Hydrotropic Solubilization of Poorly Water-Soluble Drugs

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Received 1 February 2010; accepted 26 April 2010

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.22241

ABSTRACT: The solubilizing ability of two aromatic hydrotropes, *N*,*N*-diethylnicotinamide (DENA) and *N*,*N*-dimethylbenzamide (DMBA), was investigated using a set of 13 poorly soluble, structurally diverse drugs. The number of aromatic rings in the solute molecule has a very strong effect on the solubility enhancement produced by either hydrotrope. However, although solubility enhancements in the order of 1000- to 10,000-fold were obtained with each of the hydrotropic agents, important differences were found between the two. DMBA is more hydrophobic and undergoes more extensive self-association than DENA, as determined by vapor osmometry. As a result, DMBA is generally a more powerful solubilizer of hydrophobic drugs. DENA, on the other hand, is more polar and its self-association is essentially limited to dimer formation. However, despite being less hydrophobic, DENA is an extremely powerful solubilizer of paclitaxel, a highly hydrophobic compound. Such a result is attributed to the higher hydrogen bonding ability of DENA over DMBA and the very high hydrogen bonding ability of paclitaxel. These observations in turn illustrate the strong interplay between specific and hydrophobic interactions on the observed solubilization by hydrotropic agents. © 2010 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:3953–3965, 2010

Keywords: solubility; solubilization; hydrotropic effect; osmometry; hydrophobicity; hydrogen bonding

INTRODUCTION

The number of potential drug candidates has been increasing by the advent of genomics, combinational chemistry and high throughput screening, forcing R&D organizations to accelerate attrition of compounds that do not have a high probability of successful development. Many of these new compounds are highly hydrophobic and poorly water-soluble. Solubility is one of the most important physicochemical properties for drug development since low solubility can hinder development of parenteral products and severely limit the bioavailability of orally administered dosage forms. Independently of the intended route of administration of a drug candidate, the requisite preclinical and toxicology studies make it necessary to prepare investigational formulations at relatively high concentrations. Such formulations are often obtained by using strong organic cosolvents like DMSO, which pose toxicological liabilities of their own and are not acceptable for use in clinical formulations.[1] There is a need for finding powerful solubilizing systems that are also suitable for a wide range of poorly soluble drugs. In addition to enabling preclinical studies, a desirable solubilizing system should also be acceptable for formulations used in the clinic. The solubilizing properties of hydrotropes can be exploited for this purpose. However, the development of a hydrotropic solubilizing system suitable for clinical formulations poses two important hurdles. One has to do with the ability of any given hydrotropic system to work with a wide variety of drug molecules. Another hurdle has to do with how to exploit the hydrotropic effect without the accompanying issue of high doses of the hydrotrope in the formulation. In this report, we focus on the former issue, and present an investigation of the solubilizing attributes of a hydrotropic system. The latter issue will be the subject of a subsequent report.

Hydrotropy is a molecular phenomenon whereby adding a second solute (the hydrotrope) results in an increase in the aqueous solubility of poorly soluble solutes.[2,3] Solubility enhancement is one of the advantages of hydrotropes.[3–7] Hydrotropic solutions can be used to extract hydrophobic drugs without the need of organic solvents.[8] Among many existing hydrotropes, nicotinamide is the most widely studied

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Journal of Pharmaceutical Sciences, Vol. 99, 3953–3965 (2010)

in the pharmaceutical field. However, the level of solubility enhancement obtainable with nicotinamide is typically less than 500-fold, which is not sufficient for a number of poorly soluble, hydrophobic drugs. In order to develop a more general solubilization vehicle, a more powerful hydrotrope is needed. Lee et al. screened more than 60 hydrotrope candidates and found that nicotinamide derivatives are excellent hydrotropes of the model poorly soluble drug, paclitaxel. In particular, *N*,*N*-diethylnicotinamide (DENA) was the best hydrotrope, which enhanced the solubility of paclitaxel by five orders of magnitude at 3.5 M concentration.[3] Lee's report also suggested that a good hydrotrope should have high water-solubility while maintaining hydrophobicity. In other words, an effective hydrotropic solubilization depends on the balance between these two counteracting effects. Based on these findings, we hypothesize that a nonionic hydrotrope of high aqueous solubility with a phenyl ring in its structure would make a very good solubilizer for a large number hydrophobic drugs. One such hydrotrope is *N*,*N*-dimethylbenzamide (DMBA) and was chosen as the second hydrotrope in this study. In this report, we compare the solubilizing attributes DENA and DMBA, in order to compare their ability to make good solubilizers for a wide variety of hydrophobic drugs. In so doing, we compare the (hydrotropic) solubilizing effect of phenyl ring, with a delocalizing electron cloud, versus that of a pyridinyl ring, where the electron cloud is highly polarized.

Although definitive studies are yet to be made, we can say that the term hydrotropy does not imply a specific solubilization mechanism. The broad range and functionality of hydrotropes has led to various suggested hydrotropic solubilization mechanisms, including complexation,[4,6,7,9,10] self-aggregation,[3,11–15] changes in the nature or structure of the solvent,[16–18] etc. For aromatic hydrotropes such as nicotinamide, sodium salicylate and sodium *p*-toluenesulfonate, two main mechanisms have been proposed. One is stacking complexation, and the other is self-aggregation. Strong evidence of complexation between a drug and nicotinamide is that the complexation constants ($K_{1:1}$ and $K_{1:2}$) can be obtained from phase-solubility data.[4,7] On the other hand, by showing temperature effects on the degree of self-association, Coffman et al.[12] argued that nicotinamide can solubilize riboflavin through a self-aggregation mechanism where aggregates of nicotinamide grow by stepwise monomer addition. At low concentrations, dimerization predominates, whereas at higher concentrations, trimerization, tetramerization, and so on, become the predominant equilibria.[19] DENA and DMBA can be classified as a nonionic aromatic hydrotropes, like nicotinamide. We employ vapor pressure osmometry to characterize their behavior in aqueous solution and to elucidate their hydrotropic mechanism. Osmometry has been used to assess aggregation behavior in aqueous solutions. Ts'o et al.[20,21] reported on the interaction and association of nitrogenated bases and nucleosides in aqueous solution by this method. Coffman and Kildsig[12,19] characterized the self-association of nicotinamide by osmometry and light scattering.

MATERIALS AND METHODS

Materials

The model drugs used in this study cover a wide range of hydrophobicity and a diversity in molecular structures. The solutes included in this study are griseofulvin, clofibrate, nifedipine, glybenclamide, progesterone, dihydroanthracene, felodipine, anthracene, fenofibrate, itraconazole, probucol, coenzyme Q10, and paclitaxel. Paclitaxel was obtained from Samyang Genex (Daejeon, South Korea). All other model drugs, as well as DENA and DMBA, were purchased from Sigma-Aldrich (St. Louis, MO). The chemical structures of the solutes used in this study are shown in Figure 1.

Solubility Measurements

Excess drug was added to screw-capped vials containing a fixed volume of the hydrotropic solution. The solution was incubated at 37°C for 3 days using a thermostatic shaker (Max Q 4000, Barnstead/Labline, Melrose Park, IL). Aliquots from each sample were taken and filtered through a 0.2 μm nylon syringe filter (Alltech, Deerfield, IL) that was prewarmed at 37°C. Hydrotropes are soluble agents that must be present at relatively high concentrations in order to exert their solubilizing effect, so that the filtration step does not alter the effective concentration of DENA or DMBA. The filtered aliquot was diluted with acetonitrile and assayed by reverse phase (RP)-HPLC analysis.

HPLC Analysis

For HPLC analysis, a binary gradient mode was employed to enhance the resolution. The drug concentration was determined with a C_{18} RP analytical column (Waters, Milford, MA) using Agilent 1100 series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with photodiode detector. Before running, all samples were filtered through a 0.2 μm nylon syringe filter (Alltech). Key parameters of the HPLC analysis methods are summarized in Table 1. Drug concentration was calculated from a standard curve of each drug. Measurements were conducted in triplicate and values reported correspond to the average value.

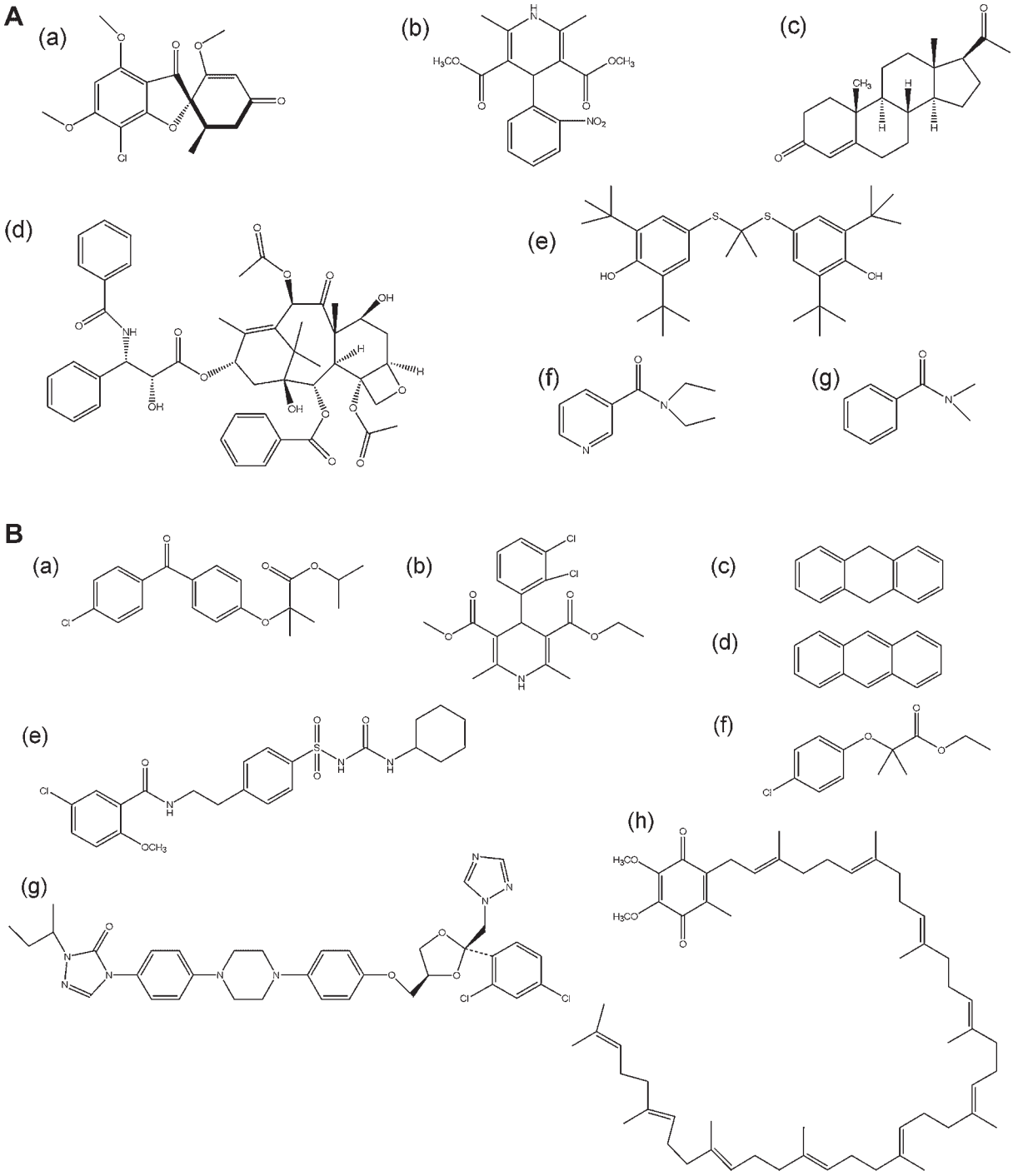

Figure 1. (A) Chemical structures of the five model compounds, and two hydrotropes: (a) griseofulvin, (b) nifedipine, (c) progesterone, (d) paclitaxel, (e) probucol, (f) DENA, (g) DMBA. (B) Chemical structures of eight additional poorly soluble compounds: (a) fenofibrate, (b) felodipine, (c) dihydroanthracene, (d) anthracene, (e) glybenclamide, (f) clofibrate, (g) itraconazole, (h) coenzyme Q10.

Osmometry Measurements

The vapor pressures of aqueous hydrotrope solutions were measured with a VAPRO5520 type vapor pressure osmometer (Wescor, Inc., Logan, UT) at room temperature. The instrument was calibrated with the standard NaCl solutions (100, 290, and 1000 Osm) supplied by the manufacturer in sealed ampoules. The average of three measurements was used for each reported measurement.

Table 1. Key Parameters of the HPLC Methods of Model Compounds

Compound	Initial Mobile Phase (Volume Parts)	Injection Volume (μL)	Wavelength (nm)
Griseofulvin	70 water, 30 acetonitrile	20	293
Glybenclamide	95 water, 5 acetonitrile	20	233
Nifedipine	70 water, 30 acetonitrile	20	240
Progesterone	70 water, 30 acetonitrile	20	254
Clofibrate	70 water, 30 acetonitrile	20	223
Dihydroanthracene	30 water, 70 acetonitrile	100	250
Anthracene	30 water, 70 acetonitrile	20	251
Felodipine	70 water, 30 acetonitrile	20	237
Paclitaxel	70 water, 30 acetonitrile	20	227
Fenofibrate	30 water, 70 acetonitrile	20	280
Itraconazole	70 water, 30 acetonitrile	20	263
Probucol	10 water, 90 acetonitrile	20	254
Coenzyme Q10	40 THF, 55 acetonitrile, 5 water[a]	20	275

[a]Except coenzyme Q10, a binary gradient mode was used.

Data Analysis

Multivariate data analysis was done using the program, Soft Independent Modeling of Class Analogy (SIMCA). Principal component analysis (PCA) was performed to obtain the overview on the data set. PCA loading plot shows correlation between variables.

Theoretical

Association Model

The approach assumes that the self-association of a hydrotrope occurs stepwise through monomer addition. As the total concentration of the hydrotrope increases, dimers are first formed from the free molecules, trimers are then formed by the addition of a free molecule to an existing dimer, and so on.[19] Higher order associations are formed through sequential monomer addition to existing (lower number) aggregates in solution. Aggregate formation steps are all equilibrium processes, each associated with an association constant. There are no assumptions regarding the magnitude of the association constants. According to this model, the physical properties of the associating solution changes smoothly with concentration. The association constants of dimerization, trimerization and tetramerization, K_2, K_3, and K_4, respectively, are defined as:

$$K_2 = [A_2]/[A_1]^2$$
$$K_3 = [A_3]/[A_2][A_1]$$
$$K_4 = [A_4]/[A_3][A_1]$$

Calculation of Association Constants

The experimentally measured osmolality must be related to stoichiometric molality and osmotic coefficient

$$\underline{m} = \nu \varphi m \tag{1}$$

where $\underline{m}$ is the measured osmolality, φ the molar osmotic coefficient, and m the stoichiometric molality. For nonelectrolytes, ν is equal to unity. Using Eq. (1), φ can be experimentally obtained at different concentrations. Once φ is obtained, the activity coefficient can be calculated by using Ts'o treatment of the Gibbs–Duhem equation.[21] This procedure is based on the relationship between the activity (γ) and osmotic (φ) coefficients:

$$\ln \gamma = (\varphi - 1) + \int_0^m (\varphi - 1) \mathrm{d} \ln m \tag{2}$$

The osmotic coefficient can be expressed in terms of m, in the form of an empirical polynomial expression in Eq. (2):[22]

$$\varphi = 1 + a_1 m + a_2 m^2 + a_3 m^3 + a_4 m^4 + a_5 m^5 + a_6 m^6 \tag{3}$$

so that the coefficients can be obtained from least squares fitting. Integration of the resulting expression gives[20,21]

$$\ln \gamma = 2\, a_1 + (3/2) a_2 m^2 + (4/3) a_3 m^3 + (5/4) a_4 m^4 + (6/5) a_5 m^5 + (7/6) a_6 m^6 \tag{4}$$

Based on Eq. (4), the activity coefficient can be calculated at any given hydrotrope concentration, m, by using the following relation:

$$\ln x_1 = \frac{m_1}{m} = (\varphi - 1) + \int_0^m (\varphi - 1) \mathrm{d} \ln m \tag{5}$$

From the equality of Eqs. (2) and (5), it follows that the mole fraction of the monomer, x_1, is numerically equivalent to the activity coefficient, as originally

shown by Ts'o et al.:[21]

$$\gamma = x_1 = \frac{m_1}{m} \tag{6}$$

In the stepwise association model, where dimers, trimers, and tetramers exist in solution

$$m = m_1 + 2K_2(m_1)^2 + 3K_2K_3(m_1)^3 + 4K_2K_3K_4(m_1)^4 \tag{7}$$

From Eqs. (6) and (7), Coffman and Kildsig[19] derived the following relationship between γ and m_1, in terms of the association constants:

$$\frac{m}{m_1} = 1/\gamma = 1 + 2K_2(m_1) + 3K_2K_3(m_1)^2 + 4K_2K_3K_4(m_1)^3 \tag{8}$$

According to Eq. (8), a plot of $1/\gamma$ versus m_1, when fitted to a polynomial, provides the values of the association constants K_2, K_3, and K_4.

RESULTS

Solubilization of Hydrophobic Drugs by Hydrotropes

Insight into the mechanism of hydrotropic solubilization and solubilization capacity of hydrotropes can obtained from the effect of hydrotrope concentration. We measured the solubilization of several poorly soluble drugs in aqueous solution by hydrotropes over a wide range of hydrotrope concentrations. Figure 2 shows the solubilization data of five model drugs (probucol, paclitaxel, progesterone, nifedipine, and griseofulvin) in water, as a function of the concentration of DENA and DMBA. These five drugs were chosen here as the solutes because of their poorly aqueous solubility (less than 14 μg/mL) and a broad hydrophobicity range (using their octanol–water partition coefficients, $2 < \log P < 10$). The solubilization effect of the hydrotropes does not produce a linear or monotonic profile in all cases. The legend in Figure 2 lists the solutes in decreasing order of hydrophobicity. For the two most hydrophobic solutes, probucol and paclitaxel, the solubilization curves exhibit a sigmoidal profile, suggesting strong cooperative intermolecular interactions involved in their solubilization process. A noteworthy feature in Figure 2a is the presence of flat kink in the solubilization profiles below but near the 1 M DENA concentration. The persistence of this feature with different solutes indicates that it is the result of a property of the hydrotrope, rather than of the mixture. This in turns points toward a situation where one particular association equilibrium (with its corresponding association constant) predominates over the rest. As sated, hydrotropy can include aggregation of the hydrotrope itself, and this point will be further addressed later in this report. The minimum hydrotropic concentration (MHC) of the hydrotropes used in this study is roughly the same when solutes are present in solution, as observed in other studies.[11] Both DENA and DMBA produced large solubilization factors for all five solutes included in Figure 2. Based on these results, we expanded the set to include five additional poorly soluble drugs covering an even wider range of hydrophobicity. The additional solutes are clofibrate, glybenclamide,

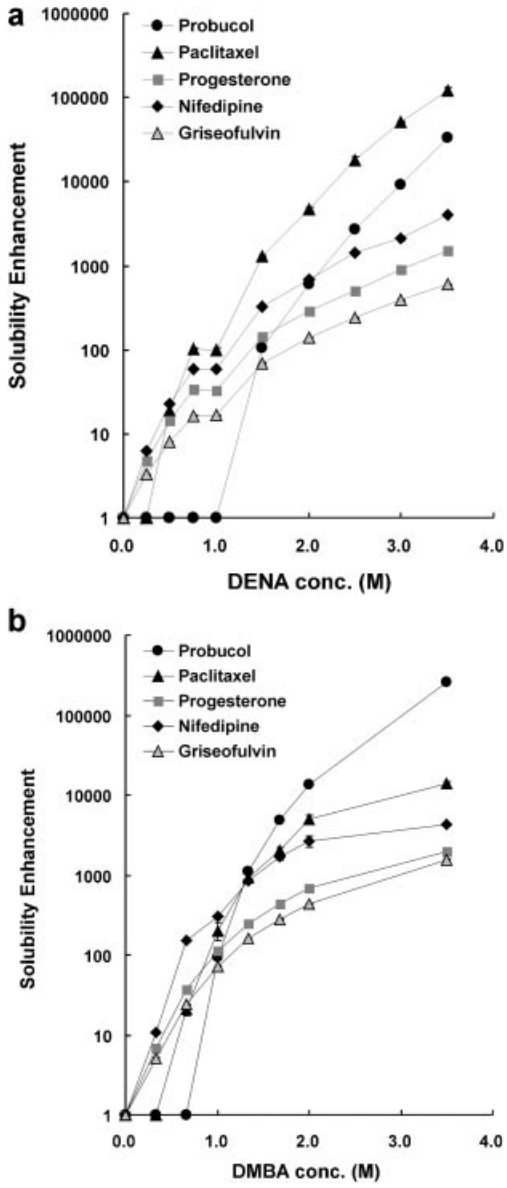

Figure 2. Solubility enhancement for the first five model compounds (griseofulvin, nifedipine, progesterone, paclitaxel, and probucol) as a function of concentration of (a) DENA and (b) DMBA. Data are means ± SD from three measurements.

felodipine, fenofibrate, and coenzyme Q10. The hydrocarbon chain in coenzyme Q10 is so large that its calculated $\log P$ value exceeds the value of 20. While such a high value may not be realistic, there is no doubt that by including such a solute in the study, the solute set covers a very wide range of hydrophobicity.

The octanol–water partition coefficient ($\log P$) value is generally used to assess the hydrophobicity of organic compounds. While a very useful hydrophobicity index, $\log P$ has some limits as illustrated in the case of coenzyme Q10, for example. In this report, we use solubility-derived data to assess hydrophobicity. The aqueous solubility of organic nonelectrolytes is given by the following expression:

$$\log X_w = -\frac{\Delta S_f(T_m - T)}{2.303RT} - \log \gamma_w \qquad (9)$$

where X_w is the (mole fraction) aqueous solubility, ΔS_f the entropy of fusion 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.[23] The first term on the right-hand side of Eq. (9) is the ideal solubility, which is entirely determined by the melting properties of the crystalline solute. The term $\log \gamma_w$, is the deviation from ideal mixing behavior, and precisely quantifies the degree to which the hydrophobicity of the drug limits its solubility in water.[23] In this study, we use the experimentally obtained value of $\log \gamma_w$ as the numerical indicator for drug hydrophobicity. Accordingly, $\log \gamma_w$ values for the model drugs obtained from Eq. (9) are listed in Table 2. Based on $\log \gamma_w$ values, the order of hydrophobicity of the drugs is coenzyme Q10 > probucol > fenofibrate > paclitaxel > felodipine > clofibrate > progesterone > nifedipine > glybenclamide > griseofulvin. The solubility enhancement of each of these ten poorly soluble drugs was determined in aqueous solutions containing 3.5 M of either DENA or DMBA, and the results are shown in Figure 3. Both hydrotropes produced large increases in the solubility for all ten model drugs, with the only exception of coenzyme Q10 by DENA. Solubility enhancements (relative to the aqueous solubility) as high as 255,000- and 122,000-fold were observed with DMBA and DENA, respectively. Even if the degree of solubilization is not the same, the two hydrotropes produced similarly shaped solubilization patterns, as shown in Figure 3. In general, DMBA solubilized these poorly soluble drugs to a greater degree than DENA with the exception of paclitaxel, for which DENA is a remarkably good solubilizer. Even though it is highly hydrophobic compared with other drugs ($\log \gamma_w = 6.15$), paclitaxel has a large number of hydrogen bond donors (HBD = 4) and acceptors (HBA = 14) in its structure. The hydrogen bonding ability of the pyridine ring in DENA, absent in DMBA (which has a phenyl ring) is likely the reason for the remarkable ability of DENA to solubilize paclitaxel. Hydrophobic interactions can be one of the main driving forces for the solubilization of lipophilic drugs. However, the solubility enhancement produced by DENA and DMBA does not show a clearly discernible relationship with $\log \gamma_w$. This result indicates that solubilization of drugs by DENA and DMBA is not solely the result of hydrophobic interactions. Nonetheless, the data in Table 3 and Figure 3 show something of a trend. In Figure 3, the points corresponding to the maximum observed solubility enhancement are encircled by a ring drawn with a dotted line. The four encircled solutes (felodipine, fenofibrate, probucol, and paclitaxel) in the graph correspond to those compounds that appear at the bottom of Table 3, where the solutes are first sorted by increasing number of aromatic rings in their molecular structure (n), and then by increasing order of hydrophobicity. In gross terms, we can say that

Table 2. Solubilities and Important Parameters of Model Compounds

	Solubility (μg/mL)	Solubility (mol/L)	$\frac{\Delta S_f(T_m-T)}{2.303RT}$	$\log \gamma_w$	$\log P$
Griseofulvin	14[40]	0.04	3.60[41]	2.54	2.0[42]
Glybenclamide	4[43]	0.0081	2.66[44]	4.18	4.7[45]
Nifedipine	5[46]	0.014	2.31[47]	4.28	2.5[48]
Progesterone	7[49]	0.022	1.21[50]	5.18	3.87[51]
Clofibrate	77[52]	0.29	0	5.28	3.3[b]
Dihydroanthracene	2.33[53]	0.013	0.92[a]	5.72	4.2[c]
Anthracene	0.089[54]	0.0005	2.01[a]	6.04	4.25[55]
Felodipine	0.5[41]	0.0013	1.55[41]	6.08	5.0[41]
Paclitaxel	0.3[3]	0.00035	2.05[38]	6.15	3.98[56]
Fenofibrate	0.3[57]	0.00078	0.92[58]	6.91	5.24[57]
Itraconazole	0.001[59]	1.42×10^{-6}	3.38[59]	7.21	5.66[60]
Probucol	0.006[41]	1.16×10^{-5}	1.60[41]	8.08	10[61]
Coenzyme Q10	0.0007[41]	8.11×10^{-7}	1.49[41]	9.34	21[41]

[a] Measured by DSC for this study.
[b] DrugBank (source: PhysProp).
[c] PubChem Substance.

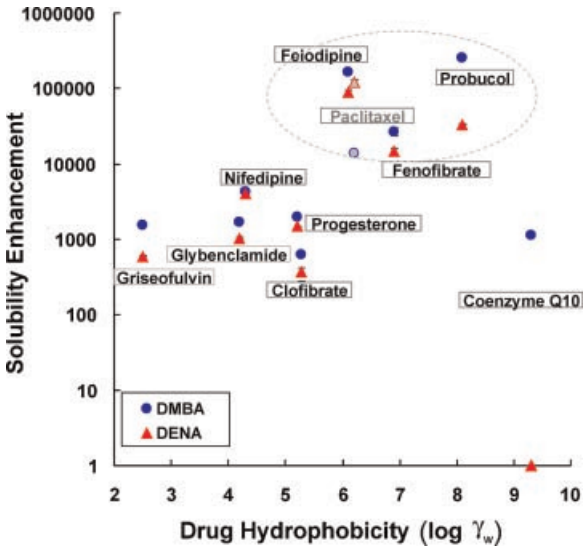

Figure 3. Solubility enhancement for 10 poorly soluble model drugs (griseofulvin, glybenclamide, nifedipine, clofibrate, progesterone, felodipine, paclitaxel, fenofibrate, probucol, and coenzyme Q10) as a function of the aqueous activity coefficient ($\log \gamma_w$), representing the drug hydrophobicity. Data are means ± SD from three measurements. Drugs were dissolved in 3.5 M DENA and in 3.5 M DMBA solutions.

increasing hydrophobicity and number of aromatic rings in the molecule of the solute favor hydrotropic solubilization. However, although it is not completely clear which of the two effects, if any, predominates, the available data suggest that the latter plays a major role. Note for example that the solutes progesterone, griseofulvin, clofibrate, coenzyme Q10, and glybenclamide cover a very wide range of hydrophobicity (over seven orders of magnitude). However, the level of solubility enhancement for all these drugs (save coenzyme Q10 with DENA) is roughly of the same order (~1000-fold). The fact that such a large span on hydrophobicity does not result in a commensurately large span in solubility enhancement suggests that the effect of n may be the stronger one. In order to further test this notion, we included additional solutes in the study. Anthracene and dihydroanthracene have each three rings in their structure. The difference between them is that all three rings in anthracene are aromatic, while only two of the rings in dihydroanthracene are aromatic (see Fig. 1). By including these two compounds, it is possible to explore the effect of a change in the aromatic character of the solute without an accompanying change in the number of rings present in the molecule. Additional insight into the interplay between hydrophobicity of the solute and the number of aromatic moieties in its structure can be gained by adding a solute like itraconazole to the solute set. Itraconazole is a drug whose $\log \gamma_w = 7.21$, places it in between the most hydrophobic solutes with $n = 2$ in Table 3 ($\log \gamma_w = 6.91$ and 8.08 for fenofibrate and probucol, respectively). An important difference however, is that $n = 3$ for itraconazole. The solubilization data for anthracene, dihydroanthracene, and itraconazole are shown in Figure 4. The solubility enhancement for anthracene (all three aromatic rings) is significantly greater than that of dihydroanthracene with both DENA and DMBA. We should point out that neither of these three-ring hydrocarbons have functional groups in their structure, such that none of them is capable of engaging in specific interactions. Therefore, the difference in solubilization enhancement between anthracene and dihydroanthracene is the result of the difference in the aromatic character of their constituting rings. The data in Figure 4 also show that itraconazole exhibits greater solubility enhancement than other solutes of similar hydrophobicity but having fewer

Table 3. Properties of Ten Model Drugs

$\log \gamma_w$	Number of Aromatic Rings (n)	Drug
5.18	0	Progesterone
2.54	1	Griseofulvin
5.28	1	Clofibrate
9.34	1	Coenzyme Q10
4.18	2	Glybenclamide
4.28	2	Nifedipine
6.08	2	Felodipine
6.91	2	Fenofibrate
8.08	2	Probucol
6.15	3	Paclitaxel

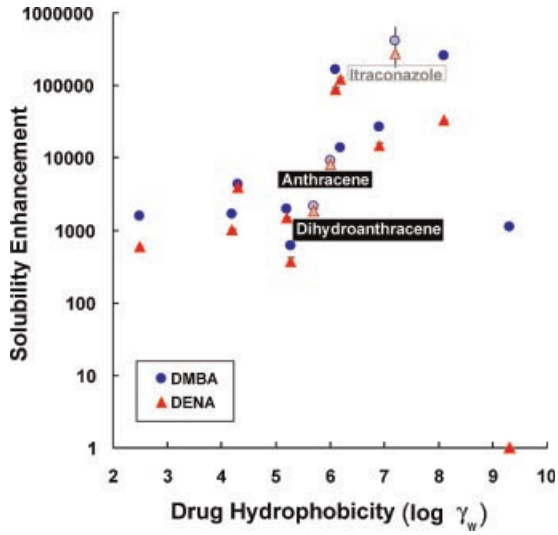

Figure 4. Solubility enhancement of 13 poorly soluble solutes including itraconazole, anthracene, and dihydroanthracene as a function of $\log \gamma_w$. Data are means ± SD from three measurements. Drugs were dissolved in 3.5 M DENA and in 3.5 M DMBA solutions.

aromatic rings in their structure. In fact, itraconazole is the solute for which hydrotropic solubilization is the greatest of the entire set. The number of aromatic rings plays a defining role on the observed solubility enhancement with both DENA and DMBA.

Even though the data in Figure 4 strongly support the notion that the number of aromatic rings in the solute has a strong effect on solubility enhancement by the hydrotropes, an objective analysis encompassing the entire data set would be more informative. Multivariate analysis can serve this purpose quite well, since it looks into the variability of the data as a whole, irrespectively of its source, and can be applied to the entire available data set, that is, all 13 model compounds. Even though the limited number of solutes included in the study (N = 13) prevents us from getting a full predictive model, multivariate analysis can nevertheless provide very useful information. The multivariate analysis in this study included groups of variables reflecting different properties of the solutes:

Size and structural attributes: Number of aromatic rings (n), number of pyridine rings, number of non-pyridine rings, molecular weight (MW), length (L), and compactness (MW/L) of the solute.
Melting properties: Melting point, heat of fusion (ΔH_f), entropy of melting (ΔS_f).
Polarity and hydrophobicity: $\log P$, $\log \gamma_w$, HBD, and HBA.
Solubility and solubilization: $\log S$, $\log X_w$, and $\log S/S_o$.

The analysis showed that with the exception of coenzyme Q10, the different model solutes can be considered as part of the same group. The results of the principal component analysis (PCA) are presented in Figure 5. The closer two variables fall in the PCA graph, the closer the correlation between them. For example, $\log P$ and $\log \gamma_w$, both of which are measures of hydrophobicity appear near each other. The results show that the degree of solubility enhancement ($\log S/S_o$) exerted by both DENA and DMBA has stronger correlation with the number of aromatic rings in the solute molecule than with any other variable. The cluster made by these variables is encircled, in gray, in the figure. It is widely accepted that planar molecular geometry of the solute favors hydrotropic interactions. Nevertheless, a noteworthy result from Figure 5 is that the effect is strong enough as to predominate over the direct effect of hydrophobicity ($\log \gamma_w$ and $\log P$), whose projections fall farther away from $\log S/S_o$ in the PCA plot. Hydrotropy and its resulting solubilization are no more than a mechanism of reduction of the free energy of mixing. In this sense, hydrophobicity of the solute is ultimately the driving force. However, here we have

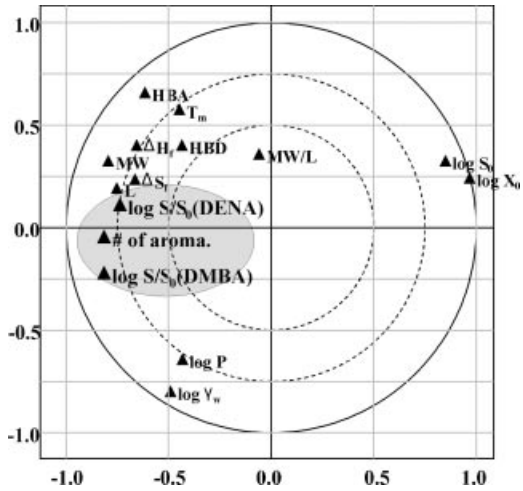

Figure 5. Principal component analysis loading plot of the model compounds, except coenzyme Q10, and 15 variables. The variables belong to four categories: size and structural attributes; melting properties, polarity and hydrophobicity; solubility and solubilization (see text).

a situation where a geometrical aspect (i.e., the presence of aromatic rings in the solute molecule) acts as an enabler and modifier of the driving force, and hence of the resulting solubilization effect. Another important factor is the nature of the aromatic ring present (phenyl vs. pyridinyl), such a factor is differentiating enough as to displace, in opposite directions, the effect of DENA from that of DMBA in relation to $\log S/S_o$, as seen in Figure 5.

Hydrotropic Solubilization Mechanism of DENA and DMBA

DENA shows a more clearly distinguishable MHC than DMBA but neither of the two hydrotropes shows a linear or monotonic profile in Figure 2. The particular shape of the pattern is a reflection of the self-association behavior of the hydrotrope.[11] We investigated this aspect using vapor pressure osmometry. When hydrotropes associate, the effective number of particles in solution decreases, thus producing a measurable osmotic effect. If there are no aggregates in solution, a graph of an osmotic molarity (osmolarity) versus molarity gives a straight line with a slope of 1, according to Eq. (1). Plots of osmolarity versus molarity for DENA and DMBA are shown in Figure 6. The graph shows the self-association of DENA and DMBA, evidenced by the departure from the reference straight line. The osmolarity of DENA could not be measured for concentrations exceeding 3.25 M due to instrumental limitations. By adapting the stepwise association model described above, the association constants were obtained. First, the molar osmotic coefficients

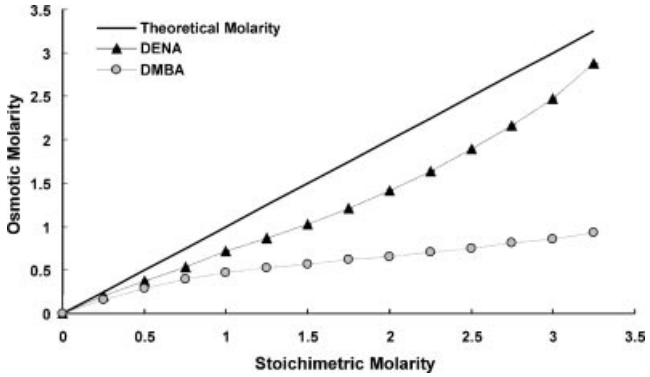

Figure 6. Plot of osmotic molarity versus stoichiometric molarity for DENA and DMBA: comparison of experimental data to theoretical monomer curve. Reported osmotic data are the average ± SD from three measurements.

(φ) of DENA and DMBA were calculated according to Eq. (1) and are summarized in Table 4. Molar activity coefficient (γ) of DENA and DMBA were then calculated according to Eq. (4) and are listed in Table 5. The fitted coefficients for calculating molar activity coefficients were obtained by plotting the osmotic coefficient versus the hydrotropic molar concentration as shown in Figure 7. The data from both DENA and DMBA were fitted to sixth degree polynomials in order to obtain an explicit expression for the osmotic and activity coefficients (Eqs. 3 and 4, respectively). Once the activity coefficients are obtained, the association constants can be calculated from the best fit to Eq. (8). The corresponding plots obtained from DENA and DMBA are shown in Figure 8. The

Table 4. Experimental Value of Osmotic Coefficients (φ) at 25°C

	Experimental Osmotic Coefficients	
Conc. (M)	DENA	DMBA
0	1	1
0.25	0.807 ± 0.034	0.640 ± 0.031
0.5	0.756 ± 0.004	0.572 ± 0.006
0.75	0.714 ± 0.005	0.529 ± 0.032
1	0.713 ± 0.010	0.468 ± 0.012
1.25	0.692 ± 0.026	0.419 ± 0.021
1.5	0.684 ± 0.014	0.375 ± 0.001
1.75	0.690 ± 0.022	0.353 ± 0.003
2	0.705 ± 0.045	0.329 ± 0.005
2.25	0.730 ± 0.018	0.313 ± 0.007
2.5	0.756 ± 0.048	0.299 ± 0.002
2.75	0.785 ± 0.055	0.296 ± 0.007
3	0.825 ± 0.055	0.284 ± 0.001
3.25	0.885 ± 0.015	0.287 ± 0.001
3.5	—[a]	0.307 ± 0.004
3.75	—[a]	0.318 ± 0.002
4	—[a]	0.379 ± 0.001

[a]Data of DENA could not be obtained over 3.25 M because of instrumental limitation. Values are the means ± SD from three measurements.

Table 5. Activity Coefficients at 25°C Computed from the Fitted Osmotic Coefficients

Conc. (M)	DENA	DMBA
0.25	0.660	0.511
0.5	0.529	0.348
0.75	0.464	0.273
1	0.422	0.230
1.25	0.390	0.197
1.5	0.365	0.171
1.75	0.349	0.149
2	0.340	0.132
2.25	0.338	0.119
2.5	0.340	0.110
2.75	0.344	0.103
3	0.352	0.098
3.25	0.375	0.093
3.5	—[a]	0.088
3.75	—[a]	0.085
4	—[a]	0.088

[a]Data of DENA could not be obtained over 3.25 M because of instrumental limitation.

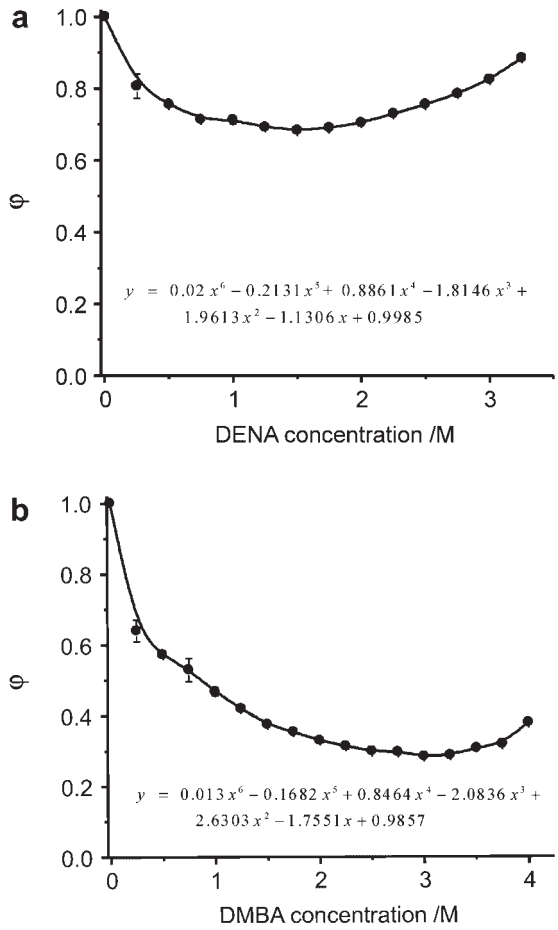

Figure 7. Plot of the osmotic coefficient (φ) versus molar concentration of (a) DENA and (b) DMBA and the fitted polynomial expressions. The φ values were used from the data in Table 4.

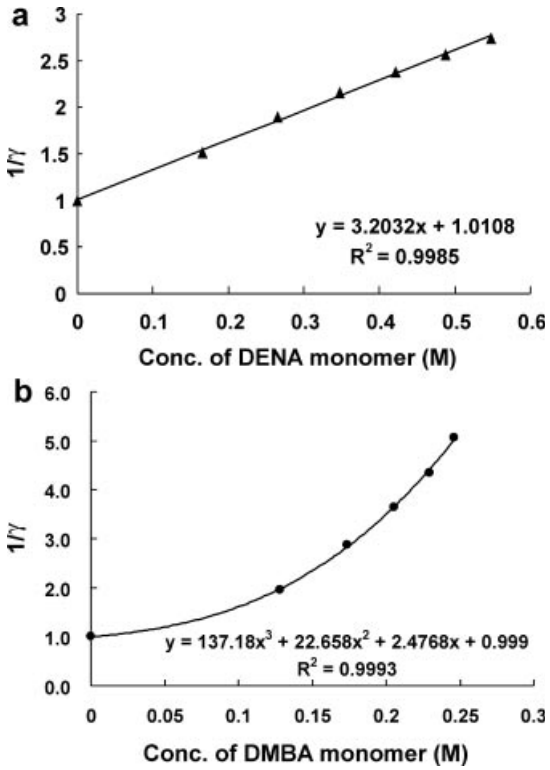

Figure 8. Plots of $1/\gamma$ versus monomer concentration of (a) DENA and (b) DMBA and their fitted polynomial expressions. The values of γ were used from the data in Table 5.

self-association properties of DENA and DMBA are significantly different. The self-association behavior of DENA can be properly described by the formation of exclusively dimers, whereas description of the self-association of DMBA requires the presence of dimers, trimers, and tetramers. Accordingly, the association constant of dimerization of DENA was $1.6\,M^{-1}$, whereas the association constants for dimer, trimer and tetramer formation of DMBA were 1.238, 6.1, and $4.54\,M^{-1}$, respectively. The higher aggregation tendency of DMBA is the result of its more hydrophobic nature and offers a suitable explanation for its higher solubilizing ability, compared with DENA, for the majority of the solutes. Being more hydrophobic than DENA, DMBA showed higher solubility enhancement of poorly soluble drugs at substantially lower concentrations, as seen in Figure 2. Several studies report that aromatic molecules dissolved in water tend to self-associate.[24–28] Aromatic stacking interactions are found abundantly in nature and the classical example is the stacking of nucleic bases.[24] The more hydrophobic a hydrotrope, the lower the concentration at which it can begin to aggregate.[13] The results of this study show two different effects at play behind the observed solubilization by hydrotropes. On the one hand, a structural feature of the solute, such as the presence and number of aromatic moieties has a very strong effect on its solubilization by hydrotropes. Such an effect is strong enough to somewhat mask the role of solute hydrophobicity in the PCA plot. On the other hand, despite the significance of the aromatic character of the solute, hydrophobicity remains an important factor. As long as the target solute is a hydrophobic drug, the more hydrophobic the hydrotrope (with its enhanced self-aggregation properties in aqueous media) the more powerful its solubilizing ability, whether the solute has an aromatic moiety or not. The presence of an aromatic moiety enhances the solubilization effect: for two equally hydrophobic solutes, the hydrotropic effect is much greater for the one with (more) aromatic rings in its structure. Furthermore, the comparison between DENA and DMBA reveals a very important aspect: the ability to fine-tune hydrotropic solubilization through hydrogen bonding and specific interactions. The hydrogen bonding properties of DENA make it an extremely powerful solubilizer for paclitaxel, a highly hydrophobic drug, despite the more favorable (higher) hydrophobicity of DMBA. The multivariate analysis in Figure 5 is informative in the sense that it shows a general relationship. The aromatic nature of the solute plays such a strong role in hydrotropic solubilization, as to mask the effect of hydrophobicity, and this applies to the generality of hydrophobic drugs. Furthermore, even if qualitative, this general result points toward the possibility of finding predictive correlations for the solubilization of drugs by hydrotropes. The solubility of hydrophobic compounds is related to the molecular surface area of the solute,[29] and solubility estimations are significantly improved if the total surface area (TSA) is broken down into its polar and nonpolar components.[30] In an analogous way, we can expect that the contribution of a molecular descriptor such as the aromatic surface area, quantifying the aromatic character of the solute, will yield correlations of hydrotropic solubilization. However, to be predictive, polar surface area (PSA) methods require inclusion of the hydrogen bonding properties of the solute.[31] We surmise that the general relationship involving the number of aromatic moieties in the solute can be turned into predictive correlations by incorporating polar, hydrophobic and aromatic surface area parameters, scaled by the hydrogen bonding properties of the solute.

Hydrotropes are powerful solubilizing agents of hydrophobic drugs. They are also versatile in the sense that make it possible to take advantage of structural aspects of the solute such as the presence of aromatic rings, hydrophobicity, hydrogen bonding ability and specific interaction properties. Furthermore, solubility enhancement with the use of hydrotropes can achieve several orders of magnitude. Despite these advantages, however, and as mentioned above, the use of hydrotropic agents poses an

important pharmaceutical hurdle. In order to be effective, hydrotropes need to be present at nonnegligible concentrations. A situation that may lead to hydrotrope induced toxicity.[32] The presence of sizable concentrations of free hydrotrope in the formulation is a serious consideration since it imposes restrictions as to the type and number of hydrotropic structures acceptable in pharmaceutical applications. Published toxicology information is rather limited for DENA[33–35] and extremely limited for DMBA.[36] However, it is reasonable to expect that the use of plain hydrotropic agents as solubilizing excipients in pharmaceutical formulations is bound to present serious challenges at best. Therefore, for hydrotropy to become a fully exploitable phenomenon in pharmaceutical applications, it is necessary to address the potential risks associated with the systemic absorption of the free hydrotrope, while still taking advantage of its solubilization properties. In other words, a system is needed where the hydrotrope is let to exert its solubilizing effect while being effectively prevented from being systemically absorbed. A viable approach for such a system is one where the hydrotrope is turned nonbioavailable through covalent linkage to a polymeric matrix. The development of polymeric hydrotropic micelles,[37–39] whose hydrophobic core hosts a covalently linked hydrotrope is likely to serve this purpose. We propose that by covalently linking hydrotropes to polymeric micelles, it will be possible to expand and exploit hydrotropy as a practical solubilizing tool for drugs, first in support of drug discovery efforts, but eventually for preclinical and clinical formulations. However, investigations on polymeric micelles with new and existing hydrotropes such as DMBA and DENA need to be first undertaken, and this will be the subject of a subsequent report.

CONCLUSIONS

This study identified that the two aromatic hydrotropes, DENA and DMBA, are good solubilizing agents for a variety of poorly soluble drugs. DMBA showed higher solubilization capacity and more nonspecific solubilization tendency than DENA. Vapor osmometry data suggest that the difference in solubilization power between the two agents is the result of their differences in self-aggregation properties. Such differences are, in turn, the result of the different hydrophobicity between DENA and DMBA. As in the general case, aromatic moieties in the solute significantly favor the hydrotropic effect. However, comparison between DENA and DMBA shows that the specific interactions of the particular hydrotrope add a whole dimension suitable for the optimization of solubilization strategies. A good portion of poorly soluble drugs are hydrophobic and have aromatic rings in their structure. The hydrotropic agents studied here can be expected to make strong solubilizers (by orders of magnitude) for a wide variety of poorly soluble drugs. The results of this study lead us to hypothesize that it should be possible to identify a small set of hydrotropic moieties whose solubilization properties encompass the wide range of chemical diversity of drugs in general, and further research on this area is warranted.

ACKNOWLEDGMENTS

This work was supported in part by NIH through GM065284 and the Korea Research Foundation Grant funded by the Korean Government (KRF-2008-357-D00072).

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