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ORIGINAL CONTRIBUTION

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Probing the micellization of diblock and triblock copolymers of poly(L-lactide) and poly(ethylene glycol) in aqueous and NaCl salt solutions

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Abstract The micelle formation of a series of amphiphilic block copolymers in aqueous and NaCl solutions was studied by a fluorescent probe technique using pyrene as a "model drug". These copolymers were synthesized from poly (ethylene glycol) (PEG) and L-lactide by a new calcium ammoniate catalyst. They had fixed PEG block lengths (44, 104 or 113 ethylene oxide units) and various poly(L-lactide) (PLLA) block lengths (15–280 lactide units). The critical micelle concentration (cmc) was found to decrease with increasing PLLA content. The distinct dissimilarity of the cmc values of diblock and triblock copolymers based on the same block length of PEG provided evidence for the different configurations of their micelles. It was also observed that the introduction of NaCl salt significantly contributed to a decrease in the cmcs of the copolymers with short PEG and PLLA blocks, while it had less influence on the cmcs of copolymers with long PEG or PLLA blocks. The dependence of partition coefficients ranging from 0.2×10^5 to 1.9×10^5 on the PLLA content in the copolymer and on the micelle configuration was also discussed. The contribution of NaCl salt to increasing the partition of pyrene into a micellar phase was observed.

Keywords Fluorescence · Micelles · Poly(ethylene glycol) · Poly(L-lactide)

Introduction

In the last 2 decades, there was great interest in microcontainers or micelles prepared from the amphiphilic block copolymer polylactide (PLA)–poly(ethylene glycol) (PEG) as prospective drug delivery systems. They showed no toxicity [1], prolonged circulation time in vivo owing to the shielding effect of PEG chains against the phagocytosis of the mononuclear phagocytic system [2, 3, 4], and sustained release of drug molecules owing to the controlled degradation of the biodegradable polymers. The incorporated drug molecules in microcapsules can be released by a diffusion mechanism plus degradation of the biodegradable polymers [5].

Various techniques were used to evaluate the physicochemical characteristics of these micelles. It was shown by both solid-state ^{13}C NMR and liquid-state ^{1}H NMR that PLA–PEG could self-assemble to form "core–shell" micelles with a central solidlike core of a PLA block and a solvated and hydrophilic corona of a PEG block [4, 6, 7]. The stability of PLA–PEG aqueous dispersions to added salt was studied by measuring the phase-separation temperature [8]. Static light scattering and dynamic light scattering were utilized to investigate the dependence of the aggregation number and hydrodynamic radius of PLA–PEG micelles on the PLA block length [6].

Although PLA–PEG micelles can be used as drug carriers under an extremely dilute condition by taking advantage of their considerably low critical micelle concentration (cmc) values, those cmcs are too low to be detected effectively by light scattering techniques or surface tension measurements and have not been investigated systematically [8, 10, 11, 12]. The fluorescent probe technique has proved to be an effective method to study the micellizations of nonionic and ionic amphiphilic micellar systems [13], such as poly(styrene-*b*-ethylene oxide) (PS–PEO) [14, 15], poly(oxyethylene-*b*-oxypropylene-*b*-oxyethylene) [16], poly(styrene-*b*-sodium acrylate) [17, 18], giving proper information on the partition of the probe between the micellar and water phases [15].

The study reported here is devoted to investigating in detail the respective influences of poly(L-lactide) (PLLA) and PEG block lengths on the cmc values of diblock and triblock PLLA–PEG copolymers in aqueous and salt solutions by a fluorescence technique. The partition coefficient of pyrene as a "model drug" between the micellar and water phase was measured and the relationship of the partition coefficient to the PLLA content in the copolymers and to the micelle configuration is evaluated.

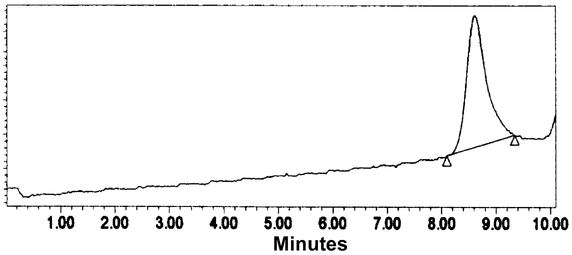

Fig. 1 Gel permeation chromatogram of poly(L-lactide) (*PLLA*$_{81}$)–poly(ethylene glycol) (*PEG*$_{104}$)–PLLA$_{81}$ in tetrahydrofuran

Experimental

Materials

A series of diblock and triblock copolymers were synthesized by ring-opening polymerization of L-lactide (Aldrich Co.) in the presence of methoxy PEG (MeOPEG) and PEG (Aldrich Co.), respectively, using a new catalyst, calcium ammoniate, as reported by Piao et al. [19]. PEG and MeOPEG were dried under reduced pressure for several hours prior to use to remove traces of water. The L-lactide was purified by recrystallizing it four times from dried ethyl acetate. The purified L-lactide and PEG or MeOPEG of correct ratio were put into a flame-dried glass reactor and dried xylene was injected in as a solvent. A known amount of calcium ammoniate was transferred into the reactor and the reaction was carried out at 80 °C under stirring for 24 h. The resulting copolymers were dissolved in methylene chloride and precipitated into methanol, followed by centrifugation and drying in a vacuum at room temperature for 24 h.

The molecular composition of the block copolymers obtained, i.e., the molar ratio of PLLA to PEG, was determined in $CDCl_3$ with a Unity-400 NMR spectrometer, based on the integral intensities of the peaks corresponding to the PLLA block (–CH, δ 5.1 ppm) and to the PEG block ($–OCH_2CH_2$, δ 3.65 ppm) (Fig. 2), and the degrees of polymerization (DP) of the PLLA blocks were calculated from this ratio and the M_n of PEG or MeOPEG used. The molecular weight and the molecular weight distribution, M_w/M_n, were determined using a Waters 410 gel permeation chromatograph with tetrahydrofuran (THF) eluant at a flow rate of 1.0 ml/min at 25 °C (Fig. 1). The results are collected in Table 1, where the subscripts stand for the DP of the blocks.

Preparation of the micelle systems

The block copolymer micelle systems were prepared as follows [15].

1. Copolymer solutions: A copolymer (0.1 g) was first dissolved in THF (around 4 ml) in a 100-ml volumetric flask and 40 ml doubly distilled water was added with gentle agitation, followed by removing THF (25 °C, with a water aspirator) for 2 h.
2. Pyrene solution: A predetermined amount of pyrene solution in acetone (0.1 mg/l) was added into a series of volumetric flasks

Fig. 2 ^1H NMR spectrum of PLLA$_{81}$–PEG$_{104}$–PLLA$_{81}$ in $CDCl_3$

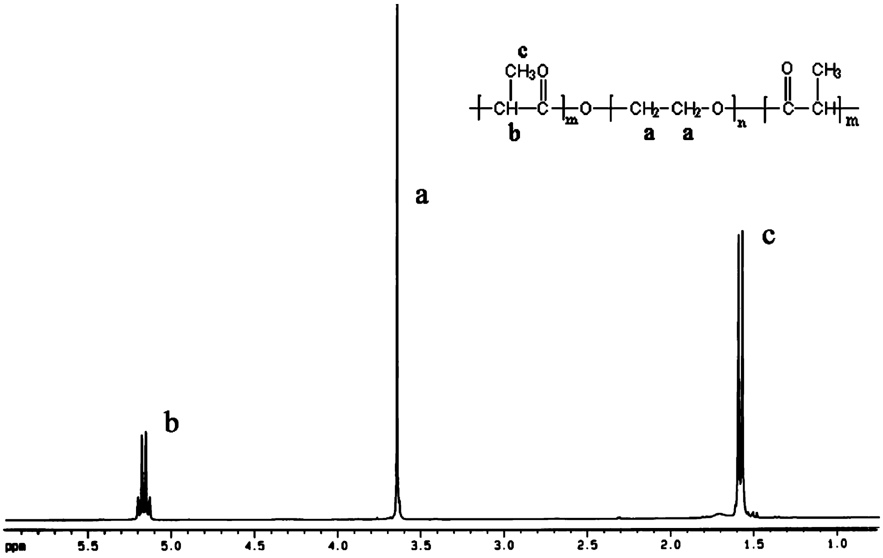

Table 1 Molecular characteristics of diblock poly(L-lactide) (*PLLA*)–methoxy poly(ethylene glycol) (*MeOPEG*) copolymers and triblock poly(L-lactide) (*PLLA*)–poly(ethylene glycol) (PEG)–(*PLLA*) copolymers

Sample (diblock)[a]	M_w/M_n[b]	PLLA weight percent	Sample (triblock)[a]	M_w/M_n[b]	PLLA weight percent
MeOPEG$_{44}$–PLLA$_{15}$	1.23	35	PLLA$_{17}$–PEG$_{44}$–PLLA$_{17}$	1.17	55
MeOPEG$_{44}$–PLLA$_{27}$	1.27	49	PLLA$_{28}$–PEG$_{44}$–PLLA$_{28}$	1.25	67
MeOPEG$_{44}$–PLLA$_{61}$	1.27	69	PLLA$_{44}$–PEG$_{44}$–PLLA$_{44}$	1.09	76
MeOPEG$_{44}$–PLLA$_{113}$	1.14	80	PLLA$_{63}$–PEG$_{44}$–PLLA$_{63}$	1.12	82
MeOPEG$_{113}$–PLLA$_{23}$	1.26	25	PLLA$_{11}$–PEG$_{104}$–PLLA$_{11}$	1.19	26
MeOPEG$_{113}$–PLLA$_{39}$	1.23	36	PLLA$_{33}$–PEG$_{104}$–PLLA$_{33}$	1.23	51
MeOPEG$_{113}$–PLLA$_{96}$	1.35	58	PLLA$_{81}$–PEG$_{104}$–PLLA$_{81}$	1.19	72
MeOPEG$_{113}$–PLLA$_{133}$	1.28	66	PLLA$_{140}$–PEG$_{104}$–PLLA$_{140}$	1.25	82

[a] The subscripts stand for the degrees of polymerization of the blocks. They were determined by ^{1}H NMR in $CDCl_3$
[b] Determined by gel permeation chromatography measurement

and the acetone was evaporated completely. The solid pyrene left in each flask was of such amount that when the flask was filled with a solution to the calibration mark, the pyrene concentration in the final solution was 6×10^{-7}mol/l, similar to the saturation solubility of pyrene in water at 22 °C [13, 20].

3. Aqueous micelle systems: The copolymer solution and a balance amount of doubly distilled water were added into the volumetric flasks containing the solid pyrene consecutively. The desired copolymer concentration from 10^{-5} to 0.8 g/l was achieved by predetermining the volume of the copolymer solution.
4. Salt-containing micelle systems: Before the final dilution with doubly distilled water, a given amount of NaCl solution was added to the copolymer/pyrene solution to give a final NaCl concentration of 0.9%.
5. Finally the flasks were thermostated at 30–40 °C for about 2 h to equilibrate the pyrene partition between the water and the micelles, and subsequently were cooled overnight to room temperature.

Fluorescence measurement

Steady-state fluorescence spectra were obtained with a Perkin Elmer LS50B luminescence spectrometer. Square quartz cells of 1.0 cm×1.0 cm were used. For fluorescence emission spectra, the excitation wavelength was set at 339 nm, and for excitation spectra, the detection wavelength was set at 390 nm. The spectra were recorded with a scan rate of 30 nm/min.

Results and discussion

Block copolymer synthesis

A series of diblock and triblock copolymers were synthesized by ring-opening polymerization of L-lactide in the presence of MeOPEG or PEG and catalyzed by calcium ammoniate. The macromolecular initiators MeOPEG and PEG carried one and two hydroxyl end groups in each molecular chain and thus resulted in diblock (MeOPEG–PLLA) and triblock (PLLA–PEG–PLLA) copolymers, respectively, according to the polymerization mechanism of the system [19]. The molecular weights $M_n=2{,}000$ and 5,000 (corresponding to DP = 44 and 113, respectively) of MeOPEG and $M_n=2{,}000$ and 4,600 (DP = 44 and 104, respectively) of PEG were chosen so that the PEG block could be excreted easily after the PLLA block had biodegraded, because the copolymers were expected to be used for medical purposes. With a given MeOPEG or PEG length, various PLLA lengths were obtained by adjusting the monomer-to-initiator ratio, as shown in Table 1. With the four series of block copolymers obtained, it is possible to evaluate the influence of the absolute and relative lengths of the PEG or PLLA blocks on the micellization and to make comparison between diblock copolymers and triblock copolymers. Because the ring-opening polymerization catalyzed by calcium ammoniate exhibited a character of “quasi-living polymerization” [19], the copolymers obtained showed quite narrow molecular weight distributions, with M_w/M_n in the range 1.1–1.3. Their narrow and single gel permeation chromatography profiles (Fig. 1) and straightforward NMR spectra (Fig. 2) showed that the copolymers obtained were target diblock and triblock copolymers.

cmc measurement

Pyrene has been employed as an effective fluorescent probe for the micellization of various systems. Because of its photophysical sensitivity to the change of environmental polarity [21, 22], the micellization is always accompanied by noticeable changes in its fluorescence spectra and fluorescence lifetime, such as the redshift of the low-energy band of L_a ($S_2 \leftarrow S_0$), the increase in the lifetime of the fluorescence decay, and the vibrational fine structure changes [14]. Fluorescence excitation spectra of 6×10^{-7} mol/l pyrene in water in the presence of PLLA$_{44}$–PEG$_{44}$–PLLA$_{44}$ of various concentrations are shown in Fig. 3. Obviously the fluorescence intensity increases tremendously and the maximal peak position shifts from 333 to 335 nm with increasing polymer

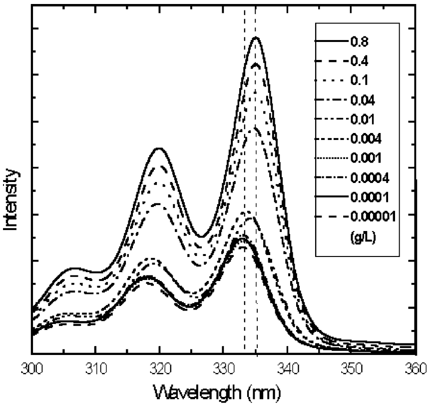

Fig. 3 Excitation spectra of pyrene in water for $PLLA_{44}$–PEG_{44}–$PLLA_{44}$ at various concentrations at $\lambda_{em} = 390$ nm

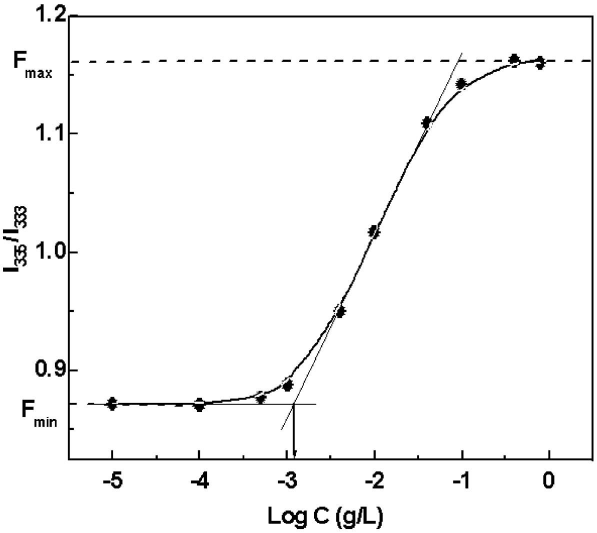

Fig. 4 Intensity ratio I_{335}/I_{333} of pyrene excitation spectra versus log$c$ for $PLLA_{44}$–PEG_{44}–$PLLA_{44}$

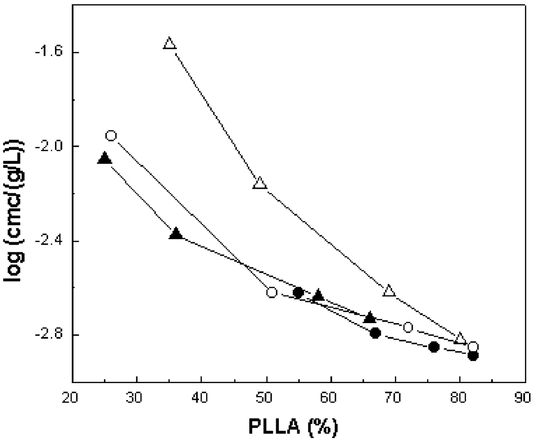

Fig. 5 The effect of PLLA content on the critical micelle concentration (*cmc*) values of various copolymers with PEG block lengths of methoxy PEG (*MeOPEG*$_{44}$) (*open triangles*), MeO-PEG_{113} (*closed triangles*), PEG_{44} (*closed circles*), and PEG_{104} (*open circles*)

concentration, although the apparent pyrene concentration remains unchanged. This peak shift is attributed to the formation of micelles in the system and the pyrene probe molecules which enter the micelle phase show much stronger fluorescence than in the water phase. On the basis of this peak shift, the cmc can be determined [15]. As seen from the plot of the fluorescence intensity ratio of I_{335}/I_{333} of the excitation spectra versus the logarithm of the concentration in Fig. 4, an S-shaped curve is obtained. It reflects the whole process of micellization and the intersection of the lower horizontal tangent and the slope tangent corresponds to the cmc of the system (as shown by the arrow in Fig. 4).

It is also found that the wavelength of the maximal peak position in the excitation spectrum using pyrene as a probe in the PLLA–PEG micelle system (335 nm) is smaller than the peaks in the poly(ϵ-caprolactone)–PEG system (336.5nm) and in the PS–PEO system (338 nm) [15, 19]. This indicates the dependence of the maximal peak position on the relative hydrophobic nature of the micelle cores formed, i.e., the more hydrophobic the micelle core, the larger the maximal peak shift.

Dependence of cmc on block lengths of the copolymers

The micelle formation of amphiphilic block copolymers is the result of two competitive functions: the hydrophilic function of the soluble block, which contributes to keeping copolymer molecules dispersing stably in water, and the hydrophobic function of the insoluble block, which inclines to draw copolymer molecules out of water and to make them aggregate. It is important to evaluate the influences of the relative and absolute lengths of the two blocks on the micellization of the amphiphilic block copolymers.

The cmc is plotted against the weight percent of the PLLA block in Fig. 5 for the four series of samples listed in Table 1. For a given PEG length, the cmc of the copolymer decreases with increasing PLLA content, i.e., with increasing PLLA block length. This is understandable because the hydrophobic PLLA block has the function of causing aggregation. In the region of PLLA content lower than about 50%, the cmc is very sensitive to both the PEG block length and the PLLA content (relative length of PLLA to PEG), while in the region of PLLA content higher than about 50%, the cmc declines slowly with increasing PLLA content. The difference caused by the PEG block length becomes less and less,

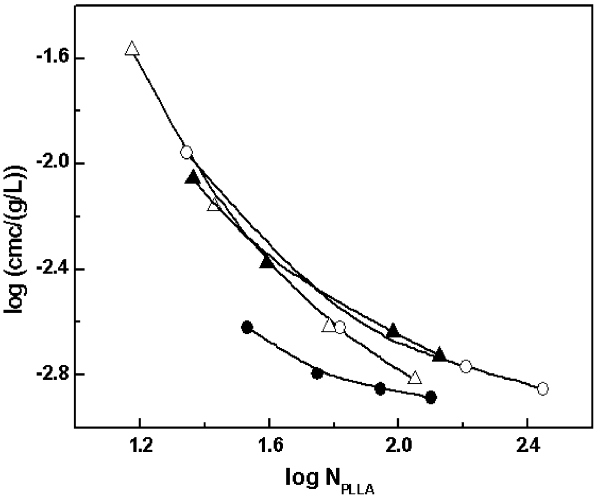

Fig. 6 Influence of the PLLA block length on the cmc values of various diblock and triblock PLLA–PEG copolymers of MeO-PEG_{44} (*open triangles*), $MeOPEG_{113}$ (*closed triangles*), PEG_{44} (*closed circles*), and PEG_{104} (*open circles*)

indicating that the PLLA content is the predominant factor over the absolute PEG length in this region.

Among the diblock MeOPEG–PLLA series, the cmc values of the $MeOPEG_{44}$ series are always higher than those of the $MeOPEG_{113}$ series for a given PLLA content, although apparently the latter has a longer hydrophilic PEG block. This fact leads to the conclusion that the absolute length of the PLLA block plays a more important role in determining the micellization than that of the PEG block for the diblock series. In order to make this point clearer, the cmc data are plotted against the DP (DP, N_{PLLA}) of the PLLA block in Fig. 6, where N_{PLLA} designates the total DP of the two PLLA blocks in the case of the triblock. It is shown in Fig. 6 that the cmc always decreases with increasing N_{PLLA} for the four series of samples examined and for the two series of diblock copolymers, the absolute PEG length does not make much difference in general. Over a wide range, the $MeOPEG_{44}$ series exhibit lower cmcs than the MeO-PEG_{113} series for the shorter PEG block of the former. Among the triblock series, the PEG_{104} series has a higher cmc than the PEG_{44} series owing to the hydrophilic nature of the PEG chains no matter what the PLLA content (Fig. 5) or for a given N_{PLLA} (Fig. 6). This difference from the diblock series is assumed to be related to the different micelle configurations between the diblock series and the triblock series, as discussed later.

Among the four series, the $MeOPEG_{44}$–PLLA diblock and the PEG_{44}–PLLA triblock have the same PEG block length, but the cmc of the former is much higher than that of the latter for a given N_{PLLA} (Figs. 5, 6). This is attributed to the different micelle configurations of the diblock and triblock copolymers. As shown in Fig. 7, because of the amphiphilic structure of the diblock and triblock copolymers, the diblock copolymer trends to form a starlike micelle [6, 23, 24], while the triblock copolymer tends to form a flowerlike micelle [25]. Obviously, the soluble PEG blocks in a diblock copolymer extend into water more freely and deeply than that in a triblock copolymer and thus possess more probability of interaction with water molecules by hydrogen bonding. As a result a diblock copolymer requires a higher concentration to aggregate into micelles than a tirblock copolymer of the same PEG block length.

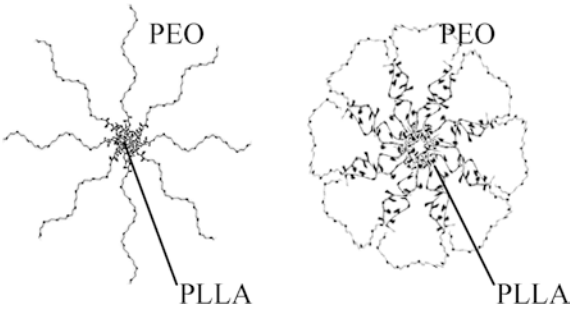

Fig. 7 Configurations of diblock and triblock PLLA–PEG copolymers: “star” and “flower” models

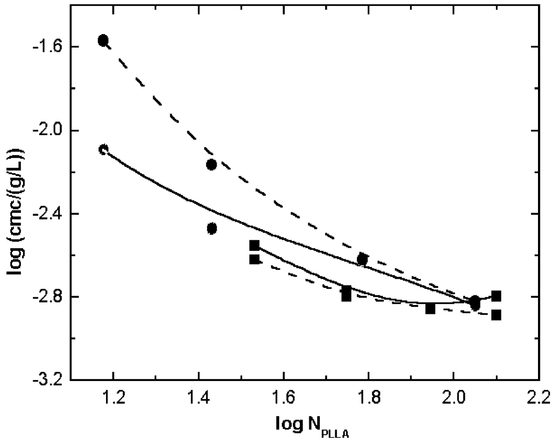

Fig. 8 The cmc values of the copolymers with $MeOPEG_{44}$ (*circles*) and PEG_{44} (*squares*) blocks in aqueous (*dashed line*) and salt (*solid line*) solutions

Influence of NaCl on the cmc

Usually cmc values of nonionic amphliphilic block copolymers are not markedly influenced by the presence of a salt. For most of the copolymers examined in this study, the addition of NaCl of 0.9% (the NaCl concentration in human body) did not cause an appreciable change in the cmcs if other conditions were fixed; however, the MeO-PEG_{44}–PLLA copolymer was an exception. As shown in Fig. 8, the addition of NaCl caused a significant reduction of the cmcs, especially for those containing short PLLA blocks. Explanations of the salt effect on water and surfactant solutions have been advanced [26, 27, 28, 29]. On one hand, NaCl modifies the water solution properties as a water "structure maker" and makes water more polar (salting-out effect) [26, 27]. On the other hand, this cmc reduction may be attributed to the interaction or even coordination of the cationic Na^+ ions with the ether groups [28, 29, 30, 31]. This interaction leads to weakening of the interaction between the PEG chain and the surrounding water molecules and thus to lowered cmcs. The copolymers with long PLLA blocks did not show significant lowering of the cmc in NaCl solution, because in this case the length of the PLLA blocks itself was the predominant factor in determining the cmc compared with the role of PEG blocks.

As shown in Fig. 8, the two curves for the triblock PEG_{44} series almost overlap and they both are lower than the two curves for the $MeOPEG_{44}$ series. This may be attributed to the flowerlike micelle configuration of the triblock copolymers. In this configuration, PEG blocks play a less important role than PLLA blocks as pointed out earlier and thus the addition of NaCl and its interaction with PEG chains do not result in a significant difference to the cmc.

Partition coefficients of pyrene

As a hydrophobic molecule, pyrene prefers incorporation into the hydrophobic part of a micelle. As cited earlier, pyrene was more favorable to partition into the PS core of PS–PEO block copolymers than into sodium dodecyl sulfate micelles [15]. The cmc measurements with pyrene as a fluorescent probe are actually based on the partition of pyrene between the water and micellar phases. Therefore the measurement of the partition coefficient of pyrene as a model drug may provide information on its affinity to the micelle phase or micelle cores, and thus provide information on the chemical composition and structure of the micelle cores.

It has been suggested that a simple partition equilibrium exists between the PS micelle core phase and the water phase for the PS–PEO system [15]. Owing to the similarity between PS–PEO and PLLA–PEG, we presume that such analysis can also be applied to PLLA–PEG micelle systems:

$$\frac{[\mathrm{Py}]_\mathrm{m}}{[\mathrm{Py}]_\mathrm{w}}=\frac{K_\mathrm{v}V_\mathrm{m}}{V_\mathrm{w}}, \tag{1}$$

$$\frac{[\mathrm{Py}]_\mathrm{m}}{[\mathrm{Py}]_\mathrm{w}}=\frac{K_\mathrm{v}\chi_\mathrm{PLLA}(c-\mathrm{cmc})}{1000\rho_\mathrm{PLLA}}, \tag{2}$$

where $[\mathrm{Py}]_\mathrm{m}$ and $[\mathrm{Py}]_\mathrm{w}$ are the apparent concentrations of pyrene in the micellar and aqueous phases, respectively, V_m and V_w are the real volumes of the two phases, K_v is the equilibrium partition coefficient of pyrene between the micelle core and water phase, c is the total copolymer concentration in grams per liter, χ_PLLA is the weight fraction of PLLA in the copolymer, and ρ_PLLA is the density of the PLLA core, which is assumed to be that of bulk PLLA (1.2 g/ml). Obviously the K_v value is a measure of the affinity of pyrene to the PLLA core [15].

Experimentally, the ratio of pyrene in the micellar and water phases is determined by the following equation:

$$\frac{[\mathrm{Py}]_\mathrm{m}}{[\mathrm{Py}]_\mathrm{w}}=\frac{F-F_\mathrm{min}}{F_\mathrm{max}-F}, \tag{3}$$

where $F=I_{335}/I_{333}$ and F_min and F_max are the minimum and maximum ratios of I_{335}/I_{333} in the lower and higher flat regions as indicated in Fig. 4. With F, F_min, and F_max determined experimentally and from Eqs. (3) and (2), the plot of $[\mathrm{Py}]_\mathrm{w}/[\mathrm{Py}]_\mathrm{m}$ versus $1/(c\text{–cmc})$ should be a straight line through the origin. Its slope is $1{,}000\rho_\mathrm{PLLA}/K_\mathrm{v}\chi_\mathrm{PLLA}$, and K_v is thus calculated. Figure 9 demonstrates such a group of straight lines for the $MeOPEG_{44}$ diblock series. Obviously the K_v value is a function of the PLLA block length. The dependence of K_v on the PLLA weight percent for the four series of PLLA–PEG block copolymers is shown in Fig. 10. K_v tends to increase with increasing PLLA percentage in the block copolymers. This is understandable because pyrene likes to enter the PLLA micellar cores.

Among the four series of block copolymers, the $MeOPEG_{44}$ series exhibits the lowest K_v values (Fig. 10). The curve for the $MeOPEG_{113}$ series is above that for the $MeOPEG_{44}$ series, indicating again that in addition to the relative composition, the absolute length of the PLLA blocks affects the partition of pyrene in the two phases. Comparison of the PEG_{44} series triblock and the

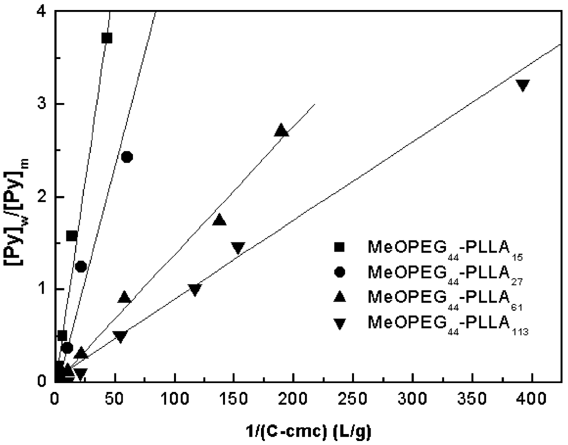

Fig. 9 $[\mathrm{Py}]_\mathrm{w}/[\mathrm{Py}]_\mathrm{m}$ versus $1/(c\text{–cmc})$ for the $MeOPEG_{44}$ series diblock copolymers

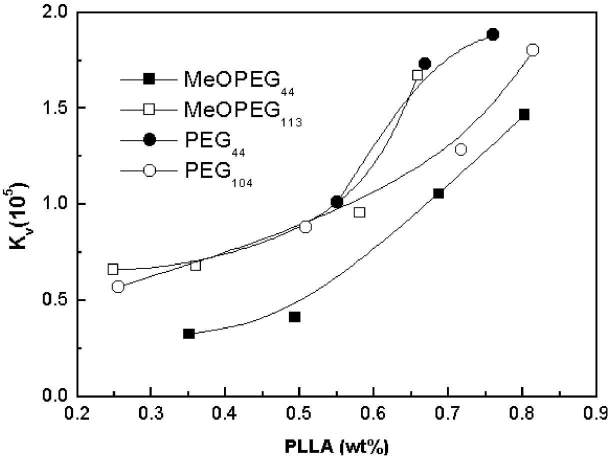

Fig. 10 K_v versus PLLA weight percent for the four series of PLLA–PEG block copolymers

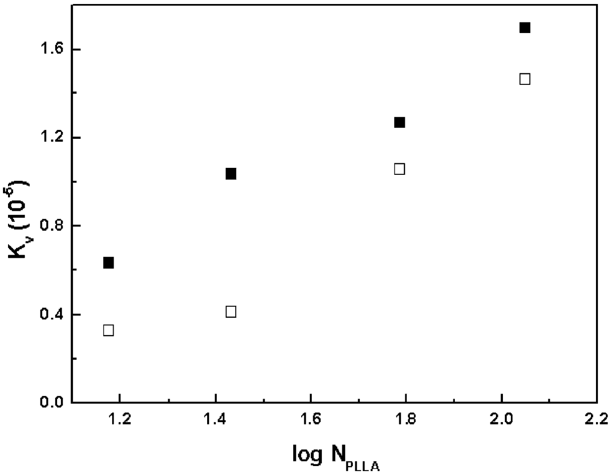

Fig. 11 K_v versus logN_{PLLA} for MeOPEG$_{44}$ series diblock copolymers in aqueous (*open squares*) and 0.9% NaCl (*closed squares*) solutions

MeOPEG$_{44}$ series diblock indicates that a flowerlike micelle has a higher K_v value than a starlike micelle of the same composition.

All the K_v values of the four series of block copolymers range from 0.2×10^5 to 1.9×10^5 (dimensionless). They are less than those of PS–PEO (K_v ranges from 2×10^5 to 4×10^5). This implies that it is less favorable for pyrene to partition into the PLLA core because it is less hydrophobic than the PS core. Of course, similar conjugated structures of pyrene to PS and possible π–π interaction between them are responsible for the special affinity between pyrene and PS chains.

The influence of NaCl on the K_v values of the MeOPEG$_{44}$ series diblock copolymers is shown in Fig. 11. Obviously higher K_v values are determined in the salt solution. As pointed out earlier, the Na^+ and Cl^- ions themselves and the induced polarity in the water system give the driving force for more pyrene molecules to enter the PLLA cores.

Conclusions

Four series of PLLA–PEG diblock and triblock copolymers were prepared by ring-opening polymerization of L-lactide in the presence of PEG macromolecular initiators by using calcium ammoniate catalyst. The influences of the PLLA and PEG block lengths on the micellization of the block copolymers were evaluated. It was found that the role of the PLLA block is more significant than that of the PEG block in micelle formation when the PLLA block is long enough. Added NaCl can decrease the cmc values of diblock copolymers containing short PLLA blocks. Usually triblock copolymers have a lower cmc than diblock ones, because they form flower-shaped micelles that are different from the star-shaped micelles aggregated by diblock ones. Increasing the PLLA content in the copolymer contributes to increasing the partition coefficient of pyrene as a model drug between micellar and aqueous phases. The introduction of NaCl contributes to the increase in the partition coefficients of pyrene.

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References

1. Herold DA, Keil K, Bruns DE (1989) Biochem Pharmacol 38:73
2. Gref R, Minamitake Y, Peracchia MT, Trubetskoy V, Torchilin V, Langer R (1994) Science 263:1600
3. Verrecchia T, Spenlenhauer G, Bazile DV, Murry-Brelier A, Archimband Y, Veillard M (1995) J Controlled Release 36:49
4. Stolnik S, Heald CR, Neal J, Garnett MC, Davis SS, Illum L, Purkiss SC, Barlow RJ, Gellert PR (2001) J Drug Targeting 9:361
5. Peracchia MT, Gref R, Minamitake Y, Domb A, Lotan N, Langer R (1997) J Controlled Release 46:223
6. Riley T, Stolnik S, Heald CR, Xiong CD, Garnett MC, Illum L, Davis SS, Purkiss SC, Barlow RJ, Gellert PR (2001) Langmuir 17:3168
7. Heald CR, Stolnik S, Kujawinski S, De Matteis C, Garnett MC, Illum L, Davis SS, Purkiss SC, Barlow RJ, Gerllert PR (2001) Langmuir 18:3669
8. Hagan SA, Coombes GA, Garnett MC, Dunn SE, Davies MC, Illum L, Davis SS, Harding SE, Purkiss S, Gellert PR (1996) Langmuir 12:2153

9. Heald CR, Stolnik S, De Matteis C, Garnett MC, Illum L, Davis SS, Leermakers FAM (2001) Colloids Surf 179:79
10. Tanodekaew S, Pannu R, Heatley F, Attwood D, Booth C (1997) Macromol Chem Phys 198:927
11. Yasugi K, Nagasaki Y, Kato M, Kataoka K (1999) J Controlled Release 62:89
12. Nagasaki Y, Okada T, Scholz C, Iijima M, Kato M, Kataoka K (1998) Macromolecules 31:1473
13. Kalyanasundaram K, Thomas JK (1977) J Am Chem Soc 99:2039
14. Zhao CL, Winnik MA, Riess G, Croucher MD (1990) Langmuir 6:514
15. Wilhelm M, Zhao CL, Wang YC, Xu RL, Winnik WA (1991) Macromolecules 24:1033
16. Kabanov AV, Nazarova IR, Astafieva IV, Batrakova EV, Alakhov VY, Yaroslavov AA, Kabanov VA (1995) Macromolecules 28:2303
17. Astafieva I, Zhong XF, Eisenberg A (1993) Macromolecules 26:7339
18. Astafieva I, Khougaz K, Eisenberg A (1995) Macromolecules 28:7127
19. Piao LH, Dai ZL, Zhang XF, Deng MX, Chen XS, Jing XB (2003) Polymer 44:2025
20. Dong DC, Winnik MA (1984) Can J Chem 62:2560
21. Nakajima A (1971) Bull Chem Soc Jpn 44:3272
22. Nakajima A (1976) J Mol Spectrosc 61:467
23. Halperin A (1987) Macromolecules 20:2943
24. Zhang L, Eisenberg A (1995) Science 268:1728
25. Zhao Y, Liang HJ, Wang SG, Wu C (2001) J Phys Chem 105:848
26. Frank HS, Evans MS (1945) J Chem Phys 13:507
27. Kabalnov A, Olsson U, Wennerström H (1995) J Phys Chem 99:6220
28. Florin E, Kjelllander R, Eriksson JC (1984) J Chem Soc Faraday Trans 1 80:2911
29. Alexandridis P, Holzwarth JF (1997) Langmuir 13:6074
30. Wyttenbach T, Von Helden G, Bowers MT (1997) Int J Mass Spectrom 165:377
31. Johansson P (2001) Polymer 42:4367