Journal of Colloid and Interface Science 387 (2012) 275–284

Contents lists available at SciVerse ScienceDirect

Journal of Colloid and Interface Science

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Poly(ethylene oxide)–poly(styrene oxide)–poly(ethylene oxide) copolymers: Micellization, drug solubilization, and gelling features

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ARTICLE INFO

Article history:
Received 18 May 2012
Accepted 27 June 2012
Available online 3 August 2012

Keywords:
Block copolymer
Polymeric micelle
Phase behavior
Drug delivery system
Release kinetics

ABSTRACT

Two new poly(ethylene oxide)–poly(styrene oxide) triblock copolymers (PEO–PSO–PEO) with optimized block lengths selected on the basis of previous studies were synthesized with the aim of achieving a maximal solubilization ability and a suitable sustained release, while keeping very low material expense and excellent aqueous copolymer solubility. The self-assembling and gelling properties of these copolymers were characterized by means of light scattering, fluorescence spectroscopy, transmission electron microscopy, and rheometry. Both copolymers formed spherical micelles (12–14 nm) at very low concentrations. At larger concentration (>25 wt%), copolymer solutions showed a rich phase behavior, with the appearance of two types of rheologically active (more viscous) fluids and of physical gels depending on solution temperature and concentration. The copolymer behaved notably different despite their relatively similar block lengths. The ability of the polymeric micellar solutions to solubilize the antifungal drug griseofulvin was evaluated and compared to that reported for other structurally-related block copolymers. Drug solubilization values up to 55 mg g^{-1} were achieved, which are greater than those obtained by previously analyzed poly(ethylene oxide)–poly(styrene oxide), poly(ethylene oxide)–poly(butylene oxide), and poly(ethylene oxide)–poly(propylene oxide) block copolymers. The results indicate that the selected SO/EO ratio and copolymer block lengths were optimal for simultaneously achieving low critical micelle concentrations (cmc) values and large drug encapsulation ability. The amount of drug released from the polymeric micelles was larger at pH 7.4 than at acidic conditions, although still sustained over 1 day.

1. Introduction

Advances in materials science and nanotechnology offer novel approaches to address formulation issues and to regulate drug biodistribution and release patterns [1,2]. Block or graft-copolymers consisting of hydrophilic and lipophilic domains are able to form polymeric micelles and nanocompartmentalized particles, via self-assembly in an aqueous environment, that exhibit a long circulation half-life due to the stabilization provided by the hydrophilic shell. These core–shell-type nanostructures are particularly suitable to host poorly-soluble drugs and to target them to the required tissue or cells [3–9]. As a consequence, the local drug bioavailability and the safety of the treatment are improved [10–12].

Probably, the most widely studied amphiphilic triblock copolymers are those composed of hydrophilic PEO blocks and hydrophobic propylene oxide (PPO) blocks, which can be classified in two families: the linear poloxamers (Pluronics®), and the X-shaped poloxamines (Tetronic®) [13–16]. The reasons for their popularity can be summarized in: (i) commercial availability in a very broad range of compositions (i.e., a wide variety of molecular weights, block lengths, and PEO/PPO ratios); (ii) proven solubilization capacity and sustained drug release; (iii) high biocompatibility of most varieties; (iv) enhancement of drug transport across cellular barriers; and (v) approval of some varieties by US FDA and EMA to be used in pharmaceutical formulations and medical devices [13–16]. Nevertheless, PEO–PPO block copolymers still present a number of limitations that could curtail their application, such as (i) limited stability of the self-assembled nanostructures upon dilution in the bloodstream, particularly for derivatives with high EO/PO ratios, (ii) incomplete micellization of the unimers, and (iii)

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0021-9797/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jcis.2012.06.090

variability from batch to batch in micellar sizes, drug delivery capacities, and release profiles.

To overcome some of these limitations, more hydrophobic block copolymer counterparts with similar architecture, but with the PPO segment replaced by a more hydrophobic one, such as poly(butylene oxide) (PBO), poly(styrene oxide) (PSO) or phenylglycidyl ether (PG), have been developed by the Attwood and Booth's group in collaboration with us during last years [17–21]. Polystyrene oxide-based block copolymers are of particular interest due to (i) their ability to self-assemble at very low concentrations into micelles with improved solubilization ability and stability [22,23] and (ii) the low glass transition temperatures (ca. 40 °C) of the core-forming block, which enables the incorporation of drugs at temperatures that are compatible with thermolabile agents [22,24,25]. In general, triblock PSO-based block copolymers show larger solubilization capacity of hydrophobic drugs if compared to commercially available Pluronics® or Tetronic® copolymers thanks to their more hydrophobic cores, although such an enhancement depends on copolymer structure, block length ratios, micellar shape, and drug affinity for the block-forming micellar core [18,22,23]. Some of these factors are also key in providing suitable polymeric chain solubility and stability; in fact, when designing styrene-oxide copolymers for enhancing drug solubility by increasing/decreasing the length of the hydrophobic/hydrophilic block, the copolymer chain solubility, the micelle stability, and/or the drug solubilization capacity have been found to be compromised [18,24]. Shorter EO and longer SO block lengths typically have led to reduced polymeric chain solubility, whereas copolymers with longer EO blocks and/or very short SO block self-assemble at high concentrations and form micelles with lower drug entrapment abilities [20].

In the present work, we report on the synthesis, the characterization of the self-assembling properties, and the drug solubilization and release profiles of two new triblock PEO–PSO copolymers, $EO_{33}SO_{14}EO_{33}$ and $EO_{38}SO_{10}EO_{38}$ (the subscripts denoting the block lengths) using fluorescence spectroscopy, light scattering, transmission electron microscopy (TEM), and rheometry. The main goals of the present work were to target optimized block lengths and hydrophilic/hydrophobic block molar ratios of the copolymers on the basis of previous studies to simultaneously achieve a compromise between polymer chain solubility and micelle formation at very low copolymer concentrations and to study the effect of subtle differences on the copolymer block lengths to obtain a micellar core with a suitable size for hosting great amounts of a poorly-soluble drug such as the antifungal griseofulvin, used as a model for comparison purposes with other block copolymer structures. The micellar systems based on $EO_{33}SO_{14}EO_{33}$ and $EO_{38}SO_{10}EO_{38}$ block copolymers largely reach these goals, improving griseofulvin encapsulation and release. Hence, these results prove the potential benefits of this class of copolymers as components of drug delivery systems improving the performance of Pluronic and Tetronic block copolymers, while exhibiting the biocompatibility, cytocompatibility, and capacity of inhibiting efflux pumps of the latter [26].

2. Experimental

2.1. Materials

$EO_{33}SO_{14}EO_{33}$ and $EO_{38}SO_{10}EO_{38}$ copolymers were synthesized as previously described [27,28]. Briefly, high vacuum and ampule techniques were used to eliminate unwanted moisture. Initiation of the bifunctional precursor was potassium hydroxide and 1,2-butanediol partly in the form of its potassium salt. The mole ratio OH/OK was ~9, and this being chosen to achieve a suitable polymerization rate. The monomers were distilled and dried immediately before use. Styrene oxide was added to the ampule by syringe, and for the second stage of polymerization, ethylene oxide was distilled through the vacuum line. The polymerization of styrene oxide at 85 °C was slow, taking as long as 8 weeks. Weight-averaged (M_w) to number-averaged (M_n) molecular weight ratios were determined at 25 °C using a Waters gel permeation chromatography (GPC) system equipped with a 1515 isocratic pump and a 2410 refractive index detector (Waters, Milford, MA). Chloroform was used as the eluent, and monodisperse PEO was employed as standard. M_n values were estimated from ^{1}H NMR spectra recorded on a Bruker ARX400 spectrometer (Bruker, Milton, ON, Canada) in deuterated chloroform. Table 1 summarizes the molecular characteristics of both copolymers. Water was double distilled and degassed before use. Pyrene and griseofulvin were from Sigma–Aldrich.

2.2. Methods

2.2.1. Characterization of block copolymer micelles

2.2.1.1. Fluorescence measurements. Values of cmc were obtained from pyrene fluorescence measurements at 37 ± 0.1 °C (Cary Eclipse fluorescence spectrophotometer, Agilent., Germany) as described by Lee et al. [29]. Stock solutions were prepared by dissolving the copolymers in water for 24 h before being diluted to the desired concentrations within the range 1–50 g dm^{-3}. Pyrene dissolved in acetone was added to the copolymer solution and, after acetone evaporation, was allowed for equilibration during 24 h. The final copolymer solution contained 3×10^{-7} M pyrene. The fluorescence spectrum (λ_{exc} = 335 nm) was the average of three scans and was corrected for scattering using an equivalent blank solution before determining the ratio I_1/I_3 of the first and third vibronic peaks. Reproducibility was better than 2%.

2.2.1.2. Dynamic and static light scattering measurements. DLS and SLS intensities were measured at 37 °C by means of an ALV-5000F (ALV-GmbH, Germany) instrument with vertically polarized incident light (λ = 488 nm) supplied by a diode-pumped Nd:YAG solid-state laser (Coherent Inc., CA, USA) and operated at 2 W and combined with an ALV SP-86 digital correlator with a sampling time of 25 ns to 100 ms (for DLS). The intensity scale was calibrated against scattering from toluene. Measurements were made at a scattering angle $\theta = 90°$ to the incident beam, as appropriate for particles smaller than the light wavelength. Solutions were filtered through Millipore Millex filters (Triton free, 0.22 μm porosity) directly into cleaned scattering cells and let to equilibrate at 37 °C for 30 min before measurement. Experiment duration was in the range 5–10 min, and each experiment was repeated at least two times. The correlation functions from DLS runs were analyzed by the CONTIN method to obtain the intensity distributions of decay rates (Γ) [30]. From the decay rate distributions, the apparent diffusion coefficients ($D_{app} = \Gamma/q^2$, $q = (4\pi n_s/\lambda)\sin(\theta/2)$) were derived, being n_s the refractive index of solvent. Values of the apparent hydrodynamic radius ($r_{h,app}$, radius of the hydrodynamically equivalent hard sphere corresponding to D_{app}) were calculated from the Stokes–Einstein equation

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

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

Static light scattering data were analyzed in terms of scattering theory for hard spheres [31–33] whereby the interparticle structure factor (S) in the equation

$$K^*c/(I - I_s) = 1/SM_w \quad (2)$$

was approximated by