Estimation of the Increase in Solubility of Drugs as a Function of Bile Salt Concentration

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Purpose. The objective of this study was to develop a model to predict the extent to which bile salts can enhance the solubility of a drug, based on the physicochemical properties of the compound. The ability to predict bile salt solubilization of poorly soluble drugs would be a key component in determining which drugs will exhibit fed vs. fasted differences in drug absorption.

Methods. A correlation between the logarithm of the octanol/water partition coefficient [log P] of six steroidal compounds and their solubilities in the presence of various concentrations of sodium taurocholate at 37°C, $log\ [SR] = 2.234 + 0.606\ log\ [P]$ ($r^2 = 0.987$) where SR is the ratio of the solubilization capacity of the bile salt to the solubilization capacity of water for the drug, was used to predict the solubility of five further compounds with diverse structures. The solubilities of the compounds in presence of sodium taurocholate were then measured.

Results. The predicted solubilities were within 10% of the experimentally observed solubilities for griseofulvin, cyclosporin A and pentazocine. The model overpredicted the solubility of phenytoin and diazepam in 15 mM sodium taurocholate solution by a factor of 1.33 and 1.62 respectively.

Conclusions. The expected increase in solubility as a function of bile salt concentration can be estimated on the basis of the partition coefficient and aqueous solubility of the compound.

KEY WORDS: poorly soluble drugs; solubility; bile salts; sodium taurocholate; partition coefficient.

INTRODUCTION

An important determinant of drug absorption from the gastrointestinal tract (GIT) is the rate at which the drug goes into solution. Food intake exerts a complex influence on the bioavailability of drugs from the GIT. Although for some drugs the effects of food on drug absorption are due to physical or chemical interaction with specific food components (1,2), in many cases, the changes in GI physiology parameters associated with conversion from the fasted to the fed state are likely to be the main source of differences in availability. The absorption of poorly soluble drugs such as griseofulvin (3), phenytoin (4) and danazol (5), has been found to increase in the presence of food. One possible explanation for this phenomenon is that delayed gastric emptying caused by the presence of food in the stomach allows more time for disintegration and dissolution of these drugs/formulations before they enter the small intestine. A second possibility is that the increase is related to the sharp increase in bile concentration in the small intestine in response to ingestion of the meal. On an average, the bile salt concentrations in the duodenum and upper jejunum are approximately 2–3 times higher postprandially (10–15 mM; range 3–35 mM) (6,7), as compared to fasting conditions (5 mM, range 0–14 mM) (7,8). Thus, the absorption of drugs showing dissolution-rate limited uptake may be enhanced, when administered in the fed state, due to the surfactant effects of bile components.

Bile salt enhanced dissolution has been demonstrated *in vitro* for compounds that exhibit food related increases in absorption, e.g. griseofulvin (9), danazol (10,11) and phenytoin (12). The dissolution rate (DR) of a compound can be described by a modification of the Noyes-Whitney equation (13)

$$DR = dc/dt = A\ D\ (Cs)/V\ h \qquad (1)$$

The bile salts may decrease the interfacial energy between the drug and the dissolution medium, thereby increasing the effective surface area (A) available for dissolution (9). They may also increase the solubility of the drug (Cs) via micellar solubilization. Typically, wetting effects predominate at bile salt concentrations below the CMC, and solubility effects tend to prevail above the CMC (14); however, the dominant mechanism by which the dissolution rate increases may vary from compound to compound (10,11).

It is currently not possible to identify, on an *a priori* basis, which compounds will be more bioavailable if dosed in the fed state. Development of a model for predicting the extent to which bile salts can enhance the dissolution of drugs, based on physicochemical properties of the drug such as partition coefficient, melting point, aqueous solubility etc., could aid in the selection of dosing strategy and formulation design for compounds which exhibit dissolution-rate limited absorption. A first step in developing a predictive model is to establish a means of predicting increases in solubility of drugs as a function of bile salt concentration.

MATERIALS AND METHODS

Hydrocortisone (lot 38F0863), betamethasone 17-valerate, griseofulvin (lot 70H-0418) and phenytoin (lot 16F-0215) were purchased from Sigma Chemical Co. (St. Louis, Missouri). Betamethasone (lot 8001) and dexamethasone were donated by The Upjohn Co. (Kalamazoo, Michigan), cyclosporine A (lot 88095.01) by Sandoz Inc. (East Hanover, New Jersey), danazol (WIN-17757) and pentazocine (lot ZA) by Sterling-Winthrop Inc. (Collegeville, Pennsylvania), triamcinolone by Lederle Laboratories (Pearl River, New York) and diazepam (lot 227-070) by Hoffman La Roche (Nutley, New Jersey). Table I gives a summary of the physicochemical properties of the compounds studied.

Sodium taurocholate (lot 112H-5002, Sigma Chemical Co., St. Louis, Missouri) was chosen as the model bile salt

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Table I. Physicochemical Properties of the Compounds Studied

Compound	Aqueous solubility (μg/ml)[a]	log P[b]	Melting point[i]	pKa	Mol. weight
Triamcinolone	168	1.025[c,d]	270	N/A	394.44
Hydrocortisone	326	1.55[c,d]	219	N/A	362.47
Dexamethasone	92	1.89[c,d]	270	N/A	392.47
Phenytoin	27.1	1.92[e]	296	8.06[j]	252.30
Betamethasone	63	1.97[c,d]	233	N/A	392.47
Griseofulvin	29.9	2.18[f]	220	N/A	352.71
Diazepam	65.2	2.82[f]	133	3.3[k]	284.75
Cyclosporine A	6.6	3.00[g]	150	N/A	1202.60
Betamethasone 17-valerate	5.9	3.60[c,d]	183	N/A	476.58
Pentazocine	44.9	4.03[h]	148	8.95[l]	285.43
Danazol	1.05	4.53[e]	225	N/A	337.46

[a] Determined experimentally at 37°C.
[b] Octanol/water partition coefficient.
[c] Tomida, 1978 (15).
[d] Caron, 1984 (16).
[e] Determined experimentally by HPLC method (16).
[f] Leo, 1971 (17).
[g] Taylor, 1993 (18).
[h] Wilson, 1984 (19).
[i] Merck Index.
[j] Schwartz, 1977 (20).
[k] Barret, 1973 (21).
[l] Osol, 1980 (22).
N/A—Not applicable.

because cholic acid is one of the major bile acids in the small intestine. Unlike glycine conjugated bile salts which have pKa values typically in the range of 4.3–5.3, the pKa of sodium taurocholate is approximately 2 (23). This ensures that taurocholate will be completely ionized at pH 5.5 and therefore will not precipitate at the concentrations used. The concentrations of the bile salt chosen (0–30 mM) span the usual concentration ranges in the fasted and fed states. The CMC of sodium taurocholate, as determined by surface tension experiments at 37°C and ionic strength of 0.1M, was 3 mM.

Solubility Measurements

Solubility studies were conducted in McIlvaine Buffer (pH 5.5, ionic strength 0.1M). The buffer consisted of 0.05M citric acid (Columbus Chemical Industries, Columbus, WI), 0.1M disodium hydrogen phosphate (Aldrich, Milwaukee, WI) and the desired concentration of sodium taurocholate. The ionic strength (IS) was adjusted to 0.1M (representative of physiologic IS) with sodium chloride (Mallinckrodt, Paris, KY). The final pH of 5.5 was verified with an Orion Research Model 811 pH meter (Orion Research, Cambridge, MA). pH 5.5 represents a luminal pH intermediate between the fasted and fed states (24). Pentazocine solubility experiments were also conducted in pH 7.0 McIlvaine Buffer (0.033M citric acid, 0.067M Na_2HPO_4, IS 0.1M), in order to determine the effect of extent of ionization on the increase in solubilization via bile salt micelles.

Screw cap vials containing an excess of the drug and 20 ml of the corresponding bile salt solution were shaken on an orbital rotating mixer (Adam's Nutator, Becton Dickinson, Parsippany, New Jersey) in an oven maintained at 37°C (Theleco Precision Oven, Precision Scientific Co., Chicago, Illinois). All determinations were made in triplicate. Samples were taken at 4, 8, 24 and 48 h, to ensure that the equilibrium solubility had been reached. The samples were filtered through a 0.4 μM polycarbonate filter (Nucleopore, Pleasanton, California), and diluted appropriately prior to analysis.

HPLC Assay

Danazol and betamethasone 17-valerate were analyzed using a Partisil 10 ODS 10 μm column (250 mm × 4.6 mm; Whatman Inc., Clifton, New Jersey). The mobile phases consisted of 30% water: 30% methanol: 40% acetonitrile for danazol and 75% methanol: 25% water for betamethasone 17-valerate. Flow rates of 1 ml/min and 1.2 ml/min (Spectroflow 400, Kratos Analytical, Ramsey, New Jersey) resulted in retention times of 8 and 5 min. for danazol and betamethasone 17-valerate, respectively. The absorbance was measured at 280 nm (Spectroflow 773 Absorbance Detector, Kratos Analytical, Ramsey, New Jersey) for danazol and at 242 nm for betamethasone 17-valerate. Phenytoin, diazepam and cyclosporine were analyzed using a 250 mm × 4.6 mm Spherisorb ODS-2 column with 10 μ packing (Alltech, Deerfield, Illinois). The mobile phases consisted of acetonitrile/water (40:60 v/v) for phenytoin, 45% methanol: 50% water: 5% acetonitrile for diazepam, while acetonitrile/water (70:30 v/v) for cyclosporine. Flow rates of 0.8, 2 and 2 ml/min resulted in retention times of 8, 12 and 10 min for phenytoin (228 nm), diazepam (228 nm) and cyclosporine (215 nm), respectively. Samples were injected via an automatic sampler (AN-728 autosampler, Anspec, Ann Arbor, Michigan)

and injector (two-position electric value actuator, Valco Instruments Co. Inc., Houston, Texas). The signal was recorded and integrated with a model D-2000 Chromato-Integator (Hitachi Ltd, Tokyo). The method of external standards was used to convert the measured peak heights to concentration units. Calibration curves for standard solutions had excellent correlation coefficients in all cases (R^2 values of 0.99 were typical).

UV Assay

Betamethasone, dexamethasone, hydrocortisone, triamcinolone, griseofulvin and pentazocine were analyzed by a scanning UV spectrophotometer (Perkin-Elmer Lambda 7, Danbury, Connecticut). Standard curves for each bile salt concentration/pH/drug combinations were constructed and the peak wavelength determined. The Beer-Lambert law was closely obeyed for each compound at all bile salt levels (R^2 values of 0.99 were typical).

RESULTS AND DISCUSSION

Development of the Model

In an attempt to predict the enhancement of solubilities in the presence of bile salts, the effect of sodium taurocholate on the solubility of a series of six structurally similar steroids viz. hydrocortisone, triamcinolone, dexamethasone, betamethasone, betamethasone 17-valerate and danazol, was investigated at seven different taurocholate concentrations (0, 0.1, 1, 3.75, 7.5, 15 and 30 mM). The results of these studies have been previously reported (10). There was no significant increase in solubility of these steroids at the fasted state levels (0 mM to 7.5 mM) of taurocholate. Increases in solubility above the CMC i.e., as the bile salt concentration increased from the fasted to the fed state levels (15 mM), were greater for the more lipophilic compounds.

In order to predict the degree of solubilization by bile salts, solubility curves were constructed for these steroids as a function of taurocholate concentration. For concentrations below the CMC, there was no increase or very little increase in solubility of each steroid with increase in taurocholate concentration. For concentrations above the CMC, the solubilities increased linearly as a function of bile salt concentration. From the slope of the linear parts of the solubility profiles, the solubilization capacities (moles drug/mole bile salt) of the bile salt micelles were calculated. It was observed that the solubilization capacity of the bile salt, SCbs, was on the same order of magnitude for all compounds studied. However, the solubilization capacity of water, SCaq, (moles drug/mole water), varied widely among the compounds. It therefore appears that the driving force for bile salt solubilization of steroids is determined by their hydrophobicity, rather than their affinity for bile salt micelles.

When the logarithm of the solubilization ratio, (SR = SCbs/SCaq) was plotted against the logarithm of the octanol/water partition coefficients for the steroids, a linear relationship was observed with

$$log\ SR = 2.23 + 0.61\ log\ P \quad (r^2 = 0.99) \qquad (2)$$

From this relationship, it was determined that the solubility of the steroids at any given taurocholate concentration and the corresponding increase in solubility [C_{sx}/C_{so}] can be predicted from the equation

$$C_{sx} = C_{so} + (SC_{bs})\ (MW)\ ([NaTC]) \qquad (3)$$

where C_{sx} is the solubility (μg/ml) in the presence of taurocholate, C_{so} is the aqueous solubility (μg/ml) in the absence of bile salts, (SC_{bs}) is the solubilization capacity of the bile salt for the drug (predicted from the partition coefficient using the regression equation (2), and the solubilization capacity of water for the drug), MW is the molecular weight of the compound, and [NaTC] is the concentration of sodium taurocholate in mM.

Verification of the Model

To determine whether this relationship holds for compounds other than the steroids, the model was used to predict the solubilities of 5 non-steroidal compounds (griseofulvin, phenytoin, diazepam, cyclosporine A and pentazocine) in the presence of sodium taurocholate. For example, the solubility of griseofulvin in 15 mM NaTC was predicted by first calculating the solubilization ratio from the partition coefficient of griseofulvin (equation 2) to be SR = 3595. Next, the aqueous solubility of griseofulvin (30 μg/ml) was converted to moles griseofulvin/mole water, to give the solubilization capacity of water, SCaq = 14×10^{-7}. Since the solubilization ratio, SR, is defined as SR = SCbs/SCaq, the solubilization capacity of the bile salt for griseofulvin was then calculated by taking the product of SR and SCaq, resulting in SCbs = 5×10^{-3}. At a concentration of 15 mM sodium taurocholate, the solubility of griseofulvin was predicted from equation 3 to be 56 μg/ml. The experimentally observed solubility (n = 3) was 55 μg/ml, in excellent agreement with the prediction based on the steroid data.

The solubilities of griseofulvin, phenytoin, diazepam and cyclosporine A at various concentrations of sodium taurocholate were similarly predicted. The solubilities predicted from equation 3 at 15 mM taurocholate are compared to the experimentally determined solubilities in Table II. It is seen that the model predicts the solubility of griseofulvin and cyclosporine A quite accurately, however, it overpredicts the solubility of phenytoin and diazepam in 15 mM bile salt solution by a factor of 1.33 and 1.62 respectively.

In order to predict the solubility of the ionizable drug pentazocine, the measured solubility at the pH of interest seems to be required, in addition to the solubility in water and octanol/water partition coefficient. The experimental solubilities at pH 5.5 (6.85 mg/ml) and pH 7.0 (0.93 mg/ml) were used in place of Cso (solubility in water) in equation (3), to calculate the predicted solubilities at 15 mM taurocholate. The experimental and predicted solubilities were found to be quite similar (Table II). When we attempted to use the Henderson Hasselbalch equation (equation 4)

$$Cso' = Csi\ \{1 + [H^+]/Ka\} \qquad (4)$$

where Csi is the intrinsic solubility of the free base, [H^+] is the hydrogen ion concentration and Ka is the dissociation

Table II. Comparison of Predicted vs. Experimental Solubility of Non-steroidal Compounds at 15 mM Sodium Taurocholate

Compound	Predicted solubility (μg/ml)	Experimental solubility (μg/ml)
Cyclosporine A	26.40	26.43
Griseofulvin	55.78	55.14
Phenytoin	44.88	33.67
Diazepam	192.50	118.70
Pentazocine (pH 5.5)	7424.18	7723.05
Pentazocine (pH 7.0)	1509.17	1658.02

constant, to predict the solubility of pentazocine at the pH of interest (Cso'), it overpredicted the solubility by more than an order of magnitude. At pH 5.5, Cso' was predicted to be 108 mg/ml, as compared to the experimentally determined value of 6.85 mg/ml. Since the initial and final pH at equilibrium for the solubility determination was 5.5, the probable explanation for the overprediction of Henderson Hasselbalch equation in terms of pentazocine solubility is that a salt is formed, the solubility of which becomes limiting to the apparent aqueous solubility. Thus the use of a measured rather than a calculated solubility at the pH of interest as the basis point for predictions is appropriate.

As for the steroidal compounds, there was very little solubilization of non-steroids at taurocholate concentrations below the CMC. Above the CMC, the solubilities increased linearly with bile salt concentration and solubilization ratios could be calculated in a manner analogous to that used for the steroids. The results are plotted in Fig. 1. The log [SR] for cyclosporine A, griseofulvin, pentazocine at both pH 5.5 and pH 7.0, fall on or very close to the prediction line based on the steroids. The model does not fit quite so well for diazepam and phenytoin. The solubilization ratio calculated

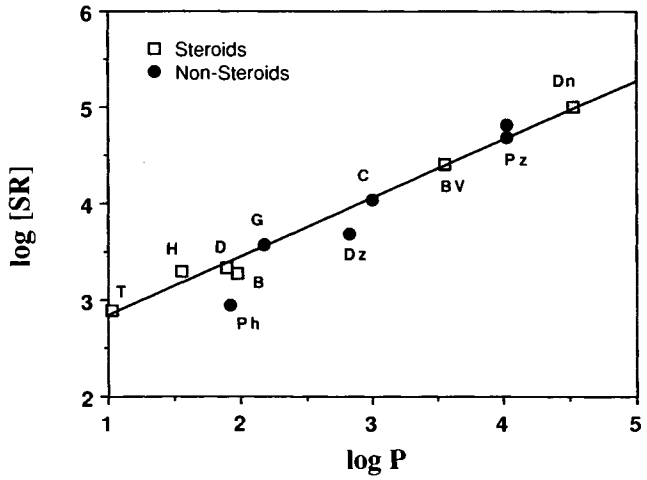

Fig. 1. Log [Solubilization Ratio] in aqueous solution taurocholate solutions as a function of log [octanol/water partition coefficient] for the non-steroidal compounds (circles; Ph = phenytoin, G = griseofulvin, Dz = diazepam, C = cyclosporin A, Pz = pentazocine), along with the prediction line based on steroid data (squares; T = triamcinolone, H = hydrocortisone, D = dexamethasone, B = betamethasone, BV = betamethasone 17-valerate, Dn = danazol); log [SR] = 2.23 + 0.60 log [P].

from the partition coefficient by utilizing equation 2, seems to overpredict the actual solubilization ratio and hence the actual solubilization capacity of the bile salt micelles for these two compounds, to some extent.

When the log SR of all the eleven compounds (steroids and non-steroids) was regressed against log P, a linear relationship was still observed

$$\log SR = 2.09 + 0.64 \log P$$

but the correlation coefficient was slightly reduced, $r^2 = 0.951$.

There have been many previous attempts to correlate solubilization by surfactants with physicochemical properties of organic compounds (25). Attwood and Florence state that "No simple relationship exists between any single property of a solubilizate and its maximum additive concentration in a given surfactant." Polarity and polarizibility, chain length and chain branching, molecular size, shape and structure have been shown to affect solubilization of a compound in a surfactant system. Several authors have noted an increase in the tendency for solubilization by a surfactant with an increase in lipophilicity. Where they exist, correlations tend to hold only for a narrow range of compounds. A linear relationship has been reported between the lipophilicity of the solubilizate, expressed as either its octanol/water or ether/water partition coefficient (P) and its partition coefficient between micelles and aqueous phase (Pm), for substituted barbituric acids solubilized by polyoxyethylene stearates (26), for steroids by long-chain polyoxyethylene nonionic surfactants (27) and for a series of benzoic acid derivatives solubilized by polysorbate 20 (28). Tomida et al. noted a similar linear free energy relationship between log [Pm] and log [P], for the solubilization of a set of benzoic acid derivatives by polyoxyethylene lauryl ether (29). Three parallel lines were required to represent log [Pm] vs. log [P] data adequately, although most derivatives could be represented by one of these lines. The additional lines were required for the nitro and cyano substituents and for the dicarboxylic acid derivatives, indicating the solubilization of these derivatives in different regions of the micelle. Similarly, the solubilization of several steroids by polyoxyethylene lauryl ether micelles could be represented by two lines when plotted as log [Pm] against log [P]; one line representing steroids possessing a fluorine atom and the other line representing steroids without this substituent (15). Fahelelbom and co-workers found a linear relationship between log [Pm] and log [P], for the micellar solubilization of clofazimine analogues in Triton X, an ionic surfactant and sodium lauryl sulfate, a non-ionic surfactant (30).

Our results show that the increase in solubility as a function of bile salt concentration can generally be predicted simply on the basis of the partition coefficient and aqueous solubility of the compound. The accuracy of the prediction is good for compounds as structurally diverse as the steroids, for cyclosporine A, griseofulvin and pentazocine, and is a reasonable estimate for phenytoin and diazepam. It should be additionally noted that only for highly lipophilic drugs is solubilization by sodium taurocholate important: a log P value of greater than 2.5 is required for a doubling of the solubility at 15 mM sodium taurocholate.

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REFERENCES

1. J. P. Griffin. Drug interactions occurring during absorption from the gastrointestinal tract. *Pharmac. Ther.* 15:79–88 (1981).
2. A. Melander. Influence of food on the bioavailability of drugs. *Clin Pharmacokinet.* 3:337–351 (1978).
3. R. G. Crounce. Human pharmacology of griseofulvin: The effect of fat intake on gastrointestinal absorption. *J. Invest. Dermatol.* 37:529–532 (1961).
4. T. Hamaguchi, D. Shinkuma, T. Irie, Y. Yamanaka, Y. Morita, B. Iwamoto, K. Miyoshi, and N. Mizuno. Effect of a high-fat meal on the bioavailability of phenytoin in a commercial powder with a large particle size. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 31(7):326–330 (1993).
5. W. N. Charman, M. C. Rogge, A. W. Boddy, and B. M. Berger. Effect of food and a monoglyceride emulsion formulation on danazol bioavailability. *J. Clin. Pharmacol.* 33:381–386 (1993).
6. O. Fausa. Duodenal bile acids after a test meal. *Scand. J. Gastroent.* 9:567–570 (1974).
7. A. Tangerman, A. vanSchaik, and E. W. vander Hook. Analysis of conjugated and unconjugated bile acids in serum and jejunal fluid of normal subjects. *Clin. Chem. Acta.* 159:123–132 (1986).
8. T. L. Peeters, G. Vantrappen and J. Janssens. Bile acid output and the interdigestive migrating motor complex in normals and in cholecysectomy patients. *Gastroent.* 79:678–681 (1980).
9. T. R. Bates, M. Gibaldi, and J. L. Kanig. Rate of dissolution of griseofulvin and hexoestrol in bile salt solutions. *Nature.* 210:1331–1333 (1966).
10. V. Bakatselou, R. Oppenheim, and J. Dressman. Solubilization and wetting effects of bile salts on the dissolution of steroids. *Pharm. Res.* 8:1461–1469 (1991).
11. L. J. M. Naylor. *Dissolution mechanisms of poorly soluble compounds in simple and mixed micelle systems.* Doctoral Thesis. The University of Michigan. (1993).
12. D. Shinkuma, T. Hamaguchi, M. Koboyashi, N. Mizuno, and Y. Noboru. Influence of bile on the gastrointestinal absorption of phenytoin in rats. *Chem. Pharm. Bull.* 33:5023–5027 (1985).
13. A. A. Noyes, and W. R. Whitney. The rate of solution of solid substances in their own solution. *J. Am. Chem. Soc.* 19:930–934 (1897).
14. H. Weintraub, and M. Gibaldi. Physiological surface active agents and drug absorption IV: Effect of premicellar concentrations of surfactant on dissolution rate. *J. Pharm. Sci.* 58:1368–1372 (1969).
15. H. Tomida, T. Yotsuyanagi, and K. Ikeda. Solubilization of steroid hormones by polyoxyethylene lauryl ether. *Chem. Pharm. Bull.* 26:2832–2837 (1978).
16. J. C. Caron, and B. Shroot. Determination of partition coefficients of glucocorticosteroids by high performance liquid chromatography. *J. Pharm. Sci.* 73:1703–1706 (1984).
17. A. Leo, C. Hansch, and D. Elkins. Partition coefficient and their uses. *Chem. Rev.* 71:525–616 (1971).
18. N. Taylor, A. E. Mark, P. Vallat, R. M. Brinne, B. Testa, and W. F. van Gunterent. Solvent-dependent conformation and hydrogen-bonding capacity of cyclosporine A: evidence from partition coefficients and molecular dynamics simulations. *J. Med. Chem.* 36:3757–3764 (1993).
19. T. D. Wilson. Pentazocine. In *Analytical Profiles of Drug Substances.* vol. 13, 1984, pp. 361–416.
20. P. A. Schwartz, C. T. Rhodes, and J. W. Cooper. Solubility and ionization characteristics of phenytoin. *J. Pharm. Sci.* 66:994–997 (1977).
21. J. Barrett, W. F. Smyth, and I. E. Davidson. An examination of acid-base equilibria of 1,4-benzodiazepines by spectrophotometry. *J. Pharm. Pharmacol.* 25:387 (1973).
22. A. Osol. *Remington's Pharmaceutical Sciences.* 16th edition. Mack Publishing Co., Easton, Pennsylvania, 1980.
23. M. C. Carey, and D. M. Small. Micelle formation by bile salts: Physical, chemical and thermodynamic conditions. *Arch. Intern. Med.* 130:506–527 (1972).
24. J. B. Dressman, R. R. Berardi, L. C. Dermentzoglou, T. L. Russell, S. P. Schmaltz, J. L. Barnett, and K. M. Jarvenpaa. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm. Res.* 7:756–761 (1990).
25. D. Attwood and A. T. Florence. *Surfactant Systems, Their Chemistry, Pharmacy and Biology,* Chapman and Hall, London, 1983.
26. A. A. Ismail, M. Wafik Gouda, and M. M. Motawi. Micellar solubilization of barbiturates I. Solubilities of certain barbiturates in Polysorbates of varying hydrophobic chain length. *J. Pharm. Sci.* 59:220–224 (1970).
27. B. W. Barry and D. I. D. El Eini. Solubilization of hydrocortisone, dexamethasone, testosterone and progesterone by long chain polyoxyethylene surfactants. *J. Pharm. Pharmacol.* 28:210–218 (1976).
28. J. H. Collet and I. Koo. Interaction of substituted benzoic acids with polysorbate 20 micelles. *J. Pharm. Sci.* 64:1253–1255 (1975).
29. H. Tomida, T. Yotsuyanagi, and K. Ikeda. Solubilization of benzoic acid derivatives by polyoxyethylene lauryl ether. *Chem. Pharm. Bull.* 26:2824–2831 (1978).
30. K. M. S. Fahelelbom, R. F. Timoney, and O. I. Corrigan. Micellar solubilization of clofazimine analogues in aqueous solutions of ionic and nonionic surfactants. *Pharm. Res.* 10:631–634 (1993).