Hydrotropic Polymers: Synthesis and Characterization of Polymers Containing Picolylnicotinamide Moieties

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ABSTRACT: Our previous studies on low molecular weight hydrotropes showed that nicotinamide derivatives increased the aqueous solubility of paclitaxel by several orders of magnitude. We were interested in knowing whether the polymeric forms of those low molecular weight hydrotropes could maintain hydrotropic properties. *N*-Picolylnicotinamide (PNA) was one of the best hydrotropes identified for paclitaxel, and polymers based on PNA were synthesized and tested for their hydrotropic properties. The pendent hydrotropic PNA moieties were attached to the polymer backbone through either an oligo(ethylene glycol) or a phenyl group as a spacer. The PNA moiety was bound to the polymer backbone either at the 2-position or at the 6-position of the pyridine ring of nicotinamide to result in poly(2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(2-VBOPNA)) or poly(6-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(6-VBOPNA)), respectively. The ability of PNA-containing polymers to increase the aqueous solubility of paclitaxel was examined by measuring the concentration of dissolved paclitaxel in various polymer concentrations. The PNA-containing polymers increased the water solubility of paclitaxel by more than 3 orders of magnitude, and the hydrotropic property of the polymers was pronounced even at low polymer concentrations. P(2-VBOPNA) showed a higher hydrotropic property than P(6-VBOPNA). At the polymer concentration of 40 mg/mL, the water solubility of paclitaxel was enhanced up to 700-fold, depending on the type of polymer used. On the other hand, PNA displayed an efficient solubilizing ability at above 100 mg/mL. Fluorescence study indicated that the hydrotropic polymers formed noncovalent molecular assemblies through the self-association of pendent hydrotropic PNA moieties at much lower concentration range ($(2.1-4.6) \times 10^{-2}$ mg/mL) than PNA (22 mg/mL). This observation supports the high solubilization abilities of hydrotropic polymers for paclitaxel. These results suggest a hydrotropic property of the PNA-based polymers operates under the same mechanism as PNA itself. The cross-linked networks of PNA-based hydrotropic polymers (i.e., hydrotropic hydrogels) were as effective as water-soluble polymers in solubilizing paclitaxel. This study shows that hydrotropic polymers and hydrogels that are prepared based on low molecular weight hydrotropic agent are as effective as the low molecular weight counterpart.

Introduction

Hydrotropes (or hydrotropic agents) are a diverse class of water-soluble compounds that, at high concentrations, enhance water solubilities of poorly soluble solutes.[1−4] Some examples of hydrotropes are nicotinamide and its derivatives, anionic benzoate, benzosulfonate, and neutral phenols such as catechol and pyrogallol, which are characterized by bulky, planar, and compact moieties.[4−6] Hydrotropes have attracted growing interest due to their unique solution properties in aqueous solutions as well as technical applicability in various fields including drug delivery and separation technologies.[7−11] Hydrotropes self-associate and form noncovalent assemblies of nonpolar microdomains to solubilize hydrophobic solutes at the minimal hydrotrope concentration (MHC) and above.[2,4] The self-aggregates of hydrotropes differ from surfactant self-assemblies in that they form planar or open-layer structures instead of forming compact core−shell type aggregates.[3,4]

In the field of drug delivery, poor aqueous solubility of hydrophobic drugs has been one of the important issues in designing clinically useful formulations, since poor aqueous solubility leads to poor bioavailability.[12,13] Hydrotropes have been used to increase aqueous solubilities of poorly soluble drugs. In many cases, the water solubilities of poorly soluble drugs have been increased by 2−4 orders of magnitude simply by mixing with hydrotropes in water.[14−16] Despite this advantage, application of low molecular weight hydrotropes in drug delivery has not been practical, because it may result in absorption of a significant amount of hydrotropes themselves into the body along with the drug. One approach to prevent coabsorption of hydrotropes, e.g., from the gastrointestinal tract after oral administration, is to make polymeric hydrotropic agents (hydrotropic polymers). To date, various polymeric solubilizing systems, such as polymeric micelles and micelle-like aggregates from polymeric amphiphilies, have been used to increase the solubility of poorly water-soluble drugs.[17−26] Hydrotropic polymers are expected to provide an alternative approach for increasing aqueous solubility of poorly soluble drugs.

Low molecular weight hydrotropes often exhibit a higher selectivity in solubilizing guest hydrophobic molecules than surfactant micelles do.[2,4] Thus, identification of structures of hydrotropes for effective solubilization of a specific drug molecule is important.[27] Recently, we have examined a number of candidate hydrotropes for solubilization of paclitaxel.[28] Of the 100 potential candidates examined, structures based on nicotinamide were identified to be good hydrotropes for enhancing the aqueous solubility of paclitaxel. The hydrotropic properties of various nicotinamide derivatives for paclitaxel were largely dependent on the

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substituents on the amide nitrogen of nicotinamide. Of various nicotinamide derivatives, *N*-picolylnicotinamide (PNA) was one of the best hydrotropes, which increased the water solubility of paclitaxel by 3−5 orders of magnitude over its intrinsic solubility in pure water (0.3 μg/mL as measured in our laboratory).[28] Thus, PNA was a good candidate for synthesis of hydrotropic polymers. In this work, we describe the synthesis and characterization of hydrotropic polymers based on PNA. The preliminary work on the hydrotropic property of PNA-based hydrogels is also reported.

Experimental Section

Materials and Equipment. 2-Hydroxynicotinic acid, 6-hydroxynicotinic acid, 1,1′-carbonyldiimidazole (CDI), 3-picolylamine, 4-vinylbenzyl chloride, acrylic acid, 1,3-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), 2-(2-(2-chloroethoxy)ethoxy)ethanol (CEEE), ammonium persulfate (APS), 2,2′-azobis(isobutyronitrile) (AIBN), and ethylene glycol dimethacrylate (EGDMA) were purchased from Aldrich Co. and used without further purification. 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPD) was purchased from Wako Chemicals USA, Inc., and used as received. Paclitaxel was obtained from Samyang Genex Corp. (Taejeon, South Korea). Tetrahydrofuran (THF) was distilled from Na/benzophenone under N_2, prior to use. *N*,*N*-Dimethylformamide (DMF) was vacuum-distilled over anhydrous magnesium sulfate. Acetone, *n*-hexane, diethyl ether, methylene chloride, chloroform, methanol, and ethanol were of the reagent grade. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker ARX300 spectrometer at 300 and 75 MHz, respectively. Elemental analysis was performed on a Perkin-Elmer series II CHNS/O analyzer 2400. UV−vis spectra were obtained using a Beckman DU 640 spectrophotometer. Electrospray ionization mass spectrometry (ESI-MS) assay was carried out on a FinniganMAT LCQ (ThermoFinnigan Corp, San Jose, CA). The electrospray needle voltage was set at 4.5 kV, the heated capillary voltage was set to 10 V, and the capillary temperature was 225 °C. A typical background source pressure was 1.2×10^{-5} Torr as read by an ion gauge. The sample flow rate was approximately 10 μL/min. The drying gas was nitrogen. The LCQ was scanned to 2000 amu for these experiments. Molecular weights and molecular weight distributions were determined using a GPC equipped with an Agilent 1100 series RI detector, quaternary pump, and three PLgel 5 μm Mixed-D columns in set. The eluent was DMF containing Bu_4NBr (0.1% w/v) with a flow rate of 0.6 mL/min. The molecular weights were calibrated with polystyrene standards. Glass transition temperatures were recorded on a TA Instruments DSC 2920 differential scanning calorimeter at a heating rate of 20 °C/min.

2-Hydroxy-*N*-picolylnicotinamide (2-HPNA) (1). 2-HPNA was synthesized following a one-pot two-step synthetic procedure. To a stirred suspension of 2-hydroxynicotinic acid (30 g, 0.216 mol) in THF (700 mL) was added CDI (34.8 g, 0.216 mol) in one portion. The reaction mixture was stirred at reflux under nitrogen. After 24 h, 3-picolylamine (35 g, 0.324 mol) was added dropwise to the stirred suspension of *N*-(2-hydroxynicotinyl)imidazole in THF at reflux. The reaction was maintained for 24 h under nitrogen. After cooling the reaction mixture to room temperature, the pale yellow precipitates was filtered, washed with diethyl ether, and dried in vacuo to yield 2-HPNA. Yield 83%; mp= 198−200 °C; λ_{max}(THF) = 330 nm. ^{1}H NMR (DMSO-d_6): δ 4.54 (d, J = 5.8 Hz, 2H), 6.47 (m, 1H), 7.33 (dd, J = 4.8, 7.7 Hz, 1H), 7.69 (m, 2H), 8.34 (m, 1H), 8.44 (d, J = 4.8, 1H), 8.53 (s, 1H), 10.20 (t, J = 5.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 39.9, 106.3, 120.1, 123.5, 134.9, 135.1, 139.6, 144.1, 148.1, 148.8, 162.2, 163.5. ESI-MS, m/z = 230 ([M + H]$^+$). Anal. Calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 62.84; H, 4.86; N, 18.14.

2-(Hydroxyethoxyethoxyethoxy)-*N*-picolylnicotinamide (HEEEPNA) (2). 2-HPNA (30 g, 0.131 mol) was dissolved in a mixture of ethanol (80 mL) and KOH (18.36 g, 0.327 mol) in distilled water (20 mL). The solution was heated to 80 °C, and CEEE (33.1 g, 0.196 mol) was then added dropwise. The reaction mixture was stirred for 24 h under nitrogen. After cooling to room temperature, the solvent was evaporated, and the crude reaction mixture was extracted with chloroform (3 × 300 mL) against distilled water. The solution was dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure, and the product was isolated by column chromatography on a silica gel using THF/*n*-hexane. Yield 70%; λ_{max}(THF) = 331 nm. ^{1}H NMR (DMSO-d_6): δ 3.44−3.53 (m, 8H), 3.68 (t, J = 5.3 Hz, 2H), 4.17 (t, J = 5.3 Hz, 2H), 4.53 (d, J = 5.8 Hz, 2H), 4.55 (t, J = 5.3 Hz, 1H), 6.49 (m, 1H), 7.34 (dd, J = 5.0, 7.7 Hz, 1H), 7.71 (m, 1H), 7.97 (dd, J = 2.2, 6.1 Hz, 1H), 8.34 (dd, J = 2.2, 7.7 Hz, 1H), 8.44 (dd, J = 1.1, 4.4 Hz, 1H), 8.54 (d, J = 2.2 Hz, 1H), 10.15 (t, J = 5.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 49.1, 60.1, 67.4, 69.5, 69.6, 69.7, 72.3, 105.7, 119.5, 123.5, 134.8, 135.2, 143.3, 144.1, 148.1, 148.9, 161.2, 163.4. ESI-MS, m/z = 362 ([M + H]$^+$). Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.55; H, 6.34; N, 11.65.

2-(2-(Acryloyloxy)ethoxyethoxyethoxy)-*N*-picolylnicotinamide (ACEEEPNA) (3). To a stirred solution of HEEEPNA (17.43 g, 0.0482 mol) and acrylic acid (13.9 g, 0.193 mol) in THF (200 mL) were added DCC (39.80 g, 0.193 mol) and DMAP (2.36 g, 0.019 mol) in THF (300 mL). The reaction mixture was stirred at room temperature for 24 h under nitrogen. After filtration of dicyclohexylurea, ACEEEPNA was isolated by column chromatography on a silica gel using THF/*n*-hexane. Yield 72%; λ_{max}(THF) = 330 nm. ^{1}H NMR (DMSO-d_6): δ 3.49 (m, 4H), 3.56 (t, J = 5.0 Hz, 2H), 3.68 (t, J = 5.5 Hz, 2H), 4.14−4.19 (m, 4H), 4.53 (d, J = 5.8 Hz, 2H), 5.91 (dd, J = 1.7, 9.9 Hz, 1H), 6.15 (dd, J = 9.9, 17.1 Hz, 1H), 6.30 (dd, J = 1.7, 17.1 Hz, 1H), 6.48 (t, J = 6.9 Hz, 1H), 7.33 (dd, J = 5.0, 7.7 Hz, 1H), 7.71 (m, 1H), 7.95 (dd, J = 2.2, 6.6 Hz, 1H), 8.34 (dd, J = 2.2, 7.2 Hz, 1H), 8.44 (dd, J = 1.7, 5.0 Hz, 1H), 8.54 (d, J = 2.2 Hz, 1H), 10.16 (t, J = 5.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 49.1, 63.3, 67.4, 68.2, 69.6, 105.6, 119.5, 123.4, 128.1, 131.6, 134.8, 135.2, 143.3, 144.0, 148.1, 148.9, 161.2, 163.4, 165.4. ESI-MS, m/z = 416 ([M + H]$^+$). Anal. Calcd for $C_{21}H_{25}N_3O_6$: C, 60.71; H, 6.07; N, 10.11. Found: C, 60.36; H, 5.97; N, 10.00.

Poly(2-(2-(acryloyloxy)ethoxyethoxyethoxy)-*N*-picolylnicotinamide) (P(ACEEEPNA)). To a solution of ACEEEPNA (5 g, 0.012 mol) in DMF, AIBN as an initiator (0.061 g, 3 mol % to monomer) was added. The reaction mixture was degassed with a stream of nitrogen for 30 min. The polymerization was carried out at 70 °C for 24 h. After the reaction, the solution was dialyzed against distilled water (pH 2.5) for 30 h using a dialysis membrane (Spectra/Por; MWCO, 1000), followed by drying in vacuo at 60 °C.

2-(4-Vinylbenzyloxy)-*N*-picolylnicotinamide (2-VBOPNA) (4). The suspension of 2-HPNA (9 g, 0.039 mol) and K_2CO_3 (13.57 g, 0.098 mol) in dry acetone was heated to 70 °C. 4-Vinylbenzyl chloride (12 g, 0.079 mol) was then added dropwise to the suspension. The reaction was maintained for 20 h under nitrogen. After the end of this period, the crude reaction mixture was filtered to obtain a thick brown liquid. The product 2-VBOPNA was isolated by column chromatography with THF/*n*-hexane on a silica gel. Yield 75%; mp = 75−77 °C; λ_{max}(THF) = 252 nm. ^{1}H NMR (DMSO-d_6): δ 4.53 (d, J = 6.2 Hz, 2H), 5.21 (dd, J = 1.0, 11.0 Hz, 1H), 5.21 (s, 2H), 5.77 (dd, J = 1.0, 17.7 Hz, 1H), 6.55 (m, 1H), 6.67 (dd, J = 11.0, 17.7 Hz, 1H), 7.27−7.32 (m, 4H), 7.41 (m, 2H), 7.70, (m, 1H), 8.19 (dd, J = 2.4, 6.7 Hz, 1H), 8.38 (m, 1H), 8.44 (dd, J = 1.4, 4.8 Hz, 1H), 8.55 (d, J = 1.4 Hz, 1H), 10.14 (t, J = 6.2, 1H). ^{13}C NMR (DMSO-d_6): δ 40.1, 52.0, 106.5, 114.5, 120.1, 123.4, 126.3, 128.0, 134.8, 135.2, 136.0, 136.1, 136.6, 143.2, 143.5, 148.1, 148.9, 161.2, 163.3. ESI-MS, m/z = 346 ([M + H]$^+$). Anal. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 73.21; H, 5.59; N, 12.09.

Poly(2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(2-VBOPNA)). To a solution of 2-VBOPNA (5 g, 14 mmol) at the concentration of 1.0 M in distilled water (pH 2), APS (67.5 mg, 0.3 mmol), an initiator, was added. The mixture was degassed by nitrogen gas bubbling for 30 min. The reaction mixture was maintained for 24 h at 80 °C under nitrogen. At the end of

this period, the polymer was isolated by the dialysis using a membrane (Spectra/Por, molecular weight cutoff (MWCO), 1000) against distilled water (pH 2.5). The solution of P(2-VBOPNA) was then dried at 60 °C in vacuo.

6-Hydroxy-*N*-picolylnicotinamide (6-HPNA) (5). Synthesis of 6-HPNA was performed in an identical procedure utilized for 2-HPNA, except that 6-hydroxynicotinic acid was used instead of 2-hydroxynicotinic acid. Yield 85%; mp = 182−184 °C; λ_{max}(THF) = 260 nm. ^{1}H NMR (DMSO-d_6): δ 4.42 (d, J = 5.8 Hz, 2H), 6.35 (d, J = 9.6 Hz, 1H), 7.31 (dd, J = 4.8, 7.7 Hz, 1H), 7.68 (m, 1H), 7.88 (dd, J = 2.8, 9.6 Hz, 1H), 8.03 (d, J = 2.8 Hz, 1H), 8.52 (d, J = 1.8 Hz, 1H), 8.85 (t, J = 5.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 40.2, 112.1, 119.1, 123.4, 135.0, 135.1, 137.5, 139.0, 148.1, 148.8, 162.3, 163.7; ESI-MS, m/z = 230 ([M + H]$^+$). Anal. Calcd for $C_{12}H_{11}N_3O_2$: C, 62.87; H, 4.84; N, 18.33. Found: C, 62.49; H, 4.79; N, 18.19.

6-(4-Vinylbenzyloxy)-*N*-picolylnicotinamide (6-VBOPNA) (6). Synthesis of 6-VBOPNA was performed in an identical manner used for 2-VBOPNA, except that 6-HPNA was used instead of 2-HPNA. Yield 70%; mp = 136−138 °C; λ_{max}(THF) = 256 nm. ^{1}H NMR (DMSO-d_6): δ 4.44 (d, J = 5.8 Hz, 2H), 5.13 (s, 2H), 5.22 (dd, J = 1.1, 11.0 Hz, 1H), 5.79 (dd, J = 1.1, 17.7 Hz, 1H), 6.46 (d, J = 9.4 Hz, 1H), 6.69 (dd, J = 11.0, 17.7 Hz, 1H), 7.27 (d, J = 8.4 Hz, 2H), 7.33 (m, 1H), 7.43 (d, J = 8.4, 2H), 7.68 (m, 1H), 7.89 (dd, J = 2.8, 9.4 Hz, 1H), 8.45 (m, 1H), 8.52 (d,, J = 1.6 Hz, 1H), 8.85 (t, J = 5.8 Hz, 1H). ^{13}C NMR (DMSO-d_6): δ 40.3, 51.5, 112.6, 114.5, 118.6, 123.4, 126.3, 128.0, 134.9, 135.2, 136.1, 136.4, 136.5, 137.8, 141.4, 148.1, 148.9, 161.3, 163.5. ESI-MS, m/z = 346 ([M + H]$^+$). Anal. Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 73.04; H, 5.43; N, 12.07.

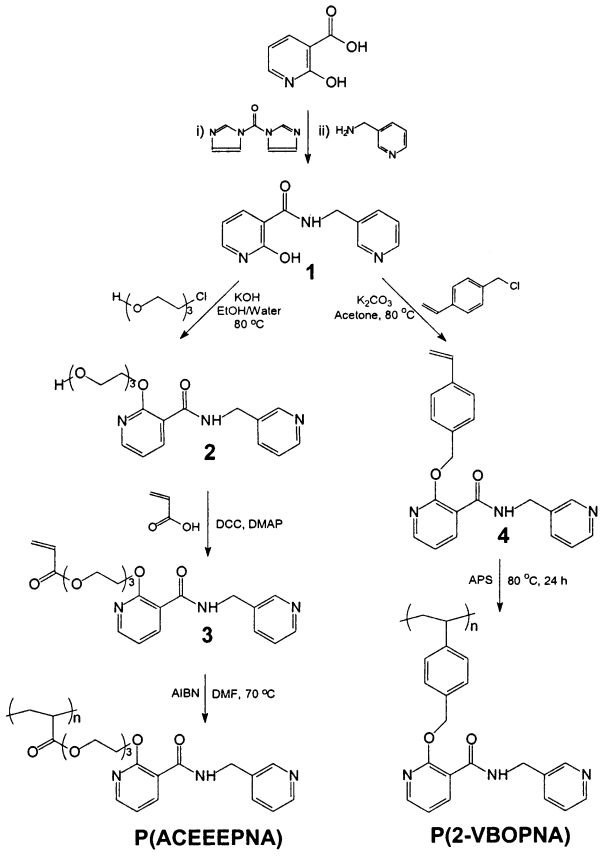
Poly(6-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(6-VBOPNA)). Polymerization of 6-VBOPNA was performed in an identical manner utilized for the synthesis of P(2-VBOPNA), except that 6-VBOPNA was used instead of 2-VBOPNA.

Paclitaxel-Loaded Hydrogels. Paclitaxel (10 mg) was added to 1 mL aqueous solutions (pH 2) of 2-VBOPNA or 6-VBOPNA. The concentration of 2-VBOPNA and 6-VBOPNA was varied from 230 to 470 mg/mL. The mixture was stirred and equilibrated for 24 h at 37 °C. The 24 h equilibrium step can be skipped if excess paclitaxel is present. The paclitaxel/monomer suspension was filtered using a 0.2 μm nylon membrane. To the filtered solution was added ethylene glycol dimethacrylate (EGDMA), a cross-linker, at the concentration of 6 mol % to the monomer. After degassing with dry nitrogen for 30 min, AAPD, a water-soluble initiator (1 mol % to the monomer), was added, and the solution was placed in an oil bath at 60 °C. The polymerization was performed in a sealed tube (i.d. = 10 mm) for 24 h.

Swelling Measurements. Paclitaxel-dissolved P(2-VBOPNA) hydrogels were synthesized at a fixed monomer concentration of 410 mg/mL, and various amounts of EGDMA as a cross-linker (6, 10, and 15 mol % to 2-VBOPNA) were used to control the cross-linking density. The hydrogels were dried at room temperature for 48 h and further dried under vacuum for another 48 h at room temperature. The swelling ratios of disk-shaped gels with 10 mm diameter and 2 mm thickness were measured in distilled water (pH 2) at 37 °C after removing excess water from the gel surface with filter paper. The swelling ratio was defined as a ratio of the weight of a swollen hydrogel (W) and the weight of a dried gel (W_0).

Solubility Studies. Excess paclitaxel was added to screw-capped vials each containing a fixed volume of individual hydrotrope solution. This mixture in the vials was stirred using a magnetic stirring bar at 37 °C. The samples were taken at 24 h, filtered through a 0.2 μm nylon membrane, and analyzed for paclitaxel using HPLC. The concentration of paclitaxel was determined by an isocratic reverse-phase HPLC (Agilent 1100 series, Agilent Technologies, Wilmington, DE) using a Symmetry column (Water Corp., 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 and linked to ChemStation software for data analysis. The paclitaxel concentrations in the samples were obtained using a calibration curve. The HPLC assay for paclitaxel was based on a linear standard curve obtained using the concentration range 0.2−200 μg/mL, and the detection limit was 0.05 μg/mL.

Scheme 1. Synthetic Route for Hydrotropic Polymers



Fluorescence Measurements. Fluorescence spectra of PNA and PNA-containing polymers were recorded on a Spex FluoroMax-2 spectrofluorometer at room temperature. For the emission spectra of PNA and P(6-VBOPNA), excitation and emission slit widths were set at 5.0 nm. The excitation wavelength was 261 nm. For the emission spectra of P(ACEEE-PNA) and P(2-VBOPNA), excitation and emission slit widths were set at 2.0 and 1.0 nm, respectively. The sample solutions were excited at 330 nm. The emission of all the samples was monitored at 380 nm and accumulated with an integration of 3 s/nm. The concentration of PNA aqueous solutions was in the range 0.2−290 mg/mL. For polymers, sample solutions in doubly distilled water were diluted to obtain concentration ranged from 3.5×10^{-4} to 1 mg/mL. The samples were degassed by gentle bubbling of nitrogen for 30 min before measurements.

Results

Synthesis and Characterization of Hydrotropic Polymers. Hydrotropic polymers based on the structure of PNA were synthesized as illustrated in Scheme 1. Synthesis of the PNA-based hydrotropic polymers is divided into two different approaches for incorporation of the PNA moiety to the polymer backbone. The first approach involves synthesis of polymers by incorporating an oligo(ethylene glycol) spacer of three ethylene glycol units between the polymer backbone and the pendent PNA moiety. 2-HPNA (**1**) was synthesized through a one-pot two-step synthetic process, where 2-hydroxynicotinic acid is converted to the corresponding acid imidazolide by reaction with CDI, and the acid imidazolide is reacted further with 3-picolylamine to form 2-HPNA (**1**). Selective reactivity of the CDI chem-

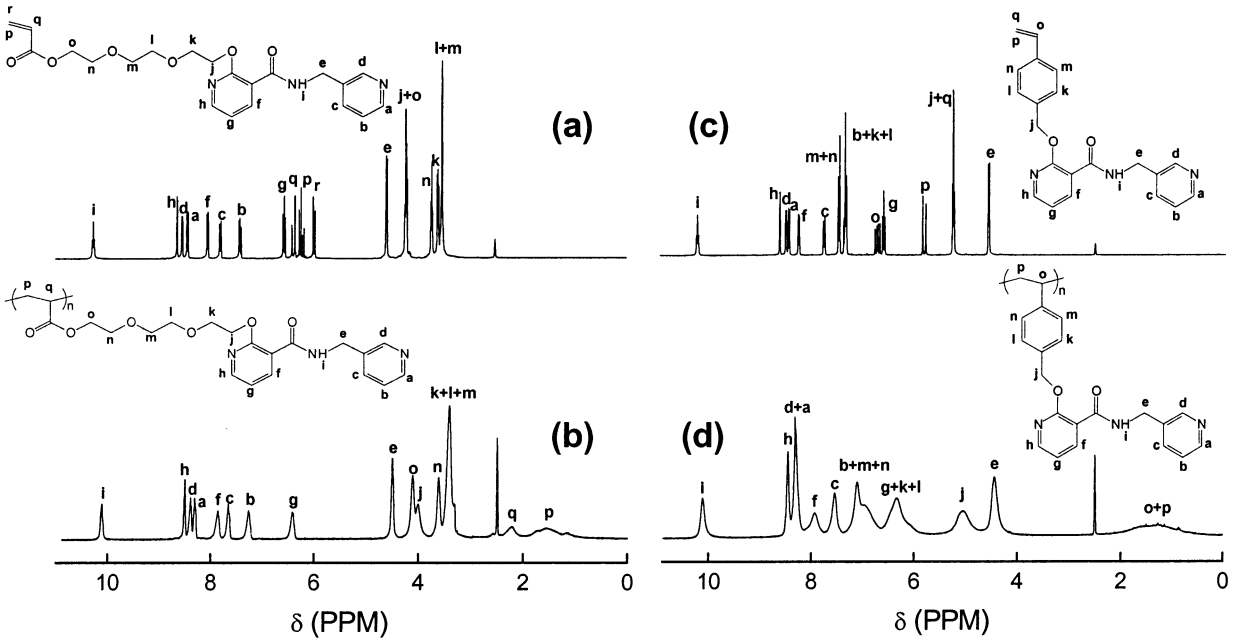


Figure 1. ^{1}H NMR spectra of ACEEEPNA (a), P(ACEEEPNA) (b), 2-VBOPNA (c), and P(2-VBOPNA) (d) in DMSO-d_6.

istry[29,30] allows a one-pot synthesis of 2-HPNA, where the acid imidazolide formed in situ and reacted with 3-picolylamine without purification. P(ACEEEPNA) possessing oligo(ethylene glycol) as a spacer between the polyacrylate backbone and the hydrotropic PNA moiety was synthesized by free-radical polymerization of ACEEEPNA (**3**) using AIBN as an initiator in DMF. ^{1}H NMR spectra show the characteristic resonance peaks of ACEEEPNA (Figure 1a) and P(ACEEEPNA) (Figure 1b). Polymerization of the monomer was confirmed by disappearance of the resonance peaks of vinyl protons of ACEEEPNA at 5.91−6.30 ppm in the ^{1}H NMR spectrum and the vinyl carbon signals at 128.1 and 131.6 ppm in the ^{13}C NMR spectrum (data not shown).

In the second approach of polymer synthesis in Scheme 1, hydrotropic polymers with a phenyl spacer were prepared by attaching the PNA moiety in different orientations to the polymer backbone. These polymers are poly(2-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(2-VBOPNA)) and poly(6-(4-vinylbenzyloxy)-*N*-picolylnicotinamide) (P(6-VBOPNA)), which have hydrotropic PNA moieties bound to the polymer backbone through the 2- and 6-positions, respectively, of the pyridine ring of nicotinamide. For synthesis of P(2-VBOPNA), 2-HPNA (**1**) was reacted with 4-vinylbenzyl chloride to produce 2-VBOPNA (**4**). The same synthetic procedure was employed for P(6-VBOPNA), except that 6-HPNA (**5**) synthesized from the reaction of CDI-activated 6-hydroxynicotinic acid with 3-picolylamine was utilized instead of 2-HPNA (**1**). The characteristic resonance peaks of 2-VBOPNA and P(2-VBOPNA) are shown in parts c and d of Figure 1, respectively. The molecular weights, molecular weight distributions, and glass transition temperatures of polymers are summarized in Table 1. Thermal analyses showed no crystalline melting for all polymers. P(ACEEEPNA) exhibited a glass transition at −7 °C due to the presence of the oligo(ethylene glycol) unit. The glass transition temperatures of P(2-VBOPNA) and P(6-VBOPNA) were 75 and 107 °C, respectively. Orientation of the pendant PNA moiety resulted in significant difference in the glass transition temperature.

Table 1. Properties of Hydrotropic Polymers

polymers	conv (%)	M_n[a]	M_w/M_n[a]	T_g (°C)[b]
P(ACEEEPNA)	78.5	29 000	1.32	−7
P(2-VBOPNA)	86.8	41 000	1.75	75
P(6-VBOPNA)	85.5	54 000	1.74	107

[a] Estimated by GPC. [b] Measured by DSC at a heating rate of 20 °C/min.

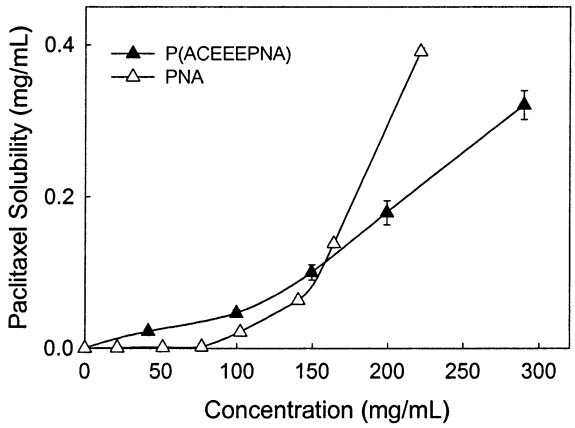


Figure 2. Enhancement in the aqueous solubility of paclitaxel as a function of the concentrations of P(ACEEEPNA) and PNA ($n = 3$).

Solubilization of Paclitaxel by Hydrotropic Polymers. Enhancement of the aqueous paclitaxel solubility as a function of the concentrations of P(ACEEEPNA) and PNA is shown in Figure 2. It is interesting to note that the 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. The enhancements of the water solubility of paclitaxel at 21, 50, and 102 mg/mL of PNA were 1.3-, 10-, and 70-fold, respectively. PNA below 21 mg/mL did not increase the water solubility of paclitaxel to any significant degree. The aqueous solubility of paclitaxel was not noticeable until the PNA concentration reached 100 mg/mL (Figure 2). In contrast to PNA,

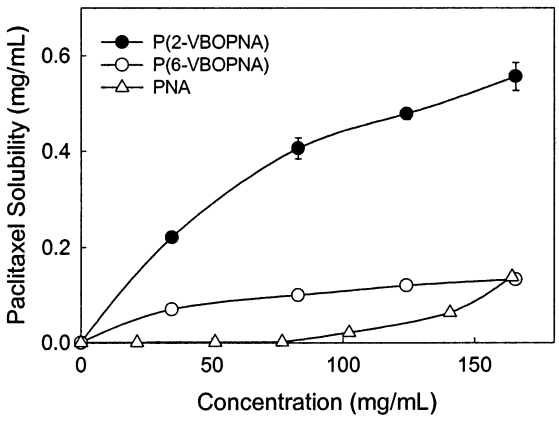


Figure 3. Enhancement in the aqueous solubility of paclitaxel as a function of the concentrations of P(2-VBOPNA), P(6-VBOPNA), and PNA ($n = 3$).

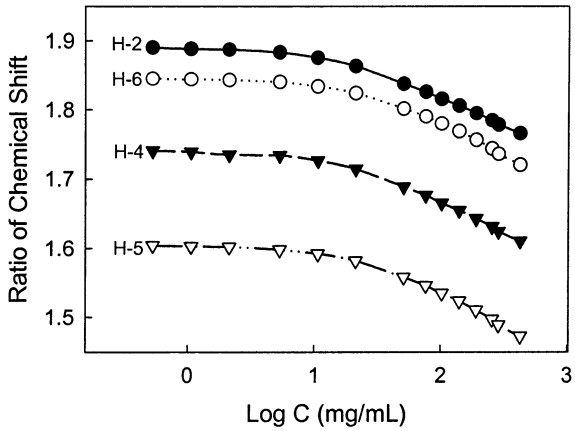


Figure 4. Ratio of chemical shifts of nicotinamide protons to the chemical shift of HDO protons as a function of the PNA concentration. (H-2, H-4, H-5, and H-6 indicate the proton position of the nicotinamide ring of PNA.)

P(ACEEEPNA) solubilized paclitaxel to a significant extent even at the low concentration range, e.g., less than 50 mg/mL. The water solubility of paclitaxel was enhanced approximately 100-fold in the presence of 40 mg/mL P(ACEEEPNA).

Figure 3 shows solubility enhancement of paclitaxel in aqueous solutions of P(2-VBOPNA) and P(6-VBOPNA). Both polymers 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 and 0.13 mg/mL, respectively, at the maximum polymer concentration of 165 mg/mL. The maximum concentration corresponds to the solubility limit of hydrotropic polymers. Compared with P(ACEEEPNA) having a hydrophilic oligo(ethylene glycol) spacer, P(2-VBOPNA) and P(6-VBOPNA) have the lower solubility limit due to hydrophobic phenyl spacers. 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 (Figure 2), while P(2-VBOPNA) and P(6-VBOPNA) showed negative curvatures in the aqueous paclitaxel solubility as a function of the polymer concentration (Figure 3). 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 a better stacking ability to solubilize paclitaxel.

Although PNA was one of the identified structures for effective solubilization of paclitaxel, its self-association behavior in an aqueous phase has not been clearly understood. For this reason, the behavior of hydrotropic polymers in an aqueous solution was investigated in association with the solution behavior of PNA in water. One of the characteristics that many hydrotropes share is self-association to form a loose, noncovalently assembled structure, at above the minimal hydrotrope concentration (MHC). Such assembled structure provides specific domains for solubilization of hydrophobic solutes. Figure 4 shows a concentration dependence of the ratio of chemical shifts of nicotinamide protons to the chemical shift of HDO protons in D_2O. At the lower concentration range (<10 mg/mL) of PNA, changes in the chemical shift ratios of nicotinamide protons are negligible. As the concentration of PNA increases, the ratios of chemical shifts of all protons of the nicotinamide ring exhibit a substantial decrease. Thus, it might be suggested that PNA self-associates via the vertical plane-to-plane stacking interaction of the aromatic rings. The pyridine protons of the picolyl group were also found to participate in association, which was supported by the shielding effect at the identical concentration range (data not shown). The mean value of MHC, which is the threshold concentration of self-aggregate formation, can be determined from the inflection points in Figure 4. The MHC value of PNA in the aqueous media was estimated 22 mg/mL. Interestingly, this MHC value is consistent with the concentration (21 mg/mL) where PNA begins to exhibit the solubilizing ability for paclitaxel in aqueous solutions.

Hydrotropic polymers having pendent PNA moieties are expected to undergo self-association by intra- and intermolecular interaction of the PNA moieties, leading to the formation of an assembled structure in aqueous solutions. NMR spectroscopy was found not to be a useful technique for the behavior of the polymers in aqueous solutions since the chemical shifts of the peaks of polymer-bound PNA protons could not be accurately pinpointed due to the peak broadening. Thus, the behavior of the hydrotropic polymers in water was investigated using fluorescence spectroscopy. The fluorescence intensity has been previously used to estimate the extent of stacking of heterocyclic bases in aqueous solutions. Intramolecular aromatic interaction in stacked conformation leads to the loss in quantum yield or the fluorescence quenching.[31,32] Figure 5 shows changes in fluorescence intensity at 380 nm as a function of the concentration of hydrotropic polymers and PNA. The fluorescence intensity of PNA increased as its concentration increased to about 20 mg/mL. However, the fluorescence of PNA was strongly quenched upon further increase in the concentration. This behavior confirmed self-association of PNA in a stacked conformation, and the critical concentration, where association begins to occur, is in accordance with the concentration of 22 mg/mL observed with NMR measurements. The emission of P(ACEEEPNA) originating from pendent PNA moieties undergoes self-quenching at the inflection concentration of 4.6×10^{-2} mg/mL. This indicates that association of P(ACEEEPNA) by intra- and intermolecular plane-to-plane interaction between PNA moieties occurs at much lower concentration than that of PNA.

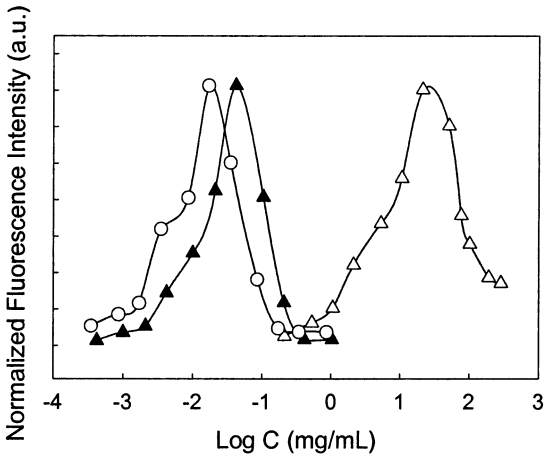


Figure 5. Fluorescence intensities as a function of the concentrations of PNA (△), P(ACEEEPNA) (▲), and P(6-VBOPNA) (○) in aqueous solutions.

For aqueous solutions of P(2-VBOPNA) and P(6-VBOPNA) having a phenyl spacer, similar trends in the changes of emission intensity were observed as the polymer concentration was increased. Interestingly, the hydrotropic polymers P(2-VBOPNA) and P(6-VBOPNA) bearing a phenyl spacer undergo self-association at the concentration range lower than that by the polymer with an oligo(ethylene glycol) spacer. The MHC values of P(2-VBOPNA) and P(6-VBOPNA) were estimated to be 2.5×10^{-2} and 2.1×10^{-2} mg/mL, respectively.

Hydrotropic Hydrogels. Once it was found that polymeric form of PNA maintained the hydrotropic property, the next question was whether the cross-linked hydrotropic polymers would still be hydrotropic. The loading of paclitaxel into the hydrogels was carried out by first solubilizing paclitaxel in aqueous solutions of 2-VBOPNA and 6-VBOPNA, followed by the in situ cross-linking reaction to form hydrogels. The modified hydrotropes, 2-VBOPNA and 6-VBOPNA, have limited water solubility at basic pH. For the hydrogel synthesis in aqueous media, acidic pH (pH 2) was employed to make modified hydrotropes freely water-soluble since nitrogen atoms in pyridine rings were fully protonated at pH 2. In this condition, polymers were also freely water-soluble. The equilibrated mixture of paclitaxel and 2-VBOPNA or 6-VBOPNA was filtered through a 0.2 μm nylon membrane to obtain a transparent solution without any insoluble paclitaxel particles. The transparency (or optical density) of the paclitaxel-dissolved monomer solution was maintained even after hydrogel synthesis. This shows that cross-linking of the polymer chains did not alter the hydrotropic property of the polymers. Consequently, the paclitaxel solubility in hydrogels was determined by the increased water solubility of paclitaxel through the hydrotropic solubilization by 2-VBOPNA or 6-VBOPNA. Since the polymer chains in the cross-linked network are not as free as individual polymer chains in solution, decrease in hydrotropic property was expected. As shown in Figure 6, however, paclitaxel solubility in hydrogels increased as the concentration of 2-VBOPNA or 6-VBOPNA used in hydrogel synthesis increased. At the concentrations used in the experiment, the paclitaxel solubility increased up to 1.62 mg/mL, which is more than 5000 times increase in paclitaxel solubility in pure water. The higher hydrotropic property of P(2-VBOPNA) than P(6-VBOPNA) was also maintained in the hydrogels. Thus,

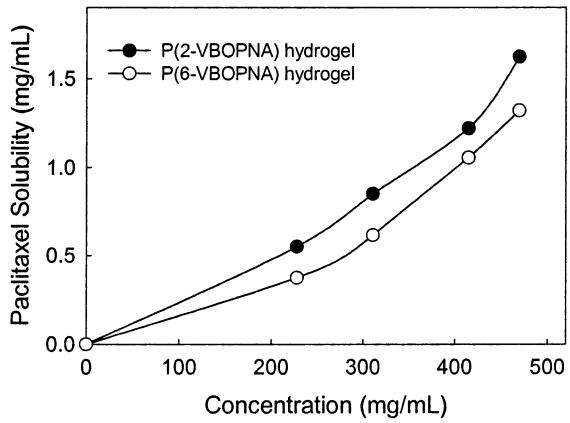


Figure 6. Paclitaxel solubility in PNA hydrogels as a function of the concentrations of 2-VBOPNA and 6-VBOPNA ($n = 3$).

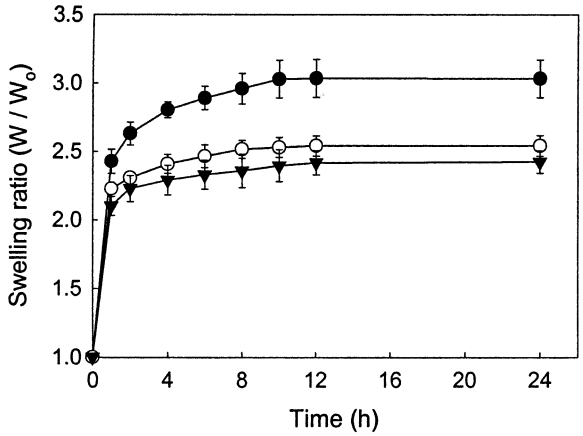


Figure 7. Swelling kinetics of paclitaxel-loaded P(2-VBOPNA) hydrogels cross-linked with EGDMA at the concentration of 6 (●), 10 (○), and 15 mol % (▼) ($n = 3$).

it appears that hydrotropic polymers are still effective in the cross-linked networks.

Since hydrogels absorb water and swell in the presence of abundant water, it was interesting to know whether the swelling of the hydrotropic hydrogels would alter the paclitaxel solubility. The swelling of hydrogels means lowering of the concentration of hydrotropic polymers, which in turn lowers the solubility of paclitaxel. Figure 7 shows the swelling kinetics of paclitaxel-loaded P(2-VBOPNA) hydrogels with different cross-linking density. The paclitaxel solubility in the P(2-VBOPNA) hydrogels before swelling was 1.22 mg/mL. The equilibrium swelling of the hydrogels was reached after about 12 h, and the amount of water uptake decreased as the cross-linking density increased, as expected. At equilibrium swelling, the turbidity increase of hydrogels was observed when the cross-linking density was 6 or 10 mol % to the monomer. This indicates precipitation of paclitaxel upon dilution of the polymer concentration by swelling of the hydrogels. It is interesting to note that the transparency of the hydrogel with the cross-linking density of 15 mol % was preserved even after equilibrium swelling. This result indicates that the water solubility of paclitaxel can be maintained by minimizing the dilution of polymer concentration in the hydrogel through the increase of cross-linking density.

Discussion

Although the use of hydrotropes is one of the easiest ways of increasing water solubility of poorly soluble

drugs, they have not been used widely. The main reason may be the coabsorption of a significant amount of low molecular weight hydrotropes along with the drug. Our approach in this work was to polymerize low molecular weight hydrotropes to prevent their absorption into the body. The question was whether the polymeric forms of hydrotropes could maintain hydrotropic properties of their monomeric counterparts. Another question was concerned with solubilization mechanism by polymeric hydrotropes since, for most hydrotropic systems, the mechanism of solubilization is not clear.

Polymeric hydrotropes possessing PNA moieties maintained the hydrotropic property of PNA itself. Hydrotropic polymers increased the water solubility of paclitaxel by more than 3 orders of magnitude at the concentration range tested. The solubility increase by P(ACEEEPNA) exhibits a positive deviation from linearity, which is one of the characteristics of hydrotropic solubilization by low molecular weight hydrotropes, as in the case with PNA.[33] The positive deviation of the solubility increase from the linearity implies the formation of higher order complexes as a result of the specific phenomenon, such as the stepwise self-association of hydrotropes.[34] Thus, 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 the 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. Interestingly, P(2-VBOPNA) possessing PNA moieties at the 2-position of the pyridine ring of nicotinamide showed much more pronounced hydrotropic property than P(6-VBOPNA) with PNA bound at the 6-position. It has been reported that the structural and geometric features of hydrotropes modulate their self-assembly and hydrotropy.[6] For example, the hydrotropic properties of *o*-, *m*-, and *p*-hydroxybenzoate, as well as 2,4-, 2,5-, and 2,6-dihydroxybenzoates, are markedly different.[35] Even this minor structural difference affects the operation of hydrotropy. Thus, the binding orientation of the hydrotropic moiety to polymer backbone is expected to be one of the significant factors in controlling the hydrotropic property of the hydrotropic polymers.

Like many other hydrotropes, nicotinamide was found to self-associate in aqueous solution, primarily as dimers and trimers.[36,37] Using NMR spectroscopy, Rasool et al. reported that nicotinamide self-associated via vertical stacking of aromatic rings, which was supported by shielding of protons of nicotinamide upon increase in the concentration.[27] In addition, Chan et al. suggested that the NMR spectroscopy was a useful tool in supporting a model of the vertical plane-to-plane stacking of heterocyclic rings of purine and 6-methylpurine in an aqueous phase.[38] Fluorescence quenching experiment suggested that the hydrotropic polymers formed noncovalent stack-type assemblies through the self-association of pendent hydrotropic PNA moieties at much lower concentration range ($(2.1-4.6) \times 10^{-2}$ mg/mL) than free PNA (22 mg/mL). In general, the low molecular weight hydrotropes display efficient solubilizing ability beyond relatively high MHC values that ranged from 20 to 200 mg/mL or higher, depending on their classes.[2,4] 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. Self-association behavior of the PNA-based hydrotropic polymers, as shown by the fluorescence measurements, strongly supports the high solubilizing abilities of the polymeric hydrotropes for paclitaxel at low concentration range as compared with that of PNA. On the basis of 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 assemblies of hydrotropic polymers are reminiscent of polymeric micelles or micelle-like aggregates from amphiphilic polymers, which have been widely exploited to enhance the water solubility of poorly soluble drugs.[17−26] There are, however, important differences. The most notable difference between polymeric hydrotropes and other polymeric systems, e.g., polymeric micelles, is that hydrotropic polymers form assemblies through plane-to-plane stacking interaction as compared with formation of hydrophobic cores by polymeric micelles.[39−41] In addition, the molecular structure of hydrotropic polymers can be tailor-made for specific drugs based on the identified low molecular weight hydrotropes.

We noted that hydrotropic polymers bearing a phenyl spacer underwent self-association at the lower concentration range than a polymer with an oligo(ethylene glycol) spacer. In a recent report on the oligosaccharide-carrying polystyrene derivatives by Kobayashi et al., the hydrophobic interaction of the phenyl groups between the polymer main chain and pendent oligosaccharide units was found to facilitate the gathering of the attached hydrophilic oligosaccharide moieties.[42] Thus, it is likely that the interaction between PNA moieties in hydrotropic polymers having a phenyl spacer is more favored by intra- and intermolecular hydrophobic interactions between the phenyl groups adjacent to PNA moieties compared with the hydrotropic polymer with a hydrophilic and flexible oligo(ethylene glycol) spacer. This finding indicates that the property of the spacer group between the polymer backbone and the hydrotrope moiety can be adjusted to control the minimum association concentration of hydrotropic polymers.

Hydrotropic hydrogels present advantages over water-soluble hydrotropic polymers. One of them is that the hydrogels, due to their cross-linked nature, maintain the local polymer concentration even in the presence of abundant water. Hydrotropic hydrogels may swell but will not dissolve and dilute the local polymer concentration as much as non-cross-linked polymers do. For example, if dried hydrotropic polymer powder is introduced into water, the polymer molecules will dissolve away from the matrix, leading to lowering of hydrotropic property. On the other hand, hydrotropic hydrogels are expected to maintain most of their hydrotropic properties. For this reason, it was important for us to confirm that cross-linked polymeric hydrotropes were also hydrotropic. In our approach, paclitaxel was first dissolved in the monomer solution before cross-linking polymerization. If the stack-type assemblies of PNA moieties were disrupted by cross-linking process during the hydrogel synthesis, paclitaxel would precipitate out from water due to the loss of its solubilization source. The

formed hydrogels maintained their transparency, indicating that paclitaxel remained dissolved in the hydrogels. This observation suggests that the layered structure of PNA moieties was maintained during cross-linking polymerization.

In this study, paclitaxel was added to the monomer solutions before polymerization, but the hydrotropic hydrogels can be made first, purified, and subsequently loaded with poorly soluble drugs. One advantage of using hydrotropic hydrogels is that no organic solvents are necessary for loading of poorly soluble drugs. The unique property of the hydrotropic hydrogels could find novel applications in many areas. The details on the properties of the hydrotropic hydrogels, such as the swelling kinetics and the drug releasing behavior, are currently under investigation.

Conclusions

Polymeric hydrotropes were synthesized based on *N*-picolylnicotinamide (PNA), which was found to be an effective hydrotrope for solubilization of paclitaxel. Hydrotropic polymers self-associated to form noncovalent assemblies at the much lower concentration range ($(2.1-4.6) \times 10^{-2}$ mg/mL) than PNA (22 mg/mL) through the intra- and intermolecular plane-to-plane stacking between the pendent PNA moieties. The PNA-based hydrotropic polymers were effective in increasing the water solubility of paclitaxel by more than 3 orders of magnitude. The data in this study showed that hydrotropic polymers could be synthesized on the basis of the identified structures of low molecular weight hydrotropes. The observation that the cross-linked network of hydrotropic polymers maintains the hydrotropic property allows design of hydrotropic macromolecules in various sizes and shapes.

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