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Hydrotropic polymer micelles as versatile vehicles for delivery of poorly water-soluble drugs

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ABSTRACT

Polymer micelles have been used widely for delivery of poorly water-soluble drugs. Such drug delivery, however, has been based primarily on hydrophobic interactions. For better drug loading and improved stability, hydrotropic polymer micelles were used. To develop a versatile polymer micelle for solubilizing various poorly soluble drugs, two different hydrotropic agents were examined. The solubilizing properties of two hydrotropic agents, *N,N*-diethylnicotinamide (DENA) and *N,N*-dimethylbenzamide (DMBA), in polymeric form were investigated for their ability to solubilize five drugs with low aqueous solubility covering a wide range of hydrophobicity and molecular structures. The hydrotropes were covalently linked to the hydrophobic block of a block copolymer that also had a hydrophilic poly(ethylene glycol) (PEG) block.
The solubilizing capacity of the polymeric hydrotropes was compared with that of the non polymeric hydrotropes, as well as of two conventional (non hydrotropic) copolymer systems. The solubilizing capacity of polymeric hydrotropes reflects combined effects of the micellar solubilization by the hydrophobic micelle core and hydrotropic solubilization. Because of the highly localized configuration, hydrotropes in the polymeric form are more powerful solubilizers than in the monomeric (non-polymeric) solution. It is possible to produce 1~3 orders of magnitude increase in solubility with polymeric hydrotropes at the 1% (w/v) level. Of the two hydrotropic polymeric systems in this study, the DENA-based system is highly specific, whereas the DMBA-based system is a general solubilizer of hydrophobic drugs. An additional advantage of polymeric hydrotropes over the non-polymeric form is absence of high concentrations of free hydrotropes in the formulation. Solubilization vehicles based on polymeric hydrotropes are expected to provide a new and versatile means of preparing formulations for various poorly soluble drugs and drug candidates without using organic solvents. This advantage is accompanied with the inherent controlled release property of the hydrotropic polymer micelles, making them ideal for pharmaceutical formulations used in drug candidate screening and toxicology studies.

1. Introduction

Polymer micelles have been used widely as nanosized vehicles for solubilizing various poorly soluble drugs, e.g., a large portion of anticancer drugs, and also for delivery of the drug to the target site [1–5]. To this end, a wide variety of polymer micelles have been developed and some of them are currently under clinical studies [6,7]. Despite extensive studies on polymer micelles, the drug loading, i.e., solubilization of poorly soluble drugs, is mainly based on hydrophobic interactions between the drug and the micelle core. Thus, not all poorly soluble drugs solubilize in a given polymer micelle to the same extent. The ability of polymer micelles to solubilize various poorly soluble drugs has not been studied in detail. For effective use of polymer micelles as targeted drug delivery systems, better understanding on drug solubilization in polymer micelles is required.

Many drugs and drug candidates are poorly soluble, and it is one of the main hurdles for developing clinically useful pharmaceutical formulations [8–10]. This problem is particularly severe at the drug discovery stage, where large numbers of compounds need to be formulated and tested for potency and specificity [11]. Drug discovery screening requires solubilizing vehicles that work for a large number of organic compounds of diverse chemical structures. The common approaches of dissolving poorly soluble drugs include use of organic solvents, such as dimethylsulfoxide (DMSO) which is rather toxic [11], and surfactants. Surfactants enhance solubility of hydrophobic solutes via micelle formation at the concentrations above the critical micelle concentration (CMC). Surfactant-based formulations also present some toxicity issues, e.g., Cremophor EL which can lead to hypersensitivity reactions in human and animals [12] and polysorbate 80 which has been associated with potential hepatitis and renal failure

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[13]. Surfactants and organic cosolvents are often used in combination. There is an urgent need for finding powerful solubilizing systems that are also suitable for a wide range of poorly soluble drugs. In addition to enabling drug discovery studies, a desirable solubilizing system is one that is also acceptable for preclinical and toxicology studies, and of course for ultimate clinical applications. In this study, hydrotropic polymer micelles were examined as solubilizing agents for various poorly soluble drugs.

Hydrotropes dissolved in water can produce high degree solubility enhancement for drugs [14]. However, there are two important obstacles to developing a hydrotropic solubilizing system suitable for clinical or even toxicology formulations. One hurdle pertains to the ability of any given hydrotropic agent to effectively solubilize a broad range of hydrophobic drug molecules. The second obstacle is the toxicology concerns of cosolvents and surfactants shared by the use of freely dissolved hydrotropes. Hydrotropes are organic compounds dissolved in the vehicle at sizable concentrations. Freely dissolved hydrotropes in a formulation are subject to systemic absorption in the body following administration. However, hydrotropes offer an advantage in this regard in relation to cosolvents and surfactants in that it is possible to covalently link hydrotropes to polymeric chains in such a way that their solubilizing power can be exploited without the issues of systemic absorption [15–17]. Two hydrotropic agents, *N,N*-diethylnicotinamide (DENA) and *N,N*-dimethylbenzamide (DMBA), are effective solubilizers of a wide range of poorly soluble drugs. The pyridinyl ring or phenyl ring in each agent results in different and potentially complementary solubilizing abilities between the two. Their potential systemic absorption from the formulation is an undesirable situation. Such systemic absorption can be precluded by covalently linking the hydrotropes to a polymeric matrix.

Polymeric micelles by amphiphilic diblock copolymers are generally more stable and safer than conventional surfactant micelles [18]. Polymer micelles have been studied as a drug carrier of a hydrophobic drug by hydrophobic interaction with the drug [18–23]. Polymeric forms of hydrotropes are amphiphilic block copolymers containing hydrotropes in their hydrophobic core [15,24,25]. This study investigated the properties of PEG-*b*-PDENA and PEG-*b*-PDENA for their ability to solubilize different drugs. As free hydrotropes, DENA showed considerably greater solubilizing capacity for paclitaxel, whereas DMBA showed better ability to solubilize a wide diversity of chemical structures [14]. Five model drugs used in this study are probucol, paclitaxel, progesterone, nifedipine and griseofulvin. The solubilization capacity of hydrotropic polymeric micelles was compared with that of conventional polymeric micelle, such as a block copolymer of PEG and poly(lactic acid) (PEG-*b*-PLA), as well as of that of the amphiphilic random copolymer, poly[(2-methacryloylethyl phosphocholine)-*co*-(*n*-butyl methacrylate), designated as PMB30W.

2. Materials and methods

2.1. Materials

Griseofulvin, nifedipine, progesterone and probucol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Paclitaxel was obtained from Samyang Genex (Daejeon, South Korea). PEG-*b*-PDENA (with a block ratio of 5K5K) was synthesized by atom transfer radical polymerization (ATRP) from a macro-initiator, brominated PEG (PEG-Br, MW 5000), as reported previously [15,24]. The block ratio of 5K5K refers to a molecular weight of 5000 for the hydrophilic PEG and 5000 for the hydrophobic portion of the copolymer, respectively. PMB30W (MW 50,000) was synthesized using the monomer provided by Professor Kazuhiko Ishihara following the method described in his publications [26,27]. PEG-*b*-PLA (a block ratio of 2K2K) was synthesized by ring opening polymerization of D,L-lactide with monomethoxy PEG (MW 2000) as a macroinitiator and stannous octoate as a catalyst [28].

2.2. Synthesis of PEG-b-PDMBA

PEG-Br, a macro-initiator, was synthesized using a previously reported method [15,24] to synthesize PEG-*b*-PDMBA (block ratios of 5K2K, 5K3.5K, and 5K5K). Monomeric 2-(4-vinylbenzyloxyl)-*N,N*-dimethylbenzamide (VBODMBA), was synthesized by reacting 2-hydroxy-*N,N*-dimethylbenzamide (HDMBA) with 4-vinylbenzyl chloride in acetone at 70 °C and was isolated using column chromatography with n-hexane/THF on a silica gel. HDMBA was in turn obtained by reacting 2-hydroxybenzoic acid (salicylic acid) and 1,1′-carbonyldiimidazole (CDI) in THF and adding dimethylamine to the suspension of *N*-(2-hydroxyphenyl) imidazole in THF. PEG-*b*-PDMBAs (block ratios of 5K2K, 5 K3.5 K, and 5K5K) were synthesized by ATRP method of VBODMBA monomers using PEG-Br (MW 5000) as an initiator. Cupper (I) bromide was used as a metal catalyst and *N,N,N′,N′,N″*-pentamethydiethyl enetriamine (PDMETA) as a ligand. By adding pre-determined amounts of VBODMBA, the hydrophobic block lengths were controlled. The hydrotropic amphiphilic block copolymers, were purified several times using proper solvents. Molecular weights were analyzed by ^{1}H NMR.

2.3. Solubility measurements

Excess drug solute was added to screw-capped vials containing a fixed level of 1% (w/v) solubilizing polymer solutions in the absence of any organic solvent. Usually 1 mg of a drug was added to the solution and allowed the drug to dissolve for several hours. If the solution became clear, then additional solute was added. To reach equilibrium the solution was incubated at 37 °C for 3 days using a thermostatic shaker (Max Q 4000, Barnstead/Labline, Melrose Park, IL). Aliquots from the samples were filtered through a 0.2 μm-pored nylon syringe filter (Alltech, Deerfield, IL) that was pre-warmed at 37 °C. The filtered aliquot was diluted with acetonitrile and assayed by reverse phase HPLC analysis.

2.4. HPLC analysis

A binary gradient mobile phase was employed for the analysis. The column used was a C_{18} RP analytical column (Waters, Milford, MA, USA) using an Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with photodiode detector. Before analysis, all samples were filtrated through a 0.45 μm-pored syringe filter. The injection volume was 20 μL and the flow rate of mobile phase was 1.0 mL/min. Drug concentration was calculated from a standard curve for each drug. Solubility values reported are the average of three measurements.

2.5. Data analysis

Principal component analysis (PCA) was performed using the program SIMCA-P+ (version 10.0.4, Umetrics AB, Umea, Sweden).

2.6. Fluorescence measurements

Blank polymer micelles were prepared by dialysis for 24 h against deionized water. The dialysate of each polymer was then filtered through a 0.2 μm-pored nylon syringe filter (Alltech, Deerfield, IL), lyophilized and dried under vacuum. To determine the critical micelle concentration (CMC) of the hydrotropic polymers, pyrene was used as probe. A volume of pyrene dissolved in acetone, equivalent to 2.4 μmol of pyrene was spiked into vials. After evaporating acetone overnight under the hood, 4 mL aliquots of aqueous polymer solution of various concentrations (ranging from 2.5×10^{-4} to 1 mg/mL) were added to the vials (to make the final pyrene concentration of 6×10^{-7} M). All samples were incubated at 60 °C for an hour and further shaken at 37 °C for a day. The fluorescence spectra were

obtained using a LS-50 Fluorescence Spectrometer (PerkinElmer, USA) at room temperature. The pyrene excitation spectra were monitored in the wavelength range of 300 ~ 360 nm and at 390 nm for emission using a 5 nm slit width. The fluorescence spectra were conducted following a procedure described elsewhere [19,24,29]. From the pyrene emission spectra (excitation at 339 nm), the intensity ratio (I_{341}/I_{334}) or the intensity ratios (I_{339}/I_{334}) were plotted as a function of their hydrotropic polymer concentrations. A red shift of the fluorescence excitation spectra (for example, from 334 nm to 341 nm or to 339 nm) with the increased concentration of the polymer indicates the transfer of pyrene molecules from water environment to the hydrophobic micellar core. The point of a sharp change in the intensity ratio corresponds to the CMC of the micelle forming agent.

3. Results and discussion

3.1. Polymeric hydrotropes

Fig. 1 shows the chemical structures of the polymeric hydrotropes. The structures of the other two conventional polymer micelle formers, PEG-*b*-PLA and PMB30W, included for comparison in this study, are also shown. The molecular weights of PEG-*b*-PDMBA with different hydrophobic block lengths are listed in Table 1. In the ^{1}H-NMR analyses, the ratio of ethylene proton of PEG (O—CH_2CH_2—) and of the poly(2-(4-vinylbenzyloxy)-*N*,*N*-dimethylbenzamide) (PDMBA) backbone (—CH_2CH_2—) were used to assess the corresponding molecular weights. The same chain length of 5K was used for the hydrophilic PEG block in the two types of polymeric hydrotrope used in this study. However, the different sizes of the hydrophobic blocks PDENA and PDMBA were used. Block ratios of 5K5K and 5K2K were used for PEG-*b*-PDENA and PEG-*b*-PDMBA, respectively. DMBA is more hydrophobic than DENA [14] so that a block ratio of 5K5K results in freely water soluble PEG-*b*-PDENA but insoluble PEG-*b*-PDMBA.

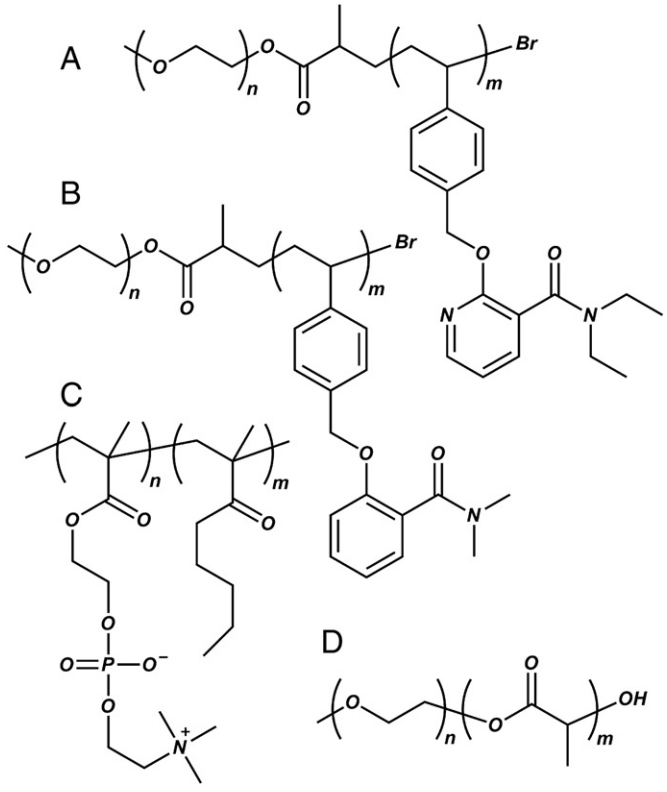

Fig. 1. Chemical structures of the block copolymers used as solubilizing agents. (a) PEG-*b*-PDENA, (b) PEG-*b*-PDMBA, (c) PMB30W, and (d) PEG-*b*-PLA.

Table 1
Characterization data of PEG-*b*-PDMBA, PEG-*b*-PDENA and PEG-*b*-PLA.

Polymer	Mn (g/mol)[a]	Mn (g/mol)[b]	CMC[c] (μg/mL)
PEG-*b*-PDMBA (5K2K)	7000	7400	3.6
PEG-*b*-PDMBA (5K3.5K)	8500	9000	2.5
PEG-*b*-PDMBA (5K5K)	10,000	10,400	2.5
PEG-*b*-PDENA (5K5K)	10,000	10,000	36
PEG-*b*-PLA (2K2K)	4000	4400 [15]	3.8 [15]

[a] Molecular weight added to synthesize this polymer.
[b] Calculated from peak integration of ^{1}H NMR spectra.
[c] Critical micelle concentration determined by fluorescence measurements.

Even at a block ratio of 5K3.5K, PEG-*b*-PDMBA is barely soluble in water. A block ratio of 5K2K is necessary for obtaining freely water soluble PEG-*b*-PDMBA and that is the block ratio used in this study.

3.2. Solubilization of hydrophobic drugs by hydrotropic polymers vs. hydrotropes

Five drugs (probucol, paclitaxel, progesterone, nifedipine and griseofulvin) were used as test solutes for investigating the solubilization properties of the polymeric hydrotropes. These drugs are poorly soluble in water (<14 μg/mL) and cover a broad range of hydrophobicity. The calculated octanol–water partition coefficient (log P) ranges from 2 to 10. In addition, data on the solubilization of these drugs by the free (non polymeric) DENA and DMBA are available [14]. The chemical structures of the solutes used in this study are shown in Fig. 2.

The log P value is commonly employed to assess the hydrophobicity of organic compounds. In this study, solubility-derived data were used as the measure of hydrophobicity of the solute. Specifically, the activity coefficient (γ_w) of the drug in a saturated solution in water was used. The values of log P and log γ_w for hydrophobic organic compounds are highly correlated [30]. Since log P values can be readily calculated from the molecular structure, they are very useful for making estimates when the solubility of the drug is unknown. The magnitude of log γ_w, on the other hand, is the precise measure by which the hydrophobicity of the drug limits its solubility in water. However, the solubility of the drug in water must be known to calculate the value of log γ_w. Here we use the solubility of the solute in water as reference for the solubilization by polymeric hydrotropes and use log γ_w as the measure of hydrophobicity of the drugs. The relationship between the aqueous solubility and the activity coefficient is given by:

$$\log X_w = -\frac{\Delta S_m(T_m - T)}{2.203RT} - \log \gamma_w \quad (1)$$

where X_w is the (mole fraction) aqueous solubility, ΔS_m is the entropy of melting of the crystalline solute, T_m and T are the absolute melting and experimental temperatures, respectively, R is the gas constant and γ_w is the activity coefficient of the solute in water [14,31]. The log γ_w values for the model drugs obtained according to Eq. (1) are listed in Table 2. Based on the aqueous activity coefficient, the order of hydrophobicity of the drugs is probucol > paclitaxel > progesterone > nifedipine > griseofulvin.

One of the objectives of this study is to investigate the solubilizing properties of the polymeric hydrotropes for poorly soluble drugs in general, and to compare the performance of the polymeric hydrotropes with those expected from the use of cosolvents and surfactants. To this effect, a concentration of 1% (w/v) of the polymeric hydrotropes was chosen to assess their solubilizing power. Aqueous vehicles are highly desirable when solubilizing drugs for screening of *in vivo* activity or toxicology studies of drug candidates. The concentration used in this study results in a solubilizing vehicle that is 99% aqueous. In addition,

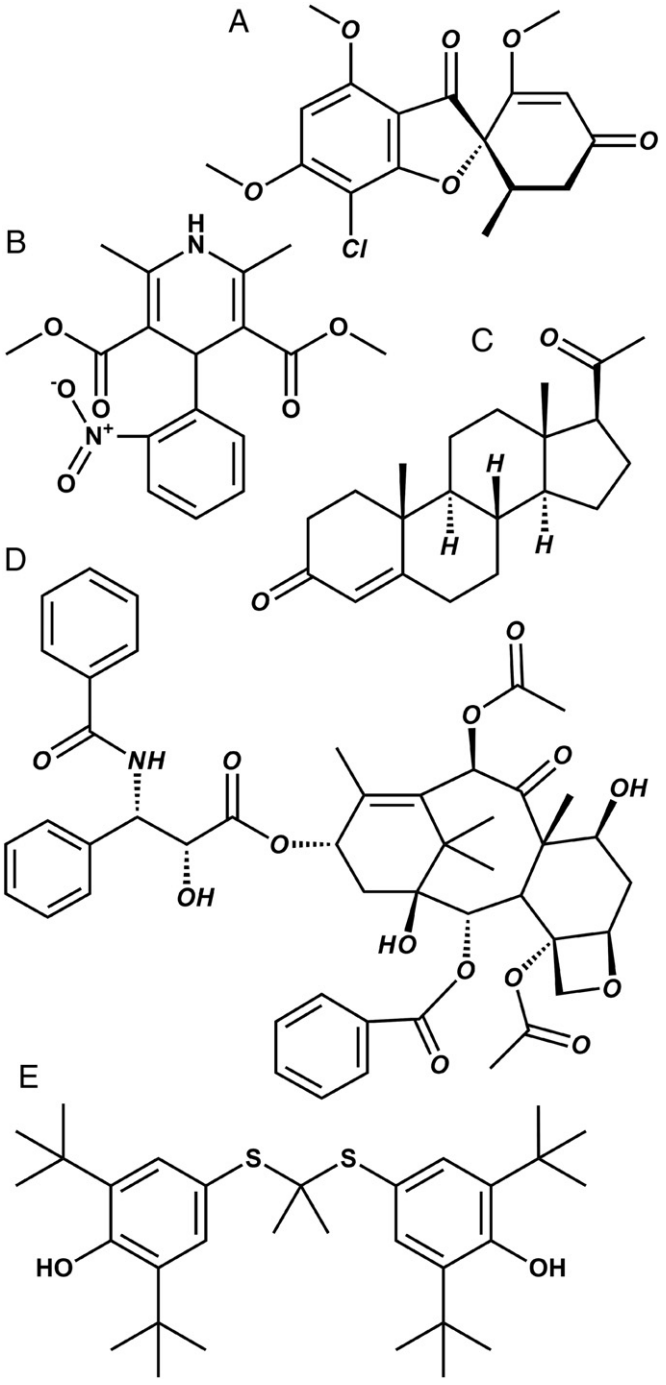

Fig. 2. Chemical structures of the model hydrophobic drugs used in the study: (a) griseofulvin; (b) nifedipine; (c) progesterone; (d) paclitaxel; and (e) probucol.

the 1% (w/v) concentration represents an important level for purposes of comparison with cosolvents and with surfactants. In the case of organic cosolvents, concentrations of 1% (w/v or v/v) become impractically low. At 1% concentration, an organic cosolvent mixed with water (even a strong solvent like DMSO) is bound to produce a minimal, if not negligible, solubility enhancement. Organic cosolvents need to be present at significantly higher concentrations to produce significant solubilization effects [44]. When using surfactants on the other hand, the concentrations used are lower than those of cosolvents. Concentrations of 1% or lower are common when using surfactants as solubilizing agents.

The solubility enhancement achieved by 1% (w/v) PEG-*b*-PDENA is shown in Fig. 3A for the five model dugs, as a function of the

Table 2
Solubility and relevant parameters of the model solutes.

	Solubility (μg/mL)	Number of aromatic rings	$\frac{\Delta S_f(T_m-T)}{2.303RT}$	log γ_w	log P
Griseofulvin	14 [32]	1	3.60 [33]	2.54	2.0 [34]
Nifedipine	5 [35]	2	2.31 [36]	4.28	2.5 [37]
Progesterone	7 [38]	0	1.21 [39]	5.18	3.87 [40]
Paclitaxel	0.3 [41]	3	2.05 [24]	6.15	3.98 [42]
Probucol	0.006 [33]	2	1.60 [33]	8.08	10 [43]

hydrophobicity of the solute. With the notable exception of paclitaxel, for which the solubility enhancement is about 6000-fold, 1% PEG-*b*-PDENA produces a small (griseofulvin, nifedipine and progesterone) or negligible (probucol) solubility enhancement. A practical way of assessing the solubilizing power of additives of different chemical structures is to look into the solubilization enhancement in relation to the concentration of the additive. As vehicles for testing drug candidates, the desirable situation is to have a solubilizing vehicle that consists of water, as much as possible. For this reason, this study compared the solubilization properties of monomeric (freely dissolved) and polymeric hydrotropes by looking at the solubility enhancement produced, relative to the concentration (% w/v). The DENA in polymeric form is a more effective solubilizer than as the freely dissolved hydrotrope. While Fig. 3A shows the solubility enhancement obtained with 1% of polymeric DENA, Fig. 3B and C shows the range of freely dissolved DENA necessary to obtain similar solubility enhancement. For the less hydrophobic drugs (Fig. 3B), dissolved DENA needs to be present at about 5% to produce similar solubility enhancement as 1% of the polymeric form (PEG-*b*-PDENA). For the more hydrophobic drugs (Fig. 3C) the difference is more drastic. The case of paclitaxel is unusual in the sense PEG-*b*-PDENA is a particularly effective solubilizer for this compound [41]. At the 1% level, PEG-*b*-PDENA increases the solubility of paclitaxel by nearly 6000-fold, whereas free DENA at 5% produces no appreciable solubilization of the same drug. Free DENA levels of roughly 30% are necessary to produce similar solubility enhancement for paclitaxel as compared with 1% of the polymeric form. For highly hydrophobic probucol, DENA is not an effective solubilizer; neither 1% PEG-*b*-PDENA nor 25% free DENA solubilizes probucol. Fig. 3C shows a sharp threshold concentration, clearly marking the hydrotrope level at which the solubilization effect changes from negligible to appreciable. This type of profile points toward a solubilization mechanism involving molecular association (and corresponding association constants) between the solute and the hydrotrope [14]. The marked difference between the solubilization effects of the free and polymeric hydrotrope raises the question as to whether the increased solubilization capacity of the polymeric hydrotrope in relation to the freely dissolved species is a result of the configuration of the hydrotropic agent (free vs. polymeric) or an effect of the solubilizing ability of the block copolymer itself. Data from the second hydrotrope sheds light on this question.

The solubility enhancement produced by 1% (w/v) polymeric DMBA (PEG-*b*-PDMBA) in aqueous solution is plotted for the five drugs in Fig. 4A. Comparison of Figs. 3A and 4A shows significant effect of the specific type of a hydrotrope linked to the polymeric matrix. In general, PEG-*b*-PDMBA is a stronger solubilizer than PEG-*b*-PDENA, with the exception of paclitaxel, for which the latter is a somewhat stronger solubilizer. Another important difference between PEG-*b*-PDENA and PEG-*b*-PDMBA is that the extent of solubilization by the latter shows a definitive trend with the hydrophobicity of the drug, as seen in Fig. 4A. PEG-*b*-PDMBA is an increasingly effective solubilizer as the hydrophobicity of the solute increases. The results in Fig. 4 also show that free DMBA needs to be present at levels 5 to 25 times higher than polymeric DMBA in order to produce similar solubility enhancement. The solubilization results

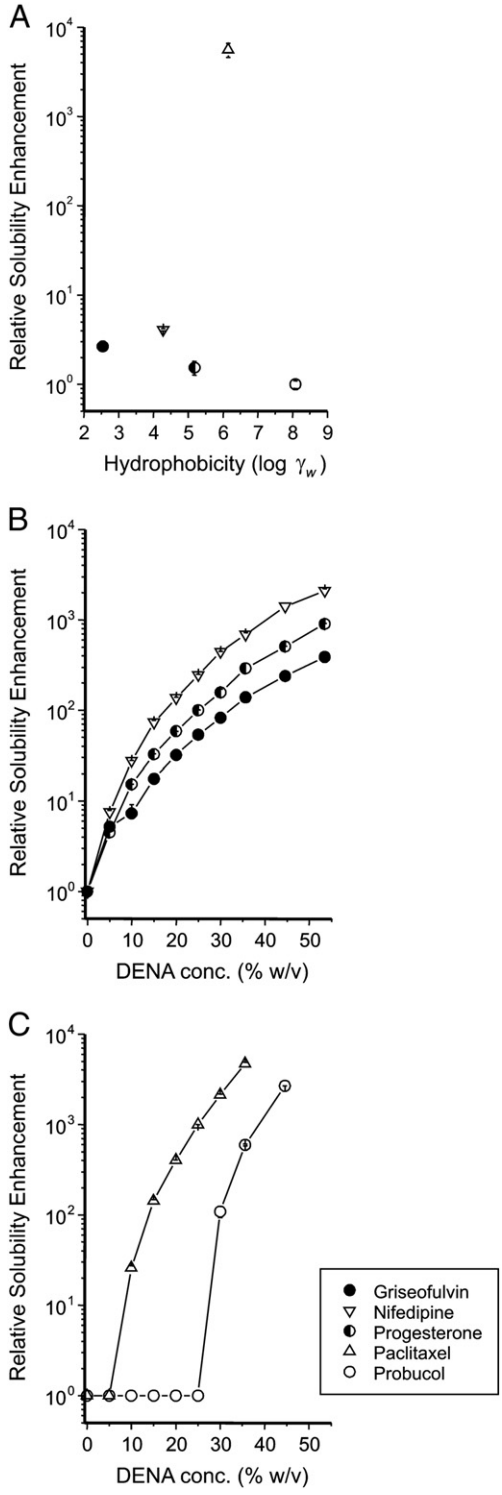

Fig. 3. Solubilizing properties of DENA in free and polymeric form. (A) Relative solubility increase, as a function of the hydrophobicity of the solute (log γ_w), produced 1% (w/v) PEG-*b*-PDENA dissolved in water as the vehicle. (B) Solubility enhancement for the griseofulvin, nifedipine and progesterone as a function of the concentration of the freely dissolved DENA. (C) Solubility enhancement for the two most hydrophobic solutes (paclitaxel and probucol) as a function of the concentration of the freely dissolved DENA.

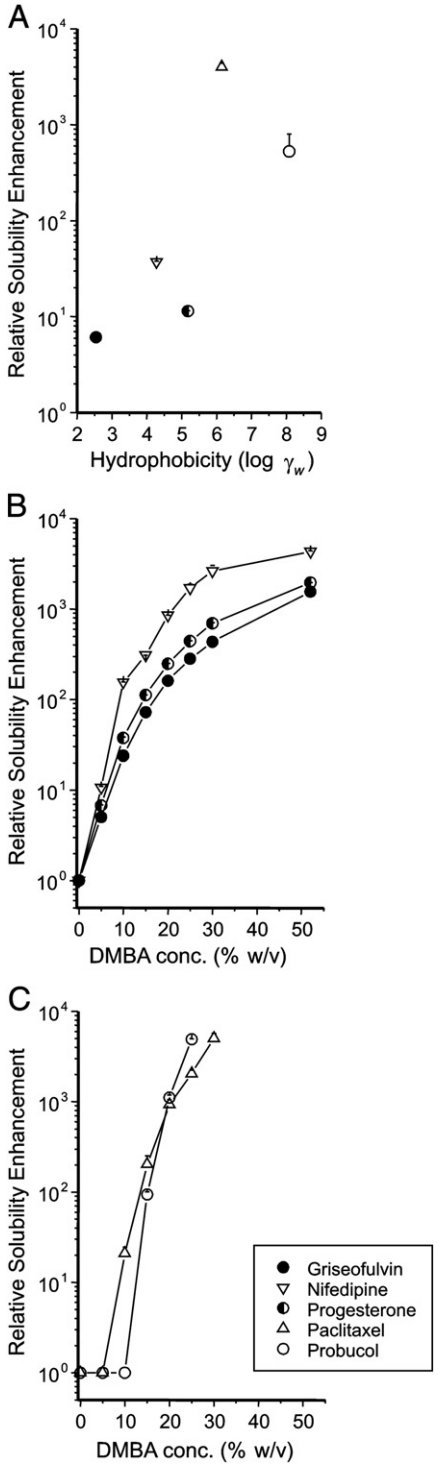

Fig. 4. Properties of DMBA in free and polymeric forms. (A) Relative solubility increase, as a function of the hydrophobicity of the solute (log γ_w), produced by 1% (w/v) PEG-*b*-PDMBA dissolved in water as the vehicle. (B) Solubility enhancement for griseofulvin, nifedipine and progesterone as a function of the concentration of the freely dissolved DMBA. (C) Solubility enhancement for paclitaxel and probucol as a function of the concentration of the freely dissolved DMBA.

strongly suggest that it is the presence of the hydrotrope, more than the block copolymer chain, that bears the solubilization capacity of the polymeric hydrotrope. It should be pointed out, however, that on the molar basis the backbone block copolymer enhances the solubilizing properties of the hydrotropes. In a 1% solution of either PEG-*b*-PDENA or PEG-*b*-PDMBA, only a small fraction of that 1% of total additive is made of the actual hydrotrope. This means that even though the hydrotrope plays a determining role on the solubilizing power of the polymeric species, the polymer backbone may also enhance the overall solubilizing ability. It is possible that the vinylbenzyloxy (VBO) spacer contributed to the observed

solubilizing effect, but additional studies would be needed to verify this possibility.

3.3. Comparison of solubilization capacity with amphiphilic polymers

The hydrotropic polymer systems used in this study form micellar dispersions under the conditions used. However, micellar dispersions of polymers, even when free from any hydrotropes, are themselves effective solubilizing agents. Therefore, to understand the solubilization mechanism of hydrotropic polymeric micelles, we need to investigate the effect of the dual character, i.e., the micellar and hydrotropic, nature of such systems. We explore this question on the following two sections. We compared the solubilization capacity of polymer micelles which are made of non-hydrotropic, amphiphilic copolymers. For this purpose, two amphiphilic polymers were selected; one is poly[(2-methacryloylethyl phosphocholine)-*co*-(*n*-butyl methacrylate) (PMB30W) which is an amphiphilic random copolymer; and the other is poly(ethylene glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA, 2K2K) which is a well known conventional amphiphilic block copolymer [15]. PMB30W consists of 0.3 mole fraction units of 2-methacryloyloxyethyl phosphocholine (MPC) as hydrophilic part and 0.7 mole fraction units of *n*-butyl-methacrylate (BMA) as hydrophobic part (MW = 50,000). This polymeric system was chosen based on its reported ability to solubilize paclitaxel to 1 mg/mL at 4.5% (w/v) in aqueous solution [45]. The solubility enhancements of five model drugs by these two polymers, as well as the two polymeric hydrotropes at 1% (w/v) aqueous concentrations are shown in Fig. 5. PMB30W showed a close to linear relationship between solubility enhancement and hydrophobicity of the drugs (log γ_w). In other words, PMB30W becomes a better solubilizer the more hydrophobic the solute. The conventional amphiphilic block copolymer, PEG-*b*-PLA also showed close to linear relationship with solute hydrophobicity. Based on their solubilization profiles, PMB30W and PEG-*b*-PLA act as general solubilizers of hydrophobic drugs. Of our hydrotropic solubilizers, PEG-*b*-PDENA is highly specific; it is the most powerful solubilizer for paclitaxel but it is less effective than the other solubilizers for the rest of the drugs. On the other hand, PEG-*b*-PDMBA seems to share solubilizing attributes with both the traditional amphiphilic copolymers and with PEG-*b*-PDENA. Polymeric DMBA shares to some extent the specificity toward paclitaxel with polymeric DENA, while sharing the attributes of a general solubilizer for hydrophobic drugs with PMB30W and PEG-*b*-PLA. PEG-*b*-PDMBA containing DMBA as a hydrophobic core solubilized all five model drugs to similar to or greater than PMB30W and PEG-*b*-PLA. The apparent combination of hydrophobic (non specific) and specific interactions of PEG-*b*-PDMBA with the solutes suggests a link between the structure of the solute and its solubilization by polymeric hydrotropes.

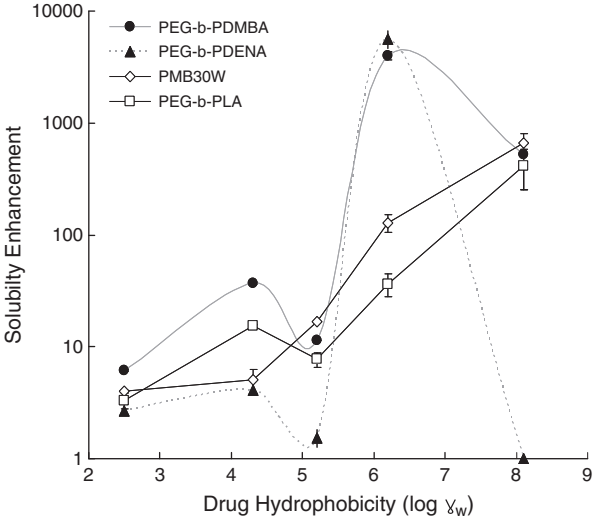

Fig. 5. Solubility enhancement of the five model drugs produced by 1% (w/v) concentrations of the block copolymers PEG-*b*-PLA (2K2K) and PMB30W, compared with the polymeric hydrotropes PEG-*b*-PDENA (5K5K) and PEG-*b*-PDMBA (5K2K). (Average ± SD, n = 3).

3.4. Solubilization Mechanism of PEG-b-PDMBA and PEG-b-PDENA

To investigate the solubilization mechanism of polymeric forms of hydrotropes, their CMC values were determined and compared with that of PEG-*b*-PLA. The CMC values were obtained by fluorescence using pyrene as probe and the results for PEG-*b*-PDENA (5K5K) and PEG-*b*-PDMBA (5K2K) are shown in Fig. 6. The CMC values of new polymers, PEG-*b*-PDMBA (5K2K, 5K3.5K, and 5K5K) are listed in Table 1. Not surprisingly, polymers with a longer PDMBA block have lower CMC values due to greater proportion of the hydrotropic block. The data in Fig. 6 indicate that at a concentration of 1% (w/v) in water, both hydrotropic polymers are present predominantly in micellar form. Therefore, polymeric hydrotropes and amphiphilic block copolymers share micellar solubilization as the primary mechanism for solubility enhancement of hydrophobic drugs. However, the strong effect of the presence of the hydrotrope shown in Figs. 3–5 suggests an additional component to the solubilization mechanism of polymeric hydrotropes.

The correlation between the structural properties of the solutes and the extent of solubilization produced by hydrotropic and an amphiphilic block polymer was investigated. The presence of aromatic moieties in the solute molecule has been shown to have an important effect on the ability of both DENA and DMBA as freely dissolved hydrotropes [14]. It is likely that the type of specificity exhibited by polymeric hydrotropes but not by the conventional amphiphilic block copolymers is the result of the favorable interactions of the hydrotropes with the aromatic moieties of the solutes. Multivariate analysis similar to that previously carried out for the freely dissolved hydrotropes was used [14]. Several variables were chosen and divided into four categories, as listed in Table 3.

Fig. 7 shows the resulting Principal Component Analysis (PCA) plot. The closer two variables are in the PCA plot, the closer the correlation between them. For example, log S_o and log X_w, the aqueous solubility expressed in μg/mL and mole fraction units, respectively, in quadrant 1 fall near each other in the same area. It is noteworthy that the solubility enhancements by the two polymeric forms of two hydrotropes, PEG-*b*-PDENA and PEG-*b*-PDMBA show

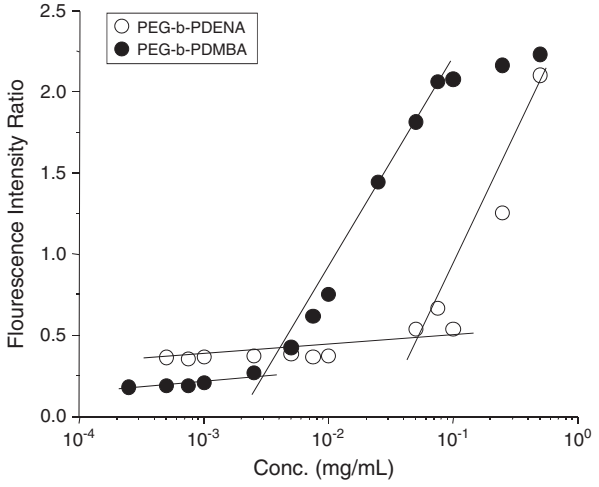

Fig. 6. Plot of the fluorescence intensity ratios (I_{339}/I_{334} and I_{341}/I_{334}) from pyrene excitation spectra vs. concentrations of PEG-*b*-PDMBA (5K2K) and PEG-*b*-PDENA (5K5K). The break in the slope corresponds to the critical micelle concentration.

Table 3
The four categories used for multivariate analysis.

Category	Variables
1. Size and structural attributes	Number of aromatic rings (n), molecular weight (MW), length (L), and compactness (MW/L) of the solute
2. Melting properties	Melting point, heat of melting (ΔH_m) and entropy of melting (ΔS_m)
3. Polarity and hydrophobicity of the solutes	log P, log γ_w, number of hydrogen bonding donors (HBD) and number of hydrogen bonding acceptors (HBA)
4. Solubility and solubilization	log S, log X_w, and log S/S_o

stronger correlation with the number of aromatic rings (# of aroma in quadrant 2) in the solute than with its hydrophobicity (log P). In contrast, the solubility enhancement produced by the amphiphilic random copolymer, PMB30W, shows a strong correlation with the hydrophobicity of drugs. The results of PMB30W are consistent with those from an additive that solubilizes through non-specific hydrophobic interactions. The PCA results from the polymeric DENA and DMBA obtained here are consistent with the PCA results from the freely dissolved hydrotropes [14]. These results show that when linked to the polymeric backbone, DENA and DMBA maintain their hydrotropic solubilization characteristics. In other words, the self-association of free hydrotropes, which is the main mechanism of hydrotropic solubilization, is maintained after polymerization. As a free hydrotrope, DMBA showed higher solubilization capacity and it may be related to its higher aggregation tendency due to higher hydrophobicity in relation to DENA [14]. The presence of the covalently linked hydrotropes in the polymeric hydrotropic systems offers two simultaneous solubilization effects: micellar and hydrotropic solubilization. The combination results in a synergistic solubilization mechanism. The hydrotrope immobilized in the hydrophobic core of the micelle becomes a more effective solubilizer than the same moiety freely dissolved in an aqueous environment. Polymeric hydrotropes can be optimized through judicious hydrotrope selection for a specific solute and for families of structurally related compounds. Solubilizing agents of "wide spectrum" (i.e., useful for a large number of drugs and drug candidates) are highly desirable. It is anticipated that a small number (3 to 4) of carefully selected hydrotropes, conjugated as analogs of PEG-*b*-DMBA, will result in a family of polymeric hydrotropes capable of solubilizing a wide range of drugs of varying hydrophobicity and chemical structures. Such a set of hydrotropic polymer micelle systems can be used to load and deliver various different poorly soluble drugs more effectively. One additional advantage of such systems is that they can be used to formulate new drug candidates that cannot be dissolved in traditional formulations, thus, the same formulation can, in principle, be used from the basic preformulation studies all the way to clinical studies.

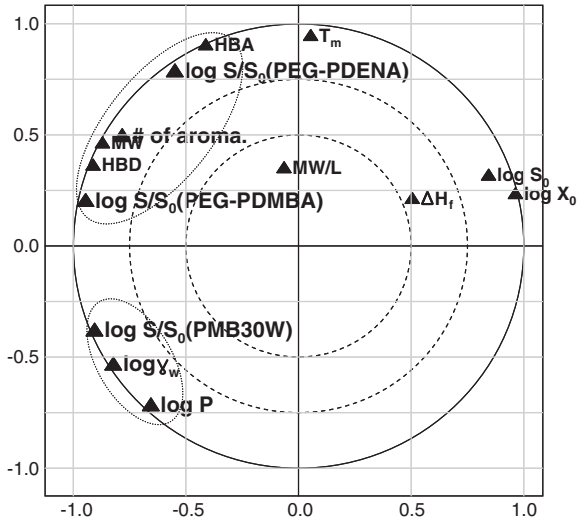

Fig. 7. Principal component analysis loading plot of the five model drugs and variables. The variables belong to four categories: Size and structural attributes; melting properties, polarity and hydrophobicity; solubility and solubilization.

4. Conclusion

This study identified that certain hydrotropes in polymer micelle forms can be good solubilizers of poorly soluble drugs that have a broad range of hydrophobicity. The polymeric form of DMBA (PEG-*b*-PDMBA) showed higher solubilization capacity for different drugs than the polymeric form of DENA (PEG-*b*-PDENA) which is highly specific for paclitaxel. Due to the highly localized hydrotrope concentration inside the hydrophobic core of the polymer micelles, polymeric forms of hydrotropes can solubilize the same drug at a substantially lower concentration than the corresponding freely dissolved hydrotrope. Conventional copolymers, e.g., PMB30W and PEG-*b*-PLA, provide only non-specific hydrophobic interactions. Hydrotropic polymer micelles like PEG-*b*-PDMBA also exhibit non-specific hydrophobic interactions. However, PEG-*b*-PDMBA also exhibits additional specific interactions with drug molecules involving the aromatic moieties of the drug. As a result, PEG-*b*-PDMBA exhibited stronger solubilizing capacity than PMB30W and PEG-*b*-PLA or freely dissolved DMBA. The results of this study support the hypothesis that it should be possible to identify and produce a small set of hydrotropic polymer micelles, based on the polymeric hydrotrope concept, whose solubilization properties encompass the wide range of chemical diversity of drugs in general.

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