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Self-Assembly of Poly(γ-benzyl *L*-glutamate)-*block*-poly(ethylene glycol) and Poly(γ-benzyl *L*-glutamate)-*graft*-poly(ethylene glycol) Blend in Ethanol

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Poly(γ-benzyl *L*-glutamate)-*block*-poly(ethylene glycol) (PBLG-*block*-PEG) and poly(γ-benzyl *L*-glutamate)-*graft*-poly(ethylene glycol) (PBLG-*graft*-PEG) were synthesized by a standard *N*-carboxyl-γ-benzyl-*L*-glutamate anhydride method and by an ester exchange reaction, respectively. The self-association behaviours of PBLG-*block*-PEG/PBLG-*graft*-PEG blend in ethanol were investigated by transmission electron microscopy and viscometry. It was showed that the polypeptide copolymer blend could self-assemble to form polymeric micelles with a core-shell structure in the shape of tree-like. Effects of the testing temperature and the weight ratio of PBLG-*block*-PEG to PBLG-*graft*-PEG on the critical micelle concentration of the polypeptide copolymer blend in ethanol were studied.

Key Words: Polypeptide copolymer blend, Self-assembly, Critical micelle concentration, Morphology.

INTRODUCTION

Amphiphilic copolymers composed of hydrophobic and hydrophilic chains could form micelle structures with the hydrophobic inner core and the hydrophilic outer shell in aqueous media[1-4]. Based on their potential application and academic interest in many interdisciplinary field, the self-assembly behaviours of amphiphilic molecules have received much attention both experimentally and theoretically[1]. The nanoscale structure holds a range of potential applications[5-7] such as carriers of catalysts, protein simulation, macromolecular conformational study, nanoreactors, *etc*..

The polymeric micelles formed by block or graft copolymers consisting of polypeptide segments and hydrophilic polymer chains have attracted more attention due to their potential application such as drug delivery[2,8,9]. Kwon *et al.*[10] have reported that poly(β-benzyl *L*-aspartate) (PBLA)/poly(ethylene oxide) (PEO) diblock copolymers could self-assemble to form polymeric micelles consisting of an outer shell of poly(ethylene oxide) and an inner core of poly(β-benzyl *L*-aspartate) in aqueous medium. Cho *et al.*[11] have reported the formation of polymeric micelles composed of poly(γ-benzyl *L*-glutamate) and poly(ethylene oxide) in aqueous medium and the drug delivery system based on the core-shell nanoparticles with poly(γ-benzyl *L*-glutamate) (PBLG) as the hydrophobic inner core and poly(ethylene oxide) as the hydrophilic outer shell. Compared with pure block or graft copolymers, polypeptide copolymer blends have received little attention. In the present work, the self-association behaviours of PBLG-*block*-PEG/PBLG-*graft*-PEG blend in ethanol were investigated by TEM and viscometry. It was revealed that the polypeptide copolymer blend could self-assemble to form polymeric micelles with a core-shell structure in the shape of tree-like. Effects of the testing temperature and the weight (wt) ratio of PBLG-*block*-PEG to PBLG-*graft*-PEG on the critical micelle concentration (CMC) of the polypeptide copolymer blend in ethanol were investigated.

EXPERIMENTAL

Amine-terminated α-methoxy-ω-amino poly(ethylene glycol) (AT-PEG, M_w = 5000) and poly(ethylene glycol methyl ether) (mPEG, M_w = 350) were purchased from Sigma Inc. (USA) and used without further purification. Hexane, tetrahydrofuran (THF) and 1,4-dioxane are of analytical grade and dried with sodium to remove water before use. All other solvents are of analytical grade and used without further purification.

Synthesis of polypeptide copolymers: PBLG-*block*-PEG was prepared by a standard *N*-carboxyl-γ-benzyl-*L*-glutamate anhydride (NCA) method[2,9]. Molecular weight of the PBLG-*block*-PEG copolymer was estimated by NMR method[2]. The molecular weight of PBLG-*block*-PEG used in the study was about 70000.

Poly(γ-benzyl *L*-glutamate) was also prepared by a standard *N*-carboxyl-γ-benzyl-*L*-glutamate anhydride (NCA) method[2,9]. The molecular weight of PBLG was estimated from

the intrinsic viscosity measured in dichloroacetic acid[12]. The molecular weight of PBLG homopolymer is about 60000. PBLG-*graft*-PEG was obtained by the ester exchange reaction of PBLG homopolymer with mPEG in 1,2-dichloroethane with *p*-toluenesulfonic acid as a catalyst according to the method described in the documents[1]. The grafting percentage of PBLG-*graft*-PEG was 11.3 % calculated according to the document[1,2]. Fig. 1 shows the schematic representations of PBLG-*block*-PEG structure and PBLG-*graft*-PEG structure.

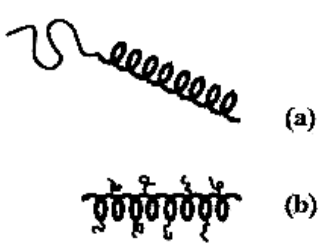


Fig. 1. Schematic representations of (a) PBLG-*block*-PEG structure and (b) PBLG-*graft*-PEG structure

Preparation of polypeptide copolymer micelles: The obtained polypeptide copolymer samples were first dissolved in $CHCl_3$ to make a 2 g/L polymer solution. Subsequently, a given volume of ethanol was added into the polymer $CHCl_3$ solution with stirring. The formation of the polypeptide copolymer micelles occurred, as indicated by the appearance of turbidity in the solution, when about 20 vol. % ethanol was added. After 2 h, the addition of ethanol was continued until the polymer concentration in the micelle solution was about 0.2 g/L[13]. The micelle solution was kept overnight and then dialyzed against ethanol using dialysis membranes (3500 molecular weight cut-off) to remove the $CHCl_3$ for 48 h at room temperature. It was preferred that ethanol was exchanged at intervals of 10-12 h. The solution was diluted with ethanol to the desired concentration.

Test methods: The morphology of the micelles was obtained by TEM (JEM-1200-EXII). Drops of micelle solution were placed on a carbon film coated copper grid and then were dried at room temperature. Before the observations, the sample was stained by aqueous phosphotungstic acid solution (1.0 wt %). The TEM bright field imaging was performed with 120 kV accelerating voltage.

Viscosity measurements of the micelle solution were made in an Ubbelohde viscometer, which was placed in a thermostatically controlled bath with a precision of ± 0.1 ºC. The measurements were repeated at least three times and the times obtained were arithmetically averaged, then converted to the relative viscosity (η_γ), η_γ was further converted to the specific viscosity (η_{sp}). The experiments were carried out by diluting the micelle solution step by step. The curve of η_{sp}/C *versus* the concentration (C) of the micelle solution was drawn. By analyzing the curve of η_{sp}/C-C, the critical micelle concentration of the polypeptide copolymer blend in ethanol could be obtained[14].

RESULTS AND DISCUSSION

Effects of the testing temperature on the critical micelle concentration of the polypeptide copolymer blend in ethanol: Fig. 2 shows the effects of testing temperature on the critical micelle concentration of PBLG-*block*-PEG/PBLG-*graft*-PEG (wt ratio: 1:1) blend in ethanol. Fig. 2 showed the critical micelle concentration of the polypeptide copolymer blend in ethanol decreases with increasing the testing temperature. Price *et al.*[15] reported the increase of the testing temperature promotes the interaction of PBLG segments in both PBLG-*block*-PEG and PBLG-*graft*-PEG by accelerating the moving of the polypeptide blocks, suggesting that the critical micelle concentration of the polypeptide copolymer blend debases with the increase of the testing temperature. The phenomenon proves that the testing temperature could affect the self-association behaviour of the polypeptide copolymer blend in ethanol.

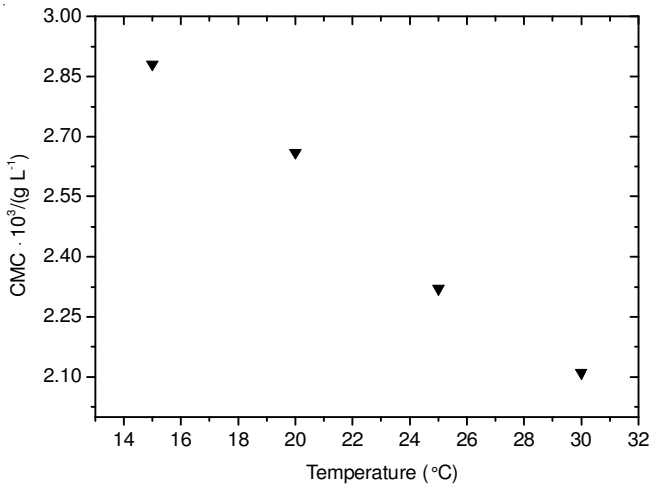


Fig. 2. Plot of the critical micelle concentration of PBLG-*block*-PEG/PBLG-*graft*-PEG (wt ratio: 1:1) blend in ethanol with different testing temperatures

Effects of PBLG-*graft*-PEG wt content on the critical micelle concentration of the polypeptide copolymer blend in ethanol: Fig. 3 presents the the effects of PBLG-*graft*-PEG wt content on the critical micelle concentration of polypeptide copolymer blend in ethanol. As is shown in Fig. 3, with the increase of PBLG-*graft*-PEG wt content, the critical micelle concentration of the polypeptide copolymer blend in ethanol increases. Compared with PBLG-*block*-PEG, PBLG-*graft*-PEG contains more hydrophilic PEG segments, the increase of the PBLG-*graft*-PEG content promotes the hydrophilicity of the polypeptide copolymer blend and accordingly increases the critical micelle concentration of the polypeptide copolymer blend. This situation also demonstrates that the PBLG-*graft*-PEG content could affect the self-assembly of the polypeptide copolymer blend in ethanol.

Micelle morphologies of PBLG-*block*-PEG, PBLG-*graft*-PEG and PBLG-*block*-PEG/PBLG-*graft*-PEG blend in ethanol: Fig. 4 indicates the morphologies of the micelles formed by PBLG-*block*-PEG, PBLG-*graft*-PEG and PBLG-*block*-PEG/PBLG-*graft*-PEG blend (wt ratio: 1:1) in ethanol. As seen from Fig. 4, the morphologies of the micelles formed by the three polypeptide copolymers were spherical shape, spindle shape and tree-like shape, respectively. As described in document[1], the difference of the micellar morphology could be attributed to the difference of their molecular architectures. This situation proves that the different molecular architectures

could also affect the self-association behaviour of the polypeptide copolymers.

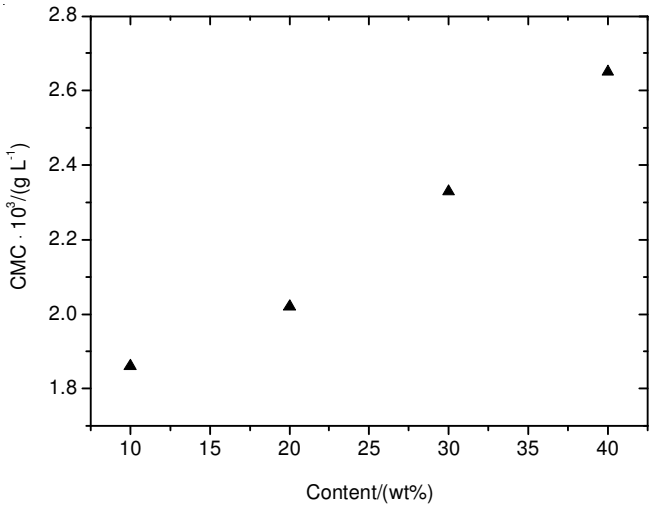
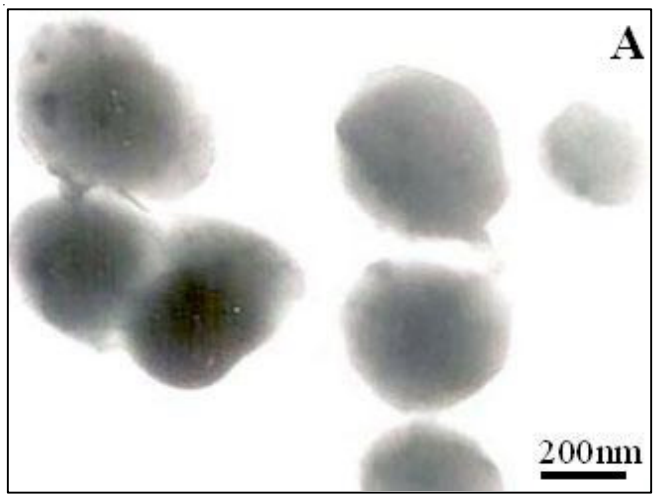


Fig. 3. The plot of the critical micelle concentration of PBLG-*block*-PEG/PBLG-*graft*-PEG blend in ethanol with different PBLG-*graft*-PEG wt contents, where the testing is 25 ºC



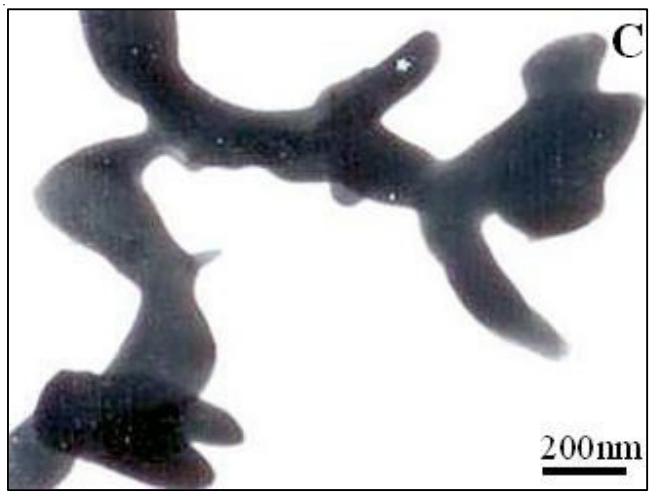


Fig. 4. TEM photographs of the micelles formed by (a) PBLG-*block*-PEG, (b) PBLG-*graft*-PEG and (c) PBLG-*block*-PEG/PBLG-*graft*-PEG (wt ratio: 1:1) blend

Conclusion

PBLG-*block*-PEG and PBLG-*graft*-PEG were synthesized. The self-association behaviours of PBLG-*block*-PEG/PBLG-*graft*-PEG blend in ethanol were investigated by TEM and viscometry. TEM observation reveals that the polypeptide copolymer blend could self-assemble to form polymeric micelles with a core-shell structure in the shape of tree-like in ethanol. Viscosity measurements show that the increase of the testing temperature promotes the formation of the micelles of the polypeptide copolymer blend and the augment of PBLG-*graft*-PEG content increases the critical micelle concentration of the polypeptide copolymer blend.

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