Polymer Micellar Aggregates of Novel Amphiphilic Biodegradable Graft Copolymer Composed of Poly(aspartic acid) Derivatives: Preparation, Characterization, and Effect of pH on Aggregation

Tong-Ying Jiang,[1] Zhong-Yan Wang,[1] Lin-Xi Tang,[1] Feng-Kui Mo,[1] Chuang Chen[2]

[1]*School of Pharmacy, Shenyang Pharmaceutical University, Shenyang 110016, People's Republic of China*
[2]*Liaoning Dasheng Pharmaceutical Co. Ltd., Shenyang, 110136, People's Republic of China*

Received 19 March 2005; accepted 27 April 2005
DOI 10.1002/app.22818
Published online 19 December 2005 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Novel amphiphilic biodegradable graft copolymer based on poly(aspartic acid) was prepared by attaching monomethoxy polyethylene glycol (mPEG) as hydrophiphic segment to poly(aspartic acid-*g*-octadecylamine) (PASP-*g*-ODA) as hydrophobic backbone. The chemical structures of amphiphilic copolymers were confirmed by FTIR and ^{1}H NMR spectroscopy. The polymeric micelles were prepared with solvent evaporation and their physicochemical properties in aqueous media were characterized by dynamic light scattering (DLS) and fluorescence spectroscopy. These micelles were confirmed to be pH-sensitive by measuring optical transmittance of micelle solution and the size of micellar aggregates. The number average diameter of polymeric micelles prepared in medium at pH 2.5 was larger than that in neutral and basic medium and showed a bimodal size distribution because of the protonation of carboxyl groups in backbone. Furthermore, the polymeric micelle can load water-insoluble drug (podophyllotoxin), and the drug release from micelles showed a pH-dependency.
 J Appl Polym Sci 99: 2702–2709, 2006

Key words: amphiphilic polymer; micelles; poly(aspartic acid); pH-sensitivity; drug delivery systems; podophyllotoxin

INTRODUCTION

Recently, polymer micelles or self-aggregates of hydrophobized water-soluble polymers have been extensively studied as novel types of carriers for drug delivery system. The amphiphilic copolymers containing incompatible hydrophobic and hydrophilic segments in aqueous milieu give rise to two distinctly separated phases: hydrophobic inner core and hydrophilic outer shell. Core segregation from aqueous milieu, which is the direct driving force for micellization, proceeds through intermolecular association force, including hydrophobic and electrostatic interaction, metal complexation, and hydrogen bonding of constituents of copolymers, while the hydrophilic palisades surrounding the inner core is the crucial factor to the stability of polymeric micelle. Among the different polymer-based drug delivery systems, polymeric micelles represent a promising delivery vehicle for poorly water-soluble pharmaceutical active ingredients and are widely used in pharmaceutical formulation to enhance drug solubility, stability, and biopharmaceutical properties, namely permeability across membranes and permanence in the circulation.[1–3]

Polymeric micelles, which have natural protein or synthesized peptides on their periphery or interior, have recently been developed for utility in the field of gene and drug delivery.[4–6] Poly(aspartic acid) has fully biodegradable, water-soluble properties and toxicological suitability such as lack of toxicity, antigenicity, and immunogenicity and has become an attractive candidate for drug carriers.[7] Amphiphilic block copolymers based on poly(aspartic acid) and monomethoxy polyethylene glycol (mPEG) have been synthesized by melt polycondensation reaction of mPEG and *N*-(benzyloxycarboxyl)-L-aspartic acid anhydride.[8] Amphiphilic graft copolymers based on poly(2-hydroxyethyl aspartamide) have been synthesized by the aminolysis of polysuccinimide (PSI) with amine-terminated poly(ethylene oxide) chains and aminoethanol.[9] Although the active flanking carboxylic groups of poly(aspartic acid) can react with some chain segments or groups to adjust backbone amphiphilic properties or to carry active substances,[10,11] while few studies were reported on the attaching of mPEG to poly(aspartic acid-*g*-octadecylamine) (PASP-*g*-ODA) by esterification based on hydroxyl of mPEG and carboxyl of PASP-*g*-ODA.

Moreover, the *pK*-values of conventional polymers based on a weak acid (carboxylic group) range typi-

Correspondence to: F.-K. Mo (fengkuimo@yahoo.com.cn).

cally from 4 to 6, so the aggregates of polymers with carboxylic group usually have pH-sensitivity.[12] For example, the self-aggregates of poly(aspartic acid) grafted with long alkyl chain showed pH-sensitive aggregation behavior. In acidic range (below pH 3.2), large aggregates were formed by the attractive interaction between protonated aspartic acid unit in PASP backbone, which results in the increase in turbidities and effective diameter.[6]

Podophyllotoxin (PODO), which is a highly lipophilic aryltetralin lactone and virtually insoluble in water, was selected as a model drug. PODO has a wide array of effects on biological systems, including inhibition of viral replication, nucleoside transportation into mammalian cells, and microtubule assembly during mitosis.[13,14] Clinicians and research scientists have long speculated that PODO's lackluster therapeutic index could be a result of its insolubility and unpredictable systemic behavior.[15]

The PASP-*g*-ODA with 21% degree of substitution (DS) of octadecylamine (ODA) was water-insoluble polymer, based on which we synthesized amphiphilic graft copolymer PASP-*g*-ODA-*g*-PEG by conjugating mPEG with PASP-*g*-ODA. Carboxyl groups in the backbone may endow PASP-*g*-ODA-*g*-PEG with pH-sensitive property that may affect the state of aggregates in aqueous environment. Thus, we monitored the formation of stable self-aggregates in aqueous environment and the effect of pH on the self-aggregation of PASP-*g*-ODA-*g*-PEG in aqueous solution. Moreover, we prepared the polymeric micelle loading PODO to investigate the capability of PASP-*g*-ODA-*g*-PEG to carry water-insoluble drug and properties of drug release.

EXPERIMENTAL

Materials

L-Aspartic acid was obtained from BBI (Ontario, Canada) and was used without further purification. Monomethoxy polyethylene glycol (mPEG; weight average molecular weight (M_w) = 5000) was obtained from Fluka (Buchs, Switzerland) and was used without further purification. *N*, *N*′-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), ODA, and phosphoric acid (85%) were purchased from Shanghai Chemical Industry Company (China). *N*, *N*-Dimethylformamide (DMF) and other chemicals were purchased from Liaoning Chemical Industry Company (China) and were of analytical grade. DMF was dried using Molecular Sieve 4 Å. Benzene was dried with sodium metal and distilled.

Preparation of PSI and PASP-*g*-ODA

General structures of the PASP derivatives are shown in Figure 1. PSI was prepared by polycondensation of L-aspartic acid in the presence of phosphoric acid at 180°C.[16] The structure of PSI was confirmed by FTIR and ^{1}H NMR analysis, which were in agreement with the published data.[16] The weight average molecular weight (M_w) of PSI was 37,200 g mol^{-1}, which was determined by viscosimetric method following Mark–Houwink equation $[\eta] = 1.32 \times 10^{-2} \times M^{0.76}$.[17]

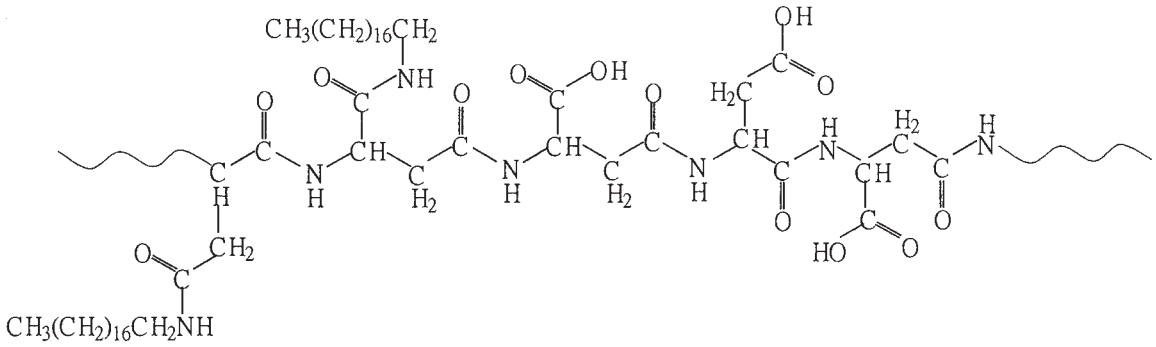
PASP-*g*-ODA was prepared by the method of the literature with little modification.[6] PSI (1.45 g) was dissolved in DMF (15 mL) and was heated to 70°C. The ODA (1.35 g) was added to the mixture. The reaction mixture was stirred at 70°C for 24 h and cooled to ambient temperature. Insoluble product was removed by filtration. The clear filtrate was added dropwise to 1*M* NaOH solution to hydrolyze the remaining succinimide unit of PSI. After vigorous stirring at ambient temperature for 3 h, the reaction mixture was precipitated in excess methanol twice. The precipitate was collected by filtration and dispersed in distilled water. The obtained mixture was acidified with 3 mL hydrochloric acid (12*M*) and stirred for 1 h at ambient temperature. The insoluble substance was filtered and washed with distilled water until the filtrate proved to be neutral when tested with pH paper. The product was dried in vacuo at 60°C. The structure of PASP-*g*-ODA was characterized by FTIR spectrophotometry and ^{1}H NMR analysis.

IR (cm^{-1}): 3400–2500 (—COON), 2930–2850 (—CH_2—, —CH_3), 1730 (—COOH), 1658 (—CO—NH—) and 1541 (—CO—NH). ^{1}H NMR, δ/ppm: 0.84 (t, 3H, —CH_3), 1.23 (s, 29.7H, —(CH_2)$_{15}$—CH_2CH$_3$), 2.6–2.8 (m, 9.3H, —CO—CH—CH_2—CO—NH—), 4.5–4.7 (m, 4.6H, —NH—CH (CO) CH$_2$).

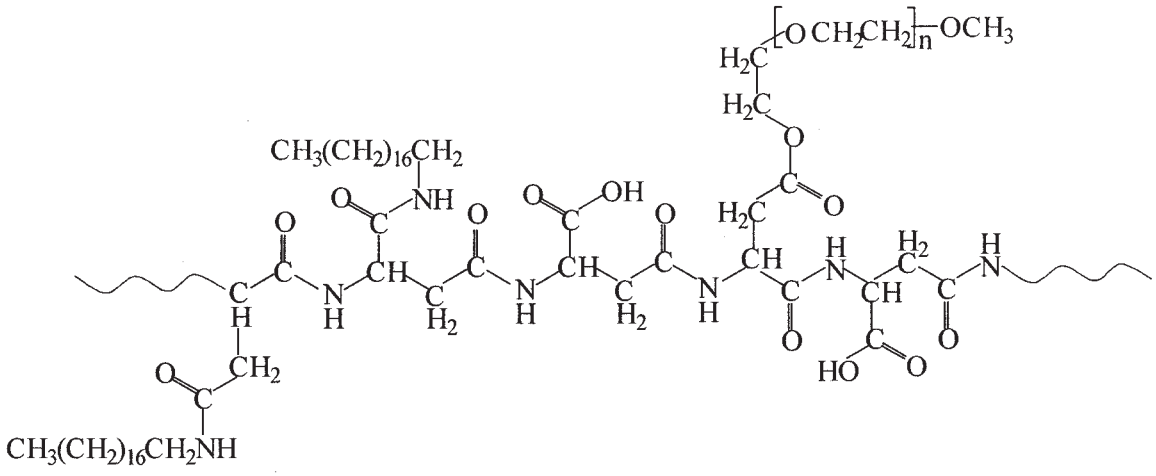
Preparation of PASP-*g*-ODA-*g*-PEG

All polymers synthesized are listed in Table I. A general procedure is described below. PASP-*g*-ODA, mPEG and DMAP were dissolved in anhydrous DMF (15 mL) and dry benzene (30 mL), and benzene was then removed in vacuo. DCC dissolved in dry DMF (5 mL) was added dropwise to the solution at 0°C. The reaction mixture was stirred at ambient temperature for 24 h. The insoluble precipitate was filtered out and the clear filtrate was dialyzed against distilled water using dialysis bag (M_w cutoff: 12,000–14,000 g mol^{-1}) for 48 h with successive exchange of fresh distilled water. The product solution was filtered through 0.8 μm film and freeze-dried. The structure of PASP-*g*-ODA-*g*-PEG was characterized by FTIR spectrophotometry and ^{1}H NMR analysis.

IR (KBr, cm^{-1}): 3400–2500 (—COON), 2930–2850 (—CH_2—, —CH_3), 1740 (—CO—OCH$_2$—), 1730 (—COOH), 1648 (—CO—NH—), 1550 (CO—NH) and 1105 (O—C—O). ^{1}H NMR, δ/ppm: 0.85 (t, 3H, —CH_3), 1.23 (s, 30H, —(CH_2)$_{15}$—CH_2CH$_3$), 2.6–2.8 (m, 9.4H, —CO—CH—CH_2—CO—NH—), 3.7 (d, 25.6H, —CH_2CH$_2$O—), 4.5–4.7 (m, 4.7H, —NH—CH (CO) CH$_2$).



PASP−g−ODA



PASP−g−ODA−g−PEG

Figure 1 Molecular structure of PASP-*g*-ODA and PASP-*g*-ODA-*g*-PEG.

Structural characterization

Infrared spectra were recorded with a Bruker IFS 55 FTIR spectrometer, using KBr plates. PASP-*g*-ODA and PASP-*g*-ODA-*g*-PEG were dissolved in DMSO-d_6. ^{1}H NMR spectra were measured using Bruker ARX-300 spectrometer (300 MHz).

Preparation of polymeric micelles with solvent evaporation

PASP-*g*-ODA-*g*-PEG (60 mg) was dissolved in methanol (20 mL) in a round-bottom flask by slight heating. Distilled water (30 mL) was added to the solution in a dropwise manner. Methanol was evaporated under

TABLE I
Preparation and Physicochemical Properties of PASP-g-ODA-g-PEG Copolymers

PASP-g-ODA-g-PEG	I	II	III	IV
Starting mPEG, M_w	5000	5000	5000	5000
Starting PSI, M_w	37,200	37,200	37,200	37,200
PASP-g-ODA (g)	1.68	1.68	1.68	1.68
mPEG (g)	0.75	1.5	3	4
DCC (g)	0.062	0.124	0.248	0.331
DMAP (g)	0.018	0.036	0.072	0.097
DS of mPEG, Feed (mol %)	1.5	3	6	8
DS of mPEG, ^{1}H NMR (mol %)	1.2	2.7	5.1	6.5
CMC ($\times 10^{-3}$g · L^{-1})	0.98	4	11	—
Mean diameter (nm) of micelle	70.4 ± 13.6	39.6 ± 5.3	14.1 ± 1.7	—

vacuum at 30°C and the mixture was concentrated to 16 mL, followed by sonication using a bath-type sonicator model at room temperature for 20 min. The micellar solution was filtered through 0.45 μm film to remove dust and the filtrate was freeze-dried.

Measurement of fluorescence spectroscopy

Critical micelle concentration (CMC) of PASP-*g*-ODA-*g*-PEG copolymers was estimated by measuring fluorescence spectroscopy using pyrene, a hydrophobic fluorescence probe that preferentially partitions into the hydrophobic core of the micelle.[18] Samples for spectroscopic analysis were prepared as follows:

A pyrene stock solution ($5 \times 10^{-3}M$) was prepared in acetone and stored at 4°C until used. The pyrene solution in acetone was added to distilled water to make a pyrene concentration of $12.0 \times 10^{-7}M$. The solution was then distilled under vacuum at 40°C to remove acetone. The polymer solutions of various concentrations (10^{-4}–1 g $\cdot$ L^{-1}) were prepared in distilled water (or aqueous solution at pH 2.5) with solvent evaporation as earlier. The polymer solutions were mixed with the acetone-free pyrene solution and the final concentration of pyrene in each sample solution was adjusted to $6.0 \times 10^{-7}M$, which is nearly equal to its solubility in water at 25°C. The resulting mixture was stirred at 50°C for 6 h to equilibrate the pyrene and the micelles and left to cool overnight at room temperature. The obtained solution was then used to measure the steady-state fluorescence spectra at ambient temperature using spectrofluorometer (Shimadzu F-7000 spectrofluorimeter, Shimadzu, Tokyo, Japan). The emission wavelength used for excitation spectra was 390 nm. The widths of slits were chosen to be 1.5 and 1.5 nm for excitation and emission, respectively.

Measurement of the size distribution

The size distribution was measured at room temperature with dynamic light scattering (DLS; Nicomp 380ZLS) with a He–Ne laser at a scattering angle of 90°. Each aggregate solution of 1 g $\cdot$ L^{-1} concentration was used for measuring the size distribution.

Measurement of optical transmittance

To investigate the property of the polymeric micelle solution at various pH, the micelle solutions of PASP-*g*-ODA-*g*-PEG (1 g $\cdot$ L^{-1}) were prepared according to the earlier method except adding solutions at various pH prepared from diluted HCl or NaOH instead of distilled water to the polymer methanol solution and then the optical transmittance of micelle solutions was measured at 500 nm (Shimadzu UV-2201, Japan). The optical transmittance of micelle solutions of PASP-*g*-ODA-*g*-PEG with various DS of mPEG was also measured at 500 nm.

Physical loading of PODO in PASP-*g*-ODA-*g*-PEG micelle

PASP-*g*-ODA-*g*-PEG (60 mg) (mPEG 5.1%) and PODO (20 mg) were dissolved in 20 mL of methanol by slight heating and 30 mL of distilled water (solutions at pH 2.5) was added dropwise to the stirring solution. Methanol was evaporated at 30°C via rotary evaporation and the mixture was reduced to 16 mL and sonicated using a bath-type sonifer at room temperature for 20 min. Each micellar solution was filtered through a 0.45 μm film to remove dust and precipitated PODO and the filtrate was freeze-dried. To measure the content of the drug loaded, a known amount of freeze-dried product was dissolved in DMF and the solution was assayed with HPLC method to determine the content of the loaded drug. The HPLC condition is as follows: a reverse-phase column Diamonsil™ (Dikma Technologies) C18 column 200 $\times$ 4.6 mm, 5 μm, mobile phase of methanol/water(50/50 v/v) and a UV detector set at 300 nm with a flow rate of 1 mL $\cdot$ min^{-1}.

Drug release experiment

Freeze-dried product (50 mg) was dissolved in 10 mL of distilled water, and the solution was sealed in a dialysis bag (M_w cutoff: 12,000–14,000), which was followed by incubating in 50 mL of release medium (containing 45 mL of phosphate buffered at pH 2.2 or 7.0 and 5 mL alcohol) at 37°C, respectively. The release of PODO from micelles was tested under mechanical shaking (60 rpm). The whole incubation medium was replaced by fresh medium so as to maintain a sink condition at predetermined sampling time intervals. The amount of PODO released into the removed incubation solution was analyzed with HPLC method.

RESULTS AND DISCUSSION

Synthesis and structural characterization

A graft copolymer, PASP-*g*-ODA-*g*-PEG, was synthesized in three steps. PSI was aminolyzed with ODA and the remaining succinimide units of PSI were hydrolyzed with NaOH solution. After synthesizing precursors of PASP-*g*-ODA, the mPEG was attached to PASP-*g*-ODA by esterification between hydroxyl of mPEG and carboxyl of PASP-*g*-ODA. The structures of PASP-*g*-ODA and PASP-*g*-ODA-*g*-PEG are shown in Figure 1.

The feed ratio and main physicochemical properties of a series of PASP-*g*-ODA-*g*-PEG are summarized in Table I. The copolymer with various DS of mPEG

could be achieved by varying the feed ratio of mPEG to PASP-*g*-ODA in the esterification. The FT-IR spectrum of PASP-*g*-ODA showed the characteristic absorption peak of carboxyl group at 1730 cm^{-1} and between 3400 and 2500 cm^{-1} and the characteristic absorption peak of amide at 1658 and 1541 cm^{-1}. In the FT-IR spectrum of PASP-*g*-ODA-*g*-PEG, the above absorption peaks had no obvious change, but the sharp peak of ether at 1105 cm^{-1} appeared, at the same time, the peaks between 2850 and 2930 cm^{-1} for methylene became strong. In addition, the inconspicuous shoulder peak at 1740 cm^{-1} was attributed to the formation of the ester. These results indicate that mPEG has been attached to PASP-*g*-ODA backbone. The structures of PASP-*g*-ODA and PASP-*g*-ODA-*g*-PEG were also confirmed by ^1H NMR analysis. In the spectrum of PASP-*g*-ODA, The peaks at 0.84 and 1.23 ppm were assigned to methyl and methylene of ODA, and the peaks at 2.6–2.8 ppm and 4.5–4.7 ppm belonged to methylene and methine protons of backbone, respectively. The DS of ODA was calculated by comparing the integral area of the peak of methyl of ODA at 0.84 ppm with one at 4.5–4.7 ppm. The DS of ODA was expressed as DS = (octadecylalkyl groups/polymer repeating unit) × 100%. Comparing to the spectrum of PASP-*g*-ODA, the peaks at 3.7 ppm belonged to methylene of mPEG in the spectrum of PASP-*g*-ODA-*g*-PEG. The DS of mPEG was calculated by comparing the integral area of the peak at 3.7 ppm with one at 4.5–4.7 ppm. The DS of mPEG was expressed as DS = (polyethylene glycol/polymer repeating unit) × 100%.

Physicochemical properties of polymeric micelles

Fluorescence measuring was used to monitor the formation of polymeric micelles in aqueous media.

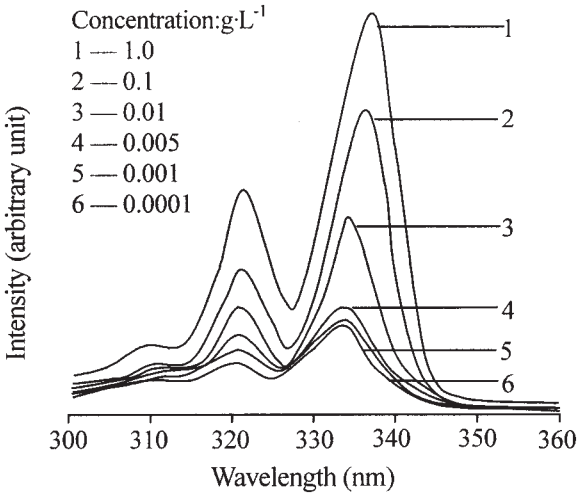


Figure 2 Excitation spectra of pyrene (6.0×10^{-7} mol · L^{-1}) in distilled water in the presence of PASP-*g*-ODA-*g*-PEG (emission wavelength was 390 nm).

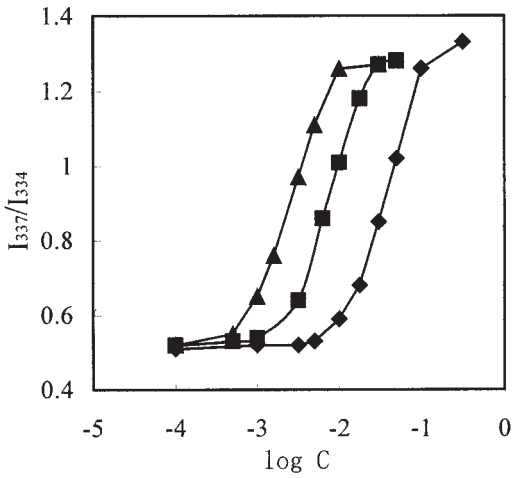


Figure 3 Plots of the intensity ratio I_{337}/I_{334} from pyrene excitation spectra *vs.* the log C of PASP-*g*-ODA-*g*-PEG series in distilled water. (▲) DS of mPEG = 1.2%, (■) DS of mPEG = 2.7%, (◆) DS of mPEG = 5.1%.

Pyrene was used as a fluorescence probe because it is a condensed aromatic hydrocarbon that is highly hydrophobic and sensitive to the polarity of the surrounding environment. Below the CMC, the pyrene is solubilized in water, a medium of high polarity. When micelles are formed, pyrene partitions preferentially into the hydrophobic domain afforded by the micellar core, and thus experiences a nonpolar environment. Consequently, numerous changes such as an increase in the fluorescence intensity and a red shift of excitation spectrum are observed.[12,19] The fluorescence excitation spectra of the pyrene at various PASP-*g*-ODA-*g*-PEG concentrations are shown in Figure 2. At low concentration of the polymeric micellar solution, changes in the total fluorescence intensity and the shift of the band at 334 nm were insignificant. As the concentration of the polymer increases, however, an increase in the total fluorescence intensity and a red shift of spectrum were evidently observed. The band for pyrene at 334 nm shifted to 337 nm with increasing polymer concentration, which demonstrated that pyrene molecules gradually transferred into less polar micellar core. The CMC, which is the threshold concentration of self-assembly by intra- and/or intermolecular association, was determined from the change of the intensity ratio of I_{337}/I_{334} against the polymer concentration in the pyrene excitation spectrum. The curves of intensity ratios I_{337}/I_{334} against the copolymers concentration are plotted in Figure 3. CMC of copolymers with various DS of mPEG (listed in Table I) was taken from the intersection of two straight lines: a horizontal line with an almost constant value of the ratio (I_{337}/I_{334}) in the lower concentration range and a tangent to the steep upward section of the sigmoidal curve. The higher CMC values corresponding to higher ratio of mPEG were attributed to the increase of

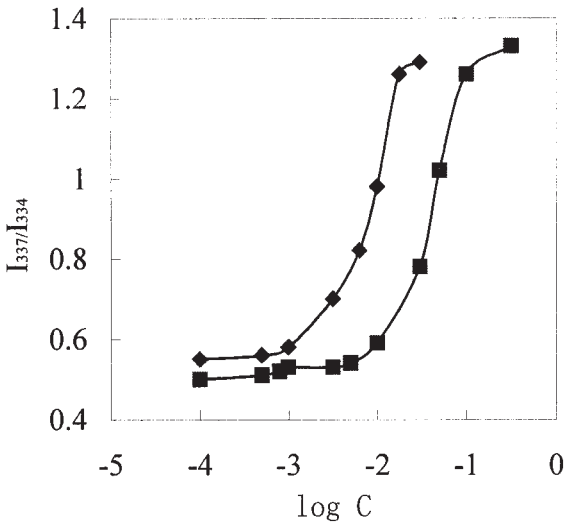


Figure 4 Plots of the intensity ratio I_{337}/I_{334} from pyrene excitation spectra *vs*. the log C of PASP-*g*-ODA-*g*-PEG series in (■) distilled water and (◆) solution at pH 2.5.

hydrophilic moiety. This result is similar to the report of Yasugi K. et al. that CMC of block copolymers of poly(L-lactide) (PLA)/poly(ethylene glycol) (PEG) increased with decreasing weight ratio of PEG to PLA segment.[20] Clearly, the increase in the hydrophilic portion reduced the chances of hydrophobic interactions between the copolymer chains and resulted in the formation of weaker hydrophobic cores. Figure 4 shows the changes of the intensity ratios I_{337}/I_{334} in solution at pH 2.5 and in distilled water containing various PASP-*g*-ODA-*g*-PEG (DS of mPEG = 5.1) concentration. It can be seen that the CMC of PASP-*g*-ODA-*g*-PEG in solution at pH 2.5 is lower than that in distilled water. The phenomenon is considered as the result of the increase in hydrophobic moiety that derives from protonation of carboxyl groups at a lower pH region. The result is similar to the report elsewhere that the CMC of block copolymers of PLA/PEG modified with sulfonamide in borate buffer (pH 9.0) were slightly higher than that of PLLA/PEG without modification, which is attributed to the enhanced hydrophilicity as the result of the ionization of sulfonamide groups at a higher pH region.[12]

The properties of PASP-*g*-ODA-*g*-PEG micellar solutions were investigated by measuring the optical transmittance and particle size. Optical transmittance changes of polymeric micellar solutions prepared in solutions at various pH are shown in Figure 5. The optical transmittances for copolymers with various DS of mPEG had no obvious differences at each pH points. The number average diameters of polymeric micelles that were determined by DLS decreased from 70.4 to 14.1 nm (as Table I) with the increasing DS of mPEG from 1.2 to 5.1 (mol %). The result is consistent with other reports that stronger hydrophobicity produced larger particle size.[4,21]

The pH-sensitivity of polymeric micelles was examined by measuring optical transmittance of micellar solution, particle size, loading drug, and drug release as a function of pH of medium used for preparing micellar solution. Each polymeric micelle had a phase transition around pH 4 irrespective of DS of PEG as shown in Figure 5. Below pH 4, the transmittance of micellar solution was lower because of the formation of larger aggregates. Figure 6 shows the effect of pH on diameter of polymeric micelle. We noted that the size of micelles increases with decreasing pH for the same concentration of PASP-*g*-ODA-*g*-PEG (mPEG 1.2%). The number average diameters of polymeric micelles prepared in solution at pH 2.5 showed a bimodal size distribution. The small one is located near 50–100 nm, while the larger one varies between 200 and 300 nm. The larger one may be secondary aggregates from primary small aggregates. The formation of larger aggregates could be attributed to the loss of the polarity of backbone resulting from protonation of carboxyl groups and attractive interaction between protonated aspartic acid units in or between backbones. Polymeric micelles prepared in distilled water or basic solution show single-model distribution. This can be attributed to the fact that a part or whole of deprotonated carboxyl group endows each aggregates with negative charge; therefore, self-aggregation of polymer can be stabilized by charge repulsion.

When PODO was incorporated into the micelles in solution at pH 2.5 and in distilled water, the drug contents loaded by micelles were 8.7 ± 1.2 wt % and 7.2 ± 0.9 wt %, respectively. Figure 7 shows the cumulative PODO release from PASP-*g*-ODA-*g*-PEG micelles against pH 2.2 and 7.0 releases medium. It can be seen that the rapid release during the first 1 h is similar at pH 2.2 and 7.0, while 41 wt % of PODO was

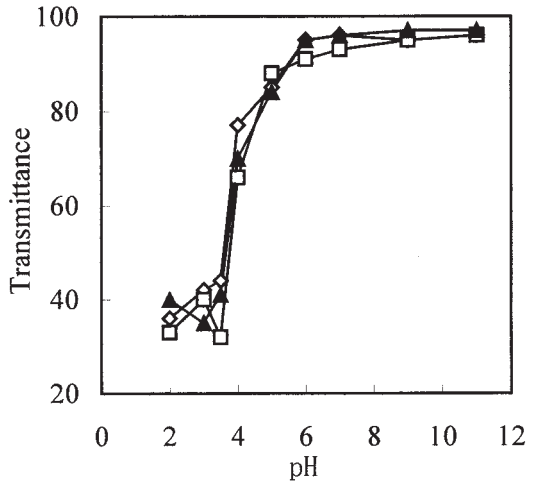


Figure 5 Optical transmittance change of PASP-*g*-ODA-*g*-PEG solution with various DS of mPEG *vs*. pH of solution used for preparing micelles. (□) DS (mPEG) = 1.2%, (◇) DS (mPEG) = 2.7%, (▲) DS (mPEG) = 5.1%.

released at pH 2.2 and 57 wt % of PODO at pH 7.0 when the release time reaches 3 h. These results suggest that drugs absorbing the surface of micelles resulted in the rapid release, but other factors controlled drug release have no evident differences during the first 1 h; therefore, the release rate of PODO is near.

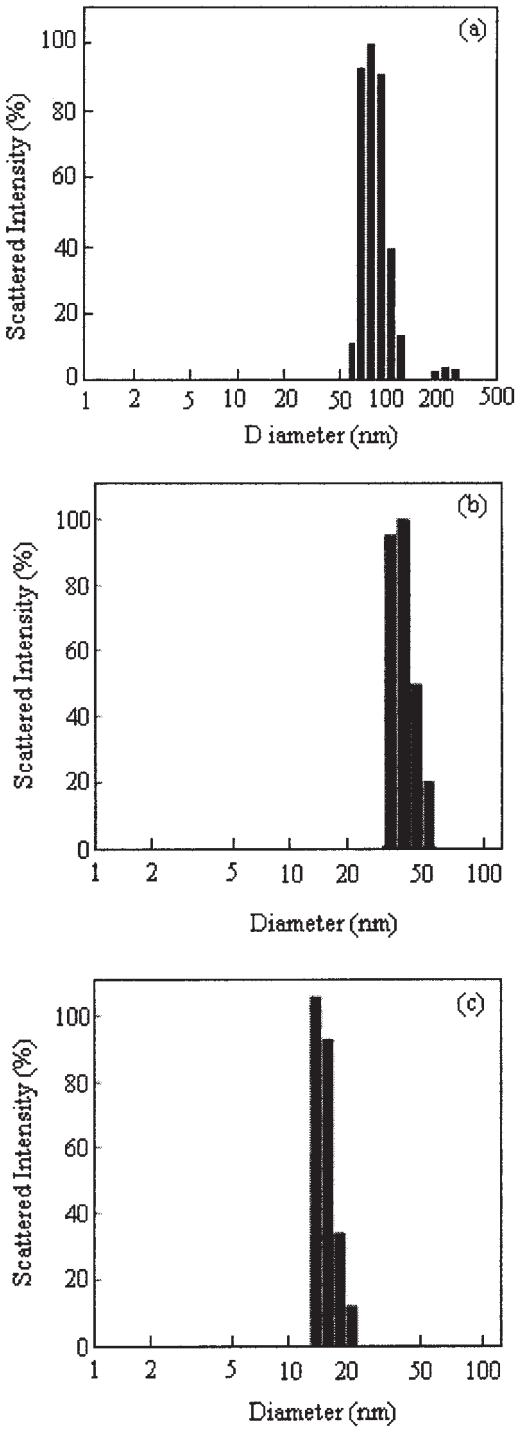


Figure 6 Dependence of the micellization of the graft copolymer on pH in aqueous solution (DS of mPEG = 1.2%, the concentration of the copolymer in aqueous solution is 1.0 g L^{-1}).(a) pH 2.5 solution, (b) distilled water, (c) pH 10.5.

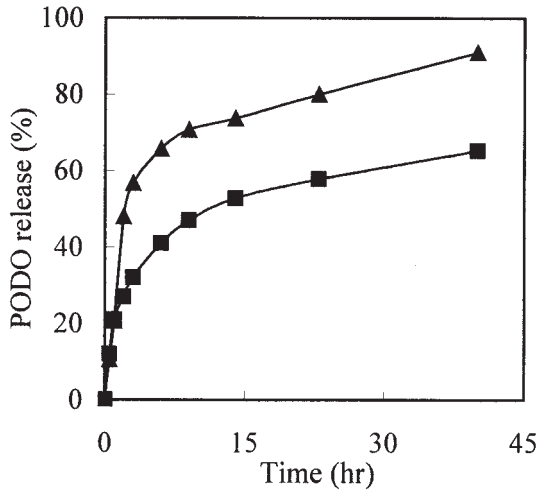


Figure 7 Release profiles of PODO from polymeric micelle against release medium (■) pH 2.5 and (▲) pH 7.0 at 37°C.

After the second replacement of release medium, carboxyl group of polymeric micelle may experience deprotonation at 7.0 or protonation at pH 2.2 to the maximum degree and the structure of polymeric micelle have fine changes. The rapid drug release between 1 and 3 h at pH 7.0 is the result of deprotonation of the most carboxyl group and the release of drug loaded in hydrophobic domain constructed by protonated carboxyl group. This indicates that the drug release from PASP-*g*-ODA-*g*-PEG micelles showed a pH-dependency and the drug release rate could be adjusted by varying pH of the release medium.

CONCLUSIONS

Novel amphiphilic biodegradable graft copolymer, PASP-*g*-ODA-*g*-PEG, was prepared by controlled grafting mPEG to PASP-*g*-ODA backbone by esterification. Increasing the ratio of mPEG to PASP-*g*-ODA induced the decrease in diameter of micelle and increase in CMC of amphiphilic polymer. The polymeric micelles can form in solutions at various pH. Because of the protonation or deprotonation of carboxyl groups in backbone, the PASP-*g*-ODA-*g*-PEG micelle in the aqueous media showed the pH-sensitivity, i.e., the diameter of micellar aggregates and the turbidity of micellar solution were larger in acid than those in neutral and basic medium and the content of PODO loaded by the polymeric micelle in acid solution was larger than that in neutral. Furthermore, the drug release from PASP-*g*-ODA-*g*-PEG micelles showed a pH-dependency.

References

1. Mira, F. F.; Mariella, P.; Françoise, M. W. J Controlled Release 2003, 93, 59.
2. Lasic, D. Nature 1992, 335, 279.

3. Afsaneh, L.; John, S.; Glen, S. K. Adv Drug Delivery Rev 2002, 54, 169.
4. Kang, H.; Kim, G. D.; Han, S. H.; Chang, I. S. J Controlled Release 2002, 81, 135.
5. Harada, A.; Togawa, H.; Kataoka, K. Eur J Pharm Sci 2001, 13, 35.
6. Kang, H. S.; Shin, J. D.; Yang, J. W. Polym Bull 2000, 45, 39.
7. Matsuyama, M.; Kokufuta, F.; Kusumi, T.; Harada, K. Macromolecules 1980, 13, 196.
8. Won, C. Y.; Chu, C. C.; Lee, J. D. Polymer 1998, 39, 6677.
9. Caldwell, G.; Neuse, N. W.; Perlwitz, A. G. J Appl Polym Sci 1997, 66, 911.
10. Harada, A.; Kataoka, K. Macromolecules 1998, 31, 288.
11. Yokoyama, M.; Fukushima, S.; Uehara, R.; Okamoto, K.; Kataoka, K.; Sakurai Y.; Okano, T. J Controlled Release 1998, 50, 79.
12. Han, S. K.; Na, K.; Bae, Y. H. Colloids Surf A 2003, 214, 49.
13. Greenspan, E. M.; Colsky, J.; Schoenbach, E. B.; Shear, M. J.; J Natl Cancer Inst 1954, 14, 1257.
14. Sackett, D. L. Pharmacol Ther 1993, 59, 163.
15. Stahelin, H. F.; Wartburg, A. V. Cancer Res 1991, 51, 5.
16. Neri, P.; Antoni, G.; Benvenut, F.; Cocola, F.; Gazzei, G. J Med Chem 1937, 16, 893.
17. Vlasák, J.; Rypáček, F.; Drobník, J.; Saudek, V. J Polym Sci Polym Symp 1979, 66, 59.
18. Malmsten, M.; Lindman, B. Macromolecules 1992, 25, 5440.
19. Kim, I. S.; Kim, S. H. Int J Pharm 2000, 245, 67.
20. Yasugi, K.; Nagasaki, Y.; Kato, M.; Kalsoka, K. J Controlled Release 1999, 62, 89.
21. Nah, J. W.; Jeong, Y. I.; Cho, C. S. J Polym Sci Part B: Polym Phys 1998, 36, 415.