Dyes and Pigments 97 (2013) 347–352

Contents lists available at SciVerse ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Spectroscopical properties of a DTAF-labeled hydrophilic–hydrophobic copolymer in water and surfactant micelles

Alina Iovescu [a], Monika Gosecka [b], Teresa Basinska [b], Adriana Bãran [a], Gabriela Stîngã [a], Stanislaw Slomkowski [b], Dan Florin Anghel [a,*]

[a] Laboratory of Colloids, "Ilie Murgulescu" Institute of Physical Chemistry, Romanian Academy, Spl. Independentei 202, Sector 6, 060021 Bucharest, Romania
[b] Department of Engineering of Polymer Materials, Center of Molecular and Macromolecular Studies, Polish Academy of Sciences, ul. H. Sienkiewicza 112, 90-363 Lodz, Poland

ARTICLE INFO

Article history:
Received 4 October 2012
Received in revised form
21 December 2012
Accepted 2 January 2013
Available online 16 January 2013

Keywords:
Poly(styrene-co-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol)
5-(4,6-Dichlorotriazinyl)aminofluorescein
Label
Micelle
Absorption spectroscopy
Fluorescence

ABSTRACT

Emulsion copolymerization of hydrophilic α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol macromonomer with styrene yields core–shell microspheres and a water-soluble copolymer fraction. This copolymer has a higher content of hydrophilic polyglycidol attested by ^{1}H NMR. Further information is acquired by labeling the copolymer with 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF) and making absorption and fluorescence studies. The acquired data on aqueous solution of labeled copolymer without (L) and with micelles of dodecyltrimethylammonium chloride (LD), sodium dodecylsulfate (LS) and hexaethyleneglycol mono *n*-dodecyl ether (LE) reveal that the dianionic and anionic tautomers of the DTAF-label are apparent and concordant with the recorded spectrophotometric pK_a values. The emission intensities, quantum yields and lifetimes obey the sequence $LE < LS < L < LD$. They are along with the increasingly deprotonated forms of the fluorophore and unveil that the micelle-label interactions depend on the surfactant.

1. Introduction

Polymer microspheres tailored for medical diagnostics should contain hydrophilic surface to prevent the nonspecific adsorption of proteins [1–3]. Emulsion polymerization of styrene and α-tert-butoxy-ω-vinylbenzyl-polyglycidol macromonomer did yield nearly monodisperse microspheres with polyglycidol enriched surface [4,5], which succeeded in diagnostic tests [2,3]. The hydroxyl groups from polyglycidol repeating units allow for derivatization with fluorophores, which should improve the microspheres detection. In the particles synthesis a fraction of water-soluble poly(styrene-co-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol) copolymers, P(S-co-PGL), is however obtained. These copolymers are not incorporated into the microspheres surface layer, but resemble it and deserve investigation in aqueous solution. To envision the spectral behavior of labeled particles, we found convenient to consider the water-soluble copolymers after their fluorescent modification. Therefore, we recovered the water-soluble material, characterized it by ^{1}H NMR and DLS and further labeled with 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF).

The DTAF label is a derivative of fluorescein, a fluorophore largely used in biosciences [6,7]. It is well documented that the signals in the emission spectra of fluorescein and related compounds are dependent on pH, salt [8], temperature [9], organic solvent [10], surfactants [11–14], cyclodextrins [15]. Alkylated fluoresceins were found to attach to CTAB, SDS and TX-100 micelles, while fluorescein did bind only to the former, and the binding was stronger for the derivatives with longer alkyl chains [12]. Fluorescently labeled polymers bearing fluorescein or its derivatives also reported on the label environment [16,17] and the interactions herein [18].

This paper unveils the photophysical properties of DTAF-labeled P(S-co-PGL) in water and surfactant micelles, the latter being the simplest biomimetic aggregates. Three surfactants with the same hydrophobic part, but different head groups are used to form a selective microenvironment for the fluorescent tag. The investigation reveals important differences in the spectral behavior of DTAF-labeled copolymer which depend on surfactant nature. The

Abbreviations: P(S-co-PGL), poly(styrene-co-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol); DTAF, 5-(4,6-dichlorotriazinyl)aminofluorescein; L, aqueous DTAF-labeled poly(styrene-co-α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol); LD, L with dodecyltrimethylammonium chloride micelles; LS, L with sodium dodecylsulfate micelles; LE, L with hexaethyleneglycol mono *n*-dodecyl ether micelles.

* Corresponding author. Tel.: +4021 316 79 12x32; fax: +4021 312 11 47.
E-mail addresses: danflorin.anghel@gmail.com, adan@icf.ro (D.F. Anghel).

0143-7208/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.dyepig.2013.01.003

results align to our previous interest in microspheres for medical diagnosis, being useful to prefigure the behavior of fluorescent particles at biological interfaces. We have to mention that the copolymer is original and according to our knowledge there is no report in the literature either on it or on its fluorescent counterpart in water or surfactant micelles.

2. Experimental part

2.1. Materials

The water-soluble P(S-co-PGL) copolymer was isolated from suspension of microspheres produced by emulsion copolymerization of styrene and α-*tert*-butoxy-ω-vinylbenzyl-polyglycidol (PGL) macromonomer ($M_n = 2780$ and $M_w/M_n = 1.09$). The synthesis was carried out according to the procedure described elsewhere [4]. Briefly, the recipe consisted of styrene (10 g), PGL (0.3 g), $K_2S_2O_8$ (0.2 g) and water (125 mL). The macromonomer was dissolved in water and then styrene was added. This mixture was placed into reactor, degassed by purging of argon and stirred at room temperature at 400 rpm. After 45 min of stirring, temperature was raised up to 70 °C and $K_2S_2O_8$ was added. The polymerization carried out for 27 h under stirring and continuous flow of argon produced suspension of microspheres. Centrifugation of the above mentioned suspension resulted in particle deposition. Yield of particles was 94%. The collected supernatant containing the water soluble copolymer fraction (0.38 g, as gravimetrically determined) was concentrated using vacuum evaporator and placed into the dialysis bag (MWCO 12,000–14,000, pore diameter ca. 25 Å, VISKING Dialysis Tubing). The subsequently dialysis against three times distilled water was carried out for 3 days (fresh portions of water were exchanged every 24 h). Then, the sample was completely dried yielding the water-soluble copolymer.

The P(S-co-PGL) copolymer was labeled with 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF) from Invitrogen, according to the following procedure: 0.15 g of copolymer was dissolved in 2.5 mL of buffer solution of sodium carbonate (pH = 9.6) in 25 mL glass flask equipped with a stirring bar. Then, 2 mL of 3 times distilled water was added together with 0.5 mL of dimethyl sulfoxide (DMSO). After dissolution of copolymer, 2 mL of DMSO solution containing 3.33×10^{-3} g/mL of DTAF was added. The solution was incubated for 24 h at room temperature with continuous stirring in a dark place. Finally, the solution was dialyzed in a similar way as the parent copolymer. Then, the labeled polymer was dried using a vacuum system and characterized by UV–Vis and fluorescence spectroscopy. The amount of label determined by UV–Vis was of 0.87 mol %.

Reagent grade chemicals from Fluka (sodium dodecylsulfate, SDS and fluorescein), Aldrich (dodecyltrimethylammonium chloride, DTAC) and Nikko Chemicals (hexaethyleneglycol mono *n*-dodecyl ether, $C_{12}E_6$) were used without purification. Their molarities in the investigated aqueous mixtures were above the respective critical micellar concentration (cmc), being of 1×10^{-2} M for SDS, 4×10^{-2} M for DTAC and 1×10^{-4} M for $C_{12}E_6$. The natrium hydroxide (NaOH) and hydrochloric acid (HCl) used for pH titrations were reagent grade and purchased from Merck. Millipore water was used for preparation of aqueous solutions.

2.2. Methods

The ^{1}H NMR spectrum of P(S-co-PGL) copolymer was recorded on a Bruker AC-200 (200 MHz) spectrometer. The copolymer was dissolved in D_2O with TMS as internal standard.

The gel permeation chromatography (GPC) analysis of the P(S-co-PGL) copolymer was performed in DMF at a flow rate of 0.8 mL/min, in thermostated conditions at 70 °C. The calibration was based on polystyrene standards. The chromatograph was equipped with a Knauer HPLC pump, model K-501, two Polymer Standards Service (PSS) columns: PSSGram 100 Å and PSSGram 3000 Å, and an interferometric refractometer detector Wyatt/Optilab 903 (Wyatt Technology Corporation).

Hydrodynamic diameter of aggregates (micelles) formed by copolymer in water was determined by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments). The same method was used to estimate the hydrodynamic diameter of the SDS, DTAC and $C_{12}E_6$ micelles, at their respective concentrations presented above.

The UV–Vis absorption measurements were carried out using a Carry 100 Bio spectrophotometer. The pK_a values of the labeled copolymer, with or without surfactant micelles, were spectrophotometrically determined as the inflection point of the absorption maximum (λ_{max}) variation with pH. The pH titrations were done under continuous stirring using HCl (0.1 M) and NaOH (0.01 and 0.1 M) aqueous solutions.

The pH was monitored at 25 °C with an ORION pH-meter, model 420 A, calibrated with standard buffers. The pH readings were made when the electrode signal remained stable.

Fluorescence measurements were performed on an Edinburgh Instruments F920 spectrofluorimeter. The spectra were corrected. The fluorescence quantum yields were calculated according to the procedure described elsewhere [19]. Fluorescein in 0.01 M NaOH solution was used as a standard, taking its quantum yield equal to 0.92 [20]. The fluorescence lifetimes measurements were carried out using the time-correlated single photon counting technique. The excitation source was a picosecond pulsed diode laser at 470.0 nm. Fluorescence emission was collected at 515 nm. The instrumental diffusion was measured using a Ludox solution. The time-dependent fluorescence intensity was fitted to a model with one or two exponentials using the Marquardt–Levenberg algorithm. The goodness-of-fit criterion (χ^2) and the residual curve were checked each time in order to obtain reliable lifetime values. There were accepted a minimal value approaching 1 for χ^2 and residual data looking like random noise distributed around zero.

The UV–Vis and fluorescence spectra were recorded at 25 °C using cells of 1 cm path length.

Unless otherwise specified, the labeled copolymer concentration was 4.7×10^{-4} M (0.04 mg/mL) in all the measurements. It provides a fluorophore concentration of 4×10^{-6} M.

3. Results and discussion

3.1. Characterization of parent P(S-co-PGL) copolymer

The investigated copolymer P(S-co-PGL) with chemical structure shown in Scheme 1 was characterized by ^{1}H NMR. Table 1 shows the chemical shifts of proton signals in the ^{1}H NMR spectrum of P(S-co-PGL). From the integration of signals corresponding to *tert*-butyl groups of polyglycidol chain-end (separated signal of 9 protons), and knowing that there are 5 protons in styrene phenyl ring and 4 protons of phenyl ring in each of polyglycidol constitutional units, the fraction of overall phenyl rings corresponding to polystyrene contribution is calculated by equations (1) and (2):

$$5f_{Ph} + 4f_M = 1 \quad (1)$$

and thus

$$f_{Ph} = (1 - 4f_M)/5 \quad (2)$$

where f_M and f_{Ph} denote fraction of protons in phenyl ring from macromonomer and fraction of protons from phenyl rings of