Original Article

Mahidol Univ J Pharm Sci 2015; 42 (4), 169-177

Effect of hydrophobic nanoparticle cores on the characteristics of poly(ε-caprolactone)-co-d-α-tocopheryl polyethylene glycol 1000 succinate nanoparticles loaded with camptothecin

Y. Sirithananchai[1], VB. Junyaprasert[1,2], K. Sakchaisri[3], J. Suksiriworapong[1,2,]*

[1] *Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand*

[2] *Center of Excellence in Innovative Drug Delivery and Nanomedicine, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand*

[3] *Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand*

Abstract

The clinical efficacy of camptothecin (CPT), a potent antitumor drug, is restricted by its insolubility in aqueous and organic solvents and its instability in physiological fluid. The incorporation of drug in nanoparticles can minimize these problems. Since CPT exhibits hydrophobic properties, the nanoparticle core with different hydrophobicity may affect the characteristics of nanoparticles. This study aimed to investigate the effect of hydrophobicity of the nanoparticle core on the characteristics of poly(ε-caprolactone)-co-d-α-tocopheryl polyethylene glycol 1000 succinate (PCL-TPGS) nanoparticles for the entrapment of CPT. The result found that the increasing hydrophobicity of the nanoparticle core significantly enlarged the particle size of blank nanoparticles from 40 nm to 164 nm while it decreased the polydispersity index (PdI) from 0.518 to 0.242 and the zeta potential (ZP) from -1.79 mV to -22.9 mV. After drug loading, the particle size increased but the ZP remained almost unchanged as compared to the blank nanoparticles. The initial fed amount of CPT at 0.1 mg yielded the highest %entrapment efficiency (%EE). The increasing amount of CPT dramatically decreased %EE from 94.03-96.73% to 18.96-22.53% but slightly increased %drug loading from 0.073-0.077% to 0.085-0.092%. The results suggested that the hydrophobicity of the PCL-TPGS nanoparticle core affected the characteristics of nanoparticles. From these results, the 3:1 PCL-TPGS nanoparticles loading with 0.1 mg of CPT showed the desirable size (smaller than 200 nm), narrow PdI, and highest %EE. Therefore, this formulation will be chosen for the development of CPT delivery system.

Keyword: Camptothecin, Poly(ε-caprolactone), d-α-Tocopheryl polyethylene glycol 1000 succinate, Nanoparticles, Hydrophobic

1. INTRODUCTION

Camptothecin (CPT) is a plant alkaloid isolated from an oriental tree, *Camptotheca acuminata*. CPT shows the significant antitumor activity against various cancers, including lung, ovarian, breast, pancreas, gliomas, and stomach. It inhibits the activity of DNA topoisomerase I, which is required for replication and transcription of cell cycle.[1,2] However, the clinical efficacy of CPT is significantly restricted by its insolubility in water and most organic solvents. Moreover, CPT is spontaneously and rapidly inactivated due to hydrolysis of its active lactone form resulting in an inactive carboxylate form (Figure 1). Although this reaction is reversible, its equilibrium favors the carboxylate form at the physiological condition.

Several strategies have been proposed primarily to increase the solubility of CPT as well as to improve the stability of CPT in

***Corresponding author:** E-mail: jiraphong.suk@mahidol.ac.th

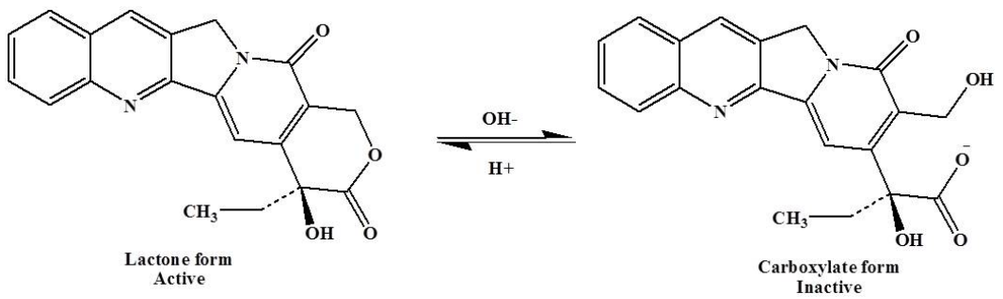

Figure 1. Reversible transformation of active lactone camptothecin to inactive carboxylate camptothecin.

physiological fluid. CPT has been incorporated to several kinds of drug carrier systems to improve drug solubility and stability.[3] Until now, various kinds of nanocarriers for CPT have been studied for example, polymeric micelles, dendrimers, lipid-based nanoparticles, and microspheres.[2, 4-6] Among these carriers, the polymeric nanoparticles have been extensively investigated due to many advantages. They can enhance drug solubility and stability in the biological environment by entrapping hydrophobic drugs in the compatible hydrophobic core.[2, 7] Meanwhile, the coverage of these particles by the hydrophilic part of polymer chain can avoid the mononuclear phagocyte system (MPS) leading to the prolonged circulation time. In addition, the nanoparticles can also enhance the absorption of drugs into the tumor tissues by the enhanced permeability and retention (EPR) effect.[8]

Various types of copolymers have been used for the fabrication of nanoparticles. Among the most commonly used biodegradable synthetic polymers, the aliphatic polyesters including poly(ε-caprolactone) (PCL) exhibit attractive properties such as biocompatibility, nontoxicity, and nonimmunogenicity.[9-11] In addition, these polymers have been approved by the United States Food and Drug Administration (U.S. FDA) for pharmaceutical and biomedical applications. Over the decades, PCL has been extensively studied as biomaterials. However, the use of PCL is restricted due to the high degree of crystallinity and slow biodegradation rate. Additionally, the high hydrophobicity of PCL accelerates the removal of PCL particles from blood circulation.[11, 12] Therefore, the modification of PCL with hydrophilic polymer is necessary to solve these issues.

D-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS) is a water-soluble derivative of vitamin E. Its structure is composed of hydrophobic alkyl chain of vitamin E and hydrophilic part of PEG.[13] It has been approved by U.S. FDA as water-soluble vitamin E nutritional supplement and drug delivery vehicle.[14] Due to many attractive characteristics of TPGS including anti-cancer property, P-glycoprotein inhibitory activity, and solubilizing effect for both water-soluble and water-insoluble compounds, the addition of TPGS to the PCL chain may modify the properties of PCL. The entrapment of CPT by PCL-TPGS nanoparticles may thus improve the solubility and stability of CPT. However, one major factor affecting the loading capacity of nanoparticles is the compatibility between nanoparticle core and drug. It has been reported that the entrapment of hydrophobic drugs could be enhanced by increasing the hydrophobic nanoparticle core.[15] Therefore, it is of our interest to investigate the different hydrophobic PCL chains of PCL-TPGS copolymers on the characteristics and loading capacity of the nanoparticles for CPT. The ratios of hydrophobic to hydrophilic segments of PCL-TPGS copolymers were varied at 1:1, 2:1, 3:1, and 4:1 and these copolymers were employed to prepare the nanoparticles. In addition, the initial amount of drug fed was varied to observe the effect on the loading capacity of the system.

2. MATERIALS AND METHODS

2.1 Materials

CPT was purchased from Xi'an Lyphar

Biotech Co., Ltd. (Shaanxi, China). TPGS and poloxamer 407 (P407) were gifted from BASF (Ludwigshafen, Germany). Acetonitrile (ACN), methanol, and tetrahydrofuran (THF) were of high-performance liquid chromatography (HPLC) grade (Burdick & Jackson, Ulsan, Korea). Glacial acetic acid was purchased from RCI Labscan Co., Ltd. (Bangkok, Thailand). Sodium acetate trihydrate (CH_3COONa) was obtained from VWR international BVBA (Leuven, Belgium). Sterile water for injection was purchased from Thai Nakorn Patana (Bangkok, Thailand). Triethylamine (TEA, $(C_2H_5)_3N$) was purchased from Carlo Erba reagents (Val de Reui, France).

2.2 Nanoparticle preparation

The nanoparticles were prepared by nanoprecipitation method.[16, 17] Briefly, PCL-TPGS (54 mg) with an increasing hydrophobic to hydrophilic ratio (1:1, 2:1, 3:1, and 4:1) and TPGS (6 mg) were dissolved in 10 mL of THF. The solution was added to 10 mL of water containing P407 0.6% w/v. THF was evaporated at room temperature and the resultant was centrifuged at 1,370×g to eliminate aggregates. For CPT-loaded nanoparticles, various amounts of CPT (0.1, 0.3, and 0.5 mg) were dissolved with PCL-TPGS polymer in THF and the nanoparticles were prepared as previously described. The nanoparticles were kept as a dispersion form for further analysis.

2.3 Particle size analysis

The particle size and size distribution (PdI) of nanoparticles were determined by photon correlation spectroscopy (PCS) using Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The samples were measured after dilution with deionized water to obtain a suitable scattering intensity and performed at an angle of 173°, 25°C. All mean particle size and PdI values were attained by averaging values of 10 measurements.

2.4 Zeta potential (ZP) analysis

The surface charge of nanoparticles, defined as the value of electrical potential at the shear plane of particles, was determined by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). All measurements were performed at 25°C. The measurement of each sample was repeated three times and an average value was reported.

2.5 Determination of drug loading efficiency

The loading efficiency of nanoparticles was evaluated in terms of percent drug loading (%DL) and percent entrapment efficiency (%EE). The measurement of %DL and %EE was performed by direct and indirect methods. For the indirect method, the nanoparticle dispersion was centrifugally filtered at 14,811×g for 15 min through Microcon Ultracel YM-30 tube (MW cut-off 3000 Da, Millipore, Schwalbach, Germany). The amount of drug in filtrate was analyzed by HPLC. For the direct method, the lyophilized sample was dissolved in ACN and acetate buffer. The precipitate was filtered through 0.22 μm nylon syringe filter. The clear solution was analyzed by HPLC. The amount of drug present in the nanoparticles was calculated by the different amount of drug found in the filtrate and the lyophilized sample. The %DL and %EE were calculated according to the equations below.

$$\%DL = \frac{\text{Amount of drug in lyophilized sample-Amount of drug in filtrate}}{\text{Weight of lyophilized nanoparticles}} \times 100 \quad (1)$$

$$\%EE = \frac{\text{Amount of drug in lyophilized sample-Amount of drug in filtrate}}{\text{Weight of drug fed initially}} \times 100 \quad (2)$$

2.6 Percent yield (%yield)

The yield of nanoparticles was determined by recording the dry weight of nanoparticles after lyophilization. After preparation, 3 mL of nanoparticles was transferred into the accurately weighed vial and then frozen at -80°C overnight. Subsequently, the frozen nanoparticles were lyophilized for 24 h. Finally, the weight of dried product was recorded. The %yield was calculated by the following equation.

$$\%Yield = \frac{\text{Weight of lyophilized nanoparticles}}{\text{Initial weight of all components}} \times 100 \quad (3)$$

2.7 HPLC Analysis

The analysis of CPT was performed by HPLC method[18] using Shimadzu HPLC machine (Japan) equipped with DGU-20A5 Degasser, LC-20AD Pump, SIL-10AF Autosampler, and SPD-20A UV/VIS detector. A reversed-phase C18 column (Phenomenex® Gemini-NX 5µ C18 110Å, 150×4.6 mm, with a guard column) was used as a stationary phase. The mixture of ACN and acetate buffer pH 5.5 with 1% v/v TEA at the ratio of 21:79 v/v was utilized as a mobile phase. CPT was eluted through the gradient mode of mobile phase which was varied from 21 to 45% for 5 min. Eluent was pumped through the column at a flow rate of 1 mL/min. The injection volume was 20 µL and the detection wavelength was 370 nm. Calibration curves of lactone and carboxylate forms of CPT were constructed at concentrations of 0.025, 0.1, 1, 5, 10 and 20 µg/mL with R^2 of at least 0.9995. The relative standard deviation of interday and intraday precisions was less than 2%. The %recovery of CPT was in the acceptable range of 98.5-101.5%.[19]

2.8 Statistical analysis

The results are expressed as the mean ± standard deviation (SD) from at least 3 measurements. The one way ANOVA or student's t-test was determined using SPSS program for multiple or pair comparisons, respectively. The statistical difference is considered at the probability level of 0.05.

3. RESULTS AND DISCUSSION

3.1 Blank nanoparticles

The PCL-TPGS nanoparticles were prepared using TPGS in combination with P407 as stabilizers. The hydrophobic to hydrophilic ratio of PCL-TPGS copolymers and the initially fed amount of CPT were investigated since these may affect the properties of the nanoparticles. It was postulated that the PCL-TPGS nanoparticles formed by aggregating PCL and vitamin E segments inside the nanoparticle core surrounded by the hydrophilic PEG segment. Without any stabilizer, the nanoparticles tended to aggregate resulting in micron-sized particles. Furthermore, we also found that the emulsification efficiency of sole TPGS was inadequate to form the stable nanoparticles (data not shown). Therefore, the combination of TPGS and P407 were used as stabilizers. Upon the polymer aggregation, TPGS acting as an emulsifier inserted vitamin E part to the core of nanoparticles and tethered the PEG chain to the aqueous environment.[9] Meanwhile P407 physically adsorbed on the surface of nanoparticles and formed the steric stabilizing layer surrounding the nanoparticles preventing the aggregation tendency of particles. The combination of TPGS and P407 could synergistically facilitate the formation of PCL-TPGS nanoparticles. The results of blank PCL-TPGS nanoparticles are summarized in Figure 2. Various hydrophobic to hydrophilic ratios of PCL-TPGS copolymers at 1:1, 2:1, 3:1, and 4:1 resulted in the %hydrophobic chain of 27.43%, 52.73%, 65.54%, 72.20%, respectively, as calculated by nuclear magnetic resonance spectroscopy.

It was discovered that the smallest particles with hydrodynamic diameter of 40±5 nm was obtained from 1:1 PCL-TPGS formulation. However, this formulation had the widest size distribution (0.518 ± 0.009). As shown in Figure 2A and 2B, the increasing hydrophobic to hydrophilic ratio from 1:1 to 4:1 led to the significant increment of particle size from 40 ± 5 nm to 164 ± 1 nm (p-value < 0.05) and the narrow PdI from 0.518 ± 0.009 to 0.242 ± 0.020, respectively. All polymers used in this study contained the same TPGS part. Hence the different hydrophobicity of PCL-TPGS stemmed from the different chain length of PCL. The increasing hydrophobic to hydrophilic ratio from 1:1 to 4:1 increased the PCL chain length and thus increased the hydrophobicity of polymer. The longer hydrophobic PCL chain enlarged the size of nanoparticles which agreed well with the previous report.[20] The 1:1 and 2:1 PCL-TPGS nanoparticles had higher PdI value than the 3:1 and 4:1 nanoparticles probably due to the fact that the small particles tended to aggregate upon the nanoparticle formation in order to reduce the free energy of particles.[21] The longer hydrophobic to hydrophilic ratio

of PCL-TPGS copolymers tended to form the more compacted hydrophobic core of nanoparticles and had the greater thermodynamic stability than those formed by shorter hydrophobic chain of PCL-TPGS.[22] Therefore, the 3:1 and 4:1 nanoparticles had less free energy than the 1:1 and 2:1 PCL-TPGS nanoparticles resulting in the lower PdI value. These results were in consistent with the previous report.[15] It was established that the particle formation was primarily affected by the nature and the length of hydrophobic block whereas the hydrophilic block had only a slight effect. The ZP of all polymer ratios showed the negatively charged surface. The ZP decreased from -1.8 ± 1.0 mV to -22.9 ± 2.0 mV when increasing the hydrophobicity from 1:1 to 4:1. It was obvious that the longer hydrophobic PCL segment provided the higher negatively surface charge. This was probably due to the presence of carbonyl groups of PCL segments on the nanoparticle surface.[23] At 1:1 and 2:1 hydrophobic to hydrophilic ratios, the ZP was almost zero due to the PEG segment enabling to cover almost completely the surface of nanoparticles. The obtained data agreed well with the literatures reporting that the lowering of absolute ZP value was possibly due to the adsorption of polymers on the surface of nanoparticles.[24, 25] The %yield of all blank formulations was almost 100% (Table 1).

Table 1. The %yield of blank and CPT-loaded PCL-TPGS nanoparticles (mean ± SD, n = 3)

Formulation	Hydrophobic to hydrophilic ratio			
	1:1	2:1	3:1	4:1
Blank nanoparticles	99.67 ± 0.64	100.00 ± 0.21	99.98 ± 0.12	100.10 ± 0.09
CPT-loaded nanoparticles (0.1 mg)	99.30 ± 1.22	98.29 ± 0.85	99.86 ± 0.36	99.90 ± 0.63
CPT-loaded nanoparticles (0.3 mg)	99.82 ± 0.81	100.37 ± 1.08	99.21 ± 2.01	98.75 ± 2.68
CPT-loaded nanoparticles (0.5 mg)	99.72 ± 1.39	99.14 ± 1.45	99.24 ± 1.98	99.79 ± 0.37

3.2 CPT-loaded nanoparticles

The particle size, PdI and ZP of CPT-loaded nanoparticles are illustrated in Figure 2. After drug loading, almost all CPT-loaded nanoparticles exhibited larger size than the blank nanoparticles. The ZP values of drug-loaded 1:1 and 2:1 PCL-TPGS nanoparticles became more negative possibly due to the presence of drug in either lactone or carboxylate form on the surface of nanoparticles. The overall %EE and %DL were ranged from 18.96 to 96.73% and 0.073 to 0.092%, respectively, as shown in Figure 3. The %yield of CPT-loaded nanoparticles was found to be in the range of 98.29-100.37% (Table 1).

Effect of hydrophobic to hydrophilic ratio of polymers

At the same amount of CPT, the particle size increased with the longer hydrophobic PCL segment (p-value < 0.05). The particle size of CPT-loaded nanoparticles enlarged from 33 ± 5 nm to 343 ± 15 nm with the increasing hydrophobicity of nanoparticle core. This result was in agreement with the blank nanoparticles and the previous report[20] as aforementioned. The particle sizes of CPT-loaded 1:1, 2:1, 3:1, and 4:1 PCL-TPGS nanoparticles were in the range of 33-54 nm, 80-85 nm, 166-199 nm, and 287-343 nm, respectively. Similar to the blank nanoparticles, the PdI values of all CPT-loaded nanoparticles tended to decrease with increasing hydrophobic to hydrophilic ratio of nanoparticle core from 0.742 ± 0.079 to 0.172 ± 0.003 (p-value > 0.05). However, the 3:1 and 4:1 nanoparticles showed the narrow size distribution of around 0.200 probably due to the aggregation tendency of more hydrophobic polymers resulting in the uniformity of particle

size as mentioned previously.[22] The surface charge of most nanoparticles remained almost unchanged as compared to the blank nanoparticles suggesting that all CPT molecules were entrapped inside the nanoparticles. The increment of hydrophobic to hydrophilic ratio of PCL-TPGS polymer insignificantly affected %EE and %DL (p-value > 0.05) as showed in Figure 3.

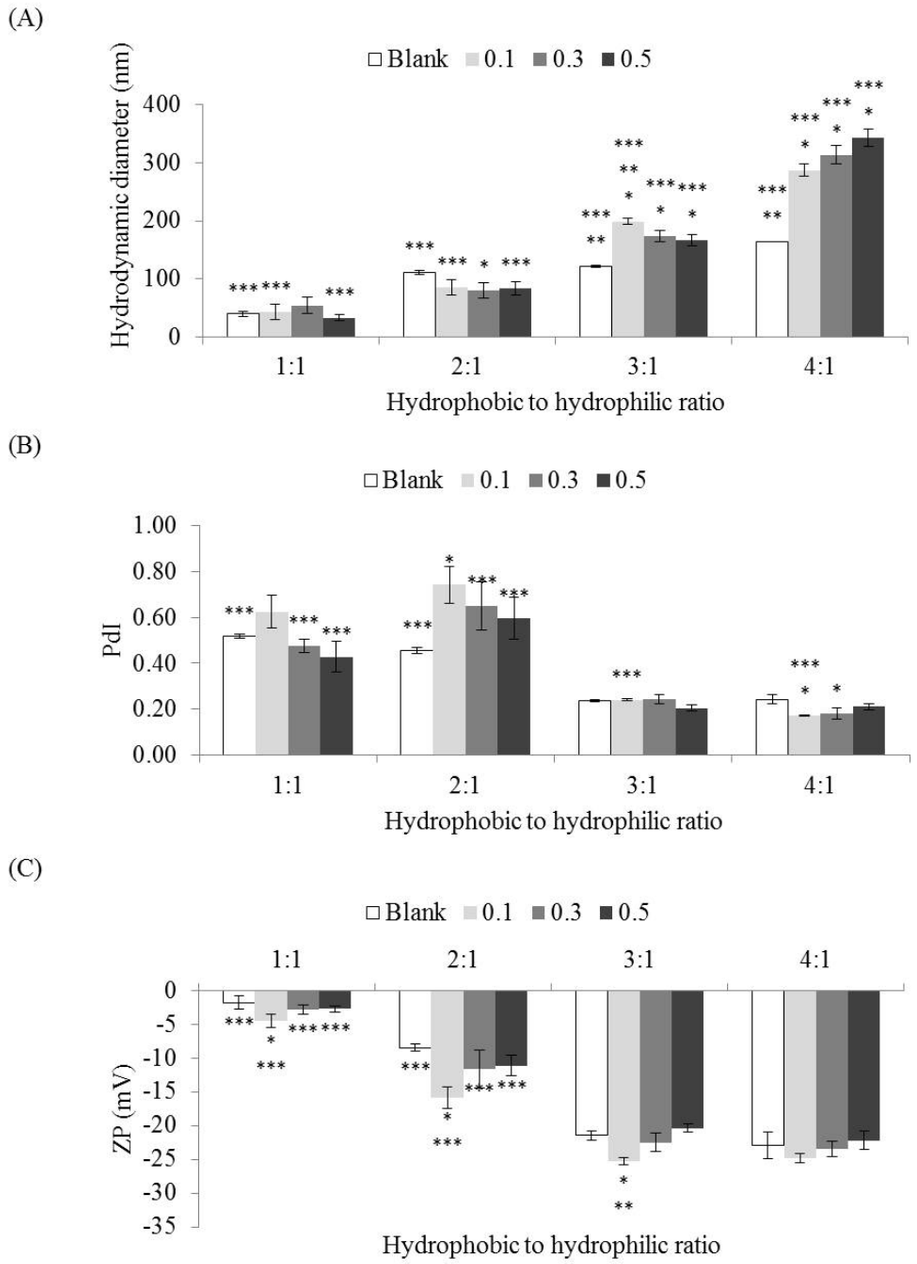

Figure 2. The hydrodynamic diameter (A), polydispersty index (PdI, B), and zeta potential (ZP, C) of blank and CPT-loaded PCL-TPGS nanoparticles. An error bar indicates the standard deviation from at least three measurements. *Significant difference comparing between blank and CPT-loaded nanoparticles at the same hydrophobic to hydrophilic ratio of polymer and the same amount of CPT. **Significant difference comparing among all amounts of CPT at the same hydrophobic to hydrophilic ratio of polymer. ***Significant difference comparing among various hydrophobic to hydrophilic ratios of polymer at the same amount of CPT.

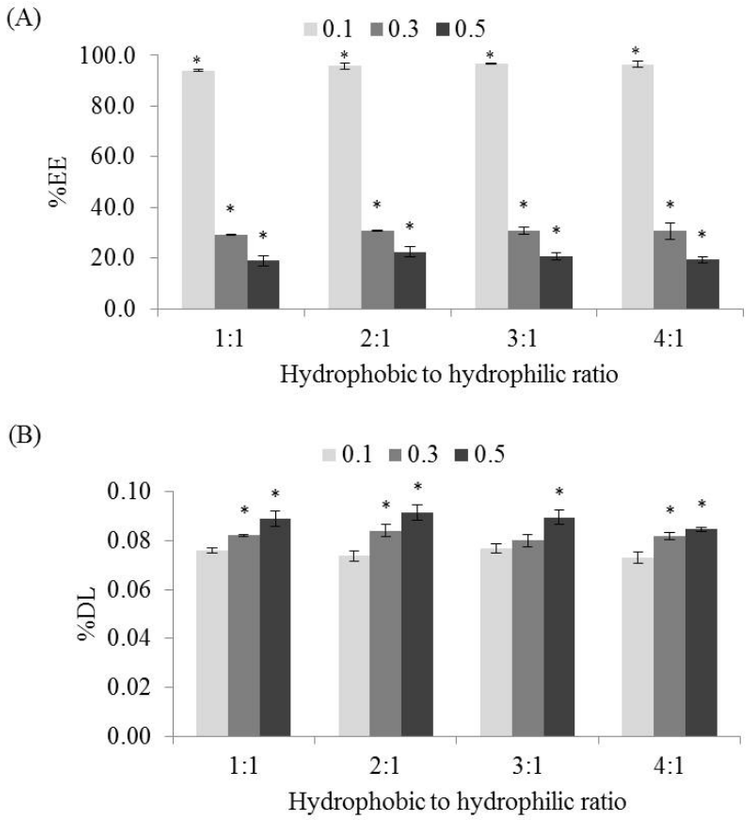

Figure 3. The percentages of entrapment efficiency (%EE) (A) and drug loading (%DL) (B) of CPT-loaded PCL-TPGS nanoparticles. An error bar indicates the standard deviation from at least three measurements. *Statistically significant difference comparing among various amounts of CPT at the same hydrophobic to hydrophilic ratio of polymer.

Effect of initially fed amount of CPT

In this study, the amount of polymer was set constant so the increasing amount of CPT from 0.1 to 0.5 mg increased the drug to polymer ratio. In Figure 2, the increasing amount of CPT insignificantly affected the hydrodynamic diameter, PdI, and ZP values of the nanoparticles. However, the increasing drug to polymer ratio had much effect on the loading efficiency. The highest %EE was obtained when CPT was initially fed at 0.1 mg. The increasing CPT amount to 0.3 and 0.5 mg dramatically reduced %EE from 94.03-96.73% to 29.42-30.90% and 18.96-22.53%, respectively (p-value < 0.05). However, it increased %DL from 0.073-0.077% to 0.080-0.084% and 0.085-0.092%, respectively (p-value < 0.05). The %yield of all nanoparticles remained almost constant when increasing amount of CPT (Table 1). The initial fed amount of CPT at 0.1 mg yielded the highest %EE and provided the %DL approaching the theoretical value. This result indicated that these PCL-TPGS nanoparticles reached the maximum capacity for the entrapment of CPT at 0.1 mg of initially fed amount of drug. Therefore, 0.1 mg of CPT was selected for the development of CPT delivery system.

From these results, it can be concluded that the hydrophobicity of PCL-TPGS nanoparticles had impacts on the particle size, size distribution, and surface charge of the nanoparticles while the amount of CPT loading affected the loading efficiency of the system.

4. CONCLUSION

The results suggested that the hydrophobicity of PCL-TPGS nanoparticle core affected the characteristics of nanoparticles. The increasing hydrophobic to hydrophilic ratio of PCL-TPGS polymer enlarged the particle sizes of blank and CPT-loaded nanoparticles. In the meantime, it reduced the size distribution and resulted in more negative surface charge. Nevertheless, it slightly affected the loading efficiency for the entrapment of CPT. The increasing amount of CPT lowered %EE and %DL. The loading efficiency was limited by the fed amount of CPT. From these results, the 3:1 PCL-TPGS nanoparticles loading with 0.1 mg of CPT showed the desirable size (less than 200 nm), narrow PdI, and highest %EE. Therefore, this formulation will be chosen for further development of CPT delivery system.

5. ACKNOWLEDGEMENTS

We would like to acknowledge the financial support from the Thailand Research Fund, the Office of the Higher Education Commission and Mahidol University (Grant No. MRG5680013), from the 60th Year Supreme Reign of his Majesty King Bhumibol Adulyadej Scholarship and from the Office of the Higher Education Commission and Mahidol University under the National Research Universities Initiative.

REFERENCES

1. Martins S, Tho I, Reimold I, Fricker G, Souto E, Ferreira D, et al. Brain delivery of camptothecin by means of solid lipid nanoparticles: Formulation design, *in vitro* and *in vivo* studies. Int J Pharm. 2012:439 (1–2):49-62.
2. Watanabe M, Kawano K, Yokoyama M, Opanasopit P, Okano T, Maitani Y. Preparation of camptothecin-loaded polymeric micelles and evaluation of their incorporation and circulation stability. Int J Pharm. 2006:308(1-2):183-9.
3. Martins SM, Sarmento B, Nunes C, Lucio M, Reis S, Ferreira DC. Brain targeting effect of camptothecin-loaded solid lipid nanoparticles in rat after intravenous administration. Eur J Pharm Biopharm. 2013:85(3, Pt A):488-502.
4. Morgan MT, Nakanishi Y, Kroll DJ, Griset AP, Carnahan MA, Wathier M, et al. Dendrimer-encapsulated camptothecins: Increased solubility, cellular uptake, and cellular retention affords enhanced anticancer activity *in vitro*. Cancer Res. 2006:66(24): 11913-21.
5. Yang SC, Lu LF, Cai Y, Zhu JB, Liang BW, Yang CZ. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release. 1999:59(3):299-307.
6. Dora CL, Alvarez MS, Trentin AG, de Faria TJ, Fernandes D, da Costa R, et al. Evaluation of antimetastatic activity and systemic toxicity of camptothecin-loaded microspheres in mice injected with B16-F10 melanoma cells. J Pharm Pharm Sci. 2006:9(1):22-31.
7. Kawano K, Watanabe M, Yamamoto T, Yokoyama M, Opanasopit P, Okano T, et al. Enhanced antitumor effect of camptothecin loaded in long-circulating polymeric micelles. J Control Release. 2006:112(3):329-32.
8. Kobayashi H, Watanabe R, Choyke PL. Improving conventional enhanced permeability and retention (EPR) effects; what is the appropriate target? Theranostics. 2014: 4(1):81-9.
9. Bernabeu E, Helguera G, Legaspi MJ, Gonzalez L, Hocht C, Taira C, et al. Paclitaxel-loaded PCL-TPGS nanoparticles: *In vitro* and *in vivo* performance compared with Abraxane®. Colloids Surf B Biointerfaces. 2014:113:43-50.
10. Torchilin VP. Passive and active drug targeting: Drug delivery to tumors as an example. In: Schäfer-Korting M, editor. Drug delivery, Handbook of Experimental Pharmacology. Heidelberg: Springer Berlin Heidelberg; 2010. (197) p. 3-53.
11. Smythe E, Warren G. The mechanism of receptor-mediated endocytosis. Eur J Biochem. 1991:202(3):689-99.
12. Demeule M, Regina A, Che C, Poirier J,

Nguyen T, Gabathuler R, et al. Identification and design of peptides as a new drug delivery system for the brain. J Pharmacol Exp Ther. 2008:324(3):1064-72.

13. Guo Y, Luo J, Tan S, Otieno BO, Zhang Z. The applications of vitamin E TPGS in drug delivery. Eur J Pharrm Sci. 2013:49(2):175-86.
14. Rao JP, Geckeler KE. Polymer nanoparticles: Preparation techniques and size-control parameters. Prog Polym Sci. 2011:36(7): 887-913.
15. Kuskov AN, Voskresenskaya AA, Goryachaya AV, Artyukhov AA, Shtilman MI, Tsatsakis AM. Preparation and characterization of amphiphilic poly-N-vinylpyrrolidone nanoparticles containing indomethacin. J Mater Sci Mater Med. 2010:21(5):1521-30.
16. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm. 1989:55(1):R1-R4.
17. Lince F, Marchisio DL, Barresi AA. Strategies to control the particle size distribution of poly-ε-caprolactone nanoparticles for pharmaceutical applications. J Colloid Interface Sci. 2008:322(2):505-15.
18. Warner DL, Burke TG. Simple and versatile high-performance liquid chromatographic method for the simultaneous quantitation of the lactone and carboxylate forms of camptothecin anticancer drugs. J Chromatogr B Biomed Sci Appl. 1997:691(1):161-71.
19. Commission British Pharmacopoeia. British Pharmacopoeia 2009. United Kingdom: Stationary Office, 2008.
20. Choi C, Chae S, Kim T, Jang M, Cho C, Nah J. Preparation and characterizations of poly(ethylene glycol)-poly(ε-caprolactone) block copolymer nanoparticles. Bull Korean Chem Soc. 2005:26(4):523-8.
21. Allen C, Yu Y, Maysinger D, Eisenberg A. Polycaprolactone-b-poly(ethylene oxide) block copolymer micelles as a novel drug delivery vehicle for neurotrophic agents FK506 and L-685,818. Bioconjug chem. 1998:9(5):564-72.
22. Gaucher G, Dufresne MH, Sant VP, Kang N, Maysinger D, Leroux JC. Block copolymer micelles: Preparation, characterization and application in drug delivery. J Control Release. 2005:109(1–3):169-88.
23. Ma Y, Huang L, Song C, Zeng X, Liu G, Mei L. Nanoparticle formulation of poly(ε-caprolactone-co-lactide)-d-α-tocopheryl polyethylene glycol 1000 succinate random copolymer for cervical cancer treatment. Polymer. 2010:51(25):5952-9.
24. Abdelbary AA, Li X, El NM, Elassasy A, Jasti B. Effect of fixed aqueous layer thickness of polymeric stabilizers on zeta potential and stability of aripiprazole nanosuspensions. Pharm Dev Technol. 2013:18(3):730-5.
25. Nakarani M, Patel P, Patel J, Patel P, Murthy RSR, Vaghani SS. Cyclosporine A-nano-suspension: Formulation, characterization and *in vivo* comparison with a marketed formulation. Sci Pharm. 2010:78(2):345- 61.