Pharmaceutical Research, Vol. 7, No. 4, 1990

Report

Intestinal Absorption of Drugs. III. The Influence of Taurocholate on the Disappearance Kinetics of Hydrophilic and Lipophilic Drugs from the Small Intestine of the Rat

Fred G. J. Poelma,[1] Ria Breäs,[1] and Josef J. Tukker[1,2]

Received February 2, 1989; accepted October 26, 1989

The influence of sodium taurocholate (TC) on the intestinal absorption of drugs was studied *in vivo* in a chronically isolated internal loop in the rat. The hydrophilic drugs paracetamol (PA) and theophylline (TP) and the lipophilic drugs griseofulvin (GF) and ketoconazole (KE) were used as model drugs. The drug concentrations were kept below the saturation concentration. Absorption kinetics of the drugs were evaluated on the basis of disappearance rates of the drug from luminal solutions in the intestinal loop. Concentrations of TC above the critical micelle concentration (CMC) did not affect the absorption rate of the hydrophilic drugs PA and TP; the barrier function of the intestinal wall for PA and TP was not altered in the presence of taurocholate. The addition of concentrations of TC above the CMC in the perfusion solution resulted in a reduction of the absorption rate of GF and KE. The reduction in the absorption kinetics of GF in the presence of TC correlated well with the reduction of the drug-free fraction in solution due to micellar solubilization. For KE this relation was less clear. It was not possible to determine, on the basis of the phase-separation model, to what extent the fraction of the drug incorporated in TC micelles contributes to the overall diffusion of GF and KE across the preepithelial diffusion barrier. It was concluded that TC exhibits only a minor, if not negligible, effect on the barrier function of the aqueous diffusion barrier adjacent to the intestinal wall.

KEY WORDS: absorption; small intestine; hydrophilic/lipophilic drugs; isolated intestinal loop; taurocholate.

INTRODUCTION

Bile salts can improve the bioavailability of poorly water-soluble lipophilic drugs by increasing the dissolution rate of the drug in the GI tract (1–5). On the other hand, the mechanism by which bile salts can affect the transport of the dissolved compound from the GI lumen into the systemic circulation is only poorly understood. The influence of bile salts on the transfer of the dissolved drug across the absorption barrier (a combination of a preepithelial diffusion resistance, including mucus, and the intestinal wall) has been ascribed to an alteration of the barrier function (6,7) or to a decreased thermodynamic activity of the drug caused by incorporation in micelles (6,8,9). The barrier function is reportedly affected via a multitude of interactions between bile salts and the mucous layer or the cellular membranes (7,10–12).

The discrepancy in data on the influence of bile salts on drug absorption reported in the literature can be ascribed partly to the different experimental techniques (*in vitro, in situ,* and *in vivo*). All investigational methods have one or more serious drawbacks, e.g., poorly defined absorption areas, the use of anaesthesia, gradual disintegration of the isolated intestinal segments and membranes. In this study the absorption kinetics of drugs are evaluated in an animal model, which offers several opportunities in the study of absorption kinetics under well-defined conditions: (i) a constant absorption surface (and intact blood supply), (ii) the possibility for a crossover experimental scheme, (iii) a complete control over the contents of the intestinal segment, and (iv) the absence of anaesthesia.

The purpose of the present study was to gain more insight into the mechanism by which taurocholate (TC) affects the rate of transport of a solute from the lumen across the preepithelial aqueous diffusion barrier and the intestinal wall in the small intestine of the rat. In this report several model drugs with varying lipophilicity were tested. Paracetamol (PA) and theophylline (TP) were chosen as hydrophilic compounds. Griseofulvin (GF) and ketoconazole (KE) were selected on the basis of their lipophilic character and poor bioavailability (13,14).

The absorption kinetics of the model compounds were established on the basis of the disappearance of the drug from the perfusion solution. The consequences of solubilization in micellar TC solutions on the absorption of the respective model compounds were evaluated on the basis of the phase separation model (15).

[1] Department of Pharmaceutics, Faculty of Pharmacy, University of Utrecht, Croesestraat 79, 3522 AD Utrecht, The Netherlands.
[2] To whom correspondence should be addressed.

0724-8741/90/0400-0392$06.00/0

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade. Ketoconzaole was a gift from Janssen Pharmaceutica, Beerse, Belgium. Griseofulvin was supplied by Aldrich Chemical Co., Milwaukee, Wis. Paracetamol and theophylline monohydrate were obtained from OPG, Utrecht, NL, and from Brocacef, Maarssen, NL, respectively. Taurocholate was supplied by Sigma Chemical Co., St. Louis, Mo.

A pH-meter (Type E632, Metrohm Herisau, Switzerland), a constant-temperature water bath (Thermomix 1420, B. Braun, FRG), a spectrophotometer (Pye Unicam PU 8600, Cambridge, UK), HPLC system (Model 710B Waters Intelligent Sample Processor and Model 440 UV Absorbance Detector, Waters Associates, Milford, MA), and a peristaltic pump (VRX-22, Verder, Düsseldorf, FRG), were used.

Experiments *in Vitro*

Partition Coefficient Determination of the Model Compounds

The lipophilic nature of a compound can be described by its partition over a highly lipophilic solvent phase and an aqueous phase. For the aqueous phase the same medium was applied as in the absorption experiments (perfusion solution). The aqueous phase was phosphate-buffered saline [66 m*M* sodium phosphate, 88 m*M* sodium choloride, pH 7.4; due to the extremely low solubility of the base KE at pH 7.4, for KE the pH was adjusted to 6.5 (pK_a of KE)]; *n*-octanol was used as the lipophilic phase.

The partition coefficients were determined at 21°C by shaking 2.0 ml of the octanol phase with 2.0 ml of the aqueous phase for 30 min. The concentration of the model drug in the aqueous phase before the addition of the octanol phase is similar to the concentration which is used in the absorption experiments (Table I) and is below the saturation concentration of the drugs in the aqueous phase. After centrifugation the drug concentrations in the aqueous and the octanol phase were measured by high-performance liquid chromatography (HPLC). The experimental characteristics of the various HPLC methods are outlined in Table II. The partition coefficient (P) was calculated from

$$P = C_{oc}/C_{aq} \quad (1)$$

where C_{oc} is the concentration of the drug in the octanol phase and C_{aq} the concentration in the aqueous phase.

Solubility of the Model Compounds

The solubility of GF, PA, or TP was determined in phosphate-buffered saline (66 m*M* sodium phosphate, 88 m*M* sodium chloride, pH 7.4) by shaking suspensions of each compound for 24 hr at 37°C. The solubility of KE was determined on phosphate-buffered saline at pH 6.5 (pK_a of KE). After centrifugation (30 min, 4000 rpm), the concentration of the drug in the supernatant was determined directly by a HPLC method (Table II). The buffer solutions used for the solubility determinations were identical to the perfusion buffers.

The solubilization of the model drugs by TC was determined by measuring the solubility of the respective compounds in the same buffer solutions as mentioned above with TC concentrations above the critical micelle concentration (CMC), and the fraction of the drug free in solution was calculated.

Evaluation of the in Vitro *Data*

The drug solution with taurocholate micelles is considered to consist of two separate phases: (i) an aqueous phase with a fraction of the drug free in solution and (ii) a micellar phase with the remaining fraction of the drug solubilized in micelles.

The fraction of the drug solubilized in micelles (s) and the fraction of the drug free in solution (f) is calculated from the solubility data with Eq. (2) and Eq. (3):

$$s = (C^+ - C^-)/C^+ \quad (2)$$

$$f = 1 - s \quad (3)$$

where C^+ is the solubility of the drug in the solution with micelles and C^- is the solubility in the same medium without micelles. It is assumed that s is constant and independent of the drug concentration for a particular concentration micelles and that the partition of the solute between the aque-

Table I. Physicochemical Characteristics of the Model Compounds[a]

Drug	M	pK_a	Concentration (10^{-5} M)	pH	log P ± SD (n = 3)
PA	151	9.5	331	7.4	0.2 ± 0.2
TP	180	8.6	253	7.4	0.1 ± 0.1
GF	353	>9	1.42	7.4	1.3 ± 0.1
KE	531	6.5	0.94	6.5	1.5 ± 0.2

[a] M is the molecular weight. pK_a of PA, TP, and GF from Ref. 14; pK_a of KE from Ref. 17; The concentrations of the model compounds in the aqueous phase for the log P determinations and in the perfusion solutions (in phosphate-buffered saline); log P is the logarithm of the partition coefficient of the compound between the *n*-octanol phase and the aqueous phase under the conditions described in the text.

Table II. HPLC Methods for the Determination of Griseofulvin (GF), Ketoconazole (KE), Paracetamol (PA), and theophylline (TP)

Drug	Stationary phase	Eluent	Wavelength of detection (nm)
PA/TP	LiChrosorb RP18	Methanol/water/ acetic acid 15/85/1 (v/v/v)	254
GF	LiChrosorb RP18	Methanol/water 3/2 (v/v)	254
KE	Chrom-Sep Hypersil/ODS	Acetonitrile/water/ diethylamine 48/55/0.02 (v/v/v)	254

ous phase and the micellar phase is not rate limiting; it is also assumed that only one type of micelle exists in the micellar solutions.

If the fraction of the drug free in solution is considered as the only driving force for absorption, it is expected that during absorption experiments with drug solutions, the reduced concentration of the free drug due to micellar encapsulation will result in a proportional decrease in the absorption rate of the drug, provided that the absorption barrier is not affected by the micelles.

Another approach is to take into account the contribution of the fraction of drug solubilized in micelles to the overall diffusion of the solute across the aqueous boundary layer adjacent to the intestinal wall (15). Thus, according to this phase-separation model, the diffusion of a solute in a micellar solution can be described by a combination of the diffusion coefficient of the fraction solubilized (s) and of free (f) drug. Now, the apparent diffusion coefficient of a drug in a micellar solution (D_{app}) is defined by the following expression:

$$D_{app} = s \cdot D_{mic} + f \cdot D_{free} \quad (4)$$

where D_{mic} and D_{free} are the diffusion coefficients of the drug in micelles and the free drug, respectively.

Absorption Experiments

Chronically Isolated Internal Loop

In this study a chronically isolated intestinal loop in the rat, as described earlier (16), is used to study the absorption kinetics of the model compounds. This model offers the opportunity to perfuse an isolated segment of the intestine of the rat with a constant volumetric flow of drug solution under well-defined conditions in a conscious animal; an additional advantage of this model is that more than one experiment can be performed in the same animal over an extended period of time. Briefly, an intestinal segment of approximately 8 cm (approximately 15 cm proximal to the ileocecal junction) was isolated with intact blood supply. The loop remained in the peritoneal cavity. The perfusion solution could enter and leave the segment via two Delrin cannulas in the abdominal wall. The head–tail connection of the remaining intestine was restored by end-to-end anastomosis. After surgery the rat was placed into a restriction cage and supplied with water and food. After recovery from the operation (2–4 days) the rat was ready for use in perfusion experiments.

Perfusion Technique

Before starting the perfusion, the intestinal loop was cleaned by rinsing it for 30 min with sailne at a flow rate of 1.0 ml/min. During perfusion experiments, the perfusion solution was pumped through a heat exchange device to bring the solution to body temperature just before entering the rat. Perfusions were performed in a recirculating mode (perfusion volume, 60 ml) at a rate of 1.0 ml/min. All perfusion experiments were performed between 10 AM and 5 PM. The pH of the perfusion solution remained within narrow limits (±0.1) during the perfusion experiments. The perfusions were performed at a pH of 7.4, except for KE; the pH of this perfusion solution was 6.5 [pK_a of KE (17)]. The concentration of the drugs in the perfusion solutions was below the saturation concentration of the respective drugs in the buffer solution without micelles. All perfusions with and without micelles in the medium were performed with the same concentration of the particular model compound (Table I). The perfusion solutions were freshly prepared shortly before starting the experiments. During the absorption experiments samples were taken from the perfusion solution at 0, 0.5, 1, 1.5, 2, 2.5 and 3 hr after the start of the perfusion and directly analyzed by HPLC. The absorption kinetics of the drugs were evaluated on the basis of disappearance rates of the drug from the perfusion solution. The absorption of the drugs in the presence of TC was compared with the absorption of the drug from the same medium without TC, in the same intestinal segment in the same rat.

Evaluation of the Absorption Data

The disappearance of the respective model compounds from the perfusion solution can be described by first-order kinetics. The time dependence of the concentration in the perfusion solution, C, can be written as

$$\ln(C_t/C_o) = -k_{dis} \cdot t \quad (5)$$

C_o and C_t are the concentrations of the model compound in the perfusion solution at time 0 and t, respectively. The disappearance rate constant, $k_{dis}(hr^{-1})$ was calculated from plots of $\ln(C_t/C_o)$ vs t by linear regression.

The disappearance rate constants are determined in a crossover experimental scheme, therefore the effect of the various concentrations of TC on the absorption of the model drug can be described by the ratio (r_{obs})

$$r_{obs} = k_{dis\ with\ TC}/k_{dis\ without\ TC} \quad (6)$$

The 95% confidence interval (CI) of the mean ratio r_{obs} was calculated [two-sided t test for one sample at the 5% level (18)].

When the absorption profiles of GF and KE in the presence of taurocholate are evaluated on the basis of the phase-separation model [Eq. (4)], assuming that TC micelles contribute to the mass transfer of the drug across the aqueous boundary layer, it is expected that r_{obs} is similar to the calculated ratio (r_{cal}):

$$r_{cal} = D_{app}/D_{free} \quad (7)$$

RESULTS AND DISCUSSION

Partition Coefficient of the Model Drugs

To describe the lipophhilic nature of the model drugs, both the n-octanol/water partition coefficient (P) and the extent of solubilization in TC were measured. The log P value, the molecular weight (M), and the pK_a of the various drugs are given in Table I.

The log P values are indicative of the hydrophilic or lipophilic nature of a compound, the hydrophilic compounds

having lower log P values than lipophilic compounds. The log P values for paracetamol and theophylline were 0.2 and 0.1, respectively (Table I). This indicates that these drugs are more hydrophilic than griseofulvin and ketoconazole, with log P values of 1.3 and 1.5, respectively.

Solubilization of the Model Drugs by Taurocholate

The solubilization of the model drugs by taurocholate was determined by measuring the solubility of the drugs in solutions with TC concentrations above the CMC. The fraction of the drug solubilized in TC micelles (s) and the fraction of the drug free in solution (f) were calculated from the solubility data with Eq. (2) and Eq. (3) and are given in Table III.

The hydrophilic compounds PA and TP were not solubilized at taurocholate concentrations of 10 and 20 mM TC. The lipophilic nature of GF and KE is demonstrated by their solubilization in TC. The extent of solubilization (s) of GF and KE in TC micelles is dependent on the concentration of TC. The s value for KE at a TC concentration of 20 mM was higher than the s value for GF at 20 mM TC: $s = 0.87$ and $s = 0.64$, respectively. The more lipophilic nature of KE compared to GF was also reflected in their respective log P values (Table I).

Effect of Taurocholate on the Absorption Kinetics of the Model Compounds

Paracetamol and Theophylline

In Table IV the results of the absorption experiments with PA and TP with and without 10 mM TC in the perfusion solution are presented. The absorption of the hydrophilic compounds paracetamol and theophylline was not affected by taurocholate (Table IV; r_{obs} is not significantly different from 1). This is in agreement with the fact that these compounds were not solubilized by TC (Table III), which would lead to a decreased thermodynamic activity of the drugs and a reduction in the absoprtion rate. The major absorption barrier for hydrophilic compounds is considered to be located in the cellular membranes of the intestinal wall, while on the other hand, the aqueous boundary layer adjacent to the intestinal wall is considered the highest resistance in the uptake of lipophilic compounds (19).

These results suggest that the cellular membranes of the intestinal wall are not affected by TC. One may comment on the choice of paracetamol and theophyllin as hydrophilic model compounds since the uptake of these drugs in the GI tract is possibly too effective to be further promoted by a TC effect on cellular membranes. It is also possible that the rate-limiting absorption barrier of these drugs is not located in the cellular membranes of the intestinal wall. On the other hand these results are consistent with those of Muranishi *et al.* (20), who used even higher concentrations of bile salts (40 mM) and found enhanced intestinal permeability of the poorly absorbable hydrophilic drugs streptomycin and gentamycin only in the presence of taurocholate or glycocholate–fatty acid mixed micelles, and not with bile salts alone. Contrary to solely bile salts, mixed micelles composed of bile salts and fatty acids or monoglycerides can be used for the improvement of the bioavailability of poorly absorbable (hydrophilic) drugs especially in the colorectal area, as was reviewed by Muranishi (21).

Table III. Solubilization of the Model Compounds in Taurocholate[a]

Drug	Solution	Solubility (10^{-5} M) ± SD	n	s	f
PA	Buffer	117 ± 13[b]	9		
	10 mM TC	117 ± 9[b]	8	0.00	1.00
	20 mM TC	121 ± 3[b]	8	0.03	0.97
TP	Buffer	54 ± 5[b]	3		
	10 mM TC	52 ± 4[b]	3	≈0.00	1.00
	20 mM TC	51 ± 2[b]	3	≈0.00	1.00
GF	Buffer	2.8 ± 0.4	12		
	10 mM TC	4.8 ± 0.6	9	0.41	0.59
	20 mM TC	7.8 ± 0.2	6	0.64	0.36
KE	Buffer	1.3 ± 0.2	13		
	10 mM TC	2.7 ± 0.4	10	0.52	0.48
	20 mM TC	9.8 ± 1.4	7	0.87	0.13

[a] s indicates the fraction of the drug solubilized in taurocholate; f indicates the fraction of the drug free in solution.
[b] Solubility in 10^{-3} M.

Griseofulvin and Ketoconazole

In contrast with the hydrophilic drugs, the absorption of the lipophilic drugs was dramatically influenced by taurocholate. As reflected in r_{obs} (Table IV), the absorption of GF and KE was reduced in the presence of 10 and 20 mM taurocholate.

D_{app} for GF in 10 and 20 mM TC solutions was calculated according to Eq. (4) based on the s values presented in Table IV and based on data obtained by de Smidt *et al.* (5). They found diffusion coefficients for griseofulvin in saline (D_{free}) and values for D_{mic} in 10 mM TC and in 20 mM TC of 8.0, 2.4, and $2.2 \cdot 10^{-10}$ m^2/sec, respectively. The calculated values of D_{app} for GF are given in Table IV. The values of D_{app} for KE in 10 and 20 mM TC solutions were calculated in the same manner (Table IV), assuming that the values of D_{free} and D_{mic} for KE are equal to the respective values for GF. The values for r_{cal} were calculated according to Eq. (7) and are given in Table IV.

Griseofulvin

The reduction of the absorption rate of griseofulvin in the presence of TC micelles was in agreement with the decreased fraction of the drug free in solution (f) for perfusions with both 10 mM TC and 20 mM TC. If micelle-mediated transport of GF across the aqueous boundary layer and mucus is considered, in the presence of 10 mM TC, an absorption rate of 71% ($r_{cal} = 0.71$) of the absorption rate of GF without TC is expected. The ratio calculated from the observed absorption data ($r_{obs} = 0.61 \pm 0.10$; CI, 0.51–0.71) was not significantly different from r_{cal}. In the presence of 20 mM TC, r_{obs} was statistically different from the expected ratio, if micelle-mediated transport is considered: $r_{obs} = 0.36 \pm 0.04$ (CI, 0.30–0.42) versus $r_{cal} = 0.54$ (Table IV). An

Table IV. Influence of Taurocholate on the Absorption of Paracetamol, Theophylline, Griseofulvin, and Ketoconazole[a]

Drug	Perfusion solution	r_{obs} (±SD)	n	f	r_{cal}	D_{app} (10^{-10} m^2/sec)
PA	10 m*M* TC	1.02 ± 0.23	7	1.00	—	—
TP	10 m*M* TC	0.86 ± 0.24	6	1.00	—	—
GF	10 m*M* TC	0.61 ± 0.10	6	0.59	0.71	5.70
	20 m*M* TC	0.36 ± 0.04	4	0.36	0.54	4.29
KE	10 m*M* TC	0.51 ± 0.18	6	0.48	0.62	4.98
	20 m*M* TC	0.32 ± 0.13	4	0.13	0.37	2.95

[a] $r_{obs} = k_{dis+}/k_{dis-}$; n is the number of experiments; f is the fraction of the drug free in solution; r_{cal} is calculated assuming that the drug bound to TC micelles is also contributing to the mass transfer; D_{app} is the calculated overall diffusion coefficient of the drug in the presence of TC assuming micelle-mediated transport.

explanation for this discrepancy might be that either micelle-mediated transport is less important than predicted or the barrier function of the aqueous diffusion resistance adjacent to the intestinal wall is altered in the presence of 20 m*M* TC.

Ketoconazole

The reduction in the absorption of KE in the presence of 10 m*M* TC (r_{obs} = 0.51 ± 0.18; CI, 0.32–0.70) and 20 m*M* TC (r_{obs} = 0.32 ± 0.13; CI, 0.11–0.53) was not significantly different from the expected absorption profiles, based on the fraction of drug free in solution (f) or based on micelle-mediated transport (r_{cal}) (Table IV).

Because of the scatter in the observed absorption data (r_{obs}) caused by intra- and interanimal variation (e.g., different mucus production), it was not possible to determine to what extent TC micelles contribute to the overall diffusion of ketoconazole and griseofulvin across the preepithelial diffusion barrier. It is possible that the diffusion coefficients for KE free in solution, and KE incorporated in micelles deviate from the respective diffusion coefficients for GF, and this will then lead to deviating absorption profiles for KE compared to GF. In general, the reduced absoprtion rate of GF and KE in the presence of TC micelles was in agreement with the reduction of the fraction of the free drug in solution (except for KE at a concentration of 20 m*M* TC; see Table IV). The effect of taurocholate on the barrier function of the aqueous diffusion resistance adjacent to the intestinal wall, which is considered to be the rate-limiting absorption barrier for lipophilic compounds, seems negligible compared to the effect of micellar solubilization by TC, leading to a reduced absorption rate of GF and KE.

CONCLUSIONS

The results presented in this report show that the hydrophilic compounds paracetamol and theophylline were not solubilized at concentrations of 10 and 20 m*M* taurocholate. Taurocholate (10 m*M*) did not exert an effect on the absorption of paracetamol and theophylline, which is in accordance with the expectation, since the concentration free in solution of both drugs (driving force for absorption) was not decreased by taurocholate. These results suggest that the barrier function of the cellular membranes of the intestinal wall, which are suggested to be the rate-limiting absorption barrier for these drugs, is not affected by taurocholate.

The lipophilic compounds griseofulvin and ketoconazole were solubilized in micellar solutions of 10 and 20 m*M* taurocholate. The reduction in the absorption of griseofulvin in the presence of 10 and 20 m*M* taurocholate was in agreement with the decrease in the fraction free of the drug in solution due to micellar solubilization. For ketoconazole, the relation between the decrease in the absorption kinetics and the decreased fraction of the drug free in solution due to micellar solubilization was less clear than for griseofulvin in the presence of taurocholate. Because of the scatter in the absorption data, caused by variation within rat and between rats, it was not possible to determine to what extent TC micelles contributed to the overall diffusion of griseofulvin and ketoconazole across the aqueous boundary layer and mucus adjacent to the intestinal wall (phase-separation model).

Apart from the reduction of the fraction of the free drug in solution by taurocholate (for lipophilic drugs), no distinct effect of taurocholate on the transport of the drugs across the preepithelial diffusion barrier or the intestinal wall of the small intestine was found. The lack of an effect of taurocholate on cellular membranes is apparently in contradiction with the published data on the promoting effect of bile salts on drug absorption. This is possibly due to the different experimental conditions, such as the type and concentration of the bile salt used, the model compound, the experimental model, the use of lipoidal adjuvants (mixed micelles), and the intestinal absorption area (ileum, colon, rectum).

REFERENCES

1. T. R. Bates, M. Gibaldi, and J. L. Kanig. *J. Pharm. Sci.* 55:191–199 (1966).
2. T. R. Bates, M. Gibaldi, and J. L. Kanig. *Nature* 210:1331 (1966).
3. S. Miyazaki, H. Inoue, T. Yamahira, and T. Nadai. *Chem. Pharm. Bull.* 27:2468–2472 (1979).
4. M. Gibaldi and S. Feldman. *J. Pharm. Sci.* 59:579–589 (1970).
5. J. de Smidt, J. C. A. Offringa, and D. J. A. Crommelin. Submitted for publication.
6. K. Kakemi, H. Sezaki, R. Konishi, T. Kimura, and M. Murakami. *Chem. Pharm. Bull.* 18:275–280 (1970).
7. M. Yonezawa. *Nihon. Univ. J. Med.* 19:125–141 (1977).
8. T. Yamaguchi, C. Ikeda, and Y. Sekine. *Chem. Pharm. Bull.* 34:3836–3843 (1986).
9. T. Kimura, H. Sezaki, and K. Kakemi. *Chem. Pharm. Bull.* 20:1656—1662 (1972).
10. M. R. Lewin, S. H. El Masri, and C. G. Clark. *Eur. Surg. Res.* 11:392–398 (1979).
11. B. L. Slomiany, M. Kosmala, S. R. Carter, S. J. Konturek, J. Bilski, and A. Slomiany. *Comp. Biochem. Physiol.* 87:657–663 (1987).

12. D. A. Whitmore, L. G. Brookes, and K. D. Wheeler. *J. Pharm. Pharmacol.* 31:277–283 (1979).
13. W. J. Meyler, H. W. Mols-Thürkow, and H. Wesseling. *Eur. J. Clin. Pharmacol.* 16:203–209 (1979).
14. E. F. Reynolds (ed.). *The Extra Pharmacopoeia.* 28th Ed., Pharmaceutical Press, London, 1982.
15. D. Stilbs. *J. Coll. Int. Sci.* 87:385–394 (1982).
16. F. G. J. Poelma and J. J. Tukker. *J. Pharm. Sci.* 76:433–436 (1987).
17. Product Information of Janssen Pharmaceutica, Beerse, Belgium.
18. S. Bolton. *Pharmaceutical Statistics.* (*Drugs and the Pharmaceutical Sciences,* Vol. 25), Marcel Dekker, New York. 1982.
19. I. Komiya, J. Y. Park, A. Kamani, N. F. H. Ho, and W. I. Higuchi. *Int. J. Pharm.* 4:249–262 (1980).
20. S. Muranishi, N. Muranushih, and H. Sezaki. *Int. J. Pharm.* 2:101–111 (1979).
21. S. Muranishi. *Pharm. Res.* 2:108–118 (1985).