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Physicochemical characteristics of poly(2-ethyl-2-oxazoline)/poly(ε-caprolactone) block copolymer micelles in water

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Summary

Polymeric micelles have found numerous applications in the area of biomedical engineering, including the delivery of bioactive molecules. Block copolymers, typical polymeric amphiphiles, form micelles in aqueous phases containing a hydrophobic inner core and hydrophilic outer shell. The unique characteristics of polymeric micelles are mainly controlled by the chemical structure and length of the hydrophilic and hydrophobic blocks. In this study we hypothesized that the solubility parameter values of the organic solvents typically used to dissolve the polymeric amphiphiles and eventually removed from an aqueous phase could significantly influence the characteristics of the resultant micelles in an aqueous phase. Poly(2-ethyl-2-oxazoline)-*b*-poly(ε-caprolactone) (PEtOz-*b*-PCL) was synthesized and dissolved in various organic solvents to prepare micelles in water. Their various physicochemical characteristics were investigated by the dynamic light scattering method and fluorescence spectroscopy.

Keywords

Polymeric micelles, solubility parameter, microviscosity, drug delivery

Introduction

Polymeric micelles have attracted considerable attention to date in the areas of separation technology and drug delivery applications due to their unique physicochemical characteristics and potential for biomedical applications [1-5]. Block copolymers, typical amphiphilic polymers, form micelles in an aqueous phase containing a hydrophobic core that can serve as a micro-reservoir. A hydrophilic outer shell can control the solubility of the polymeric micelles and the interactions of the

micelles with external environments [6, 7]. The high potential of polymeric micelles as a drug delivery carrier lies in the size of the micelles (i.e., the nanometer size scale) and their core-shell structure, which can mimic naturally occurring transport systems in the body [8]. Polymeric micelles especially have found useful applications in the delivery of anti-cancer drug molecules that are often insoluble in aqueous phases, and can be incorporated into the hydrophobic cores of polymeric micelles [9, 10].

A variety of block copolymers with various chemical and physical characteristics have been designed and synthesized for drug delivery applications. The most frequently studied polymeric micelles are derived from poly(ethylene oxide) (PEO), as PEO is one of the safe polymers in clinical uses with a hydrophilic nature, and can be derived with hydrophobic blocks such as polyesters, polystyrene, poly(amino acids), poly(propylene oxide), and polyalkanes [11-17]. Poly(2-ethyl-2-oxazoline) (PEtOz) also has potential for drug delivery applications, as it has the capability to form hydrogen bonds with poly(carboxylic acids) such as poly(acrylic acid) and poly(methacrylic acid) [18], which may allow further structural modification of the hydrophilic outer shell of PEtOz-derived block copolymer micelles [19]. We have previously reported the synthesis of amphiphilic block copolymers based on hydrophilic poly(2-ethyl-2-oxazoline) (PEtOz) and poly(ε-caprolactone) (PCL), a biodegradable hydrophobic block. In brief, the living cationic ring-opening polymerization of 2-ethyl-2-oxazoline provided a facile synthetic route for the preparation of hydroxyl-terminated PEtOz, which then initiated the ring-opening polymerization of ε-caprolactone in the presence of a Lewis acid catalyst. The micellar characteristics of these PEtOz/PCL block copolymers in an aqueous phase were found to be predominantly regulated by the ratio of hydrophilic to hydrophobic blocks, as well as the length of the hydrophobic blocks [19, 20].

Typically, when polymeric micelles are prepared, amphiphilic polymers are dissolved in an organic solvent and mixed with water, and this process is then followed by either rotary evaporation or dialysis against water to remove the organic solvent remaining in the water. It was hypothesized that the organic solvents used for the preparation of block copolymer micelles can influence the resultant micellar characteristics in water (e.g., size, rigidity of inner core). In this context, various organic solvents that possess different values of the solubility parameter were used to prepare PEtOz-*b*-PCL micelles in water, and the physicochemical characteristics of the micelles in water were investigated by determination of their size and steady-state fluorescence anisotropy using the dynamic light scattering method and fluorescence spectroscopy, respectively.

Experimental

Materials

2-Ethyl-2-oxazoline and ε-caprolactone were purchased from Aldrich, and were dried and vacuum distilled over calcium hydride. Methyl *p*-toluenesulfonate was obtained from Aldrich and vacuum distilled. Stannous octoate was obtained from Sigma and used without further purification. Pyrene and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Aldrich and used as received. A diblock copolymer of 2-ethyl-2-oxazoline/ε-caprolactone (PEtOz-*b*-PCL) was synthesized as previously reported [19]. In brief, a solution of 2-ethyl-2-oxazoline (60.0 g, 605 mmol) and methyl *p*-toluenesulfonate (2.25 g, 12 mmol) in acetonitrile (200 mL) was stirred at reflux for

30 h under a nitrogen atmosphere. After cooling to room temperature, 0.1 N methanolic potassium hydroxide was added to introduce a hydroxyl group at the end of the PEtOz chain. The polymer was then filtered through the silica gel, and followed by precipitation into diethyl ether and vacuum drying at 40 ^{o}C to obtain a powder form of PEtOz-OH. At room temperature, ε-caprolactone (0.58 g, 6 mmol) was added to a solution of PEtOz-OH (2.0 g) in dry chlorobenzene (15 mL) under a nitrogen atmosphere. The temperature was raised at reflux, and stannous octoate (4 mg) was added under the nitrogen atmosphere. The reaction stopped after 30 h, and the resultant block copolymer was isolated by precipitation into diethyl ether and dried in a vacuum at 40 ^{o}C.

Sample Preparation

To prepare a micellar solution, double-distilled water (20 mL) was added in a dropwise manner to a mildly stirred block copolymer solution dissolved in an organic solvent (1 mL) such as methanol, ethanol, tetrahydrofuran (THF), acetone, or acetonitrile. The organic solvent was then removed using a rotary evaporator at 30 °C for 2 h. The micellar solution in double-distilled water was diluted to obtain a constant concentration of 4 mg/mL. It was considered that the amount of residual organic solvents in the resultant micelles after rotary evaporation was negligible, as no remarkable peaks of the solvents were observed in NMR spectra (data not shown). A pyrene solution was added to the block copolymer solution to determine a critical micelle concentration (cmc). The final pyrene concentration of the samples was 6.0×10^{-7} M. All of the samples were sonicated for 10 min, and were allowed to stand for one day before fluorescence measurements. For the measurements of steady-state fluorescence anisotropy, samples were prepared by adding 4 μL of a DPH solution (2.1×10^{-3} M in THF) to the 5 mL of the block copolymer solution in water. The final DPH concentration of the samples was 1.7×10^{-6} M, and the samples were degassed by gentle bubbling of nitrogen for 30 min before measurements.

Measurements

^{1}H NMR spectra of the PEtOz/PCL block copolymer were obtained on a Bruker AC 250 spectrometer. Molecular weights and molecular weight distribution were determined using a GPC equipped with a Waters Associates 410 RI detector, 510 HPLC pump, and μ-Styragel columns with pore sizes of 10^{2}, 500, 10^{3}, and 10^{4} Å. The mobile phase was THF, and the molecular weight was calibrated with polystyrene standards. Dynamic light scattering experiments were performed with an argon ion laser system (Lexel Laser Model 95) tuned at a wavelength of 488 nm. The sample solution was filtered through a 0.45-μm filter (Millipore) directly into the pre-cleaned cylindrical cell (10 mm in diameter). The intensity autocorrelation was measured at a scattering angle (θ) of 90° with a Brookhaven BI-9000AT digital autocorrelator at 25 ± 0.1 ^{o}C. When the difference between the measured and the calculated baselines was less than 0.1 %, the correlation function was accepted. A nonlinear regularized inverse Laplace transformation technique (CONTIN) was used to obtain the distribution of decay constants [21, 22]. The mean diameter (d) of micelles was evaluated by the Stokes-Einstein equation. Fluorescence anisotropy of a sample solution containing DPH was measured using the L-format geometry of detection. Fluorescence anisotropy (r) was calculated from the following relationship:

$$r = \frac{(I_{VV} - I_{VV}{}^{s}) - G(I_{VH} - I_{VH}{}^{s})}{(I_{VV} - I_{VV}{}^{s}) + 2G(I_{VH} - I_{VH}{}^{s})} \quad (1)$$

where I^s is the contribution of scattered light from a sample solution in the absence of DPH, G (= I_{VH}/I_{HH}) is an instrumental correction factor, and I_{VV}, I_{VH}, I_{HV}, and I_{HH} refer to the resultant emission intensity polarized in the vertical or the horizontal detection planes (second subindex) when excited with vertically or horizontally polarized light (first subindex) [23]. The excitation wavelength was 360 nm, and the emission was measured at 430 nm.

Results and discussion

A hydrophilic block of poly(2-ethyl-2-oxazoline) (PEtOz) was first polymerized by ring-opening polymerization, and the methyl tosylated-PEtOz with the oxazolinium living end group was terminated by methanolic potassium hydroxide, resulting in the introduction of the hydroxyl group at the chain end. The hydroxyl group of PEtOz-OH (M_n = 4000) was then used for the ring-opening polymerization of ε-caprolactone in the presence of stannous octoate to produce PEtOz-*b*-PCL. The molecular weight and composition of the block copolymer were determined by using GPC and ^{1}H NMR, respectively. The number-average molecular weight of PEtOz-*b*-PCL was 5200 (M_w/M_n = 1.13). The ^{1}H NMR spectrum of PEtOz-*b*-PCL shows the characteristic resonance peaks of PEtOz-*b*-PCL (Figure 1) [19]. The molar ratio of ε-caprolactone to 2-ethyl-2-oxazoline in the PCL and PEtOz blocks was determined to be 0.28, calculated from the peak integration ratio of methyl protons in each block.

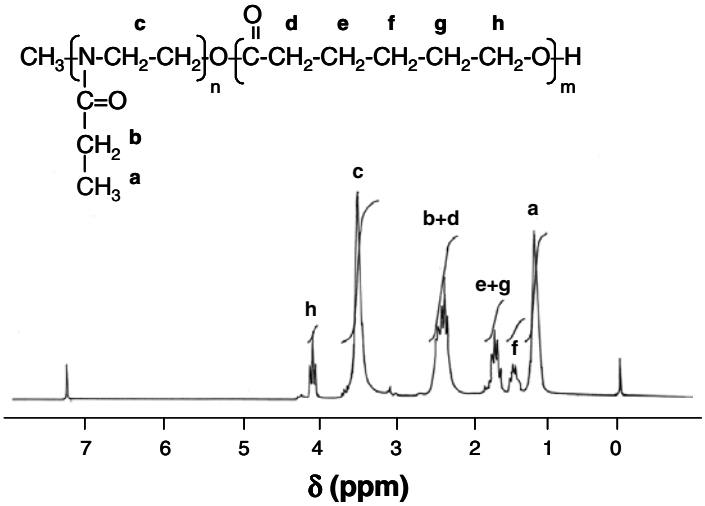

Figure 1. ^{1}H NMR spectrum of PEtOz-*b*-PCL in $CDCl_3$.

The amphiphilic nature of the block copolymer, consisting of the hydrophilic PEtOz and hydrophobic PCL blocks, led to micelle formation in water. The characteristics of

the block copolymer micelles in water were investigated by fluorescence spectroscopy and the dynamic light scattering method. We first dissolved PEtOz-*b*-PCL in a small volume of THF and mixed it with water, followed by removal of the THF by rotary evaporation, as previously described. The critical micelle concentration (cmc) value of PEtOz-*b*-PCL in water was 7.8×10^{-3} mg/mL, which was much lower than those of low molecular weight surfactants (e.g., 2.3 mg/mL for sodium dodecyl sulfate in water) and was comparable with other polymeric amphiphiles [14-16]. The mean diameter of the polymeric micelles was less than 50 nm.

Next, we investigated the effect of the organic solvents used to prepare block copolymer micelles in water. The effect of cosolvent on the micellization has been frequently investigated [24-26]. We hypothesized that the solubility parameter (δ) of an organic solvent could significantly influence the structure of hydrophobic cores during micelle formation, as well as the characteristics of the resultant polymeric micelles. We chose several organic solvents that have solubility parameter values in the range of 18.6 – 29.7 $MPa^{1/2}$, and the solubility parameter values of THF and acetone were very close to that of poly(ε-caprolactone) (δ = 19.3 $MPa^{1/2}$) [27]. The values of the solubility parameters of the various organic solvents used in this study are listed in Table 1. The contribution of dispersion force to the solubility parameter values of organic solvents used in this experiment appeared to be very close to each other (e.g., 15.2 – 16.5 $MPa^{1/2}$), unlike the polar contribution (e.g., 8.6 – 25.5 $MPa^{1/2}$) [28]. The polar contribution was calculated from the following relationship [29]:

$$\delta^2 = (\delta_d)^2 + (\delta_p')^2 \qquad (2)$$

where δ_d represents dispersion force and δ_p' indicates the polar contribution, including hydrogen-bonding capacity and polarity and induction interactions.

Table 1. Solubility parameter values of the organic solvents used in micelle formation.

Solvent	δ ($MPa^{1/2}$)	δ_d ($MPa^{1/2}$)	δ_p' ($MPa^{1/2}$)
THF	18.6	16.5	8.6
acetone	20.3	15.5	13.1
acetonitrile	24.3	15.4	18.8
ethanol	26.0	15.8	20.6
methanol	29.7	15.2	25.5

Changes in the sizes of the micelles prepared from various organic solvents are three-dimensionally plotted in Figure 2a. The contour map of this plot clearly indicates that the polar contribution to the solubility parameters of organic solvents is much more critical in controlling the size of the resultant micelles in water, rather than the contribution of dispersion force. The mean diameter of the block copolymer micelles decreased as the polar contribution of the solubility parameters increased (Figure 2b).

This could be attributed to the decreased solubility of the hydrophobic blocks in organic solvents with a high polar contribution to the solubility parameter, resulting in the formation of small micelles. It was considered that the critical water content could not significantly influence the characteristics of the resultant block copolymer micelles used in this study, as excess amount of water was initially added to the block polymer solution in organic solvent (>95% water content for all experiments before rotary evaporation).

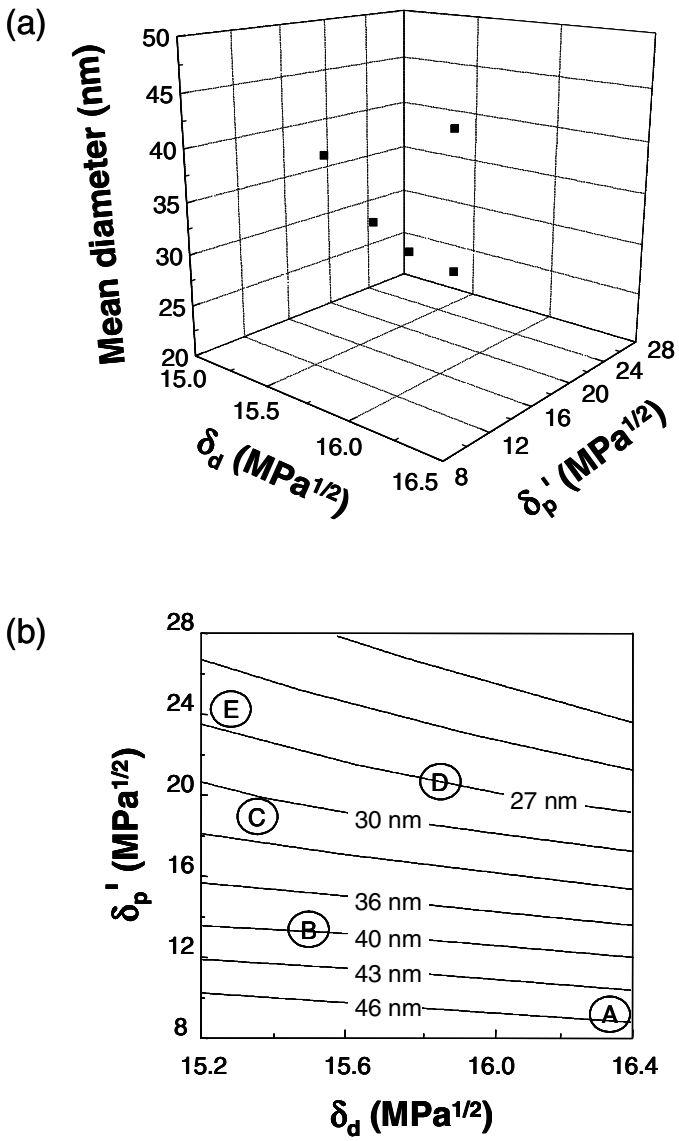

Figure 2. (a) 3-D plot and (b) contour map of the mean diameter of PEtOz-*b*-PCL micelles as a function of δ_d and δ_p' of the organic solvents (A, THF; B, acetone; C, acetonitrile; D, ethanol; E, methanol).

We also determined the microviscosity of the micellar core region by measuring the steady-state fluorescence anisotropy originated from the depolarization of DPH fluorescence, which was closely related to the rotational diffusion of DPH. The anisotropy value (r), a good indicator of the rigidity of the micellar inner core, changes in parallel with the microviscosity, as the rotational diffusion of DPH is increasingly hindered. The anisotropy values (r) measured for the PEtOz-*b*-PCL block copolymer micelles increased with the increasing polar contribution of the solubility parameters of the organic solvents, indicating more rigid inner core formation (Figure 3). This finding was quite consistent with the results from the size changes of the polymeric micelles. It was attributed that the high polar contribution of an organic solvent induced the decreased solubility of hydrophobic blocks of the polymeric amphiphiles in water, and caused the formation of small and rigid core-containing polymeric micelles. The anisotropy values of the PEtOz-*b*-PCL micelles are comparable to those of poly(1-decene-*co*-maleic acid) (0.225) and poly(1-octadecene-*co*-maleic acid) (0.273) [30]. It was considered that the effect of residual organic solvents remaining in the core after solvent removal by rotary evaporation could be negligible, as very small amount of organic solvents was used to dissolve the polymeric amphiphiles and the characteristics of the resultant polymeric micelles prepared either by solvent evaporation method or by dialysis against water for three days were very similar (data not shown).

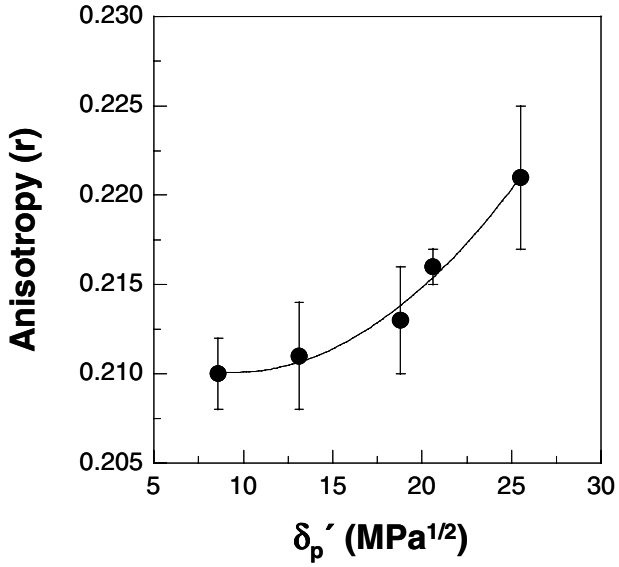

Figure 3. Changes in fluorescence anisotropy values of DPH as a function of the polar contribution of the solubility parameter (δ_p') of organic solvents used for preparation of PEtOz-*b*-PCL micelles in water.

Conclusions

Poly(2-ethyl-2-oxazoline)/poly(ε-caprolactone) block copolymer was synthesized and used to prepare micelles in water. PEtOz-*b*-PCL was first dissolved in various organic

solvents and mixed with water, followed by rotary evaporation to remove the organic solvents and to prepare micelles in water. The physicochemical characteristics of the polymeric micelles were significantly affected by the values of the solubility parameters of the organic solvents. Specifically, the polar contribution to the solubility parameters of the organic solvents controlled the size of the micelles and the rigidity of the inner cores of the micelles. This approach to controlling the size and rigidity of micelles by using organic solvents with various solubility parameters may suggest a useful means to formulate stable and efficient delivery carriers from polymeric amphiphiles for drug delivery applications.

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