP}}_{\mathrm{PEO}} = \overline{\mathrm{DP}}_{\mathrm{PEEGE}}\frac{I_{3.4\text{–}3.8\ \mathrm{ppm}} - 7}{4} \quad (4)$$

where $I_{3.4\text{–}3.8\ \mathrm{ppm}}$ is the area of the complex peak at 3.4–3.8 ppm (Fig. 2(b)). Finally, The DP of the PL block was calculated as follows:



Scheme 1. The synthesis of PEEGE-PEO precursor.



Scheme 2. The synthesis of PEEGE-PEO-PL triblock copolymers.

$$\overline{DP}_{PL} = \overline{DP}_{PEEGE}\frac{I_{[CH_3^L]}}{I_{[CH_3^{EEGE}]}} \quad (5)$$

where $I_{[CH_3^L]}$ and $I_{[CH_3^{EEGE}]}$ represent the area of the peak of the methyl group protons of the lactic units (at 1.5 ppm) and the area of the protons of one of the methyl groups of the EEGE units (at 1.1 ppm or at 1.25 ppm), respectively (Fig. 2(c)).

3.3. Synthesis of PG-PEO-PL block copolymers

The ethoxyethyl groups of the PEEGE block were successfully cleaved to hydroxyl ones by using a mild and efficient method, adopted from the low-molecular weight organic chemistry of tetrahydropyranyl ethers [21]. This allowed us to obtain series of polyfunctional PG-PEO-PL triblock copolymers, in which the degrees of polymerization of the different blocks match exactly those of the PEEGE-PEO-PL precursors (Fig. 3). Apparently the PG-PEO-PL copolymers represent a very useful precursor to hydroxyl-functional micelles composed of a biodegradable PL core and a highly hydrophilic PG-PEO shell. The chemical composition of the block copolymers presumes the formation of hairy micelles in aqueous solution [28] (see Scheme 3). On the other hand, the PG-PEO-PL copolymer can serve as a polyfunctional macroinitiator as well—new bio-degradable block-graft copolymers can be obtained through 'grafting from' the hydroxyl groups of polyglycidol block.

Table 1

Composition and molecular weight characteristics of the PEEGE block copolymers

Composition (NMR)	$\bar{M}_n$ (NMR)	$\bar{M}_w/\bar{M}_n$ (SEC)
$EEGE_7$	1000	1.25
$EEGE_7EO_{29}$	2300	1.10
$EEGE_{16}$	2300	1.18
$EEGE_{16}EO_{137}$	8300	1.13
$EEGE_{16}EO_{246}$	13,000	1.35
$EEGE_4$	600	1.48
$EEGE_4EO_{159}$	7500	1.45
$EEGE_7EO_{29}L_{13}$	3200	1.18
$EEGE_{16}EO_{137}L_{17}$	9500	1.32
$EEGE_4EO_{159}L_{25}$	9300	1.22
$EEGE_{16}EO_{137}L_{40}$	11,000	1.24
$EEGE_{16}EO_{246}L_{44}$	16,000	1.33

3.4. Aqueous solution properties

The PG-PEO-PL copolymers contain relatively short PL blocks, which allow them easily to dissolve in water. Hence the functionalized micelle dispersions were prepared by the direct dissolution method, avoiding the use of toxic organic solvents and the relatively complicated procedure of solvent evaporation or dialysis against water. The area of micellization of linear ter-polymers has not been yet sufficiently explored and there is no systematic research presented up to date [28]. In our case only the PL block is hydrophobic and one can assume that the very number of lactic units within the macromolecule is the major factor responsible for the anticipated association properties such as cmc, the aggregation number (N_{agg}), and micelle dimensions.

3.4.1. Cmc measurements

The cmc values of the PG-PEO-PL copolymers (Table 2) were obtained by hydrophobic dye solubilization (Fig. 4(a)) and SLS (Fig. 4(b)) techniques. Cmcs were not noticeably affected by the temperature of up to 40 °C and therefore only the results obtained at 25 °C will be discussed. Previously

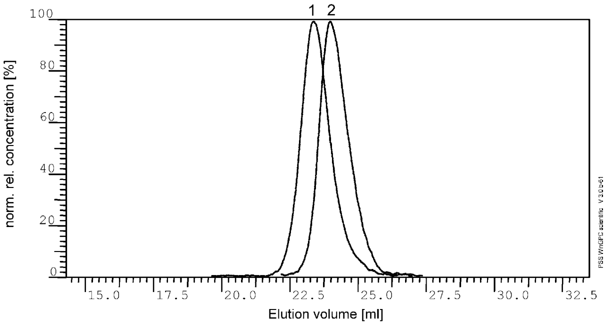


Fig. 1. SEC traces of (2) $EEGE_7EO_{29}$ and (1) $EEGE_7EO_{29}L_{13}$.

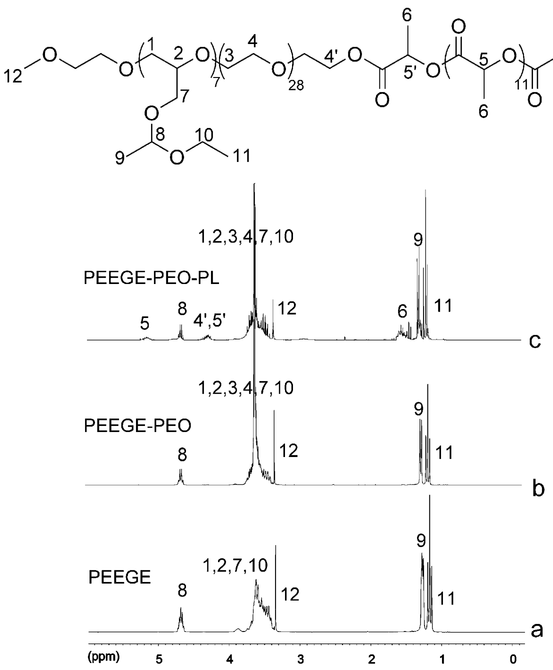


Fig. 2. ^{1}H NMR spectra of (a) PEEGE, (b) PEEGE-PEO precursor, and (c) PEEGE-PEO-PL triblock copolymer.

the DPH solubilization method was used extensively for the determination of cmc and cmt [22] of Pluronic copolymers, and recently, for amphiphilic block copolymers containing hydrophobic PL block(s) [29]. At the same time while evaluating the data obtained from SLS it was possible to derive the cmc values of the copolymers from the Debye plots taken within relatively broad concentration regions. As shown in Fig. 4(b), where only the extrapolated values at zero scattering angles are plotted, at lower concentrations the value of K_c/R_0 is affected by the presence of unassociated macromolecules (unimers) in the system and a break in the linearity of the plot occurs indicating the cmc value [30]. This behavior is typical for a closed association process such as micelle formation [31].

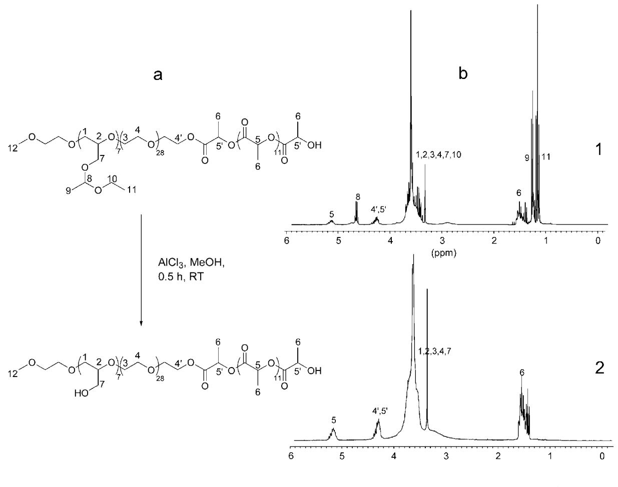
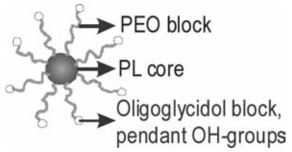


Fig. 3. (a) Reaction of deprotection of the ethoxyethyl groups. (b) ^{1}H NMR spectra of $EEGE_7EO_{29}L_{13}$ (1) and $G_7EO_{29}L_{13}$ (2) in $CDCl_3$.



Scheme 3. Supposed model of the PG-PEO-PL micelle.

The values of the cmcs obtained by SLS are lower but correlate well with those obtained by the DPH solubilization. As can be expected, the cmcs of the copolymers were affected predominantly by the number of the hydrophobic lactide units—the higher the DP of the PL block, the lower the cmc. The only exception from this observation is the copolymer with the highest content of hydrophilic units—$G_{16}EO_{246}L_{44}$, where the overall hydrophilicity results in a weaker ability for self-association.

The free energies of micellization, $\Delta G^0_{\mathrm{mic}}$, which provide us with a valuable information about the ability of the PG-PEO-PL copolymers to self-associate were calculated by the following equation, valid for nonionic surfactants [22]

$$\Delta G^0_{\mathrm{mic}} = RT \ln X_{\mathrm{cmc}} \tag{6}$$

where R is the gas constant, T is the absolute temperature, and X_{cmc} is the cmc in mole fraction. X_{cmc} was calculated from the cmc values obtained by static light scattering.

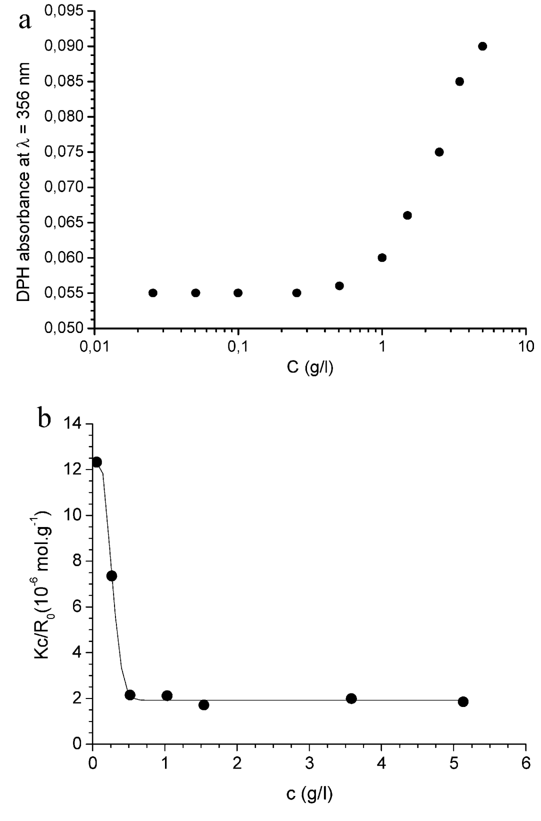


Fig. 4. Cmc determination of PG-PEO-PL copolymers: (a) variation of the absorbance of DPH at 356 nm with $G_{16}EO_{137}L_{17}$ concentration; (b) concentration dependence of SLS at zero scattering angles from $G_{16}EO_{137}L_{17}$ aqueous solution.

Table 2
Cmc values of PG-PEO-PL

Composition	$\bar{M}_n$ (NMR)	Lactic units content (mol%)	Cmc (g/l)		$X_{cmc}\times10^6$ (mol fr.)	ΔG^0_{mic} (kJ/mol)
			UV	SLS	SLS	
$G_{16}EO_{137}L_{17}$	8350	10	1.02	0.51	1.11	−31.13
$G_4EO_{159}L_{25}$	9000	13	0.99	0.50	1.00	−31.36
$G_{16}EO_{246}L_{44}$	14,500	14	0.60	0.90	1.09	−31.15
$G_{16}EO_{137}L_{40}$	9850	20	0.38	0.26	0.48	−33.04
$G_7EO_{29}L_{13}$	2700	27	1.58	1.48	9.85	−26.16

Samples $G_{16}EO_{137}L_{17}$ and $G_{16}EO_{137}L_{40}$ represent a suitable pair to estimate the role of the hydrophobic lactic unit's content. The sample $G_{16}EO_{137}L_{40}$ possesses almost twice more lactic units than $G_{16}EO_{137}L_{17}$, which leads to a lower ΔG^0_{mic} value, i.e. its micellization is more spontaneous. This is clearly indicated by the twice lower values of either the cmc or X_{cmc} as well. ΔG^0_{mic} for three of the samples—$G_{16}EO_{137}L_{17}$, $G_4EO_{159}L_{25}$, and $G_{16}EO_{246}L_{44}$, is almost the same regardless the remarkable difference in the number of lactic units, which at first sight will significantly affect the self-association ability, as it was shown by others [32]. These samples posses a relatively low and comparable mole content of lactic units (10–14 mol%) and differ only in their molecular weights. Therefore, it is feasible to assume that in the specific case of the so-called 'hairy' micelles where the number of hydrophilic units is much greater than the number of hydrophobic ones, as in our case, ΔG^0_{mic} depends not only on the number of lactic units, but also on their molar content. Moreover, due to the relatively long hydrophilic PG-PEO blocks, and at the same time due to the low molar content of lactic units, these copolymers are less capable of self-associate, which results in higher values for ΔG^0_{mic} when compared to sample $G_{16}EO_{137}L_{40}$ with 20 mol% of lactic units.

3.4.2. Dynamic light scattering

In order to obtain the values of the apparent hydrodynamic radii (R_h) of the functionalized micelles DLS measurements were performed on aqueous micellar dispersions. The latter were prepared at single polymer concentration of 2 g/l, which was invariably higher than the cmcs from Table 2. The distribution of the apparent hydrodynamic radii for all of the micelle dispersions was monomodal (as demonstrated in Fig. 5) and no peaks assigned for unassociated molecules (unimers) were observed. R_h values of the micelles are collected in Table 3. Generally, under similar environment conditions the dimensions of the micelles increase when increasing the molar content of lactic units of PG-PEO-PL copolymers.

Table 3
Influence of the lactic units content over R_h values of the functionalized micelles

Code	Lactic units content (mol%)	R_h (nm)
$G_{16}EO_{137}L_{17}$	10	12
$G_4EO_{159}L_{25}$	13	17
$G_{16}EO_{246}L_{44}$	14	36
$G_{16}EO_{137}L_{40}$	20	40

Fig. 6 shows the influence of the number of lactic units upon R_h of copolymers with different, but comparable in each series degree of polymerization (DP) of the hydrophilic blocks. The PG-PEO-PL copolymers possess the highest DP of the hydrophilic block of ca. 160, and they are compared to diblock PEO-PL copolymers obtained by Riley [4] with $DP_{EO}=113$ and to diblock PEO-PL copolymers reported by Tanodekaew [32] with short hydrophilic blocks with DP_{EO} below 50. It is obvious that the copolymers with longer hydrophilic blocks, especially PG-PEO-PL, exhibit a more abrupt increase in their hydrodynamic radii while increasing the number of lactic units. Apparently, the three dependencies from Fig. 6 are likely to intercept at DP_L of ca. 15 with corresponding R_h of 10 nm.

3.4.3. Static light scattering

The results from the static light scattering for the aqueous functionalized micelle dispersions were obtained by taking into account the data points estimated only at concentrations above cmc, and thus the non-desired influence on the overall scattered light by the unimers was avoided. A typical Zimm plot for PG-PEO-PL aggregates is shown on Fig. 7, from which the corresponding values for the apparent weight average molecular weights of the micelles, $\bar{M}_w^{app}$, the

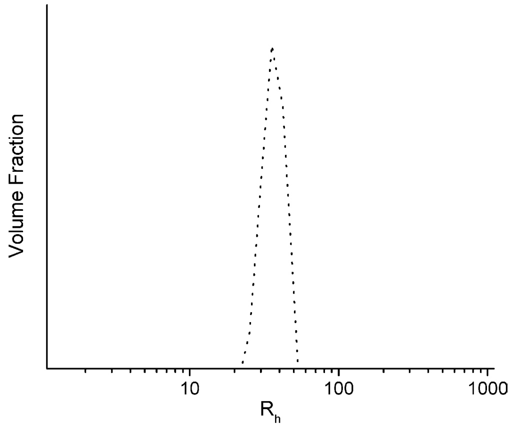


Fig. 5. Normalized distribution of the apparent hydrodynamic radius for a 2 g/l aqueous solution of copolymer $G_{16}EO_{137}L_{40}$ at 25 °C.

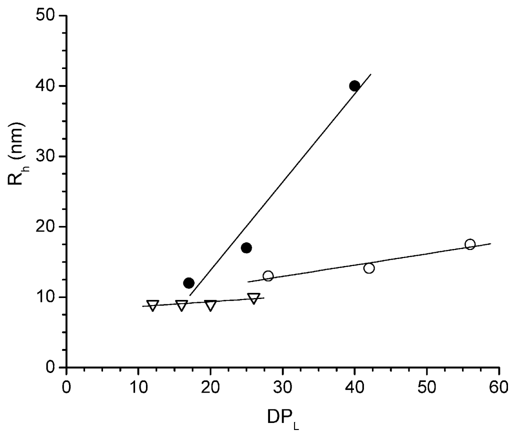


Fig. 6. Plot of the apparent hydrodynamic radii versus DP_L of: PG-PEO-PL copolymers (filled circles), PEO-PL copolymers [4] (hollow circles), and PEO-PL copolymers [32] (hollow triangles).

apparent radii of gyration, R_g, and the second virial coefficients, A_2, were obtained.

The mass average aggregation number N_{agg} of the micelles was obtained from the following relationship:

$$N_{agg} = \bar{M}_w^{app}/\bar{M}_w^{uni} \tag{7}$$

where the weight average molecular weight of the unimers, $\bar{M}_w^{uni}$, was calculated from the polydispersity index obtained by SEC multiplied by the $\bar{M}_n$ values obtained by NMR. The SLS results for PG-PEO-PL copolymers are summarized in Table 4.

As can be seen from Fig. 7, there is a noticeable curvature of the angular dependency of the light scattering intensity within the low angle region. This situation usually occurs either when large particles ($R_g > \lambda/20$ nm) populate the system, or when the shape of these particles is not truly spherical [33]. Indeed, the R_g values of the functionalized micelles are relatively high and vary from 23 to 68 nm. Furthermore, the combination of R_g and R_h yields the quantity R_g/R_h which provides useful information about the particle density and shape [34,35]. In our case the R_g/R_h value varies from 1.58 to 2.06 (Table 4), which is consistently higher than the values typical for spherical micelles in the range of 1.0–1.3 [34]. With R_g/R_h close to 2 the PG-PEO-PL micelles most likely possess elongated or rod-like shape [33].

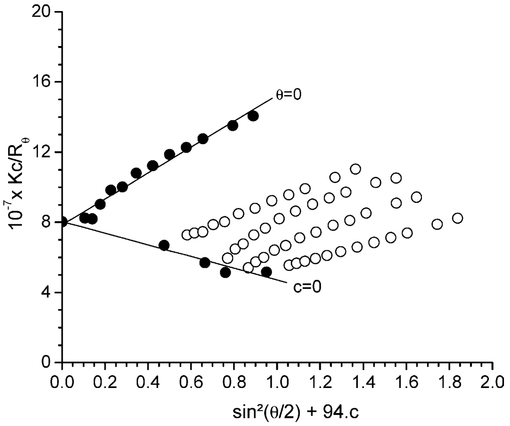


Fig. 7. Zimm plot for aqueous solutions of $G_{16}EO_{246}L_{44}$.

The values of N_{agg} rise with increasing the number of L units for copolymers $G_{16}EO_{137}L_{17}$, $G_4EO_{159}L_{25}$, and $G_{16}EO_{137}L_{40}$, which have hydrophilic blocks of comparable length. This tendency fits well with the results obtained previously for diblock PEO-PL copolymers [4,32]. The micelles formed by copolymer $G_{16}EO_{246}L_{44}$ possess a lower value for N_{agg} compared to $G_{16}EO_{137}L_{40}$ despite the presence of a longer hydrophobic PL block. This behavior can be explained after evaluating the surface area available per copolymer unit (S_t/N_{agg}) at the outer boundary of the micelle. S_t/N_{agg} is directly connected with the ability for free motion of each of the shell forming blocks [4]—the higher the S_t/N_{agg} value, the more free movement of the PG-PEO chains.

In accordance with the elongated shape of the micelles, as confirmed from the R_g/R_h ratio, S_t was derived by using the following equation valid for the surface area of prolate ellipsoid of rotation (also known as 'cigar' type):

$$S_t = 2\pi(b^2 + ab \arcsin(e)/e) \tag{8}$$

where a and b are the major and minor axes of the ellipsoid, and $e = ((1 - b^2)/a^2)^{1/2}$ [36]. The values of a and b were obtained by applying an iterative procedure. Starting from initial estimates, the values of a and b were varied in a consecutive manner with the view to determine values of the theoretically calculated $R_{h,theor}$ (see Table 5) of prolate ellipsoid following relationship (9), within an accepted tolerance of the experimentally measured values of the apparent R_h [37].

$$R_{h,theor} = \frac{(a^2 - b^2)^{1/2}}{\ln\left(\dfrac{a + (a^2 - b^2)^{1/2}}{b}\right)} \tag{9}$$

Once the values of a and b were obtained, they were double checked by calculating the theoretical radii of gyration of prolate ellipsoid particles, $R_{g,theor}$, as follows [37]:

$$R_{g,theor} = (a^2 + 0.4b^2)^{1/2} \tag{10}$$

The values of the theoretical $R_{g,theor}$ and experimental values of R_g agree well, and therefore the model used can be considered as feasible (Table 5).

Presumably, longer shell-forming blocks would require higher S_t/N_{agg} in order to obtain higher freedom of motion within the micelle shell. The value of S_t/N_{agg} increases in the samples with higher number of both the EO and the L units (Table 5). The S_t/N_{agg} values of the micelles formed by copolymers $G_{16}EO_{137}L_{40}$ and $G_{16}EO_{246}L_{44}$ are quite close—300 and 296 nm^2, respectively, although the second copolymer possesses much longer hydrophilic blocks. Accordingly, in the case of longer PG-PEO blocks

Table 4
Static light scattering results for PG-PEO-PL copolymers

Code	d*n*/d*c* (ml g^{-1})	$\bar{M}_w^{app} \times 10^{-5}$ (g mol^{-1})[a]	$\bar{M}_w^{uni} \times 10^{-5}$ (g mol^{-1})[b]	N_{agg}	R_g (nm)	R_g/R_h	$A_2 \times 10^5$ (mol ml g^{-2})
$G_7EO_{29}L_{13}$	0.106	5.40	0.038	142	49	–	−0.04
$G_{16}EO_{137}L_{17}$	0.106	4.35	0.125	34	23	1.92	1.80
$G_4EO_{159}L_{25}$	0.103	5.50	0.113	48	35	2.06	6.70
$G_{16}EO_{137}L_{40}$	0.096	9.40	0.136	69	68	1.70	3.50
$G_{16}EO_{246}L_{44}$	0.094	11.70	0.213	55	57	1.58	−1.60

[a] Apparent weight average molecular weight (SLS).

[b] Calculated from $\bar{M}_n$ (NMR) and PDI (SEC).

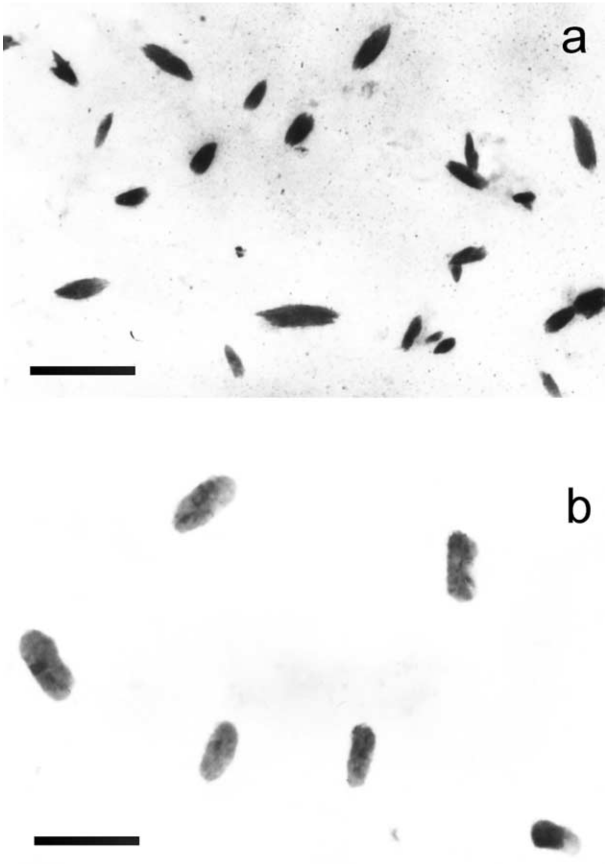


Fig. 8. TEM micrographs of particles prepared from micellar dispersion of copolymers (a) $G_{16}EO_{137}L_{17}$ and (b) $G_{16}EO_{137}L_{40}$ (each bar is 200 nm).

aggregates with higher curvature and respectively lower N_{agg} are preferably formed, thus overcoming the unfavorable effect of the increasing lateral repulsion of the PG-PEO chains. This implies that the length of the hydrophobic PL block of $G_{16}EO_{246}L_{44}$ is insufficient for obtaining stable micelles of higher N_{agg}. In addition the high values of S_t/N_{agg} indicate that very loosely packed micellar assemblies have been formed and therefore the micellar shell is highly swollen by the solvent molecules. This is more evident in the micelles of $G_{16}EO_{246}L_{44}$, which shell cannot entirely prevent the unfavorable interactions between the micellar core and the solvent, and thus a negative value for A_2 (Table 4) is obtained.

3.4.4. TEM measurements

The transmission electron micrographs of two samples differing only by the number of lactic units are shown in Fig. 8. In both cases relatively large elongated structures have been observed. The aggregates from copolymer $G_{16}EO_{137}L_{17}$ were found to be smaller, and as seen in Fig. 8(a), at least two populations of particles of different size, but with similar prolate ellipsoidal shape can be found. Note that the protocol of the preparation of the samples by solvent evaporation from the grid most likely favors a secondary aggregation within the system, and this option can be taken into account in this case. The particles formed by the copolymer $G_{16}EO_{137}L_{40}$, which PL hydrophobic block is longer, are invariably larger and relatively uniform, but still possess a shape of prolate ellipsoids (Fig. 8(b)).

4. Conclusions

New PEEGE-PEO-PL triblock copolymers with relatively low lactic unit content (10–27 mol%) were synthesized via multi mode anionic polymerization. The quantitative cleavage of the ethoxyethyl groups of the short PEEGE block leads to highly functional PG-PEO-PL amphiphilic triblock copolymers, which self-associate into

Table 5
Theoretically calculated values for a, b, S_t, $R_{h,theor}$ and $R_{g,theor}$ for prolate ellipsoids and experimentally determined values for R_h and R_g of the PG-PEO-PL micelles

Sample	a (nm)	b (nm)	$R_{h,theor}$ (nm)	R_h (nm)	$R_{g,theor}$ (nm)	R_g (nm)	S_t (nm^2)	S_t/N_{agg} (nm^2)
$G_{16}EO_{137}L_{17}$	23	7	11.79	12	23.42	23	1647	48
$G_4EO_{159}L_{25}$	34.5	10	17.29	17	35.07	35	3520	73
$G_{16}EO_{137}L_{40}$	65	30	40.95	40	67.71	68	20,723	300
$G_{16}EO_{246}L_{44}$	54	28	36.22	36	56.83	57	16,321	296

micelles after direct dissolution in water. The cmc values varied between 0.26 and 1.48 g/l depending on the composition of the copolymers. The R_h values for PG-PEO-PL copolymers were larger than the R_h of diblock copolymers of PEO-PL with a similar length of the PL blocks, but with shorter hydrophilic blocks. The dynamic and static light scattering showed that the R_g/R_h ratio for the functionalized micelles was between 1.58 and 2.06 indicating that the micelles were of non-spherical shape. Prolate ellipsoide model was used for calculating the surface area available per copolymer unit of the micelles, which in some cases turned to be very high, indicating for higher freedom of motion of the shell-forming blocks. One possible limitation of the present study that may appear is the lack of evaluation of the effect of the polyglycidol blocks over the shape of the aggregates obtained, since no previous results for pure PEO-PL copolymers that form non-spherical micelles in water were reported. At the same time, it is interesting that even a copolymer with a very short PG block ($G_4EO_{159}L_{25}$) is able to self-associate into a non-spherical micelle. Further investigations on micellar systems formed by pure PEO-PL copolymers of similar molecular weights and composition probably will elucidate this concern.

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