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Organization of poly(2-ethyl-2-oxazoline)-block-poly(2-phenyl-2-oxazoline) copolymers in water solution

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ABSTRACT

Diblock copolymers were prepared from 2-ethyl-2-oxazoline and 2-phenyl-2-oxazoline via living cationic polymerization using sequential addition of the monomers. Copolymer assemblies in aqueous solutions and on surface were studied with respect to changes in the hydrophilic-hydrophobic balance induced by increasing the length of the hydrophobic poly(2-phenyl-2-oxazoline) segment while the hydrophilic poly(2-ethyl-2-oxazoline) chain was kept constant with an average of 60 monomer units. The copolymer with a short segment of four 2-phenyl-2-oxazoline units assembled into highly hydrated aggregates that decreased twice in size after drying. Their structure was destroyed and network morphologies were formed upon spin-coating. The increase of the length of the hydrophobic segment resulted in aggregates that dissociated to micelle-sized particles when subjected to mechanical shear by spin-coating or filtering. These observations imply that the aggregates are multi-core structures originating from the assembly of primarily formed micelles. The copolymer self-assembly was evidenced by a combination of techniques: DLS, SLS, AFM and SEM.

1. Introduction

Polymer-based self-assembly systems represent a significant advance in the construction of supramolecular entities that may be useful for a wide range of applications such as drug delivery, separation technologies, dispersion stabilization, etc. [1–3]. Typical examples are the copolymers comprised of two blocks of differing nature. In selective solvents, i.e. solvents where only one of the blocks is soluble, these copolymers are able to form a variety of structures, including spherical, cylindrical, and lamellar micelles as well as micelle clusters. For practical considerations, the amphiphilic block copolymers have attracted the greatest interest due to their spontaneous self-organization in aqueous media [4,5].

Poly(2-oxazoline)s with methyl or ethyl side groups are nonionic and water-soluble polymers. Side groups longer than propyl or aryl side groups impart increased hydrophobicity to the polyoxazolines. Living cationic polymerization provides a convenient method for the preparation of poly(2-substituted-2-oxazoline)s, including homopolymers, di-, tri- and tetrablock or gradient copolymers [6–9]. Amphiphilic characteristics of poly(2-oxazoline)s can be induced through a variation of the kind of side groups, control of the block lengths and of composition. Self-organization of such materials in solution or on surfaces has been reported. Assemblies of different size and morphologies were detected and some trends in solution or surface behavior of the amphiphilic co-poly(2-oxazoline)s were observed [10–17].

Poly[(2-methyl-2-oxazoline)-b-(2-n-nonyl-2-oxazoline)] diblock copolymers that dissolve directly in water at room temperature have been observed to give rise to large metastable aggregates, unimers and micelles [10]. By means of fluorescence correlation spectroscopy, the hydrodynamic radii of the unimers ($R_h = 1.2 \pm 0.5$ nm), micelles ($R_h = 13.2 \pm 2$ nm) and aggregates ($R_h = 140 \pm 20$ nm) could be measured simultaneously. Annealing above 40 °C resulted in the dissolution of the metastable aggregates into stable micelles ($R_h = 11 \div 12$ nm).

Recently, microwave-assisted cationic ring-opening polymerization of 2-oxazolines has been exploited for the synthesis of libraries of di-, tri- and tetrablock poly(2-oxazoline)s [11–13]. Aqueous micelles were prepared from tetrablock ter- and quarterpolymers of 2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx), 2-phenyl-2-oxazoline (PhOx), and 2-n-nonyl-2-oxazoline by dissolving the polymers in organic solvents, followed by addition of deionized water and dialysis to eliminate the organic solvent [14]. It was observed that the nature of the nonselective starting solvent, as well as the initial concentration of the polymer in this solvent, had little effect on the characteristics of the final micelles in water. The size of the micelles (from 7 to 20 nm) depended mainly on the copolymer

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composition. The obtained micelles had a broad size distribution in solution and different morphologies (spherical or rice grain morphologies) visualized with both AFM and TEM experiments.

The micellization on surfaces has been reported for series of block copoly(2-oxazoline)s in a non-selective solvent [15,16]. The micelles did not exist in the initial solution but were formed during the spin-coating process by the evaporation of the solvent inducing the precipitation of the less soluble block. The increase in the fraction of this block caused the micelle morphology to change from a spherical to an elongated structure and further to a semi-continuous network of worm-like micelles.

Amphiphilic monoalkyl end-capped poly(2-methyl-2-oxazoline)s (PMeOx-C12 and PMeOx-C18), with molar masses varying from 5500 to 8500 g/mol, were prepared [17]. Critical micelle concentrations (cmc) of their aqueous solutions determined by fluorescence technique were in the range of 0.007–0.4 mg/mL and were dependent on the hydrophilic/lipophilic balance.

Diblock copolymers composed of PEtOx and polyester blocks, i.e. poly(L-lactide), poly(ϵ-caprolactone) or poly(1,3-trimethylene carbonate) were prepared [18,19]. These block copolymers assembled in aqueous solutions and formed multicore structures with mean diameters in the range of 108–210 nm with a narrow size distribution. The authors assumed that these species were formed by the association of individual micelles but such core-shell micelles were not detected in the solutions.

Poly(2-oxazoline)s contain the amide unit in the backbone and are therefore called “pseudopeptides” [20]. The potential of these polymers in bio-oriented applications has been evidenced by studies reporting a good biocompatibility of this class of polymers [21,22]. Recently, it was found that the hydrophilic members of the family did not accumulate in tissues and were rapidly cleared from the blood [23].

In view of future application, specifically in pharmacy and biomedicine as potential alternative to PEG, the systematic study on the structure-property relationships for poly(2-oxazoline)s is important. Therefore, some particular aspects such as the concentration range of stability of the nanocolloidal solutions of diblock poly(2-oxazoline)s, the effect of solution processing, etc. need detailed study. The present investigation has been undertaken to contribute an insight into the aggregation behavior of poly(2-oxazoline) diblock copolymers in relation to the copolymer composition and block length ratio.

2. Experimental

2.1. Materials

2-Phenyl-2-oxazoline (Aldrich) and 2-ethyl-2-oxazoline (Aldrich) were dried over NaOH and distilled prior to use. They were stored under nitrogen. Methyl tosylate (Aldrich) was distilled and stored under nitrogen. Nitrobenzene and *N*,*N*-dimethylacetamide were dried according to standard procedures and distilled before use. Methanol was distilled prior to use while diethyl ether and tetrahydrofuran (THF) were used as received.

2.2. Preparation of poly(2-ethyl-2-oxazoline)-block-poly(2-phenyl-2-oxazoline) diblock copolymers

Polymerizations were carried out in Schlenk reactors. The reactors were heated at 100 °C under reduced pressure and then cooled to room temperature under a flow of nitrogen. The reagents for the synthesis of the first block – the initiator methyl tosylate, solvent (solvent:monomer = 3:1 v/v) and EtOx – were added under a nitrogen atmosphere. *N*,*N*-dimethylacetamide, or nitrobenzene in the case of the highest PhOx load, was used as the solvent. The reaction system was cooled down to 0 °C and stirred at this temperature for 2 h. Subsequently, the reactor was placed in an oil bath at 80 °C under constant stirring. When the monomer conversion exceeded 0.9 (as determined by gas chromatography), PhOx was added. The reaction temperature was raised to 90 °C and the mixture was left under stirring overnight.

The temperature was then raised to 130 °C and the stirring was continued until the conversion of the second monomer ceased to increase. The conversion of both comononers exceeded 95%. After cooling to room temperature, the polymerizations were terminated by addition of methanolic KOH (active centers: KOH = 1:1.3) and stirring of the mixture for 2 h. The product was precipitated in diethyl ether, re-dissolved in THF and left in a fridge for one night. The solution was filtered to remove the salt and then concentrated, after which the product was isolated by precipitation in diethyl ether. The copolymers were purified by dialysis (MWCO 3000) in a methanol solution for 24 h.

Three copolymers were synthesized and were denoted $PEtOx_{60}$-b-$PPhOx_4$, $PEtOx_{59}$-b-$PPhOx_{12}$ and $PEtOx_{65}$-b-$PPhOx_{37}$. Here, the indices correspond to the average degree of polymerization (DP) of the blocks.

2.3. Preparation of the copolymer solutions

The copolymers were dissolved in methanol that had been filtered through a 0.02 μm Whatman ANOTOP membrane. Stock solutions with a concentration of 2 mg/mL were prepared and filtered through 0.2 μm Whatman ANOTOP membranes. Deionized and distilled water filtered through a 0.02 μm Whatman ANOTOP membrane was added drop-wise to a predetermined volume of the copolymer stock solution in methanol under constant stirring. After the addition of water, methanol was removed under reduced pressure at 25 °C. The volume was adjusted with water to obtain solutions with concentrations: (i) from 2.00 mg/mL to 0.50 mg/mL for $PEtOx_{60}$-b-$PPhOx_4$, (ii) from 0.50 mg/mL to 0.05 mg/mL for $PEtOx_{59}$-b-$PPhOx_{12}$ and (iii) from 0.10 mg/mL to 0.01 mg/mL for and $PEtOx_{65}$-b-$PPhOx_{37}$. These solutions were subjected to dynamic light scattering (DLS) and static light scattering (SLS) analysis. The copolymer assemblies were visualized by atomic force microscopy (AFM) and scanning electron microscopy (SEM). Measurements were also performed for the same solutions filtered through 0.45 μm Whatman ANOTOP membranes.

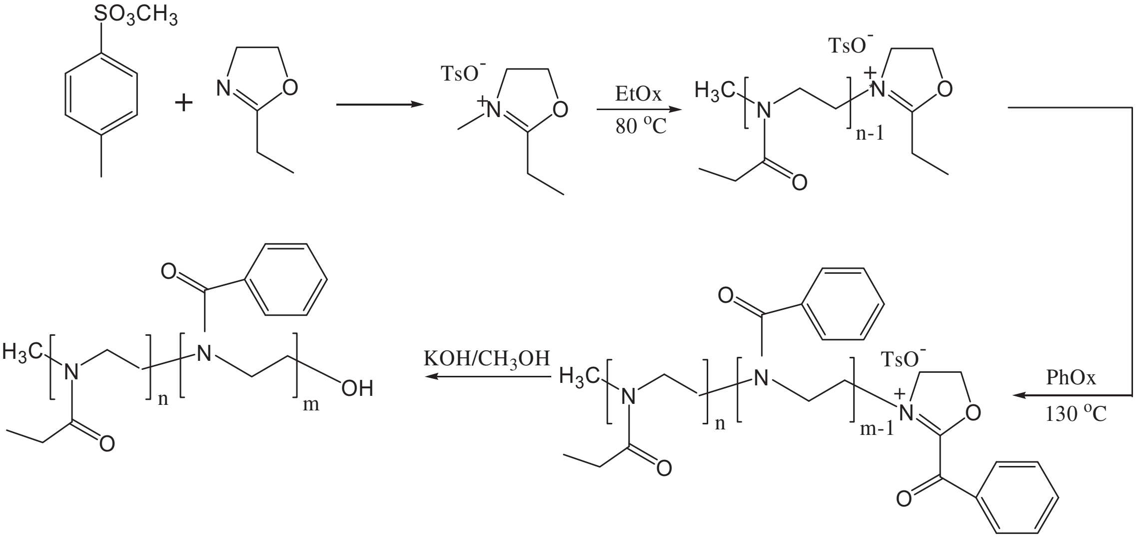
2.4. Measurements

^{1}H NMR spectra of the copolymers were recorded at 25 °C on a Varian UNITY/INOVA spectrometer operating at 300 MHz and with acetone-d_6 as the solvent.

The molar mass and dispersity of the obtained products were determined by size exclusion chromatography coupled to a multi-angle light scattering detector DAWN EOS from Wyatt Technologies ($\lambda = 690$ nm) as well as to a Δn-1000 RI WGE Dr. Bures refractive index detector ($\lambda = 620$ nm). Polymer Standard Service (PSS) SDV columns $1 \times 10^5 + 1 \times 10^3 + 2 \times 10^2$ Å were used for the measurements and THF was employed as the mobile phase. The measurements were carried out at a temperature of 35 °C. The nominal flow rate of the eluent was 1 mL/min. Chromatograms were collected and evaluated using the ASTRA 4.73 software from Wyatt Technologies and WINGPC 7.00 software from Polymer Standard Service. The specific refractive index increments (*dn*/*dc*) for the copolymer samples were estimated assuming the simple additivity relationship:

$$dn/dc = \phi_A(dn/dc)_A + \phi_B(dn/dc)_B \quad (1)$$

where ϕ_i is the mass fraction of the corresponding block obtained on the basis of the ^{1}H NMR data and the refractive index increments



Scheme 1. Synthesis of the PEtOx-b-PPhOx diblock copolymers.

of the corresponding homopolymers in THF: $dn/dc = 0.10$ mL/g for PEtOx and $dn/dn = 0.147$ mL/g for PPhOx. The latter values were measured with the differential refractive index detector.

Dynamic light scattering (DLS) measurements were performed on a Brookhaven BI-200 goniometer with a vertically polarized incident light of wavelength $\lambda = 632.8$ nm supplied by a He–Ne laser operated at 35 mW. The device was also equipped with a Brookhaven BI-9000 AT digital autocorrelator. Light scattering measurements were performed at angles to the incident beam ranging from 40° to 130°. The autocorrelation functions from DLS were analyzed by the constrained regularized CONTIN method in order to obtain distributions of relaxation rates (Γ). The decay rates provided distributions of the apparent diffusion coefficient ($D = \Gamma/q^2$), where q is the magnitude of the scattering vector, $q = (\frac{4\pi n}{\lambda})\sin(\frac{\Theta}{2})$. The apparent hydrodynamic radii were obtained from the Stokes–Einstein equation:

$$R_h^{\text{app}} = kT/(6\pi\eta D) \tag{2}$$

Here, k is the Boltzmann constant, η is the viscosity of water at temperature T, and D is the diffusion coefficient at a fixed concentration. The dispersity of the particle sizes was given as $\mu_2/\overline{\Gamma}^2$, where $\overline{\Gamma}$ is the average relaxation rate, and μ_2 is its second moment. The values of the radii of gyration, R_g, were obtained from the partial Zimm plots, which can be described by the following equation:

$$\frac{1}{I_{\text{ex}}} = C\left(1 + \frac{R_g^2 q^2}{3}\right) \tag{3}$$

Here, I_{ex} is the excess of the scattered light.

For the AFM analyses, a multimode instrument equipped with a NanoScope 3D controller (MultiMode, Veeco Instruments Inc., USA) operating in tapping mode in air with standard 125 μm single-crystal silicon cantilevers (Model TESP; Veeco Instruments Inc., USA) was used. The piezoelectric scanner had a scan range of approximately 10 × 10 μm^2. The solutions were spin-coated onto glass wafers in air at 500 rpm for 30 min. All samples were imaged at room temperature and measured 24 h after coating.

Scanning electron microscopy (SEM) was conducted on a Jeol JSM-5510 at 10 kV. The specimens for the SEM observations were prepared by evaporating aqueous solutions of the samples on clean glass slides washed with deionized water. To obtain charge dissipation during the SEM imaging, a thin layer of gold was sputter-coated on the samples with the aid of a JEOL JFC-1200 fine coater.

3. Results and discussion

3.1. Synthesis and characterization of the block copolymers

The amphiphilic feature of the targeted block copolymers was attained by carrying out a sequential ring-opening polymerization of EtOx and PhOx, resulting in copolymer species composed of a hydrophilic PEtOx and hydrophobic PPhOx blocks, respectively. The polymerization reactions were carried out according to Scheme 1. The living polymerization was terminated by addition of methanolic KOH. The block copolymers were characterized by means of NMR and SEC. The results are summarized in Table 1.

The SEC-MALLS revealed monomodal molar mass distributions for all copolymers. The molar mass dispersity values were below 1.25 and decreased with the increase of the copolymer molar mass. This observation was consistent with other research group's findings [24] concerning concentration effects in the polymerization of EtOx in *N*,*N*-dimethylacetamide.

Absolute molar masses were determined based upon the chromatographic data obtained from the MALLS and RI detectors, and the values were found to be in good agreement with those estimated on the basis of monomer conversion and the initial monomer/initiator feed ratio. Taking into account the ^{1}H NMR and SEC data copolymer compositions and chain lengths could be determined. The copolymers contained hydrophilic blocks of similar length (59–65 monomer units) while the average DP of the hydrophobic chain varied from 4 to 37, which corresponded to a mass fraction of the PPhOx block of between 9% and 46%.

Table 1
Molar masses and compositions of PPhOx-b-PEtOx diblock copolymers.

Copolymer	[PhOx]:[EtOx][a]	$M_{n,conv}$[b]	$M_{n,SEC}$	M_w/M_n	PPhOx (mass %)[c]
PEtOx$_{60}$-b-PPhOx$_4$	0.07	6600	6500	1.25	9
PEtOx$_{59}$-b-PPhOx$_{12}$	0.20	8000	7600	1.17	23
PEtOx$_{65}$-b-PPhOx$_{37}$	0.57	12500	11900	1.10	46

[a] The mole ratio of PhOx and EtOx units according the ^{1}H NMR data.
[b] The number average molar mass calculated on the basis of the initial feed ratio and monomer conversion determined by GC.
[c] The mass fraction of the PPhOx block calculated on the basis of the copolymer composition (NMR data).

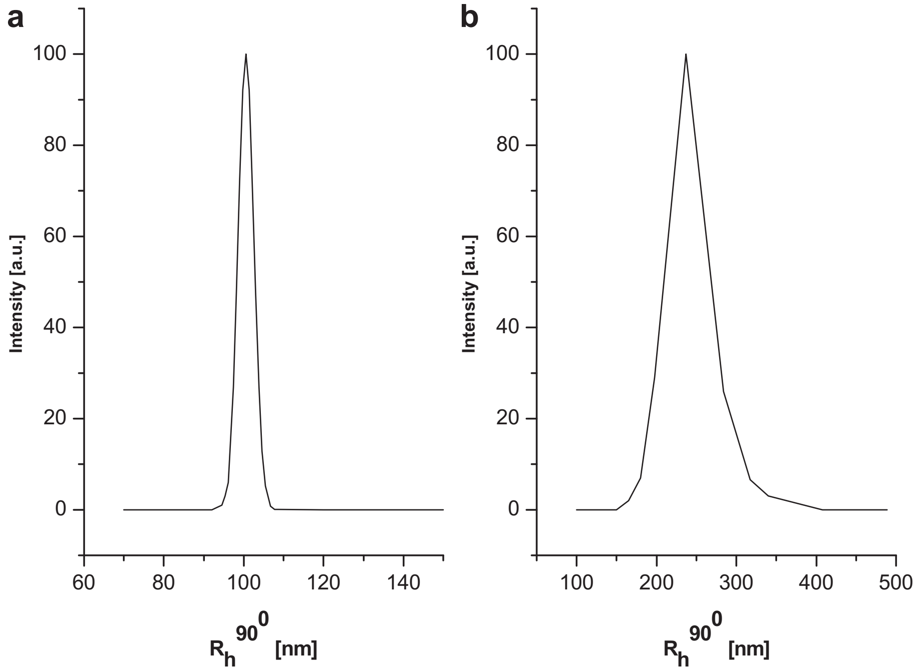


Fig. 1. The distribution of the hydrodynamic radii of PEtOx$_{60}$-b-PPhOx$_4$ aggregates in aqueous solutions measured at 25 °C and at scattering angle $\Theta = 90°$ with concentrations: a) 0.50 mg/mL and b) 2.00 mg/mL.

3.2. *Copolymer aggregation*

Attempts to obtain nano-colloidal solutions directly in water failed for all copolymers. For this reason, the copolymer samples were first dissolved in methanol at a concentration of 2 mg/mL; a concentration chosen since precipitation would occur upon addition of water for higher concentrations of the PEtOx$_{65}$-b-PPhOx$_{37}$ methanolic solution. Methanol is a good solvent for both block, and ensures a complete solubilization of the copolymers, which was also confirmed by DLS measurement. Deionized and distilled water filtered through a 0.02 μm membrane was added to a predetermined volume of the copolymer stock solution in methanol in order to trigger the association process. After this addition of water, methanol was gradually removed by evaporation under reduced pressure. The so-prepared aqueous solutions were divided into two parts, the first of which was characterized without further processing (denoted below as "prior to filtering"). The second set of solutions (denoted as "after filtering") was filtered through 0.45 μm membranes prior to the measurements.

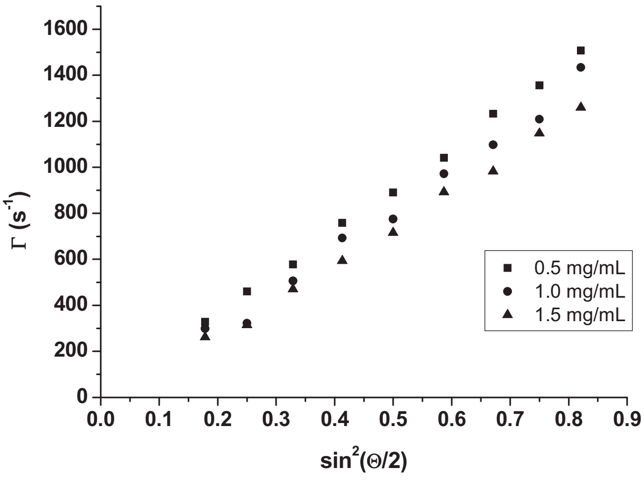


Fig. 2. Relaxation rate (Γ) of the aggregates as a function of $\sin^2(\Theta/2)$ for PEtOx$_{60}$-b-PPhOx$_4$ solutions with concentrations given in the inserted window.

3.2.1. *Aggregation of PEtOx$_{60}$-b-PPhOx$_4$*

Aqueous solutions of PEtOx$_{60}$-b-PPhOx$_4$ were prepared in concentrations of 0.50, 0.75, 1.00, 1.50 and 2.00 mg/mL. Solutions with concentrations lower than 0.5 mg/mL were not measured because the intensity of the scattered light was too low. The same reason limited the concentration ranges for the two other copolymer solution series. Fig. 1 displays distribution curves of the hydrodynamic radii of PEtOx$_{60}$-b-PPhOx$_4$ aggregates in aqueous solutions of concentration 0.5 mg/mL and 2.00 mg/mL, measured at 25 °C and at a scattering angle $\Theta = 90°$. According to these DLS measurements, a single population of aggregates was found for each of the five solutions prior to filtering. The filtering of the solutions through a 0.45 μm membrane led to no measurable light scattering. The possible reason was the dissociation of aggregates under the action of shear force into not detectable unimers, and/or absorption of the polymers on the filter material.

The size distribution of the particles was narrow as indicated by dispersity values lower than 0.11.

The R_h^{app} values were calculated using Eq. (2) and the mean diffusion coefficient values obtained from the slope of the linear fits of the relaxation rate dependence on $\sin^2(\Theta/2)$ at the fixed solution

Table 2
Values of the apparent hydrodynamic radii, R_h^{app}, and radii of gyration, R_g, of the copolymer aggregates in the aqueous PEtOx$_{60}$-b-PPhOx$_4$ solutions prior to filtering. Measurements were performed at 25 °C.

Concentration (mg/mL)	R_h^{app} (nm)	R_g (nm)	R_g/R_h^{app}
2.00	235	[a]	–
1.50	108	[a]	–
1.00	101	[a]	–
0.75	97	171	1.76
0.50	92	129	1.40

[a] R_g was too high to be determined from Zimm plot.

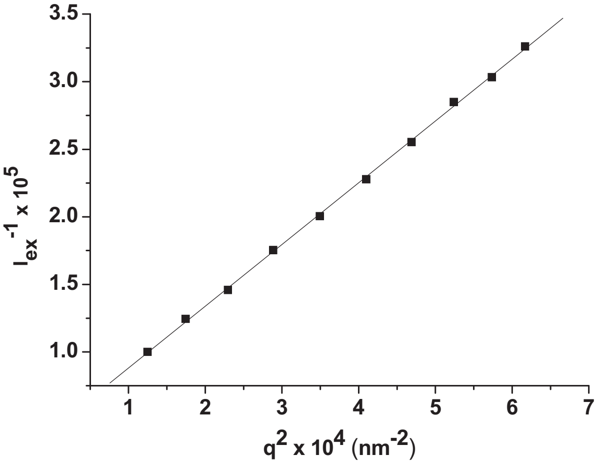


Fig. 3. The partial Zimm plot for a PEtOx$_{60}$-b-PPhOx$_4$ solution with a concentration of 0.75 mg/mL.

concentrations (Fig. 2). Table 2 contains the values of the apparent hydrodynamic radius, R_h^{app}. R_h^{app} turned out to depend upon the concentration of the polymer in solution. In the concentration range from 0.50 mg/mL up to 1.50 mg/mL a linear dependence of the increase in the size of the aggregates on the copolymer concentration was measured. These nano-colloidal solutions were stable for more than two months. At concentrations higher than 1.50 mg/mL, particles of larger dimensions were formed and visible post-aggregation occurred after two weeks.

Another parameter obtained from the light scattering measurements was the radius of gyration, R_g (also listed in Table 2). This parameter was calculated from the linear fit of the angular dependence of the quantity I_{ex}^{-1} (Fig. 3). R_g values of 129 and 171 nm were obtained, depending upon the concentration. Correspondingly, the R_g/R_h^{app} ratio increased (Table 2). This most probably indicates changes of the morphology to the more elongated shape, as can be seen in Fig. 4b. However, one has to keep in mind that the ratio R_g/R_h^{app} is a quotient of a true and a relative value. It reflects the shape, but is also influenced by the changes of swelling of the macromolecules by the solvent.

The tendency was confirmed by SEM and AFM analyses. First the surfaces for measurements were obtained without applying a spin-coating technique. The micrographs in Fig. 4 show aggregates of PEtOx$_{60}$-b-PPhOx$_4$ after drying of solutions with concentrations of 0.50 mg/mL and 1.00 mg/mL. As can also be observed, the aggregates were uniform in size. The evaporation of the absorbed water from the hydrophilic compartments of the particles caused a decrease to almost half their size measured in solution. This fact implies that the aggregates were highly hydrated in aqueous solution.

Table 3
Values of the apparent hydrodynamic radii, R_h^{app}, and radii of gyration, R_g, for the copolymer aggregates in the aqueous PEtOx$_{59}$-b-PPhOx$_{12}$ solution prior to filtering. Measurements were carried out at 25 °C.

Concentration (mg/mL)	R_h^{app} (nm)	R_g (nm)	R_g/R_h^{app}
0.50	145	[a]	–
0.30	121	178	1.47
0.20	92	94	1.02
0.10	88	90	1.02
0.08	73	76	1.04
0.05	75	76	1.01

[a] R_g was too high to be determined from a Zimm plot.

When spin-coating was used to prepare samples for SEM measurements a semi-continuous film was formed on the glass surface and no individual particles were detected.

The observations for the PEtOx$_{60}$-b-PPhOx$_4$ aqueous solutions imply a formation of aggregates rather than regular core-shell structures. Typically, the size of core-shell micelles does not exceed several tens of nanometers. Similar tendency has been observed for other amphipilic block copolymers. PEO-based amphiphilic diblock copolymers form large aggregates in aqueous solution [25–27]. This phenomenon was explained with secondary aggregation due to strong hydrophobic interactions resulting in various assemblies such as large core-shell micelles, onion-like aggregates or loose clusters of small micelles [26]. In the present case, the hydrophobic segment of PEtOx$_{60}$-b-PPhOx$_4$ was too short in order to play a dominant role in the aggregation process. It can therefore be assumed that the PEtOx block itself significantly influenced the solution properties and the macromolecular interactions. Nagpal et al. [28] explained the formation of large aggregates from diblock PEtOx-polydimethylsiloxane copolymers with the tendency of PEtOx to aggregate in water, and the aggregation being more pronounced at lower molar masses [29].

3.2.2. *Aggregation of PEtOx$_{59}$-b-PPhOx$_{12}$*

Nano-colloidal PEtOx$_{59}$-b-PPhOx$_{12}$ solutions were obtained in the concentration range from 0.50 mg/mL to 0.05 mg/mL. Aggregates were detected in all of the solutions (concentrations: 0.50, 0.30, 0.20, 0.10, 0.08 and 0.05 mg/mL) and monomodal relaxation rate distributions were observed. The dispersity of the particle sizes, measured as $\mu_2/\overline{\Gamma}^2$, did not exceed 0.10. Moreover, the aggregates displayed good stability upon storage. A repetition of the measurements after six weeks provided identical values for the hydrodynamic diameters

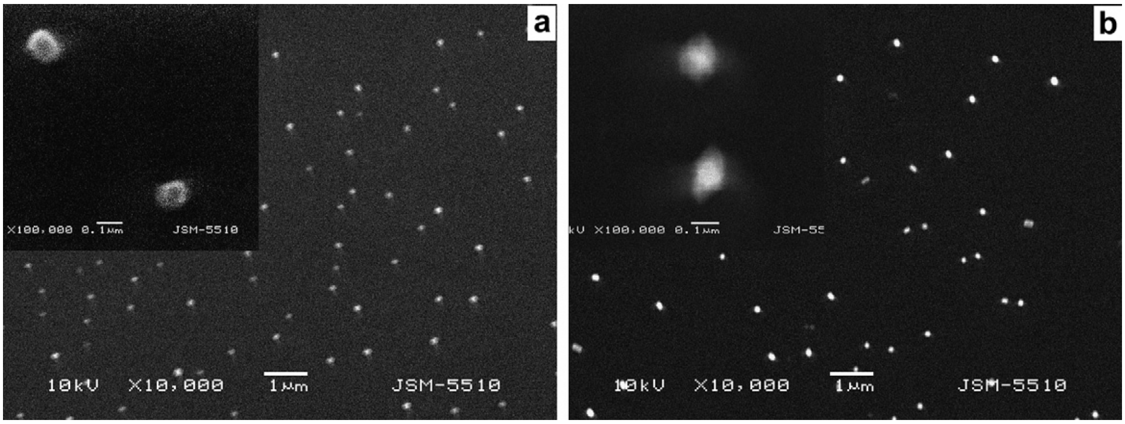


Fig. 4. SEM images of the aggregates obtained from PEtOx$_{60}$-b-PPhOx$_4$ after drying of aqueous solutions with concentrations of (a) 0.50 mg/mL and (b) 1.00 mg/mL.

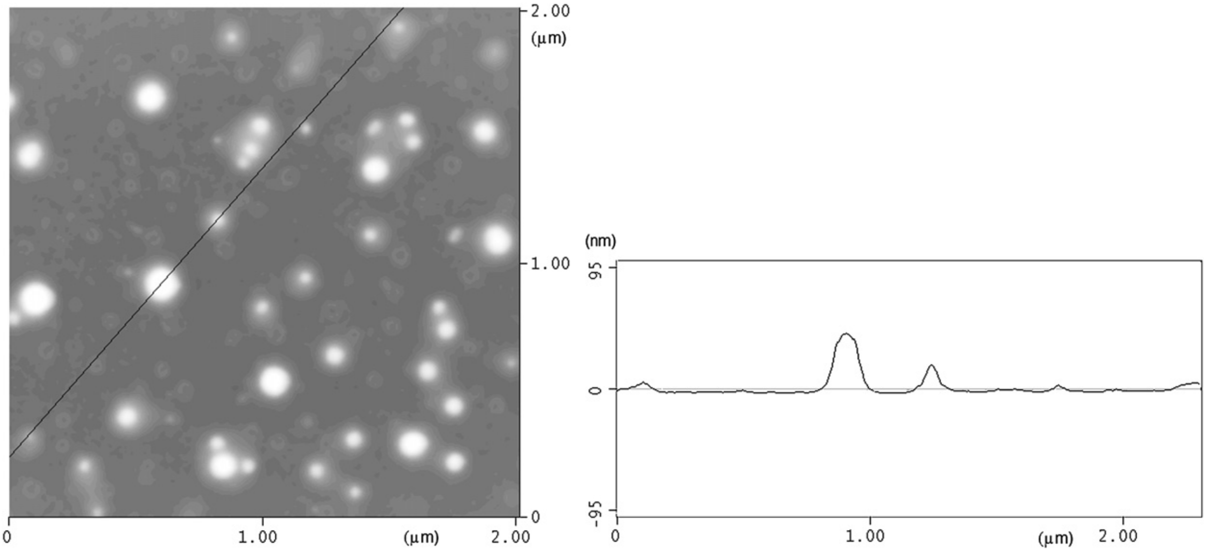


Fig. 5. A tapping-mode AFM image and the cross-sectional profiles of aggregates obtained after spin-coating of an aqueous PEtOx$_{59}$-b-PPhOx$_{12}$ solution with a concentration of 0.10 mg/mL.

of the aggregates. The threefold increase in the length of the hydrophobic segment (from 4 to 12 PhOx units) caused a shift in the concentration range of stable aggregate formation by one order of magnitude (see Tables 2 and 3). Similarly to the PEtOx$_{60}$-b-PPhOx$_{4}$ solutions, no particles were detected after filtration of PEtOx$_{59}$-b-PPhOx$_{12}$ solutions through a 0.45 µm membrane.

Data corresponding to the apparent hydrodynamic radii and the radii of gyration are summarized in Table 3. The common trend for the PEtOx$_{59}$-b-PPhOx$_{12}$ solutions consisted in an increase of the particle size with the increase of the copolymer concentration. The size of the aggregates grew gradually, and above 0.20 mg/mL a significant increase in both R_h^{app} and R_g was noticed: the R_h^{app} value was doubled with an increase in concentration from 0.08 mg/mL to 0.50 mg/mL. The corresponding values of the apparent radius of gyration, R_g, behaved in a similar manner. According to the R_g/R_h^{app} ratios, aggregates with a spherical shape existed in the solutions with concentrations equal to or lower than 0.20 mg/mL.

As for PEtOx$_{60}$-b-PPhOx$_{4}$, the aggregates formed in the PEtOx$_{59}$-b-PPhOx$_{12}$ solutions were also larger than the core-shell type micelles. Similar results have been reported before for amphiphilic diblock copolymers composed of hydrophilic PEtOx-blocks with an average DP of 54 and a hydrophobic poly(1,3-trimethylene carbonate) blocks varying in DP from 8 to 39 [19]. The size of the aggregates formed from these copolymers ranged from 199 to 210 nm. The authors suggested that the aggregates might be formed by the association of the individual micelles due to hydrophobic interactions between the cores of the micelles.

The PEtOx$_{59}$-b-PPhOx$_{12}$ aggregates were visualized by AFM and Fig. 5 displays a tapping-mode AFM image obtained after spin-coating of an aqueous PEtOx$_{59}$-b-PPhOx$_{12}$ solution with a concentration of 0.10 mg/mL.

Individual particles of different size can be clearly seen. According to the cross-sectional profiles the diameter of the larger aggregates was approximately 140 nm while those of the smaller particles were 2 to 4 times smaller. At higher magnification the morphology of the smaller particles is better seen. They are spherical structures which vertical distance of the periphery and centre differs significantly that could be assigned to hydrophilic

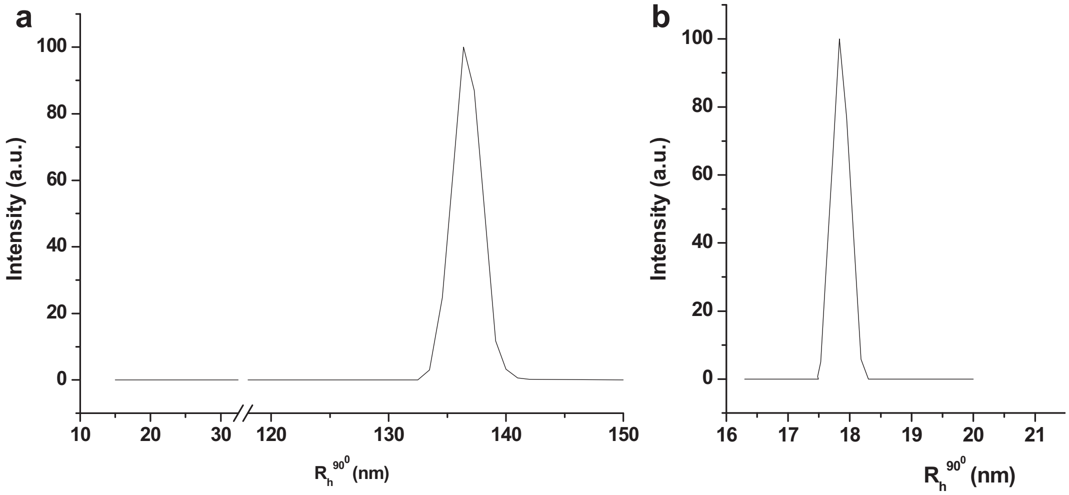


Fig. 6. The distribution of the hydrodynamic radius, measured at 25 °C and at a scattering angle $\Theta = 90°$, of the assemblies formed in PEtOx$_{65}$-b-PPhOx$_{37}$ aqueous solutions with a concentration of 0.06 mg/mL: a) prior to filtering and b) after filtering through a 0.45 µm membrane.

and hydrophobic compartments, respectively. The visualization of more than one population of species, contrarily to the DLS measurements, means disintegrating of the aggregates born in solution to smaller structures, some of which micelle-sized. These observations support the assumed morphology of the aggregates in solution as multicore particles that could be destroyed under shear force.

3.2.3. Aggregation of PEtOx$_{65}$-b-PPhOx$_{37}$

The non-filtered solutions of PEtOx$_{65}$-b-PPhOx$_{37}$ within the concentration range from 0.1 to 0.01 mg/mL were investigated by DLS. The intensity of the scattered light was found to be high, even for the most diluted sample, i.e., the one with a concentration of 0.01 mg/mL. A single population of particles was detected for each of the solutions prior to filtering. As an example the distribution of the hydrodynamic radius of the aggregates in a solution with a concentration of 0.06 mg/mL is shown in Fig. 6a. Linear angular dependencies of the Γ-function were measured for all solutions (Fig. 7a) and the values of R_h^{app} were obtained based on eq. (2). With the increase of the concentration, the average size of the particles increased from 58 nm to 156 nm (Table 4).

Subsequently, the solutions were filtered through a 0.45 μm Whatman ANOTOP membrane and measured again. The Γ-function was monomodal and linear (Fig. 7b) indicating the presence of a single population of small particles with $R_h^{app} < 20$ nm (Fig. 6b and Table 4). This was in contrast to the results on the PEtOx$_{59}$-b-PPhOx$_{12}$ and PEtOx$_{60}$-b-PPhOx$_{4}$ copolymers.

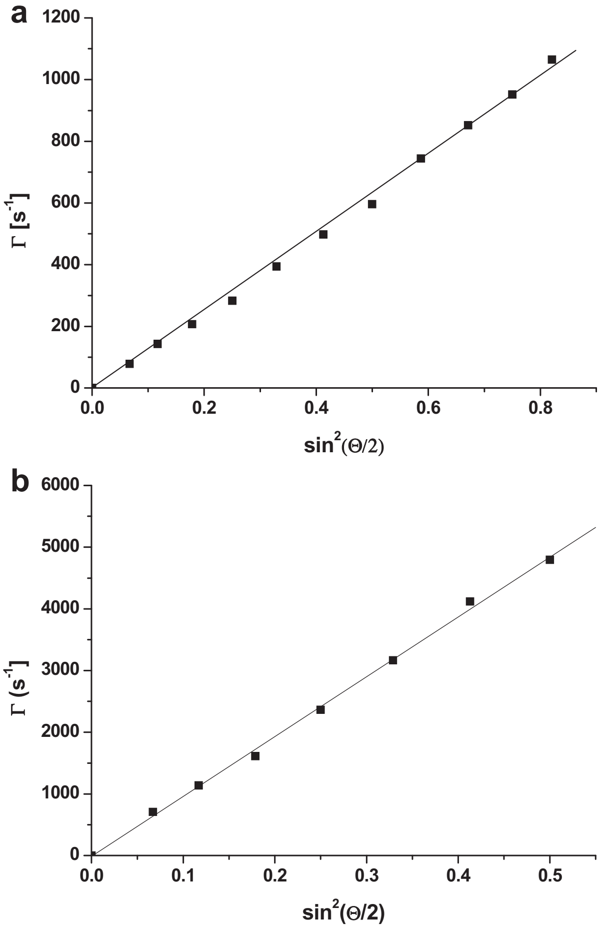


Fig. 7. Relaxation rate (Γ) as a function of $\sin^2(\Theta/2)$ for PEtOx$_{65}$-b-PPhOx$_{37}$ solutions with concentration 0.06 mg/mL: (a) prior filtering and (b) after filtering through 0.45 μm membrane.

Table 4
Values of the apparent hydrodynamic radii, R_h^{app}, of the copolymer aggregates in aqueous PEtOx$_{65}$-b-PPhOx$_{37}$ solutions measured at 25 °C.

Concentration (mg/mL)	Prior to filtering R_h^{app} (nm)	After filtering[a] R_h^{app} (nm)
0.10	156	19
0.08	139	19
0.06	130	17
0.03	121	15
0.01	58	[b]

[a] Solutions filtered through 0.45 μm Whatman ANOTOP membranes.
[b] Low intensity of the scattered light.

SLS measurements were also performed and demonstrated a lack of angular dependence of the quantity I_{ex}^{-1}, thus implying R_g values of particles smaller than 15 nm.

AFM measurements were performed on non-filtered as well as on filtered solutions spin-coated onto glass wafers and dried. Fig. 8 displays tapping-mode AFM images obtained after spin-coating of the aqueous PEtOx$_{65}$-b-PPhOx$_{37}$ solution with a concentration of 0.06 mg/mL. For the non-filtered solution, the coexistence of two populations of spherical particles – micelles and aggregates – could be visualized (Fig. 8a). The size of the aggregates could be determined by AFM as an area with a horizontal distance of 150 nm and a vertical distance of about 60 nm. The population of micelles is clearly seen in both AFM images – before (Fig. 8a) and after (Fig. 8b) filtration of the solution. The horizontal diameters measured from the cross-sectional profiles of the particles in the two images were of the same values (37 nm in average). The sizes of the micelles determined by AFM were in agreement with the micelle characteristics as measured by DLS for the copolymer solution after filtering (Fig. 6b). Both AFM and DLS measurements indicate that long hydrophobic block of PPOx promotes the formation of stable micellar structures of copolymer chains.

The difference between AFM and DLS observations prior to and after filtering needs to be addressed. The amount and sizes of smaller particles seen by AFM indicate that they would be detected with enough sensitivity by DLS if they were present in the solution as separate species prior to filtering. The plausible explanation is that large aggregates, probably consisting of many micelles glued together, dissociate into micelles only under the action of shearing rate at filtering or spin-coating. The possibility of existence of clusters of small micelles was discussed previously by Winnik et al. [26]. The influence of spin-coating process on the morphology of micelles formed was observed before [15].

Spherical micelles of similar size from amphiphilic multiblock poly(2-oxazoline)s in water were observed by Hoogenboom et al. [14]. Though one of the copolymers PMeOx$_{25}$-b-PEtOx$_{26}$-b-PPhOx$_{26}$ had a content of hydrophobic units (45 mass %) close to that of the PEtOx$_{65}$-b-PPhOx$_{37}$ copolymer discussed in the present investigation the two copolymers displayed different aggregation behavior. The hydrophilic chain of the triblock copolymer contained PMeOx as an outer segment. This increased the hydrophilicity of the triblock copolymer and overcame the aggregation tendency of its micelles [14].

The present study and the examples taken from the cited references for amphiphilic block poly(2-substituted-2-oxazoline)s display the major influence of the hydrophilic-hydrophobic balance on the structure and behavior of the copolymer aggregates and the necessity to select the proper experimental conditions for their detection. An indicative example was the case with the PEtOx$_{60}$-b-PPhOx$_{4}$. Upon drying of the copolymer solution with spin-coating a semi-continuous network was formed on the glass surface. Individual particles were visualized only without applying a spin-coating technique. On the contrary, for the PEtOx$_{65}$-b-PPhOx$_{37}$ the

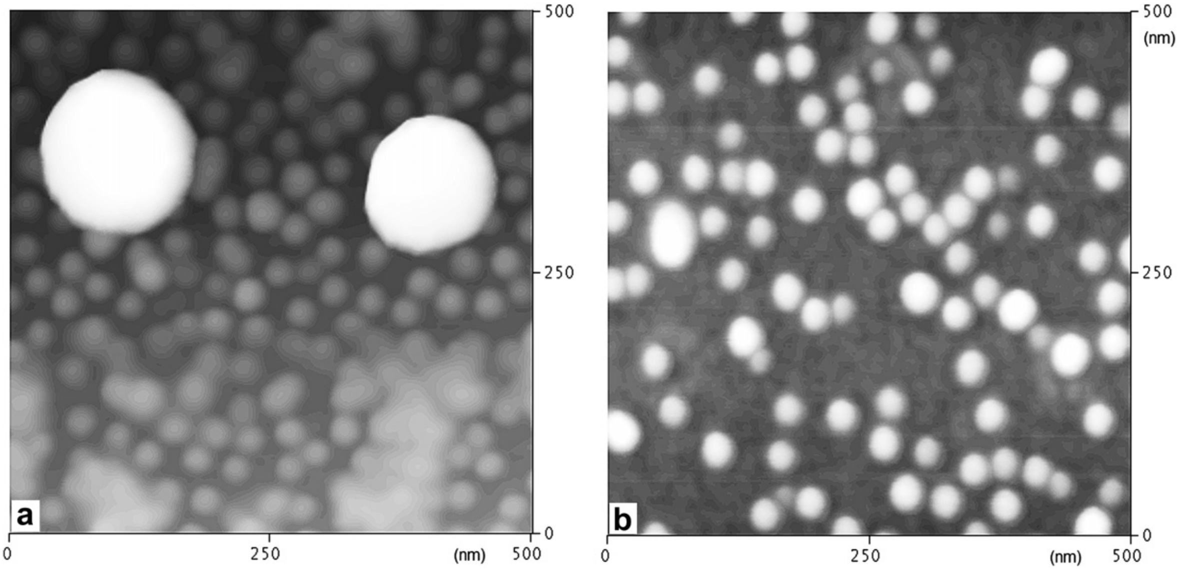


Fig. 8. Tapping mode AFM images obtained after spin-coating of the aqueous PEtOx$_{65}$-b-PPhOx$_{37}$ solution with a concentration of 0.06 mg/mL: a) prior to filtering and b) after filtering through a 0.45 μm Whatman ANOTOP membrane.

spin-coating of the copolymer solution helped to destroy the polymer aggregates and to visualize core-shell micelles.

4. Conclusions

Diblock copolymers derived from the commercially available monomers EtOx and PhOx were synthesized via living cationic copolymerization with sequential addition of the monomers. Next to the well studied oxazolines (see Introduction) for the first time the self-association behavior in aqueous media of copolymers comprised of hydrophilic PEtOx chains and strongly hydrophobic PPhOx blocks has been reported. The results clearly indicate a major influence of the copolymer composition on the aggregation process of the studied diblock poly(2-oxaoline)s and the concentration range of application of the obtained nanocolloidal systems. At low fractions (<10 mass %) of the hydrophobic segment, the copolymer self-organization resulted in highly hydrated particles. They could be visualized as individual particles after simple evaporation of the solvent whereas spin-coating destroyed them and network morphologies were observed by AFM. Upon increasing the length of the hydrophobic chain, the range of stable nanocolloidal solutions shifted to lower concentrations, as well as, more stable self-assembling structures were formed. The particles responded in a different manner to solution processing. Copolymers with almost equal mass fractions of the hydrophobic and hydrophilic blocks formed stable micelles that self-assembled into aggregates. The latter could be disintegrated to the former core-shell structures, being subjected to spin-coating on a glass wafer or filtering through membranes with an appropriate pore size. Application of the mechanical shear (spin coating or filtering) causes the aggregates to dissociate.

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