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Aqueous Solubilization of Paclitaxel Using Hydrotropic Polymer Micelle

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Abstract. Hydrotropic block copolymers, consisting of a hydrophilic poly(ethylene glycol) (PEG) block and a hydrotropic polymer, poly(2-(4-(vinyl benzyloxy)-*N,N*-diethylnicotinamide)) [P(VBODENA)], block, were synthesized by atom transfer radical polymerization (ATRP) for aqueous solubilization of paclitaxel, a representative poorly water-soluble drug. These polymers showed an excellent solubilizing effect for paclitaxel in aqueous media in comparison with the corresponding hydrotropic agent and a control micelle (PEG-PLA) and such effect was significantly dependent on the polymer concentration and composition. Paclitaxel could be solubilized into polymer micelles in aqueous media without use of an organic solvent. Due to their promising properties such as micellar characteristics and hydrotropic solubilization, the hydrotropic polymer micelle system can be useful for formulation of paclitaxel and other poorly soluble drugs.

Introduction

Many drugs have often encountered serious problems in formulations and bioavailability due to low water-solubility and this limits their clinical applications [1]. Paclitaxel is a well-known anticancer agent that is effective for the treatment of a variety of tumors. Due to poor solubility of paclitaxel, cosolvents, such as Cremophor EL and ethanol, are widely used in formulations [2]. While such cosolvents are useful in increasing the solubility, the use of cosolvents comes with toxic side effects and a stability problem such as precipitation by dilution [3]. Many approaches have been tried to enhance the solubility of paclitaxel in aqueous media, but with limited successes. Polymer micelle systems, which are self-assemblies of amphiphilic block copolymers in aqueous media, have been broadly studied as a promising formulation for hydrophobic drugs [4]. They can solubilize the hydrophobic drugs into their hydrophobic core during their self-assembling process, mainly via hydrophobic interaction. Their hydrophilic outer shell may reduce undesirable interactions in biological environments and aggregation between themselves. In spite of good colloidal and solubilizing properties, however, it has been reported that polymer micelle systems show low physical stability with the loaded drug and their stability become worse with increasing drug loading [3, 5, 6]. Furthermore, the use of organic solvents, such as acetonitrile and acetone, should be often accompanied for effective drug loading and this limit the use of polymer micelles for the drugs labile to organic solvents.

Hydrotropic agents are diverse class of water-soluble compounds which at high concentrations can enhance the water-solubility of poorly soluble solutes via self-association to form non-polar microdomains [7]. We have developed hydrotropic polymer micelles by introducing diethylnicotinamide (DENA), one of hydrotropic agents effective for solubilizing paclitaxel, into the polymeric structure. In the previous report, the hydrotropic polymer micelle, consisting of poly(ethylene glycol) as a hydrophilic segment and hydrotropic polymer as a hydrophobic segment,

was observed to show a higher solubilizing capacity with a prolonged physical stability than other typical polymer micelles [7, 8].

In this study, we have synthesized various kinds of hydrotropic block copolymers with different block lengths and compositions and evaluated their micellar characteristics and solubilizing capacity for paclitaxel in aqueous media. Their solubilizing capacity has been compared with the poly(ethlylene glycol)-polylactide micelle that is known to show the highest loading content for paclitaxel in the literatures [5, 9].

Experimental

Materials. Monomethoxy poly(ethylene glycol) (MPEG, Mn=2000 and 5000), 2-hydroxynicotinic acid, 1,1'-carbonyl diimidazole, diethylamine, triethylamine, Copper(I) bromide, and 4-vinylbenzyl chloride were purchased from Aldrich. D,L-Lactide (Polysciences) was purified by recrystallization. Paclitaxel was obtained from Samyang Genex Co. (Korea). All solvents used for synthesis were dried or distilled before use. The other chemicals used were of reagent grade.

Synthesis of PEG-poly(2-(4-(vinylbenzyloxy)-N,N-diethylnicotinamide)) diblock copolymer (PEG-P(VBODENA)). PEG-P(VBODENA) block copolymers were synthesized by atom transfer radical polymerization (ATRP) of a hydrotropic monomer, a vinyl monomer containing DENA group (VBODENA). MPEG-Br as a macroinitiator and VBODENA were synthesized by a series of chemical reactions according to the previously reported method [3, 5]. The molar ratio between MPEG-Br and VBODENA was properly varied to obtain various block copolymers.

Synthesis of PEG-poly(D,L-Lactide) diblock copolymer (PEG-PLA). PEG-PLA diblock copolymers that have been widely studied for solubilization of poorly soluble drugs, including paclitaxel, were synthesized for comparison in terms of aqueous solubilization of paclitaxel. The block polymers were synthesized by ring opening polymerization of D,L-lactide in the presence of MPEG according to the previously reported method [10].

Characterization. The chemical compositions of the polymers synthesized were confirmed from ^{1}H-NMR spectra (JNM-AL400, 400MHz). Molecular weights and molecular weight distribution were obtained using a GPC equipped with a Waters 2414 RI detector, 515 HPLC pump, and set of three Styragel column. Fluorescence spectra (JASCO FP-6500) were recorded using pyrene (6 x 10^{-7} M) as a fluorescence probe to determine critical micelle concentrations (CMCs). Transmission electron microscopy (TEM, JEOL JEM-2000EX, Japan) was performed to observe the size and shape of a drug-solubilized micelle.

Aqueous solubilization of paclitaxel. Excess of paclitaxel was added to screw-capped vials containing a fixed volume of the polymer solution in distilled water. The mixture was stirred using a magnetic bar for 24 h at 37^{o}C. An aliquot of the sample was collected, and was filtered through a 0.2 μm membrane filter. The filtrate was diluted with acetonitrile (1:1 v/v), and the concentration of paclitaxel was determined by an isocratic reverse-phase HPLC (Agilent 1100 series, Agilent Technologies, DE) using a Symmetry column (Waters) at 25^{o}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 and linked Chem-Station software for date analysis.

Release of paclitaxel solubilized in polymer micelles. Release of paclitaxel from polymer micelles was examined using a 0.8 M sodium salicylate solution as a hydrotropic release medium that can keep a sink condition. The drug-solubilized polymer micelle was introduced into a dialysis membrane bag (MWCO=12,000-14,000), and the whole bag was placed in 40 ml of 0.8 M sodium salicylate solution (37 ^{o}C) for release experiments. Sampling was done from the media outside the dialysis bag at predetermined time intervals. The released paclitaxel were determined by HPLC.

Result and Discussion

Synthesis of PEG-P(VBODENA) diblock copolymers. Various block copolymers with different block lengths and compositions were successfully synthesized by ATRP. Synthetic results are summarized in Table 1.

Table 1. Synthetic results of PEG-P(VBODENA) diblock copolymers

No	Sample name	M_n [a)]	M_n [b)]	PDI	CMC(mg/mL)
1	PEG_{2000}-$P(VBODENA)_{800}$	2,760	5,755	1.027	0.0550
2	PEG_{2000}-$P(VBODENA)_{1400}$	3,400	8,171	1.055	0.0247
3	PEG_{5000}-$P(VBODENA)_{2400}$	7,350	12,244	1.046	0.0136
4	PEG_{5000}-$P(VBODENA)_{5200}$	10,230	12,376	1.041	0.0053
5	PEG_{5000}-$P(VBODENA)_{7000}$	12,050	13,704	1.023	0.0048
6	PEG_{2000}-PLA_{1000}	3,000	6,524	1.009	-
7	PEG_{2000}-PLA_{2000}	4,000	7,325	1.170	0.0038

a) calculated from the peak integration of ^{1}H-NMR spectra, b) measured by GPC

ATRP is a well-known method useful for polymerization of various monomers in controlled way. As shown Table 1, various P(VBODENA) blocks with different MWs were synthesized by varying the feed ratio of VBODENA. Figure 1 shows that the MWs of experimentally synthesized P(VBODENA)s were almost consistent with the theoretical values calculated from the feed ratios between VBODENA and PEG-Br. The MWs of P(VBODENA) increased proportionally to the feed ratios of VBODENA. PEG-P(VBODENA)s were observed to have low polydispersity and their CMCs decreased with the increasing block length of P(VBODENA).

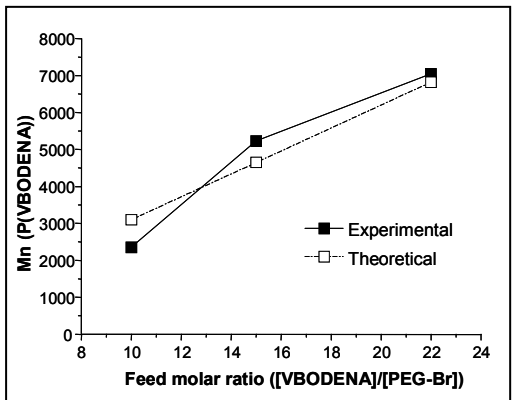

Figure 1. Molecular weight dependence of P(VBODENA) block on the feed ratio of VBODENA to PEG-br.

Aqueous solubilization of paclitaxel. Paclitaxel has an extremely low solubility in water (~ 0.0003 mg/ml). The change in the solubility was observed in the presence of DENA, PEG-PLA, and PEG-P(VBODENA) as a function of concentration (Fig. 2). At a given concentration range (0~10 wt%), DENA did not have any effect on the solubility, but in case of PEG_{2000}-PLA_{2000} the solubility of paclitaxel increased up to 0.92 mg/ml. On the other hand, the use of hydrotropic block copolymers was much more effective in solubilizing the drug and the solubility was observed to be significantly dependent on the block structure of the polymers. For PEG_{2000}-$P(VBODENA)_{1400}$ with the similar block length to PEG-PLA, the solubility was 1.66 mg/ml. The solubility increased up to about 4 mg/ml when PEG_{5000}-$P(VBODENA)_{7000}$ was used. Therefore, PEG-P(VBODENA)s could be considered to have a superior solubilizing capacity than corresponding hydrotropic agent, DENA, and PEG-PLA. In addition, it was noted that the hydrotropic block length could be an important factor for solubilization of paclitaxel. At the same concentrations, a longer P(VBODENA) block led to higher solubility. The paclitaxel-solubilized polymer solution was observed by TEM. As shown in Figure 3, spherical nanoparticles with the size of 30~40 nm and monodisperse size distribution were observed. The particle size was almost similar to the micellar size from the corresponding polymer without the drug [5]. Because there was no paclitaxel crystallite observed outside the nanoparticles, paclitaxel was confirmed to be solubilized in the polymer micelles. Thus, the solubility increase of paclitaxel can be attributed to the synergistic effect of micellar characteristics and hydrotropic solubilization of PEG-P(VBODENA) copolymers.

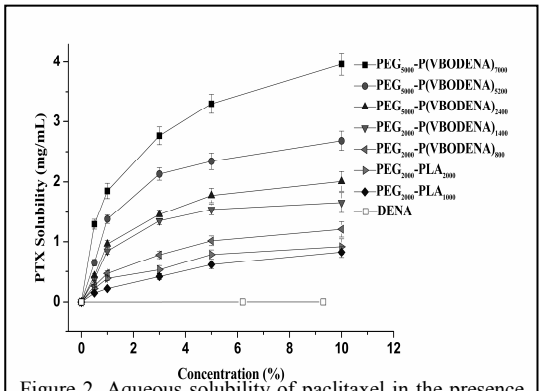

Figure 2. Aqueous solubility of paclitaxel in the presence of DENA, PEG-PLA, and PEG-P(VBODENA) at 37 °C.

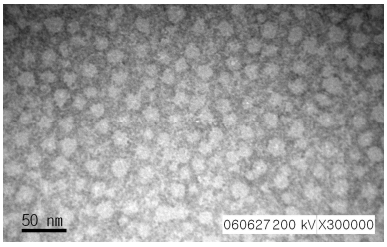

Figure 3. TEM picture of paclitaxel-solubilized PEG_{5000}-$P(VBODENA)_{5200}$.

Release of paclitaxel. Different amounts of paclitaxel was solubilized in the aqueous PEG_{5000}-$P(VBODENA)_{7000}$ solutions (10 wt%). Polymer micelles containing 0.4, 0.7, and 1.1 wt% of paclitaxel were filtered through a 0.45 μm syringe filter before lyophilization. For release experiments 20 mg of each micelle was used. Figure 4 shows the release profiles of PEG_{5000}-b-$P(VBODENA)_{7000}$ micelle. The polymer micelles released almost all drug within 24 h, regardless of the solubilized drug amounts. These results were consistent with the previous results from the hydrotropic polymer micelles, where the drug was loaded by a dialysis method using acetonitrile [5]. As a result, the paclitaxel could be solubilized into polymer micelle without the use of organic solvent and also released in aqueous media from the micelle.

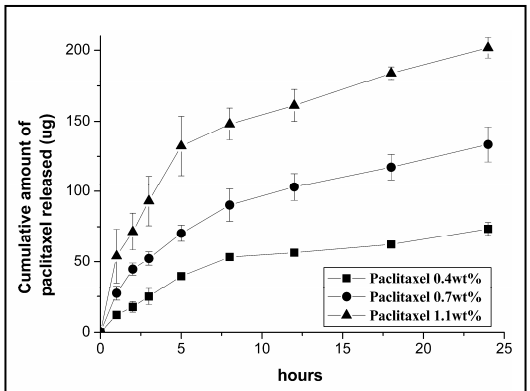

Figure 4. Release kinetics of paclitaxel from PEG_{5000}-$P(VBODENA)_{7000}$ micelles in 0.8 M sodium salicylate solution at 37 °C.

Conclusions

Hydrotropic block copolymers, consisting of a hydrophilic PEG block and a hydrotropic P(VBODENA) block, were successfully synthesized with controlled block structure by ATRP. They exhibited excellent solubilizing effect for paclitaxel in aqueous media. The block structure, especially the block length of P(VBODENA), significantly affects their solubilizing properties. Such an increase in the aqueous solubility of paclitaxel may be contributed to the synergistic effect of the micellar characteristics and the presence of hydrotropic agents in their polymer composition. The hydrotropic polymer micelle system for paclitaxel can be formulated in solution or powder state with a process excluding the use of any organic solvent. It presents a good alternative approach for solubilization of paclitaxel and other poorly soluble drugs.

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