Poly(aspartic-acid) Derivatives as Polymeric Micelle Drug Delivery Systems

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ABSTRACT: A novel amphiphilic biodegradable graft copolymer based on poly(aspartic acid) (PASP) was prepared by coupling monomethoxy poly(ethylene glycol) as the hydrophilic segment with PASP-*g*-octadecyl (PASP-*g*-OD) as the hydrophobic backbone. The critical micelle concentration of the copolymer was obtained by measuring the conductivity and fluorescence spectroscopy. Polymeric micelles were prepared with the solvent evaporation method, and the size distribution and morphology of the polymeric micelles were characterized by dynamic light scattering and transmission electron microscopy. The ability of the PASP derivatives to solubilize hydrophobic drugs was investigated using methotrexate (MTX), oleanolic acid, and podophyllotoxin (PODO) as models. The results demonstrated that drug solubility depends on the copolymer concentration, copolymer composition, as well as the physicochemical property of the drug. A stability study performed with PODO loaded by polymeric micelle solution demonstrated that the micelle carrier could protect the drug from being destroyed in a pH 7.4 buffer solution; however, the micelle did not protect it in a pH 9.5 buffer solution. Pharmacokinetic studies were carried out by intravenous administration of MTX loaded by polymeric micelle solution and MTX solution. The results demonstrated that the polymeric micelle prolonged the drug permanence in blood circulation with a mean residence time of about 2.37 times and increased the systemic bioavailability with the area under the drug concentration–time curve by about 2.34 times. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 101: 2871–2878, 2006

Key words: graft copolymers; polymeric micelles; self-assembly; drug delivery system; poly(aspartic acid) derivatives; solubility

INTRODUCTION

Polymer micelles of amphiphilic copolymers have been extensively utilized for drug carrier systems to enhance drug solubility, stability, and biopharmaceutical properties, that is, the permeability across membranes and permanence in blood circulation.[1–3] This drug carrier system is composed of two distinctly separated phases: a hydrophobic inner core and a hydrophilic outer shell. Core segregation from aqueous milieu, which is the direct driving force for micellization, is made by association of the hydrophobic moiety of the amphiphilic copolymer because of their cohesive association, including hydrophobic interaction, electrostatic interaction, and hydrogen bonding. The hydrophilic shell surrounding the inner core plays a role in stabilizing the polymeric micelle and avoiding uptake by the reticuloendothelial system (RES).

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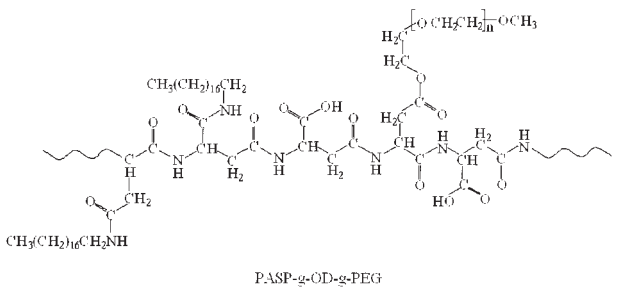
Polymeric micelles have predominant characteristics such as reduced toxic side effects of anticancer drugs via polymeric micelle formation and selective targeting, solubilization of hydrophobic drugs, stabilization of drugs, long circulation times in blood, favorable biodistribution, and lower interactions with the RES.[4–6] In fact, various types of drugs such as insoluble drugs,[7,8] a metal complex cisplatin,[9] and DNA[10] can be successfully incorporated into polymeric micelles by both chemical conjugation and physical entrapment.

α,β-Poly(aspartic acid) (PASP) derivatives possess low toxicity, biodegradability, biocompatibility, and low cost of preparation, which are the main requisites for pharmaceutical applications. Their multifunctional character can afford a variety of modifications following simple chemical procedures, and tailormade PASP derivatives with different physicochemical properties can be obtained. These properties make them a good candidate for obtaining micelle drug delivery systems. Kang et al. reported that PASP sodium salt with different degrees of substitution (DS) of the octadecyl (OD) group can form self-aggregates with a size of <30 nm in a neutral or basic aqueous medium,



Scheme 1 The molecular structure of PASP-*g*-OD-*g*-PEG.

whereas the aggregates can form large aggregates in an acid aqueous medium.[11] We found that PASP grafted with 21% DS of the OD chain was completely insoluble in water. Therefore, in this work, we coupled the hydroxyl of monomethoxy poly(ethylene glycol) (mPEG) with the carboxyl of PASP-*g*-OD to prepare the amphiphilic graft copolymer, PASP-*g*-OD-*g*-PEG. To evaluate the potential of the new PASP derivative copolymers as micelle drug carriers, the critical micelle concentration (CMC) values were measured. The solubilizations of poorly soluble drugs [methotrexate (MTX), oleanolic acid (OA), and podophyllotoxin (PODO)] as well as the stabilization of PODO by the polymeric micelle were investigated. Moreover, we investigated the *in vivo* pharmacokinetic property of MTX loaded by the polymeric micelle.

EXPERIMENTAL

Materials

L-Aspartic acid (99.9%) was obtained from Bio Basic, Inc. (Markham, Ontario, Canada) and used without further purification. mPEG (weight-average molecular weight = 5000) was obtained from Fluka (Buchs, Switzerland) and used without further purification. *N*,*N*′-Dicyclohexylcarbodiimide, 4-dimethylaminopyridine, octadecylamine (ODA), and phosphoric acid (85%) were purchased from Shanghai Chemical Industry Company (Shanghai, China). *N*,*N*-Dimethylformamide and other chemicals were analytical grade purchased from Shenyang Chemical Industry Company (Shenyang, China). The copolymers of PASP-*g*-OD-*g*-PEG (Scheme 1) were prepared and purified according to previously described preparation details.[12] Briefly, the copolymers were synthesized according to the following steps: polysuccinimmide (PSI) was obtained by thermal polycondensation of *L*-aspartic acid; PSI was aminolyzed with ODA and the remaining succinimide units of PSI were hydrolyzed with NaOH solution to obtain the precursor, PASP-*g*-OD; and mPEG was attached to PASP-*g*-OD by esterification between the hydroxyl of mPEG and the carboxyl of PASP-*g*-OD. Copolymers (I, II, and III) with a suitable DS of mPEG (listed in Table I) were selected as experimental materials.

CMC measurement

PASP-*g*-OD-*g*-PEG solutions with an increasing concentration were prepared and used to measure the conductivity. A known mount of PASP-*g*-OD-*g*-PEG was dissolved in 20 mL of methanol, and then the methanol was evaporated under a vacuum at 40°C within 30 min to prepare the polymer film. One hundred milliliters of distilled water was added to the film and it was sonicated for 5 min, incubated at 50°C for 10 min, and vortexed for 90 s. The obtained solution was filtered through a 0.45-μm film, and the filtrate was used to prepare the solutions with an increasing concentration followed by equilibrating overnight. The conductivity was measured at 25°C using a model DDS-11 conductivity meter (Instrument Factory, Shanghai, China).

Surface activity and the formation of micelles can be confirmed by measuring the CMC of PASP-*g*-OD-*g*-PEG using the fluorescent probe technique. Samples for spectroscopic analysis were prepared as follows: a pyrene stock solution (5×10^{-3} *M*) was prepared in acetone and stored at 4°C until use. The pyrene solution in acetone was added to distilled water to obtain a pyrene concentration of 12.0×10^{-7} *M*. The solution was distilled under a vacuum at 40°C to remove the acetone. Polymer solutions of various concentrations

TABLE I
Physicochemical Properties of PASP-*g*-OD-*g*-PEG Copolymers

PASP-*g*-OD-*g*-PEG[a]	I	II	III
DS of mPEG (mol %)[b]	1.2	2.7	5.1
CMC ($\times 10^{-3}$ mg/mL)[c]	0.98	4	11
Size of micelle (nm)	70.9 ± 13.6	39.6 ± 5.3	14.2 ± 1.7
Size of micelle loading MTX (nm)	81.2 ± 10.6	46.6 ± 9.6	24.2 ± 5.1
PODO concentration (mg/mL)[d]	1.37 ± 0.27	1.19 ± 0.14	0.88 ± 0.21

[a] The DS of OD in PASP-*g*-OD-*g*-PEG samples was 21% for all.
[b] The DS of mPEG (mol %) was calculated based on ^{1}H-NMR.
[c] The CMC was obtained by the fluorescent probe technique.
[d] The standard deviation values (±SD) were calculated on the basis of five experiments.

(10^{-4}–1 g/L) were prepared in distilled water with the solvent evaporation method as above. The polymer solutions were mixed with the acetone-free pyrene solution and the final concentration of pyrene in each sample solution was adjusted to 6.0×10^{-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 Shimadzu RF-5301 PC spectrofluorometer (Shimadzu, Tokyo). The emission wavelength used for the excitation spectra was 390 nm. The widths of the slits were chosen to be 1.5 and 1.5 nm for excitation and emission, respectively.

Solubility of polymer micelles

MTX, OA, or PODO and PASP-*g*-OD-*g*-PEG (1.2% mPEG) were dissolved in 20 mL of methanol. The methanol was evaporated under a vacuum at 40°C in 30 min. Then, 15 mL of distilled water was added to the polymer–drug film. The solutions were sonicated for 5 min, incubated at 50°C for 20 min, vortexed for 90 s, and kept equilibrating. Each micellar solution was filtered through the 0.45-μm film to remove unloaded drug and the filtrate was used to measure the content of drug loaded by the micelle solution with the HPLC method. The HPLC conditions were a reverse-phase column Diamonsil (Dikma Technologies) C_{18} column (200 × 4.6 mm, 5 μm) and a UV detector. For PODO, the mobile phase of methanol/water (50/50 v/v) was used with a detected wavelength set at 300 nm; for MTX, the mobile phase of acetonitrile/phosphate buffer solution (0.05*M*, pH 3.0, 14/88 v/v) was used with a detected wavelength set at 310 nm; and for OA, the mobile phase of methanol/water (88/15 v/v) was used with a detected wavelength set at 220 nm.

Stability of PODO loaded by polymeric micelles

The test samples of PODO loaded by PASP-*g*-OD-*g*-PEG (1.2% mPEG) micelles were prepared using 0.02*M* phosphate buffer at pH 5.5, 7.4, or 9.5 instead of distilled water. PODO solutions (as control) were also prepared by dissolving PODO in 0.02*M* phosphate buffer solution/alcohol (95/5 v/v). The sample solutions were maintained at 20°C. At scheduled times the solutions were sampled and assayed by HPLC.

Size and morphology of polymeric micelles

The size distribution was measured at room temperature with dynamic light scattering (DLS, Nicomp 380ZLS) with an He–Ne laser at a scattering angle of 90°. Micellar solutions with a concentration of about 1.0 mg/mL were used for measuring the size distribution.

The morphology of the polymeric micelles was characterized using transmission electron microscopy (TEM). A drop of PASP-*g*-OD-*g*-PEG solution (about 1.0 mg/mL) with or without drugs was placed on a carbon membrane coated on a 300 mesh copper grid for TEM. Excess fluid was removed from the grid surface with filter paper and the surface was freeze-dried. The grid was loaded in the TEM apparatus (JEM-2000EXII) and pictures were taken at an original magnification of 58,000 times.

Pharmacokinetics study

Mice (male and female, 3 weeks old, 20 ± 1 g) were provided by the Animal Center of Shenyang Pharmaceutical University. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University. The MTX micelle solution was prepared using the solution containing 0.02*M* phosphate buffer of pH 7.2 and 0.15*M* NaCl instead of distilled water. A 0.4-mL volume equivalent to 1.2 mg of PASP-*g*-OD-*g*-PEG and 120 μg of MTX was administered to the mice by intravenous injection into the tail vein. As a control, 0.4 mL of MTX solution (300 μg/mL) was also administered to the mice by intravenous injection. At 1/20, 1/6, 1/2, 3/4, 1, 2, 3, 5, 7, 9, 12, and 24 h time points, blood samples (approximately 0.5 mL) were obtained by retrobulbar puncture and collected into heparinized tubes at the same time. A suitable volume of tinidazole (standard) solution in methanol was added to 400 μL of the blood. The blood samples were centrifuged and 100 μL of supernatant was drawn and stored at −20°C until assay. Then, 100 μL of methanol was added to the thawed plasma sample to precipitate the proteins by vortexing for 90 s and centrifuging for 15 min at 4000 rpm. The supernatant (100 μL) was dried under a nitrogen flow. The dry product was dissolved in 50 μL of the mobile phase and centrifuged for 15 min at 4000 rpm, and 10 μL of the supernatant was analyzed by HPLC.

The noncompartmental pharmacokinetic parameters such as the area under the drug concentration–time curve (AUC), biological half-time ($t_{1/2}$), mean residence time (MRT), total clearance (CL), and apparent distribution volume at steady state (V_{ss}) were calculated based on the reported method.[13] Levels of statistical significance ($p < 0.05$) were assessed using the Student *t* test between the two means for unpaired data. All results were expressed as mean ± standard deviation.

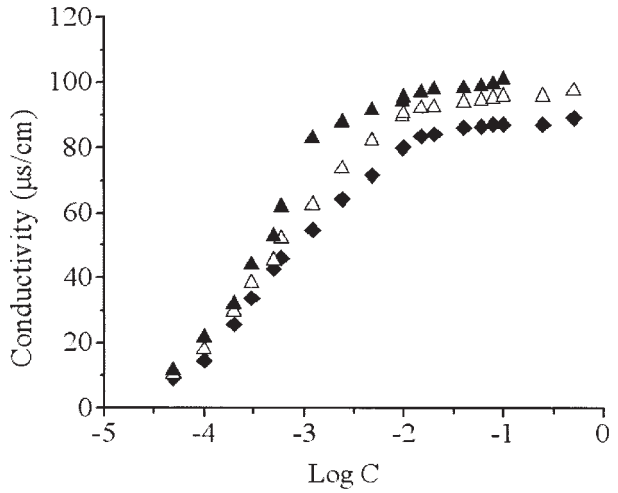


Figure 1 The concentration dependence of the conductivity of PASP-*g*-OD-*g*-PEG aqueous solutions; log *C*, the concentration of PASP-*g*-OD-*g*-PEG in solutions. DS of mPEG = (♦) 5.1, (△) 2.7, and (▲) 1.2%.

RESULTS AND DISCUSSION

Measurement of CMC

In deionized water, the carboxylic acid groups of PASP are partially ionized and the copolymer possesses a negative charge. Thus, the conductivity of the PASP-*g*-OD-*g*-PEG solution shows a correlation with the concentration of the solution. Figure 1 depicts the concentration dependency of the conductivity of the copolymer solution. A change in slope was observed in the conductivity curves at a specific copolymer concentration. The conductivity value increased with the increase in polymer concentration on the whole; however, it increased more gradually when the concentration was above the specific concentration. The copolymer concentration corresponding to the turning point in plots is its CMC. The measured CMC values of copolymers I, II, and III were 1.1, 4.3, and 12×10^{-3} mg/mL, respectively. Moreover, in the case of the same concentration solutions of three copolymers, the conductivity slightly increased with the decrease in the DS of mPEG of the copolymer because of the increase in the ionized carboxylic acid groups of PASP.

Fluorescence measurement was used to monitor the formation of polymeric micelles in aqueous media. 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 redshift of the excitation spectrum were observed. At low concentration of the polymeric micellar solution, the changes in the total fluorescence intensity and the shift of the band at 334 nm were insignificant. As the concentration of the polymer increased, however, an increase in the total fluorescence intensity and a redshift of spectrum were evident. The band for pyrene at 334 nm shifted to 337 nm with increasing polymer concentration, which demonstrated that pyrene molecules gradually transferred into the 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} versus the polymer concentration in the pyrene excitation spectrum. The curves of the intensity ratios I_{337}/I_{334} versus the copolymer concentrations are plotted in Figure 2. The CMC values of the copolymers with various DS of mPEG (listed in Table I) were basically consistent with the results obtained by measuring the conductivity, which were taken from the intersection of two straight lines (a horizontal line with an almost constant value of the I_{337}/I_{334} ratio in the lower concentration range and a tangent to the steep upward section of the sigmoidal curve). It can be noted that, for a given type of hydrophobic segment, the CMC values increase with the increasing DS of mPEG. This result is similar to the report that the CMC of poly(*L*-lactide) (PLA)/PEG increased with a decreasing weight ratio of PEG to the PLA segment.[14] Clearly, the increase in the hydrophilic portion reduced the chances of cohesive interactions between the copolymer chains and resulted in the formation of weaker hydrophobic cores.

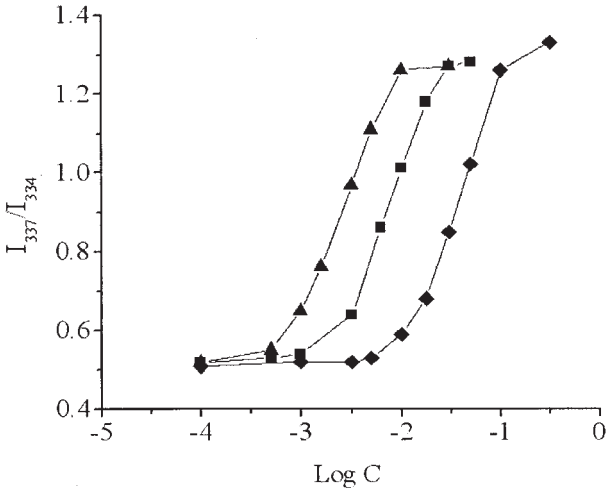


Figure 2 Plots of the intensity ratio (I_{337}/I_{334}) from pyrene excitation spectra versus the log *C* of the PASP-*g*-OD-*g*-PEG series in distilled water. DS of mPEG = (▲) 1.2, (■) 2.7, and (♦) 5.1%.

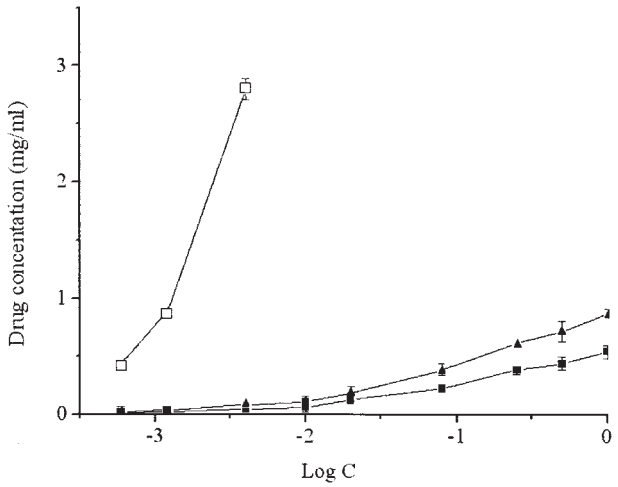


Figure 3 Solubility profiles of insoluble drugs in polymeric micelle solutions of (□) MTX, (▲) OA, and (■) PODO. The standard deviation values (±SD) were calculated on the basis of five experiments.

Solubility studies

In order to evaluate the applicability of PASP-*g*-OD-*g*-PEG as drug carriers, solubility studies were carried out using MTX, OA, and PODO as models. These drugs were selected because their poor water solubility makes them difficult to be properly formulated or limits their bioavailability.

The solubility studies used excessively high drug amounts to guarantee drug saturation conditions. The solubilization experiments of various drugs were undertaken in a distilled water medium, where each drug was insoluble in water and stable during the experimental procedures. In order to investigate the dependence of the solubility of the drugs on the copolymer concentration, PASP-*g*-OD-*g*-PEG with 2.7% DS of mPEG was used for the experiment, and the result is reported in Figure 3. A significant increase in solubility was observed for polymer concentrations above the CMC (4×10^{-3} mg/mL) and the extent of solubilization depended on the polymer concentration. The solubility of MTX, OA, and PODO in distilled water without polymer at 20°C was found to be about 21, <0.1, and <0.2 μg/mL, respectively. It was calculated that the drug concentration in the polymeric micelle solution (3 mg/mL) was 120 mg/mL in the case of MTX, 0.66 mg/mL for OA, and 1.19 mg/mL for PODO. These data indicate that the solubilization of drugs depends on the physicochemical properties of the drugs, namely, the hydrophilic/hydrophobic partition index, molecular volume, and hydrophobic/polar surface areas. Indeed, these parameters indicate the interaction of the drug with the two main components of the PASP-*g*-OD-*g*-PEG micelle: hydrophobic and hydrophilic moieties.[15] Note that the solubilization of the MTX by polymeric micelles was more evident than that of OA and PODO. The result may be attributed to the special physicochemical property of MTX and PASP-*g*-OD-*g*-PEG, that is, MTX has amino groups and PASP-*g*-OD-*g*-PEG has carboxyl groups in the side chain. In addition to the hydrophobic interaction and hydrogen bonding (as OA and PODO) between PASP-*g*-OD-*g*-PEG and MTX, the amino protonization of MTX and the electrostatic interaction between MTX and PASP-*g*-OD-*g*-PEG are two main reasons contributing to the significant increase in solubility of MTX.

Furthermore, the percentage of PODO loading decreased with increasing DS of mPEG in the case of the same polymeric concentration (3 mg/mL), and the data are listed in Table I. This result is consistent with the fact that the higher DS of mPEG results in the stronger hydrophilic property and forms a smaller particle size, providing the smaller drug storage.[16]

Stability studies

The stability of the PODO loaded by PASP-*g*-OD-*g*-PEG micelle solutions at various pHs was evaluated to investigate the effect of the polymeric micelle carrier in preventing the drug from being destroyed. Buffer solutions at pH 7.4 and 5.5 mimic the conditions found in the blood stream and in lysosomes, respectively, whereas a buffer solution at pH 9.5 is a rigorous condition for drug stability. Therefore, the hydrolysis stability of PODO loaded by polymeric micelles in such solutions was investigated. PODO was used because its trans lactone ring readily configures to its thermodynamically more stable cis-epimer or undergoes hydrolytic degradation in a weak basic medium and therefore loses biological activity. The content–time course profiles of PODO in the presence of polymeric micelles and without polymeric micelles at different pH values are reported in Figure 4.

During the 30 h of incubation, in the samples in the absence of PASP-*g*-OD-*g*-PEG micelles, the samples incubated in pH 5.5 buffer solution did not show a decrease of the PODO content, whereas both samples at pH 7.4 and 9.5 showed decreases of about 30 and 80%, respectively. The PODO content change curve showed, in fact, that PODO is destroyed more rapidly in a higher pH incubation medium. In comparison, the samples in the presence of polymer micelles (starting PODO concentration = 0.66 mg/mL and PASP-*g*-OD-*g*-PEG concentration = 3 mg/mL) showed different content change curves. At pH 5.5, the curve of the PODO micelle solution is similar to that of the PODO solution, which indicated PODO is stable under this condition. At pH 7.4, the PODO micelle samples hardly showed a decrease of PODO content during 30 h, which should be attributed to the protection of the micelles. At pH 9.5, the obvious decrease in drug content should be mainly due to degradation. Poly-

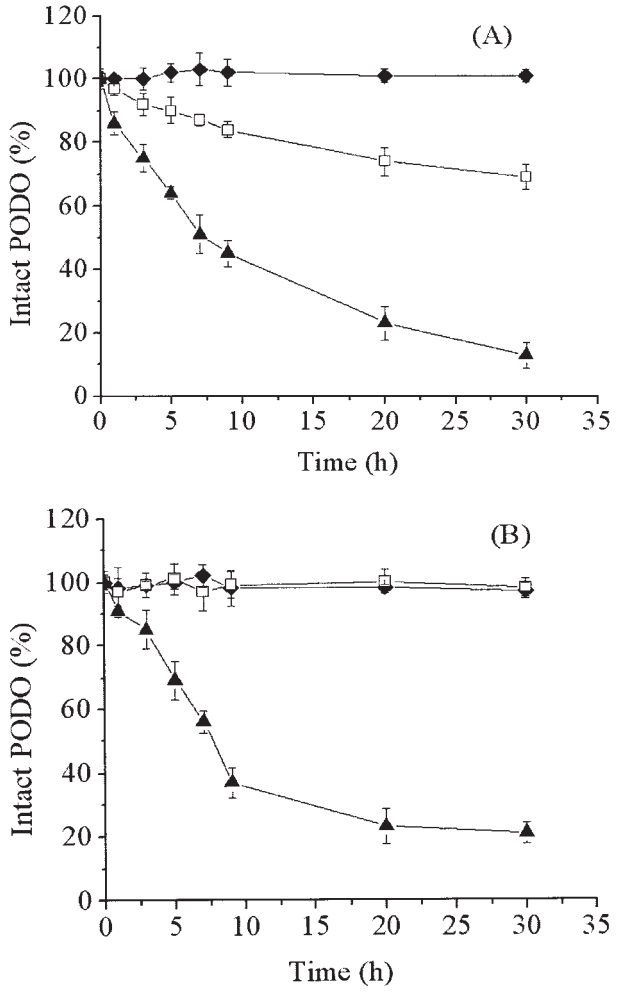


Figure 4 The stability of the (A) PODO solution and (B) PODO loaded by micelles at various pH values of (♦) 5.5, (□) 7.4, and (▲) 9.5. The standard deviation values (±SD) were calculated on the basis of five experiments.

meric micelles may experience slight changes in the basic solution because of the deprotonation of the carboxyl group of PASP-*g*-OD-*g*-PEG, and therefore the PODO loaded by the micelles was released into the medium and lost the protection of the micelles. From the results about the effect of the polymeric micelle on drug stability at various pH values, we concluded that the polymeric carriers only perform the stabilization in some ranges of pH values.

Morphology and size distribution of polymeric micelles

The micelle size versus the DS of mPEG is shown in Table I. The number-average diameters of the blank polymeric micelles decreased from 70.9 to 14.2 nm with the increase of the DS of mPEG from 1.2 to 5.1 mol %. The particle sizes were dependent on the hydrophilic moiety (i.e., the more hydrophilic moiety resulting in the smaller particle size).[17] DLS measurements carried out on micelles loading MTX confirmed that the integrity of the micelle was preserved during the loading process and the micelle size was not altered substantially, although a slight increase in micelle diameter compared with the blank micelle was noted in all cases (see Table I, Fig. 5). The result is consistent with the report that the size of aggregates or nanoparticles is larger with drug loading than without.[16] Figure 5 shows the particle size distributions of blank polymeric micelles and polymeric micelles loading MTX based on the number average by DLS. As expected, the graft copolymers formed small micelles with a narrow distribution.

The morphologies of polymeric micelles were observed by TEM (see Fig. 6). They showed almost spherical shapes without large asymmetries with sizes ranging from 30 to 70 nm in diameter, which is valuable in safe administration. Furthermore, the size is comparable to the dimension of viruses; thus, the micelles may be able to penetrate the sinusoidal and fenestrated capillaries that have approximately 100-nm pores.

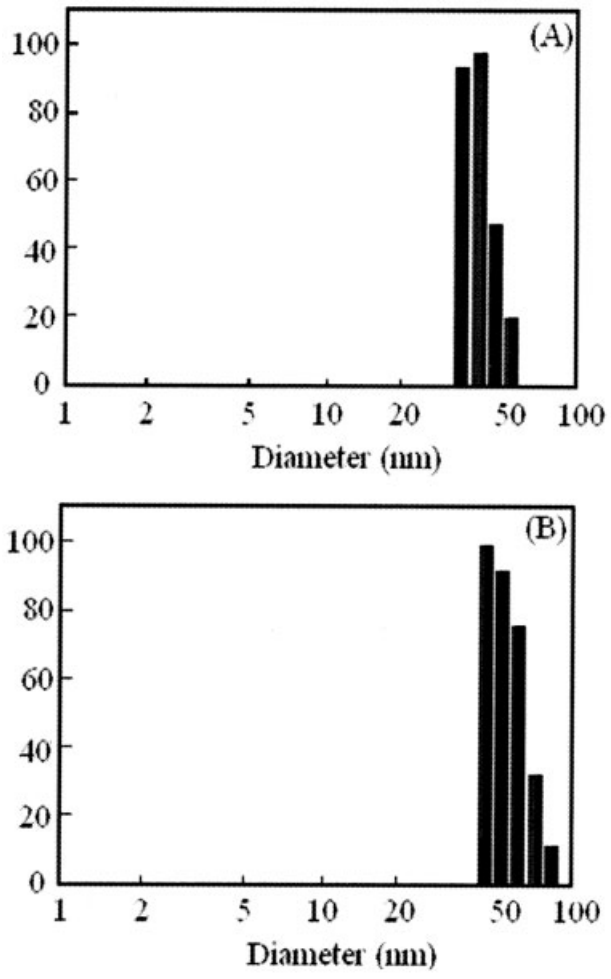


Figure 5 The particle size distributions of micelles: (A) blank polymeric micelles and (B) polymeric micelle loading MTX.

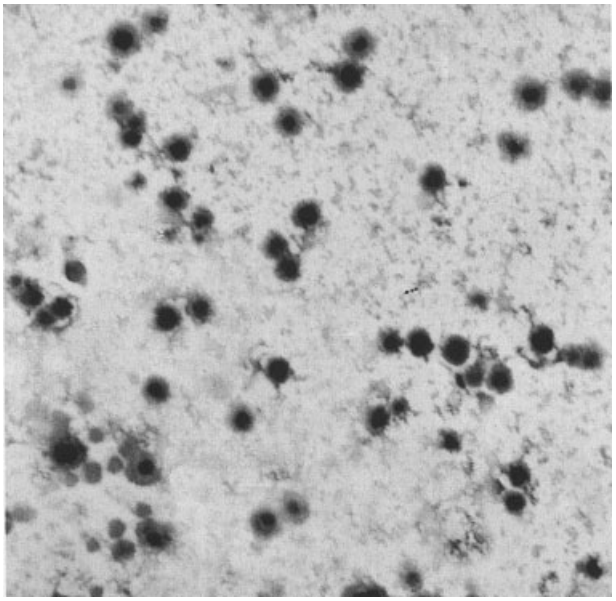


Figure 6 A TEM photograph of polymeric micelles (original magnification ×58,000).

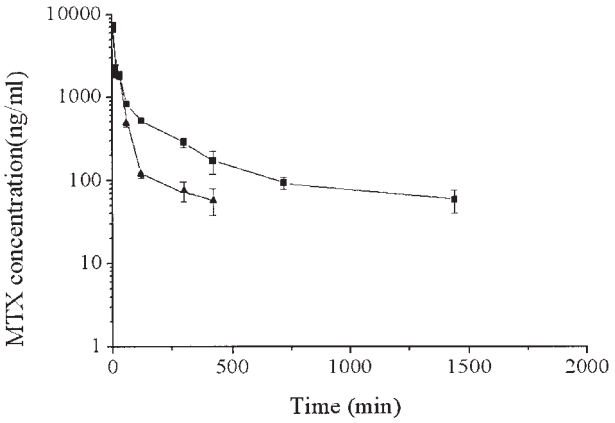


Figure 7 Plasma concentration–time curves of MTX after intravenous administration. (■) MTX loaded by polymeric micelles and (▲) MTX solution. The standard deviation values (±SD) were calculated on the basis of six experiments.

Pharmacokinetic studies

MTX, an antineoplastic agent, is routinely used in the treatment of acute lymphoblastic leukemia, psoriasis, choriocarcinoma, and related trophoblastic tumors. However, it is reported to have toxic side effects to normal cells, drug resistance, and leucopoenia and nephrotoxicity. Among many attempts to reduce the adverse effects and improve the specificity and selectivity, polymeric micelles have significant advantages based on longer circulation of the loaded drug and enhanced permeation retention effect. Therefore, MTX was selected as the model drug to investigate the influence of PASP-*g*-OD-*g*-PEG on the *in vivo* pharmacokinetic behavior of the drug.

To examine the influence of PASP-*g*-OD-*g*-PEG on the *in vivo* pharmacokinetic behavior of MTX, the drug loaded in a PASP-*g*-OD-*g*-PEG micelle was intravenously injected into mice and the time course profile of the drug in plasma was evaluated. Figure 7 shows the plasma cencentration–time profiles of MTX after administration of MTX micelle solution and MTX aqueous solution.

The noncompartmental pharmacokinetic parameters (listed in Table II) were calculated based on the observed plasma levels of MTX. The $t_{1/2}$ of the MTX micelle was found to be significantly longer compared with that of free MTX (2.39 and 1.01 h, respectively), which indicates that the entrapment in the polymeric micelle delays the MTX clearance from blood circulation. Nevertheless, the V_{ss} obtained with free MTX (66.96 mL) is similar to that of the MTX micelle (64.93 mL). This indicates that the drug loading in the polymeric micelle affects the rate but not the extent of drug distribution. Moreover, the smaller CL for the MTX micelle (19.03 mL/h) compared to that of free MTX (44.80 mL/h) showed that the micelle reduces drug elimination and yields an increase in the MTX systemic bioavailability and the AUC was found to be 6.31 μg h/mL^{-1} compared with 2.69 μg h/mL^{-1} for free MTX (μg/mL h). The hydrated outer shell formed by PEG can protect the MTX loaded in the inner core from recognition and uptake by RES and significantly prolonged the MTX loaded permanence in the blood circulation. In addition, enzymes in blood or tissues can degrade MTX whereas polymeric micelles can offer higher protection. The investigation of the tissue distribution, extent of excretion, and reabsorbance of the drug polymeric micelle at the renal tubule site should be undertaken and reported to further explain the above results.

CONCLUSION

The CMC values obtained by measuring the conductivity were basically consistent with those by measuring the fluorescence spectroscopy. Increasing the ratio of mPEG to PASP-*g*-OD induced the increase in the

TABLE II
Comparison of Pharmacokinetic Parameters Between MTX Solution and MTX Micelle

Parameter	MTX solution	MTX micelle
$t_{1/2}$ (h)	1.01 ± 0.11	2.39 ± 0.17[a]
AUC (μg/mL h)	2.69 ± 0.16	6.31 ± 0.52[a]
MRT (h)	1.45 ± 0.16	3.42 ± 0.26[a]
V_{ss} (mL)	66.96 ± 9.28	64.93 ± 6.24
Cl (mL/h)	44.8 ± 2.55	19.03 ± 1.59[a]

MRT, mean residence time.
[a] These values were significantly different from the solution ($p < 0.05$).

CMC of the copolymer and the decrease in the number-average diameter of micelles. The CMC values also showed the copolymers can form micelles in solutions with different sizes. The micelles showed almost spherical shapes without large asymmetries with sizes ranging from 30 to 70 nm in diameter, which was similar to the results obtained by DLS. Indeed, mPEG and ODA promote the PASP-*g*-OD-*g*-PEG arrangement into supramolecular structures, which can entrap hydrophobic molecules, providing for their solubilization and protection from degradation. Furthermore, the micelle carrier can significantly prolong the permanence of MTX in circulation.

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