

## Polymeric Micelle Formation of Multiblock Copolymer Composed of Poly( $\gamma$ -benzyl L-glutamate) and Poly(ethylene oxide)

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Multiblock copolymers consisting of poly( $\gamma$ -benzyl L-glutamate) (PBLG) as the hydrophobic part and poly(ethylene oxide) (PEO) as the hydrophilic part (GEG) were synthesized and characterized. GEG polymeric micelles were prepared by the dialysis technique. Particle size distributions based on intensity, volume, and number-average were  $22.6 \pm 11.9$  nm,  $23.5 \pm 4.6$  nm, and  $23.7 \pm 3.7$  nm, respectively. It was observed that particle size and size distribution of GEG polymeric micelles changed significantly with the choice of initial solvent. Transmission electron micrographs (TEM) showed the polymeric micelles to be spherically shaped, with sizes ranging from 20 nm to 40 nm in diameter. Fluorescence spectroscopy measurements suggested that GEG block copolymers were associated in water to form polymeric micelles, and the critical micelle concentrations (CMC) value of the block copolymers was  $0.0094$  g/L. Further evidence of micelle formation of GEG block copolymers and limited mobility of the PBLG chain in the core of the micelle was obtained with  $^1\text{H}$  NMR in  $\text{D}_2\text{O}$ .

### Introduction

Polymeric micelles formed in aqueous systems have attracted considerable attention over the last decade.<sup>1-3</sup> Due to their amphiphilicity, block or graft copolymers, containing hydrophobic and hydrophilic components, exhibit surfactant behavior and form micelles.<sup>4-6</sup> In aqueous solutions, block or graft copolymers form structures comprising hydrophobic cores surrounded by water-soluble polar groups that extend into the aqueous medium. The block copolymers are known to form micellar structures at very low values of critical micelle concentrations (CMC) compared with surfactants of low molecular weight.<sup>7</sup> The block copolymer micelles are stable and slowly dissociate to free polymeric chains.<sup>8-9</sup>

Wilhelm *et al.* studied micelle formation of poly(styrene) (PS) and poly(ethylene oxide) (PEO) di- or triblock copolymers in water by fluorescence technique using pyrene as a hydrophobic probe. They determined CMC from the fluorescence and excitation spectra as pyrene partitions between aqueous and micellar environments.<sup>7</sup> These methods were also used by Kwon *et al.* to study the polymeric micelle formation of poly( $\beta$ -benzyl L-aspartate) (PBLA) and PEO diblock copolymer in water.<sup>10</sup> To investigate the micellar structure and behavior of PBLA/PEO diblock copolymer in water, Kwon *et al.* also used  $^1\text{H}$  NMR<sup>10</sup> and they reported that characteristic signals of PBLA segment of diblock copolymeric micelles in  $\text{D}_2\text{O}$  decreased to low and broad peaks compared with the observations in  $\text{CDCl}_3$ , which indicated the rigid structure and limited mobility of the PBLA core. In other reports, micellar solutions of diblock copolymers of PEO and PS exhibited  $^1\text{H}$  NMR signals for the PS block, indicating the core of the micelle was in a glassy state.<sup>11</sup> However,  $^1\text{H}$  NMR studies on micellar solutions of

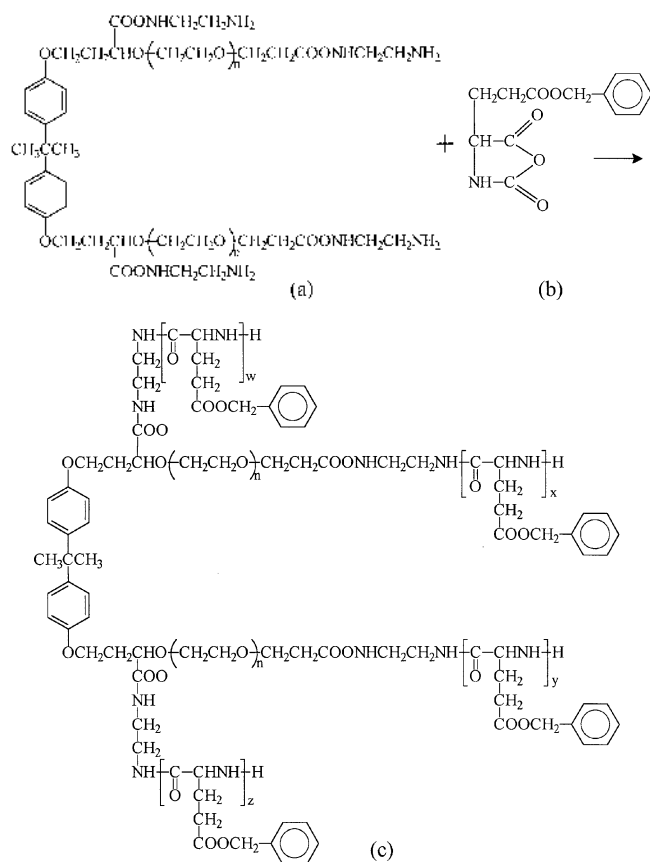
diblock copolymers of PEO and poly(propylene oxide) (PPO) suggest that the PPO core was in a liquid like state.<sup>12</sup>

Polymeric micelles composed of block copolymers have highly investigated as drug delivery drug vehicles because of their characteristic advantages, such as small size, thermodynamic stability, solubilization of hydrophobic molecules and a preventive against interaction with proteins and cells.<sup>13</sup> Especially, from the ability of their solubilization of hydrophobic molecules into the micellar core, hydrophobic drugs can be easily physically incorporated in polymeric micelles.<sup>14</sup> Polymeric micelles of block copolymers have been reported primarily for di- or triblock copolymers. Our group studied the synthesis of multiblock copolymers composed of poly( $\gamma$ -benzyl L-glutamate) (PBLG) and PEO and their polymeric micelle or core-shell type nanoparticle formation in aqueous system and report that the hydrophobic drug was physically entrapped in polymeric micelles and their drug release rate showed a pseudo zero-order pattern.<sup>15-17</sup> There have been reports of the polymeric micelle formation of multiblock copolymers in aqueous media with little understanding of the behavior.

For this study, we prepared multiblock copolymers composed PBLG/PEO/PBLG (abbreviated GEG), and their polymeric micelle formation and behavior in water were investigated using dynamic light scattering (DLS), a transmission electron microscope (TEM), fluorescence spectroscopy, and  $^1\text{H}$  NMR.

### Experimental Section

**Materials.** Bis[poly(ethylene oxide) bis(amine)] (BPEOBA; M.W.=20000) and  $\gamma$ -benzyl L-glutamate were purchased from Sigma Chem. Co. (St. Louis, MO). The



**Figure 1.** Synthesis scheme of GEG multiblock copolymer.

structure of Bis[poly(ethylene oxide) bis(amine)] is shown in Figure 1(a), according to the manufacturers report. Triphenylmethyl, *n*-hexane, dimethylformamide (DMF), dimethylacetamide (DMAc), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), 1,4-dioxane, and dichloromethane were purchased from Aldrich Chem. Co. Inc. (Milwaukee, WI). All chemicals used were of reagent or spectrophotometric grade.

**Synthesis of block copolymer.**  $\gamma$ -benzyl L-glutamate N-carboxyanhydride (BLG-NCA) was prepared according to the method proposed by Goodman and Hutchison.<sup>18</sup> The block copolymer (Figure 1(c)) was synthesized by the method reported previously.<sup>15,16</sup> The synthesis scheme is shown in Figure 1. In brief, the block copolymer was obtained by polymerization of BLG-NCA (b) initiated by the BPEOBA (a) in dichloromethane, at a total concentration of BLG-NCA and BPEOBA of 3% (W/V), for 72 hrs at room temperature. The reaction was progressed until the characteristic IR peak of BLG-NCA ( $1850\text{ cm}^{-1}$ ,  $1780\text{ cm}^{-1}$ ,  $915\text{ cm}^{-1}$ ) was no longer observed. The reaction mixture was poured into a large excess of diethyl ether to precipitate the block copolymer. The reaction mixture may contain unreacted BPEOBA and block copolymers. Since BPEOBA cannot be precipitated from the mixture of dichloromethane and diethyl ether (although the latter is a non-solvent for BPEOBA), the unreacted BPEOBA was removed by filtration with glass filter and the block copolymer was obtained

as precipitants. The resulting block copolymer was washed twice with diethyl ether and then dried *in vacuo* for 48 hrs.

**Preparation of polymeric micelles.** Polymeric micelles were prepared by the previously reported method.<sup>17,19</sup> Twenty mg of GEG block copolymer were dissolved in 10 ml of various solvent. The solution was stirred at room temperature and solubilized entirely. To form polymeric micelles, the solution was dialyzed using a molecular cutoff 12000 g/mol dialysis tube against 1.0 L  $\times$  3 of distilled water for 3 hrs. The distilled water was exchanged at intervals of 3–6 hrs during 2 days. The solution was used for analysis or freeze-dried for storage at 4  $^{\circ}\text{C}$  for the following analysis.

**$^1\text{H}$  NMR spectroscopy.**  $^1\text{H}$  NMR spectra of the copolymer for characterization of GEG block copolymer were measured in  $\text{CDCl}_3$  using 300 MHz  $^1\text{H}$  FT-NMR (Bruker). For approved micellar structure of GEG block copolymer micelles,  $^1\text{H}$  NMR spectra were measured in  $\text{CDCl}_3$  and  $\text{D}_2\text{O}$ . The polymeric micelle concentration was 0.5 wt.-% in  $\text{CDCl}_3$  and  $\text{D}_2\text{O}$ .

**Dynamic light scattering (DLS) measurements.** DLS was measured with a S4700 (Malvern Instruments, England) with an argon laser beam at a wavelength of 488 nm at 20  $^{\circ}\text{C}$ , and the sample concentration was 1.0 g/L. A scattering angle of  $90^{\circ}$  was used.

**Transmission electron microscope (TEM) observation.** The morphology of the polymeric micelles was observed using a TEM (JEOL, JEM-2000 FX II, Japan). A drop of polymeric micelle suspension in aqueous solution was placed on a carbon film coated on a copper grid for TEM and freeze-dried. The specimen on the copper grid was not stained. Observation was done at 80 kV.

**Measurement of fluorescence spectroscopy.** To investigate the fluorescence spectroscopy characteristics, GEG block copolymer solutions were adjusted to the various concentrations of block copolymers.

CMC of the GEG block copolymers was estimated to ascertain the potential of micelle formation by the measurement of fluorescence spectroscopy (Shimadzu F-7000 spectrofluorometer, Shimadzu Co. Ltd., Tokyo, Japan), using pyrene as a probe.<sup>7,20,21</sup> To obtain sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 mL vials and the acetone evaporated. The amount was adjusted to give a pyrene concentration in the final solution of  $6.0 \times 10^{-7}$  M. Ten ml of various concentrations of block copolymer solutions were added to each vial and then heated for 3 hrs at 65  $^{\circ}\text{C}$  to equilibrate the pyrene and the micelles and then left to cool overnight at room temperature. For the fluorescence spectra, the excitation wavelength was 339 nm. The emission wavelength was 390 nm for the excitation spectra. Excitation and emission bandwidths were 1.5 and 1.5 nm, respectively.

## Results and Discussion

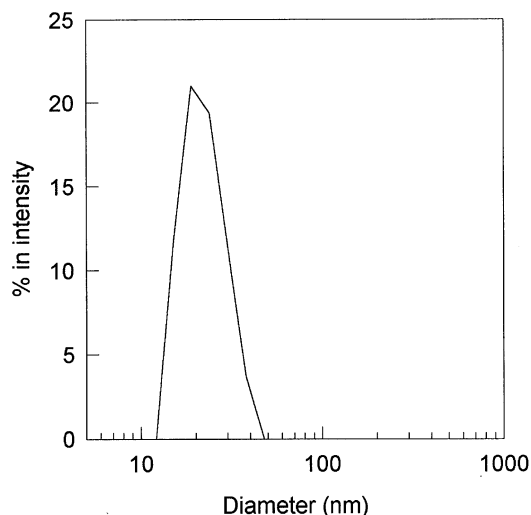
$\gamma$ -BLG NCA was initiated with amine-terminated PEO in dichloromethane solution for GEG block copolymers as shown in Figure 1. It may be assumed that the polymeriza-

tion 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 reported previously.<sup>15-18</sup> The number-average molecular weights,  $M_n$ , of the copolymer were determined by the intensity ratio of the <sup>1</sup>H NMR peaks of the methylene protons ( $\delta = 3.7$  ppm) of PEO block and the methyl protons ( $\delta = 5.1$  ppm) of PBLG block. The  $M_n$  of the GEG block copolymer was determined to be 27500, and the weight content of PEO was approximately 72.7 wt.-%.

Generally, AB type diblock copolymers and ABA type triblock copolymers exhibit micellar behavior in selective solvent. For finite concentrations larger than the CMC, the AB type di- or ABA type triblock copolymers associated into spherical micelles, which consisted of a meltlike inner-core of insoluble B blocks and exterior corona of soluble A blocks swollen by the solvent. On the other hand, BAB type block copolymers also form micelles in the selective solvent.<sup>22,23</sup> Saito and Ishizu<sup>23</sup> report that BAB type block copolymers comprising poly(2-vinyl pyridine-*b*-styrene-*b*-2-vinyl pyridine) form polymeric micelles in selective solvent, which is good for A sequences but not for B sequences, as AB type diblock copolymers. In this case, BAB type block copolymers form flower type or bridged flower type polymeric micelles whose shells form a loop structure.<sup>22,25</sup> Also, unlike with PEO-PPO or PEO-PPO-PEO, aqueous suspensions of the reversed block copolymer architecture, PPO-PEO-PPO, show rather different phase behavior.<sup>24,26</sup> This system is also dominated by spherical micelles in a wide region of the phase diagram.<sup>24</sup> Mortensen<sup>24,26</sup> reports that aqueous solutions of PPO-PEO-PPO associate into a homogeneous phase constituting an interconnected network of micelles in which micellar cores of hydrophobic PPO are interconnected by hydrophilic PEO strands.

Halperin<sup>25</sup> suggests that multiblock copolymers form molecular micelles incorporating blocks belonging to a single copolymer. These molecular micelles composed of multiblock copolymers are expected to be structurally similar to be architecture to BAB type triblock copolymers whose coronal blocks form loops anchored to the innercore. In GEG block copolymers, which have a molecular architecture similar to BAB type triblock copolymer, flower type or bridged flower type polymeric micelles in water are expected.

In a previous report,<sup>17</sup> it is observed that the GEG block copolymeric micelles show increased particle size and size distribution with an increased in the molecular weight of polymers. GEG block copolymers of relatively higher molecular weight than in this study have a small particle size, about 40-100 nm, and lower molecular weight of block copolymer, resulting in decreased particle size. It is shown however, that size distributions of GEG polymeric micelles with high molecular weight is not narrow, which may be due to the secondary aggregation. But, in this study, of GEG block copolymers of lower molecular weight, polymeric micelles have relatively narrow size distribution without secondary aggregation, and the particle size was smaller.

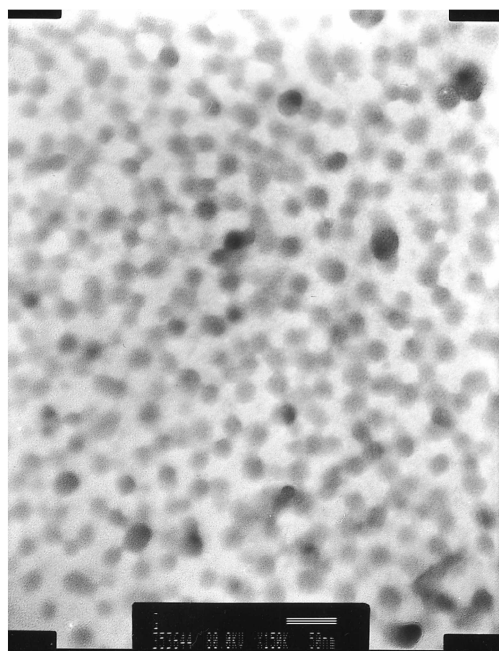


**Figure 2.** Particle size distribution of GEG polymeric micelles prepared from DMF as the initial solvent.

GEG polymeric micelles were prepared using DMF as reported previously.<sup>17</sup> The particle size of GEG block copolymer was  $22.6 \pm 11.9$  nm,  $23.5 \pm 4.6$  nm, and  $23.7 \pm 3.7$  nm, respectively. Figure 2 shows the particle size distribution of GEG polymeric micelles (prepared from DMF) based on intensity average by DLS. In this result, GEG polymeric micelles have a small particle size without secondary aggregation as a monomodal distribution, which was typically observed in the di- or triblock copolymer micelles. Also, the preparation of polymeric micelles was further investigated using various solvents because the initial solvent used to prepare micelles in water drastically affects the stability of the polymeric micelles. The effect of solvent on the particle size of GEG polymeric micelles is summarized in Table 1. When DMF and DMAc were used as the initial solvent to prepare micelles in water, the particle size of the polymeric micelles was relatively smaller than with other solvents. In the study of polymeric micelles using PBLA and PEO diblock copolymers, La *et al.*<sup>27</sup> also report that small and narrow particle size distribution was obtained using DMAc as the initial solvent for preparation of polymeric micelles. But, in the case of DMF as the initial solvent for micelle formation, the results showed increased particle size and secondary aggregation, which is the opposite of our results. Of course, a direct comparison cannot be performed because different polymer characteristics and molecular architectures were

**Table 1.** Effect of solvent on the particle size of GEG polymeric micelles

Solvent	Particle size (nm)		
	Intensity average	Volume average	Number average
DMF	$22.6 \pm 11.9$	$23.5 \pm 4.6$	$23.7 \pm 3.7$
DMAc	$18.2 \pm 11.6$	$15.1 \pm 4.3$	$13.5 \pm 1.0$
DMSO	$46.8 \pm 2.9$	$48.1 \pm 1.4$	$47.7 \pm 1.9$
1,4-Dioxane	$153.2 \pm 29.2$	$152.7 \pm 39.3$	$151.5 \pm 37.9$
THF	$99.7 \pm 23.8$	$100.1 \pm 47.0$	$99.6 \pm 46.2$

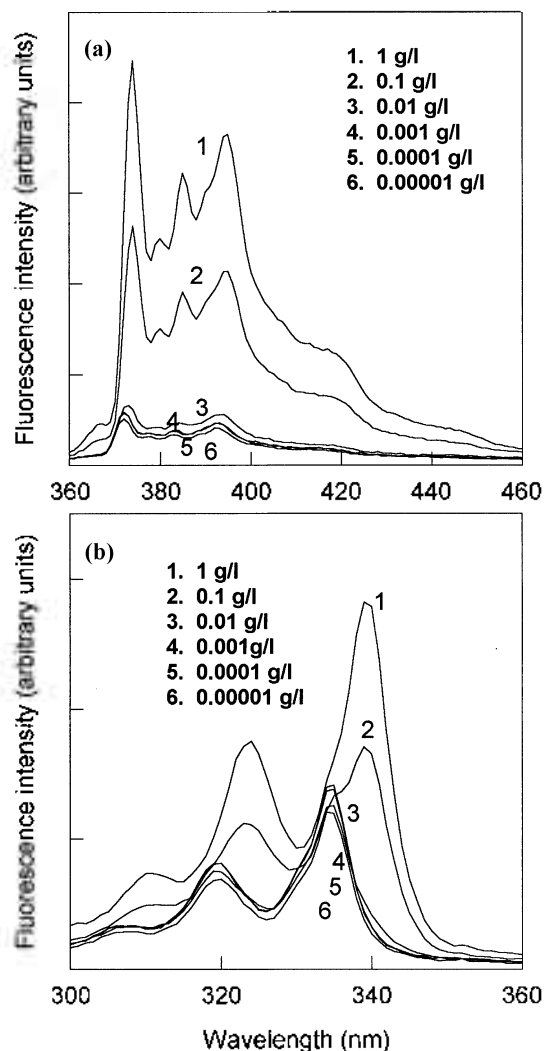


**Figure 3.** Transmission electron microscopy (TEM) photograph of GEG polymeric micelles (bar = 50 nm).

used. It was thought that these differences might be due to the solubility parameters and molecular orientation of each polymer in the organic solvent. THF and 1,4 dioxane resulted in increased particle size. These results might be due to the differences in miscibility between solvent and aqueous solution or the solubility parameters of polymers in the solvents.

Morphological observations of GEG polymeric micelles prepared by using DMF as the initial solvent were performed using TEM without staining as shown in Figure 3. Their shapes are spherical, with sizes ranging between 20-40 nm with almost uniform particle size. GEG polymeric micelles formed by dialysis are small and spherical in shape.

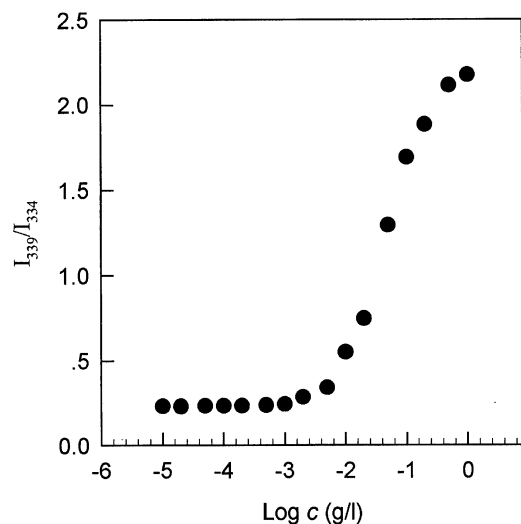
The formation of micelles was confirmed by a fluorescence probe technique using pyrene as a hydrophobic probe as reported previously.<sup>7,16,17</sup> Figure 4 shows the emission (a) and excitation (b) spectrum of pyrene in the presence of various concentrations of GEG block copolymers. Pyrene is preferentially partitioned into hydrophobic cores with a change in the photophysical properties of the molecules. The total fluorescence intensity and vibrational structure of monomer fluorescence increased with increasing concentrations of GEG polymeric micelles, as shown in Figure 4(a). This result indicates micelle formation of GEG block copolymer in water.<sup>7</sup> In the excitation spectrum, a red shift was observed with increasing concentration of GEG block copolymer, as shown in Figure 4(b). A red shift of pyrene in the excitation spectrum was observed in the study of micelle formation of PS-PEO block copolymers.<sup>7</sup> The (0,0) bands in the pyrene excitation spectra, which is at 334 nm in water, were shifted to 339 nm upon addition of GEG block copolymer. The intensity ratio  $I_{339}/I_{334}$  takes the value characteristic



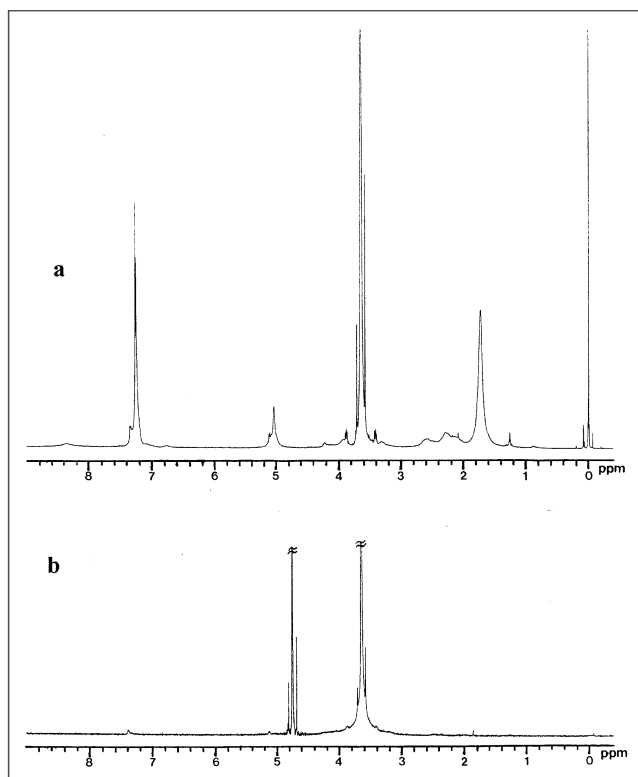
**Figure 4.** Fluorescence spectra of pyrene ( $6 \times 10^{-7}$  M)/GEG against concentration of GEG in distilled water (excitation wavelength: 339.0 nm) (a) and excitation spectra of pyrene/GEG against concentration of GEG in distilled water (Emission wavelength: 390.0 nm) (b).

of pyrene in water at low concentrations and the value of pyrene entirely in the hydrophobic domain. A plot of  $I_{339}/I_{334}$  versus  $\log c$  is shown in Figure 5. A flat region in the low concentration extreme and a sigmoidal region in the cross-over region were noted. This result indicates that signal change in the region of 0.0094 g/L ( $3.4 \times 10^{-7}$  M) can be linked to the CMC values of GEG block copolymer.

Further evidence of micelle formation of GEG block copolymer and limited mobility of the PBLG chain in the core of the micelle was obtained with  $^1\text{H}$  NMR in  $\text{CDCl}_3$  and  $\text{D}_2\text{O}$ . As shown in Figure 6(a) of  $\text{CDCl}_3$ , where micelle formation is not expected, characteristics of the protons of the benzyl group and the carbon atom adjacent to the benzyl group of the PBLG segment were observed in peaks of 7.2-7.4 and 5.0-5.2 ppm, respectively. On the other hand, in  $\text{D}_2\text{O}$  (Figure 6(b)), these peaks were not observed. This shows the restricted motion of these protons within the micellar core and that the PBLG core of the GEG polymeric micelles is a



**Figure 5.** Plot of the intensity ratio  $I_{339}/I_{334}$  from pyrene excitation spectra versus  $\log c$  for block copolymers against concentration of GEG in distilled water.



**Figure 6.**  $^1\text{H}$  NMR spectroscopy of GEG polymeric micelles in  $\text{CDCl}_3$  (a) and  $\text{D}_2\text{O}$  (b).

very rigid structure. This behavior of GEG micelles is in contrast with low molecular amphiphiles and PEO-PPO-PEO block copolymers, which typically exhibit liquid-like cores and relatively higher mobility. Also, in agreement with our results, Kwon *et al.*<sup>10</sup> report PBLA/PEO diblock copolymer has a rigid PBLA core. But, in their results, peaks of 7.4 ppm and 5.2 ppm were not unobservable, suggesting that PBLA/PEO diblock copolymer micelles may have a rela-

tively less rigid core compared with GEG polymeric micelles.

## Conclusions

The block copolymer based on PBLG as the hydrophobic part and PEO as hydrophilic part was synthesized and characterized. GEG polymeric micelles were prepared by dialysis technique. Particle size distribution in DMF based on intensity, volume, and number-average was  $22.6 \pm 11.9$  nm,  $23.5 \pm 4.6$  nm, and  $23.7 \pm 3.7$  nm, respectively. Also, particle size and size distribution of GEG polymeric micelles changed significantly with initial solvent. From the TEM observations, GEG polymeric micelles were spherically shaped and the sizes ranged between 20-40 nm in diameter. Fluorescence spectroscopy measurement suggested that GEG block copolymers were associated in water to form polymeric micelles, and the CMC value of the block copolymers was 0.0094 g/L. Further evidence of micelle formation of GEG block copolymers and limited mobility of the PBLG chain in the core of the micelle was obtained with  $^1\text{H}$  NMR in  $\text{D}_2\text{O}$ .

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