

단 신

소수성부분 PBLG와 친수성부분 PEO로 구성된 Hexablock 공중합체의 미셀형성

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Micelle Formation of the Hexablock Copolymer Consisting of Poly(γ -benzyl L-glutamate) as the Hydrophobic Part and Poly(ethylene oxide) as the Hydrophilic Part

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INTRODUCTION

Amphiphilic block copolymer, containing hydrophobic and hydrophilic components, exhibits surfactant behavior¹⁻³ and may form micelles. In aqueous media, these materials contain the structures comprised of hydrophobic cores surrounded by water-soluble polar groups which extend into the medium. The block copolymers are known to form micellar structures at very low values of critical micelle concentrations (CMC) than those for low molecular weight surfactants.⁴ Also, the block copolymer micelles are stable and dissociated slowly to free polymeric chains.^{5,6}

Ringsdorf et al. first reported that the poly-

meric micelles formation can be used to achieve the sustained release of drug from the polymer-drug conjugate.⁷ Recently, AB block copolymer micelles composed of poly(β -benzyl L-aspartate) (PBLA) and poly(ethylene oxide) (PEO) were used as vehicles to carry hydrophobic drugs which were conjugated with the polymer⁸ or entrapped physically in the polymer.⁹

Novel drug carrier systems such as polymeric micelles, nanoparticles, liposomes and modified particles have been considerably attracted because of the potential usage in site-specific or targeted drug delivery system.¹⁰⁻¹⁴ Advantages of these systems are biodistribution of drugs and prevention of undesirable side-ef-

fects.^{15,16} However, they have been restricted by the factors such as blood circulation, structurally fragile, damage by attract of reticuloendothelial systems and lower loading efficiency. To overcome these difficulties, core-shell type polymeric carriers or polymeric micelles have been proposed and intensively investigated in recent years.^{10,17} The major advantages of the system are thermodynamic stability, solubilization of hydrophobic drugs, modified biodistribution, stable storage and ability to deliver drugs with low interactions with biocomponents such as proteins and cells.¹⁸ Recently, the diafiltration method was developed for the simple preparation of micelles and also applied to the drug delivery system.¹²

In this study, we wish to report micelle formation of poly(γ -benzyl L-glutamate)(PBLG)/PEO/PBLG hexablock copolymer by the diafiltration method in water. It is expected that the PBLG/PEO/PBLG block copolymer micelles are applicable to the passive-targetable drug carriers.

EXPERIMENTAL

Materials. PBLG/PEO/PBLG (abbreviated as GEG) block copolymers were prepared by polymerization of γ -benzyl L-glutamate N-carboxyanhydride (γ -BLG NCA) initiated with amine-terminated PEO in a methylene dichloride solution by the method described previously.¹⁹⁻²¹ Clonazepam (CZ) was obtained from ROCHE, Switzerland. Dimethylformamide (DMF), reagent grade, was used as received.

Preparation of CZ-Block Copolymer Incorporated Micelles. The 20 mg of PBLG/PEO/PBLG was dissolved in 4 mL of DMF and sub-

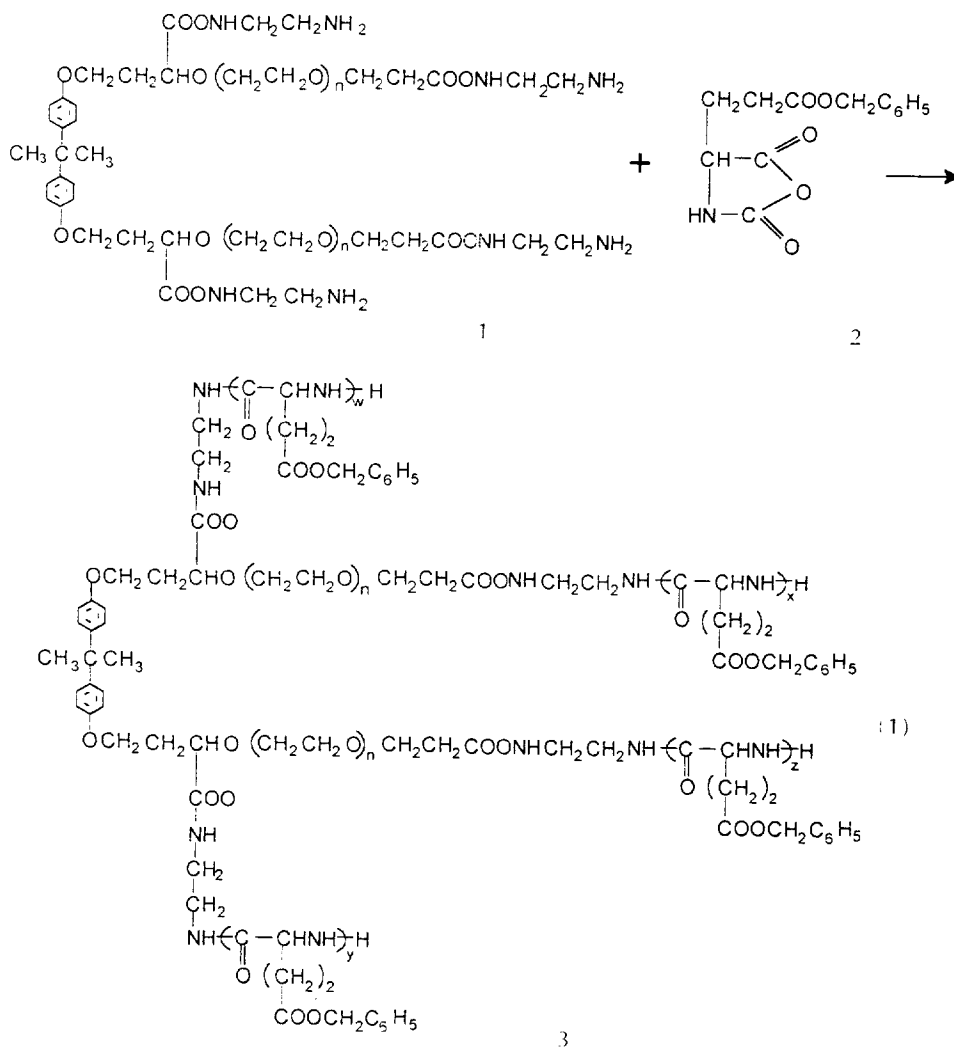
sequently 20 mg of CZ was added. The solution was stirred at room temperature. To form micelles and to remove free CZ, the solution was dialyzed using dialysis membrane (MWCO; 12~14,000) with distilled water for 24hrs by the similar method reported previously.^{9,12} Then, the solution was freeze-dried. CZ was selected as the anticonvulsant benzodiazepine which is efficacious for the treatment of panic disorder, because it had considerably hydrophobic character (water solubility: 14.7 μ g/mL) and high interactions with proteins *in vivo*.

Measurement of Fluorescence Spectroscopy. Fluorescence measurements were carried out using CZ as probes to analyze PBLG/PEO/PBLG micelles in doubly distilled water. Emission spectra were measured at varying copolymer concentrations by fluorometer (Shimadzu RF-5000). Analysis of the concentration of CZ were done with excitation wavelength of 306 nm. The effects of sodium dodecyl sulfate (SDS) on the fluorescence of CZ were investigated. All of the fluorescence experiments were carried out at room temperature.

Measurement of Surface Tension. The surface tension of the GEG-3 (PEO contents : 75.3 mol%) was measured by the Wilhelmy method using platinum plate at 25°C (KYOWA CBVP-A3).²² The CMC value of the GEG-3 was estimated by the inflection point which was obtained by the surface tension at the interface between chloroform and water at the various concentrations of the copolymers.

RESULTS AND DISCUSSION

Block copolymers(3) were prepared by polymerization of γ -BLG NCA(2) in methylene



Scheme 1

chloride solution as shown in Scheme 1. Amine-terminated PEO(1) was used as the initiator. It is assumed that the polymerization mechanism is the primary-amine mechanism in which the initiator amine undergoes a nucleophilic addition to the C-5 carboxyl group of the NCA, as suggested by Goodman et al.²³ Compositions and molecular weights of the copolymers are listed in Table 1. The copolymer composition and the molecular weight were estimated from

Table 1. Characterization of PBLG/PEO/PBLG (GEG) Block Copolymers

Sample	Content of monomeric units in mol %		\bar{M}_n
	PBLG	PEO	
GEG-1	80.9	19.1	441,900
GEG-2	48.2	51.8	112,600
GEG-3	24.7	75.3	52,600

* Molecular weights and compositions were estimated by NMR measurement.

* M. W. of PEO: 20,000.

peak intensities of the phenyl proton signal of the PBLG block and the methylene proton sig-

nal of the PEO block in the NMR spectrum. Assuming that all the amine groups of PEO participate in the polymerization, the number-average molecular weights, \overline{M}_n , of the copolymers are calculated from the copolymer composition and the molecular weight of PEO chains. Also, it is possible that heterogeneity in the block chain lengths of PBLG may hold for the block copolymers.

In Fig. 1 the emission spectra of CZ are shown in aqueous solutions. CZ displays its characteristic spectrum which shows peak at ca. 348 nm and broad band between 400 nm and 500 nm, indicating the existence of excimer of CZ in the solution. The shape of the spectrum did not change with the concentration of CZ in the aqueous solution.

In Fig. 2 the emission spectrum of CZ is shown in the presence of various concentrations of GEG-3. It is regarded that by bringing CZ and PBLG/PEO/PBLG into an aqueous milieu from a good solvent for both species (in this case DMF), CZ could be entrapped in polymeric micelles. And this was done using a simple dialysis procedure. As the DMF is removed, PBLG/PEO/PBLG micelles form and have a prospect of incorporating CZ. Furthermore, CZ was preferentially partitioned into hydrophobic microdomains with a concurrent change in the molecule's photophysical properties.²⁴ The total fluorescence intensity was increased and another peak at ca. 370 nm appeared with increasing the concentration of GEG-3. This may reflect a change in the vibrational structure of CZ monomer emission upon micellization of GEG-3. The preferential partitioning of CZ into hydrophobic domains was used to determine micropolarities (hydrophobicity of the micellar cores) of molec-

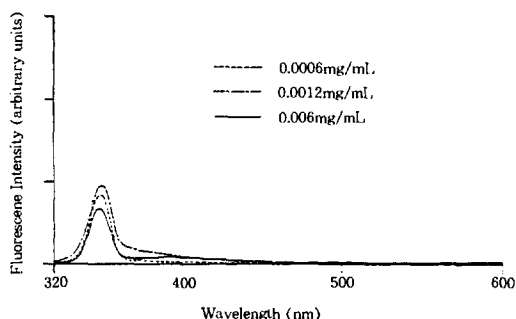


Fig. 1. Fluorescence emission spectra of CZ against concentration of CZ in distilled water. Excitation wavelength : 306 nm.

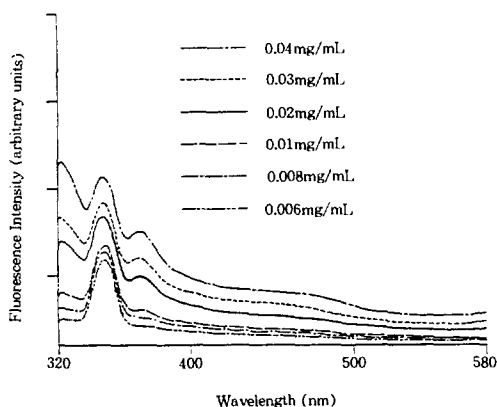


Fig. 2. Fluorescence emission spectra of CZ/GEG-3 against concentration of GEG-3 in distilled water. Excitation wavelength : 306 nm.

ular assemblies, indicating that CMC of the GEG-3 block copolymer. It is already reported that the use of the fluorescence probe pyrene, the emission characteristics of which change with the onset of association of the block copolymer.²⁵ The CMC for this polymer, as determined by fluorescence probe technique, is 0.02 mg/mL (3.8×10^{-7} mol). The CMC for this polymer was also determined by the interfacial surface tension between chloroform and water as shown in Fig. 3. The CMC value (0.025 mg/mL : 4.8×10^{-7} mol) of the polymeric micelle is almost same as that of the fluorescence probe technique.

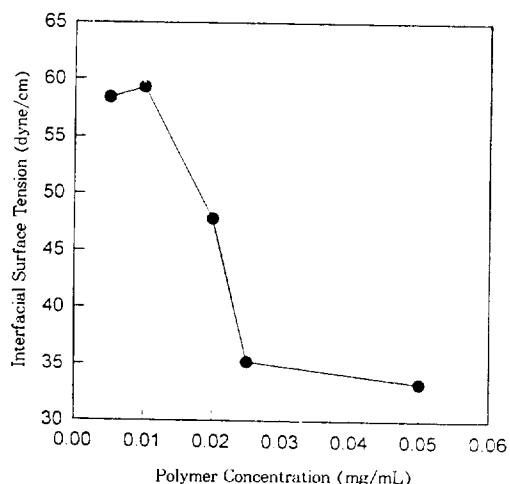


Fig. 3. Interfacial surface tension of GEG-3 against concentration of GEG-3 at the water/CHCl₃ interface.

Table 2. The CMC Values for the Block Copolymer Micelles

Sample	CMC(mol)	
	Fluorescence	Surface tension
GEG-1	2.3×10^{-8}	—
GEG-2	1.8×10^{-7}	—
GEG-3	3.8×10^{-7}	4.8×10^{-7}

The CMC values for the polymeric micelles according to the composition of the copolymers are shown in Table 2. As shown in Table 2, the CMC for the polymers decreased with increasing PBLG chain length as the hydrophobic part in the block copolymer. It is thought that the block copolymer micelles can be easily formed with an increase of hydrophobic components.

When SDS was added to the aqueous solutions of CZ/GEG-3, the decrease in fluorescence intensity at ca. 370 nm was evident as shown in Fig. 4, indicating the partial disruption of the GEG-3 micelles containing CZ. In contrast, the fluorescence peak of the free CZ at 348 nm disappeared by the addition of SDS, indicating that strong interactions between CZ and

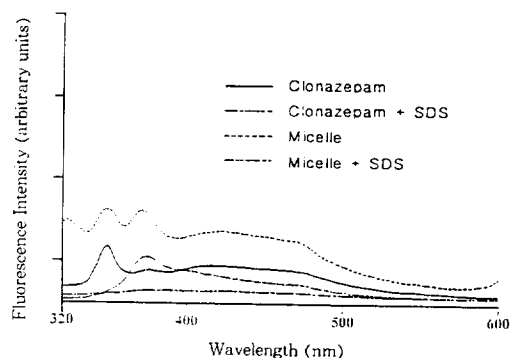


Fig. 4. Fluorescence emission spectra of CZ and CZ/GEG-3 in distilled water with and without SDS (20 mg/mL). Excitation wavelength : 306 nm.

SDS.

The polymeric micelles formed by diafiltration are small and show spherical structures which may aggregate or grow into core-shell type micelles comprising inner-core as the hydrophobic domains and outer-shell as the hydrophilic ones. Also, the micelles do not show large asymmetries because large deviations from the spheroidal shapes are normally energetically and entropically unfavored.¹²

In summary, the hexablock copolymer based on PBLG as the hydrophobic part and PEO as the hydrophilic one was synthesized and characterized. The study using the fluorescent probes suggested that PBLG/PEO/PBLG copolymers associate in water to form micelles and the CMC values of the block copolymers decreased with increasing PBLG chain length in the block copolymers. The shape of the micelles observed by electron microscopy, size of the polymeric micelles determined by dynamic light scattering and release of CZ from polymeric micelles will be reported in the near future.

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