[Chem. Pharm. Bull. 36(7)2544—2550(1988)]

Intestinal Absorption of *N,N'*-Dimethylcarbamoylmethyl 4-(4-Guanidinobenzoyloxy) Phenylacetate Methanesulfonate in Rats

TOSHIAKI NISHIHATA,*[,a,1)] YUKIKO SAITOH,[b] and KIYOSHI SAKAI[b]

Faculty of Pharmaceutical Sciences, Osaka University,[a] 1–6 Yamadaoka, Suita, Osaka 565, Japan and Osaka University of Pharmaceutical Sciences,[b] 2–10–65 Kawai, Matsubara, Osaka 580, Japan

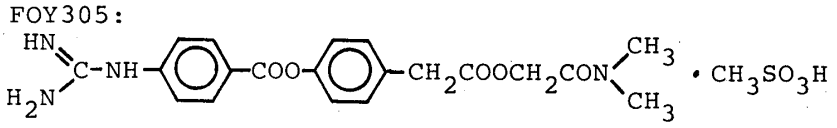
(Received October 26, 1987)

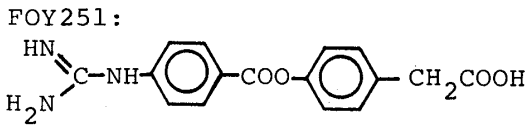
The intestinal absorption of *N,N'*-dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy) phenylacetate methanesulfonate (FOY305®) in rats was investigated using a rat intestinal loop method. Plasma drug concentrations and the disappearance of the drug from the intestinal lumen were measured. The absorption of FOY305 after administration into a rectal loop was greater than those after administration into variously positioned small intestinal loops. The low absorption of FOY305 after administration into the small intestine was due to the rapid degradation of FOY305 by esterase activity in the small intestinal lumen.

Keywords——FOY305®; intestinal absorption; loop method; esterase; metabolites; HPLC

It has been reported that pancreatitis can be induced by proteolytic enzymes such as trypsin.[2–5)] In fact, there have been many reports showing that proteolytic enzyme inhibitors provide protection against pancreatitis.[6–10)]

Recently, *N,N'*-dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy) phenylacetate methanesulfonate (FOY305®)[11)] (Fig. 1) was synthesized and found to display strong proteolytic enzyme-inhibitory activity.[12,13)] A protective effect of FOY305 against experimental pancreatitis in rats has been reported.[14)] It has also been reported that FOY305 was metabolized





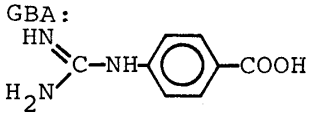


Fig. 1. Structures of FOY305, FOY251 and GBA

to *p*-guanidino benzoate (GBA) (Fig. 1) mostly *via* 4-(4-guanidinobenzoyloxy) phenylacetate (FOY251®) (Fig. 1), which also is a proteolytic enzyme inhibitor.[15] For long-term treatment of chronic pancreatitis, an oral or rectal dosage form is desired rather than an injection form.

In the present study, we investigated the intestinal absorption of FOY305 in rats to obtain fundamental information necessary for the development of an oral or rectal dosage form of FOY305.

Experimental

Materials——FOY305, FOY251 and GBA were supplied by Ono Pharmaceutical Co. (Osaka, Japan). Esterase (from porcine liver; 200 units per mg of protein) was obtained from Sigma Inc. (St. Louis, U.S.A.). Other reagents used were of analytical grade.

Animals——Male Sprague–Dawley rats, 350 to 400 g, were fasted for 16 h prior to experiments but water was given *ad libitum*. During experiments, rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and were kept on a warm surface (electric hot plate) at 38 °C to maintain body temperature.

Intestinal Absorption Study in Rats——The absorption study was performed by using an *in situ* intestinal loop method[16] with duodenum, jejunum, ileum and rectum (including part of the colon). The loop length was about 6 cm, FOY305 (40 μmol) was suspended in 2 ml of distilled water containing 50 mg of sucrose as an aid to suspension formulation. After administration of the suspension into an intestinal loop, blood was collected from the right external jugular vein at designated time intervals for 3 h and was centrifuged to obtain plasma. After 3 h, each intestinal loop was excised and any residue remaining in the loop was collected by rinsing with 10 ml of distilled water.

Degradation of FOY305——Degradation of FOY305 was investigated in 0.05 M sodium phosphate buffer (pH 7.0) containing 15 U of porcine esterase/ml at 37 °C. Degradation of FOY305 in the small intestinal lumen was also investigated by using an *in vitro* rat intestinal sac method.[17] Briefly, a 20 cm intestinal sac, containing 2.5 ml of a solution of FOY305 (4 μmol/ml) was immersed in 50 ml of 0.05 M sodium phosphate buffer (pH 7.0), and after 1 h, the solution inside the sac and outside the sac were collected to measure the metabolites of FOY305.

Assay of FOY305, FOY251 and GBA——Assay of FOY305 and its metabolites were performed by means of a high-performance liquid chromatographic method as follows: A liquid chromatograph (Trirotor-II) equipped with a UV detector (UVIDEC-100-III, Japan Spectroscopic Co., Ltd., Tokyo, Japan) was used. The separation column was 4.6 mn i.d. × 15 cm in length and contained a reverse-phase column material (Ultrasphere ODS; 5 μm diameter sphere). The mobile phase was a mixture of methanol, distilled water, acetic acid, 5% w/v sodium dodecyl sulfate and 5% w/v sodium 1-heptanesulfate in volumes of 420, 350, 1, 8, and 2 ml, respectively. The flow rate was 1.0 ml/min. FOY305 and its metabolites were detected at 265 nm.

Preparation of assay samples from rat plasma and intestinal fluid was performed as follows: 0.2 ml of plasma or of intestinal fluid was mixed well with 0.8 ml of acetonitrile at 4 °C, and the mixture was shaken for 5 min at 4 °C. After centrifugation at 900 × *g* for 10 min at 4 °C, the supernatant was collected and dried under nitrogen gas at room temperature. The residue was redissolved in the mobile phase. During these processes, no degradation of FOY305, FOY251 and GBA was observed. A 100 μl sample was injected onto the column.

Statistical Analyses——Statistical analyses were performed by using Student's *t*-test.

Results and Discussion

High-Performance Liquid Chromatography (HPLC) of FOY305, FOY251 and GBA

Typical chromatograms are shown in Fig. 2. Retention times of GBA, FOY305 and FOY251 were 3.5, 6.1, and 7.0 min, respectively. With the samples from intestinal fluid, no biological component interfered with the assay (Figs. 2A, 2B and 2C). However, plasma component(s) extracted by acetonitrile interfered with the assay of GBA, as shown in Figs. 2D and 2E. Thus, in the present study, all three compounds were assayed in intestinal fluid, but only FOY305 and FOY251 were assayed in plasma samples. Calibration curves for each compound were determined in the following concentration ranges: 0.10 to 1.6 nmol/ml for GBA, 0.4 nmol/ml to 4 nmol/ml for FOY305, and 0.4 to 4 nmol/ml for FOY251. Calibration curves for each compound were prepared by plotting peak height (y) against concentration (x), and were as follows: $y=0.202x+0.06$ ($r=0.9925$) for FOY305, $y=0.164x+0.002$ ($r=0.9905$) for FOY251, and $y=0.512x+0.005$ ($r=0.9962$) for GBA.

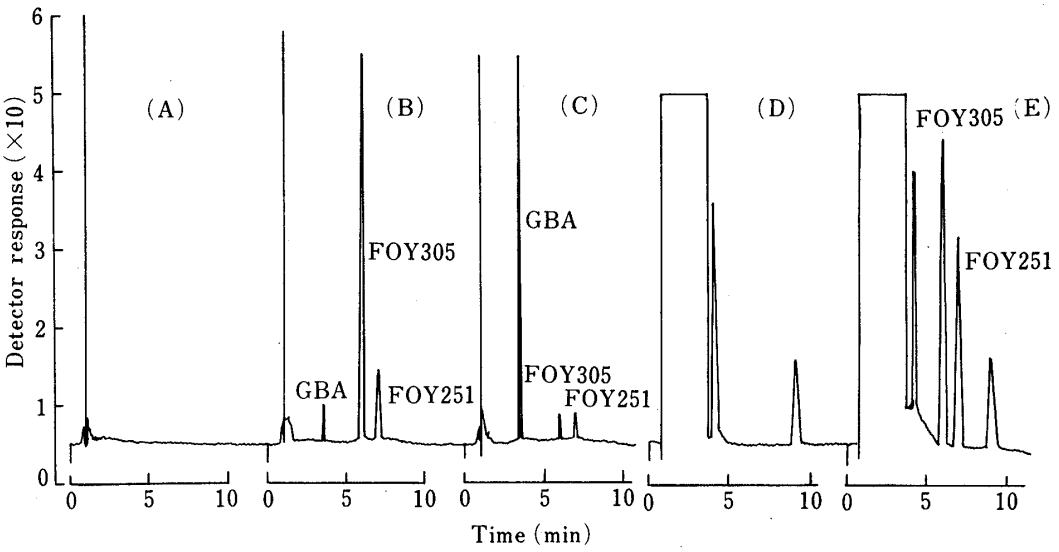


Fig. 2. Chromatograms of GBA, FOY305 and FOY251 in Intestinal Fluid after Administration of FOY305 into Rectal Loop (B) or Jejual Loop (C), or in Plasma after Administration of FOY305 into Rectal Loop (E)

A and D represent the chromatograms of intestinal fluid and plasma, respectively, before the administration.

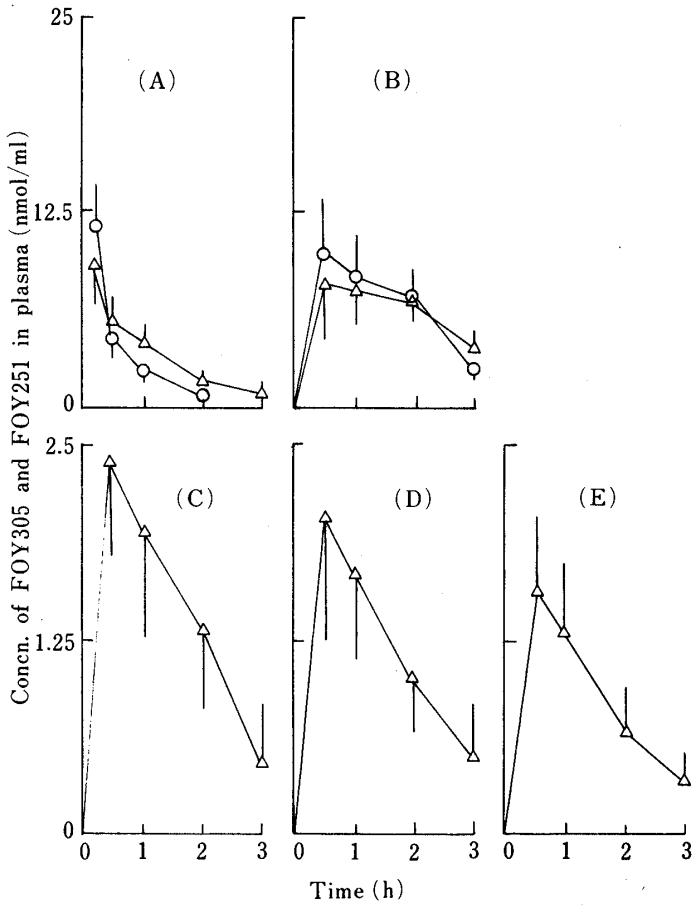


Fig. 3. Plasma Concentration of FOY305 (○) and FOY251 (△) after Administration of FOY305 at a Dose of 40 μmol/kg into Rat Intestinal Loops

B, rectum; C, duodenum; D, jejunum; E, ileum. A, represents the result after intravenous administration at a dose of 10 μmol/kg. Each value represents the mean ± S.D. ($n = 3$ to 5).

Intestinal Absorption of FOY305 after Administration into Intestinal Loops

After intravenous administration of FOY305 at a dose of 10 μmol/kg, FOY251, as well as FOY305, was detected in rat plasma (Fig. 3A). After administration of FOY305 at a dose

of 40 μmol/kg into the rectal loop, both FOY305 and FOY251 appeared rapidly in plasma (Fig. 3B). However, only FOY251 was detected in plasma when FOY305 was administered into loops of duodenum, jejunum or ileum (Figs. 3C, 3D and 3E).

Since FOY251 and FOY305 are both proteolytic enzyme inhibitors,[15] the apparent area under the curve (AUC) of plasma drug concentrations for 3 h was determined by using the sum of the molar concentrations of both compounds. Thus, AUC determined by this method should be referred to as "active AUC" of FOY305. Since it is not clear whether all degradation of FOY305 occurs *via* FOY251 or not, it is difficult to use the plasma AUC value of FOY251 alone for the estimation of relative absorption of FOY305. We did not investigate in detail the pharmacokinetics (including rate of metabolism) after administration of FOY305 through various routes, because it was difficult to sample large volume of blood frequently from small experimental animals such as rats. Thus, we could not obtain $(\text{active AUC})_{0-\infty}$; *i.e.*, the bioavailability was not determined in the present study.

To compare the absorption of FOY305 from drug concentrations in plasma after

TABLE I. Active AUC[a] (AUC) and Disappearance[b] of FOY305 from Intestinal Loops of Rats

Dose (μmol/kg)	AUC[a] (nmol·h/ml)	$[AUC]_x$ / $[AUC]_{rectum}$	Percent[b] of compound remaining in the intestinal lumen (R)			$(100-R)_x$ / $(100-R)_{rectum}$
			FOY305	FOY251	GBA	
Intravenous administration						
10	17.26±3.03	—		No measurement		—
Duodenal loop						
40	3.94±1.69	0.118	n.d.	n.d.	69.4±6.2	0.54
Jejunal loop						
40	3.35±1.03	0.100	n.d.	n.d.	72.1±7.2	0.51
Ileal loop						
40	2.59±1.15	0.077	n.d.	n.d.	77.9±7.4	0.41
Rectal loop						
40	33.50±9.29[c]	1	37.2±6.1[c]	6.1±3.2[c]	1.9±0.4[c]	1

a) Active AUC (AUC) was determined using the sum of plasma concentrations both FOY305 and FOY251. *b*) Disappearance was represented as $(100-R)$ in the table and the percent of compound remaining was determined by means of the following equation:

$$\text{percent} = (\text{molar amount of each compound}) \times 100/(\text{molar amount of FOY305 administered}).$$

Each value represents the mean±S.D. ($n=3$ to 5). n.d., not detectable. *c*) Significant difference ($p<0.01$) *versus* AUC after administration into duodenal loop.

TABLE II. Percent of FOY251 and GBA Remaining 3 h after Administration into Jejunal or Rectal Loops

	Percent of dose remaining[a]	
	FOY251	GBA
Administration of FOY251 at a dose of 20 μmol/loop		
Jejunal loop	n.d.	74.6±7.9
Rectal loop	46.9±9.6[b]	4.7±2.6[b]
Administration of GBA at a dose of 20 μmol/loop		
Jejunal loop	—	95.4±3.2
Rectal loop	—	97.2±2.6

a) Percent of dose remaining was determined as follows:

$$\text{percent} = (\text{molar amount of each compound remaining}) \times 100/(\text{molar amount of compound administered}).$$

Each value represents the mean±S.D. ($n=3$). n.d., no detectable. *b*) Significant difference ($p<0.01$) *versus* the result after administration into jejunal loop.

administration into each intestinal loop, active AUC up to 3 h after administration was employed. As shown in Table I, a high active AUC was observed after administration into the rectal loop in comparison with those after administration into small intestinal loops. The value of $[\text{active AUC}]_{\text{intestin}}\ (\text{dose})_{\text{iv}}/[\text{active AUC}]_{\text{iv}}\ (\text{dose})_{\text{intestin}}$ was calculated simply to estimate bioavailability after intestinal administration of FOY305. The value was about 0.5 for rectal administration and less than 0.1 for small intestinal administration.

The remaining amounts of drug in the loops were determined 3 h after administration and are summarized in Table I. In the rectum, the predominant residue in the loop was FOY305 (Table I). However, after administration of FOY305 into small intestinal loops, only GBA was detected at 3 h. Total recovery of FOY305, FOY251 and GBA was about 45% from the rectal loop (Table I). The recovery from other intestinal loops was about 70 to 75%; *i.e.*, about 25 to 30% of these substances disappeared from the loops.

After the administration of GBA into rectal and jejunal loops, more than 95% remained after 3 h (Table II). When FOY251 was administered into rectal and jejunal loops, 50% of FOY251, including a small amount of GBA, was recovered from the rectum, but 75% of the dose was recovered in the form of GBA from the jejunum.

From these findings, we concluded that (1) rectal (probably including colon) administration of FOY305 offers the greatest active AUC. (2) Degradation of FOY305 and FOY251 to GBA in the small intestinal lumen is rapid, but in the rectum it is slow. (3) In the rectum, FOY305 and FOY251 were well absorbed, but GBA was not. (4) GBA was also not absorbed from the small intestine.

The small active AUC of FOY305 after administration into a small intestinal loop is probably due to rapid degradation of FOY305 to GBA within the small intestinal lumen. The discrepancy in the relative absorption of FOY305 from the small intestinal loop *versus* the absorption from the rectal loop between the active AUC method and disappearance method (Table I) may be explained as follows: FOY305 and FOY251 may be degraded in the small intestinal tissues and/or liver before reaching the systemic blood circulation, *i.e.*, a first-pass effect. It has been reported[18] that rectal administration of high clearance drugs in rats completely avoided the first-pass effect in the liver. Therefore, most FOY305 may go directly to the systemic circulation after absorption *via* the rectum, without first encountering the liver.

Degradation of FOY305

Spontaneous degradation of FOY305 (10 nmol/ml) in sodium phosphate buffer was examined at pH 7.0 at 37 °C, and occurred by first-order kinetics with a degradation constant of $3.75 \times 10^{-3}\ \text{h}^{-1}$.

TABLE III. Recovery of FOY305 and Its Metabolites in the *in Vitro* Intestinal Sac Study 1 h after Starting Experiment

	Inside sac (%)[a]			Outside sac (%)[a]		
	FOY305	FOY251	GBA	FOY305	FOY251	GBA
FOY305: 10 μmol injected into sac						
Jejunum	n.d.	n.d.	71.4±7.2	1.9±0.4	10.4±2.6	17.6±4.2
Rectum	54.6±4.1[b]	4.2±2.1[b]	0.9±0.4[b]	30.6±5.2[b]	4.2±4.1	3.9±1.6[b]
GBA: 10 μmol injected into sac						
Jejunum	—	—	98.1±4.2	—	—	n.d.
Rectum	—	—	96.9±3.7	—	—	n.d.

a) Percent = (molar amount of each compound at 1 h)/(molar amount of FOY305 administered). Each value represents the mean ± S.D. ($n=4$). n.d., no detectable. *b*) Significant difference ($p<0.01$) *versus* the result in the jejunum.

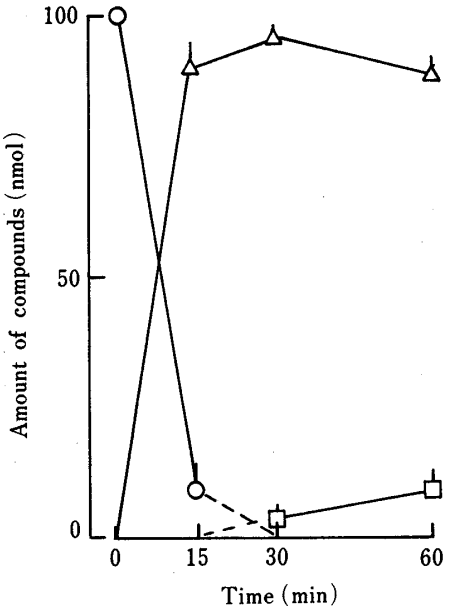


Fig. 4. Degradation of FOY305 (○) to FOY-251 (△) and further to GBA (□) in the Medium Containing Porcine Liver Esterase (15 units/ml) at 37 °C

Each value represents the mean ± S.D. ($n=3$).

This result suggests that more than 95% of FOY305 should avoid spontaneous degradation in the intestinal fluid during the 3 h study duration. However, FOY305 was degraded to FOY251 rapidly when the medium (pH 7.0) contained 15 U of esterase/ml, and only 10% of FOY305 was metabolized to GBA by this esterase after 1 h (Fig. 4).

To estimate the degradation of FOY305 in the small intestinal loops, an *in vitro* intestinal sac method was employed. As can be seen in Table III, rapid degradation of FOY305 was observed in the jejunal sac; *i.e.*, GBA appeared rapidly in the *in vitro* sac study, compared to the porcine liver esterase study. Because rapid degradation of FOY305 to GBA was seen in the jejunal lumen, the specificity of esterase activity in the jejunal lumen of rats may be different from that of porcine liver esterase and/or the total esterase activity in the rat jejunal lumen is greater than the activity used in the porcine liver esterase *in vitro* study (15 units/ml).

In the *in vitro* jejunal sac study, about 30% of the total FOYs (mainly as FOY251 and GBA) was recovered from fluid outside the sac, while about 70% of the total FOY305 (mainly GBA) was recovered from the medium inside the sac after 1 h (Table III). In the *in vitro* rectal sac study, about 40% of the total FOYs (mainly as FOY305) was recovered from fluid outside the sac and about 60% of the FOYs (mainly FOY305) was recovered from the medium inside the sac. When GBA was examined in both sacs, more than 95% was recovered from the medium inside the sac.

These results suggest that FOY305 and FOY251 may be transported through the intestinal wall, but not GBA. The appearance of GBA in the medium outside the sac may be due to degradation of FOY305 or FOY251 during passage through the small intestinal tissue. Thus, in terms of the first-pass effect, the small intestinal tissue may play a significant role in the degradation of FOY305. Thus, as earlier supposed, the discrepancy of the relative intestinal absorption of FOY305 between that estimated from active AUC value and that estimated from the disappearance may be due to first pass metabolism of FOY305 in the small intestinal tissue and in the liver.

In conclusion, a rectal formulation of FOY305 may be reasonable, if the dissolution of FOY305 in the normally small volume of rectal fluid can be accelerated. Dissolution of FOY305 is expected to be slow due to its basic character. To develop an oral dosage formulation of FOY305, some means of inhibiting the esterase activity in the small intestinal lumen may be required.

Acknowledgement This work was supported in part by a grant from Ono Pharmaceutical Co., Ltd. (Osaka, Japan).

References and Notes

1) Present address: *Pharmaceutical Chemistry Department, University of Kansas, 2065 Constant Avenue, Lawrence, Kansas 66046, U.S.A.*
2) E. L. Opie, *Am. J. Med. Sci.*, **121**, 27 (1980).
3) D. W. Elliot, R. D., Williams, and R. M. Zollinger, *Ann. Surg.*, **146**, 669 (1957).
4) K. Ohlsson and H. Tegner, *Scand. J. Gastroenterol.*, **8**, 129 (1975).
5) K. Ohlesson and A. Eddeland, *Gastroenterology.*, **69**, 668 (1975).
6) H. C. Saxena, L. S. Jensen, J. Mcgimis, and L. K., Lauber, *Proc. Soc. Exp. Biol. Med.*, **112**, 390 (1963).
7) G. M. Green and R. L. Lyman, *Proc. Soc. Exp. Biol. Med.*, **140**, 6 (1970).
8) E. M. Mcleed, J. P. Derbenwick, and J. T. Snoak, *J. Nutr.*, **102**, 469 (1972).
9) I. Ihse, B. Arnesjo, and I. Landquist, *Scand. J. Gastroent.*, **10**, 321 (1975).
10) P. Nemir, J. Hoferichter, and D. C. Drubkin, *Ann. Surg.*, **158**, 655 (1963).
11) Y. Tamura, M. Hirado, K. Okamura, Y. Minate, and S. Fujii, *Biochim. Biophys. Acta*, **484**, 417 (1977).
12) N. Kayama, K. Sakaguchi, and S. Fujii, *Gendai-Rinsho*, **12**, 1 (1980).
13) N. Kayama, K. Sakaguchi, S. Kaneko, and S. Fujii, *Gendai-Rinsho*, **12**, 11 (1980).
14) T. Okagawa, T. Obata, and S. Tsuboshima, *Gendai-Rinsho*, **12**, 35 (1980).
15) S. Ooki, K. Nishiyama, Y. Ozaki, H. Itoh, and F. Hirata, *Gendai-Rinsho*, **12**, 71 (1980).
16) T. Nishihata, H. Kamikawa, H. Takahata, and A. Kamada, *J. Pharmacobio-Dyn.*, **7**, 143 (1984).
17) A. Kamada, T. Nishihata, S. Kim, M. Yamamoto, and N. Yata, *Chem. Pharm. Bull.*, **29**, 2012 (1981).
18) D. J. A. Crommelin, J. Mooderkolk, and C. J. de Blaey, *Int. J. Pharmaceut.*, **3**, 288 (1974).