Pharmaceutical Research, Vol. 25, No. 5, May 2008 (© 2007)
DOI: 10.1007/s11095-007-9500-4

Research Paper

Quantitative Solubility Relationships and the Effect of Water Uptake in Triglyceride/Monoglyceride Microemulsions

Sagar S. Rane,[1] Yichen Cao,[1] and Bradley D. Anderson[1,2]

Received August 20, 2007; accepted November 9, 2007; published online December 19, 2007

Purpose. This paper aims to elucidate quantitative relationships between small molecule solubility/water-uptake in triglyceride/monoglyceride lipid formulations, the chemical structure of the solute, and the solvent composition.
Methods. Solubility and water uptake in tricaprylin/1-monocaprylin and tricaprylin/1-monocaprin mixtures in the "microemulsion" region at 37°C were determined with HPLC and KF coulometry, respectively. Twelve model solutes varying in hydrogen bond acidity, basicity, polarity, and molecular volume were chosen. Linear free energy relationships (LFER) (Abraham type) were implemented to obtain solvent coefficients at various monoglyceride concentrations.
Results. Profiles for both solubility and water uptake (at different water activities) in lipid mixtures containing different monoglycerides were superimposable, producing a single master curve when the monoglyceride concentrations were plotted on a molar scale. The LFER derived solvent coefficients showed a systematic dependence on the lipid composition consistent with the view that relative solubility is determined largely by the molar concentrations of individual functional groups such as glyceride ester moieties and hydroxyl groups. At low RH, water uptake increased linearly with monoglyceride concentration while cooperativity was evident in water uptake profiles at high RH.
Conclusions. This study provides a potential universal framework for predicting relative drug solubility in mixtures containing fully saturated triglycerides and monoglycerides.

KEY WORDS: linear free energy relationships; lipid formulation; microemulsions; solubility; triglycerides/monoglycerides.

INTRODUCTION

The oral route is generally the preferred method for drug administration due to its noninvasive nature, ease of use, low cost, and greater patient autonomy (1). However, numerous potential lipophilic drugs exhibit poor oral bioavailability due to their poor water solubility, despite their high membrane permeability. Indeed, poor aqueous solubility has been identified as the single largest physicochemical problem hindering oral drug absorption and lengthening drug discovery time in the current high throughput screening/combinatorial chemistry era, with more than 40% of new drug candidates belonging to this category (2).

Formulation of poorly water soluble drug candidates as solutions in lipid-based vehicles is an attractive technique for enhancing their oral bioavailability. However, selection of the optimal lipid-based delivery system for a given drug requires a consideration of numerous physicochemical and biological factors (3–10) which cannot be evaluated easily. Presently, the solubility and stability must be determined experimentally for each drug molecule/vehicle under consideration. The development of computational methods that would allow a drug candidate's solubility in a given lipid vehicle to be estimated from its chemical structure would be quite valuable in the early stages of formulation.

A number of models for predicting relative solubility in various solvent systems are available in the literature. Group contribution-based approaches (11–14) have been optimized for certain specific applications to predict, for example, vapor–liquid equilibria or octanol/water partition coefficients. Other semi-empirical models based on fitting experimental solubility data to thermodynamic expressions have been used primarily for interpolation purposes (15–20). These models require experimental data before predictive relationships can be built. Another drawback of these models is that their focus is on the general shape of the solubility curve in mixtures, rather than predicting solubility from solute and solvent structure. A more fundamental analysis based on solute–solvent interactions may yield deeper insights into solvation thermodynamics and drug solubility.

[1] Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, A323A, ASTeCC Building, Lexington, KY 40536, USA.
[2] To whom correspondence should be addressed. (e-mail: bande2@email.uky.edu)

ABBREVIATIONS: ACT, 9-Anthracenemethyl acetate; ANT, Anthracene; BEN, Benzamide; CAR, 9-Anthracene carboxylic acid; CHL, 9,10-Bis(chloromethyl) anthracene; C8, 1-monocaprylin; C10, 1-monocaprin; LFER, Linear free energy relationship; MEB, 4-Methoxy benzamide; MET, 9-Anthracene methanol; NAP, 1-Naphthalene acetic Acid; NMET, *N*-methyl benzamide; PHE, *p*-Phenylene diacetic acid; RH, relative humidity; TOL, *p*-Toluic acid; XYL, *p*-Xylylene glycol.

Linear free energy relationships (LFER) developed by Abraham *et al.* appear to be promising semi-empirical methods for predicting relative solubility once an experimental database or a "training set" is built for the solvent of interest (21–25). The Abraham approach assumes that the free energy to transfer a solute from one phase to another can be written as the sum of independent contributions to the free energy arising from different types of solute–solvent interactions. Therefore, the contribution of each type of interaction to the overall free energy of transfer appears as the product of a solute descriptor and the difference in the solvent coefficients of the two solvents involved in the transfer. The Abraham approach to LFER has also been applied to different partitioning phenomena to predict retention times on chromatographic stationary phases (26–28), drug partitioning between blood and the brain (29), and solvation and selectivity in ionic liquids (30,31). The method can be extended to virtually any process involving the transfer of solute from one phase to another (25,32).

Typical constituents of lipid formulations include long or medium chain triglycerides, long or medium chain mixed mono- and diglycerides, individual or mixed surfactants, and hydrophilic solvents (33). The focus of this paper is on triglyceride/monoglyceride/water systems. These components were selected because they are representative of the core constituents of many lipid-based self-emulsifying drug formulations and simple two- or three-component systems are more amenable to systematic analysis. Freeze-fracture electron microscopy (34) and X-ray diffraction evidence (35) suggest that monoglyceride/triglyceride/water mixtures exhibit medium-range order in the form of thermodynamically stable oriented stacks of bilayer lamellae, referred to as inverse micelles in binary (monoglyceride/water) systems or microemulsions in ternary systems. The ability of self-emulsifying drug delivery systems to form microemulsions upon mild agitation (36) may be extremely useful from a pharmaceutical perspective because microemulsions are thermodynamically stable and can potentially solubilize lipophilic drugs to a greater degree through interfacial interactions. However, the utility of each formulation will depend on whether the solvent capacity is adequately retained on dilution with aqueous phase.

Previous work in this laboratory (37,38) and elsewhere (39) suggests that solvent characteristics change systematically with solvent features such as the concentrations of polar fragments. Cao *et al.* (37) applied the linear free energy approach developed by Abraham to obtain solvent coefficients for solvation in tricaprylin/squalane mixtures. The work demonstrated that the solvent descriptors changed systematically with the concentration of triglyceride ester moieties suggesting independent contributions from the structural elements of the solvent mixture. A master equation was derived having the potential to predict triglyceride/water partition coefficients for any small molecule for which Abraham solute descriptors can be obtained. Triglycerides contain three alkyl chains and three ester groups. The length of the alkyl chains determines not only the overall hydrophobicity of the molecule, but also the molar concentration of ester groups in the solvent, and hence its polarity and hydrogen bond accepting ability. Therefore, it is not surprising that such relationships have been observed. In this work, we aim to extend this approach to a more complex system of triglyceride/monoglyceride/water and derive a master equation for predicting relative solubility. We explore whether a similar systematic dependence of the solvent coefficients on the triglyceride ester concentration, monoglyceride hydroxyl concentration, and alkyl chain lengths would exist in these mixtures.

Natural oils used in lipid vehicles also contain varying amounts of water between 300 and 9,000 ppm (40,41). Monoglycerides can retain large amounts of water, e.g. tricaprylin/1-monocaprylin mixtures in the microemulsion range may contain up to 10–15% wt/wt of water at 20°C (42). Solvated water can have a substantial effect on the solubilization capacity of lipid mixtures (37) as well as on the chemical stability of dissolved drugs. Therefore, we have also examined the uptake of water and its effect on solubilization capacity in triglyceride/monoglyceride mixtures as a function of water activity and hydroxyl and ester group concentration. To understand the effect of monoglyceride chain length or hydroxyl group concentration on solubility and water uptake, tricaprylin/1-monocaprylin and tricaprylin/1-monocaprin systems with varying concentrations of the monoglyceride were compared at 37°C. Each mixture was studied at two relative humidity conditions (i.e., ~6 and 100%). Twelve solutes were selected with widely varying intrinsic properties such as polarity, hydrogen bond donating and accepting abilities, and molecular volumes. Regression analyses were performed on the solubility data using the Abraham LFER equation to generate solvent coefficients for each lipid mixture relative to the base system, pure tricaprylin.

MATERIALS AND METHODS

Chemicals and Reagents

The twelve model solutes chosen for this investigation were mono- or di-substituted benzenes, napthalenes, or anthracenes. Structures of the model solutes employed and their melting points are shown in Table I. Anthracene and 9,10-bis(chloromethyl) anthracene (purity >95%) were purchased from TCI America (Portland, OR) and used directly without any further purification. 9-Anthracene methanol, *p*-xylylene glycol, 9-anthracene carboxylic acid, *p*-toluic acid, *p*-phenylene diacetic acid, 1-naphthalene acetic acid, 4-methoxy benzamide, benzamide and *N*-methyl benzamide were purchased from Sigma Inc. (St. Louis, MO). All of these reagents had a reported purity >98%.

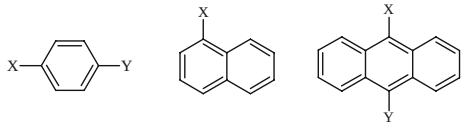
The solute 9-anthracenemethyl acetate was synthesized in our laboratory by reacting 9-anthracene methanol with excess acetic anhydride at 50°C. Its purity was >97% as determined by HPLC.

Tricaprylin (purity>99%) was purchased from Sigma Inc. (St. Louis, MO) and 1-monocaprin (purity >95%) was purchased from TCI America. 1-Monocaprylin (95.7% purity by GC) was a gift from S & J Lipids, Inc. (Ostrander, OH). Only HPLC grade solvents and de-ionized water were used for the preparation of mobile phases and solutions. Saturated KOH solution and pure water were used for the preparation of constant relative humidity chambers.

Lipid Mixtures

Tricaprylin/1-monocaprylin and tricaprylin/1-monocaprin mixtures were prepared at varying fractions of the monoglyc-

Table I. Structures of the Model Solutes, Melting Points, and Chromatographic Properties



Type: A B C

Solute[Mobile phase]	Type	Structure	Melting point (°C)	λ (nm)	Retention volume (mL)
p-Toluic acid[a]	A	X= COOH Y= CH_3	181	237	6.5
p-Phenylene diacetic acid[a]	A	X=CH_2COOH Y=CH_2COOH	250	210	2.7
p- Xylylene glycol[b]	A	X=CH_2OH Y=CH_2OH	118	218	3.1
4-Methoxy benzamide[b]	A	X= $CONH_2$ Y= OCH_3	165	252	3.1
Benzamide[c]	A	X= $CONH_2$ Y=H	128	240	3.9
N-methyl benzamide[c]	A	X= $CONHCH_3$ Y=H	79	240	4.2
1-Naphthalene acetic acid[a]	B	X=CH_2COOH	130	223	22.7
Anthracene[b]	C	X=H Y=H	214	254	5.3
9-Anthracene methyl, acetate[b]	C	X=CH_2OCOCH_3 Y=H	109	254	4.1
9- Anthracene methanol[b]	C	X=CH_2OH Y=H	161	254	4.4
9-Anthracene carboxylic acid[d]	C	X=COOH Y=H	215[f]	252	3.4
9,10 -Bis(chloromethyl) anthracene[e]	C	X=CH_2Cl Y=CH_2Cl	259[f]	258	3.2

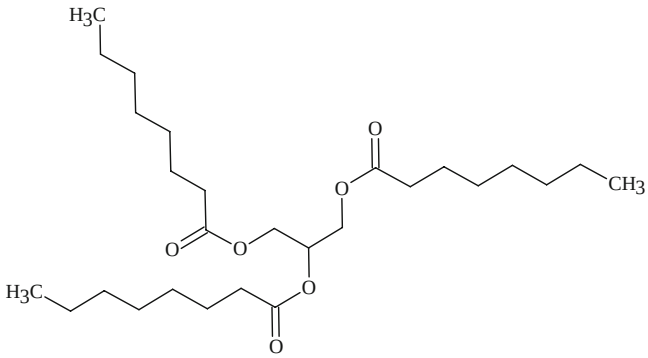
[a] 90% pH 7.0 phosphate buffer 20 mM:10% ACN
[b] 90% ACN:10% water
[c] 65% ACN:35% water
[d] 50% pH 7.0 phosphate buffer 20 mM:50% ACN
[e] 80% ACN:20% THF
[f] decomposition temperature.

eride. The structures of tricaprylin, 1-monocaprylin (C8), and 1-monocaprin (C10) are shown in Scheme 1. Tricaprylin/1-monocaprylin mixtures contained 0–40% 1-monocaprylin by weight, with an increment of 10%, while tricaprylin/1-monocaprin mixtures consisted of 0, 5, 10, 20 and 30% 1-monocaprin by weight. The above ranges of monoglyceride concentration were chosen to include monoglyceride contents representative of those in typical commercial lipid formulation mixtures, such as Labrasol and Capmul MCM (8,33). In addition, the highest monoglyceride concentration in each system was close to the highest allowed monoglyceride concentration in the mixture before precipitation or phase separation of the monoglyceride would set in under the experimental conditions. Since 1-monocaprylin is a semi-solid and 1-monocaprin is a solid at room temperature (25°C), the lipid mixtures were prepared using weight fractions. A fresh lipid mixture was made for each solubility study and mixed thoroughly by vortexing at 37°C.

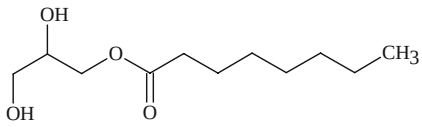
Solubility Studies

All solubility studies were conducted at 37°C. The lipid mixtures prepared above were divided into two portions, 1 ml each, and transferred to 4 ml glass vials containing excess solute which were pre-warmed to 37°C to avoid precipitation of the monoglyceride. To determine water uptake and to understand the effect of water content on solubility, two constant relative humidity chambers were used; a saturated aqueous KOH chamber (43) (~6% RH) and a chamber equilibrated with pure water (100% RH). The relative humidities were measured with a hygrometer (Fisher Scientific, RH range 2~98%). Vials were kept in the respective constant relative humidity (RH) chamber for at least 10 days. (Preliminary studies had shown that the water content of the lipid mixtures reached equilibrium in about 7 days).

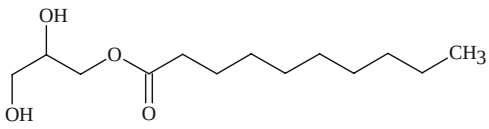
The vials were then removed from the constant relative humidity chambers, capped tightly to prevent the transfer of water, and rotated for at least 3 days in an incubator at 37°C to ensure reproducible solubility measurements. After equilibration was achieved, the vial contents were transferred to 2 ml polypropylene centrifuge tubes pre-equilibrated to 37°C. The disposable pipette tips used in the transfer were also pre-equilibrated to 37°C to avoid precipitation. The samples were centrifuged at 13,000 rpm and 37°C for 10 min and a portion



Tricaprylin



1-Monocaprylin
(C8)



1-Monocaprin
(C10)

Scheme 1. The structures of tricaprylin, 1-monocaprylin (C8), and 1-monocaprin (C10)

of the solution phase was collected for HPLC solubility analysis.

HPLC Assays

Aliquots (30–50 μL) of the lipid mixture were accurately weighed and dissolved in 6–10 ml of mobile phase. Solutions containing highly soluble solutes were diluted several fold further as needed. All samples were analyzed using a modular HPLC with a Supelco ABZ+ column (4.6 mm×25 cm; 5 μm) (Bellefonte, PA) at room temperature and variable wavelength detection (2487 Dual Wavelength Absorbance Detector, Waters, Inc., Milford, MA). The mobile phases, retention volumes, and wavelengths used for the analyses are listed in Table I. The HPLC assay for each model solute was validated over a 100-fold concentration range using at least two sets of independent standards each diluted 10× and 100×. The chromatograms were acquired and analyzed using a Model 302 Chromatography Data System (Quadrex Corp., Woodbridge, CT).

Water Content Measurements

The water content of the lipid mixtures was determined after the samples were centrifuged to remove excess solid. A Brinkmann 684 KF coulometer (Westbury, NY) was used to measure the water content. Lipid mixtures equilibrated at 6 and 100% RH were injected into the Karl–Fisher cell with a reagent composed of 70% Coulomat AG (Aldrich Chemicals) and 30% 1-dodecanol (v/v). The 1-dodecanol was found to be necessary to dissolve the lipid and to obtain reproducible results for the water content. Two Hydranal® standards (0.10 and 1.00 mg/ml water) were employed to create calibration curves for the instrument.

Determination of LFER Relationships for Water Uptake and Solubility in Triglyceride/Monoglyceride Mixtures

Data for water uptake and solubility as a function of monoglyceride concentration in the lipid mixtures were used to generate linear free energy relationships (LFER) as described by Abraham *et al.* (21–25). The solubility of a given model solute (S) in each mixture was normalized to its solubility in pure tricaprylin (S_0). The logarithm of this ratio, $\log(S/S_0)$, is proportional to the free energy of transfer of the solute between the two solvents, and can be expressed (Eq. 1) as the sum of independent contributions arising from different types of solute–solvent interactions where the contribution of each type of interaction appears as the product of a solute descriptor and the difference in the solvent coefficients of the two solvents involved in the transfer. Thus,

$$\log(S/S_0) = \Delta r R_2 + \Delta s \pi_2^H + \Delta a \Sigma \alpha_2^H + \Delta b \Sigma \beta_2^H + \Delta v V_x \quad (1)$$

where R_2 is the excess molar refraction index, π_2^H is the solute dipolarity/polarizability, $\sum \alpha_2^H$ and $\sum \beta_2^H$ are the effective hydrogen bond acidity and basicity of the solute, and V_x with units of $(cm^3/mol)/100$ is McGowan's characteristic volume (44,45). Values obtained for the various solute descriptors using the group contribution approach (46) are listed in Table II along with the abbreviations used for each solute. Each solute molecule is composed of fragments identified in ref. 46, and the descriptors of the fragments are added to obtain the descriptors for the solute molecule. The regression coefficients obtained from least-squares regression analyses of the data according to Eq. 1, Δr, Δs, Δa, Δb, and Δv, represent the differences in solvent coefficients of the lipid mixtures corresponding to each solute descriptor as the monoglyceride concentration increases relative to pure tricaprylin. The coefficient r denotes the ability of a solvent to interact with the solute through n- and π- electron pairs, i.e. through dispersive forces, s denotes the ability of the solvent to interact with dipolar/polarizable solutes, a and b are measures of the hydrogen bond basicity and acidity of the solvent respectively, and v is a descriptor for the energy of cavity formation.

Micromath Scientist version 2.02 (Micromath Co., S. L. C. UT) was employed for all the computer based least-squares regression analyses.

Choice of Solutes

The twelve solutes were chosen due to their low/limited solubility in lipids and to represent a diversity of functional groups and descriptor values. These solutes have a range of

Table II. Descriptors for All Model Solutes Obtained from Functional Group Contributions (Except Water)

Solute	Abbreviation	R_2	π_2^H	$\sum \alpha_2^H$	$\sum \beta_2^H$	V_x
p-Toluic acid	TOL	0.784	0.91	0.591	0.455	1.0726
p-Phenylene diacetic acid	PHE	0.896	1.291	1.179	0.781	1.4288
p-Xylylene glycol	XYL	1.002	1.173	0.693	0.991	1.1156
4-Methoxy benzamide	MEB	1.014	1.61	0.455	0.861	1.1724
Benzamide	BEN	0.992	1.449	0.455	0.654	0.9728
N-methyl benzamide	NMET	0.941	1.464	0.371	0.721	1.1137
1-Naphthalene acetic Acid	NAP	1.512	1.276	0.591	0.519	1.376
Anthracene	ANT	2.128	1.261	0.003	0.257	1.4544
9-Anthracenemethyl, acetate	ACT	2.176	1.668	0.003	0.592	1.9515
9- Anthracene methanol	MET	2.301	1.559	0.348	0.684	1.654
9-Anthracene carboxylic acid	CAR	2.248	1.618	0.591	0.579	1.6697
9,10-Bis(chloromethyl) anthracene	CHL	2.398	1.527	0.003	0.235	1.981
Water	–	0.39	0.4	0.35	0.38	0.17

hydrogen bond basicity, hydrogen bond acidity, molecular volume, and polarizability (Table II), which is necessary to ensure that each solvent descriptor in the model is well determined and is statistically significant. The model solutes exhibited the greatest diversity in their effective hydrogen bond acidity, α_2^H, and hydrogen bond basicity, β_2^H, parameters, followed by the molecular volume term, V_x, and the refractive index descriptor, R_2. The term that varied the least within the set of solutes was the term for the solute's dipolarity/polarizability, π_2^H . A larger set of solutes with an expanded range of dipolarity/polarizability would be beneficial in further refining the values of the solvent parameter Δs and its dependence on the monoglyceride content.

Solute Descriptors

One of the principal obstacles in the application of the LFER approach is the need to perform experimental measurements to obtain most of the solute descriptors (V_x is the only exception) before the predictive abilities can be implemented. This requires that the compound in question be available and experiments be performed, which reduces the appeal of the approach. Recently Platts *et al.* (46) have proposed an alternate strategy to obtaining each solute descriptor by adding up the individual contributions from various well defined molecular fragments. Thus, the solute descriptors can be obtained solely from chemical structure. However, this approach also relies on an experimental database, which must be large and relevant to the transfer process of interest to provide reliable estimates for each group contribution. In this study, descriptors from direct experiments were not available for all solutes employed and therefore the fragment approach was utilized (Table II).

RESULTS

The solubility and water content data generated under dry (~6% RH) and wet (100% RH) conditions in 1-monocaprylin/tricaprylin and 1-monocaprin/tricaprylin mixtures at 37°C are listed in Tables III and IV. Solubilities and water uptake in these mixtures were generated with increasing monoglyceride concentration up to 40% wt/wt 1-monocaprylin and up to 30% wt/wt 1-monocaprin. Solubilities of all twelve solutes in tricaprylin/1-monocaprylin mixtures and five representative solutes in tricaprylin/1-monocaprin mixtures are shown in Table III. Similarly, water uptake was determined for certain representative cases to explore relationships between water uptake and solute properties, as shown in Table IV.

Master Curve for Water Uptake and Solubility

The water uptake and solubility data for benzamide (BEN) and 9-anthracene carboxylic acid (CAR) in solute saturated triglyceride/monoglyceride mixtures under low (~6% RH) and high (100% RH) relative humidity conditions are shown in Figs. 1 and 2, respectively. The results show that the solubility and water uptake can be correlated with the molar concentration of polar functional groups, regardless of the chain lengths of lipids employed to generate that concentration. It is noteworthy that the solubility data

Table III. Solubilities of the Model Solutes in Tricaprylin/1-Monocaprylin and Tricaprylin/1-Monocaprin Mixtures Under Dry (~6% RH) and Wet (~100% RH) Conditions at 37°C

RH (%)	~6%						100%				
Monoglyceride (wt/wt) %	0	10	20	30	40		0	10	20	30	40
Solute	Solubility in 1-Monocaprylin/Tricaprylin mixtures (mmol/L)										
Anthracene	49.9	46.3	44.3	42.3	39.6		50.8	48.4	43.9	40.3	35.8
9-Anthracene methyl, acetate	299	296	294	297	296		313.1	298	287	271	254
9-Anthracene methanol	52.1 (1)	68.2 (1)	77.6 (3)	84.1 (3)	92.4 (2)		57.1 (0.3)	73.8 (1)	77.5 (1)	81.7 (1)	85.7 (3)
p-Xylylene glycol	20.1	33.5	54.6	80	99.8		25.1	48.1	72.6	100	135
9-Anthracene carboxylic acid	25.9 (0.3)	45.4 (0.1)	62.2 (2)	73.8 (3)	83.2 (3)		40.5 (0.3)	66.6 (0.4)	79.6 (2)	87.8 (2)	90.3 (2)
p-Toluic acid	108 (4)	170 (0.5)	229 (5)	266 (0.4)	289 (2)		162 (1)	242 (1)	304 (2)	334 (1)	365 (5)
p-Phenylene diacetic acid	0.15 (0.01)	0.94	1.95	3.27	4.32 (0.1)		0.31 (0.01)	2.68	4.56	6.59	9.17 (0.2)
1-Naphthalene acetic acid	260	385	481	563	615		376	551	674	757	825
9,10-Bis(chloromethyl) anthracene	3.28	2.94	2.74	2.41	1.98		3.95	3.26	2.63	2.19	1.59
4-Methoxy benzamide	11.2	35	63.1	93.8	122.4		15.7	44.8	81.2	131	170
Benzamide	49.7	137	227	320	411		75.2	230	368	523	667
N-methyl benzamide	331	767	1,158	1,483	1,760		472	1,220	1,821	2,665	–
Monoglyceride (wt/wt) %	0	5	10	20	30		0	5	10	20	30
Solute	Solubility in 1-Monocaprin/Tricaprylin mixtures (mmol/L)										
Anthracene	49.9	49.3	48.1	–	–		50.8	49	47.3	44.4	40.3
9-Anthracene methanol	52.1	58.3	64.1	–	–		57.1	65.4	70.6	78.7	84.8
9-Anthracene carboxylic acid	25.9	35.2	44.2	–	–		40.5	54.6	65.5	76.3	83.1
Benzamide	49.7	91	141	–	–		75.2	127	197	324	438
N-methyl benzamide	331	471	658	1,046	1,234		472	804	1,175	1,831	2,375

All units are mmol/L. The standard deviations are shown in brackets (when more than one set was used).

Table IV. Equilibrium Water Content (% wt/wt) in Tricaprylin/1-monocaprylin and Tricaprylin/1-Monocaprin Mixtures Saturated with the Model Solutes Under Dry (~6% RH) and Wet (~100% RH) Conditions at 37°C

RH. (%)	~6%					100%				
Monoglyceride (wt/wt) %	0	10	20	30	40	0	10	20	30	40
Solute	Water uptake in 1-Monocaprylin/Tricaprylin mixtures (% wt/wt)									
Anthracene	0.028 (5e−4)	0.053 (1e−3)	0.078 (5e−4)	0.105 (1e−4)	0.132 (1e−3)	0.25 (4e−3)	1.35 (0.02)	4.49 (0.03)	7.13 (0.03)	9.16 (0.1)
9-Anthracene methanol	0.033 (2e−3)	0.056 (2e−4)	0.082 (6e−4)	0.118 (1e−3)	0.149 (1e−3)	0.191 (4e−5)	1.43 (6e−3)	4.91 (0.02)	7.72 (0.06)	10.2 (8e−3)
p-Xylylene glycol	0.035 (1e−3)	0.058 (2e−3)	0.086 (4e−3)	0.125 (7e−3)	0.154 (1e−3)	0.288 (2e−3)	0.738 (2e−3)	3.94 (0.2)	6.09 (0.09)	9.07 (0.09)
9-Anthracene carboxylic acid	0.034 (1e−3)	0.056 (3e−3)	0.083 (1e−3)	0.111 (7e−4)	0.147 (5e−3)	0.319 (8e−4)	1.32 (0.02)	3.68 (0.06)	6.31 (0.02)	9.13 (0.02)
p-Toluic acid	0.035 (1e−4)	0.056 (7e−4)	0.082 (6e−4)	0.111 (5e−3)	0.14 (2e−3)	0.335 (1e−3)	1.07 (3e−3)	2.94 (0.01)	5.24 (0.02)	7.68 (0.02)
p-Phenylene diacetic acid	0.031 (1e−3)	0.052 (2e−4)	0.079 (6e−4)	0.11 (4e−4)	0.141 (4e−4)	0.254 (3e−4)	1.54 (1e−3)	3.58 (2e−3)	5.58 (0.03)	7.51 (0.09)
Benzamide	0.073 (1e−4)	0.112 (1e−3)	0.165 (2e−4)	0.218 (5e−3)	0.242 (1e−3)	0.243 (4e−3)	1.38 (9e−3)	3.61 (0.02)	4.85 (0.09)	6.44 (0.02)
N-methyl benzamide	0.075 (1e−3)	0.121 (1e−3)	0.159 (4e−3)	0.183 (7e−4)	0.201 (2e−3)	0.436 (7e−3)	1.14 (0.01)	1.92 (0.05)	2.97 (0.04)	–
No solute	–	–	–	–	–	0.239 (3e−3)	1.64 (0.09)	3.96 (0.02)	6.07 (0.03)	8.11 (0.09)
Monoglyceride (wt/wt) %	0	5	10	20	30	0	5	10	20	30
Solute	Water uptake in 1-Monocaprylin/Tricaprylin mixtures (% wt/wt)									
Anthracene	.028	0.041 (1e−3)	0.053 (4e−4)	–	–	.25	0.443 (6e−3)	1.0 (0.02)	2.83 (0.02)	4.96 (0.35)
9-Anthracene methanol	.033	0.042 (1e−4)	0.055 (1e−4)	–	–	.191	0.451 (5e−3)	1.01 (0.02)	2.77 (0.01)	4.84 (0.07)
9-Anthracene carboxylic acid	.034	0.045 (2e−3)	0.056 (1e−4)	–	–	.319	0.532 (6e−3)	1.01 (5e−3)	2.75 (0.01)	5.05 (0.02)
Benzamide	.073	0.09 (1e−3)	0.11 (2e−3)	–	–	.243	0.475 (0.02)	0.99 (0.04)	2.81 (0.05)	4.19 (1e−3)
N-methyl benzamide	.075	0.105 (2e−3)	0.123 (4e−4)	0.148 (2e−3)	0.187 (4e−4)	.436	0.705 (0.03)	1.14 (0.02)	2.11 (0.04)	2.65 (0.3)
No solute	–	–	–	–	–	.239	0.466 (1e−3)	0.931 (0.02)	2.61 (0.01)	4.51 (0.02)

Standard deviations are shown in brackets (n=2).

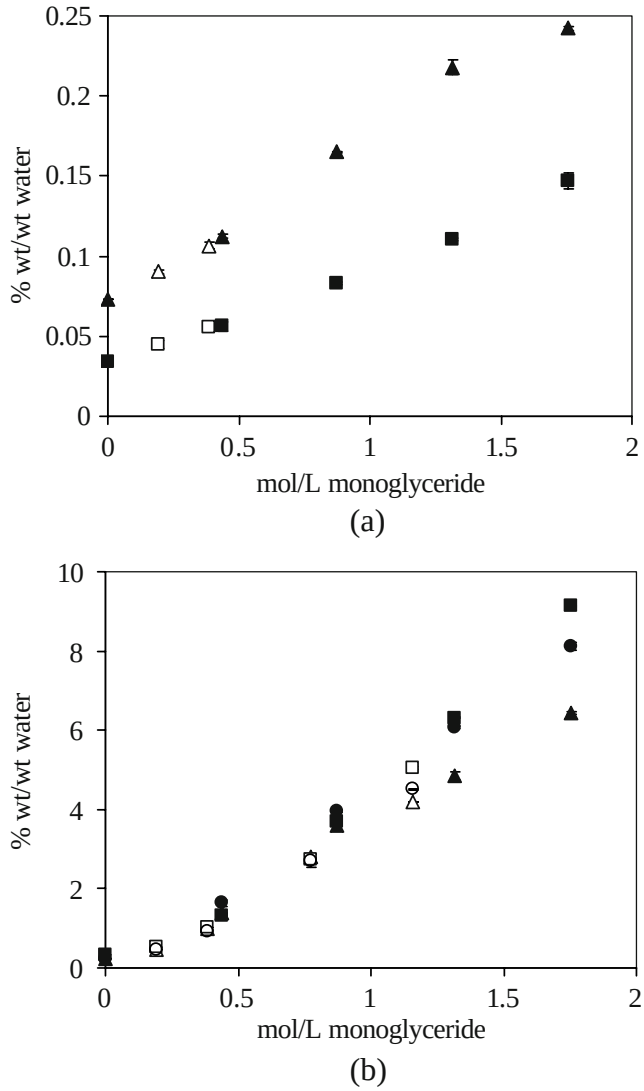


Fig. 1. Water uptake at 37°C in two triglyceride/monoglyceride mixtures saturated with various solutes from the data in Table IV at **a** low relative humidity (~6%) and **b** high relative humidity (100%). Symbols: BEN-C8(*filled triangle*); BEN-C10(*empty triangle*); CAR-C8(*filled square*); CAR-C10(*empty square*); and, in the absence of solute, C8(*filled circle*) and C10(*empty circle*). The water uptake data for a given solute superimpose onto a single curve when the monoglyceride concentration is plotted on a molar basis, regardless of the chain length of monoglyceride employed to generate that concentration. A change in the slope of water uptake *versus* monoglyceride concentration is apparent at 100% RH but not at 6% RH.

superimpose onto a single curve only when the monoglyceride concentration is plotted on a molar basis. Plots of solubility *versus* volume fraction or weight fraction did not superimpose, especially at high monoglyceride concentrations (not shown). The water uptake data for the monocaprylin and monocaprin systems also superimpose when plotted on a molar scale [Fig. 1a–b] and further illustrate the importance of the polar functional group concentration. These observations imply that the results can be extended to other triglyceride/monoglyceride mixtures.

Water uptake at low relative humidity was solute dependent in some cases, as illustrated for benzamide *versus* 9-anthracene carboxylic acid (Fig. 1a and Table IV), while solute dependent differences in water uptake were not statistically significant at high relative humidity when solute containing mixtures were compared with those containing no solute (Fig. 1b and Table IV).

In Fig. 2, four representative solutes having different physicochemical properties were chosen for displaying the solubility profiles. Among the four solutes chosen, benzamide, 9-anthracene carboxylic acid, and 9-anthracene methanol vary in their hydrogen bond donating/accepting properties while anthracene has no polar functional groups and therefore is only a weak hydrogen bond acceptor and has no hydrogen donating capability (see Table II). In addition, the anthracene derivatives are larger in molecular volume than benzamide and therefore enable the effect of molecular volume on the solubility profiles to be explored. Benzamide, 9-anthracene carboxylic acid, and 9-anthracene methanol all show increasing solubility with monoglyceride concentration indicating that solutes that have hydrogen bond accepting/donating functional groups can favorably interact with complimentary polar functional groups in the monoglyceride. In contrast, the solubility of anthracene decreases with increasing monoglyceride concentration [similar observations were made for 9-anthracenylmethyl acetate and 9,10-bis (chloromethyl) anthracene]. This trend suggests that the lipid mixture is becoming more structured with increasing monoglyceride concentration, resulting in an increasingly unfavorable energy for cavity formation, which would disfavor insertion of a large, relatively nonpolar molecule with no functional groups (or with weakly polar groups) without any compensation from new energetically favorable specific interactions. This issue will be discussed in more detail in a later section.

Relative Solvent Coefficients

The solvent coefficient differences listed in Table V and graphically illustrated in Figs. 3a–b were obtained from fits of the normalized solubility data to Eq. 1. These coefficients were obtained without including the solubility data for *p*-phenylene diacetic acid and water (see further discussion below). Each solvent coefficient changes systematically with increasing monoglyceride concentration. The coefficients Δs and Δa increase, and Δv decreases monotonically with the monoglyceride concentration. The solvent hydrogen donating parameter, Δb, is close to zero and is insensitive to the monoglyceride concentration. The parameter Δr, which is also close to zero, shows only a slight decrease with increasing monoglyceride concentration. The changes in the coefficients indicate that with increasing monoglyceride concentration the lipid mixture is better able to interact with polar and hydrogen bond donating solutes and may also be becoming more structured, as suggested by the systematic decrease in Δv. These issues will be discussed further in the Discussion section.

The normalized solubilities for each model solute in various lipid mixtures, $\log(S/S_0)$, are plotted in Figs. 4 and 5. The points are experimental values whereas the solid lines denote the fitted values obtained from Eq. 1. Some interesting observations can be drawn from Figs. 4 and 5. For most of the solutes, including benzamide, 9-anthacene methanol, *p*-xylylene glycol, 9-anthracene carboxylic acid, *N*-methyl benzamide, *p*-toluic acid, 4-methoxy benzamide, and 1-

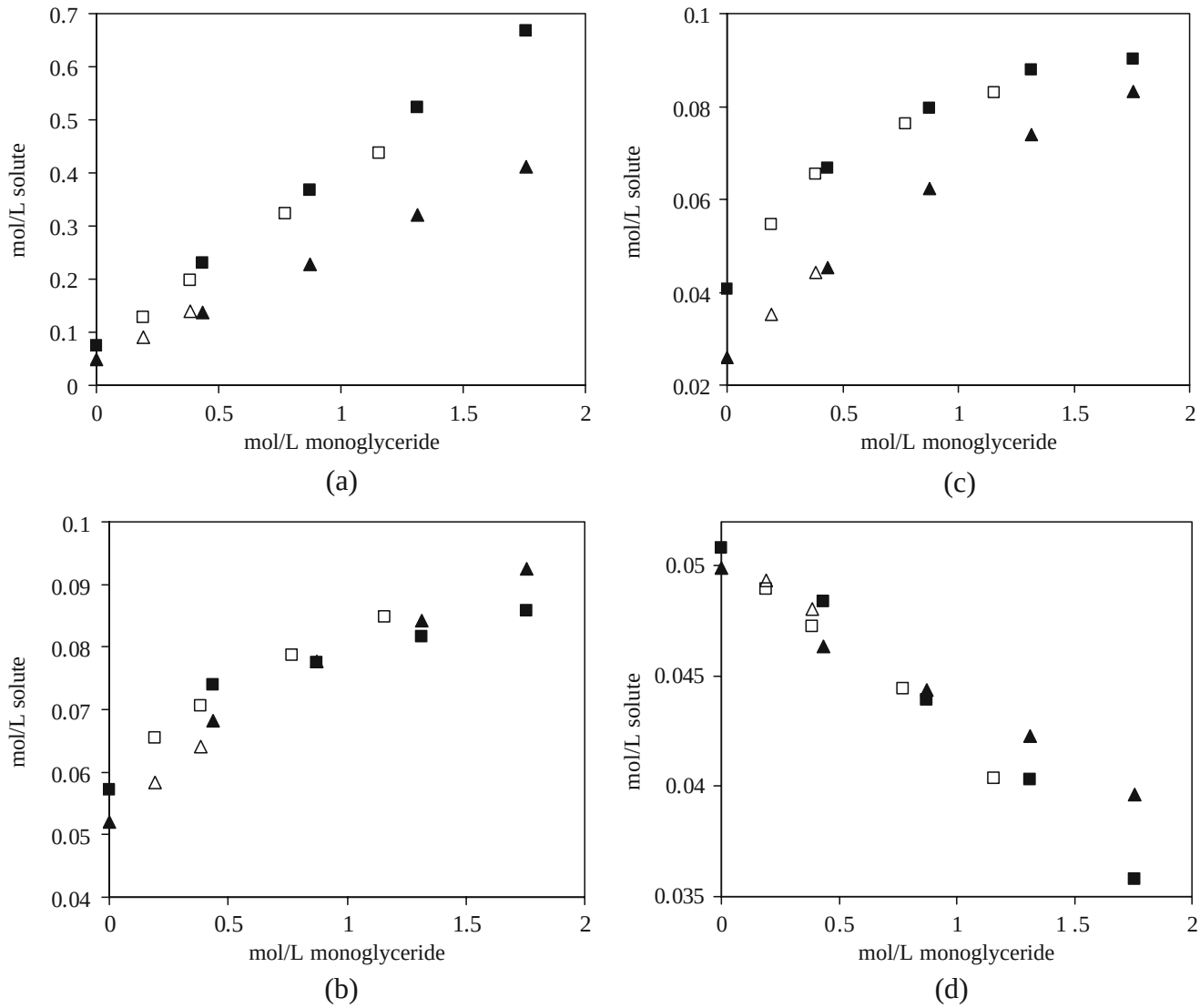


Fig. 2. Solubility profiles for representative solutes possessing a range of solute descriptor values under wet (100% RH) and dry (~6% RH) conditions at 37°C in two triglyceride/monoglyceride mixtures. Symbols: C8-DRY(*filled triangle*); C10-DRY(*empty triangle*); C8-WET (*filled square*); and C10-WET (*empty square*). Panels contain solubility data for: **a** benzamide; **b** 9-anthracene methanol; **c** 9-anthracene carboxylic acid; and **d** anthracene. The solubility data for a given solute at fixed RH superimpose onto a single curve when the monoglyceride concentration is plotted on a molar basis, regardless of the chain length of monoglyceride employed to generate that concentration. Solubility increases with monoglyceride concentration for solutes possessing strong hydrogen bond donor/acceptor groups, while the solubility of anthracene, a solute with no polar functional groups, decreases with increasing monoglyceride concentration. These plots are generated from the data in Table III.

naphthalene acetic acid, solubility increases with the monoglyceride concentration because these solutes possess strong hydrogen bond donor/acceptor groups. The solubility of 9-anthracenemethyl acetate however, is almost independent (very slightly decreasing) of monoglyceride concentration. This can be rationalized by considering the fact that the ester group on the solute cannot form strong hydrogen bonds with the lipid molecules. The solubilities of anthracene and 9,10-bis(chloromethyl) anthracene decrease significantly with increasing monoglyceride concentration, as discussed earlier. For future reference purposes, the solubilities of four solutes were also determined in *n*-decane at 37°C using the method described for lipid mixtures in this paper. The molar solubilities for various solutes in *n*-decane with standard deviations ($n=2$) are: anthracene, 0.0198 (0.0004); 9-anthracenemethyl acetate, 0.0496 (0.0008); 9-anthracene methanol, 0.0018 (0.0001); and 9-anthracene carboxylic acid 8.9×10^{-5} (1×10^{-6}).

Table V and the graphical illustration of the solvent coefficients in Fig. 3a–b indicate that the solvent coefficients in dry and wet mixtures are similar and the fit statistics reasonable. The predicted *versus* the experimental values of $\log(S/S_0)$ for the dry and wet mixtures are shown in Fig. 6a–b. The slopes of the regression lines are very close to 1.0, and the lines pass very near to the origin indicating a good fit.

Outlying Solutes and Limitations of the LFER Approach

The solubility data for water and the dicarboxylic acid, *p*-phenylene diacetic acid, were not fit well by the fragment descriptors and Eq. 1 when included with the remaining data set.

Table V. Regression Coefficients for Tricaprylin/1-monocaprylin/Water Vehicles at 37°C Generated by Fitting the Data in (A) Dry (~6% RH) and (B) Wet (100% RH) Conditions to Eq. 1

	mol/L monoglyceride	Δr	Δs	Δa	Δb	Δv	SD	R^2
A	0	0	0	0	0	0	0	0
	0.436	−0.120 (0.04)	0.570 (0.06)	0.273 (0.05)	−0.111 (0.08)	−0.316 (0.07)	0.0061	0.9923
	0.874	−0.189 (0.06)	0.770 (0.09)	0.423 (0.08)	−0.073 (0.12)	−0.415 (0.1)	0.0149	0.9921
	1.313	−0.211 (0.07)	0.903 (0.1)	0.502 (0.09)	0 (0.13)	−0.528 (0.12)	0.0195	0.9931
	1.755	−0.199 (0.08)	0.978 (0.11)	0.557 (0.1)	0.116 (0.14)	−0.650 (0.13)	0.0218	0.9941
B	0	0	0	0	0	0	0	0
	0.436	−0.093 (0.02)	0.626 (0.03)	0.244 (0.03)	−0.083 (0.04)	−0.409 (0.04)	0.0021	0.9974
	0.874	−0.169 (0.02)	0.854 (0.04)	0.362 (0.03)	−0.028 (0.05)	−0.546 (0.04)	0.0023	0.9987
	1.313	−0.214 (0.03)	1.052 (0.05)	0.403 (0.04)	0.065 (0.06)	−0.699 (0.06)	0.0042	0.9985
	1.755	−0.221 (0.03)	1.114 (0.04)	0.459 (0.04)	0.231 (0.05)	−0.817 (0.05)	0.0031	0.9992

Water and *p*-phenylene diacetic acid data were not included. SD and R^2 are the sum of squared deviations and R-squared values, respectively, the goodness-of-fit statistics. Standard deviations of the regression coefficients are shown in brackets.

This is illustrated for *p*-phenylene diacetic acid in Figs. 4c and 5c, where the experimental and model predicted (dashed lines—using the constants in Table V) solubility ratios for *p*-phenylene diacetic acid do not overlap. Possible factors that may account for this are discussed below.

DISCUSSION

Phase diagrams of self-emulsifying systems typically show that w/o microemulsions are formed at low water contents. In other regions of the phase diagram and at high water content (typically >15–20% w/w), unstable w/o or o/w emulsions may form (42,47–48). The phase diagram of a tricaprylin/1-monocaprylin/water system at 20°C is shown in Fig. 7. The region of the reverse micellar L2 phase *viz.* water-in-oil microemulsion is displayed.

Literature data showed that the monoglycerides, monocaprylin and monocaprin, have reasonably high solubility in water (49–51). In addition, the critical micelle concentration (CMC) of monoglycerides needed to form normal micelles in water is extremely low (52,53). Although monoglyceride solubility in water decreases as the chain length of the hydrophobic part increases, the CMC decreases more dramatically with the negative log of the CMC being proportional to the hydrophobic chain length (52,53). The potential for micelle formation in water complicates the determination of partition coefficients, necessitating the reliance on equilibrium solubility data in the present study. This is in contrast to an earlier study in squalane/tricaprylin mixtures (37) where partition coefficients were used to derive the solvent coefficients.

One way to envision the solvent properties of lipid-based formulation vehicles is to consider them as mixtures of glyceride ester moieties, polyoxyethylene ether functionalities, and hydroxyl groups dissolved in a relatively structured sea of saturated and partially unsaturated hydrocarbon chains of various lengths. This approach enables the use of simpler functional group approaches to describe the complex mixtures that can result when the various lipid-based vehicle components are mixed. The properties of the lipid mixtures are then directly determined by the concentration of each functional group, which also scales its contribution to the physical property under consideration.

Application of LFER Relationships to Triglyceride/Monoglyceride Mixtures

In a previous publication, Cao *et al.* (37) had successfully applied the linear free energy relationship approach as outlined by Abraham and obtained the solvent-coefficients that describe squalane/tricaprylin mixtures. Their work laid the foundation for the exploration of the LFER approach in a systematic manner using well defined and controlled systems. Literature data on solvent coefficients of various oils, such as olive oil, hexadecane, and alkanes, reveal that the coefficients appear to change systematically with the properties of the oils and correlate with the components of the system. For example, it was observed that while most of the coefficients for the hexadecane/water and alkane/water partitioning are similar to the olive oil/water partitioning, two coefficients—*s* and *a*, were markedly different for olive oil indicating the ability of olive oil to interact with dipolar/polarizable solutes and hydrogen bond donating solutes through ester functionalities in this oil. Such a correlation provides motivation to study lipid based system properties as a function of their components. In the squalane/tricaprylin system, the solvent coefficients were also found to change systematically with increasing tricaprylin content, reflecting the composition of the mixture. For example, with increasing tricaprylin content, Δs and Δa, the coefficients which describe the ability of the solvent to interact with dipolar/polarizable and hydrogen bond donating solutes, respectively, also increase, albeit non-linearly. Given these observations, the next logical step is to consider whether the LFER approach can be extended further, to more complex but compositionally well defined systems.

In this work, the LFER approach was extended to triglyceride/monoglyceride mixtures and the effect of water content on the solvent coefficients was considered. These systems are much more complex than the squalane/tricaprylin system studied previously due to the possibility of lipid self-assembly and the formation of organized phases. The squalane/tricaprylin system remains miscible and homogeneous at all compositions. However, the triglyceride/monoglyceride mixtures may not be locally homogeneous. As indicated by Fig. 7, such mixtures can exist in various phases

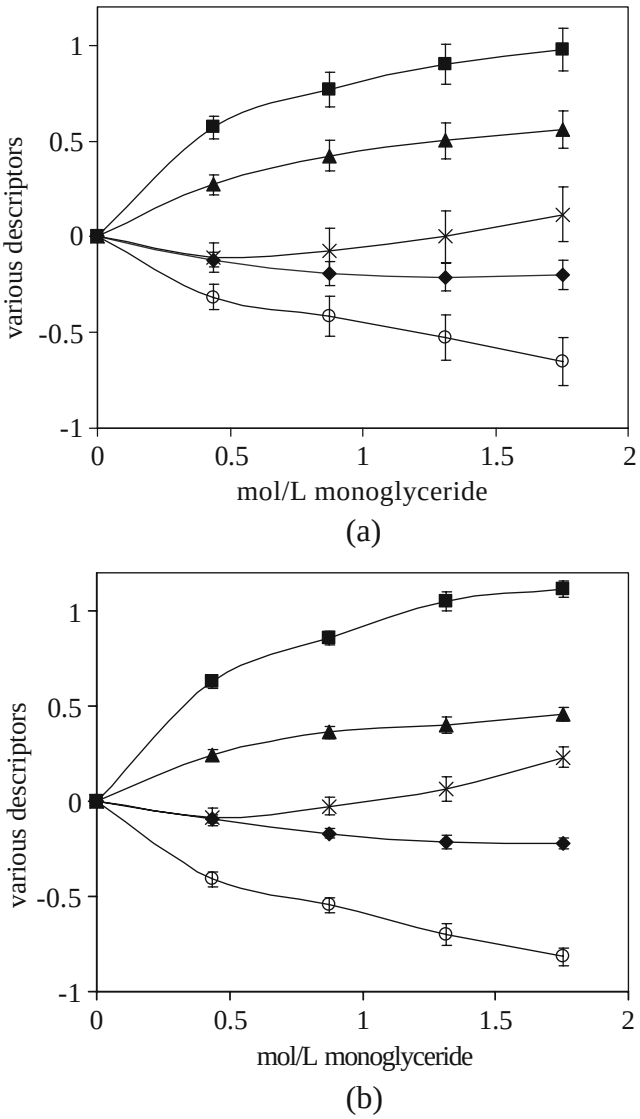


Fig. 3. Plots of solvent coefficients (from Table V) for tricaprylin/1-monocaprylin lipid mixtures as a function of 1-monocaprylin concentration at **a** ~6% RH and **b** 100% RH. Symbols: Δr, (*filled diamond*); Δs, (*filled square*); Δa, (*filled triangle*); Δb, (× *mark*); and Δv, (*empty circle*). The coefficients, Δs and Δa increase systematically with increasing monoglyceride concentration indicating that the lipid mixture is better able to interact with polar and hydrogen bond donor solutes. However, Δv decreases with increasing monoglyceride concentration, indicating that inserting a larger solute becomes progressively more difficult. The coefficients Δb and Δr are close to zero and relatively insensitive to monoglyceride concentration. The solid lines connecting the data points are used to guide the eye.

such as o/w, w/o emulsions, and liquid-crystalline phases. To simplify the analysis of these mixtures and also to focus on systems that are pharmaceutically relevant, only the "microemulsion" region (see Fig. 7) was examined in the present work.

Identification of Microemulsions

According to the definition of a microemulsion given by Danielsson and Lindman (54), microemulsions have a certain amount of organization (short range order) due to self-assembled structures (e.g., inverted micelles, lamellar phases, etc.), however, the long range order is stochastic. Thus, a microemulsion is distinguished from a homogeneous solution that is isotropic even over short distances. Triglyceride/monoglyceride microemulsions exhibit characteristics similar to the liquid-crystalline inverse lamellar L2 phase in which curved and continuous layers of triglyceride are sandwiched between layers of monoglyceride molecules. In the case of triglyceride/monoglyceride/water systems, the proposed morphology is of layers of water and polar head groups of lipids separated by continuous layers of liquid hydrocarbon chains (34,35). This stacking creates a distinct interface, and it is conceivable that certain solutes may preferentially reside at this interface due to their characteristic properties.

The phase diagram in Fig. 7 shows the boundary for the reverse micellar L2-phase in tricaprylin/1-monocaprylin/water mixtures at 20°C. The figure shows that the L2-phase extends to a very low concentration of 1-monocaprylin. Thus, other than pure tricaprylin, all the other tricaprylin/1-monocaprylin and 1-monocaprin mixtures examined in this study fall in/near the L2 phase region. Besides using Fig. 7 for reference, we also tested each lipid mixture to ensure that it was clear, optically isotropic, and thermodynamically stable, consistent with "microemulsion" formation (8). Mixtures which did not satisfy these criteria were discarded. Note that these criteria were applied to the solute and water saturated (at the corresponding water activity) lipid mixtures and not to the pure lipid mixtures. In addition, dissolution of these mixtures was also tested in oil and water to identify the continuous phase and to confirm that they were "water-in-oil" microemulsions at low RH (~6%). At high water content (i.e., at 100% RH), the microemulsions were found to be bicontinuous in oil and water. This observation is consistent with the lipid literature (55). The testing was done by adding a drop of the lipid mixture to bulk oil/water and noting the time needed for the drop to dissolve into the medium. If the continuous phase is same as the dissolving medium, the drop will dissolve immediately.

Structural Transitions in the Lipid Mixtures

Sum *et al.* recently proposed a molecular model to predict the thermodynamic and transport properties of triacylglycerols (56). Their simulation results showed that in the liquid phase, triglycerides self-assemble to form lamellar structures similar to their structure in the crystalline state. The authors noted that the residual structure of triglycerides needs to be accounted for properly to ensure that sensitive dynamic properties such as the viscosity can be calculated. X-ray scattering on liquid triglycerides (57,58) just above their melting points have also revealed that liquid triglycerides retain small domains of double layer or bilayer structure as found in their crystalline form, with the only difference being that fatty acid side chains are melted.

Lipid mixtures containing monoglycerides such as the 1-monocaprylin/benzene mixture have been shown to possess a critical micelle concentration (CMC) at which reverse micelles of monocaprylin form (59–64). However, these studies also showed that in chloroform no CMC exists because it is a very good solvent for 1-monocaprylin. A clear CMC exists only when cooperativity leads to the abrupt formation of large aggregates. If the cooperativity is weak,

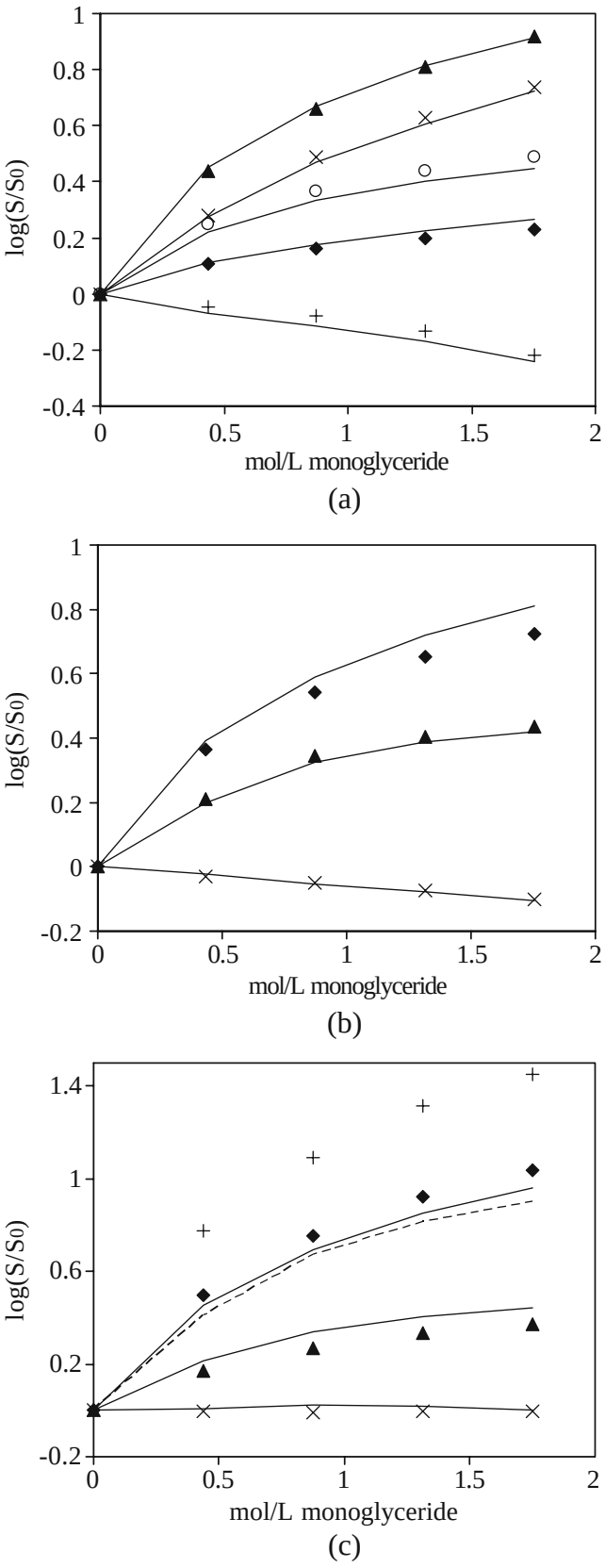


Fig. 4. Plots of experimental (Table III) and fitted values of log (S/S_0) for dry (~6% RH) lipid mixtures. Symbols: **a** MET(*filled diamond*), BEN(*filled triangle*), XYL(× *mark*), CHL(*plus mark*), and CAR(*empty circle*); **b** NMET(*filled diamond*), TOL(*filled triangle*), and ANT(× *mark*). **c** MEB(*filled diamond*), NAP(*filled triangle*), ACT(× *mark*) and PHE(*plus mark*). The *solid lines* generally show good fits of the experimental data to Eq. 1. The predicted values for the outlying molecule *p*-phenylene diacetic acid are shown with a dashed (-) line.

aggregates increasing in size form continuously such that no clear CMC is apparent (65). In the triglyceride/monoglyceride mixtures (devoid of water), a distinct CMC, if observed, would strictly denote the monoglyceride concentration at which the monoglyceride molecules abruptly form large aggregates (i.e., reverse micelles).

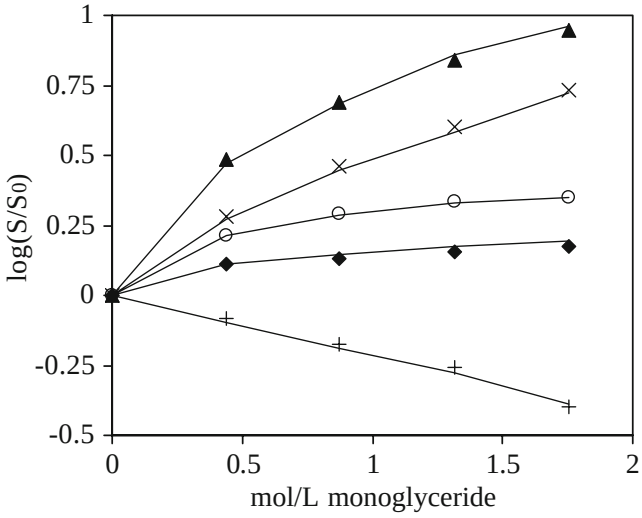
In this study, tricaprylin is a good solvent for monocaprylin and monocaprin due to their similar chemical compositions. Therefore, it is highly unlikely that a CMC exists in these systems and thus the transition is likely to be continuous. Literature data on systems that show a CMC also suggest that there may or may not be a change of slope in solubility below and above the CMC depending upon the system. If a CMC exists and a change in slope for the solubility occurs, the regions above and below the CMC would need to be treated separately.

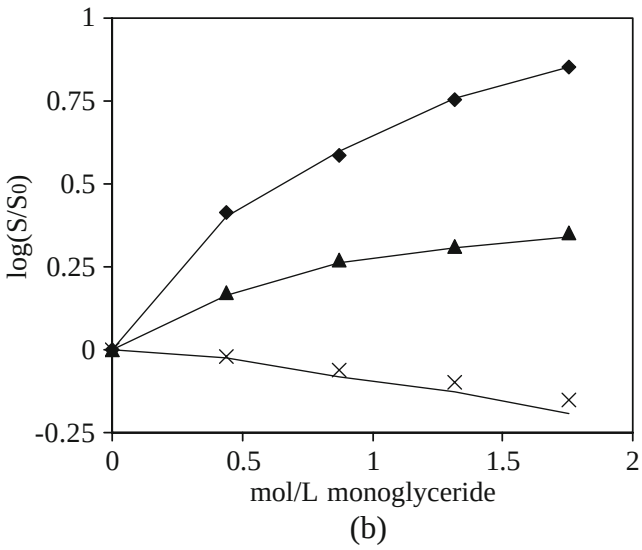
The slope of the curve for water uptake with increasing monoglyceride concentration at low water activity was used to probe for the possible existence of a CMC for monoglyceride aggregation. The results in Fig. 1(a) for water uptake in lipid mixtures under dry conditions (~6% RH) provided no evidence for a CMC at the monoglyceride concentrations explored. The absence of a distinct CMC, however, does not rule out the likelihood that lipid self-assembly occurs at the monoglyceride concentrations employed (consistent with microemulsion formation) but it does suggest that the aggregate size may increase gradually.

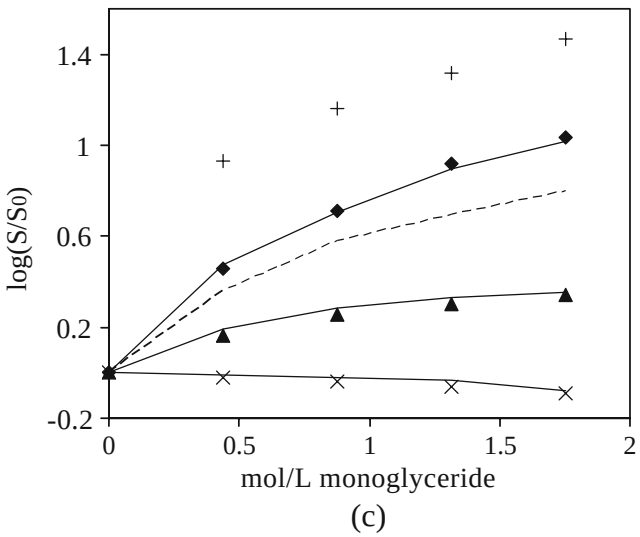
In Fig. 1b, the water uptake data at high RH (100%) exhibited a change in slope at a monoglyceride concentration <0.5 mol/L. Since Fig. 7 suggests microemulsion formation even at a monoglyceride concentration of <0.5 mol/L, the transition observed at high water activity more likely represents a structural transition from small spherical water in oil micelles or lamellar segments to extended lamellae consisting of alternating layers of water molecules and lipid head groups sandwiched between domains of lipid hydrophobic groups in the mixture. Such structural transitions involving dissolved water are well documented in the lipid literature (55), and could lead to a rise in the slope in the plot of water uptake *versus* monoglyceride concentration. More importantly, no change in the slope of the plot of solubility *versus* monoglyceride concentration was observed for any of the solutes employed in this study other than water. Therefore, there was no need to consider regions above and below an apparent transition point in the development of solubility relationships.

Master Curve for Water Uptake and Solubility

If a group contribution-based approach is valid, a master curve should be obtained when the property of interest (e.g., solubility) is plotted against the molar concentration of the relevant functional groups regardless of the method by which the functional group composition of the solvent is achieved. Figures 1 and 2 illustrate that a single master curve can be obtained for water uptake and solubility of each solute as a







◀**Fig. 5.** Plots of experimental (Table III) and fitted values of log (S/S_0) for wet (100% RH) lipid mixtures. Symbols: **a** MET (*filled diamond*), BEN(*filled triangle*), XYL(× *mark*), CHL(*plus mark*), and CAR(*empty circle*); **b** NMET(*filled diamond*), TOL(*filled triangle*), and ANT(× *mark*). **c** MEB(*filled diamond*), NAP(*filled triangle*), ACT(× *mark*) and PHE(*plus mark*). The *solid lines* generally show good fits of the experimental data to Eq. 1. The predicted values for the outlying molecule *p*-Phenylene diacetic acid are shown with a dashed (-) line.

function of the monoglyceride concentration in the lipid mixtures when the concentration of the monoglyceride is plotted on a molar scale because only the molar scale accounts for the actual functional group concentration in the mixture. If any other concentration scale (e.g., volume fraction, mole fraction, etc.) were used, the data did not superimpose (not shown). Monoglycerides having a larger difference in chain length would have produced a greater disparity. This was confirmed in a previous study by Anderson and Marra (38) which reported that solubility in various natural oils, synthetic oils, and triglycerides covering a significantly larger range of hydrophobic chain lengths (about C_4–C_{20}) falls on a single master curve when plotted against the ester group concentration when other factors such as degree of unsaturation, concentration of other functional groups, water content, etc., are kept relatively fixed.

Analysis of the Relative Solvent Coefficients

Figure 3 reveals that for both the dry and wet mixtures, Δs, the solvent coefficient relating to the ability of the solvent to interact with dipolar/polarizable solutes, and Δa, the hydrogen bond basicity of the mixture increase systematically with increasing monoglyceride concentration while Δv, the solvent coefficient related to the ease of cavity formation, decreases. The trends in Δs and Δa are consistent with the properties of ester and hydroxyl groups which are known to be better hydrogen bond acceptors than donors. The parameter reflecting the relative hydrogen bond acidity of the solvent, Δb, is very close to zero and relatively constant with increasing monoglyceride concentration. A relatively small and constant value for Δb was also observed in the squalane/tricaprylin system (37), probably reflecting the fact that the −OH groups in monoglyceride containing mixtures with tricaprylin are already hydrogen bonded to other solvent molecules and not readily available to donate hydrogen to a hydrogen acceptor group on the solute. The lipid mixture's ability to interact with solutes through *n* and π electron pairs, i.e. through dispersion forces, as measured by the Δr parameter, decreases slightly with increasing monoglyceride content and Δr is the second smallest coefficient after Δb.

The trend in Δv, which becomes progressively more negative with increasing monocaprylin content, indicates that cavity formation becomes energetically (or free energetically) more expensive in a lipid mixture containing monoglycerides, supporting the hypothesis that triglyceride/monoglyceride mixtures are more structured than the pure triglyceride. The change in Δv appears to be amplified at high relative humidity (Table V), suggesting a possible role of water in further structuring the solvent.

Analysis of Normalized Solubility Data for Different Solutes

Figures 4 and 5 and Table III establish that the solubility of most solutes in a given lipid mixture normalized by the

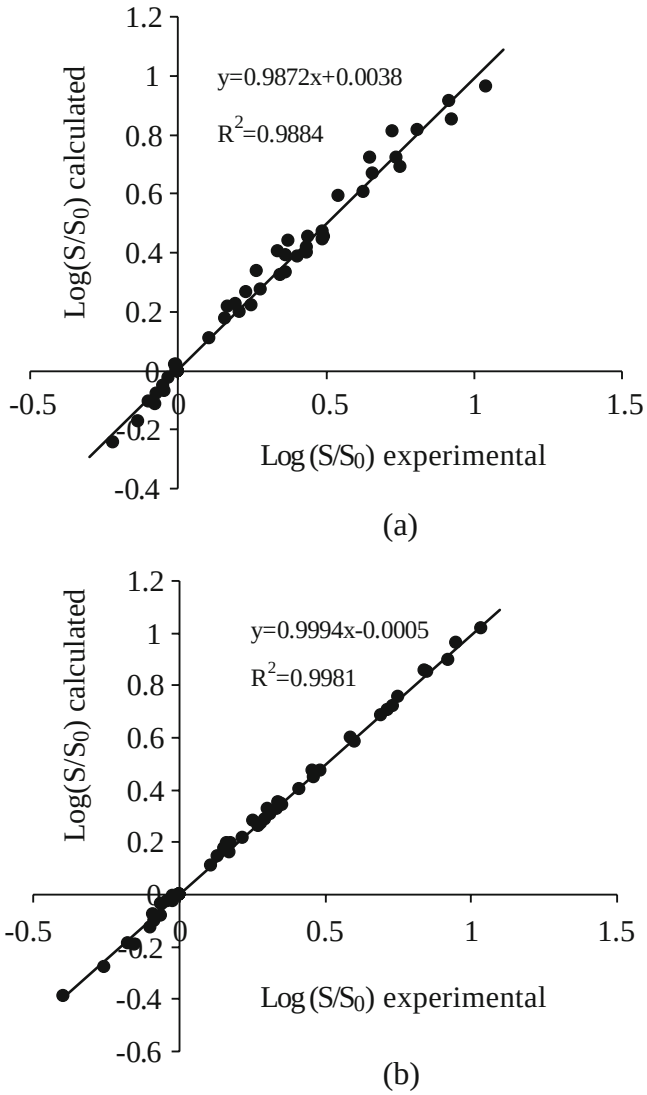


Fig. 6. Plots of model calculated and experimental log(S/S_0) values in tricaprylin/1-monocaprylin lipid mixtures as a function of 1-monocaprylin concentration at **a** ~6% RH and **b** 100% RH. The slope of the regression line is very close to 1.0 and the intercept is also close to the origin indicating good agreement of the calculated and experimental values.

solubility in tricaprylin (S/S_0) varies systematically as a function of the monoglyceride content in the lipid mixture. However, the trend in this ratio (positive or negative slope) with increasing monoglyceride concentration and the magnitude of the change depends on the nature of the solute, including its size, functional groups and their number. For example, the ratio rises most significantly for compounds that are strong hydrogen bond donors or compounds that can function as both hydrogen bond donors and acceptors, such as *p*-phenylene diacetic acid (S/S_0 ~29.6), benzamide (S/S_0 ~8.8) and *p*-xylylene glycol (S/S_0 ~5.4) (ratios are at the highest monoglyceride concentration tested under wet conditions). This observation is consistent with the abundance of hydroxyl and ester groups in the lipid mixture which can form hydrogen bonds with these solutes. As expected, the ratio is larger for the compounds that have more hydrogen bond donating or donor/acceptor groups. Thus, the ratio for *p*-phenylene diacetic acid is larger than that for 9-anthracene carboxylic acid, 1-naphthalene acetic acid, and *p*-toluic acid. Similarly the ratio for *p*-xylylene glycol is larger than that for 9-anthracene methanol. It is interesting to note that the ratio of solubilities is almost the same for 9-anthracene carboxylic acid, 1-naphthalene acetic acid and *p*-toluic acid, indicating that the free energy to transfer these molecules from tricaprylin to other tricaprylin/monoglyceride mixtures is largely dominated by the carboxylic acid functional group, and not by the phenyl, naphthyl or anthracenyl ring. This lends further credence to the argument that specific interactions such as hydrogen bonding between the relevant functional groups play the dominant role in governing relative solubility in lipid mixtures.

Normalized solubility ratios for compounds such as 9-anthracenemethyl acetate, which contains a single ester polar group (a hydrogen acceptor) depend only slightly on the lipid composition, while the solubility ratio for anthracene, which is devoid of any polar functional group, decreases with increasing monoglyceride content. This is indicative of a dominant role for the cavity term when polar, hydrogen bonding interactions are absent and again points to the possible existence of ordered structures in the triglyceride/monoglyceride mixtures. Formation of stacked lamellar structures via strong hydrogen bonding interactions in triglyceride/monoglyceride mixtures would disfavor the insertion of large solutes such as anthracene that are unable to replace the hydrogen bonds lost in creating a cavity. Albeit weak, such a "solvophobic" effect is reminiscent of the hydrophobic effect in water resulting from the strong hydrogen bonding that exists between water molecules.

Impact of Lipid Structure on Solubility Relationships

There are two kinds of structuring in triglyceride/monoglyceride lipid systems studied here that are of interest.

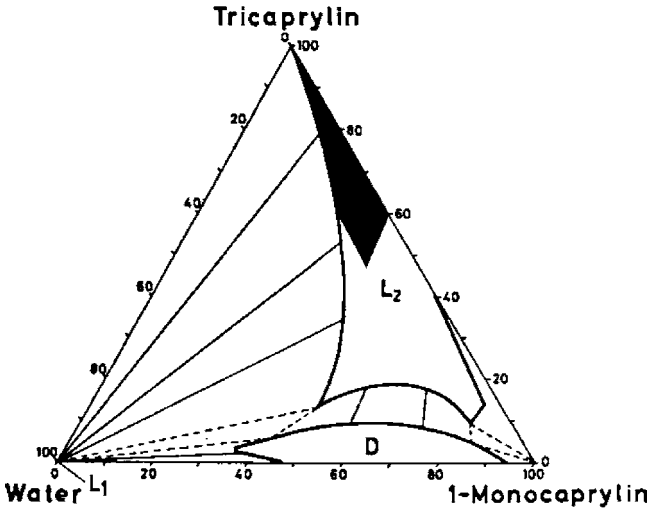


Fig. 7. Phase diagram for a tricaprylin/1-monocaprylin/water system at 20°C. L1 and L2 are regions with homogeneous isotropic solutions. *The shaded area within the L2 phase shows the microemulsion region explored in this study.* D is the region with homogenous mesomorphous phase. (From: S. Friberg and L. Mandell, Phase equilibria and their influence on the properties of emulsions. *J. Am. Oil. Chem. Soc.* 47: 149–152 (1970), reproduced with permission of the copyright owner).

The first arises from the lamellar arrangement of the lipid molecules both in pure triglycerides and triglyceride/monoglyceride mixtures. With the addition of monoglycerides, the strength of intermolecular interactions and domain sizes of the lamellae may increase with no other fundamental change in the local organization of lipid molecules. There is no abrupt thermodynamic phase change. Figure 3 shows that Δv decreases continuously with the addition of monoglyceride, suggesting that the structuring of the lipids is gradual, consistent with an increasing size of lamellar domains. There is also no evidence of cooperativity in the packing with increasing monoglyceride concentration that would abruptly change the fundamental solubility relationships. As long as the structure of lipids remains of one kind (e.g. lamellar) and only the domain size is increasing, a functional group contribution based approach may still apply. The contribution of each functional group, however, will probably be different from its contribution in an unstructured system. If the structure of lipids is fixed, proximity effects on the interaction of different functional groups in the lipids with solute functional groups may be approximately constant. Therefore, the functional group contribution based approach demonstrated for these lipid mixtures and the coefficients obtained probably represent behavior in relatively structured vehicles.

Another type of structuring in triglyceride/monoglyceride vehicles containing water is the self-association of water into clusters. This appears to mainly promote formation of a bi-continuous system at high RH and high monoglyceride concentration but may not fundamentally change the local organization of lipid molecules or alter solubility relationships significantly. This is supported by the fact that the solubility ratio for each solute is similar (though not identical) with increasing monoglyceride concentration under dry (~6% RH) and wet (100% RH) conditions (Table III). In addition, the solvent coefficients in the dry and wet case are also similar (Fig. 3, and Table V) though the change in some coefficients, particularly Δv, appears to be amplified at high water content.

Extension of Solvent Coefficients to Other Triglyceride/Monoglyceride Mixtures

As noted before, the properties of different tricaprylin/monoglyceride mixtures are similar when they are compared using a scale based on the molar concentration of monoglyceride. Thus, the results of Fig. 3a–b can be applied, in theory, to any tricaprylin/monoglyceride mixture while taking into account the molar fraction of hydroxyl groups. If however, another triglyceride is exchanged for tricaprylin, the relative solvent coefficients will probably change. Therefore, for a complete understanding of solvent coefficients for triglyceride/monoglyceride mixtures, it may be necessary to perform similar studies with more triglycerides. The previous paper by Cao *et al.* (37) showed that solvent coefficients for squalane/tricaprylin mixtures could be determined just from the concentration of the triglyceride ester groups. Therefore, one could, in principle, predict the relative solvent coefficients for other triglyceride/monoglyceride mixtures by combining the work of Cao *et al.* (37) with the results in Fig. 3a–b. However, the previous work by Cao *et al.* (37) was performed at 25°C, while this work was done at 37°C. If both studies would have been at the same temperature, obtaining a more general expression for the solubility behavior in triglyceride/monoglyceride mixtures would be straightforward.

Outliers and Limitations of the LFER Approach

As discussed in the Results section, the solubility data for *p*-phenylene diacetic acid and water were found to be poorly fit by the combined LFER analysis. In this section characteristics of the solute or solvent that may be responsible for the existence of such outliers are considered. Water was also identified as an outlier in the paper by Cao *et al.* (37). It was suggested that including water in the test set was problematic as there appears to be no consensus on the values of its descriptors. In particular, the hydrogen bond acidity parameter, $\sum \alpha_2^H$, for water appears to be solvent dependent (66). Published descriptors for "monomeric" water (67), may not be appropriate for lipid mixtures at high water content where the water may exist mainly in large clusters due to self-association. However, the water uptake could be made to fit by treating $\sum \alpha_2^H$ and $\sum \beta_2^H$ as adjustable parameters with the best fit estimates determined to be 2.7 and 2.7, respectively, indicating stronger hydrogen bonding interactions than the descriptors in Table II would suggest. Similarly, the solubility data for *p*-phenylene diacetic acid could be fit by allowing $\sum \alpha_2^H$ and $\sum \beta_2^H$ to be fitted parameters which were determined as 2.0 and 2.0, respectively. These descriptors again significantly exceed those employed in Table II, suggesting that *p*-phenylene diacetic acid exhibits a stronger tendency to hydrogen bond than expected. *p*-Phenylene diacetic acid, a dicarboxylic acid, may exhibit a strong tendency to self-associate even at very low concentrations. The self-association of carboxylic acids is known to be much stronger than that of alcohols and phenols (68). Studies by Anderson *et al.* have shown that self-association of alcohols can greatly increase their solubility in non-aqueous systems, and this can lead to deviations of the solubility from predictions based on regular solution theory (69–71). Self-association may, therefore, increase the apparent solubilities of water and *p*-phenylene diacetic acid above those expected for strictly monomeric solutes. Additional studies will be necessary to ascertain whether or not the above hypotheses can account for these outliers.

A limitation of the LFER approach when the descriptors are obtained by the fragment based approach is its inability to distinguish between topological isomers and enantiomers or to account for self-assembly of either polar solute or solvent molecules with increasing concentration. A basic assumption of the fragment based group contribution approach is that each part of a molecule acts "independently", and makes a separate contribution to the descriptors (46). Nearest neighbor effects have been taken into account, but the situation becomes complicated very quickly due to the large number of possibilities. In such situations, computer simulations may provide valuable insights because they can take into account the actual topology and stereochemical nature of the molecules involved. The lipid mixtures and solutes studied here will be treated in more detail in a subsequent paper (72) in which we conduct molecular dynamics simulations to explore the structure and solvent properties of triglyceride/monoglyceride/water mixtures.

Water Uptake and its Effect on Solubility

Cao *et al.* (37) reported that increased water uptake with increasing tricaprylin concentration in squalane/tricaprylin mixtures enhanced the solubility of the model solutes being evaluated. A portion of the increased water uptake in mixtures containing dissolved solute could be attributed to the solute, a phenomenon referred to as a "water dragging effect."

From Table IV, it is clear that the 1-monocaprylin and 1-monocaprin mixtures absorb a large amount of water at full saturation. For example, in the 40% wt/wt 1-monocaprylin mixture (devoid of water), the water content at saturation is ~10% wt/wt of the final weight. According to previous studies, this water content is close to the boundary of the microemulsion phase (42).

While the ratios of the solubilities (S/S_0) for most solutes listed in Table III are similar in wet and dry mixtures (i.e. excess water did not seem to dramatically influence the relative solubility), the solubility of benzamide is ~1.6 times higher at 100% RH than in a lipid mixture at ~6% RH at the highest monocaprylin content. Anthracene's solubility decreased with increasing water content. The practical implications of these observations are that, depending on the solute, solubility can be substantially altered by varying water content and consequently, water content may need to be carefully regulated.

CONCLUSIONS

The solubility of a series of solutes varying widely in their intrinsic properties such as hydrogen bond acidity, basicity, polarity and molecular volume were determined in triglyceride/monoglyceride mixtures under dry (~6% RH) and wet (100% RH) conditions. It was shown that the solubility and water uptake change systematically with increasing monoglyceride content, and in fact, each of these properties can be superimposed onto a single curve for a given solute when plotted *versus* monoglyceride concentration expressed on a molar scale. This finding supports the idea that even a structured lipid mixture as studied here, can be viewed as a combination of various functional groups or moieties such as ester groups, hydroxyl groups, alkyl groups etc, each of which makes an independent contribution to the overall solubility or other physical property. The successful superimposition also allows the results of this study to be extended to other triglyceride/monoglyceride mixtures beyond those explored. Linear free energy relationships, with solvato-chromic parameters of the type devised by Abraham *et al.* were identified at each solvent composition, and coefficients that describe the solvent's properties such as hydrogen bond donating/accepting ability, ability to interact through dispersion forces, energy to make a cavity, etc., were obtained. Each of the solvent coefficients was found to vary systematically with the monoglyceride content. These coefficients offer the potential to predict relative solubility in triglyceride/monoglyceride mixtures if the solute descriptors can be obtained. Two solutes—water and *p*-phenylene diacetic acid were identified as outliers in the LFER regression analysis due to their stronger hydrogen bonding tendencies than suggested by their solute descriptors. These bifunctional solutes may self-associate at relatively low concentrations, highlighting one of the potential difficulties in the universal application of fragment-based approaches. It was also found that the water uptake may increase or decrease solubility. Thus, from a practical viewpoint the effect of water on solubility needs to be carefully evaluated in the design of lipid formulations.

ACKNOWLEDGEMENTS

This work was conducted with support from a grant by Pfizer Inc. One of the authors (BDA) was a Pfizer stockholder while this study was being conducted.

REFERENCES

1. L. V. Allen Jr., N. G. Popovich, and H. C. Ansel. *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 8th ed. Lippincott Williams & Wilkins, (2004).
2. C. Lipinski. *Physicochemical properties in drug design/development. 2nd International Drug Discovery and Development Summit: Novel Concepts and Technologies to Accelerate Drug Development*: Honolulu, HI, (2003).
3. N. A. Armstrong and K. C. James. Drug release from lipid-based dosage forms. I. *Int. J. Pharm* **6**:185–193 (1980).
4. A. J. Humberstone and W. N. Charman. Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Adv. Drug Delivery Rev.* **25**:103–128 (1997).
5. R. A. Myers and V. J. Stella. Systemic bioavailability of penclomedine (NSC 338720) from oil-in-water emulsions administered intraduodenally to rats. *Int. J. Pharm.* **78**:217–226 (1992).
6. K. A. Traul, A. Driedger, D. L. Ingle, and D. Nakhasi. Review of the toxicologic properties of medium-chain triglycerides. *Food Chem. Toxicol.* **38**:79–98 (2000).
7. K. M. Wasan. Formulation and physiological and biopharmaceutical issues in the development of oral lipid-based drug delivery systems. *Drug Dev. Ind. Pharm.* **27**:267–276 (2001).
8. P. P. Constantinides, J.-P. Scalart, C. Lancaster, J. Marcello, G. Marks, M. Ellens, and P. L. Smith. Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. *Pharm. Res.* **11**:1385–1390 (1994).
9. P. P. Constantinides and J.-P. Scalart. Formulation and physical characterization of water-in-oil microemulsions containing long- *versus* medium-chain glycerides. *Int. J. Pharm.* **158**:57–68 (1997).
10. C. W. Pouton. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur. J. Pharm. Sci.* **11**:S93–S98 (2000).
11. A. Fredenslund, R. L. Jones, and J. M. Prausnitz. Group-contribution estimation of activity coefficients in nonideal liquid mixtures. *AIChE J.* **21**:1086–1099 (1975).
12. J. Gmehling, J. Li, and M. Schiller. A modified UNIFAC model. 2. Present parameter matrix and results for different thermodynamic properties. *Ind. Eng. Chem. Res.* **32**:178–193 (1993).
13. T. Fujita, J. Iwasa, and C. Hansch. A new substituent constant, π, derived from partition coefficients. *J. Am. Chem. Soc.* **86**:5175–5183 (1964).
14. A. Leo, C. Hansch, and D. Elkins. Partition coefficients and their uses. *Chem. Rev.* **71**:525–616 (1971).
15. A. Jouyban, H.-K. Chan, N. Y. K. Chew, M. Khoubnasabjafari, and W. E. Acree Jr.. Solubility prediction of paracetamol in binary and ternary solvent mixtures using Jouyban-Acree model. *Chem. Pharm. Bull.* **54**:428–431 (2006).
16. A. B. Ochsner, R. J. Belloto Jr., and T. D. Sokoloski. Prediction of xanthine solubilities using statistical techniques. *J. Pharm. Sci.* **74**:132–135 (1985).
17. W. E. Acree Jr., J. W. McCargar, A. I. Zvaigzne, and I. L. Teng. Mathematical representation of thermodynamic properties. Carbazole solubilities in binary alkane + dibutyl ether and alkane + tetrahydropyran solvent mixtures. *Phys. Chem. Liq.* **23**:27–35 (1991).

18. A. Jouyban, N. Y. K. Chew, H-K. Chan, M. Sabour, and W. E. Acree Jr. A united cosolvency model for calculating solute solubility in mixed solvents. *Chem. Pharm. Bull.* **53**:634–637 (2005).
19. S. H. Yalkowsky, and T. Roseman. In S. H. Yalkowsky (ed.), *Solubilization of Drugs by Cosolvents*, Marcel Dekker, New York, 1981, pp. 91–134.
20. A. Adjei, J. Newburger, and A. Martin. Extended Hildebrand approach: solubility of caffeine in dioxane-water mixtures. *J. Pharm. Sci.* **69**:659–661 (1980).
21. M. Abraham, P. Duce, P. Grellier, D. Prior, J. Morris, and P. Taylor. Hydrogen bonding. Part 5. A thermodynamically-based scale of solute hydrogen-bond acidity. *Tetrahedron Lett.* **29**:1587–1590 (1988).
22. M. H. Abraham, P. L. Grellier, D. V. Prior, J. J. Morris, P. J. Taylor, C. Laurence, and M. Berthelot. Hydrogen bonding. Part 6. A thermodynamically-based scale of solute hydrogen bond basicity. *Tetrahedron Lett.* **30**:2571–2574 (1989).
23. M. H. Abraham, G. S. Whiting, R. M. Doherty, and W. J. Shuely. Hydrogen bonding. Part 13. A new method for the characterisation of GLC stationary phases—the Laffort data set. *J. Chem. Soc. Perkin Trans. II* 1451–1460 (1990).
24. M. H. Abraham, G. S. Whiting, R. M. Doherty, and W. J. Shuely. A new solute solvation parameter, π_2^H , from gas chromatographic data. *J. Chromatogr.* **587**:213–228 (1991).
25. M. H. Abraham. Scales of solute hydrogen-bonding: their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* **22**:73–83 (1993).
26. M. Reta, P. W. Carr, P. C. Sadek, and S. C. Rutan. Comparative study of hydrocarbon, fluorocarbon, and aromatic bonded RP-HPLC stationary phases by linear solvation energy relationships. *Anal. Chem.* **71**:3484–3496 (1999).
27. L. C. Tan, P. W. Carr, and M. H. Abraham. Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships. I. The stationary phase. *J. Chromatogr. A.* **752**:1–18 (1996).
28. L. C. Tan, and P. W. Carr. Study of retention in reversed-phase liquid chromatography using linear solvation energy relationships. II. The mobile phase. *J. Chromatogr. A.* **799**:1–19 (1998).
29. M. H. Abraham, A. Ibrahim, Y. Zhao, and W. E. Acree Jr.. A database for partition of volatile organic compounds and drugs from blood/plasma/serum to brain, and an LFER analysis of the data. *J. Pharm. Sci* **95**:2091–2100 (2006).
30. W. E. Acree Jr. and M. H. Abraham. The analysis of solvation in ionic liquids and organic solvents using the Abraham linear free energy relationship. *J. Chem. Technol. Biotechnol.* **81**:1441–1446 (2006).
31. M. H. Abraham and W. E. Acree Jr.. Comparative analysis of solvation and selectivity in room temperature ionic liquids using the Abraham linear free energy relationship. *Green Chem.* **8**:906–915 (2006).
32. K. R. Bowen, K. B. Flanagan, W. E. Acree Jr., and M. H. Abraham. Correlating toxicities of organic compounds to select protozoa using the Abraham model. *Sci. Tot. Env.* **369**:109–118 (2006).
33. R. G. Strickley. Solubilizing excipients in oral and injectable formulations. *Pharm. Res* **21**:201–230 (2004).
34. T. Gulik-Krzywicki and K. Larsson. An electron microscopy study of the L2-phase (microemulsion) in a ternary system: triglyceride/monoglyceride/water. *Chem. Phys. Lipids* **35**:127–132 (1984).
35. K. Larsson. An X-ray scattering study of the L2-phase in monoglyceride/water system. *J. Colloid Interf. Sci.* **72**:152–153 (1979).
36. C. W. Pouton. Formulation of self-emulsifying drug delivery systems. *Adv. Drug Delivery Rev.* **25**:47–58 (1997).
37. Y. Cao, M. Marra, and B. D. Anderson. Predictive relationships for the effects of triglyceride ester concentration and water uptake on solubility and partitioning of small molecules into lipid vehicles. *J. Pharm. Sci.* **93**:2768–2779 (2004).
38. B. D. Anderson, and M. T. Marra. Chemical and related factors controlling lipid solubility. *Bull. Tech. Gatefosse.* 11–19 (1999).
39. E. Akaho, K. Iga, J. Kraal, and A. Hussain. Solubility behavior of phenolic compounds in hexane-ethyl acetate, hexane-ethyl myristate, and hexane-ethyl pivalate cosolvent systems. *J. Pharm. Sci.* **70**:1225–1228 (1981).
40. J. Kurashige, K. Takaoka, M. Takasago, Y. Taru, and K. Kobayashi. State of dissolved water in triglycerides as determined by Fourier transform infrared and near-infrared spectroscopy. *Yukagaku* **40**:549–553 (1991).
41. K. Takaoka, K. Kobayashi, M. Takahashi, and M. Sone. Studies on interaction between castor oil and dissolved water by hot wire method. *Nippon Kagaku Kaishi*. **10**:649–654 (1999).
42. S. Friberg and L. Mandell. Phase equilibria and their influence on the properties of emulsions. *J. Am. Oil. Chem. Soc.* **47**:149–152 (1970).
43. D. R. Lide. *CRC Handbook*. 82 edn. CRC press, Cleveland, OH, 2001–2002, pp. 15–25.
44. M. H. Abraham and J. C. McGowan. The use of characteristic volumes to measure cavity terms in reversed phase liquid chromatography. *Chromatographia* **23**:243–246 (1987).
45. J. C. McGowan. Estimates of the properties of liquids. *J. Appl. Chem. Biotechnol.* **28**:599–607 (1978).
46. J. A. Platts, D. Butina, M. H. Abraham, and A. Hersey. Estimation of molecular linear free energy relation descriptors using a group contribution approach. *J. Chem. Inf. Comput. Sci.* **39**:835–845 (1999).
47. M. Lindström, H. Ljusberg-Wahren, K. Larsson, and B. Borgström. Aqueous lipid phases of relevance to intestinal fat digestion and absorption. *Lipids* **16**:749–754 (1981).
48. L. Engström and A. B. Karlshamn. Aggregation and structural changes in the L2-phase in the system water/soybean oil/sunflower oil monoglycerides. *J. Dispersion Sci. Technol.* **11**:479–489 (1990).
49. N. A. Artamonova, M. I. Goryaev, Y. A. Morozova, and O. I. Efremova. Preparation and properties of monoglycerides of low molecular weight carboxylic acids. *Izvestiya Akademii Nauk Kazakhskoi SSR, Seriya Khimicheskaya* **2**:35–37 (1979).
50. E. P. Sokolova and T. G. Churyusova. Thermodynamic properties of dilute aqueous solutions of caproic acid α-monoglyceride. *Kolloidnyi Zhurnal* **37**:597–600 (1975).
51. SciFinder, version 2006; Chemical Abstracts Service: Columbus, OH, 2006; Mass solubility value for RN 502-54-5 and RN 2277-23-8 (accessed Sept 20, 2007); calculated using Advanced Chemistry Development (ACD/Labs) software, version 8.14; ACD/Labs 1994–2007.
52. P. Becher. In M. J. Schick (ed.), *Non-ionic Surfactants*, Marcel Dekker, New York, 1966, pp. 490–491.
53. W. Zwierzykowski, B. Orzecka, and K. Spich. Surface activity of some fatty acid monoglycerides. *Tenside Detergents* **13**:313–316 (1976).
54. I. Danielsson and B. Lindman. The definition of microemulsion. *Colloids Surf.* **3**:391–392 (1981).
55. F. D. Gunstone, J. L. Harwood, and F. B. Padley (eds.). In *The Lipid Handbook*, Chapman and Hall, New York, 1986, p. 332.
56. A. K. Sum, M. J. Biddy, J. J. de Pablo, and M. J. Tupy. Predictive molecular model for the thermodynamic and transport properties of triacylglycerols. *J. Phys. Chem. B.* **107**:14443–14451 (2003).
57. D. M. Small. *Handbook of Lipid Research Vol. 4. The Physical Chemistry of Lipids: From Alkanes to Phospholipids*. Plenum Press, New York, 1986, p. 367.
58. K. Larsson. In F. D. Gunstone, J. L. Harwood, and F. B. Padley (eds.), *The Lipid Handbook*, Chapman and Hall, New York, 1986, p. 337.
59. P. Becher. In M. J. Schick (ed.), *Non-ionic Surfactants*, Marcel Dekker, New York, 1966, pp. 511–515.
60. K. Kon-no, T. Jin-no, and A. Kitahara. Solubility, critical aggregating or micellar concentration, and aggregate formation of nonionic surfactants in nonaqueous solutions. *J. Colloid Interface Sci.* **49**:383–389 (1974).
61. P. Debye and W. Prins. Micellar dispersion of α-monoglycerides in benzene and chlorobenzene. *J. Colloid Sci.* **13**:86–98 (1958).
62. M. V. Flores, E. C. Voutsas, N. Spiliotis, G. M. Eccleston, G. Bell, D. P. Tassios, and P. J. Halling. Critical micelle concentrations of nonionic surfactants in organic solvents: Ap-

proximate prediction with UNIFAC. *J. Colloid Interface Sci.* **240**:277–283 (2001).
63. P. Becher. Discrepancies between micellar molecular weights by number-average and weight average methods. *Nature* **206**:611–613 (1965).
64. N. Robinson. Micellar size and surface activity of some C_{18} α-monoglycerides in benzene. *J. Pharm. Pharmacol.* **12**:685–689 (1960).
65. D. Grant and T. Higuchi. *Solubility Behavior of Organic Compounds*. Wiley Interscience, New York, 1990, pp. 236–264.
66. M. H. Abraham, A. M. Zissimos, and W. E. Acree Jr.. Partition of solutes from the gas phase and from water to wet and dry di-*n*-butyl ether: A linear free energy relationship analysis. *Phys. Chem. Chem. Phys.* **3**:3732–3736 (2001).
67. M. H. Abraham, P. L. Grellier, D. V. Prior, P. P. Duce, J. J. Morris, and P. J. Taylor. Hydrogen bonding part 7. A scale of solute hydrogen-bond acidity based on log K values for complexation in tetrachloro-methane. *J. Chem. Soc. Perkin Trans.* **2**:699–711 (1989).
68. T. L. Lemke. *Review of Organic Functional Groups: Introduction To Medical Organic Chemistry*. Lippincott Williams & Wilkins, PA, 1999.
69. B. D. Anderson, J. H. Rytting, and T. Higuchi. Solubility of polar organic solutes in nonaqueous systems: role of specific interactions. *J. Pharm. Sci.* **69**:676–680 (1980).
70. B. D. Anderson, J. H. Rytting, and T. Higuchi. Influence of self-association on the solubility of phenol in isooctane and cyclohexane. *J. Am. Chem. Soc.* **101**:5194–5197 (1979).
71. B. D. Anderson, J. H. Rytting, and T. Higuchi. Vapor pressure studies of self-association of alcohols in isooctane. I. The effect of chain length. *Int. J. Pharm.* **1**:15–30 (1978).
72. S. S. Rane and B. D. Anderson, to be published.