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# Novel micelle-forming block copolymer composed of poly (ε-caprolactone) and poly(vinyl pyrrolidone)

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**Abstract**

The well-defined poly (ε-caprolactone) (PCL)/poly(vinyl pyrrolidone) (PVP) diblock copolymers were synthesized through combining radical polymerization of VP and the controlled coordination–insertion ring-opening polymerization of CL using an aluminum alkoxide macroinitiator formed from the equimolar reaction of triethylaluminum with hydroxy-terminated PVP (PVP-OH). The molecular characterization of PCL/PVP diblock copolymers was confirmed through $^1$H NMR spectroscopy and GPC analysis. Polymeric micelles composed of PCL as a hydrophobic core and PVP as a hydrophilic shell were prepared by a diafiltration method. The micellar properties such as sizes, shapes, and critical micelle concentrations (CMC) were investigated with a dynamic light scattering (DLS) spectrometer, transmission electron microscope (TEM) and spectrofluorimeter. The sizes of micelles ranged from 30 to 80 nm in average size. The novel micelles formed from the well-defined PCL/PVP diblock copolymers seem to be feasible as novel promising carriers in biomedical and pharmaceutical applications.


*Keywords:* Poly (ε-caprolactone); Poly(vinyl pyrrolidone); Coordination–insertion ring-opening polymerization

## 1. Introduction

In recent years, the synthesis and characteristics of amphiphilic block copolymer have been attracting much attention owing to their unique molecular architecture that has different chemical nature, constituting both hydrophilic and hydrophobic blocks [1]. Appreciate choices of the different length scales, physical interactions, and solvation properties of both blocks in diblock copolymers make the use of these materials very attractive in numerous applications [2]. In particular, to improve the specific delivery of drugs with low therapeutic activity and prevent the undesirable side effects, polymeric micelles from the amphiphilic block copolymers have been the objects of growing scientific attention as delivery carriers for hydrophobic drugs in the biomedical applications [3].

Numerous water-soluble polymers have been investigated for their applications in the medicine and biotechnology. Among them, it is well known that poly (ethylene glycol)(PEG) possesses interesting physiochemical and biological properties. The absolute majority of research on drug carriers for stable long circulation and less accumulation of reticuloendothelial system (RES) has been focused on the use of PEG by far [3,4]. Recently, similar to PEG, poly(vinyl pyrrolidone) (PVP), a water-soluble polymer, is attracting attention as a material for use in the medicine and pharmaceutics because of its excellent biocompatibility with living tissue and extremely low cytotoxicity [5]. As known for many years, PVP exhibits interactions toward small molecules in solution and formed complex with a variety of these molecules because of its amphiphilic property [6]. Thus, this unique ability enables PVP to

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form a soluble complex with various hydrophilic and hydrophobic drugs. Furthermore, it has been reported that the PVP could serve to protect degradation of DNA from extracellular nucleases [7] as well as make peptides or proteins enhance more stability in vivo with substantial retention of activity, and reduction of immunogenecity [8].

Biodegradable polymers have been of particular interest in the pharmaceutical and biomedical applications because of easy removal from body after use. They are favorable for release of drugs [9], proteins [10] and genes [11] at the sustained rate, as well as fabrication of scaffold in tissue engineering [12]. In particular, poly (ε-caprolactone) (PCL) has been widely used with poly (lactide) (PLA), and poly (glycolide) (PGA) due to their attractive properties such as biodegradability, permeability, biocompatibility, and capability to be blended with various commercial polymers [9].

The synthesis method of well-defined diblock copolymers is of great critical for the preparation of novel materials. Thus, to produce diblock copolymers with well-characterized segment lengths, narrow molecular weight distribution, and no homopolymer under appreciate experimental condition, the novel model materials need to synthesize in a well-controlled way. As it has already been demonstrated by Hamitou et al. [13], aluminum alkoxides are known to be very effective initiators of the polymerization of lactones and lactides, and it has been well-established that the ring-opening of lactide and lactones using aluminum alkoxides allows the synthesis of polyester chains with predictable molecular weight and well-characterized chain end groups as a result of the polymerization mechanism.

In this work, we synthesized and characterized a novel PCL/PVP diblock copolymer through combining radical polymerization of VP and controlled coordination–insertion ring-opening polymerization of CL using aluminum alkoxides. Polymeric micelles composed of PCL as a hydrophobic core and PVP as a hydrophilic shell were prepared by a diafiltration method and the properties of the micelles were investigated. The emphasis of this study was placed on the development of a novel micelle-forming diblock copolymer for drug delivery applications.

## 2. Experimental part

### 2.1. Materials

Vinyl pyrrolidone(VP)(Aldrich) was purified by distillation under reduced pressure. ε-caprolactone (Fluka) was dried over calcium hydride for 48 h at room temperature and distilled under reduced pressure. Triethylaluminium (Aldrich), a 1 M solution in toluene, pyrene (Aldrich), and 2-mercaptoethanol(ME) were used without further purification. 2,2-azoisobutyronitrile (AIBN)(WAKO) was purified by precipitation into ice water from an acetone solution and dried under vacuum. The dialysis tubes (Spectrum, molecular weight cut off: 6000–8000, and 12,000–14,000) were used for micelle preparation.

### 2.2. Synthesis of hydroxy-terminated PVP (PVP-OH)

The PVP-OH was synthesized by the radical polymerization of VP monomer using ME as a chain transfer agent. Polymerization was performed in a 100 ml glass reactor equipped with a glass-vacuum joint and a Teflon coated stir bar. Briefly, ME (0.7 g, 9 mmol), and AIBN (0.1 g) were dissolved in VP (10.0 g, 90 mmol) monomer in the glass reactor. The dissolved solution was frozen into liquid nitrogen. The reactor was then degassed through connecting it with a vacuum pump. The reactor sealed off using Teflon tape was placed into an oil bath set at 80 °C, and then polymerization reaction was allowed to maintain for 24 h. The obtained viscous mixture was terminated through cooling it in water. The reaction product was then dissolved in methanol, and precipitated with an excess ethyl ether to give a white product. The resulting product was dried under vacuum at 40 °C for 48 h. In addition, the products were filtered using dialysis membranes from molecular weight cut-off 3500–8000 repeatedly after dissolving in water in order to obtain the PVP-OH with narrow MWD. The final product was obtained through freeze-drying.

### 2.3. Synthesis of PCL/PVP diblock copolymers

The PCL/PVP diblock copolymers were synthesized through the controlled coordination–insertion ring-opening polymerization of CL using aluminum alkoxide macroinitiators.

CL polymerization was carried out in dry $CH_2Cl_2$ with the macroinitiator formed from the equimolar reaction product of $AlEt_3$ and PVP-OH. The vessel equipped with an internal thread was treated with a solution of methylene chloride/dichrolomethylsilane (90/10 in volume) and allowed to dry at 70 °C overnight. The required amount of PVP-OH was charged into the pre-silanized vessel under nitrogen atmosphere, and then $CH_2Cl_2$ and the equimolar amount of $AlEt_3$ (1.9 M solution in toluene) was added into the flask equipped with a rubber septum using a syringe. The mixture was allowed to stir for 4 h at room temperature for emission of ethane and product of aluminum alkoxide macroinitiator ($Et_2$OPVP). CL was then added into the flask using a syringe and the reaction flask was placed into a 40 °C oil bath. After 48 h, the reaction was terminated by adding a 5-fold molar excess of 1N HCl solution with respect to $AlEt_3$. The polymer solution was washed with water up to a neutral pH, and was precipitated again with methanol/cold hexane, filtered, and then dried under vacuum for 24 h. The obtained polymer solution stored at 20 °C until use. The resulting products of PCL/PVP diblock copolymers were obtained through adjusting the molar feed ratio of CL monomers to macroinitiators.

### 2.4. Preparation of PCL/PVP polymeric micelles

Polymeric micelles composed of PCL as a hydrophobic core and PVP as a hydrophilic shell, and drug-loaded polymeric micelles were prepared by a diafiltration method. Briefly, 10 mg of PCL/PVP diblock copolymer was dissolved in 5 ml of DMF. After the above two solutions were completely mixed and dissolved through stirring at room temperature, the solution was dialyzed to remove the organic solvent using a dialysis tube (molecular weight cut off: 6000–8000 or 12,000–14,000) against 3 l of distilled water for 12 h.

### 2.5. Critical micelle concentration (CMC)

Fluorescence spectra for CMC of PCL/PVP diblock copolymer were recorded with a spectrofluorimeter (Shinmadzu F-700). Pyrene was used as hydrophobic fluorescence probe. A known amount of pyrene was added into each of a series of vials and the acetone was then evaporated. Micellar solution (5 ml) was added into the each viral where the final concentration of pyrene was $6 \times 10^{-7}$ M. The concentration of micelles in each vial ranged from 0.5 mg/1 ml to 0.001 ml. The sample solution was placed into an oven at 70 °C for 4 h and subsequently allowed to store overnight at room temperature.

The fluorescence excitation spectra were measured at the emission wavelength $\lambda_{em} = 390$ nm.

### 2.6. Measurements

Molecular weights and molecular weight distributions of PVP-OH were determined using gel permeation chromatography (GPC) (Waters Model 150-C) equipped with a Waters 410 differential refractometric detector and a TSK-gel G2000 SWXL column. The mobile phase was distilled water at a flow rate of 0.5 ml/min. Pullulan P-400, P-200, P-100, P-50, P-20, P-10 and P-5 were used as standard markers. Also, molecular weight and molecular weight distributions of diblock copolymers were determined with an ultrastyragel column (K-810, K-803, K-804, and K-806) at room temperature. $CHCl_3$ was used as the mobile phase at a flow rate of 1 ml/min and column calibration was performed with polystyrene standards. Proton nuclear magnetic resonance ($^1$H NMR) spectra were recorded at 30 °C in $CDCl_3$ using AVANCE 600 spectrometer. The sizes and size distributions of PCL/PVP polymeric micelles were measured at a 90° angle with a DLS spectrometer (ELS-8000, Ostuka Electronic Co. Ltd) equipped a vertically polarized incident beam at 633 nm by a He–Ne laser. After filtered through a Millipore filter of pore size 0.45 μm, the samples were measured at 25 °C in distilled water. The sizes and shapes of micelles were observed with a TEM (JEL 1010, JEOL, Co. Ltd.). A drop of solution containing micelles was deposited on the grid and after the excess has been taken off, a drop of the negative staining solution (2 wt% uranyl acetate) was then deposited on the grid. After a few seconds the excess fluid was drain off by applying a narrow tapered strip of filter paper to the edge of the grid and the grid was allowed to dry before observation with the TEM.

## 3. Results and discussion

The amphiphilic and degradable diblock copolymers composed of PCL as the hydrophobic part and PVP as the hydrophilic one were synthesized through combining the radical polymerization and the coordination–insertion ring opening polymerization. The radical polymerization techniques have been made to obtain end-functionalized PVP in order to widen the application of PVP in a variety of field of protein modification and gene or drug carrier [14]. Mono hydroxyl-terminated PVP (PVP-OH) as the first step was synthesized through a radical polymerization of VP using ME as a chain transfer agent and AlBN as an initiator (Fig. 2). Introduction of hydroxyl group at the end of main chain is required to produce aluminum alkoxide macroinitiators through reaction with $Et_3Al$ for sequential polymerization of CL. The molecular weights of PVP-OH were controlled by adjusting molar feed ratio of ME to VP. The chemical structure of PVP-OH obtained was confirmed by $^1$H NMR spectroscopy. Peak of methylene proton ($HOCH_2-$) at the end of PVP-OH appeared as a multiplet due to coupling with hydroxyl group at 3.64 ppm. The molecular weights ($M_n$) obtained ranged from 4400 to 10,304 with relatively low polydispersities by GPC analysis. The representative GPC chart of the PVP-OH (in water vs pullulan standard) as shown in Fig. 1 gave $M_n = 5030$ g/mol, and $M_w/M_n = 1.1$. However, it is possible to have contamination of another type polymer such as telecheric OH-PVP–PVP-OH produced by radical coupling termination because the GPC curve of the obtained polymer is bimodal as shown in Fig. 1.

Ring-opening polymerizations of lactones and lactides have been carried out through a set of polymerization methods using metal compounds such as common-used

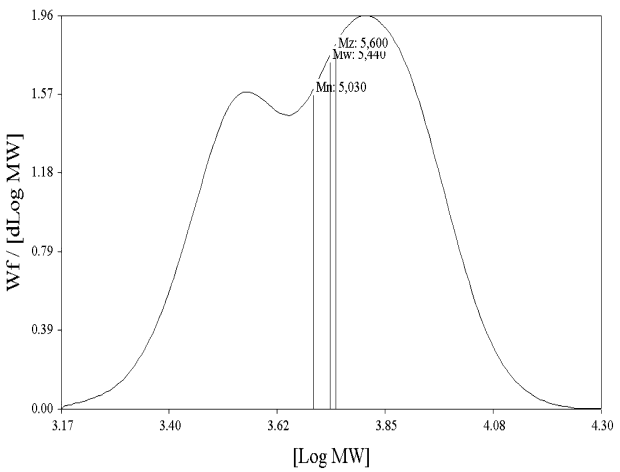


Fig. 1. GPC chart of PVP-OH.

stannous octoate ($SnOct_2$), and ionic polymerization methods. However, these polymerizations can be accompanied with side intramolecular and intermolecular transesterification reactions, resulting in degradation, and formation of linear and cyclic oligomers [15]. In contrast, it has been reported elsewhere that coordination insertion polymerization using aluminum alkoxides is more effective method for ring-opening polymerization of lactide and lactones [13]. Therefore, in this study, the PCL/PVP (abbreviated as CP) diblock copolymers as a second step were synthesized through the controlled coordination–insertion ring-opening polymerization of CL using aluminum alkoxide macroinitiator. Fig. 2 shows a series of reactions used in the synthesis of the PCL/PVP diblock copolymer. Formation of the macroinitiator was achieved through reaction of equimolar $Et_3Al$ to PVP-OH prepolymer. The sequential ring opening of CL was followed through the reaction of CL monomers with the macroinitiators. In the coordination–insertion polymerization reaction, it was reported that the used solvent, temperature, and macroinitiator structure had slightly different polymerization effect [16]. In particular, polar solvents such as THF, and $CH_2Cl_2$ made the polymerization rate significantly decrease. It is due to competition reaction between monomer and solvent for coordination to Al. In this experiment, as compared to the toluene generally used, more polar $CH_2Cl_2$ was used as an alternative due to extremely low solubility of PVP-OH in toluene. Indeed, the coordination–insertion polymerization is unusually slow, which reflects more or less a loss of reactivity of the aluminum alkoxide macroinitiators ($Et_2AlOPVP$). It is thought that the used polar $CH_2Cl_2$ and intramolecular coordination of active Al groups onto the carbonyl groups of PVP may make polymerization rate slow. Thus, these conditions taken into consideration, the polymerization temperature was made from 25 to 40 °C and the reaction was carried out for 48 h longer than 24 h. This sequential polymerization was terminated by hydrolysis of the active aluminum alkoxide bond, leading to the formation of the hydroxyl end group. As it has already been demonstrated [13,17], the ring opening polymerization of CL was initiated through aluminum alkoxides, and then propagated through a coordination–insertion mechanism, which ruptured selectively the acyl-oxygen bond of CL and inserted into aluminum-oxygen bond of the macroinitiator ($Et_2AlOPVP$). It is thought that PCL homopolymer obtained during synthesis of diblock copolymer is negligible because the practical values of PCL molecular weight determined through H NMR measurement after block copolymer synthesis were very close to the theoretical values of PCL molecular weight calculated according to the feed ratio of CL monomer to PVP-OH.

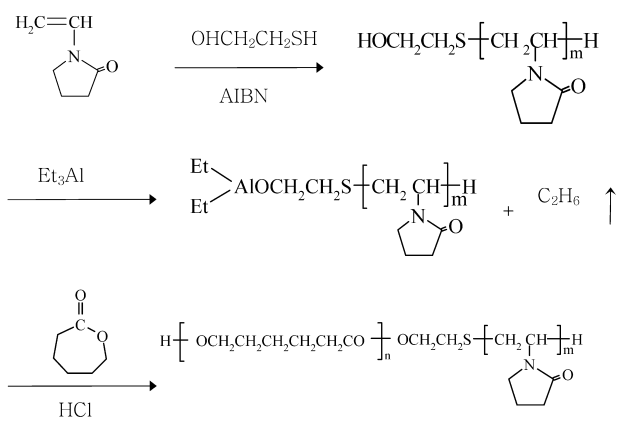


Fig. 2. Synthesis scheme of PCL/PVP diblock copolymer.

Table 1 shows a series of PCL/PVP diblock copolymers prepared. The molecular weights of the corresponding PVP-OH were fixed at each 5030, while the PCL segments were controlled through adjusting molar ratio of CL monomer to macroinitiator. The compositions of block copolymers were determined by $^1$H NMR from the signal intensites of the PVP methylene group ($\delta = 3.20$ ppm) in ring and the PCL methylene ester group ($\delta = 4.06$ *ppm*). From the compositions by $^1$H NMR and the $M_n$ of prepolymer PVP-OH block by GPC, the molecular weights of PCL block were calculated. The molecular weights of the produced polymers were almost close to those expected from the monomer-to-initiator ratio with narrow polydispersities through strict control of the reaction conditions.

Fig. 3 shows $^1$H NMR spectrum of CP-3 diblock copolymer. Typical signals of both PVP (protons: h–m) and PCL (proton: a–e) units were detected. Symbol * corresponds to residual *n*-hexane that was used in order to obtain precipitates. A distinctive signal of b is not detected in the NMR spectrum because the chemical shift of f is the same as that of b and peak intensity of f is very weak compared with that of b, resulting in an overlap between two peaks. Also, as situated next to a carbon bearing two methylene protons, signal a at 3.64 ppm appears clearly as a triplet, reflecting the fact that the living ring-opening polymerization of CL was propagated until the hydroxyl group in PCL was introduced by hydrolysis of aluminum residues. As a consequence, the $^1$H NMR data demonstrated the successful reaction of the PCL/PVP diblock copolymers.

The micelles from PCL/PVP diblock copolymer were obtained through a diafiltration method. As shown in the

Table 1
Characterization of the PCL/PVP diblock copolymer

| Sample | $M_n$ of PVP-OH ($\times 10^{-3}$)[a] | [M]/[I] | Molecular weight of PCL | | MWD[a] |
|---|---|---|---|---|---|
| | | | Calc.[b] | $M_n$[c] | |
| CP-1 | 5.03 | 22 | 2500 | 2800 | 1.15 |
| CP-2 | 5.03 | 44 | 5000 | 4300 | 1.18 |
| CP-3 | 5.03 | 100 | 11400 | 13900 | 1.35 |

[a] Measured from GPC anylsis.
[b] Calculated by feed molar ratio between ε-CL and PVP-OH.
[c] Calculated from $^1$H NMR spectra in $CDCl_3$ by using Mn value of PVP obtained by GPC analysis.

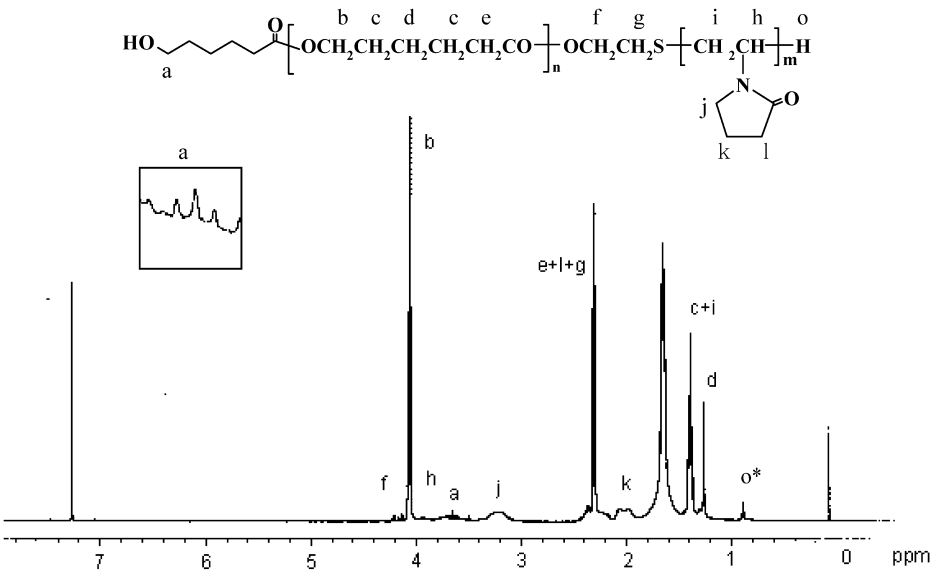


Fig. 3. $^{1}$H NMR spectrum of CP-3 diblock copolymer.

Table 2, the hydrodynamic diameters of micelles were measured with a DLS. The diameters based on number average were 32.6 ± 4.2, 42.8 ± 6.4, and 79.4 ± 12.8 nm for CP-1, CP-2, and CP-3, respectively, and their size distributions were very narrow. Also, the sizes and shapes of well-formed PCL/PVP micelles were confirmed with a TEM (Fig. 4). The TEM photograph of CP-1 micelles showed round shapes and the average size was almost similar to that in DLS measurement (Fig. 5) although significant size distributions were seen in TEM image because of the secondary aggregation of the micelles during the sample drying.

Critical micelle concentrations (CMCs) in PCL/PVP diblock copolymer solutions were determined using pyrene as previously reported [18]. Pyrene as a fluorescence probe is preferentially solubilized into the hydrophobic region. The fluorescence of the pyrene is sensitive to change of microenvironment, which permits monitoring its incorporation into hydrophobic core in micelle above CMC. When its environment changes from a polar region to a less polar one, a remarkable change occurs in its excitation spectra. The change reflects the micelle formation and partitioning of pyrene into hydrophobic environment.

Table 2
Characterization of PCL/PVP polymeric micelles

| Sample | Composition of PCL (mol%)[a] | Hydrodynamic diameter of micelles (nm)[b] | CMC (mg/l)[c] |
|---|---|---|---|
| CP-1 | 35.2 | 32.6 ± 4.2 | 10 |
| CP-2 | 45.4 | 42.8 ± 6.4 | 5 |
| CP-3 | 73.0 | 79.4 ± 12.8 | 2 |

[a] Calculated from $^{1}$H NMR spectra in $CDCl_3$.
[b] Determined by DLS measurement.
[c] Determined from pyrene intensity ratios in fluorescence excitation spectra.

Fig. 6(a) shows the excitation spectra of pyrene at different concentrations of CP-1 diblock copolymer solution. The excitation spectra of pyrene showed a critical change, indicating that the (0,0) band of pyrene shifted from 333.5 to 337.3 nm. The change suggested that pyrene molecules moved from polar water to less polar PCL core of micelle. Therefore, the CMC values of PCL/PVP diblock copolymers could be determined through the change of characteristic excitation spectra of pyrene. The plot of $I_{337.3}/I_{333.5}$ ratios versus the logarithm of CP-1 diblock copolymer concentrations is shown in Fig. 6(b). The intensity ratio ($I_{337.3}/I_{333.5}$) exhibited a negligible change at low concentration of polymer. However, as the concentration of polymer increased, the intensity ratio increased dramatically from a certain concentration of polymer, that is


Fig. 4. TEM photograph of CP-1 micelles.

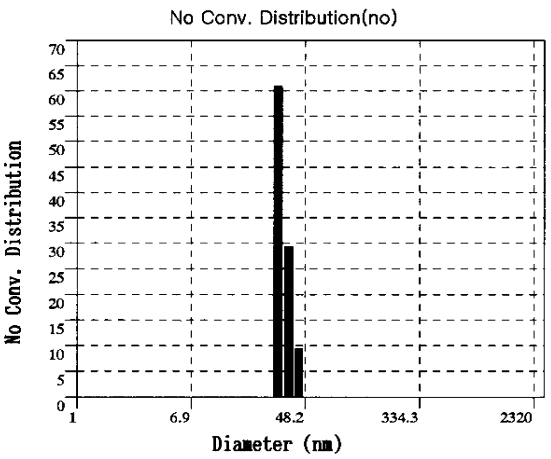


Fig. 5. Size distribution of CP-1 micelles.

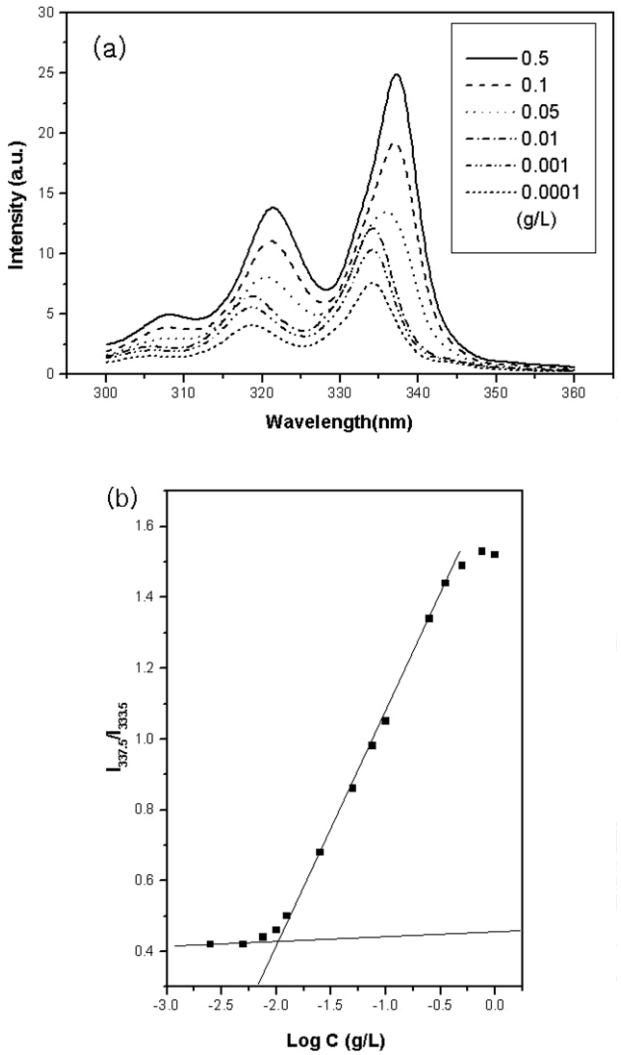


Fig. 6. Fluorescence excitation spectra (a) of pyrene ($6 \times 10^{-7}$ M) at different concentrations of CP-1 diblock copolymer solution: ($E_m = 390$ nm) and plot (b) of $I_{337.3}/I_{333.5}$ ratios versus the logarithm of CP-1 diblock copolymer concentrations.

CMC, indicating that pyrene molecules transferred from aqueous phase to hydrophobic PCL core. The CMC was determined from the concentration corresponding to the interception point of two straight lines. As summarized in Table 2, the CMC of diblock copolymer depended on the length of hydrophobic PCL segment as expected.

In conclusion, the well-defined PCL/PVP diblock copolymers were synthesized through combining the radical polymerization of VP and controlled coordination–insertion ring-opening polymerization of CL using aluminum alkoxides. Polymeric micelles composed of PCL as a hydrophobic core and PVP as a hydrophilic shell were prepared by a diafiltration method. The sizes of micelles ranged from 30 to 80 nm in average size. The novel micelles formed from the well-defined PCL/PVP diblock copolymer seem to be feasible as novel promising carriers in biomedical and pharmaceutical applications.

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