properties. European Journal of Pharmaceutics and Biopharmaceutics, 2002. **53**(2): 155-160.

19. Wissing, S.A. and Muller, R.H. *The influence of solid lipid nanoparticles on skin hydration and viscoelasticity - in vivo study.* European Journal of Pharmaceutics and Biopharmaceutics, 2003. **56**(1): 67-72.
20. Wissing, S.A. and Muller, R.H. *Cosmetic applications for solid lipid nanoparticles (SLN).* International Journal of Pharmaceutics, 2003. **254**(1): 65-68.
21. Jenning, V., Gysler, A., Schafer-Korting, M. and Gohla, S.H. *Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin.* European Journal of Pharmaceutics and Biopharmaceutics, 2000. **49**(3): 211-218.
22. Saupe, A., *Pharmaceutical and cosmetic applications of nanostructured lipid carriers (NLC): sun protection and care.* 2004, Free University of Berlin.
23. Pouton, C.W., *Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems.* European Journal of Pharmaceutical Sciences, 2000. **11**(Supplement 2): S93-S98.
24. Yang, S., Zhu, J., Lu, Y., Liang, B. and Yang, C. *Body Distribution of Camptothecin Solid Lipid Nanoparticles After Oral Administration.* Pharmaceutical Research, 1999. **16**(5): 751-757.
25. Mei, Z., Li, X., Wu, Q., Hu, S. and Yang, X. *The research on the anti-inflammatory activity and hepatotoxicity of triptolide-loaded solid lipid nanoparticle.* Pharmacological Research, 2005. **51**(4): 345-351.
26. Zara, G.P., Cavalli, R., Fundaro, A., Bargoni, A., Caputo, O. and Gasco, M.R. *Pharmacokinetics of Doxorubicin incorporated in solid lipid nanosheres (SLN).* Pharmacological Research, 1999. **40**(3): 281-286.
27. Olbrich, C., Mueller, R.H., Tabatt, K., Kayser, O., Schulze, C. and Schade, R. *Stable biocompatible adjuvants--a new type of adjuvant based on solid lipid nanoparticles: a study on cytotoxicity, compatibility and efficacy in chicken.* Altern. Lab. Anim., 2002. **30**(4): 443-58.

Chapter 4

HYDROTROPIC NANOCARRIERS FOR POORLY SOLUBLE DRUGS

Tooru Ooya[1], Sang Cheon Lee[2], Kang Moo Huh[3] and Kinam Park[4]
[1]School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Tatsunokuchi, Ishikawa, 923-1292, Japan; [2]Korea Institute of Ceramic Engineering and Technology, 233-5 Gasan-dong, Guemcheon-gu, Seoul 153-801, South Korea; [3]Chungnam National University, Dept. of Polymer Science and Engineering, Yuseong-gu Gung-dong 220, Daejeon, 305-764, South Korea; [4]Departments of Pharmaceutics and Biomedical Engineering, Purdue University, West Lafayette, IN 47907-1336, U.S.A.

Abstract: Delivery of poorly water-soluble drugs remains as one of the most difficult challenges in the pharmaceutics and drug delivery areas. One of the recent approaches of increasing the water-solubility of poorly soluble drugs has been to utilize polymeric hydrotropic agents. Hydrotropic agents in nanocarrier forms, such as dendrimers and polymer micelles, increase the water solubility by orders of magnitude. The hydrotropic nanocarriers have advantages over other carriers for their high drug loading efficiency and long-term stability.

Key words: hydrotropy, dendrimer, polymer micelle, water-solubility, paclitaxel, nanocarrier

1. INTRODUCTION

Recent advances in nanoscience and nanotechnology provide us with a great opportunity to understand biological systems in nano-scale, and such understanding has created new research fields such as "bio-nanotechnology" [1]. New designs of nanocarriers based on structural characteristics in nano-scale are expected, because the interdisciplinary fields of bio-nanotechnology undoubtedly have a great potential of new bio-functions toward regulation of biological systems. It is known that biological processes including large macromolecular self-assemblies are based on dynamic consequences of cooperativity and metastability. The assembly of large supramolecular

M.R. Mozafari (ed.), Nanocarrier Technologies: Frontiers of Nanotherapy, 51–73.

complexes produces dynamic features in a construct. Supramolecular architectures formed via non-covalent interactions are expected to play an important role in terms of dynamic functions under physiological conditions. Generally, self-assembly, such as protein folding, involves hydrophobic interaction [2], existing in water at hydrophobic interface of the systems [3]. Such self-assembly via hydrophobic interaction plays an important role for creating nanocarriers that deliver poorly water-soluble drugs [4-6].

Nanocarriers are highly useful for delivering poorly soluble drugs without drug solubilizers that may be toxic and for targeting to suitable tissues and cells. There have been various approaches for increasing the aqueous solubility of poorly soluble drugs via self-assembly. Polymeric micelles have been extensively studied as a promising drug formulation that can effectively dissolve various types of hydrophobic drugs with high drug loading capacity for increased bioavailability. Hydrophobic drugs can be dissolved or physically entrapped in the core of polymeric micelles at concentrations that can exceed their intrinsic water-solubility by orders of magnitude. Recently, our research groups have introduced new polymeric systems known as the hydrotropic polymer, hydrotropic dendrimer, and hydrotropic polymer micelle. They can increase the solubility of poorly soluble drugs by several orders of magnitude. The monomers of those hydrotropic polymers were designed based on the molecular structures of low molecular weight hydrotropic agents (or hydrotropes) which are highly effective in solubilizing poorly soluble drugs. In the case of hydrotropic dendrimers, increased density of drug-solubilizing molecules in a dendrimer contributes to the effective solubilization. The hydrotropic polymers and dendrimers have been developed into hydrotropic polymer micelles that can act as nanocarries for poorly water soluble drugs. This chapter introduces hydrotropic nanocarries based on hydrotropic polymers and dendrimers for delivery of poorly soluble drugs.

2. WHAT IS HYDROTROPIC SOLUBILIZATION?

Hydrotropy refers to an increase in water solubility caused by the addition of large amount of a second solute [7]. However, the exact meaning of hydrotropy is still unclear. There have been various theoretical and experimental studies aiming at explanation of hydrotropic solubilization [8, 9]. Most of hydrotropic molecules are composed of an aromatic ring substituted by anionic or cationic moieties. The hydrotropic molecules are usually too small to induce micelle formation. The hydrotropic molecules aggregate at a certain concentration, known as the minimum hydrotropic concentration (MHC), to increase the solubility of poorly soluble drugs significantly [10]. For example, nicotinamide has been shown to enhance the

solubilities of a wide variety of hydrophobic drugs through complexation [11-13]. Here, the aromaticity of the pyridine ring of nicotinamide and its derivatives, such as *N*-methylnicotinamide and *N,N*-diethylnicotinamide, promotes stacking of molecules through its planarity for the aggregation [12]. Another interesting phenomenon of hydrotropic solubilization is self-association of the hydrotrope in an aqueous phase. Some experimental data indicate that some hydrotropes, including nicotinamide and aromatic sulfonates, associate in aqueous solutions [7]. Studies on the nicotinamide–riboflavin system showed that the self-association of nicotinamide contributed to the solubility increase of riboflavin rather than complexation between two species [7]. According to the report of Silva et al., the micellization process does not occur in hydrotrope-water mixtures, and the the MHC of usual hydrotropes is about 1 M, which is higher than typical critical micelle concentrations (CMC) of 10^{-2}–10^{-3} M [9]. Thus, both high concentration and association are needed to induce hydrotropic solubilization.

Hydrotropes solubilize hydrophobic drugs, but it is difficult to find out the structure-activity relationship for the hydrotropic solubilization. Hydrotropic solubilization of paclitaxel has been studied using more than 60 candidate hydrotropic agents and their analogues [14]. Several effective hydrotropic structures were identified for their ability to solubilize paclitaxel, and some of them are shown in Fig. 4-1. Among them, *N,N*-diethylnicotinamide (DENA) was found to be the most effective hydrotropic agent for paclitaxel. The solubility of paclitaxel was 5~6 orders of magnitude greater than the intrinsic solubility of paclitaxel. *N*-Picolylnicotinamide (PNA), *N*-allylnicotinamide, and sodium salicylate were also found to have high solubilizing capacity for paclitaxel. This information can be used to find other hydrotropic compounds and to design new hydrotropic analogues that are effective for paclitaxel and other poorly soluble drugs.

3. DESIGN OF HYDROTROPIC POLYMERS

3.1 Hydrotropic polymers

One barrier for successful oral drug delivery using hydrotopes is the co-absorption of a significant amount of low molecular weight hydrotropes along with the drug to be delivered. Thus, there was a need for developing polymeric form of hydrotropes effective in increasing the drug solubility. The polymeric forms of the hydrotropic agents may provide various advantages, including prevention of co-absorption from the GI tract after oral administration while maintaining the beneficial hydrotropic properties.

The polymers used to increase the water solubility of hydrophobic compounds were found in the patent literature. The structures of those polymers were based on polyvinylpyrrolidone (PVP). The investigators synthesized new pyrrolidone-containing polymers of which side groups had considerably reduced steric hindrance when complexing with water insoluble organic compounds [15, 16]. These polymers increased the water solubility of poorly soluble drugs, such as furosemide, indomethacin, and triamterene. The chemical structures of these polymers were based only on PVP, and thus the structural variation of the polymer has been limited. Therefore, it is important to systematically diversify the structure of the hydrotropic polymers so that their property can be tailored for specific drug solubilization.

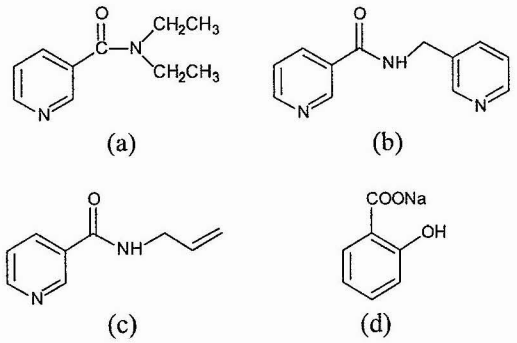

Figure 4-1. Several hydrotropic agents identified for paclitaxel solubilization: N,N-diethylnicotinamide (a), N-picolylnicotinamide (b), N-allylnicotinamide (c), and sodium salicylate (d).

3.2 Design parameters of hydrotropic polymers and structure-property relationship

Since the exact mechanisms of solubility increase of poorly soluble drugs by hydrotropes are not known, it is difficult to predict the structural requirements of hydrotropes for effective solubilization of various drugs. Furthermore, when the concept of hydrotropic solubilization is extended from simple low molecular weight species to the polymeric forms, the

structural needs for the hydrotropy are still unpredictable and become more complicated. Thus, the most rational approach for the synthesis of hydrotropic polymers is utilizing the chemical structures of the most promising low molecular weight hydrotropic agents. Hydrotropic properties of various low molecular weight molecules were examined for their ability to increase water-solubility of paclitaxel [14]. From the study, the information on structure-activity relationship and structural requirements of the hydrotropes for the paclitaxel solubilization were obtained. Hydrotropic polymers were designed based on the excellent hydrotropes, such as *N*-picolylnicotinamide (PNA) and *N,N*-diethylnicotinamide (DENA). PNA and DENA at the 3.5 M concentration increased the water solubility of paclitaxel up to 30 and 40 mg/mL, respectively.

To synthesize hydrotropic polymers, several design parameters should be considered. They are the polymer backbone, the type of hydrotropes, orientation of hydrotropic moieties to the polymer backbone, and spacer groups bridging the polymer backbone and the hydrotropic moieties. The first step for hydrotropic polymers is the modification of the hydrotropes with polymerizable groups. This step has a significant meaning since it dictates all subsequent design parameters. Estimation of hydrotropic property of modified hydrotropic agents is important since the type of the spacer group between the polymer backbone and the hydrotropic agent may significantly affect their hydrotropic property. Enhancement of aqueous solubility of paclitaxel was observed with increasing the concentration of 2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide (2-VBOPNA), 6-(4-vinylbenzyloxy) -*N*-picolylnicotinamide (6-VBOPNA), and PNA [17]. It is noteworthy that 2-VBOPNA and 6-VBOPNA, with a vinylbenzyloxy group linked to 2- and 6-position of pyridine ring, showed rather different pattern of solubilization but retained comparable hydrotropic property for the paclitaxel to that of PNA. 2-VBOPNA enhanced the aqueous solubility of paclitaxel to a larger extent than 6-VBOPNA did at a wide concentration range. This finding suggests that the linked orientation of hydrotropic moiety (e.g. PNA) to the polymerizable group is the one of the key factors deciding the hydrotropic property of the modified hydrotropes. Even though the hydrotropic moieties can be linked into the commercially available polymer backbone, the design of the polymerizable hydrotropic agent is more promising in generation of excellent hydrotropic polymers. For hydrotropic polymers to efficiently increase water-solubility of drugs, the high content of hydrotropic moieties in the polymer is essential since the hydrotropic action is presented only in the high local concentration of hydrotropic groups. The simple conjugation of the hydrotropic moieties into the polymer backbone does not guarantee the high degree of hydrotropes in the polymer structure. On the other hand, the polymerization of the hydrotropes modified with polymerizable groups can

produce the polymers with hydrotropic moieties in every repeating unit, thereby maximizing the content of the hydrotropic structure in the polymer chains. Various polymer backbones can be obtained by introducing allyloxy, acryloyl, styryl, and oligoethyleneoxyacryloyl groups to the hydrotropic moieties. PNA was used as a hydrotropic moiety for the synthesis of hydrotropic polymers with the styryl backbone [18]. High molecular weight polymers could be synthesized by radical polymerization, and the polymers had the high content of PNA groups. Fig. 4-2 shows the synthetic approach for poly (2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) P(2-VBOPNA) and Poly (2-(2-(acryloyloxy) ethoxyethoxyethoxy)-*N*-picolylnicotinamide) 'P(ACEEEPNA)'.

Effect of the design parameters on the hydrotropic properties of the polymers was examined [18]. The spacer type and the binding orientation of the hydrotropic moieties to the polymer backbone significantly affected the solubility-enhancing properties for paclitaxel. Fig. 4-3 shows the solubility increase of paclitaxel as a function of PNA and PNA-containing hydrotropic polymers. P(ACEEEPNA) retained the hydrotropic property of PNA to increase the water-solubility of paclitaxel to 0.32 mg/mL at the maximum polymer concentration of 290 mg/mL. As compared with the intrinsic water-solubility of paclitaxel (0.3 μg/ml), this is 1000-fold increase in solubility. In contrast to PNA, P(ACEEEPNA) solubilized paclitaxel to a significant extent even at the low concentration range, e.g. less than 50 mg/mL. The polymer solubilized paclitaxel even at the very low concentration range, where solubilization of paclitaxel by PNA was not observed. P(2-VBOPNA) and P(6-VBOPNA) also retained the hydrotropic property of PNA and increased the water-solubility of paclitaxel to 0.56 mg/mL and 0.13 mg/mL respectively. There was a big difference in the hydrotropic property between the polymer having an oligo(ethylene glycol) spacer and the polymers with a phenyl spacer. The hydrotropic property of P(2-VBOPNA) and P(6-VBOPNA) was much more pronounced than that of P(ACEEEPNA). P(ACEEEPNA) showed a positive curvature as its concentration increased, while P(2-VBOPNA) and P(6-VBOPNA) showed negative curvatures in the aqueous paclitaxel solubility as a function of the polymer concentration. It is interesting to notice that P(2-VBOPNA) is a much better hydrotropic polymer than P(6-VBOPNA). Apparently, the orientation of the PNA moiety in P(2-VBOPNA) has better stacking ability to solubilize paclitaxel. It appears that the PNA moieties bound to the flexible oligo(ethylene glycol) spacer are favored to form high orders of associated structures with increasing concentration. Thus, the structure and property of a spacer group between the polymer backbone and the hydrotropic moiety play a key role in modulating the solubilization profile by polymeric hydrotropes. Hydrotropic polymers can be made using the same hydrotropic moiety but with different

orientations by copolymerization of monomers obtained from the same hydrotrope. This approach can provide an opportunity of the facile interaction of hydrotropic units with paclitaxel by compensating the motional limitation of each polymer-bound hydrotropic moiety.

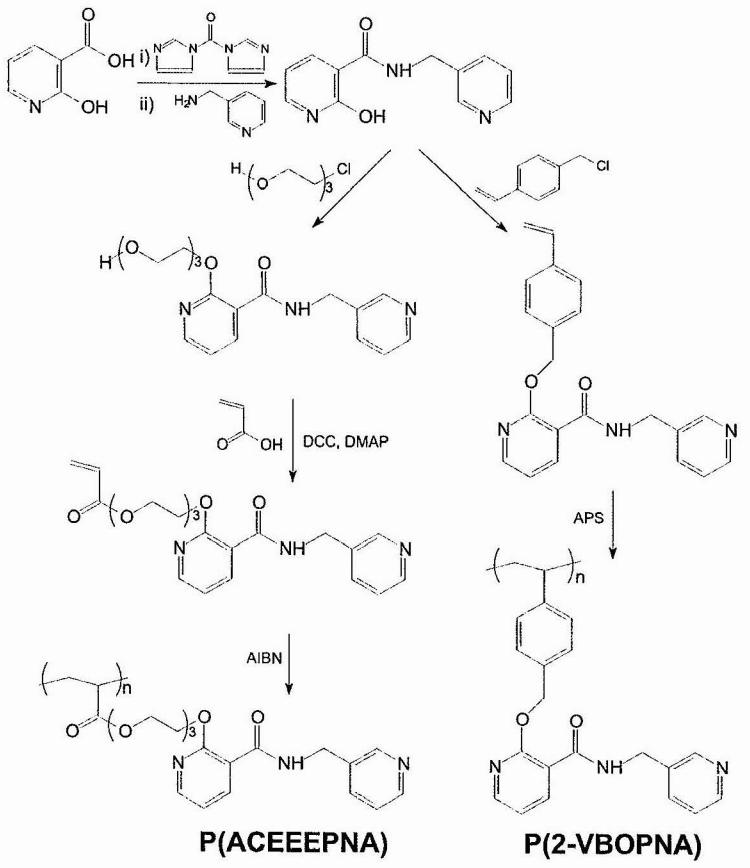

Figure 4-2. Synthetic route for P(ACEEEPNA) and P(2-VBOPNA).

Hydrotropic copolymers can also be made using two different hydrotropes as shown in Fig. 4-4. The concept of using two different hydrotropes on the same polymer backbone is based on the concept of the facilitated hydrotropy. The facilitated hydrotropy is the use of combination of different hydrotropic agents to yield higher hydrotropic property, compared to individual hydrotropes [10]. The maximum synergistic hydrotropic effect can be obtained by optimizing the factors, such as the type and length of spacers, orientations of the hydrotrope, and the use of different hydrotropes.

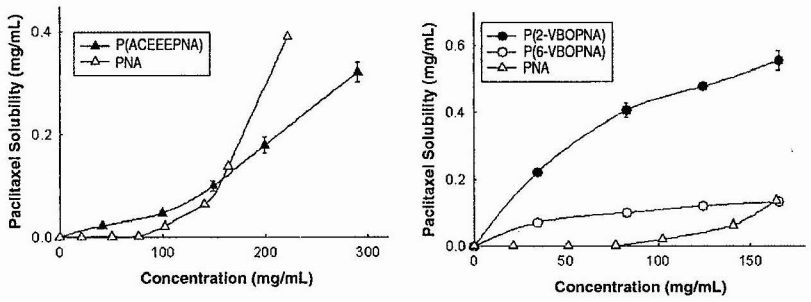

Figure 4-3. Enhancement of paclitaxel solubility as a function of PNA and PNA-containing hydrotropic polymers. (Modified from reference 18).

In general, the low molecular weight hydrotropes display efficient solubilizing ability beyond relatively high MHC values that range from 20 to 200 mg/mL or higher, depending on their classes [20]. Thus, it appears that the highly localized concentration of PNA moieties bound to the hydrotropic polymers facilitate self-association at a much lower concentration range. The lower MHC values, as compared with low molecular weight hydrotropic agents, might be one of the important characteristics of the hydrotropic polymers in that a small amount of the polymeric hydrotropes can self-associate and display the solubilizing ability for poorly water-soluble compounds. Based on the properties distinct to low molecular weight hydrotropes, one could define a hydrotropic polymer as a freely water-soluble polymer possessing the ability to increase aqueous solubility of poorly soluble compounds through association of its hydrotropic moieties. The appropriate selection and combination of the polymer backbone, the type of hydrotropes, binding orientation of hydrotropes, the spacer groups,

and facilitated hydrotropic concept may result in the solubilizing systems of high potential for successful oral formulations of poorly water-soluble drugs.

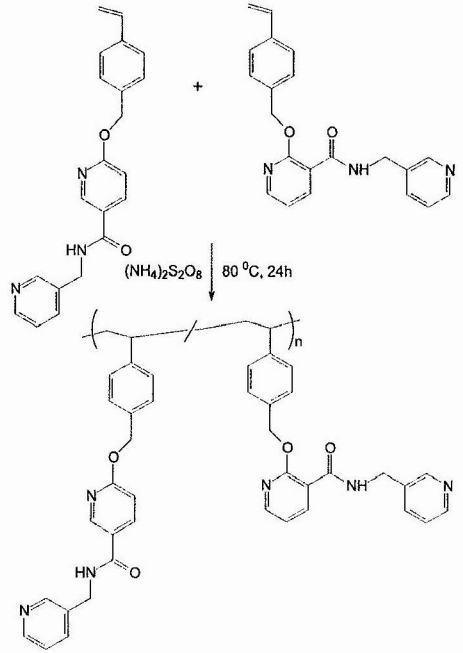

Figure 4-4. Synthetic scheme of P(2-VBOPNA-co-6-VBOPNA).

4. DESIGN OF HYDROTROPIC DENDRIMERS

4.1 Polyglycerol dendrimers as a hydrotrope

Dendrimers are nano-sized, highly branched macromolecules with monodispersed characters. Due to the nano-sized spherical shape and surface functionalities, chemical reactivity and physical properties, e.g. viscosity, are quite different from those of linear polymers [19]. Encapsulation of hydrophobic compounds in dendrimers has been extensively studied for drug delivery [20, 21]. In particular, biocompatible and/or biodegradable dendrimers have been used as drug delivery systems and tissue scaffolds [22,

23]. Polyglycerol dendrimers (PGDs) [24, 25] have a good potential as biomaterials because of high water solubility, chemical reactivity and structural similarity to poly(ethylene glycol) (PEG). So far, PEG has been used to modulate water solubility of poorly soluble drugs [26, 27]. PEG with molecular weight of 400 (PEG400) has been frequently used as a co-solvent to dissolve poorly water-soluble drugs [28, 29]. For example, PEG400 increased the aqueous solubility of ß-oestradiol by 4-5 orders of magnitude at its concentration of 80 wt% and higher [29]. It is suggested that the majority of PEG400 is believed to self-associate through hydrogen bonding mediated by water molecules at concentrations greater than 80 wt% [30]. From these observations, we hypothesized that PGD could act as a hydrotrope because the high density of ethylene glycol-like units in PGD can provide high local concentrations of PEG400.

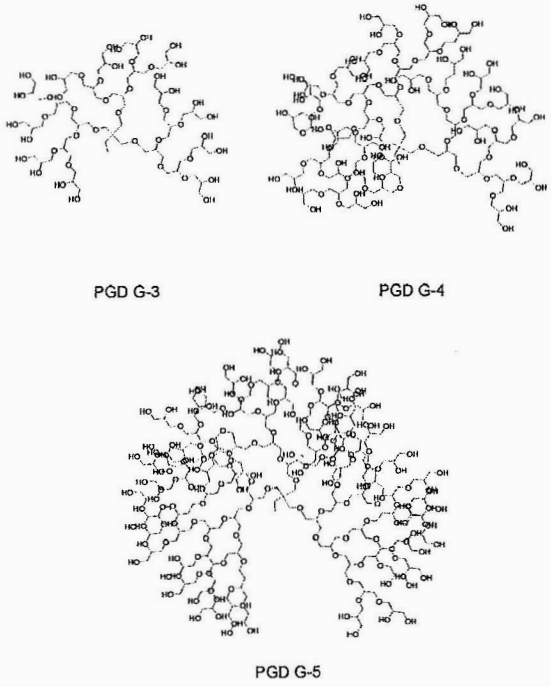

Figure 4-5. Schematic structures of G-3, G-4 and G-5 polyglycerol dendrimers. (Modified from reference 32).

Polyglycerol dendrimers (PGDs) with 4-5 generations (Fig. 4-5) were synthesized and used to investigate the effect of dendritic architecture and its generation on aqueous solubilization of paclitaxel [31]. Chemical and physical properties of the PGDs were characterized by NMR, MALDI-TOF mass, GPC, viscosity and dynamic light scattering measurements (Table 4-1). The paclitaxel solubility in all solutions of PGDs, even below 10 wt%, was much higher than that in PEG400 (Fig. 4-6). Increase in the paclitaxel solubility by PGDs was dependent on the dendrimer generation. The dendritic structure was the reason for the enhanced solubility of paclitaxel even at low concentrations. ^{1}H NMR spectra of paclitaxel before and after mixing with PGDs in D_2O suggested that the aromatic rings and some methyne groups of paclitaxel were surrounded by PGDs (Fig. 4-7). PGDs, which do not require hydrophobic segment as in polymeric micelles, provide an alternative method of hydrotropic solubilization of poorly soluble drugs.

Table 4-1. Results of MALDI-MS, GPC, and Viscosity of PGDs.

Sample Code	M_{theo} [a]	MALDI	DLS	[η]
		M_w [b]	R_h (nm)	(mL·g^{-1})
PGD G-3	1,689	1,690	1.1	1.89
PGD G-4	3,508	3,507	1.9	3.90
PGD G-5	7,104	6,959	2.4	3.06

[a] Theoretical molecular weight, [b] Determined by MALDI-TOFF mass measurements

4.2 PEGylated hydrotropic dendrimers

In addition to the hydrotropic property of PGDs, surface modification of PGDs with PEG would provide even higher local concentrations of PEG. To evaluate the effect of PEG modification on hydrotropic solubilization, PGD was PEGylated by using methoxy-PEG with Mn of 550 and 2,000 [32]. PEGylated PGD with generations 4 (PEG-G4) was synthesized by conjugating *N,N'*-carbonyldiimidazole (CDI)-activated methoxy-PEG (Mn = 550, and 2,000) to PGD-G4. The CDI-activated methoxy-PEG was allowed to react with PGD-G4 in DMSO in the presence of dimethylaminopyridine (DMAP) and the solution was stirred at 70°C under N_2 for 24h to obtain PEGylated PGD-G4s, PEG500-PGD-G4 and PEG2000-PGD-G4 (Fig. 4-8).

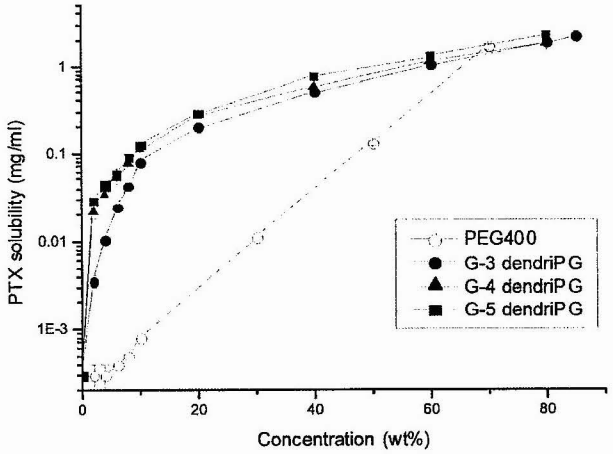

Figure 4-6. Aqueous paclitaxel (PTX) solubility as a function of the PGD concentration. (Modified from reference 31).

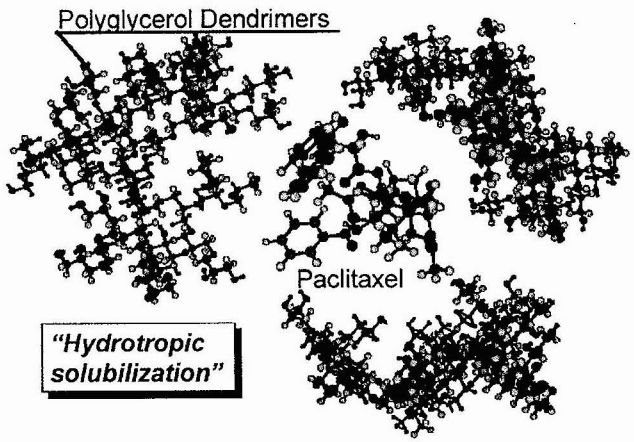

Figure 4-7. Image of hydrotropic solubilization by PGDs.

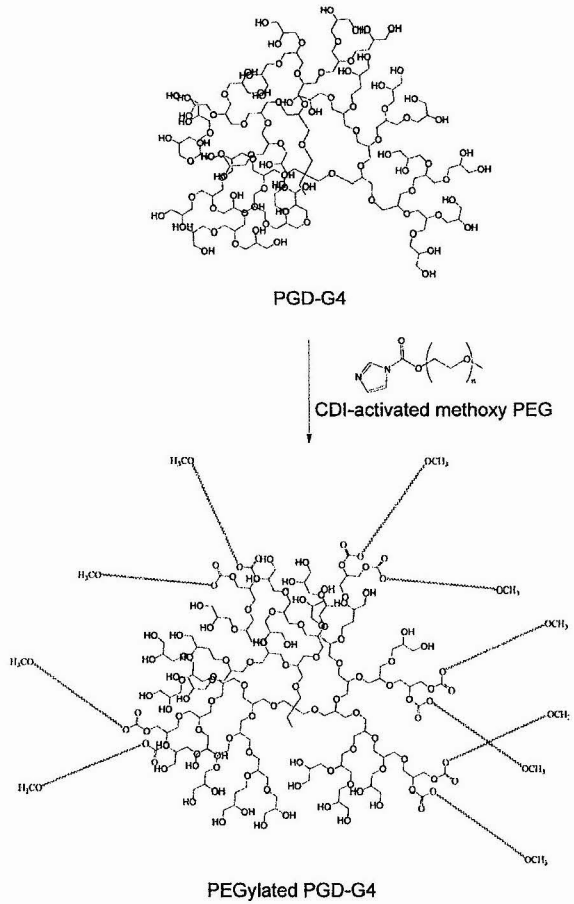

Figure 4-8. Synthesis of PEGylated polyglycerol dendrimers (Modified from reference 42).

The ability of PGD G-4 to enhance the paclitaxel solubility was grater than that of PEG 400. PEG2000-PGD-G4 solubilizes paclitaxel at the similar level. On the other hand, PEG500-PGD-G4 showed the greatest enhancement: paclitaxel solubility was considerably increased over 2 wt%. These results suggest that the methoxy-PEG550 chains grafted on the surface

of PGD-G4 contribute to interact with paclitaxel molecule. Methoxy-PEG-2000 was not effective due to the low solubility effect of PEG2000 [33]. Paclitaxel solubilities by PEGy500-PGD-G4 and PEGy2000-PGD-G4 at 10 wt% concentration each were 1,520-fold, and 408-fold higher, respectively, than the paclitaxel solubility in water (0.3 μg/ml) [14] (Fig. 4-9). This result suggests that increased local density of PEG550 due to conjugation to PGD surface provides a nano-scale space with a high concentration of PEG550.

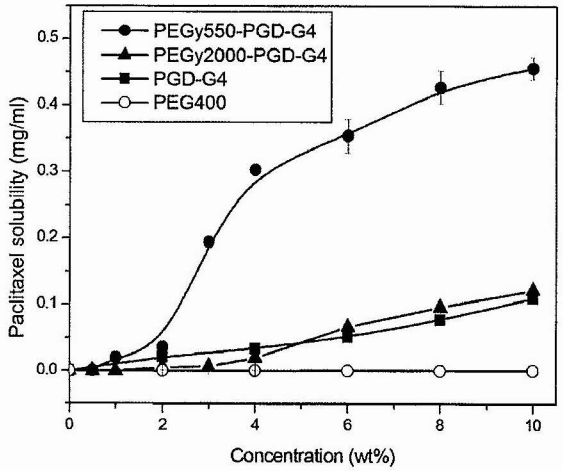

Figure 4-9. Palitaxel solubility as a function of concentration of PEG derivatives. (Modified from reference 42).

5. SELF-ASSEMBLING SYSTEMS FOR DRUG SOLUBILIZATION

Surfactants are amphiphilic molecules that are characterized by distinct polar and nonpolar regions. In aqueous media, as the concentration of surfactant increases they self-assemble to form unique soluble structures called micelles. The concentration at which micelles begin to form is called the critical micelle concentration (CMC). These micelles are normally

spherical and composed of a hydrophobic core and a hydrophilic shell. Nonpolar regions of the surfactant molecules are segregated from the aqueous exterior to form an inner core that is surrounded by a palisade of the polar regions which are in direct contact with the water.

There has been considerable interest in the interaction between hydrophobic solutes and micellar structures as a solubilization process by which solubility of the solutes can be enhanced [34]. A hydrophobic drug can be located within the micelle core during self-assembling process and becomes water-soluble as long as the micellar structures remain intact in aqueous media. There are a number of surfactants available for use as solubilizing agents. If the surfactant concentration falls below the CMC by dilution, the micellar structures can not be kept any more and a drug that was solubilized by the micelles precipitates from the aqueous medium [35]. In addition, several problems such as poor incorporation efficiency for many drugs, and thermodynamic instability, and toxicity have significantly limited their clinical applications. For these reasons, recently much interest has been placed on polymer micelles.

Amphiphilic block copolymers with diverse block structures may self-assemble in aqueous media to form micellar structures as low molecular weight surfactants do. It is generally known that polymeric amphiphiles have a much lower CMC than low molecular weight surfactants because they have more interaction sites. For this reason, micelles from amphiphilic block copolymers are more stable and so they have attracted a lot of attention as vehicles for solubilization and delivery of poorly water-soluble drugs [36-39]. They can act as water-soluble, biocompatible nano-carriers with a size of 10~100 nm and a proven efficacy of delivering poorly soluble drugs. Many advantages of using polymer micelles have been demonstrated and reviewed in the literature [37, 40, 41]. Polymer micelles with characteristic core-shell structures have successfully been used to solubilize poorly soluble drugs, such as paclitaxel [42], doxorubicin [43], cisplatin [44], amphotericin B [45], risperidone [46], and cyclosporine A [47].

5.1 Hydrotropic polymer micelles

In polymer micelle systems, the hydrophilic shell allows steric stability and thereby a long circulation of the drug, whereas the hydrophobic core are responsible for solubilizing and delivering poorly soluble drugs. Several major factors which influence the solubilizing property of polymer micelles have been studied [37]. They include the nature of the drug, nature of core-forming polymer block, block structure, and molecular weight. It has been demonstrated in many studies that the compatibility between the solubilizate (drug) and the core-forming polymer block is a critical factor to increase the

loading capacity and loading efficiency. As a result, a polymer micelle system that can solubilize a given drug most effectively may be achieved by designing the hydrophobic polymer block that form micellar core with direct interaction with drug molecules.

Hydrotropic polymers that are synthesized by polymerization of monomers containing hydrotropic groups can be used as a hydrophobic building block for constructing micellar structures. There are a number of identified hydrotropic structures that are effective to enhance the water-solubility of various poorly soluble drugs. For example, nicotinamide derivatives were found to increase the water-solubility of paclitaxel by several orders of magnitude [14]. In addition, hydrotropic property was maintained in their polymeric forms (hydrotropic polymers) [18]. Hydrotropic polymers containing *N,N*-diethylnicotinamide (DENA) group in their repeating units was used as a core-forming polymer block [48]. Amphiphilic block copolymers could be synthesized by atom transfer radical polymerization of vinyl monomers containing DENA group using poly(ethylene glycol) macroinitiator as illustrated in Fig. 4-10. Hydrotropic polymer micelles, consisting of a hydrophilic poly(ethylene glycol) shell and a hydrophobic core that contains a significant amount of hydrotropic moieties, could be obtained via self-assembling property in aqueous media.

It is commonly observed that polymer micelles show low stability in drug-loaded state and the stability becomes even lower as the drug loading content increases. In many cases, the process for incorporation of drugs into micelles requires the use of organic solvents for dissolving either or both of the drug and the polymer because the micelle-forming polymer is not easily dissolved in water. On the other hand, based on synergistic effect of the unique micellar characteristics and hydrotropic activity, the hydrotropic polymer micelles exhibited a high drug loading capacity (up to 37.4 wt% for paclitaxel for example) with enhanced long-term stability. The poor physical stability of paclitaxel-loaded micelles was overcome by introducing hydrotropic moieties into the core structure of polymer micelle. Hydrotropic micelles could maintain their colloidal stability for more than two months without drug precipitation at even higher drug loading contents than in other polymer micelles. The block copolymers are easily water-soluble and drug loading process can be performed by simple mixing in aqueous media without the use of organic solvent. The chemical composition of hydrotrope-rich core may be tailor-made to optimize and maximize the micellar property and hydrotropic activity for a specific drug. Therefore, the hydrotropic polymer micelle presents an alternative and promising approach in formulation of poorly soluble drugs.

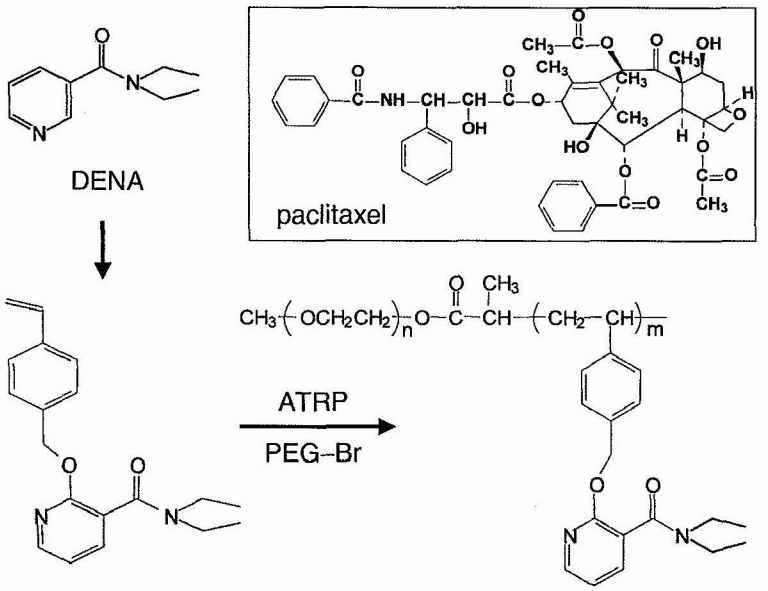

Figure 4-10. Synthetic route for amphiphilic block copolymer of poly(ethylene glycol) and hydrotropic polymer.

5.2 Hydrotropic dendrimer micelles

Instead of amphiphilic block copolymers, hydrotropic dendrimers (PGD) can be used as a building block of micelles. As mentioned in the section 4, dendritic structure of PGDs with 4-5 generations significantly increased aqueous solubility of paclitaxel. By conjugating hydrophobic moieties to PGDs, one can imagine that the hydrophobically modified PGDs form micelles, the shell part of which consists of PGDs (Fig. 4-11). As a hydrophobic moiety, cholesterol was conjugated with PGD (generation 4, G4), and its self-assembled structure was evaluated by dynamic light scattering (DLS) and atomic force microscopy (AFM) [49]. Cholesterol was selected as a hydrophobic moiety of the conjugate because cholesterol conjugated with water-soluble polymers is known to form stable aggregates in aqueous solution [50,51]. A cholesterol-conjugated PGD-G4 (Chol-PGDG4)

was synthesized by conjugating cholesterol chloroformate and PGD-G4 (Fig. 4-12). To prepare self-assembled Chol-PGDG4, distilled water was added to Chol-PGD-G4 at room temperature. Chol-PGD-G4 was immediately dissolved, and the solution was stirred for 10 min. From histogram analysis of the DLS, mean diameter of Chol-PGD-G4 at the concentration of 2.6×10^{-6} M was calculated to be 49.9-59.9 nm. Although small intensity (%) of lager diameter was observed, 81% of the scattering intensity was come from the diameter of 49.9-59.9 nm. On the other hand, the diameter of PGD G-4 itself was observed at 0.99-2.3 nm. These results suggest that the conjugation of one cholesterol group to PGD-G4 induces formation of self-assembly in water. The results of AFM indicate that the mean diameter of Chol-PGD-G4 micelle was around 20 nm.

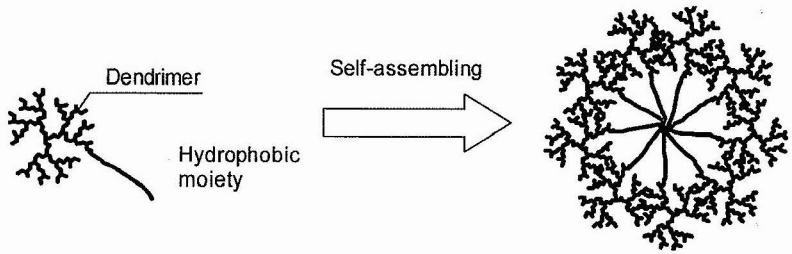

Figure 4-11. Self-assembly of hydrophobically modified PGDs to a micellar structure in aqueous solution.

From the viewpoint of the solubilizing effect of PGD-G4, association of the PGD-G4 molecules may affect the solubilizing ability. After making the Chol-PGD-G4 micelle, the solubilization test was carried out using PGD-G4 as a control. Paclitaxel solubility of Chol-PGD-G4 was almost similar to that of PGD-G4 at the concentration ranging from 0.5 to 10 wt%. This result suggests that the PGD-G4 molecules exist on outer parts of the self-assembly and act as the hydrotrope. The cholesterol group does not seem to participate in the solubilization of paclitaxel, because hydrophobic core of self-assembly can generally solubilize paclitaxel very well [52]. Presumably, once the self-assembled structure was formed, the assembly was stable in aqueous conditions due to strong hydrophobic interaction of cholesterol groups, and therefore, the outer parts consisting PGD-G4 could interact with paclitaxel.

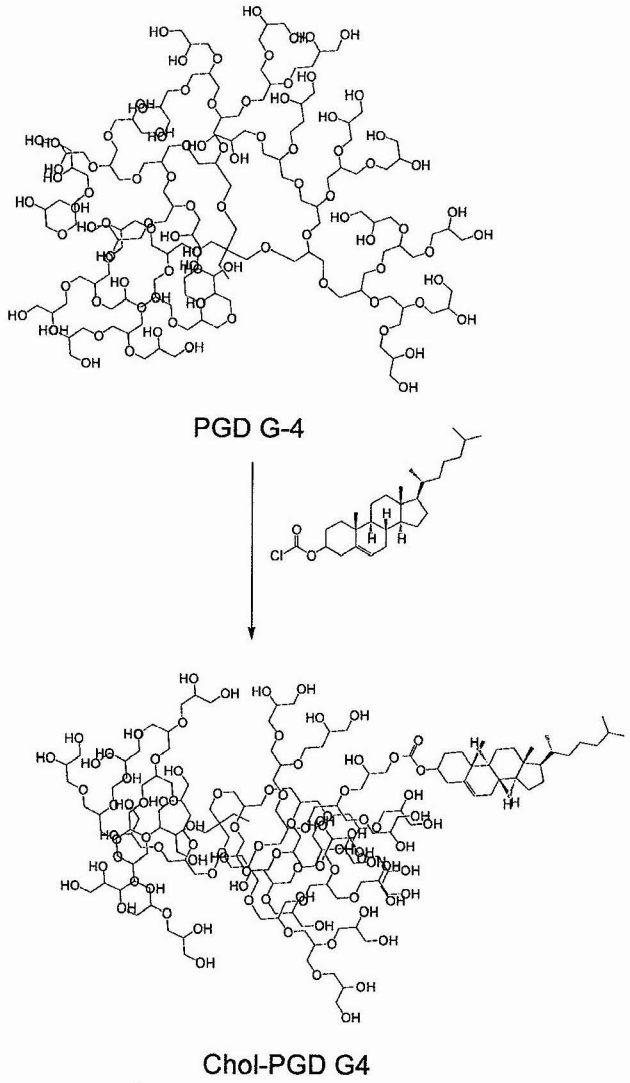

Figure 4-12. Synthetic scheme of Chol-PGDG4. (Modified from reference 49).

6. CONCLUSIONS

Hydrotropic polymers, dendrimers and micelles have been developed as nanocarriers for poorly water soluble drugs. Paclitaxel has been used as a model hydrophobic drug to show the effectiveness of solubility enhancing properties of various hydrotropic nanocarieers. Hydrotropic polymers and dendrimers have advantages for solubilization of paclitaxel in terms of increasing the local concentration of hydrotropes in aqueous media. Those compounds were developed as hydrotropic polymer or dendrimer micelles. The hydrotropic polymer micelles present unique advantages over conventional polymer micelles in that the interaction between the polymer segment and paclitaxel is based on miscibility between the two, instead of the hydrophobic interaction alone. For this reason, the hydrotropic polymer micelles in aqueous solution are more stable than the conventional polymer micelles. The hydrotropic dendrimer micelles were formed by self-assembling cholesterol-dendrimer conjugates. The dendrimer acted as micelle shells that could solubilize paclitaxel as well as dendrimer itself. The new polymer systems based on hydrotropic polymers and dendrimers provide a new approach of designing nanocarriers for poorly soluble drugs.

Acknowledgement

This study was supported in part by National Institute of Health through grant GM65284.

7. REFERENCES

1. Taton, T.A. Bio-nanotechnology: Two-way traffic, *Nature Materials* 2003, *2*, 73-44.
2. Baldwin, B.L. Making a network of hydrophobic clusters, *Science* 2002, *295*, 1657-1658.
3. Scatena, L.F., Brown, M.G., Richmond, G.L. Water at hydrophobic surfaces: weak hydrogen bonding and strong orientation effects, *Science* 2001, *292*, 908-912.
4. Jain, T.K., Morales, M.A., Sahoo, S.K., Lesile-Pelecky, D.L., Labhasetwar, V. Iron oxide nanoparticle for sustained delivery of anticancer drugs, *Mol. Pharm.* 2005, *2*, 185-193.
5. Gao, Z., Lukyanov, A.N., Singhal, A., Torchilin, V.P. Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs, *Nano Lett.* 2004, *4*, 1915-1918.
6. Srinivas, G., Discher, D.E., Klein, M.L. Self-assembly and properties of diblock copolymers by coarse-grain molecular dynamics, *Nature Materials*, 2004, *3*, 638-644.
7. Coffman, R.E., Kildsig, D.O. Hydrotropic solubilization-mechanistic studies, *Pharm. Res.* 1996, *13*, 1460-1463.
8. Kildsig, D.O., Suzuki, H., Sunada, H. Mechanistic studies on hydrotropic solubilization of nifedipine in nicotinamide solution. *Chem. Pharm. Bull* 1998, *46*, 125-130.

9. Silva, R.C.D., Spitzer, M., Silva, L.H.M.D., Loh, W. Investigations on the mechanism of aqueous solubility increase caused by some hydrotropes. *Thermochimica Acta* 1999, *328*, 161-167.
10. Balasubramanian, D., Srinivas, V., Gaikar, V.G., Sharma, M.M. Aggregation behavior of hydrotropic compounds in aqueous solution. *J. Phys. Chem.* 1989, *93*, 3865-3870.
11. Hussain, M.A., Diluccio, R.C., Maurin, M.B. Complexation of moricizine with nicotinamide and evaluation of the complexation constants by various methods. *J. Pharm. Sci.* 1993, *82*, 77-79.
12. Rasool, A.A., Hussain, A.A., Dittert, L.W. Solubility enhancement of some water-insoluble drugs in the presence of nicotinamide and related compounds. *J. Pharm. Sci.* 1991, *80*, 387-393.
13. Fawzi, M.B., Davision, E., Tute, M.S. Rationalization of drug complexation in aqueous solution by use of huckel frontier molecular orbitals. *J. Pharm. Sci.* 1980, *69*, 104-106.
14. Lee, J., Lee, S.C., Acharya, G., Chang, C.J., Park, K. Hydrotropic solubilization of paclitaxel: Analysis of chemical structures for hydrotropic property. *Pharm. Res.* 2003, *20*, 1022-1030.
15. Login, R.B., Merianos, J.J., Dandreaux, G., and Shih, J.S. Polymerizable derivatives of 5-oxo-pyrrolidinecarboxylic acid, U.S. Patent, U.S.A., 4,946,967, 1990.
16. Dandreaux, G., Login, R.B., Merianos, J.J., Garelick, P., Plochocka, K., Negrin, M. and Shih, J.S. Poly(pyrrolidonyl oxazoline), U.S. Patent, U.S.A., 5,008,367, 1991.
17. Lee, S.C, Lee, J., and Park, K. *Unpublished results.*
18. Lee, S.C., Acharya, G., Lee, J. and Park, K. Hydrotropic polymers: Synthesis and characterization of polymers containing picolylnicotinamide moieties, *Macromolecules* 2003, *36*, 2248-2255.
19. Frechet, J.M.J., Tomalia, D.A. *Dendrimers and Other Dendritic Polymers*, John Wiley and Sons, NY, 2001.
20. Kojima, C., Kono, K., Maruyama. K., Takagishi, T. Synthesis of polyamidoamine dendrimers having poly(ethylene glycol) grafts and their ability to encapsulate anticancer drugs, *Bioconjugate Chem.* 2000, *11*, 910-917.
21. Morgan, M.T., Carnahan, M.A., Immoos, C.E., Ribeiro, A.A., Finkelstrin, S., Lee, S.J., Grinstaff, M.W. Dendritic molecular capsules for hydrophobic compounds, *J. Am. Chem. Soc.* 2003, *125*, 15485-15489.
22. Ihre, H.R., Padilla De Jesus, O.L., Szoka, F.C. Jr., Frechet, J.M.J. Polyester dendritic systems for drug delivery applications: Design, synthesis, and characterization. *Bioconjugate Chem.* 2002, *13*, 443-452.
23. Padilla De Jesus, O.L., Ihre, H.R., Gagne, L., Frechet, J.M.J., Szoka, F.C.Jr. *Bioconjugate Chem.* 2002, *13*, 453-461.
24. Haag, R., Sunder, A., Stumbe, J.F. An approach to glycerol dendrimers and pseudo-dendritic polyglycerols. *J. Am. Chem. Soc.* 2000, *122*, 2954-2955.
25. Frey, H., Haag, R. Dendritic polyglycerol: a new versatile biocompatible material. *Rev. Mol. Biotech.* 2002, *90*, 257-267.

26. Leuner, C., Dressman, J. Improving drug solubility for oral delivery using solid dispersions, *European J. Pharm. Biopharm.* 2000, *50*, 47-60.
27. Sugimoto, M., Okagaki, T., Narisawa, S., Koida, Y., Nakajima, K. Improvement of dissolution characteristics and bioavailability of poorly water-soluble drugs by novel cogrinding method using water-soluble polymer. *Int. J. Pharm.* 1998, *160*, 11-19.
28. Basit, A.W., Newton, J.M., Short, M.D., Waddington, W.A., Ell, P.J., Lacey, L.F. The effects of polyethylene glycol 400 on gastrointestinal transit: Implications for the formulation of poorly-water soluble drugs, *Pharm. Res.*, 2001, *18*, 1146-1150.
29. Groves, M.J., Bassett, B., Sheth, V. The solubility of 17 β-oestradiol in aqueous polyethylene glycol 400, *J. Pharm. Pharmacol.*, 1984, *36*, 799-802.
30. Sato, T., Niwa, H., Chiba, A. Dynamical structure of oligo(ethylene glycol)s-water solutions studied by time domain reflectometry, *J. Chem. Phys.*, 1998, *108*, 4138-4147.
31. Ooya, T., Lee, J., Park, K. Hydrotropic dendrimers of generations 4 and 5: Synthesis, characterization, and hydrotropic solubilization of paclitaxel, *Bioconjugate Chem.*, 2004, *15*, 1221-1229.
32. Ooya, T., Lee, J., Park, K. Effects of ethylene glycol-based graft, star-shaped, and dendritic polymers on solubilization and controlled release of paclitaxel. *J. Controlled Release*, 2003, *93*, 121-127.
33. Mall, S., Buckton, G., Rawlins, D.A. Dissolution behaviour of sulphonamides into sodium dodecyl sulfate micelles: A thermodynamic approach. *J. Pharm. Sci.*, 1996. *85*, 75-78.
34. Myrdal, P.B., Yalkowsky, S.H. *Solubilization of drugs in aqueous media*, in *Encyclopedia of Pharmaceutical Technology*. 2002, Marcel Dekker, Inc. pp. 2458-2480.
35. Bader, H., Ringsdorf, H., Schmidt, B., Watersoluble Polymers in Medicine. *Angew. Makromol. Chem.*, 1984. *123*, 457-485.
36. Allen, C., Maysinger, D., Eisenberg, A. Nano-engineering block copolymer aggregates for drug delivery. *Colloids and Surfaces B: Biointerfaces*, 1999. *16*, 3-27.
37. Jones, M.C., Leroux, J.C. Polymeric micelles - a new generation of colloidal drug carriers. *Euro. J. Pharm. Biopharm.*, 1999. *48*, 101-111.
38. Lavasanifar, A., Samuel, J., Kwon, G.S. Poly(ethylene oxide)-block-poly(L-amino acid) micelles for drug delivery. *Adv. Drug. Del. Rev.*, 2002. *54*, 169-190.
39. Kwon, G.S. Polymeric micelles for delivery of poorly water-soluble compounds. *Crit. Rev. Ther. Drug Carr. Syst.*, 2003. *20*, 357-404.
40. Kwon, G.S., Okano, T. Polymeric micelles as new drug carriers. *Adv. Drug. Del. Rev.*, 1996. *21*, 107-116.
41. Soga, O., Van Nostrum, C.F., Fens, M., Rijcken, C.J.F., Schiffelers, R.M., Storm, G. and Hennink, W.E. Thermosensitive and biodegradable polymeric micelles for paclitaxel delivery. *J. Control. Rel.*, 2005. *103*, 341-353.
42. Ooya, T., Lee, J., Park, K., Solubility enhancement of paclitaxel by PEGylated polyglycerol dendrimers. *Controlled Release Society 31st Annual Meeting transactions*, 2004, #684.

43. Nakanishi, T., Fukushima, S., Okamoto, K., Suzuki, M., Matsumura, Y., Yokoyama, M., Okano, T., Sakurai, Y. and Kataoka, K. Development of the polymer micelle carrier system for doxorubicin. *J. Control. Rel.*, 2001, *74*(1-3), 295-302.
44. Yokoyama, M., Okano, T., Sakurai, Y., Suwa, S. and Kataoka, K. Introduction of cisplatin into polymeric micelle. *J. Control. Rel.*, 1996. *39*, 351-356.
45. Lavasanifar, A., Samuel, J., Kwon, G.S. Micelles self-assembled from poly(ethylene oxide)-block-poly(N-hexyl stearate L-aspartamide) by a solvent evaporation method: effect on the solubilization and haemolytic activity of amphotericin B. *J. Control. Rel.*, 2001, *77*, 155-160.
46. Ould-Ouali, L., Noppe, M., Langlois, X., Willems, B., Te Riele, P., Timmerman, P., Brewster, M.E., Arien, A., Preat, V. Self-assembling PEG-p(CL-co-TMC) copolymers for oral delivery of poorly water-soluble drugs: a case study with risperidone. *J. Control. Rel.*, 2005. *102*, 657-668.
47. Aliabadi, H.M., Mahmud, A., Sharifabadi, A.D., Lavasanifar, A. Micelles of methoxy poly(ethylene oxide)-b-poly(epsilon-caprolactone) as vehicles for the solubilization and controlled delivery of cyclosprine A. *J. Control. Rel.*, 2005. *104*, 301-311.
48. Huh, K.M., Lee, S.C., Cho, Y.W., Lee, J., Jeong, J.H., Park, K. Hydrotropic polymer micelle system for delivery of paclitaxel. *J. Control. Rel.*, 2005. *101*, 59-68.
49. Ooya, T., Huh, K.M., Saitoh, M., Tamiya, E., Park, K. Self-assembly of cholesterol-hydrotropic dendrimer conjugates into micelle-like structure: Preparation and hydrotropic solubilization of paclitaxel. *Sci. Tech. Adv. Mater.*, 2005, *6* (5): 452-456.
50. Akiyoshi, K., Deguchi, S., Tajima, H., Nishikawa, T., Sunamoto, J. Microscopic structure and thermoresponsiveness of a hydrogel nanoparticle by self-assembly of a hydrophobized polysaccharide. *Macromolecules* 1997, *30*, 857-861.
51. Yusa, S., Kamachi, M., Morishima, Y. Self-association of cholesterol-end-capped poly(sodium 2-(acrylamido)-2-methylpropanesulfonate) in aqueous solution. *Macromolecules* 2000, *33*, 1224-1231.
52. Liggins, R.T., Burt, H.M. Polyether–polyester diblock copolymers for the preparation of paclitaxel loaded polymeric micelle formulations. *Adv. Drug Delivery Review* 2002, *54*, 191-202.