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Journal of Controlled Release 93 (2003) 121–127
journal of controlled release
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Effects of ethylene glycol-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel☆

Tooru Ooya[a], Jaehwi Lee[b], Kinam Park[b,*]

[a] School of Materials Science, Japan Advanced Institute of Science and Technology, Tatsunokuchi, Ishikawa 923-1292, Japan
[b] Departments of Pharmaceutics and Biomedical Engineering, Purdue University, School of Pharmacy, West Lafayette, IN 47907, USA

Received 15 April 2003; accepted 7 July 2003

Abstract

New methods and pharmaceutical compositions were developed to increase the aqueous solubility of paclitaxel (PTX), a poorly water-soluble drug. Graft and star-shaped graft polymers consisting of poly(ethylene glycol) (PEG400) graft chains increased the PTX solubility in water by three orders of magnitude. Polyglycerol dendrimers (dendriPGs) dissolved in water at high concentrations without significantly increasing the viscosity and, at 80 wt.%, were found to increase the solubility of PTX 10,000-fold. The solubilized PTX was released from graft polymers, star-shaped graft polymers, and the dendriPGs into the surrounding aqueous solution. The release rate was a function of the star shape and the dendrimer generation. The availability of the new graft, star and dendritic polymers having ethylene glycol units should permit development of novel delivery systems for other poorly water-soluble drugs.

Keywords: Poly(ethylene glycol); Star polymer; Dendrimer; Paclitaxel; Solubilization

1. Introduction

Formulation of poorly water-soluble drugs has been extensively studied to improve bioavailability after oral or parenteral delivery. Previous formulations include surfactant micelles, micro- or nanoparticles, solid dispersions, complexation with cyclodextrin and mixing with co-solvents. Poly(ethylene glycol) (PEG) has been used to modulate the water solubility of poorly soluble drugs [1,2]. PEG with a molecular weight of 400 (PEG400) has been frequently used as a co-solvent to dissolve poorly water-soluble drugs [3,4]. For example, PEG400 increases the aqueous solubility of β-oestradiol by four to five orders of magnitude at a concentration of 80 wt.% and higher [4]. At these concentrations, the majority of PEG400 is believed to self-associate through hydrogen bonding mediated by water molecules [5]. Such association may alter the water structure to increase the solubility of poorly soluble drugs. However, the use of extremely high concentrations of PEG400 is not desirable. Furthermore, when a PEG400-containing drug formulation is diluted, e.g., after administration into the GI tract or injection into the blood, the solubility of poorly water-soluble drugs is expected to decrease. Thus, the usefulness of PEG may be limited. For this

☆ Presented at 11th International Symposium on Recent Advances in Drug Delivery Systems and CRS Winter Symposium, Salt Lake City, UT, March 3–6, 2003.

* Corresponding author. Tel: +1-765-494-7759; fax: +1-765-496-1903.
E-mail address: kpark@purdue.edu (K. Park).

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doi:10.1016/j.jconrel.2003.07.001

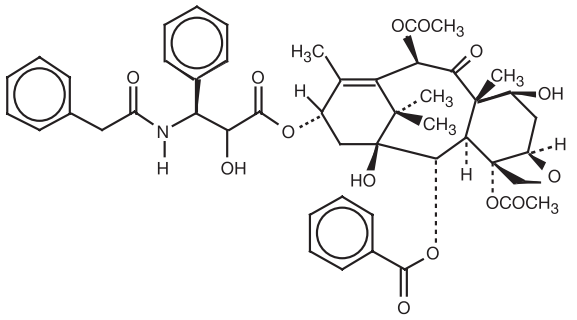

Fig. 1. Chemical structure of paclitaxel.

reason, it was of interest to design new ethylene glycol-based polymer architectures that would increase the solubility of poorly soluble drugs even under diluted conditions. Since the high concentration of PEG400 is the most important factor in solubility enhancement, it was hypothesized that the new polymer architectures should possess a high local density of ethylene glycol chains to increase the water solubility of poorly soluble drugs. Here, we report a new approach to increasing the solubility of paclitaxel (PTX) (Fig. 1), a model for poorly water-soluble drugs, by using graft, star and dendritic architectures consisting of ethylene glycol units.

2. Materials and methods

2.1. Materials

Oligo(ethylene glycol) methacrylate (OEGMA; Mn 460) was purchased from Polysciences (Warrington, PA). All other chemicals for syntheses were purchased from Aldrich (Milwaukee, WI). Paclitaxel was kindly supplied by Samyang Genex (Taejeon, South Korea). HPLC grade acetonitrile and water were used as solvents in the HPLC analysis. Macroinitiators for poly(OEGMA), five-arm star poly (OEGMA), *O*-isobutyl bromide-monomethoxy-capped OEG (OEGBr), and 1,2,3,4,6-penta-*O*-isobutyryl bromide-α-D-glucose were synthesized according to Wang and Armes and Stenzel-Rosenbaum et al. [6,7]. Poly(OEGMA) (Fig. 2a) and five-arm star poly(OEGMA) (Fig. 2b) were synthesized by atom transfer radical polymerization of OEGMA using the macroinitiators in the presence of copper(I) chloride and 2,2′-bipyridyl as previously described [8]. Polyglycerol dendrimers (dendriPGs) with generation 3 (G-3), 4 (G-4) and 5 (G-5) (Fig. 2c) were synthesized by step-by-step allylation and dihydroxylation reactions, according to Haag et al. [9].

2.2. Characterization

Chemical structures of the polymers were characterized by ^{1}H- and ^{13}C-NMR spectroscopy using a Bruker ARX 300 spectrometer. Molecular weights of poly(OEGMA) and five-arm star poly(OEGMA) were determined using a gel permeation chromatography (GPC) system equipped with an Agilent 1100 series RI detector, quaternary pump and PL aquagel-OH columns (Agilent Technologies, Wilmington, DE). A mixture of 0.1 M sodium nitrite and acetonitrile (80:20) was used as the GPC eluent. Molecular weights were calibrated using poly(ethylene oxide) standards. Molecular weights of all dendrimers were determined by matrix-assisted laser desorption ionization (MALDI) mass spectroscopy using a PerSeptive Biosystems Voyager mass spectrometer (Framingham, MA). The viscosity of the dendrimers in water was measured at 25 °C using a Cannon-Manning Semi-Micro Viscometer (Size: 50 C286, Cannon Instrument, PA).

2.3. Solubility test of paclitaxel

Excess paclitaxel (~ 10 mg) was added to screw-capped vials containing fixed volumes of the polymer solutions (2–80 wt.%) and stirred at 37 °C. Samples were taken at 24 h, filtered through 0.2-μm nylon membrane filters and analyzed for paclitaxel using HPLC. The concentration of paclitaxel was determined by isocratic reverse-phase HPLC (Agilent 1100 series) using a Symmetry column (Waters, Milford, MA) at 25 °C. The mobile phase consisted of acetonitrile–water (45:55 v/v) with a flow rate of 1.0 ml/min. A diode array detector was set at 227 nm. The paclitaxel concentrations in the samples were obtained from a calibration curve.

2.4. Paclitaxel release experiment

Paclitaxel release from the dendrimers was carried out using dialysis membranes; molecular weight cut-

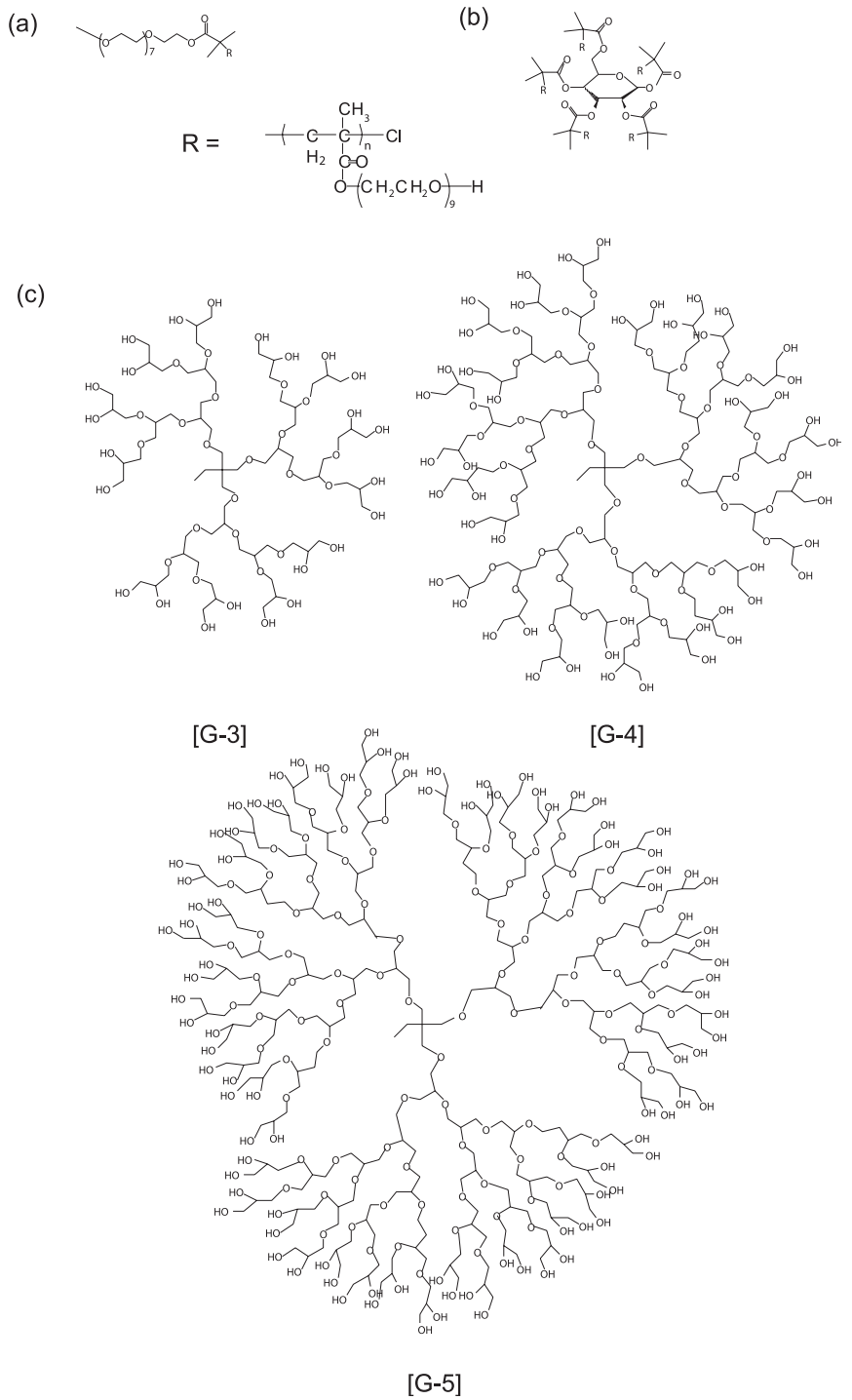

Fig. 2. Chemical structure of (a) poly[oligo(ethylene glycol) methacrylate] [poly(OEGMA)], (b) five-arm star poly(OEGMA), and (c) polyglycerol dendrimers with generation 3 (G-3), 4 (G-4) and 5 (G-5).

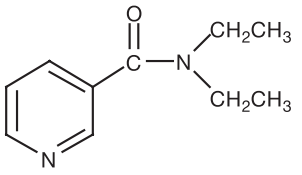

Fig. 3. Chemical structure of *N*,*N*-diethylnicotinamide.

off 1×10^3 Da. A predetermined amount of paclitaxel was dissolved in 10% (w/v) of poly(OEGMA) or five-arm star poly(OEGMA) and 80% (w/v) of dendrimer solutions. *N*,*N*-Diethylnicotinamide (Fig. 3) (1.5 M in PBS) solution was used as the release medium. *N*,*N*-Diethylnicotinamide at a concentration of 3.5 M was found to increase paclitaxel solubility from 0.3 μg/ml to 39 mg/ml [10], approximating an infinite sink without requiring simulated flow conditions [11]. Samples were taken at predetermined time intervals and assayed for paclitaxel by isocratic reverse-phase HPLC.

2.5. Dilution of paclitaxel-solubilized dendrimer solution in serum

Paclitaxel (1.5 mg) was dissolved in 80 wt.% G-5 dendrimer solution (1.0 ml) at 37 °C. After confirming complete solubilization, the solution was diluted dropwise with fetal bovine serum. The changes in the appearance of the solution were monitored visually under a light microscope.

3. Results and discussion

The ^{1}H- and ^{13}C-NMR spectra of the synthesized poly(OEGMA) and star poly(OEGMA) indicated the successful polymerization of OEGMA [8]. The number-average molecular weights (M_n) of poly (OEGMA) and star poly(OEGMA) were 10,300 and 98,700, respectively, as calculated from the M_n of linear PEG. Treatment of the star polymer with 1 M NaOH at 80 °C for 24 h resulted in a decrease in the M_n to 11,800. This indicated that poly(OEGMA) graft chains were actually linked to the glucose core as arms of the star, and the arm segment had a similar M_n to that of poly(OEGMA). The ^{1}H- and ^{13}C-NMR spectra of G-3, G-4 and G-5 dendrimers also confirmed the successful syntheses of the dendrimers [8]. The real masses of the G-3, G-4 and G-5 dendrimers determined from MALDI mass spectra were found to be 1713.1 (+Na), 3507.4 (+H) and 7069.1 ($-H_2O$), respectively. Those values were consistent with the theoretical molecular weights (G-3: 1689; G-4: 3508; and G-5: 7104). The poly(OEGMA), star poly (OEGMA), and polyglycerol dendrimers were highly water soluble.

The paclitaxel solubility in all polymer solutions was increased compared with that in PEG400. The ability to enhance the paclitaxel solubility at 10 wt.% concentration was in the increasing order: G-5, G-4, G-3 dendrimers, star poly(OEGMA) and linear poly (OEGMA) (Table 1). Poly(OEGMA) increased the paclitaxel solubility, but a much more significant effect was observed with the five-arm star poly (OEGMA), even though the molecular weight of the arm segment in the star poly(OEGMA) was similar to that of poly(OEGMA). The paclitaxel solubility in water was previously determined to be 0.3 μg/ml [10]. Thus, the paclitaxel solubility in 10 wt.% star poly(OEGMA) was 130-fold higher than the paclitaxel solubility in water. This result supports the hypothesis that increasing the density of PEG400 chains or ethylene glycol units is a key factor in enhancing the solubility of paclitaxel. From this point of view, the dendritic architecture of the polyglycerol dendrimers can be expected to further increase the density of the ethylene glycol units. Paclitaxel solubilities in G-3, G-4 and G-5 dendrimers at a 10 wt.% concentration were 270-, 370- and 430-fold higher,

Table 1
Paclitaxel solubility in different polymer solutions

Samples	MW (g/mol)	Paclitaxel solubility (mg/ml)[a]	
		10 wt.%	80 wt.%
PEG400	400	0.0004 ± 0.0001	16.17 ± 0.35
Poly(OEGMA)	10,300[b]	0.0116 ± 0.0005	–
Five-arm star poly(OEGMA)	98,700[b]	0.0397 ± 0.0028	–
G-3 dendrimer	1690[c]	0.0804 ± 0.0051	1.873 ± 0.158
G-4 dendrimer	3508[c]	0.1100 ± 0.0062	1.817 ± 0.078
G-5 dendrimer	7087[c]	0.1282 ± 0.0091	2.305 ± 0.056

The aqueous paclitaxel solubility in the absence of any polymers is 0.0003 mg/ml.

[a] Mean ± S.D. ($n = 3$).

[b] The number-average molecular weight determined by GPC from the calibration curve based on linear PEGs.

[c] Determined by MALDI mass spectra.

respectively, than the paclitaxel solubility in water. Furthermore, the viscosity of the dendrimers at various concentrations was similar to that of PEG400 (Fig. 4), so that paclitaxel solubility could even be measured at a concentration of 80 wt.%. It is known that one advantage of the dendritic architectures is the lower viscosity relative to the linear, hyperbranched architectures [12]. For this reason, the paclitaxel solubilities in G-3, G-4 and G-5 dendrimers exceeding 10 wt.% could be determined, while measurement was extremely difficult with the star poly(OEGMA) and poly(OEGMA) solutions due to their high viscosity. The dendrimers at the concentration of 80 wt.% increased the paclitaxel solubility to 1.8–2.3 mg/ml (Table 1). This is more than a four-orders-of-magnitude increase in solubility. The enhanced paclitaxel solubility is not a molecular weight effect. As shown in Fig. 5, paclitaxel solubility was not significantly increased as the molecular weight of PEG was increased up to 2000. Even with a molecular weight and concentration (50 wt.%) of the G-3 dendrimer (M_w: 1690) similar to PEG2000, the paclitaxel solubility was 11-fold higher than that in PEG2000 (Fig. 5). These results strongly suggest that high density of ethylene glycol units in the dendritic structure contributes to an increase in the paclitaxel solubility.

Fig. 6 shows the cumulative release profile of paclitaxel from binary solutions of the dendrimers (Fig. 6a), poly(OEGMA) and star poly(OEGMA)

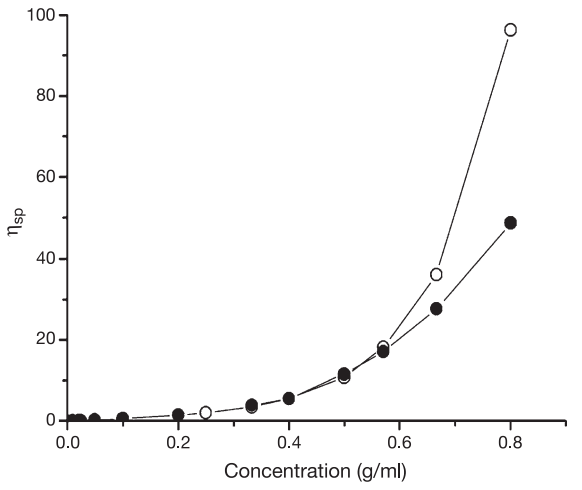

Fig. 4. Specific viscosity of the G-4 dendrimer and PEG400 in water at 25 °C. G-4 dendrimer (open circle) and PEG400 (closed circle).

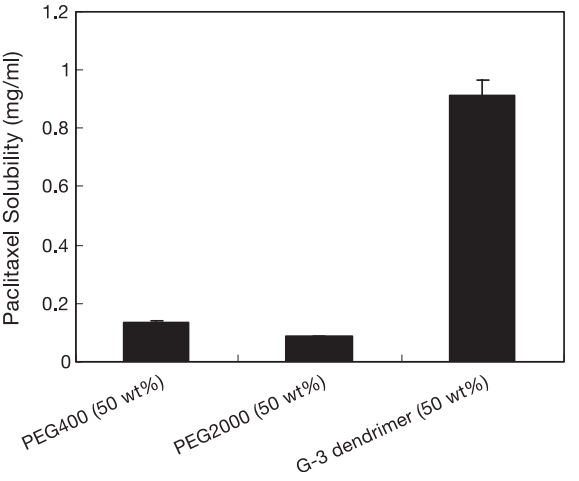

Fig. 5. Paclitaxel solubility in 50 wt.% PEG400 (M_w: 400), PEG2000 (M_w: 2000) and G-3 dendrimer (M_w: 1690).

(Fig. 6b) . All of the paclitaxel was released from the dendrimer-containing solutions during 96 h, independent of the dendrimer generation. Since the release medium (10 ml of 1.5 M *N*,*N*-diethylnicotinamide) is capable of solubilizing 10 times more paclitaxel than the amount of paclitaxel entrapped inside the dialysis chamber [10], the penetration of the release medium into the donor cell was thought to affect the release rate. To confirm the medium effect, the release of paclitaxel from its particulate state (i.e., in the absence of any polymers) entrapped inside the dialysis chamber was carried out (Fig. 6). The release rate was significantly slower than that of paclitaxel-loaded dendrimer solutions. This result indicates that the penetration of the release medium had a minor effect, if any, on enhancing the paclitaxel release rate. Even at the lower concentrations of the solubilized paclitaxel in 10 wt.% poly(OEGMA) and five-arm star poly(-OEGMA) solutions, the apparent release rate was much slower than that from the paclitaxel-loaded dendrimer solutions. Furthermore, the cumulative percentages of the released paclitaxel from the poly(-OEGMA) and five-arm star poly(OEGMA) solutions after 96 h were 54% and 26 %, respectively. Therefore, the increased paclitaxel release rates can be attributed to the solubilizing ability of the new architectural polymers.

When serum was added to the paclitaxel-solubilized dendrimer solution (80 wt.%), two phases (i.e., a

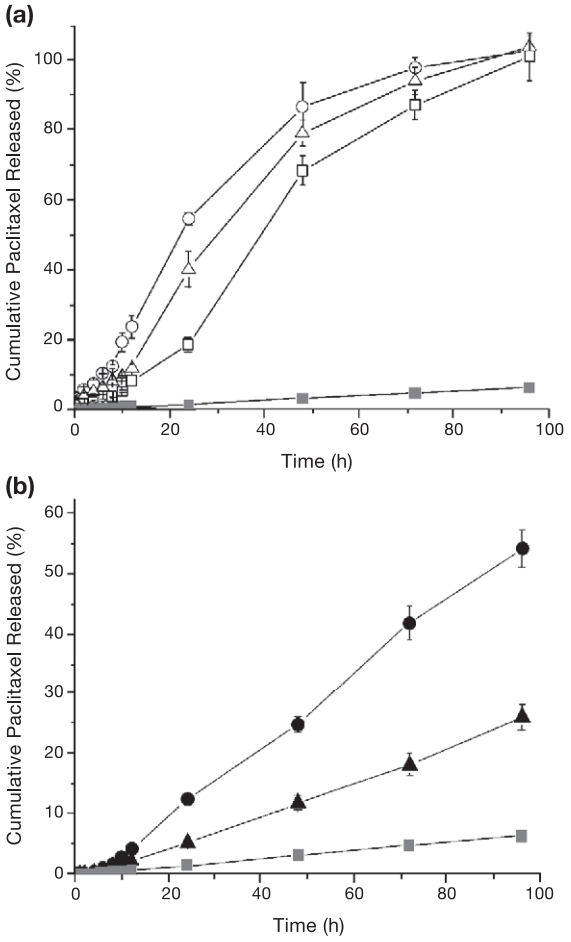

Fig. 6. Cumulative release profiles of paclitaxel that was solubilized in aqueous polymer solutions: (a) 80 wt.% of the G-5 dendrimer (open circle), 80 wt.% of the G-4 dendrimer (open triangle), 80 wt.% of the G-3 dendrimer (open square) and paclitaxel particles (closed and gray-colored square), (b) 10 wt.% of the five-arm star poly(OEGMA) (closed circle), 10 wt.% of the poly(OEGMA) (closed triangle) and paclitaxel powder (closed and gray-colored square).

watery serum phase and a dense dendrimer solution phase) were initially formed due to their difference in density. Precipitation of paclitaxel was observed at the interface between the serum and the paclitaxel-solubilized dendrimer solution. Soon, the two phases became homogeneous, and precipitation continued to occur in the serum-diluted dendrimer solution. This result indicates that paclitaxel was not entrapped into the dendritic structures. To avoid precipitation upon dilution, it is desirable to maintain the high density of ethylene glycol units of the dendrimers. Currently, we are working on the preparation of the dendrimer-cross-linked hydrogels to overcome the reduction in the high density of ethylene glycol units upon dilution. Preliminary results suggest that paclitaxel-loaded hydrogels do not cause paclitaxel precipitation and the hydrogel remains transparent even after 1 day in water. Evidently, paclitaxel is solubilized and distributed throughout the hydrogels. Nano- and micro-sized cross-linked hydrogels may be useful for delivering paclitaxel by parenteral administration, while a macroscopic hydrogel may be ideal for delivery of paclitaxel by oral administration.

4. Conclusions

The star and dendritic architectures of ethylene glycol units increased the aqueous solubility of paclitaxel by up to four orders of magnitude. Dendrimers were much more effective than PEG400 in increasing the paclitaxel solubility. This result is likely due to the increased local density of ethylene glycol units. The star and dendritic polymers consisting of ethylene glycol units are expected to be useful for both oral and parenteral delivery of paclitaxel and other poorly water-soluble drugs. A more detailed understanding on the solubilization mechanism by these polymer architectures will allow development of better polymeric systems for delivery of poorly soluble drugs.

Acknowledgements

This study was supported by National Institute of Health through Grant GM 65284, Samyang, Inc., NSF Industry/University Center for Pharmaceutical Processing Research and the Japanese Government through Monbukagakusho Grant for visiting research scholar. The authors express their appreciation to Professor Nobuhiko Yui at JAIST, Japan, for supporting this study.

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