Characterization and Comparison of Diblock and Triblock Amphiphilic Copolymers of Poly(δ-valerolactone)

Wen-Jen Lin, Chia-Ling Wang, Lee-Wei Juang

School of Pharmacy, College of Medicine, National Taiwan University, Taipei 100, Taiwan

Received 7 March 2005; accepted 8 July 2005
DOI 10.1002/app.22580
Published online 30 January 2006 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Three types of pegylated amphiphilic copolymers of poly(δ-valerolactone) (PVL) were copolymerized with methoxy poly(ethylene glycol) (MePEG) and poly(ethylene glycol) (PEG_{4000} and $PEG_{10,000}$), respectively. Pegylation of PVL allowed copolymers possessing amphiphilic property and efficiently self-assembled to form micelles with a low critical micelle concentration (CMC) in the range of 10^{-7}–$10^{-8}M$. The average molecular weight of copolymers was in the range of 10,000–20,000 Da, and the polydispersity of copolymers was about 1.7–1.8. Higher mobility of low molecular weight PEG (i.e., MePEG and PEG_{4000}) than high molecular weight $PEG_{10,000}$ allowed valerolactone ring opening more efficient in terms of PVL/MePEG and PVL/PEG_{4000} copolymers possessing longer chain length in hydrophobic domain. Pegylated PVL with low CMC and triblock structure was preferred to encapsulate drug during micelle formation. Although all of these amphiphilic copolymers exhibited controlled release character, the micelles formed by triblock copolymer possessed a more stable core-shell conformation than that by diblock copolymer, and resulted in the release of drug from triblock micelles slower than that from diblock micelles. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 1836–1841, 2006

Key words: δ-valerolactone; methoxy poly(ethylene glycol); poly(ethylene glycol); micelle

INTRODUCTION

Recently, biodegradable amphiphilic copolymers have become attractive in both fundamental research and drug delivery system. The amphiphilic copolymers comprise both hydrophobic and hydrophilic domains, which spontaneously assemble in aqueous media to form micelles with a core-shell structure above critical micelle concentration (CMC). It has been reported that the CMC of micelles formed by amphiphilic copolymers was lower than that by small molecular surfactants, and this is one of the reasons to promote the synthesis of many different types of copolymers possessing amphiphilic character so as to develop the nano-sized micellar drug carrier.[1] Nano-sized micellar carrier not only can be used to control drug release and to prevent drug degradation, but also is feasible to passively accumulate in leaky vaculature or tumor tissue.[2]

Poly(ethylene glycol) (PEG) is a biocompatible material and widely applied in pharmaceutics. Low molecular weight PEG has been used as an external phase during preparation of microparticles by hot-melt microencapsulation process.[3] Using PEG instead of toxic organic solvent, methylene chloride, for microsphere preparation is an advantage in this novel method. The stability of protein during encapsulation process has been improved by pegylation of protein with methoxy poly(ethylene glycol) (MePEG).[4] The pegylated protein shows more stablility than native protein against exposure to dichloromethane and homogenization, and also reduces surface adsorption onto microspheres. The preparation of amphiphilic copolymer via copolymerization with PEG has been reported, where PEG acts as the hydrophilic outer shell after formation of polymeric micelles. The flexibility of hydrophilic PEG chain not only prevents plasma protein adsorption onto micelle surface, but also avoids micelles uptake by reticular endothelial system and prolongs their circulation time in blood.[5,6] The cationic block copolymers consisting of a PEG block are able to spontaneously self-assemble with plasmid DNA and form a complex with a stable nature. Here, the PEG block still possesses the advantages mentioned earlier.[7]

The aim of present work was designed to investigate the impacts of pegylated copolymers on the performance of micellar carrier. The polymerized δ-valerolactone (VL) was selected to be the hydrophobic domain of micelles, and each δ-VL monomer possessed six-membered ring in structure. The exploration and application of poly(δ-valerolactone) (PVL) in drug delivery was seldom investigated. In this study, δ-VL was copolymerized with MePEG, low molecular weight PEG (PEG_{4000}), and high molecular weight

Correspondence to: W.-J. Lin (wjlin@ha.mc.ntu.edu.tw)
Contract grant sponsor: National Science Council in Taiwan; contract grant number: NSC 90–2320-B-002–105.

PEG ($PEG_{10,000}$), respectively, via a modified ring-opening copolymerization method, where PEG directly induced ring opening of δ-VL monomers.[8,9] The properties of diblock PVL/MePEG and two triblock PVL/PEG amphiphilic copolymers were characterized and their biocompatibility and degradability were studied. The influences of the type and the molecular weight of PEG on the performance and release behavior of drug-loaded micelles were investigated and compared. The stability of micellar solution in terms of their size change was further evaluated in water at 4°C for 12 weeks.

EXPERIMENTAL

Materials

$PEG_{10,000}$ and δ-VL were from Aldrich Chemical Company (WI, USA). PEG_{4000} was from Wako Pure Chemical Ind. LTD (Osaka, Japan). MePEG was from Fluka Chemical Company Inc., (Buchs, Switzerland). MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide thiazolyl blue) was from Sigma Chemical Co. (Dorset, UK).

Synthesis of diblock and triblock pvl/peg copolymers

The modified ring-opening copolymerization in the absence of external catalysts was applied to synthesize three types of copolymers, PVL/MePEG, PVL/PEG_{4000}, and PVL/$PEG_{10,000}$.[8] The feed molar ratios of VL to MePEG and VL to PEG were 100 : 1 and 200 : 1, respectively. δ-VL and MePEG or PEG were weighed and frozen in liquid N_2. The mixture was then vacuumized and immersed in an oil bath for copolymerization. The synthesized product was dissolved in dichloromethane and extracted with *n*-heptane for several times. The dichloromethane layer was collected and the solvent was removed by rotary evaporation. Finally, the copolymers were obtained and further dried at 40–45°C.

Characterization of copolymers

The molar ratio of lactone monomer to PEG block of copolymers was determined by 200 MHz 1H NMR (Bruker DPX-200, Billerca, MA). The molecular weight (MW_{peak}) and molecular weight distribution in terms of polydispersity of copolymers were determined by gel permeation chromatography (GPC). The MW_{peak} was calculated from the calibration curve of log (*MW*) versus time by using different molecular weights of polystyrene as the standards. A Styragel column (7.8 mm × 30 cm, Waters, Milford, MA) was equipped with a refractive index detector (Shimadzu RID-10A, Japan), and chloroform was the eluting solvent at a flow rate of 1 mL/min at 35°C. The melting temperature (T_m), enthalpy of fusion, and glass transition temperature (T_g) of copolymers were measured using a differential scanning calorimeter (DSC) (LT-Modulate DSC 2920, DuPont Instrument, Wilmington, DE). Each sample (~5 mg) was heated to 100°C at a rate of 10°C/min (first run), and the melting temperature and enthalpy of fusion were determined from the DSC endotherm. For measurement of the T_g, the sample was rapidly quenched and reheated to 100°C at a heating rate of 10°C/min (second run). The T_g was taken at the midpoint as the heat capacity changed.

In vitro degradation study

Each pegylated copolymer was weighed in a glass tube, melted at 70°C, and solidified in a freezer. The molded copolymer was placed in a glass screw-capped test tube containing 10 mL of pH 7.4 phosphate buffer solution and maintained at 37°C. Sample was removed at each specific time point, filtered through a 0.45 μm membrane, and washed with distilled water. The solid samples were collected and dried in vacuum at room temperature for 3 days, after which the weight and the molecular weight of the remaining copolymers were determined.

In vitro cytotoxicity of copolymers

Normal human fibroblast cell line was maintained in the minimum essential medium (MEM) containing 10% fetal bovine serum in an atmosphere containing 5% CO_2 at 37°C. Ten thousand cells in 100 μL of culture medium were plated in 96-well plates overnight. Cells were then incubated in an equal volume (100 μL) of various concentration of polymeric solution at three 10-fold dilutions ranging from 0.001 up to 0.1 mg/mL for 24 h. Each polymer concentration was carried out in triplicate. The cytotoxicity of copolymers was evaluated using MTT assay and determined with a spectrophotometer (Spectra MAX PLUS) at 550 nm. The ratio of cell survival with and without copolymer treatment was calculated.

Preparation of drug-loaded micelles

Indomethacin and pegylated copolymers were previously dissolved in acetone, after which deionized water was added slowly. The solution was then placed in a dialysis bag, immersed in 1 L of deionized water, and dialyzed for 24 h. After that, the micelle solution was sonicated and centrifuged. The average particle size of micelles was measured with a particle sizer (Coulter® N4 Plus, Haieleah, FL) at $\theta = 62.6°$. The amount of indomethacin incorporated in micelles was determined with a validated UV spectrophotometer at 318 nm (Jasco model 7800, Tokyo, Japan). The percent-

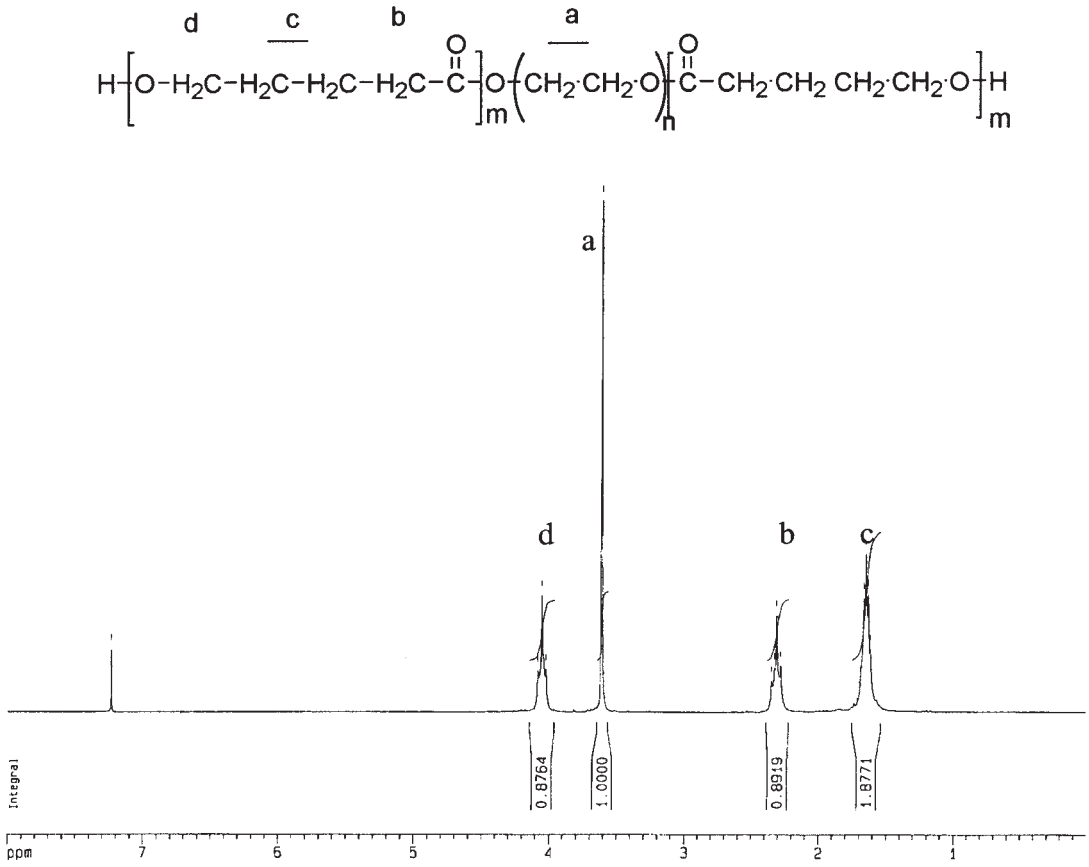


Figure 1 ^{1}H NMR spectrum of PVL/PEG$_{4000}$ copolymers.

age of drug loaded in micelles was defined as the amount of drug encapsulated in micelles to the total amount of drug added initially. The stability of micelles in terms of their particle size change was conducted in water at 4°C for 12 weeks. The particle size of micelles was measured at beginning and at 1, 2, 3, 4, 6, 8, 10, and 12 week after storage. The ratio of particle size of micelles after storage to their initial size was calculated.

In vitro release study

Drug-loaded micelles were placed in a vial containing pH 7.2 phosphate buffer solution. The vial was then sealed with the dialysis membrane (Spectrum®, CA cut off *MW* 6000–8000) and immersed in the same medium. The release of indomethacin from micelles was conducted at 37 ± 0.5°C, and the stirring speed was set at 50 rpm. Samples (1 mL) were withdrawn at specific time points for 14 days, and the concentrations of indomethacin were determined with a validated UV spectrophotometer at 318 nm.

RESULTS AND DISCUSSION

Characterization of copolymers

Three types of amphiphilic copolymers, PVL/MePEG, PVL/PEG$_{4000}$, and PVL/PEG$_{10,000}$, were synthesized in the absence of external catalysts, which avoided residing toxic substances in the final products. Figure 1 shows the ^{1}H NMR spectrum of PVL/PEG$_{4000}$. The peaks corresponding to repeat units of PVL and PEG were observed. PVL/MePEG and PVL/PEG$_{10,000}$ exhibited a spectra similar to PVL/PEG$_{4000}$. Table I lists the composition, molecular weight, polydispersity, and CMC of diblock and triblock amphiphilic copolymers. The molar ratios of VL to PEG of PVL/MePEG, PVL/PEG$_{4000}$, and PVL/PEG$_{10,000}$ were 77/1, 124/1, and 72/1, respectively. The chain length of PVL block of PVL/PEG$_{10,000}$ copolymer was shorter than that of PVL/MePEG and PVL/PEG$_{4000}$ copolymers. This result indicated that the rates of ring opening of VL by MePEG and PEG$_{4000}$ were similar to each other, but more efficient than PEG$_{10,000}$ under current copolymerization condition. In other words, higher mobility of low molecular weight PEG (i.e., MePEG and

TABLE I
Characteristics of Copolymers

Copolymer	Molar ratio of VL/(Me)PEG	MW_{peak} (g/mol)	M_w/M_n	CMC ($10^{-7}M$)
PVL/MePEG	77/1	9,300	1.8	1.83
PVL/PEG$_{4000}$	124/1	21,000	1.8	0.54
PVL/PEG$_{10,000}$	72/1	11,000	1.7	1.10

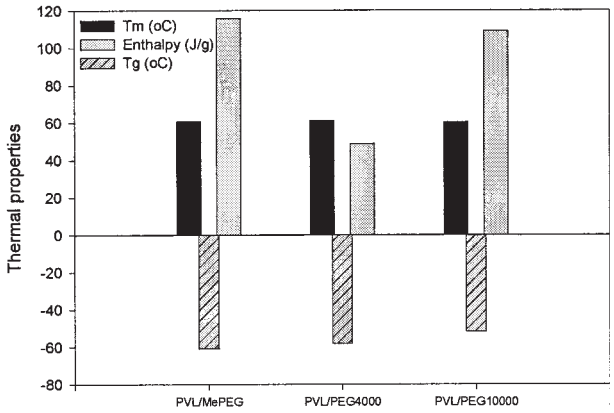


Figure 2 The thermal properties of amphiphilic copolymers.

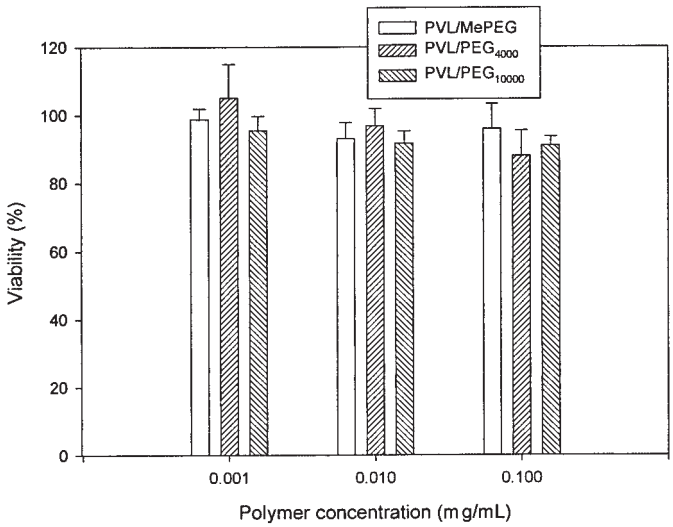


Figure 3 Cell viability in various concentrations of amphiphilic copolymers.

PEG_{4000}) than high molecular weight $PEG_{10,000}$ resulted in more efficient fission of acyl-oxygen bond of VL ring, and in terms of the molar ratio of VL monomer to PEG block was in the order of PVL/MePEG $\sim$ PVL/PEG_{4000} > PVL/$PEG_{10,000}$.[10] The molecular weights (MW_{peak}) of copolymers were in the range of 10,000–20,000 Da, and the polydispersity was about 1.7–1.8. The CMC values of PVL/MePEG, PVL/PEG_{4000}, and PVL/$PEG_{10,000}$ were measured to be 1.83, 0.54, and 1.10 $\times$ $10^{-7}M$, respectively, where the micellization efficiency and the maintenance of micelle conformation were in the order of PVL/PEG_{4000} > PVL/$PEG_{10,000}$ > PVL/MePEG. Figure 2 shows the thermal properties of copolymers. Three copolymers possessed the melting temperatures and the glass transition temperatures in the range of 60.3 $\sim$ 61.3°C and $-51.7 \sim -60.9$°C, respectively, and PVL/PEG_{4000} had the lowest enthalpy of fusion in terms of possessing the lowest crystallinity. The T_g and T_m of PVL have been reported as -66 and 62°C, respectively.[11] The melting temperatures of PEG_{4000} and $PEG_{10,000}$ were measured in our laboratory to be 58 and 64°C, respectively, which were similar to the reported values. In vitro cytotoxicity of amphiphilic copolymers was further evaluated in normal human fibroblast cells. Figure 3 shows the ratio of survival cells after being treated with 10^{-3}–10^{-1} mg/mL of copolymers. All of these copolymers showed similar in vitro cytotoxicity, and more than 90% of cells were viable after treating with copolymers. It has been reported that the copolymeric micelles with low CMC and high hydrophobic–hydrophilic ratio have positive effect on micelle stability, especially after dilution with large volume of body fluid, and this was one of the advantages of micelles formed from amphiphilic copolymers rather than from small molecular surfactants.[12] Based on the characteristics of three pegylated copolymers, it seemingly suggested that PVL/PEG_{4000} could be the best candidate as a micellar drug carrier than the other two copolymers.

Degradation of copolymers

Figure 4 shows the weight loss of two triblock copolymers, PVL/PEG_{4000} and PVL/$PEG_{10,000}$, during 360 days. The prominent weight loss was observed during the first 7 days, and there were 7.2% of PVL/PEG_{4000} and 22.1% of PVL/$PEG_{10,000}$ lost. The possible reason accounted for weight loss in the first phase could be due to the solubilization of uncopolymerized PEG polymer in aqueous medium. However, the decrease of polymer mass in the second phase was slow, and the total amounts of copolymers lost at the end of 360 days were 15.4 and 40.0% for PVL/PEG_{4000} and PVL/$PEG_{10,000}$, respectively. The change of molecular weight distribution of copolymers is shown in Figure 5. Although the GPC chromatograms showed a change of molecular weight distribution to small size direction, most of the degraded copolymers were not

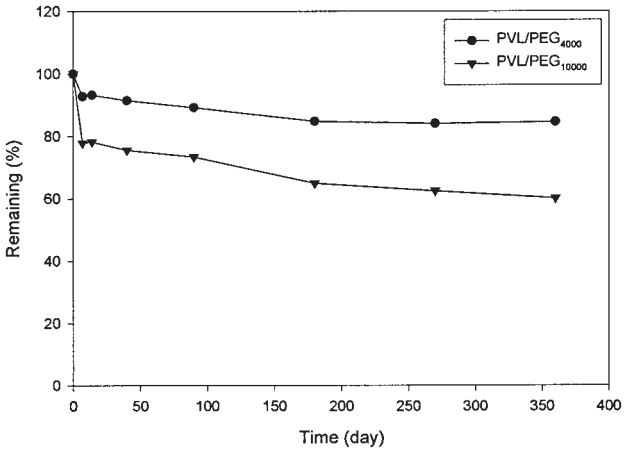
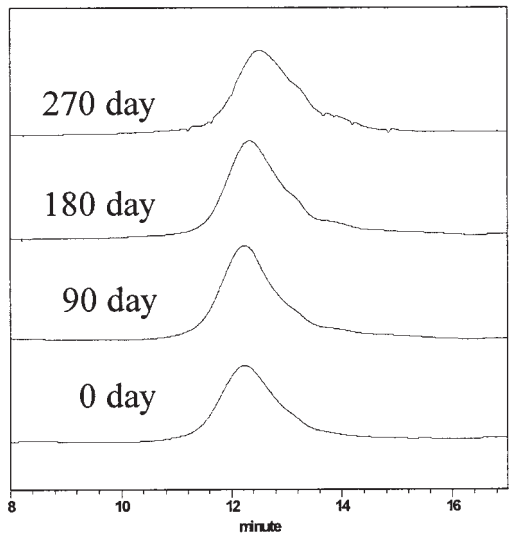
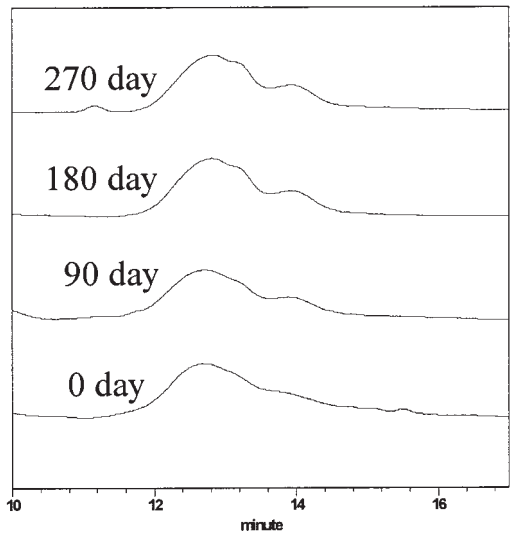


Figure 4 Weight percentage remaining of triblock copolymers.



(a)



(b)

Figure 5 Molecular weight change of triblock copolymers. (a) PVL/PEG_{4000}, (b) PVL/$PEG_{10,000}$. The chromatograms from bottom to top represented degradation at 0, 90, 180, and 270 days.

small enough to dissolve in water to account for its weight loss in the second phase. It was observed that the slope of the second phase corresponding to PVL/PEG_{4000} was slightly less steep than that of PVL/$PEG_{10,000}$. The higher initial molecular weight of PVL/PEG_{4000} than that of PVL/$PEG_{10,000}$ resulted in the degradation of PVL/PEG_{4000}, which was slower and less prominent than PVL/$PEG_{10,000}$.

In vitro release of drug-loaded micelles

Figure 6 shows the particle size distribution of PVL/MePEG micelles. The similar results were also observed from the other two copolymeric micelles. The average particle sizes of PVL/MePEG, PVL/PEG_{4000}, and PVL/$PEG_{10,000}$ micelles were 154.3 ± 7.9, 121.0 ± 10.3, and 150.0 ± 4.9 nm, respectively, and the drug encapsulation efficiency in micelles were (66.8 ± 3.3), (93.5 ± 0.5), and (76.4 ± 3.2)%, respectively. This result suggested that pegylated PVL with triblock structure and low critical micelle concentration was preferred to encapsulate drug during micelle formation. Figure 7 shows *in vitro* release of drug from three types of micelles in pH 7.2 buffer solutions. The drug release was significantly reduced by micelles when compared to the free drug, and the release of drug from diblock micelles (i.e., PVL/MePEG) was faster than that from triblock micelles (i.e., PVL/PEG). Although these amphiphilic copolymers exhibited controlled release character, the micelles formed by triblock copolymer possessed a more stable core-shell conformation than diblock copolymer did, and resulted in the release of drug from triblock micelles slower than that from diblock micelles.

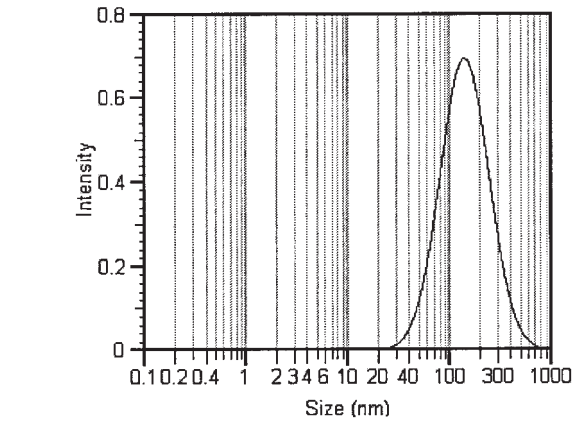


Figure 6 Size distribution of PVL/MePEG micelles.

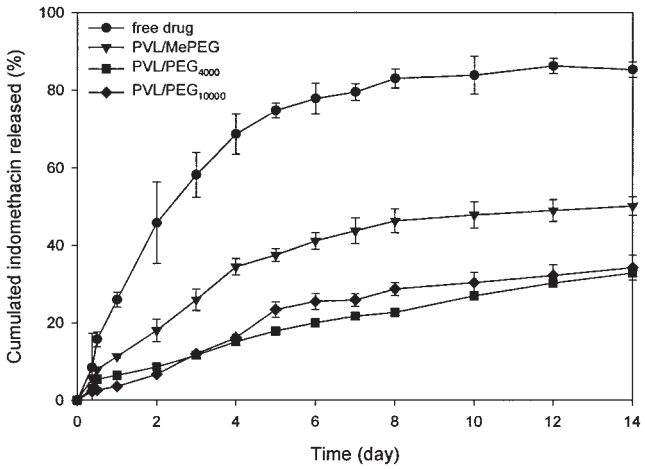


Figure 7 In vitro release of drug from micelles.

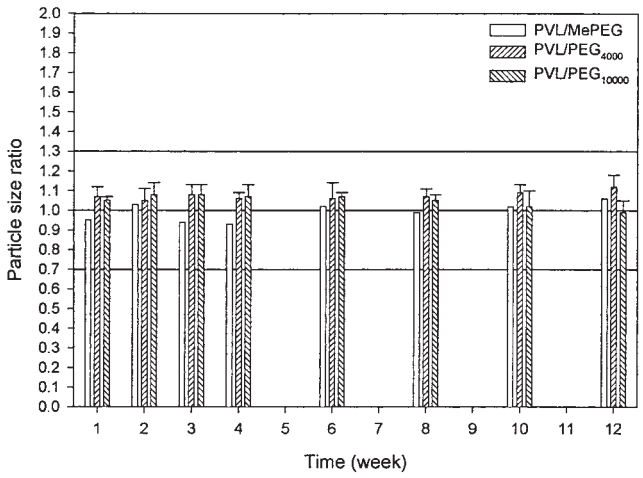


Figure 8 The change of particle size of micelles in water at 4°C.

Stability of micelles

The stability of micelles was conducted in water at 4°C for 12 weeks. The average particle sizes of micelles before and after storage were measured. The ratio of particle size of micelles after storage to its initial size in the range of 1.0 ± 0.3 indicated the stability of micellar system retained, while outside this range, a significant aggregation or dissociation occurred.[13] Figure 8 shows the change of particle size of micelles following storage for 12 weeks. All of the micelle solutions maintained their sizes within 1.0 ± 0.3 range at the end of the study, irrespective of diblock or triblock and the molecular weight of PEG in copolymers.

CONCLUSIONS

The modified ring-opening copolymerization was feasible to synthesize pegylated PVL. Higher mobility of low molecular weight PEG (i.e., MePEG and PEG_{4000}) than high molecular weight $PEG_{10,000}$ allowed VL ring opening more efficient in terms of PVL/MePEG and PVL/PEG_{4000} copolymers possessing longer chain length in hydrophobic domain. The pegylated copolymers possessed amphiphilic property, which further formed nano-sized micelles above CMC. The pegylated PVL with triblock structure and low CMC was preferred to encapsulate drug during micelle formation. Although all of these amphiphilic copolymers exhibited controlled drug release character, the micelles formed by triblock copolymer possessed a more stable core-shell conformation than diblock copolymer did, and resulted in the release of drug from triblock micelles slower than that from diblock micelles.

This work was supported by the National Science Council in Taiwan (NSC 90-2320-B-002-105).

References

1. Kwon, G. S. Crit Rev Ther Drug Carrier Syst 1998, 15, 481.
2. Yokoyama, M.; Satoh, A.; Sakurai, Y.; Okano, T.; Matsumura, Y.; Kakizoe, T.; Kataoka, K. J Controlled Release 1998, 55, 219.
3. Lin, W. J.; Flanagan, D. R.; Linhardt, R. J. Polymer 1999, 40, 1731.
4. Klibanov, A. L.; Maruyama, K.; Torchilin, V. P.; Huang, L. FEBS Lett 1990, 268, 235.
5. Stolnik, S.; Illum, L.; Davis, S. S. Adv Drug Delivery Rev 1995, 16, 195.
6. Diwan, M.; Park, T. G. J Controlled Release 2001, 73, 233.
7. Laus, M.; Sparnacci, K.; Ensoli, B.; Butto, S. O.; Caputo, A.; Mantovani, I.; Zuccheri, G.; Samori, B.; Tondelli, L. J Biomater Sci Polym Ed 2001, 12, 209.
8. Cerrai, P.; Tricoli, M.; Andruzzi, F. Polymer 1989, 30, 338.
9. Kim, S. Y.; Ha, J. C.; Lee, Y. M. J Controlled Release 2000, 65, 345.
10. Yang, G.; Zhang, L.; Feng, H. J Membr Sci 1999, 161, 31.
11. Nakayama, A.; Kawasaki, N.; Arvanitoyannis, I.; Aiba, S.; Yamamoto, N. J Appl Polym Sci 1997, 66, 741.
12. Allen, C.; Maysinger, D.; Eisengerg, A. Colloids Surf B 1999, 16, 1.
13. Saez, A.; Guzman, M.; Molpeceres, J.; Aberturas, M. R. Eur J Pharm Biopharm 2000, 50, 379.