Summary: A water-insoluble organic 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is solubilized in aqueous solutions of aggregates of amphiphilic block copolymers with hydrophobic dendritic pendants. The number (100–200) of DPPH molecules solubilized in an aggregate, which is evaluated from UV-visible absorption spectra, is in agreement with that of the DPPH radicals determined from electron paramagnetic resonance (EPR) spectroscopy. The DPPH radicals are stably solubilized without decomposition in the polymer aggregates. The radicals exhibit a single-line EPR absorption, which is narrowed by the interspin interaction, and indicates the assembly formation of DPPH radicals in polymer aggregates. These results suggest the effective utilization of the DPPH radical as a spin-probe indicator in aqueous solutions.

When DPPH is solubilized in aqueous solutions of NaAMPS-*b*-G2(n3), the polymer solutions become purple colored, which is characteristic of the DPPH radical.

Single-Line EPR Spectra from Radicals Encapsulated in Aggregates of Amphiphilic Block Copolymers with Hydrophobic Dendritic Pendants in Water

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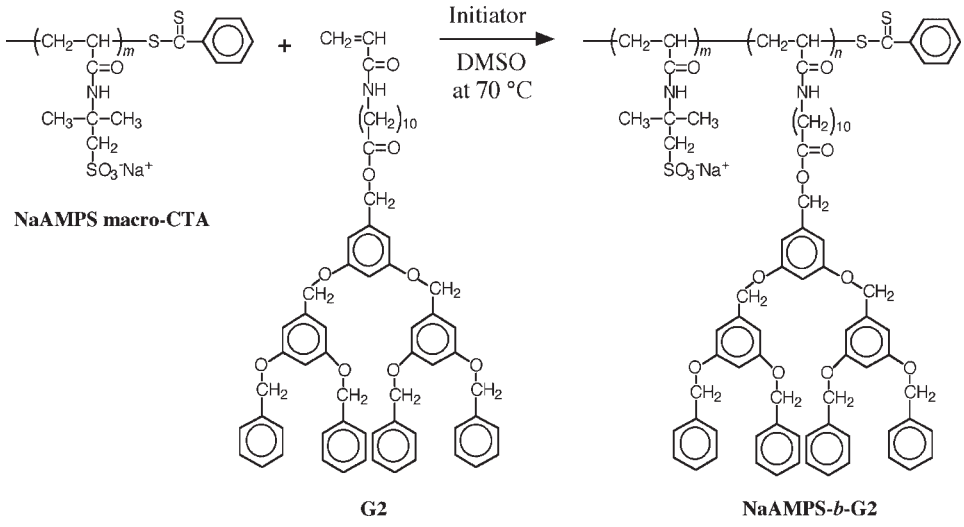
Introduction

The selective uptake of hydrophobic guest molecules into surfactant and polymer micelles in water deserves attention in connection with drug delivery, oil recovery, oleomaterial trapping, pigment protection, etc.[1–5] Uptake occurs even into dendrimers, dendron-building polymers, their aggregates, and dendritic gels.[6–11] It occurs in the hydrophobic interior in the amphiphilic architecture, but the amphiphiles must be designed with the chemical structure and size of the guests in mind.

Poly(sodium(2-(acrylamido)-2-methylpropanesulfonate)-*block*-poly(3,5-bis(3′,5′-bis-(benzyloxy)benzyloxy)benzyl-1, 1′-acrylamidoundecanoate)((NaAMPS-*b*-G2) (Scheme 1) is an amphiphilic copolymer with a hydrophilic linear polyelectrolyte block and a hydrophobic dendritic pendant block, and the polymer molecules self-aggregate into spherical particles with a diameter of ~70 nm in water, which is independent of the polymer concentration.[11] Guest dyes with the selected structure are encapsulated into the large cavity or available void within and between the hydrophobic dendron moieties in the aggregate.

Such superior structure-selective uptake in an aggregate of a copolymer with dendron moieties may also occur for different series of guest molecules, such as radicals. The radicals encapsulated into the limited spaces are expected



Scheme 1. Preparation scheme of a NaAMPS-b-G2 diblock copolymer.

to behave differently to free radicals in solution. In the present work, the uptake of water-insoluble 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals into the aggregates of copolymers in water is investigated by UV-visible absorption and electron paramagnetic resonance (EPR) spectroscopy. To the best of the author's knowledge, the uptake of water-insoluble organic radicals (including DPPH) into polymer aggregates in water has never been reported. Incidentally, the uptake of water-insoluble 1,3,5-triphenyl verdazyl and galvinoxyl radicals occurs to a much lesser extent, which indicates the selective solubilization of DPPH in the polymer aggregates.

Experimental Part

Synthesis of 3,5-Bis[3′,5′-bis(benzyloxy)benzyloxy]benzyl 11-Acrylamidoundecanoate (G2)

Sodium 11-acrylamidoundecanoate was prepared according to the method of Yeoh et al.[12] Sodium 11-acrylamidoundecanoate (3.10 g, 11.2 mmol), 3,5-bis[3′,5′-bis(benzyloxy)benzyloxy]-benzyl bromide (3.06 g, 3.79 mmol), tetra-*n*-butylammonium bromide (234 mg, 0.744 mmol), and 2,6-di-*tert*-butylcresol (18.5 mg, 0.0840 mmol) were mixed with water (40 cm^3) and chloroform (20 cm^3). After the mixture was heated at 110–115 °C for 24 h, it was diluted with chloroform (300 cm^3) and washed twice with water. The organic phase was dried over anhydrous sodium sulfate, and the solvent was removed by evaporation. The crude product was purified with chloroform as an eluent by silica gel column chromatography.

The first fraction was collected and evaporated to obtain a white solid (G2): yield 2.69 g (72.4%), m.p. 50–51 °C.

^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.12$–1.36 (m, 12H, CH_2), 1.41–1.58 (m, 2H, CH_2), 1.59–1.72 (m, 2H, CH_2), 2.32–2.41 (t, 2H, CH_2), 3.24–3.35 (q, 2H, CH_2), 4.97 (s, 4H, CH_2), 5.04 (s, 8H, CH_2), 5.05 (s, 2H, CH_2), 5.52–5.60 (br s, 1H, CON*H*), 5.60–5.66 (m, 1H, vinyl C*H*), 6.01–6.11 (m, 1H, vinyl C*H*), 6.22–6.30 (m, 1H, vinyl C*H*), 6.55 (s, 1H, Ar C*H*), 6.59 (s, 4H, Ar C*H*), 6.69 (s, 4H, Ar C*H*), 7.29–7.48 (m, 20H, Ar C*H*).

^{13}C NMR (DEPT) (126 MHz, $CDCl_3$): $\delta = 25.04$ (*C*H_2), 27.03 (*C*H_2), 29.22 (*C*H_2), 29.33 (*C*H_2), 29.37 (*C*H_2), 29.47 (*C*H_2), 29.55 (*C*H_2), 29.65 (*C*H_2), 34.39 (*C*H_2), 39.71 (*C*H_2), 65.97 (*C*H_2), 70.07 (*C*H_2), 70.19 (*C*H_2), 101.67 (Ar *C*H), 101.75 (Ar *C*H), 106.50 (Ar *C*H), 107.14 (Ar *C*H), 126.10 (vinyl *C*H_2), 127.66 (Ar *C*H), 128.13 (Ar *C*H), 128.70 (Ar *C*H), 131.17 (vinyl *C*H), 136.86 (Ar *C*), 138.58 (Ar *C*), 139.29 (Ar *C*), 160.09 (Ar *C*), 160.28 (Ar *C*), 165.61 (*C*ONH), 173.71 (*C*OO).

Block Copolymerization of G2

4-Cyanopentanoic acid dithiobenzoate was synthesized according to the method reported by Mitsukami et al.[13] The sodium 2-(acrylamido)-2-methylpropanesulfonate macro-ion chain transfer agent (NaAMPS macro-CTA) was prepared by reversible addition–fragmentation chain transfer (RAFT) controlled radical polymerization using 4-cyanopentanoic acid dithiobenzoate as a chain transfer agent.[13,14] NaAMPS macro-CTA (1.57 g, 0.0709 mmol, weight-average molecular weight as determined by static light scattering (SLS) $= 2.21 \times 10^4$, degree of polymerization = 71, molecular weight distribution as determined by gel permeation chromatography (GPC) = 1.28), G2 (2.00 g, 2.04 mmol), and 4,4′-azobis-4-cyanovaleric acid (3.96 mg, 0.0141 mmol, initiator) were dissolved in dimethyl sulfoxide (DMSO) (25 cm^3, dried over 4 Å molecular sieves and distilled under reduced pressure). The solution was degassed by Ar bubbling for 30 min and reacted at 70 °C for 24 h (Scheme 1). The clear reaction solution was poured into large excess of chloroform, and the resulting precipitate (polymer) was collected by filtration. The crude polymer (NaAMPS-*b*-G2) was purified by dissolving in methanol and reprecipitating in a large excess of chloroform, yield 0.88 g.

Characterization of NaAMPS-b-G2

^{1}H NMR spectra were obtained with a Bruker DRX-500 spectrometer operating at 500 MHz. The resonance bands of the G2 sequence were clearly observed at 4.8–5.2 and 6.5–6.8 ppm in DMSO-d_6 at 100 °C but were not discernible in D_2O at 27 °C, which suggests the association of the G2 blocks in water. The degree of polymerization of the G2 segment was calculated to be 3 from the integral area intensity ratio of the ^{1}H NMR resonance band (in DMSO-d_6 at 100 °C) for the pendent methylene protons in the NaAMPS segment and that for the benzyl protons in the G2 segment.

Fluorescence spectra were recorded on a Hitachi F-2500 spectrophotometer (excitation wavelength of 350 nm). The critical aggregation concentration (0.2 mg · cm^{-3}) was determined to be the polymer concentration at which the emission band (510 nm) of 8-anilino-1-naphthalenesulfonic acid ammonium salt hydrate (ANS) (2.0×10^{-5} M) in aqueous 0.1 M NaCl solutions of NaAMPS-*b*-G2 increased in intensity and underwent a blue-shift in wavelength.

GPC analysis was performed with a JASCO GPC-900, equipped with a refractive index detector and a Shodex 7.0 μm bead-size GF-7M HQ column. Measurements were performed at 40 °C, with a 0.6 cm^3 · min^{-1} flow rate using methanol eluent that contained 0.2 M $LiClO_4$. The system was calibrated with a poly(ethylene oxide) standard. The molecular weight distribution determined for the diblock copolymer was 1.25.

The SLS measurements (Otsuka Electronics DLS-7000) were performed at 25 °C with an Ar^+ laser (488 nm), and a refractive index increment of 0.160 cm^3 · g^{-1}. Samples were measured in aqueous 0.1 M NaCl solutions over a polymer concentration (C_p) range (2.0–10 mg · cm^{-3}). The weight-average molecular weight value (2.60×10^6) was evaluated by the extrapolation of the scattering angle θ and C_p to zero in a Zimm plot (Figure 1). The aggregation number for NaAMPS-*b*-G2 at $m = 71$ and $n = 3$ was 104.

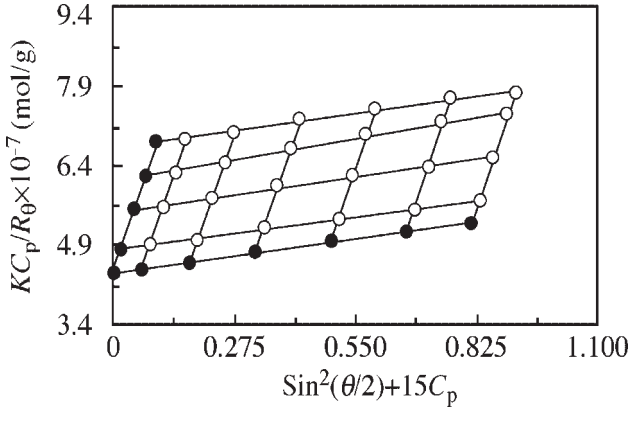


Figure 1. Zimm plot at 25 °C for aqueous 0.1 M NaCl solutions of NaAMPS-*b*-G2. Scattering angles were varied from 30° to 130° with a 20° interval. The polymer concentrations were varied from 2.0 to 10 mg · cm^{-3}. K = instrumental constant. R_θ = reduced scattering intensity.

NaAMPS-*b*-G2(n3)

$m = 71$, $n = 3$, critical aggregation concentration = 0.2 mg · cm^{-3}, aggregation mass = 2.60×10^6, aggregation number = 104.

NaAMPS-*b*-G2(n4.5)

$m = 71$, $n = 4.5$, critical aggregation concentration = 0.07 mg · cm^{-3}, aggregation mass = 3.29×10^6, aggregation number = 156.

Solubilization of DPPH

Commercial-grade DPPH and other chemicals were used without further purification. Ultra-pure water (a Millipore Milli-Q Lab purification system) was used throughout the experiments. UV-visible absorption spectra were recorded on a Shimazdu UV-2200 instrument by using a 10 mm path quartz cell. X-band EPR spectra were recorded on a JES RE2X spectrometer in air. Aqueous solutions of NaAMPS-*b*-G2 were shaken for 24 h with solids of DPPH. Insoluble solids were filtered out (Millipore filter, 0.65 μm pore size), and the filtrates were used for the measurements. For the determination of solubility, the same procedure was carried out for water without NaAMPS-*b*-G2.

Results and Discussion

When DPPH is solubilized in aqueous solutions of NaAMPS-*b*-G2(n3), the polymer solutions become purple colored, which is characteristic of the DPPH radical. The color changes from pale to dark depending on the polymer concentration, as seen in Figure 2. It can be visually confirmed from the hyperchromic tendency that the uptake of the DPPH radicals increases with increasing polymer concentration.

The solubilized DPPH has been quantified by UV-vis spectroscopy according to the procedure described elsewhere.[11] After an aqueous filtrate of NaAMPS-*b*-G2(n3) solubilized DPPH is diluted with ethanol (60%, v/v), the absorption band (523 nm) of DPPH is measured. The absorbance of an aqueous saturated solution of DPPH and an aqueous solution of NaAMPS-*b*-G2(n3) after dilution with ethanol (60%, v/v) is then subtracted from the

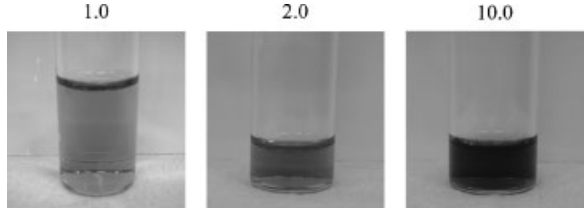


Figure 2. Aqueous solutions of DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates. Numerical values indicate polymer concentrations (in mg · cm^{-3}).

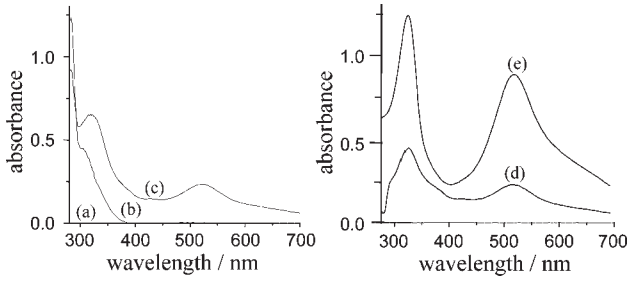


Figure 3. UV-visible absorption spectra of ethanol-diluted solutions (60% ethanol, v/v). (a) an aqueous saturated solution of DPPH, (b) an aqueous solution of NaAMPS-*b*-G2(n3) (2.0 mg · cm^{-3}), (c) an aqueous solution of DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates (2.0 mg · cm^{-3}), (d) (c) – (b) – (a), (e) an ethanol solution of DPPH (1.16×10^{-4} M).

absorbance of an aqueous solution of NaAMPS-*b*-G2(n3) solubilized DPPH. (The absorbance of an aqueous saturated solution of DPPH is negligibly low, which indicates the low solubility of DPPH in water.) Figure 3 displays typical absorption spectra and a differential spectrum. The molar concentration of DPPH in an aggregate of NaAMPS-*b*-G2(n3) is calculated from the subtracted absorbance by using the molar extinction coefficient ($\varepsilon = 7.63 \times 10^3$ M^{-1} · cm^{-1}) of free DPPH determined in an aqueous ethanol solution (60% ethanol, v/v).

The number of DPPH molecules in the aggregates, evaluated by using the aggregation number (104), are 113, 98, and 140, respectively, in aqueous solutions of NaAMPS-*b*-G2(n3) at 1.0, 2.0, and 10.0 mg · cm^{-3}. The uptake of DPPH is comparable to the aggregation number of the polymer, which indicates that the solubilization of DPPH molecules is equivalent to a factor of the polymer number in an aggregate. It should be noted that this value is larger than that (36) of Oil Yellow, which is the most strongly solubilized dye in NaAMPS-*b*-G2(n3) aggregates among the homologous dyes previously examined.[11]

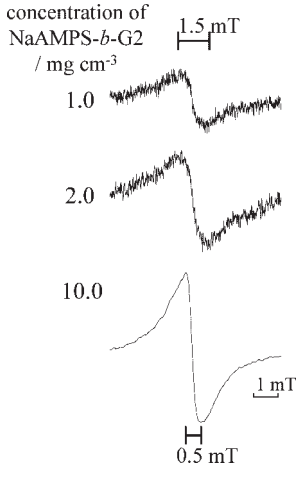


Figure 4. EPR spectra of aqueous solutions of DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates at different polymer concentrations.

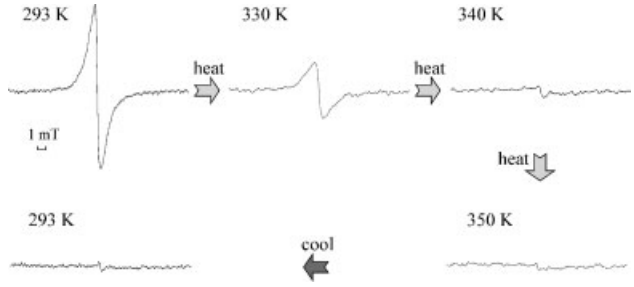


Figure 5. Temperature dependence EPR spectra of an aqueous solution of DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates (polymer concentration = 10.0 mg · cm^{-3}).

The same procedure has been applied on an aqueous solution of NaAMPS-*b*-G2(n4.5) at 2.0 mg · cm^{-3}. The uptake of DPPH is 206 molecules per polymer aggregate. This value is larger than the case of the NaAMPS-*b*-G2(n3) aggregate. The result is consistent with the fact that NaAMPS-*b*-G2(n4.5) molecules with a long dendron block are associated into larger aggregates than the NaAMPS-*b*-G2(n3) molecules. This indicates that the solubilization of DPPH in the NaAMPS-*b*-G2 aggregates is greatly related to the volume of the dendron block or, in other words, the void volume in the core of the aggregate, which is created by the dendron blocks.

The presence of DPPH radicals is confirmed by EPR spectra, which are shown in Figure 4. The spectrum of DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates in an aqueous solution at a polymer concentration of 1.0 mg · cm^{-3} consists of a single-line absorption at $g = 2.003$ with a peak-to-peak linewidth of 1.5 mT, which suggests the assembly of radicals. This EPR spectrum of DPPH is completely different from the fact that the free DPPH radicals in ethanol display hyperfine structures. With increasing polymer concentration, that is, with an increase in the solubility of DPPH, the EPR signal of DPPH increases in intensity and sharpens in shape, while the linewidth decreases to 0.5 mT. This indicates the growth of a DPPH assembly. The number of DPPH molecules in a NaAMPS-*b*-G2(n3) aggregate is evaluated from the intensity of the ESR radical to be 104 and 130, respectively, for polymer concentrations of 1.0 and 10.0 mg · cm^{-3}. These values are very close to the numbers of DPPH radicals solubilized in the aggregates. This indicates that most DPPH radicals are solubilized without decomposition in the polymer aggregates. Moreover, the single-line band with narrow linewidth that arises from the DPPH assembly can be utilized as a spin-probe indicator.

Figure 5 shows temperature dependence EPR spectra of the DPPH solubilized in NaAMPS-*b*-G2(n3) aggregates in an aqueous solution at a polymer concentration of 10.0 mg · cm^{-3}. The EPR signal weakens upon heating and disappears at 350 K. However, the signal does not recover upon cooling to 293 K. This indicates the collapse of the polymer aggregates on heating, followed by the release and decomposition of the DPPH radicals.

Conclusion

The hydrophobic organic radical, DPPH, is solubilized in aqueous solutions by encapsulation into aggregates of the amphiphilic block copolymer, NaAMPS-*b*-G2, which consist of a hydrophilic linear polyelectrolyte block and a hydrophobic block that carries a pendant dendritic moiety. The solubility is evaluated from absorption spectra of DPPH in the polymer solutions. The band intensity increases with increasing aggregate concentration and 100–200 molecules of DPPH are determined to be solubilized per aggregate, depending on the size of the dendron block. The majority of the solubilized DPPH molecules retained the character of a radical. The DPPH exhibits a single-line EPR absorption narrowed by interspin interactions, which indicates the formation of an assembly of DPPH radicals in the polymer aggregates. The EPR results indicate that the DPPH radicals attain stable solubility in water upon encapsulation by the dendron block-containing core of the polymer aggregates without remarkable decomposition (at room temperature). This report presents the possibility of the biomedical utilization of DPPH radical as a spin-probe indicator in an aqueous solution.

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