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Research Paper

The Influence of Water Content of Triglyceride Oils on the Solubility of Steroids

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Purpose. To determine if hydration of long- and medium-chain triglyceride oils (long = soybean and olive, medium = Miglyol 812) has a significant effect on the ability to solubize the model hydrophobic compounds progesterone, estradiol, and testosterone.
Methods. Soybean, olive, and Miglyol 812 oils were treated in one of two ways: hydrated or desiccated (hydrated, then dried). Solubility of ^{3}H–labeled progesterone, estradiol, and testosterone in the triglycerides was measured by liquid scintillation counting.
Results. Both hydration state and chain length of the triglycerides were shown to have a significant influence on the solubility of steroids. Solubility of estradiol hemihydrate and testosterone monohydrate in hydrated triglycerides is decreased by about 30%–40% compared with desiccated oils. The solubility of anhydrous testosterone was decreased by hydration of the oils due to conversion to the monohydrate crystalline form. In contrast, the solubility of progesterone was insensitive to the state of hydration of all oils.
Conclusions. Hydration of triglyceride oils caused a significant decrease in the solubility of steroids, which may form hydrates or hemihydrates. Results suggest the need for knowledge of the hydration state of triglyceride oils to be used as pharmaceutical excipients.

KEY WORDS: hydrates, moisture content, solubility, steroids, triglycerides.

INTRODUCTION

Because nearly 40% of all new drug candidates are classified as "poorly water-soluble," numerous methods have been developed to overcome the inherent difficulties in oral absorption of these compounds (1). Among these, much attention has focused on the incorporation of poorly water-soluble drug molecules into lipid-based formulations (2–7). Currently, the physicochemical factors controlling drug solubility in these systems are only poorly understood.

In spite of their potential as drug delivery vehicles, very few studies to date have systematically examined the solubilization properties of the components of lipid-based systems. The oil phase represents one of the most important components in many lipid-based formulations largely due to an ability to solubilize large amounts of lipophilic compounds. The effect of hydration of triglyceride oils on the solubilization of small molecules has only recently been reported (8). Cao and co-workers reported that solvation water enhanced the solubility of benzamine and *N*-methylbenzamide in squalane/tricaprylin mixtures, probably due to hydrogen bonding (8). The objective of this study is to determine if hydration of triglyceride oils has a significant effect on their ability to solubilize hydrophobic compounds. The steroids progesterone, estradiol, and testosterone were chosen to represent model poorly water-soluble compounds due to their stable nature and range in lipophilicity, in addition to the different hydration states of the three produced by recrystallization.

MATERIALS AND METHODS

Chemicals and Reagents

Soybean oil and olive oil are two long-chain triglycerides frequently used in drug delivery formulations and for this reason were chosen as representative long-chain triglycerides. Super Refined Soybean oil, USP/NP, and Super Refined Olive oil, NF/NP, were a gift from CRODA, Inc. (Parsippany, NJ, USA) and were stored under nitrogen. The extent of unsaturation of the hydrocarbon chains of olive oil is significantly greater than that of soybean oil. Miglyol 812 was chosen as the representative medium-chain triglyceride and was a gift from Sasol, Inc. (Houston, TX, USA). Triolein was chosen as a well-characterized long-chain triglyceride ($C_{18:1}$), and was purchased from Sigma-Aldrich (St. Louis, MO, USA). Progesterone (99%), 17β-estradiol (98+%), and testosterone (99+%) were purchased from Sigma-Aldrich and were recrystallized in acetone as described below. An important attribute of this series of steroids is the variation in hydration state after recrystallization. Progesterone is supplied as the anhydrous crystalline form and remains in that state after recrystallization in acetone. Estradiol is supplied in the hemihydrate form and also remains in that state after recrystallization in acetone. Testosterone is supplied in the anhydrous state but

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forms a monohydrate during the recrystallization process. Testosterone monohydrate can then be subsequently dehydrated to the anhydrous form by heating. [1,2,6,7-^{3}H]Progesterone (1 mCi/ml), [6,7-^{3}H]estradiol (1 mCi/ml), and [1,2,6,7-^{3}H]testosterone were purchased from Amersham Biosciences (Piscataway, NJ, USA). The solvents acetone and cyclohexane were purchased from Fisher Scientific (Pittsburgh, PA, USA) and Sigma-Aldrich, respectively, and were HPLC grade. Scintiverse BD liquid scintillation cocktail was purchased from Fisher Scientific. Triple distilled water was obtained by distilling house reverse osmosis water first over potassium permanganate and then over sulfuric acid. Ceramic molecular sieves (1.6 mm pellets) were purchased from Sigma-Aldrich and were dried under vacuum at 145°C for 48 h prior to use. Karl Fisher reagents (Coulomat AG, 1-dodecanol) and standards (Hydranal 0.1 and 1.0) were purchased from Sigma-Aldrich and used as received. Phosphorus pentoxide was purchased from Sigma-Aldrich and used as received.

Preparation of Triglyceride Oils

Oils used in the current studies were prepared in one of two ways: hydrated or desiccated (hydrated, then dried). Hydration of soybean, olive, and Miglyol 812 oils was accomplished by washing each of the oils with an equal volume of triple distilled water, mixing well, waiting 5 min, inducing separation of the oil and water by centrifugation, and removing the excess water. This extraction process was repeated 5 times, resulting in oils that were saturated with water. Drying of the hydrated oils to produce "desiccated oils" was carried out by the use of ceramic molecular sieves. About 15 g of oil was directly exposed to about 1.2 g of ceramic sieves with gentle shaking. A nitrogen blanket was applied during the drying process. Drying oils were sampled periodically and assayed by Karl Fisher coulometer to determine the time at which the oils had been dried to a desiccated state (<25 μg water/g oil).

Karl Fisher Titrimetry

All oils were analyzed by Coulometric Karl Fisher analysis (Brinkman 684KF model, Westbury, NY, USA) for water content in the hydrated and desiccated states. The media used was a mixture of 70 ml Coulomat AG and 30 ml 1-dodecanol. Calibration was performed with certified Hydranal water standards of 0.1 and 1.00 mg H_2O/g solution. Calibration measurements were performed in quadruplicate. For each sample, a small volume of oil was added to the anode compartment of the Karl Fisher coulometer using a syringe. The amount of material added to the coulometer was determined by weight, and water contents have been reported as μg H_2O/g oil, with all sample measurements performed in triplicate.

Solute Preparation

Preparation of Stocks of Radiolabeled Steroids

Preparation of large stocks of tracer ^{3}H-labeled solid progesterone, estradiol, and testosterone was carried out by the method of Jain *et al.* (9). Briefly, unlabeled steroid were dissolved in acetone at 48°C, followed by filtration of each solution through Whatman no. 1 filter paper into a beaker containing the radiolabeled steroid. Forty milliliters of water was added, and the solution was stirred using a magnetic stirring bar while cooling to room temperature. The solvent was allowed to evaporate under flowing nitrogen gas. The resulting specific activities of the steroids were as follows: 0.061 ± 0.002 mCi/g progesterone, 0.570 ± 0.031 mCi/g estradiol, and 0.0581 ± 0.002 mCi/g testosterone. Recrystallization of the steroids was also carried out without ^{3}H-labeling for use in blank samples and for solute characterization.

Drying of Testosterone Monohydrate

The monohydrate form of testosterone produced by recrystallization in acetone was dried to the anhydrous state by heating to 80°C for 48 h in the presence of phosphorus pentoxide (10–11). This process was carried out using both radiolabeled and non-radiolabeled testosterone monohydrate.

Solute Characterization

Recrystallized non-radiolabeled progesterone, estradiol, and testosterone were examined to verify the hydration state of solid steroids both prior to and following exposure to oils. It is assumed that the physical state of recrystallized, but non-radiolabeled, steroids is identical in crystal structure to ^{3}H-labeled counterparts, as a result of the recrystallizations being conducted identically. This assumption is necessary to avoid radiologic contamination of X-ray and thermal equipment. The X-ray diffraction pattern of recrystallized progesterone was consistent with anhydrous crystalline form (12). Differential scanning calorimetry (DSC) of recrystallized estradiol revealed two well defined endothermic peaks at 174°C and 180°C, which agrees well with values in the literature (13). Thermogravimetric analysis (TGA) of recrystallized estradiol revealed a loss of weight of 3.2%, which is consistent with the expected loss of water from an estradiol hemihydrate. The X-ray diffraction pattern of the recrystallized estradiol was in accordance with the crystal structure of the hemihydrate. DSC of recrystallized testosterone showed two well-defined endothermic peaks at 125°C and 163°C in agreement with the literature (11). TGA of recrystallized testosterone exhibited a loss of weight of 5.9%, consistent with the expected loss of water from a monohydrate of testosterone.

Solubility Studies

Soybean, olive, and Miglyol 812 oils in both the hydrated and desiccated states were exposed to excess solid, tracer-labeled ^{3}H-progesterone, ^{3}H-estradiol, or ^{3}H-testosterone in 4-ml glass vials with Teflon-lined caps. A parallel set of samples were prepared using recrystallized steroids containing no radiolabel. Samples were blanketed with nitrogen gas, sealed, and rotated at 27°C. At predetermined time points, samples containing radiolabel were withdrawn, filtered using an 0.2-μm nylon Acrodisc syringe filter, diluted with Scintiverse BD liquid scintillation cocktail, and allowed to rest overnight prior to analysis for total steroid content by liquid scintillation counting (LSC). Prior to the study, it was verified that none of the steroids showed significant adsorption to the nylon filter membrane. LSC measurements were performed on a Beckman model LS 6500 (Fullerton, CA, USA). All determinations were made in triplicate, with the %RSD be-

tween replicates of typically less than 5%. Equilibrium steroid solubility was established by monitoring the change in steroid content over time of each sample. Equilibrium was established when drug content varied by less than 5% between subsequent measurements separated by several days. After equilibrium, the excess solid in the nonradiolabeled samples was recovered and subjected to DSC and X-ray analysis.

In the case of measurement of solubility of anhydrous testosterone in hydrated oils, a slightly different procedure was followed due to the slow attainment of equilibrium. Samples of hydrated soybean, olive, or Miglyol 812 oils were exposed to excess solid, tracer-labeled ^{3}H-testosterone in the anhydrous form in glass vials with Teflon-lined caps. Blank samples were prepared with recrystallized testosterone in the anhydrous form. Samples were blanketed with nitrogen gas, sealed, and rotated at 27°C. Following rotation for 2 weeks (the time period indicated by preliminary studies for equilibrium solubility to be reached for testosterone monohydrate), the samples were filtered through an 0.2-μm Acrodisc filter to remove excess solute, and the filtrate was exposed to a few seed crystals of non-radiolabeled testosterone monohydrate (about 10 mg). Samples were then allowed to equilibrate again under nitrogen gas at 27°C. At predetermined time points (separated by several days) samples were taken as described above by filtration through a 0.2-μm Acrodisc filter and assayed by LSC. Examination of the crystals of testosterone by TGA and X-ray diffraction after exposure of the anhydrous form to hydrated and desiccated oils confirmed that the anhydrous crystalline form is converted to the monohydrate following exposure to hydrated oils, but remains in the anhydrous form following exposure to desiccated oils.

Specific Interactions

To probe the possibility of specific interactions, such as hydrogen bonding, between the steroids and triglycerides studies of steroids in cyclohexane/triolein solutions were carried out (14). Cyclohexane is a nonpolar, lipophilic hydrocarbon solvent, while triolein has the potential to act as a hydrogen bond acceptor. All three steroids can accept hydrogen bonds, but only estradiol and testosterone can potentially donate hydrogen bonds. The interaction between steroid and triglyceride molecules in cyclohexane can be written as

$$(S) + (L) \leftrightarrow (SL) \quad (1)$$

$$K_{11} = (SL)/(S)(L) \quad (2)$$

where S is free steroid, L is free triolein, and K_{11} is steroid-triolein equilibrium constant (14). If self-association of the steroids or triolein is assumed not to occur, and only 1:1 complexes of steroid-triolein are considered significant, then by mass balance the total concentration of steroid in solution (S_T) can be represented by

$$S_T = S_o + (K_{11}S_o)(L_T)/(K_{11}S_o + 1) \quad (3)$$

where S_o is the solubility of steroid in triolein (neat) and L_T is the total triolein in solution. The association constant between steroid and triolein can be calculated from a plot of (S_T) vs. (L_T). The solubility of progesterone, estradiol, and testosterone in 10 triolein/cyclohexane mixtures ranging from 0.01 M to 0.10 M triolein were carried out in the manner described above for solubility studies in hydrated and desiccated oils, with the only difference being that filtration was carried out using 0.45-μm PTFE Acrodisc filters. It was verified prior to the study that none of the steroids showed significant adsorption to the PTFE filter membrane.

RESULTS

Water Content of Oils

Table I summarizes the equilibrium water contents of hydrated and desiccated soybean, olive, and Miglyol 812 oil. Water content of oils was shown to vary significantly between the hydrated and desiccated oils, with the medium-chain triglyceride incorporating a much greater amount of water in the hydrated state than either of the long-chain triglycerides. Results indicate that ceramic molecular sieves have successfully dried triglycerides to <25 μg water/g oil (between 1:810 and 1:1530 mole ratios water to triglyceride) after exposure of the oils to sieves for about 10 days. Hydrated oils contained about 1/23, 1/21, and 1/16 mole ratios water to oil for soybean oil, olive oil, and Miglyol 812 oil, respectively.

Solubility Measurements

Presented in Table II are the equilibrium solubilities of anhydrous progesterone, estradiol hemihydrate, testosterone monohydrate, and anhydrous testosterone in soybean, olive, and Miglyol 812 oils in both the hydrated and desiccated states. In comparing the results of soybean oil to those of olive oil, it can be seen that the degree of hydrocarbon unsaturation has no significant effect on the solubility of the steroids. On the other hand, the length of the hydrocarbon chain does appear to have a significant impact on solubility. For instance, solubility of estradiol hemihydrate in hydrated Miglyol 812, is more than twice that observed in either hydrated soybean or olive oils. Greater solubility in shorter chain triglycerides is consistent with results typically seen in the literature for other solutes (15–18).

The effect of oil hydration on the solubility of steroids can also be observed in Table II. In all cases, hydrated oils dissolve significantly less estradiol hemihydrate, testosterone monohydrate, and anhydrous testosterone than do the desiccated oils. A loss of solubility of about 30–40% is seen in each case. The effect of hydration of oils on solubility of estradiol and testosterone is independent of chain length of the oil, as the same trend is seen in both hydrated long- and medium-chain triglycerides. Only progesterone solubility appears to be unaffected by the state of hydration of the oil.

Details of the possible mechanism of this observation can be examined by a comparison of the solubility of the mono-

Table I. Equilibrium Water Content of Oils (n = 12)

	Hydrated		Desiccated	
Oil	μg H_2O/g oil (SD)	Mole ratio H_2O:oil[a]	μg H_2O/g oil (SD)	Mole ratio H_2O:oil[a]
Soybean	896 (54)	1:23	<25	1:810
Olive	998 (65)	1:21	<25	1:830
Miglyol 812	2360 (93)	1:16	<25	1:1530

[a] Calculated using an average molecular weight of soybean oil of 886 g/mol (company literature, Croda, Inc.), olive oil of 872 g/mol, and Miglyol 812 oil of 471 g/ml (company literature, Sasol, Inc.).

Table II. Equilibrium Solubility of Anhydrous Progesterone, Estradiol Hemihydrate, Testosterone Monohydrate, and Anhydrous Testosterone in Triglyceride Oils of Varying Water Content

Oil	Anhydrous progesterone mg/g (SD)	Estradiol hemihydrate[a] mg/g (SD)	Testosterone monohydrate[a] mg/g (SD)	Anhydrous testosterone[a] mg/g (SD)
Soybean				
Hydrated	24.1 (1.1)	1.02 (0.02)	5.8 (0.4)	6.0 (0.4)
Desiccated	23.5 (0.9)	1.51 (0.01)	9.1 (0.2)	8.6 (0.2)
Olive				
Hydrated	20.9 (0.3)	1.01 (0.03)	5.4 (0.4)	5.2 (0.2)
Desiccated	23.9 (1.1)	1.45 (0.08)	8.5 (0.1)	8.0 (0.4)
Miglyol 812				
Hydrated	36.2 (1.2)	2.28 (0.1)	8.6 (0.3)	8.9 (0.2)
Desiccated	36.7 (0.8)	3.80 (0.1)	14.4 (0.7)	14.3 (0.4)

Soybean and olive oils represent long-chain triglycerides and Miglyol 812 represents a medium-chain triglyceride.

[a] Significant difference between hydrated and desiccated oils by *t* test ($p < 0.05$), $n = 3$.

hydrate and anhydrous forms of testosterone. In the case of the hydrated oils, no significant differences exist between the solubility results when comparing the anhydrous and monohydrate forms of testosterone as starting materials. Examination of the X-ray and TGA of the excess solid recovered from the samples showed the testosterone to be present only as the monohydrate (data not shown). For hydrated oils, no matter what crystal hydrate of testosterone is used to begin the solubility study, at equilibrium, the steroid will be in the less soluble monohydrate form. Thus, high water activity in the oil is associated with the production of the less soluble monohydrate. When considering desiccated oils, it was again found that no significant differences exist between the solubility results when comparing the anhydrous and monohydrate forms of testosterone as starting materials. X-ray and TGA examination of the solid remaining for anhydrous testosterone in desiccated oils verified the solid did remain in the anhydrous form.

Specific Interactions

Additional experiments were carried out to examine an alternative explanation of the effect of water on solubility of steroids in oils. It has been suggested that the greater solubility of many drug compounds in medium chain as compared to long chain triglycerides is due to specific interactions between the drug and the triglyceride functional groups (15). For lower molecular weight oils, the number density of hydrogen-bond acceptors is greater than the higher weight oils suggesting more opportunities exist for drug-oil hydrogen bonding. If hydrogen-bonding of the steroid to the triglyceride is a major driving force for solubilization of the drug, then the presence of a competitor for hydrogen bonding sites, such as water, could explain the diminished solubility of estradiol and testosterone in hydrated oils. It would also explain why progesterone, which cannot donate hydrogen bonds, is not influenced by the presence of water. Studies of anhydrous progesterone, estradiol hemihydrate, and testosterone monohydrate solubilization were carried out to determine if specific interactions were indeed occurring between the steroids and the model triglyceride triolein. In Fig. 1, total solubility of each of the steroids is plotted as a function of the concentration of triolein. A linear relationship with only a slightly positive slope was found between triolein concentration and solubility of each of the steroids. The association constants (K_{11}) for triolein and anhydrous progesterone, estradiol hemihydrate, and testosterone monohydrate were calculated from the above results using Eq. (3). $K_{11,\text{anhydrous progesterone}} = 2.25 \pm 0.28$, $K_{11,\text{estradiol hemihydrate}} = 10.52 \pm 0.42$, and $K_{11,\text{testosterone monohydrate}} = 2.96 \pm 0.60$. The K_{11} values for progesterone and testosterone were similar, despite the potential of only the latter to donate a hydrogen bond to triolein. The K_{11} value for estradiol does indicate a stronger association with triolein than was seen for the other two steroids, but the value is still much smaller than that typically observed in the literature for hydrogen bonding interactions (14). In addition, spectroscopic examination by Fourier-transform infrared spectroscopy (data not shown) was unsuccessful at finding evidence of hydrogen bonding between triolein and estradiol hemihydrate (19,20). These results suggest that specific interactions between the steroid molecules and the triglyceride molecules are probably very slight. It may thus be concluded that a strong competition with water for hydrogen bonding sites on the triolein does not appear to fully explain the reduced solubility of steroids in hydrated oils.

DISCUSSION

We have shown that the hydration of triglycerides has the potential to significantly alter the solubility of estradiol

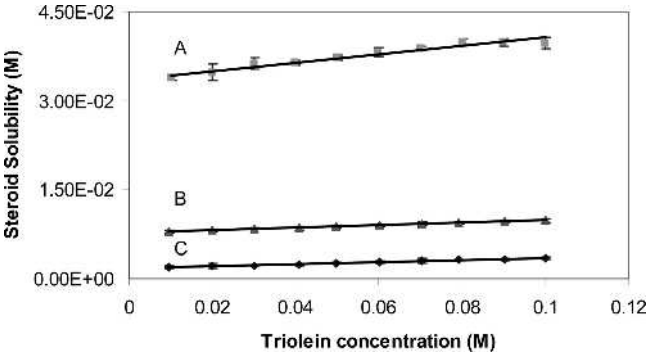

Fig. 1. Anhydrous progesterone (A), testosterone monohydrate (B), and estradiol hemihydrate (C) solubilization by cyclohexane/triolein mixtures. Mean ± SD, $n = 4$.

and testosterone but not progesterone. A strong interaction between a model triglyceride triolein and the steroid molecules is not detectable either by solubility studies in cyclohexane or by spectroscopic measurements, leading to the conclusion that solubility of estradiol and testosterone in triglycerides in the presence of water is governed by other factors. In the case of solutes capable of forming hydrates the following equilibrium exists:

$$\text{Drug} \bullet H_2O \leftrightarrow \text{Drug}_{an} + H_2O$$

where Drug • H_2O represents the hydrate, and Drug_{an} represents the anhydrous form. As is typical for this equilibrium reaction, supplying water to the system forces the reaction to the left, resulting in the formation of the less soluble hydrate. Conversely, removing water from the system favors the formation of the more soluble anhydrous form. The current findings suggest that even a small amount of water in oil is enough to shift the equilibrium in the direction of the hydrate for estradiol and testosterone. At first consideration, it may appear surprising that such low amounts of water in the hydrated oils can indeed induce hydrate formation in testosterone. In the case of hydrated soybean oil at equilibrium, water is present in slight excess over that of testosterone with about 2×10^{-5} moles of testosterone and 5×10^{-5} moles of water solubilized per gram of oil. In the case of hydrated Miglyol, water is present at a much greater excess at 1.3×10^{-4} moles per gram of oil. It should be kept in mind that although water solubility in the oils is fairly low, in the hydrated oils the water is present at unit thermodynamic activity, shifting the equilibrium to the hydrated solute. The low concentration of water in the hydrated oils may in part be responsible for the slow rate of conversion of the anhydrate to the hydrate (about 2 weeks), and so precautions should be taken to ensure that the equilibrium state is reached in solubility determinations. The extent to which this phenomenon may be observed in other solutes is not yet known. These findings are similar to those of Zhu and Grant who observed the conversion of ampicillin anhydrate to the less soluble trihydrate upon recrystallization of the solute from organic solvent-water mixtures (21).

Control of the environment of active pharmaceutical compounds has been recognized as vital in the pharmaceutical development and manufacturing processes due to the possibility of forming hydrated crystalline forms that may exhibit altered pharmaceutical properties (22). Based on our current findings, it seems that the water content of triglyceride oils used as excipients in pharmaceutical dosage forms must also be known in order to fully understand solubility results.

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REFERENCES

1. J. R. Robinson. Introduction: semi-solid formulations for oral drug delivery. *Bulletin Technique Gattefosse* **89**:11–13 (1996).
2. A. J. Humberstone and W. N. Charman. Lipid-based vehicles for the oral delivery of poorly water-soluble drugs. *Adv. Drug Del. Rev* **25**:103–128 (1997).
3. B. J. Aungst. Novel formulation strategies for improving oral bioavailability of drugs with poor membrane permeation or presystemic metabolism. *J. Pharm. Sci.* **82**:979–987 (1993).
4. A. T. Serajuddin, P. C. Sheen, D. Mufson, D. F. Berstein, and M. A. Augustine. Effect of vehicle amphiphilicity on the dissolution and bioavailability of a poorly water-soluble drug from solid dispersion. *J. Pharm. Sci.* **77**:414–417 (1988).
5. N. H. Shah, M. T. Carvajal, C. I. Patel, M. H. Infeld, and A. W. Malick. Self-emulsifying drug delivery systems (SEDDS) with polyglycolysed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. *Int. J. Pharm.* **106**:15–23 (1994).
6. R. A. Myers and V. Stella. Systemic bioavailability of penclomidine (NSC-338720) from oil-in-water emulsions administered intraduodenally to rats. *Int. J. Pharm.* **78**:217–226 (1992).
7. R. A. Schwendener and H. Schott. Lipophilic1-beta-D-arabinofuranosyl cytosine derivatives in liposomal formulations for oral and parenteral antileukemic therapy in the murine L1210 leukemia model. *J. Cancer Res. Clin. Oncol.* **122**:723–726 (1996).
8. Y. Cao, M. Marra, and B. 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).
9. U. Jain, W. Higuchi, C. Liu, P. Lee, and N. Mazer. Cholesterol thermodynamic activity determinations in bile salt-lecithin-cholesterol systems and cholesterol-rich liquid crystalline mesophase formation. *Pharm. Res.* **9**:792–799 (1992).
10. E. Shefter and T. Higuchi. Dissolution behavior of crystalline solvated and nonsolvated forms of some pharmaceuticals. *J. Pharm. Sci.* **52**:781–791 (1963).
11. A. Thakkar and N. Hall. Micellar solubilization of testosterone III: dissolution behavior of testosterone in aqueous solutions of selected surfactants. *J. Pharm. Sci.* **58**:68–71 (1969).
12. B. Haner and D. Norton. Crystal data. I. For some pregnenes and pregnadienes. *Acta Crystallogr.* **17**:1610 (1964).
13. B. Jerslev, S. Frokjaer, and P. Thorbek. Organic solid phase analysis II. Two unexpected cases of pseudopolymorphism. *Arch. Pharm. Chemi. Sci. Ed* **9**:123–130 (1981).
14. H. Fung and T. Higuchi. Molecular interactions and solubility of polar nonelectrolytes in nonpolar solvents. *J. Pharm. Sci.* **60**: 1782–1788 (1971).
15. Y. Yamaoka, R. Roberts, and V. Stella. Low-melting phenytoin prodrugs as alternative oral delivery methods for phenytoin: a model for other high-melting sparingly water-soluble drugs. *J. Pharm. Sci.* **72**:400–405 (1983).
16. B. K. Kang, J. S. Lee, S. K. Chon, S. Y. Jeong, S. H. Yuk, G. Khang, H. B. Lee, and S. H. Cho. Development of self-microemulsifying drug delivery systems (SMEDDS) for oral bioavailability enhancement of simvastatin in beagle dogs. *Int. J. Pharm.* **274**:65–73 (2004).
17. C. Malcolmson, C. Satra, S. Kantaria, A. Sidhu, and M. J. Lawrence. Effect of oil on the level of solubilization of testosterone propionate into nonionic oil-in-water microemulsions. *J. Pharm. Sci.* **87**:109–116 (1998).
18. T. R. Kommuru, B. Gurley, M. A. Khan, and I. K. Reddy. Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *Int. J. Pharm.* **212**:233–246 (2001).
19. E. Lamcharfi, G. Kunesch, C. Meyer, and B. Robert. Investigation of cyclodextrin inclusion compounds using FT-IR and Raman spectroscopy. *Spectrochimica Acta Part A* **51**:1861–1870 (1995).
20. F. Parker and K. Bhaskar. Self-association of cholesterol and its interaction with triglycerides. An IR study. *Biochemistry* **7**:1286–1290 (1968).
21. H. Zhu and D. Grant. Influence of water activity in organic solvent + water mixtures on the nature of the crystallizing drug phase. 2. Ampicillin. *Int. J. Pharm.* **139**:33–43 (1996).
22. J. De Smidt, J. Fokkens, H. Grijseels, and D. Crommelin. Dissolution of theophylline monohydrate and anhydrous theophylline in buffer solutions. *J. Pharm. Sci.* **75**:497–501 (1986).