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Synthesis and Characterization of MPEG-*b*-PDPA Amphiphilic Block Copolymer via Atom Transfer Radical Polymerization and Its pH-Dependent Micellar Behavior

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Abstract: Block copolymer micelles are generally formed via the self-assembly of amphiphilic block copolymers in an aqueous medium. The hydrophilic and hydrophobic blocks form shell and core micelles, respectively. The block copolymers of methoxy poly(ethylene glycol) (MPEG)-*b*-poly(2-diisopropylamino)ethyl methacrylate (PDPA) were synthesized via atom transfer radical polymerization, with the macroinitiator synthesized by the coupling of 2-bromoisobutyryl bromide with MPEG in the presence of a triethyl amine base catalyst. The atom transfer radical polymerization of 2-diisopropylamino)ethyl methacrylate was performed in conjunction with an *N,N,N',N'',N''*-pentamethyl-diethylenetriamine/copper bromide catalyst system, in DMF, at 70 °C. The pH induced micellization/demicellization was studied using fluorescence, with a pyrene probe. Furthermore, the pH dependent micellization was confirmed using the microviscosity method, with a dipyme fluorescence probe. The pH dependant micelle size distribution was studied using dynamic light scattering. The characterization of the synthesized polymers was established using gel permeation chromatography and from the ^{1}H-nuclear magnetic resonance spectroscopy.

Keywords: atom transfer radical polymerization, block copolymers, micelles, macroinitiator, poly(2-(diisopropylamino)ethyl methacrylate (PDPA).

Introduction

Over the past few decades, stimuli-responsive polymeric micelles have attracted considerable attention as drug carriers in drug delivery and biomedical applications. Stimuli-sensitive amphiphilic block copolymers consisting of hydrophilic and hydrophobic segments have established themselves as essential building blocks for the preparation of stimuli-responsive micelles suitable for use as drug carriers.[1-3] In an aqueous environment, the hydrophobic blocks of the copolymer are expected to segregate into the core micelle and hydrophilic block form corona or outer shell. This shell-core micelle architecture of the polymeric micelle is essential for their utility as functional materials in pharmaceutical applications. The hydrophobic micelle core functions as a microenvironment for the incorporation of various therapeutic compounds, while the corona, or outer shell, functions as a stabilizing interface between the hydrophobic core and the external medium. As a result, polymeric micelles can be used as efficient carriers for reagents with poor solubility and/or low stability in physiological environments.[4,5] Although significant progress has recently been made in the field of smart polymers, the problem of their optimum delivery at physiological pH remains a formidable challenge.[6,7] Environmentally sensitive polymers exhibit a sharp change in their behavior in response to external stimuli, such as temperature, pH, ionic strength, electric field and chemical or biochemical agents.[8] Current approaches to the development of pH responsive micelles involve either the incorporation of "ionizable" groups, including carboxylic acids,[9,10] amines,[11,12] and sulfonamides[13,14] into the copolymer. However, the number of systems that are responsive within the physiologically accessible pH range of 4.5-7.4 is quite limited.

Recent developments in the field of controlled radical techniques have led to the facile synthesis of well-defined block copolymers with a wide range of functional monomers. Atom transfer radical polymerization (ATRP) is one of the best techniques that have been developed for the synthesis of well-defined functional polymers.[15,16] In this study, we synthesized an poly(ethylene glycol) (MPEG)-*b*-poly(2-diisopropylamino)ethyl methacrylate (PDPA) (MPEG-*b*-PDPA) amphiphilic block copolymer via atom transfer radical polymerization. Recently, several PEG derivatives were used as either macroinitiators or macromonomers in ATRP[17-20] and also in anionic polymerization.[21] This amphiphilic block

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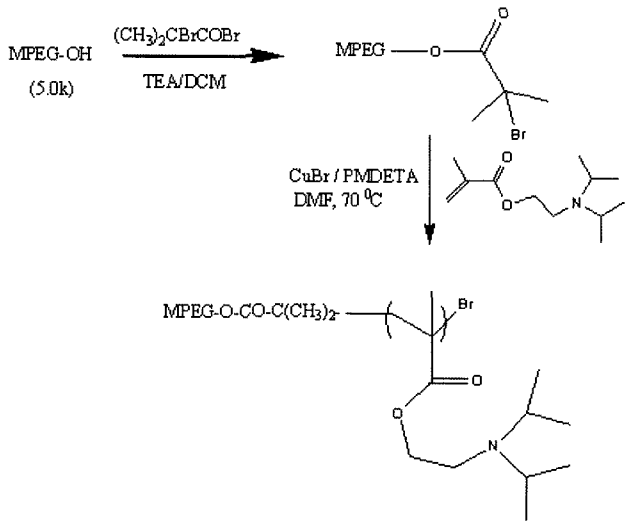
copolymer consists of a tertiary amine methacrylate. This amine methacrylate is hydrophilic at low pH due to its ionization and exhibits hydrophobic character above the critical pH value at which it becomes deionized. Diblock copolymers consisting of tertiary amine methacrylate monomers have recently been synthesized via the ATRP approach in both organic[22] and aqueous media.[16] These materials show stimuli responsive behavior in aqueous solution. In this study, the 2-diisopropylethanol amine (DPA) polymerization was carried out using an MPEG-Br macroinitiator/CuBr/PMDETA catalyst system in DMF as the solvent at 70 °C. The resulting polymers were characterized by ^{1}H-NMR and GPC and the physicochemical properties of the micelle were investigated in terms of their micellization-demicellization behavior, critical micelle concentration (CMC) and pH sensitivity by monitoring the fluorescence of pyrene at various pH conditions. Their microviscosity was studied by monitoring the fluorescence of dipyme at various pH values. The size and micelle size distributions were measured by dynamic light scattering.

Experimental

Materials. Methoxy poly(ethylene glycol) (MPEG) (M_n= 2,000, 5,000), *N,N*-dimethyl formamide (DMF) (anhydrous), dichloromethane (anhydrous), 2-bromoisobutyryl bromide, triethyl amine, Cu(I)Br, *N,N,N',N'',N'''*pentamethyldiethylenetriamine (PMDETA), pyrene, and pyrenemethanol were purchased from Sigma-Aldrich, Korea. Thionyl chloride was purchased from Fluka. 2-(Diisopropylamino) ethyl methacrylate (DPA) was purchased from Scientific Polymer Products, U.S.A. The monomer was purified by passing it through a column and then stored at -20 °C. Dipyme was synthesized following the reported procedure.[23] All other chemicals were of reagent grade and used as received. Diethyl ether, chloroform, and tetrahydrofuran were obtained from Samchun Chemical Co. (Korea) and were used as received. Dialysis was performed by using Spectra/Por^R Regenerated Cellulose Dialysis Membranes with Mw cutoff values of 3,500 and 6-8,000 against deionized water at room temperature.

Synthesis of Macroinitiator, MPEG-Br. To a dry 250 mL round bottom flask equipped with a magnetic stir bar, MPEG (M_n=5,000) was added and the flask was placed in an oil bath at 80 °C under vacuum, maintained at this temperature for 2 h and then allowed to cool to room temperature. The polymer was dissolved in DCM and then an excess amount of triethyl amine catalyst was added 5 times. The resulting reaction mixture was cooled in an ice bath and the temperature maintained in the range of 0-10 °C, followed by the addition of an excess amount of 2-bromoisobutyryl bromide 5 times in a dropwise manner. After the completion of the addition, the reaction mixture was allowed to warm up to room temperature and continuous

Scheme I



stirring was applied for 24 h. After this, the resulting solution was concentrated under vacuum, redissolved in THF, the residue of the Et_3N·HBr salt was removed by filtering and then the solution was concentrated, followed by precipitation in cold ether to obtain the macroinitiator, MPEG-Br, which was dried under vacuum at room temperature for 48 h. Further this can be used as a macroinitiator for the synthesis of MPEG-*b*-PDPA block copolymer as shown in Scheme I.

Synthesis of MPEG-*b*-DPA Diblock Copolymer. To a dry Schlenk tube equipped with a magnetic stir bar, CuBr (0.07175 g, 0.5 mmol) was added. It was then fitted with a rubber septum, was evacuated twice, and refilled with dry nitrogen. Following this, degassed DMF (6 mL) and the ligand, PMDETA (0.0865 g, 0.5 mmol), were added using a gas tight syringe. 2-(Diisopropylamino) ethyl methacrylate (2.13 g, 10.0 mmol) was then added and the reaction mixture was stirred well. The macroinitator, MPEG-Br (2.575 g, 0.5 mmol), was added to the reaction mixture and the Schlenk tube was placed in an oil bath maintained at a temperature of 70 °C. The polymerization was stopped after the desired time period. The resulting polymer was dialyzed against deionized water for 24 h at room temperature. Following this, the sample was dried in a vacuum oven at 40 °C for 48 h. The resulting polymers were characterized using ^{1}H-NMR and GPC.

Characterization. The resulting polymers were characterized by ^{1}H-NMR spectroscopy (500 MHz JNM-LA FT-NMR). The molecular weight and polydispersity index (PDI) were measured using gel permeation chromatography (GPC, Shodex-KF 802.5, KF 803L), with THF as the eluent and a flow rate of 1 mL/min. The molecular weights were calculated against low polydispersity PEG standards.

pH Sensitivity. The micellization-demicellization behavior of the pH-sensitive block copolymers was investigated by fluorescence spectroscopy using pyrene as a probe. A

buffer solution containing 1 M phosphate saline buffer was prepared. A stock solution of pyrene in THF was added to the buffer solution and the THF was removed by heating at 60 °C for a few hours. The final pyrene concentration was 1×10^{-6} M. The excitation spectrum of pyrene was recorded at 392 nm. The pK_a value of the polymer was measured by the titration method. In this case, 50 mg of the polymer was dispersed in 50 mL of distilled water and then dissolved at pH 2.0, after which the pH was adjusted to be less than 5.0 with the addition of 0.1 mL of 1 N NaOH solution, and the pH was recorded to obtain the titration profile. The pK_a values of the polymers were calculated from the derivative values of the titration curves, which corresponds to the inflection point. The micelle size was characterized by DLS (dynamic light scattering, Malvern Instrument Ltd. Series 4700) with a helium laser at 633 nm and a digital correlator. When the difference between the measured and calculated baselines was less than 0.1%, the correlation function was deemed acceptable. The scattering angle was fixed 90 °C and the temperature was adjusted to 25.8 °C. The concentration of the solution was kept at 2 mg·mL^{-1} and the micelle size was checked at various pH values.

Results and Discussion

Synthesis and Characterization of the Amphiphilic Block Copolymer of MPEG-*b*-PDPA. In this study, we investigated the synthesis of the amphiphilic block copolymers of MPEG-*b*-PDPA using the macroinitiators, MPEG (2.0k)-Br and MPEG (5.0k)-Br, via the atom transfer radical polymerization (ATRP) of DPA, and the resulting polymers were characterized by ^{1}H-NMR and GPC. The pH induced micellization-demicellization behavior was studied by fluorescence spectroscopy using pyrene as the probe and the microviscosity was determined using dipyme as the probe. In order to synthesize the pH sensitive moiety, DPA, via ATRP, an initiating site was anchored by the coupling of 2-bromoisobutyryl bromide with MPEG in the presence of triethyl amine as a base catalyst in dichloromethane at 0-10 °C.[17,24,25] The resulting product was characterized by ^{1}H-NMR (500 MHz, $CDCl_3$) spectroscopy, as shown in Figure 1. The ^{1}H-NMR spectrum showed a new peak at 1.9 ppm corresponding to the -$C(CH_3)_2$-Br methyl protons along with the methoxy PEG peaks, confirming the attachment of the initiating group. Recently, Armes *et al.* reported the ATRP of a DPA using CuBr/bipy in methanol at 20 °C[22,26,27] and Mallapragada K. Suriya *et al.* also reported the ATRP of a DPA using CuBr/NPPM in toluene at 70 °C.[28] In this study, the polymerization was carried out with the CuBr/PMDETA catalyst system in DMF as an aprotic polar solvent at 70 °C. Scheme I illustrates the ATRP, initiated by the macroinitiator, of the 2-(diisopropylamino)ethyl methacrylate block, resulting in the formation of the diblock copolymer with polydispersities in the range of 1.3-1.5. The results for these polymers are summarized in Table I. The ^{1}H-NMR spectrum (in $CDCl_3$) of the synthesized block copolymer is shown in Figure 2. It showed new peaks at 1.1 ppm (-$CH(CH_3)_2$), 2.6 ppm (-CH_2-$N(CH(CH_3)_2)$, 3.0 ppm (-CH_2-$N(CH(CH_3)_2)$, and 3.8 ppm (-OCH_2CH_2N) along with the macroinitiator peaks, confirming the synthesis of the diblock copolymer, MPEG-*b*-PDPA. The synthesis of the block copolymer was further confirmed by gel permeation chromatography in THF using PEG standard traces, as shown in Figure 3. The gel permeation chromatogram of the diblock copolymer showed an increase in the number average molecular weight from 5,000 to 7,800, indicating the formation of the block copolymer.

Table I. Molecular Weight Characteristics of MPEG-*b*-PDPA in DMF at 70 °C

Sample Code	Reaction Time (h)	M_n (SEC)	PDI
P1[a]	18	7,800	1.38
P2[b]	9	9,600	1.35
P3[b]	12	10,000	1.31
P4[b]	18	10,400	1.36
P5[c*]	18	4,900	1.44

[a]Target DP=20; [Cat]:[L]:[initiator]=[DPA], [1]:[1]:[1]. [b]Target DP=40; [Cat]:[L]:[initiator]=[DPA], [1]:[1]:[1]. [c]target DP=20; [Cat]:[L]:[initiator]=[DPA], [1]:[1]:[1]. Initiator MPEG 2.0k.

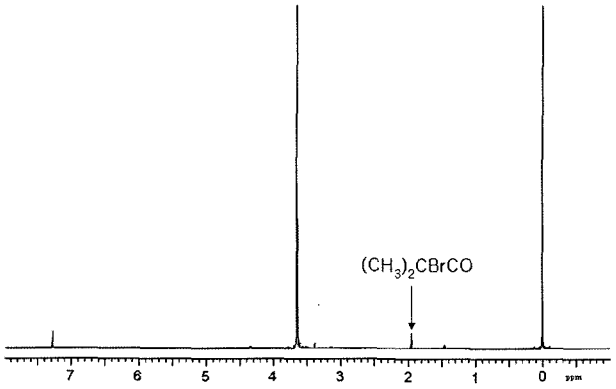


Figure 1. ^{1}H-NMR of spectrum of MPEG-Br.

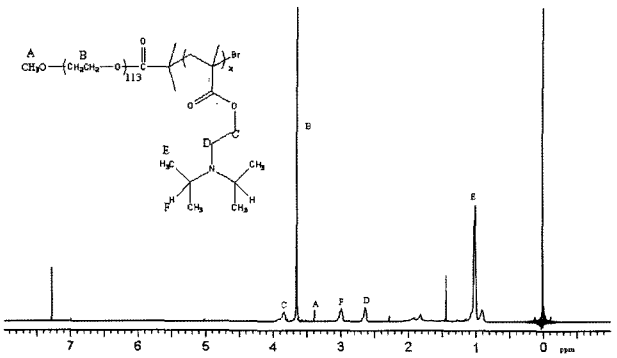


Figure 2. ^{1}H-NMR of spectrum of MPEG-*b*-PDPA.

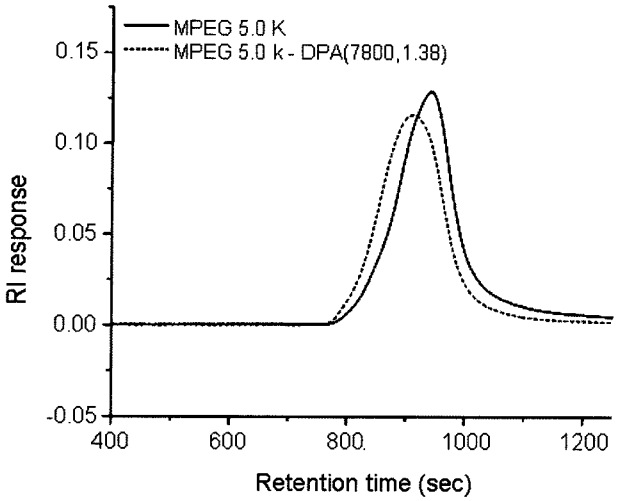


Figure 3. Gel permeation chromatograms of MPEG and MPEG-*b*-PDPA.

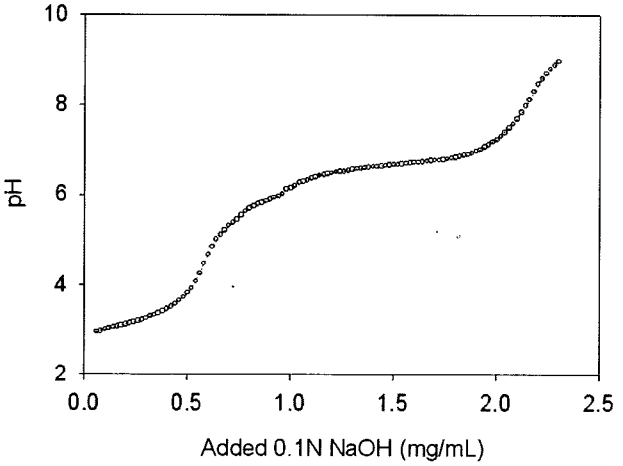


Figure 4. Titration curve of block copolymer P1.

Acid-Base Titration Curve. The amphiphilic block copolymer consisted of hydrophilic and hydrophobic blocks. Since the hydrophobic block is pH sensitive, the solution properties of the polymer solution were investigated as a function of the solution pH. Initially, the effect of the addition of acid or base to a dilute solution of the polymer on the pH was monitored. Figure 4 shows the titration curve of the block copolymer, P1, indicating that it has a pK_a value of around 6.3.

Micellization-Demicellization Behavior by Fluorescence Spectroscopy. To investigate the pH-induced micellization behaviors of the MPEG-*b*-DPA block copolymer, it was dissolved in dilute acidic PBS buffer solution at a concentration of 1 mg·mL^{-1}, since the DPA block is protonated and, hence, hydrophilic under these conditions. On adjusting the pH to around 6-7, the DPA block becomes deprotonated and forms micelles consisting of a DPA hydrophobic core and MPEG hydrophilic shell. The micellization-demicellization

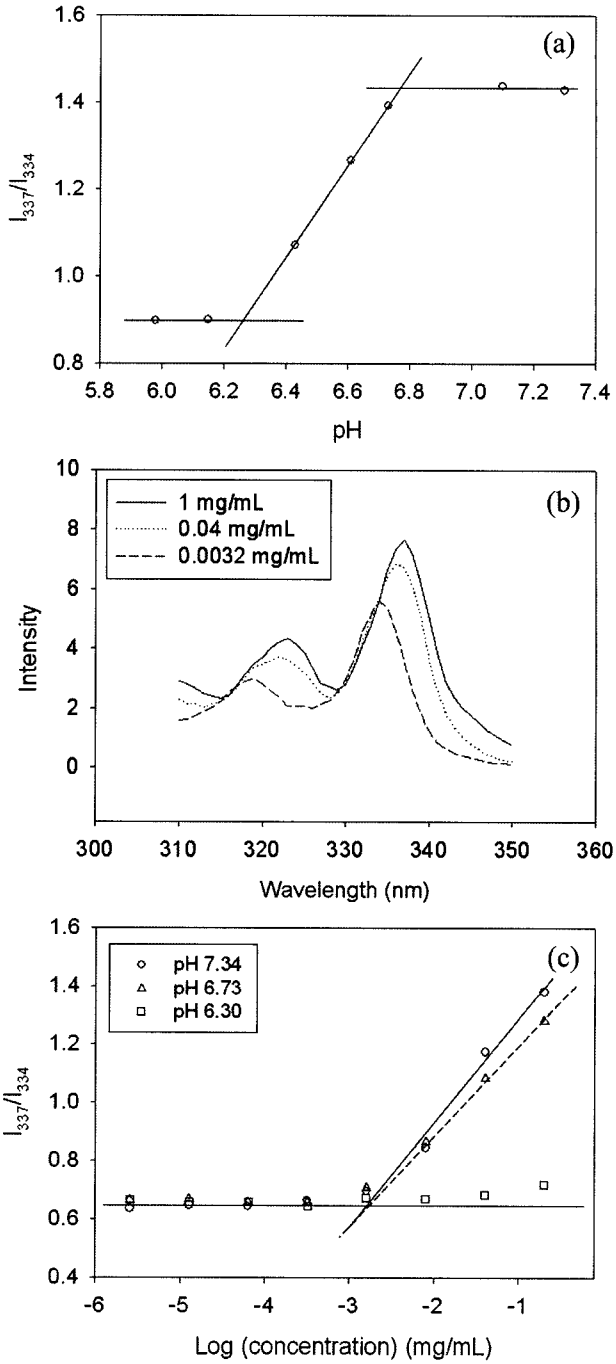


Figure 5. (a) Plots of the intensity ratio I_{337}/I_{334} (from pyrene excitation spectra) vs. pH of polymer solution. (b) Excitation spectra of pyrene in MPEG-*b*-DPDA block copolymer solution at pH 7.34. The concentration of pyrene was 1×10^{-6} M. (c) Plots of the intensity ratio I_{337}/I_{334} (from pyrene excitation spectra) vs. log (concentration).

was observed by fluorescence spectroscopy using pyrene as a probe. Pyrene fluorescence is a very sensitive technique for detecting the formation of block copolymer micelles. Pyrene is highly hydrophobic and poorly soluble in water

and, consequently, it migrates into the hydrophobic cores. Thus, a red shift is observed in the pyrene fluorescence spectra, as well as changes in the relative peak intensities in the vibrational structure.[4,29] Figure 5(a) shows the dependence of the I_{337}/I_{334} ratio on the block copolymer concentration under various pH conditions. As the solution pH is increased, the tertiary amine moiety becomes deprotonated. The critical pH for micellization was estimated from the reduction in the I_{337}/I_{334} ratio, which indicates a more hydrophobic (micellar) environment for the pyrene probe. The critical micellization pH value estimated for the block copolymer, P1, is 6.36. The MPEG 2.0-*b*-PDPA block copolymer, P5, is soluble below pH 6.3, but above pH 7.0 the polymer precipitates out from solution because the hydrophobic chain length is high. The relative balance between the hydrophilic and hydrophobic blocks is considered to be an important factor for the micellization.

pH-Dependence of Critical Micellization Concentration (CMC). The fluorescence spectroscopy was performed to investigate the micellization behavior of the synthesized block copolymers. A small intensity change in the intensity ratio of the first and third vibrational band occurs in micellar media when using a pyrene probe. Moreover the intensity ratio of I_{337}/I_{334} provides qualitative information on the number of micelles in the given system. The typical excitation spectra of pyrene in MPEG-*b*-DPDA block copolymer (P1) solution at pH 7.34 are shown in Figure 5(b). As the polymer concentration was increased from 0.0032 to 1 mg/mL the peak at 337 nm underwent noticeable increase compared that of 334 nm, which indicates that the micelle formation and increment of number of micelles. Figure 5(c) shows the dependence of I_{337}/I_{334} of the copolymer concentration at various pH conditions for the polymer (P1). The CMC were determined at pH 6.30, 6.73, and 7.34 for polymer P1. The CMC values at pH 6.73 and 7.34 are 0.002 and 0.0031 mg/mL, respectively, whereas no CMC value was observed at pH 6.30. This clearly indicates that the polymers can form micelles above pH 6.73, but cannot form micelles below pH 6.30 due to the complete ionization of the PDPA block.

The micellization-demicellization was also confirmed by the microviscosity method using fluorescence spectroscopy with dipyme as a probe. The dipyme was synthesized according to the literature reported procedure.[23] Dipyme is a molecule made of two pyrenemethylene moieties connected by an ether linkage. Figure 6(a) shows that at a low dipyme concentration, the formation of the excimer is intermolecular and the amount of excimer formed with respect to the monomer yields information about the viscosity of the environment. As a result, the measurement of the monomer to excimer intensity ratio, I_E/I_M, allows the microviscosity to be obtained for surfactant and polymeric micelles.[30] Figure 6(b) shows the effect of the pH on the fluorescence intensity ratio of dipyme, I_E/I_M, in an aqueous solution of the block

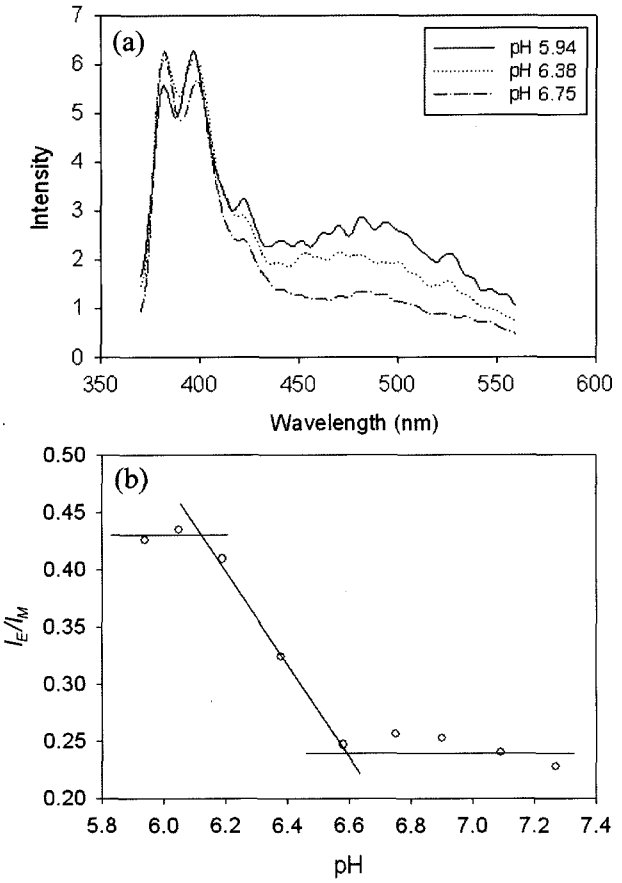


Figure 6. (a) Emission spectra of dipyme in MPEG-*b*-PDPA block copolymer (M_n=7,800). The concentration of dipyme was 1×10^{-6} M. (b) Plots of the intensity ratio I_E/I_M (from pyrene excitation spectra) vs. the pH of the polymer solution.

copolymer. The fluorescence intensity ratio, I_E/I_M, sharply decreased from 0.40-0.25 when the pH was increased above 6.2. This indicates that the PDPA acts as a hydrophobic core above pH 6.2. The critical micellization-dimicellization was observed in the pH range of 6.2-6.5.

Dynamic Light Scattering. The size and size distribution of the micelles prepared were measured by means of the dynamic light scattering (DLS) method. Figure 4 shows the potentiometric titration curve amphiphilic block copolymer of P1 (M_n=7,800). The block copolymer has a pK_a value of around 6.30. Figure 7 shows the typical pH dependent size distribution of the P1 block copolymer. It shows a mean diameter in the range of 120 to 250 nm. The DLS studies reveal that there is a considerable increase in the micelle size above pH 6.3, indicating that large aggregates rather than well defined micelles were formed.

Conclusions

The present results show that the MPEG-*b*-PDPA diblock coplymers were successfully synthesized via the atom trans-

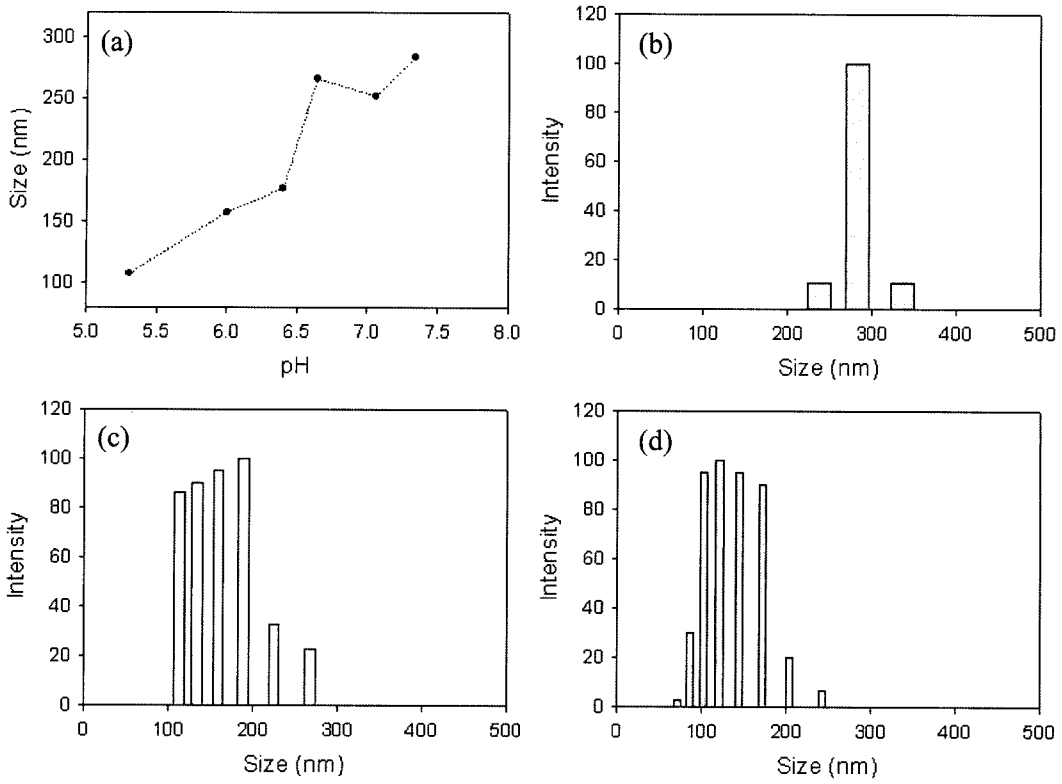


Figure 7. The micelle size distribution profile at various pH values.

fer radical polymerization of DPA and their structure was controlled by adjusting the feed ratio of monomer and initiator. The aqueous solution properties were evaluated by acid-base titration, fluorescence spectroscopy and dynamic light scattering. The amphiphilic block copolymers were found to exhibit pH sensitivity and excellent pH dependent micellization behavior in dilute solution. Furthermore, the critical micellization concentration was estimated. The DLS studies showed that there was a considerable increase in the micelle size above pH 6.3, indicating that large aggregates rather than well defined micelles were formed. These amphiphilic block copolymers are expected to find applications in the field of drug delivery.

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