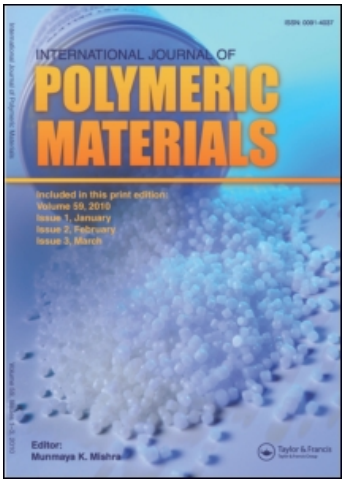

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Guoquan Zhu[a]; Qiaochun Gao[a]; Zhihe Li[a]; Fagang Wang[a]
[a] School of Materials Science and Engineering, Shandong University of Technology, Zibo, P. R. China

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Factors Influencing the Surface Morphology of Poly(γ-benzyl *L*-glutamate)-*Block*-(Random Coil Polymer) Aggregates Deposited from the Dilute Solution

Guoquan Zhu, Qiaochun Gao, Zhihe Li, and Fagang Wang

School of Materials Science and Engineering, Shandong University of Technology, Zibo, P. R. China

Poly(γ-benzyl L-glutamate)-*block*-poly(ethylene glycol) (PBLG-*block*-PEG, PEG as random coil polymer) was synthesized by a standard N-carboxyl-γ-benzyl-L-glutamate anhydride method. The surface morphology of PBLG-*block*-PEG copolymer aggregates from the dilute solution was studied by scanning electron microscopy (SEM). The effects of precipitation temperature, precipitation time, various solvent systems, and copolymer solution concentration on the surface morphologies of the polypeptide block copolymer aggregates from the dilute solution were investigated.

Keywords aggregates, dilute solution, factors, polypeptide block copolymer, surface morphology

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Address correspondence to Guoquan Zhu, School of Materials Science and Engineering, Shandong University of Technology, Zibo 255049, P. R. China. E-mail: guoquanzhu888@163.com

INTRODUCTION

Based on outstanding biocompatible and biodegradable properties, polypeptides and their copolymers have received lots of attention for their potential applications [1–22]. The synthesized polypeptides and their copolymers have been studied widely in the fields of functional biomaterials, protein simulation, polymer carriers for protein conjugates, macromolecular conformational research, catalysis, and artificial skin substrates [23–29].

Over the past decades, the amphiphilic polypeptide copolymers composed of hydrophobic polypeptide segments and hydrophilic polymer chains have attracted much interest [30–33]. Rodriguez-Hernandez and Le Commandoux have reported the formation of schizophrenic vesicles based on a zwitterionic diblock copolymer poly(L-glutamic acid)-*block*-poly(L-lysine). The hydrophobicities of the two polypeptide blocks vary with the changes in the pH value in aqueous medium, leading to the formation of vesicles with different supramolecular structures [34]. Cho et al. reported the formation of polymeric micelles composed of poly(γ-benzyl L-glutamate) and poly(ethylene glycol) in aqueous medium and the drug delivery system based on the core-shell nanoparticles with PBLG as the hydrophobic inner core and PEG as the hydrophilic outer shell [4]. Kwon et al. have reported that poly(β-benzyl L-aspartate) (PBLA)/poly(ethylene oxide) (PEO) diblock copolymers could self-assemble to form polymeric micelles with an outer shell of PEO and an inner core of PBLA in aqueous medium [7].

In order to understand the properties of polypeptides and control them, it is essential to characterize their morphology and structure, and to elucidate the relationship between their properties and the morphology and structure [35–37]. Geil et al. have reported the morphologies of polypeptide homopolymer aggregates in dilute solution [36,37]. However, to the best of our knowledge, no experimental work has so far been reported on the studies of the factors influencing the surface morphologies of polypeptide block copolymer aggregates deposited from the dilute solution. As the molecular structure of polypeptide block copolymer and homopolymer is different, it is still interesting to study the surface morphology of polypeptide block copolymer from the dilute solution. In this work, poly(γ-benzyl *L*-glutamate)-*block*-poly(ethylene glycol) (PBLG-*block*-PEG) copolymer has been synthesized. SEM technique was used to study the surface morphologies of PBLG-*block*-PEG copolymer aggregates from the dilute solution. The effects of precipitation temperature, precipitation time, various solvent systems, and copolymer solution concentration on the surface morphologies of the polypeptide block copolymer aggregates from the dilute solution were investigated.

EXPERIMENTAL

Materials

The amine-terminated α-methoxy-ω-amino poly(ethylene glycol) (AT-PEG, $Mw = 20000$) was purchased from Sigma Inc., 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 Copolymer

PBLG-*block*-PEG copolymer (shown in Figure 1) was prepared by a standard N-carboxyl-γ-benzyl-L-glutamate anhydride (NCA) method [12,18]. Briefly, PBLG-*block*-PEG copolymer was obtained by the ring-opening polymerization of γ-BLG NCA initiated by AT-PEG ($M_w = 20000$) in *1,4*-dioxane at room temperature. The reaction mixture was poured into a large volume of anhydrous ethanol. The precipitated product was dried under vacuum and then purified twice by repeated precipitation from a chloroform solution into a large volume of anhydrous methanol. The molecular weight of PBLG-*block*-PEG copolymer was estimated by nuclear magnetic resonance (NMR) measurements (Avance 550) [12,18]. It was calculated by the peak intensities of the methylene proton signal (5.1 ppm) of polypeptide and the ethylene proton signal (3.64 ppm) of PEG in the ^{1}H-NMR spectra [12]. The molecular weight of PBLG-*block*-PEG copolymer used in the study was 120000.

Polypeptide Copolymer Solution Preparation

Polypeptide copolymer solutions were prepared according to the previous documents [36,37]. Briefly, copolymer solutions containing 10^{-4} to 10^{-3} g/ml were prepared by heating a weighted amount of polypeptide copolymer in a solvent or solvent mixture at 100°C for $20 \sim 30$ min with stirring. These solutions were then filtered hot through a coarse fritted-glass disk and transferred

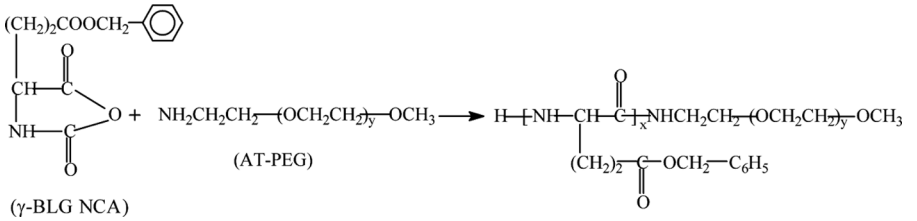


FIGURE 1: Synthesis of PBLG-*block*-PEG copolymer.

to stoppered glass tubes. Precipitations were carried out between 20 and 90°C in oil baths regulated to better than 0.5°C. In most cases precipitation was almost quantitative within 72 h. The precipitate suspension was used in SEM measurements and slightly vibrated before use.

SEM Photomicrographs

Testing specimens were obtained by depositing drops of precipitate suspension onto clean glass plates and drying them at room temperature [36,37]. Gold was sprayed on samples in vacuum. The investigation was carried out using a scanning electron microscope (Sirin 200, FEI, Holland). Acceleration voltage was 10 kV.

RESULTS AND DISCUSSION

Effects of Precipitation Temperature on the Surface Morphology of Polypeptide Copolymer Aggregates Deposited from the Dilute Solution

Figure 2 shows the photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited from 7×10^{-4} g/ml various solutions: (a) mesitylene solution at 90°C and (b) mesitylene solution at 40°C, where the precipitation time is 72 h. As can be seen from Figure 2, the surface morphologies of polypeptide copolymer aggregates from the dilute solution present fibrillar strands, and the surface morphologies are alterant with changing the precipitation temperature. As described by Sun et al. [35], PBLG segments in α-helical conformation aggregated to form fibrillar strands and PEG segments in random coil conformation are deposited on the surface of PBLG aggregates. Also seen from Figure 2, it is found that the fibrillar strands at higher precipitation temperature become thinner, shorter, and have a less aggregating density than at a lower precipitation temperature. Usually, the molecular moving ability increases with the augment of temperature and the aggregation ability relatively decreases in dilute solution, suggesting the surface morphologies of polypeptide copolymer aggregates from the dilute solution definitely depend on the precipitation temperature [36,37].

Effects of Mixed Solvent System on the Surface Morphology of Polypeptide Copolymer Aggregates Deposited from the Dilute Solution

Figure 3 presents the photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 70°C from 6×10^{-4} g/ml various

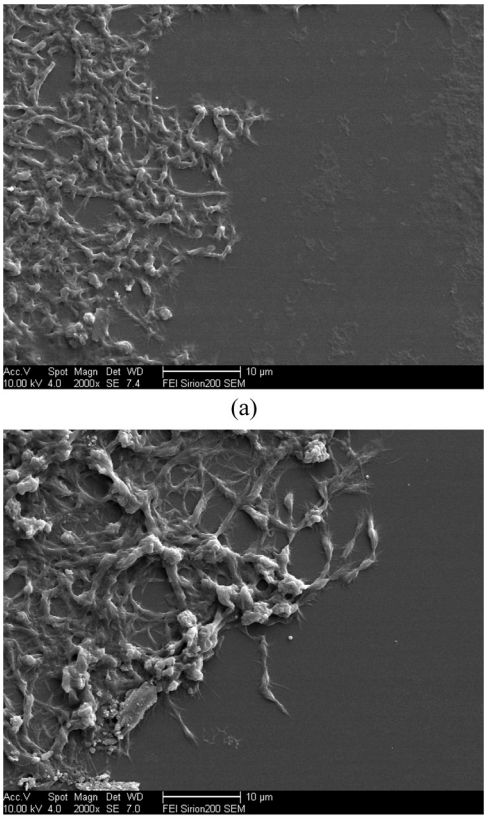


FIGURE 2: SEM photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited from 7×10^{-4} g/ml various solutions: (a) mesitylene solution at 90°C and (b) mesitylene solution at 40°C, where the precipitation time is 72 h (magnification 2000×).

solutions: (a) mesitylene/xylene (vol. ratio is 3:1), (b) mesitylene/xylene (vol. ratio is 2:1), and (c) mesitylene/xylene (vol. ratio is 1:1), where the precipitation time is 72 h. As seen from Figure 3, the surface morphologies of polypeptide copolymer aggregates in mixed solvents system show fibrillar ribbons or loops, and the surface morphologies are mutative with changing the mixed solvents components. With the increase of xylene volume content in the mixed solvents system, it is found that the fibrillar ribbons become thinner and less congregating, and the fibrillar loops become more loose, indicating that the increase of xylene volume content promotes the formation of thinner fibrillar ribbons or loose fibrillar loops. As known, different solvents hold a different speciality, suggesting that the surface morphologies of the polypeptide copolymer aggregates from the dilute solution also depend on the solvents' speciality.

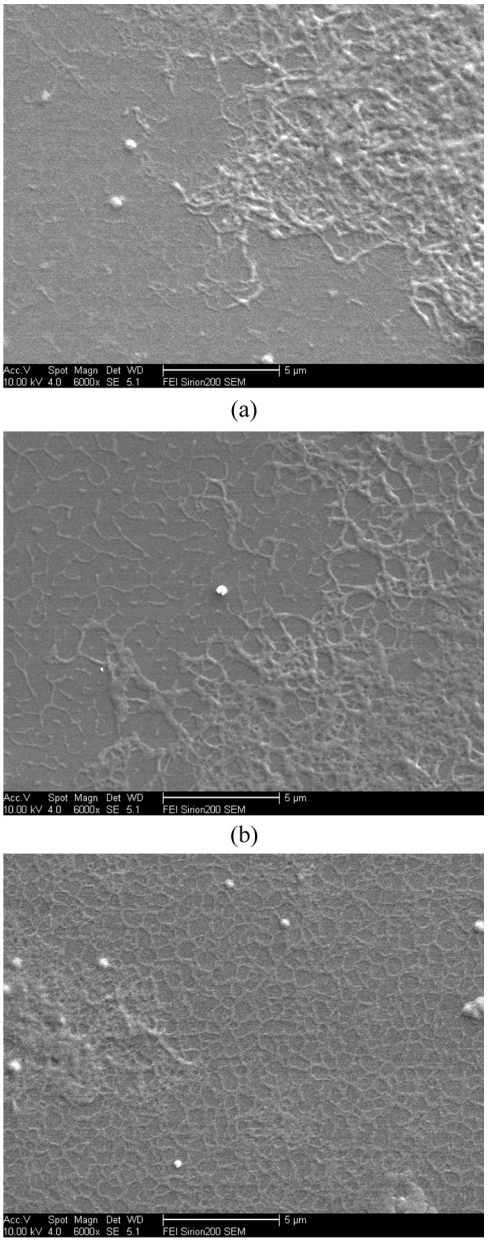
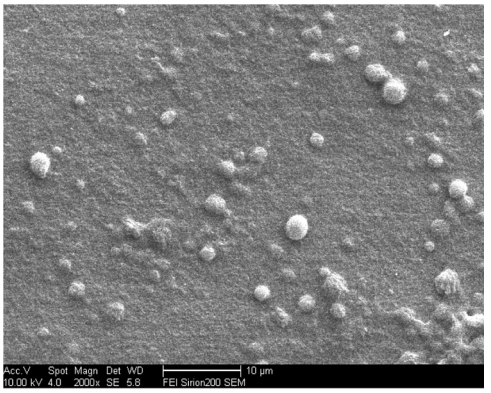
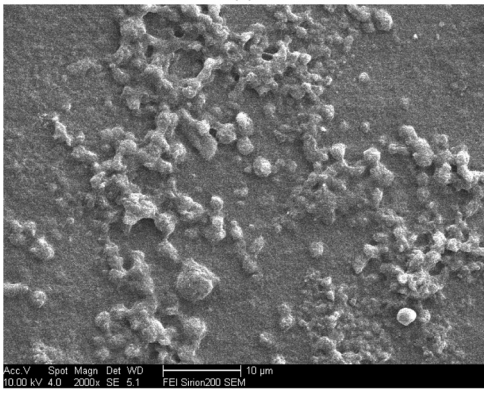


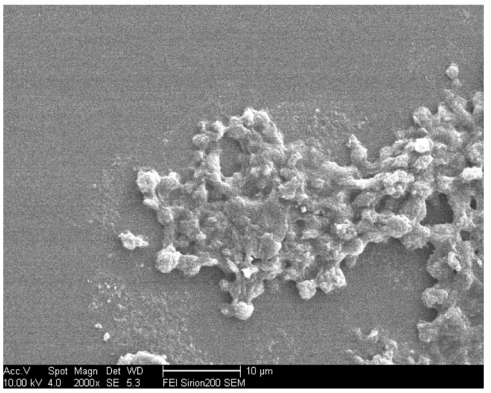
FIGURE 3: SEM photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 70°C from 6×10^{-4} g/ml various solutions: (a) mesitylene/xylene (vol. ratio is 3:1), (b) mesitylene/xylene (vol. ratio is 2:1), and (c) mesitylene/xylene (vol. ratio is 1:1), where the precipitation time is 72 h (magnification 6000×).



(a)



(b)

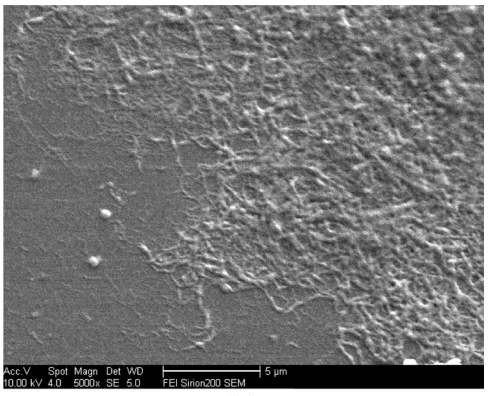


(c)

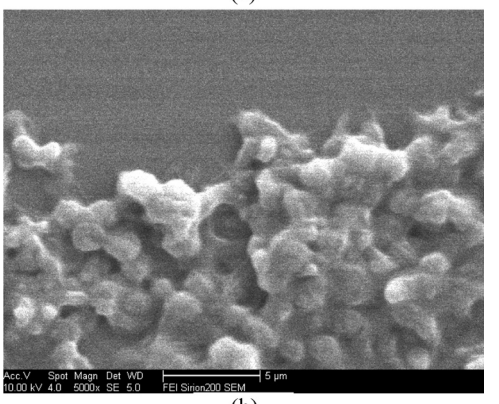
FIGURE 4: SEM photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 30°C from 7.5×10^{-4} g/ml various solutions: (a) xylene, (b) xylene/hexane (vol ratio is 19:1), and (c) xylene/hexane (vol. ratio is 9:1), where the precipitation time is 72 h (magnification 2000×).

Effects of Solvent/Nonsolvent Mixed System on the Surface Morphology of Polypeptide Copolymer Aggregates Deposited from the Dilute Solution

Figure 4 indicates the photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 30°C from 7.5×10^{-4} g/ml various solutions: (a) xylene, (b) xylene/hexane (vol. ratio is 19:1), and (c) xylene/hexane (vol. ratio is 9:1), where the precipitation time is 72 h. As is shown in Figure 4, the surface morphologies of polypeptide copolymer aggregates from solvent/nonsolvent mixed system (hexane as the nonsolvent) present various shaped spheres. With the increase of the hexane volume content in the mixed system, it is revealed that the shape of spheres becomes more irregular and the aggregation density of the spheres becomes more large, suggesting the nonsolvent promotes the aggregation of copolymer molecules. This phenomenon proved that the introduction of the nonsolvent into the mixed system changes the sur-



(a)



(b)

FIGURE 5: SEM photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 35°C from 8×10^{-4} g/ml various solutions: (a) mesitylene/xylene (vol. ratio is 3:1) after 100 h and (b) mesitylene/xylene (vol. ratio is 3:1) after 720 h (magnification 5000×).

face morphologies of the polypeptide copolymer aggregates from the dilute solution [36,37].

Effects of Precipitation Time on the Surface Morphology of Polypeptide Copolymer Aggregates from the Dilute Solution

Figure 5 reveals the photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 35°C from 8×10^{-4} g/ml various solutions: (a) mesitylene/xylene (vol. ratio is 3:1) after 100 h and (b) mesitylene/xylene (vol. ratio is 3:1) after 720 h. As seen from Figure 5, the surface morphologies of the polypeptide copolymer aggregates from the dilute solution become much different with the change of the precipitation time. With the increase of the precipitation time, it is found that the surface morphologies of the polypeptide

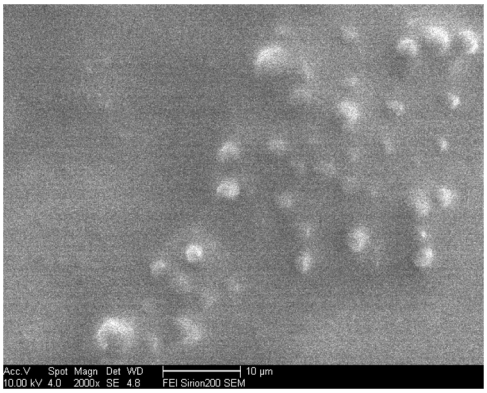


FIGURE 6: SEM photographs of the surface of PBLG-block-PEG copolymer aggregates deposited at 35°C from various solutions: (a) 1.2×10^{-4} g/ml toluene solution and (b) 9×10^{-4} g/ml toluene solution, where the precipitation time is 72 h (magnification 2000×).

aggregates from the dilute solution change from fibrillar ribbons or loops to tightly congregating irregular spheres. As the increase of the precipitation time upgrades the solvent power of a solvent and further promotes the associations of copolymer molecules, indicating that the increase of the precipitation time could exert a marked effect on the surface morphologies of the polypeptide copolymer aggregates from the dilute solution.

Effects of Copolymer Solution Concentration on the Surface Morphology of Polypeptide Copolymer Aggregates from the Dilute Solution

Figure 6 presents the photographs of the surface of PBLG-*block*-PEG copolymer aggregates deposited at 35°C from various solutions: (a) 1.2×10^{-4} g/ml toluene solution and (b) 9×10^{-4} g/ml toluene solution, where the precipitation time is 72 h. As is shown in Figure 6, the surface morphologies of the polypeptide aggregates from the dilute solution are variational with the change of the copolymer solution concentration. At lower copolymer solution concentration, the surface morphology of the polypeptide copolymer aggregates shows regular and loosely congregating spheres. At higher copolymer solution concentration, the surface morphology of the polypeptide copolymer aggregates reveals irregular and tightly congregating spheres. This phenomenon testified that the copolymer solution concentration also exerts a marked effect on the surface morphologies of the polypeptide copolymer aggregates from the dilute solution.

CONCLUSIONS

Poly(γ-benzyl-*L*-glutamate)-*block*-poly(ethylene glycol) (PBLG-*block*-PEG) copolymer was synthesized. The surface morphologies of PBLG-*block*-PEG copolymer aggregates from the dilute solution were studied by SEM technique. Experimental results demonstrated that precipitation temperature, precipitation time, various solvent system, and copolymer solution concentration all exerted marked effects on the surface morphologies of the polypeptide block copolymer aggregates from the dilute solution. The changes of these factors affected the congregating of polypeptide block copolymer segments, and further changed the surface morphologies of the polypeptide block copolymer aggregates from the dilute solution.

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