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RESEARCH PAPER

In Vivo Performance of Wax Matrix Granules Prepared by a Twin-Screw Compounding Extruder

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ABSTRACT

The in vivo performance of wax matrix granules (WMGs) prepared by a twin-screw compounding extruder was evaluated in fasted beagle dogs. In vitro dissolution behavior of the model drug, diclofenac sodium (DS), from WMGs was strongly influenced by pH in a dissolution medium due to its solubility (DS is soluble in pH 6.8 and insoluble in pH 1.2 and 4.0) and was independent of paddle rotation rate (50, 100, and 200 rpm) of the dissolution apparatus. Pharmacokinetics parameters such as mean residence time (MRT) showed a sustained action of WMGs in beagle dogs; however, the transit time of WMGs in the small intestine is found to control total drug absorption. Furthermore, the values of the area under the curve (AUC) of the plasma concentration–time curve and the maximum concentration C_{max} *significantly decreased with decreases in hydroxypropylcellulose (HPC) content in WMGs. Good correlation between one in vitro dissolution parameter (mean dissolution time, MDT) and two in vivo parameters (*AUC_{12} *and MRT) suggested that it would be possible to design WMGs with a desired in vivo performance by controlling HPC content.*

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INTRODUCTION

The effects of physiological factors such as gastrointestinal (GI) pH, GI transit time (GITT), GI motilities, and drug absorption window on in-vivo performance of drugs must be evaluated carefully in the design of sustained-release dosage forms (1–4). In particular, the GITT of the product will often control total drug bioavailability. A number of studies of GITT indicated that the transit time of the product in the small intestine (SITT) is around 4 hr in the human and is not influenced by either food intake or dosage form (5–7). On the other hand, gastric emptying time (GET) was affected significantly by these two factors, so that drug bioavailability closely correlated with GET rather than SITT (5). Furthermore, drug release should be designed to complete during the transit of the product in the drug absorption window. With this in mind, multiple-unit systems are advantageous in sustained-release dosage forms over single-unit systems because the GET of a multiple-unit system has a statistical distribution (8). However, each unit in the system has to have enough strength to withstand GI motilities because disintegration of one or several units will result in an unexpected increase of drug concentration in the blood, possibly leading to serious side effects (9,10). Accordingly, the mechanical strength of dosage forms is particularly important for accomplishing sustained action. In this portion of our series of studies, we report the dissolution behavior and release mechanism of diclofenac sodium (DS) from wax matrix granules (WMGs) prepared using a twin-screw compounding extruder. This preparation method has several advantages, such as ease of manufacturing, short manufacturing time through continuous production, low temperature, ease of sharpening small granules, and the ability to produce homogeneous granules (11). The granules prepared were found to show pH-independent dissolution profiles and high mechanical strength. Furthermore, the release of DS from WMGs was explained by the case II mechanism, in which swelling of hydroxypropylcellulose (HPC) used as a ratecontrolling agent controlled total release rate (12). The twin-screw compounding extruder has also been applied to formulate solid dispersion and other systems (13).

In the present article, we investigate in vivo performance of WMGs, especially the relationship between in vitro drug dissolution rate and bioavailability.

Table 1

Formulation of Wax Matrix Granules

Ingredient	WMG-A	WMG-B	WMG-C
Diclofenac sodium	10	10	10
Carnauba wax	60	50	30
HPC-SL	30	40	60

MATERIALS AND METHODS

Materials

The model drug DS was obtained from Sanwa Chemical Company, Limited (Tokyo, Japan). Carnauba wax, used as a matrix substance, and HPC were purchased from Noda Wax Company (Tokyo, Japan) and Nippon Soda Company (Tokyo, Japan), respectively. Voltaren® tablet (Japan Ciba-Geigy Pharmaceuticals Co., Tokyo, Japan) was used as a conventional immediate-release dosage form. Each Voltaren tablet contains 25 mg of DS. All other reagents were analytical grade.

Preparation of Wax Matrix Granules

Table 1 shows the WMG formulations investigated in the present study. A twin-screw compounding extruder (KEX 30, Kurimoto Ltd., Osaka, Japan) was used to prepare WMGs. Details of the preparation process were described in our previous papers (11,12). The WMG prepared has a cylindrical form approximately 2 mm in length and 2 mm in diameter.

Dissolution Studies

Dissolution studies were conducted according to the paddle method in the Japanese Pharmacopoeia XIII under the following conditions; sample weight, 200 mg; dissolution medium, 500 ml of pH 1.2, pH 6.8 (pH 1.2 and pH 6.8 solutions are defined as the first and the second fluid for Japanese Pharmacopoeia XIII), and pH 4.0 solutions (0.1 M acetic acid buffer solution); temperature, 37°C ± 0.5°C; paddle rotation rate, 50, 100, and 200 rpm. At predetermined intervals, 10 ml of dissolution medium were withdrawn and immediately filtrated (Fine Filter F72, Toyama Industry Co., Japan). The volume of the dissolution medium was kept at 500 ml by adding fresh medium of the same temperature. Sample solutions were

assayed for DS spectrophotometrically at 277 nm (UV-240 spectrophotometer, Shimadzu Co., Kyoto, Japan).

Mean dissolution time (MDT; hr) was calculated by Eq. 1:

$$\mathrm{MDT} = \int_0^\infty t(\mathrm{d}m/\mathrm{d}t)\mathrm{d}t \Big/ \int_0^\infty (\mathrm{d}m/\mathrm{d}t)\mathrm{d}t \qquad (1)$$

where m and $\mathrm{d}m/\mathrm{d}t$ are the amount of DS dissolved and the dissolution rate at time t, respectively.

Assay for the Plasma Concentration of Diclofenac Sodium

A modification of the high-performance liquid chromatography (HPLC) method reported by Sagara et al. (14) was used to determine DS in the plasma sample. As an internal standard, 200 μl of 0.1 M hydrochloride and 1 ml of ethylacetate containing 10 γ of diazepam were added to 0.5 ml of plasma sample. After 15 min of shaking and centrifugation at 3000 rpm for 10 min, 0.7 ml of the organic phase was removed, and the remaining solvent was evaporated under reduced pressure. The residue obtained was dissolved with 200 μl of the mobile phase (a mixture of 0.1% v/v acetic acid solution and acetonitrile, 2:3 v/v) for HPLC assay. At a flow rate of 1.0 ml/min, 50 μl of sample solution was injected into HPLC (YMC-Pack ODS-A, 150 mm × 4.6 mm, 50 μm particle size, YMC Co., Ltd., Japan), and DS was assayed at 282 nm. The HPLC system consisted of a Shimadzu LC-6A, SPD-6A, and C-R5A integrator recorder. Under these conditions, DS and diazepam used as an internal standard have retention times of 7.28 and 6.03 min, respectively. The lower detection limit of the assay was 60 ng/ml.

Bioavailability Study

Eight healthy male beagle dogs weighing between 10 and 15 kg were fasted for 24 hr prior to and until the end of experiment in the study, but were allowed free access to water. The dogs were divided into four groups of two dogs, and each group took a Voltaren tablet as the immediate-release dosage form or one of three kinds of WMGs having different dissolution profiles. Each dog took two different dosage forms at 1-week intervals. WMGs corresponding to 37.5 mg of DS were encapsulated in hard gelatin capsules (No. 0). The sample was administrated with 30 ml of water.

Blood samples were taken with heparinized syringes at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, and 12 hr after the administration of the samples and were centrifuged (3000 rpm for 20 min) to separate the plasma from the blood samples. The plasma samples were then kept frozen until assay.

Pharmacokinetic Analysis

Four pharmacokinetic parameters (the maximum drug concentration in plasma C_{max}, the time to $C_{\max}$ (T_{max}), the area under the curve (AUC) of the drug concentration–time curve from 0 to 12 hr calculated by trapezoidal rule (AUC_{12}), and mean residence time (MRT) were selected to evaluate in vivo performance of the samples. MRT (hr) was calculated from Eq. 2.

$$\mathrm{MRT} = \int_0^\infty tC_p\mathrm{d}t \Big/ \int_0^\infty C_p\mathrm{d}t \qquad (2)$$

where C_p is the DS concentration in plasma at time t.

RESULTS AND DISCUSSION

As already pointed out, pH values in the GI tract vary according to the location, food intake, and individual differences, and drug release from the dosage form should be designed with pH in the GI tract in mind (15). In particular, the influences of physiological factors on pharmaceutical properties have to be evaluated well when designing a sustained-release dosage form.

To estimate the dependency of drug release on pH, in vitro release profiles of DS from four products were investigated under three different pH conditions (pH 1.2, 4.0, and 6.8), the results of which are shown in Fig. 1. Percentages of DS released from all products in the pH 1.2 and pH 4.0 dissolution media were less than 25% at 24 hr since the dissociation constant pK_a of DS is about 4.0. Though DS was released from Voltaren and WMG-C relatively quickly compared with WMG-A and WMG-B, the differences among these four dosage forms were quite small. The solubility of DS may have played a critical role in these differences because the HPC used as the rate-controlling agent for DS release can swell and solve in all the above pH conditions. In dissolution medium with pH 6.8, 100% of the DS was released from the Voltaren tablet within 30 min.

On the other hand, release rates from the three kinds of WMGs increased in proportion to the amount of HPC in the WMG. Even the release rate of DS from WMG-C that contained 60% (w/w) HPC was found to be slower than that from Voltaren. As described in our previous

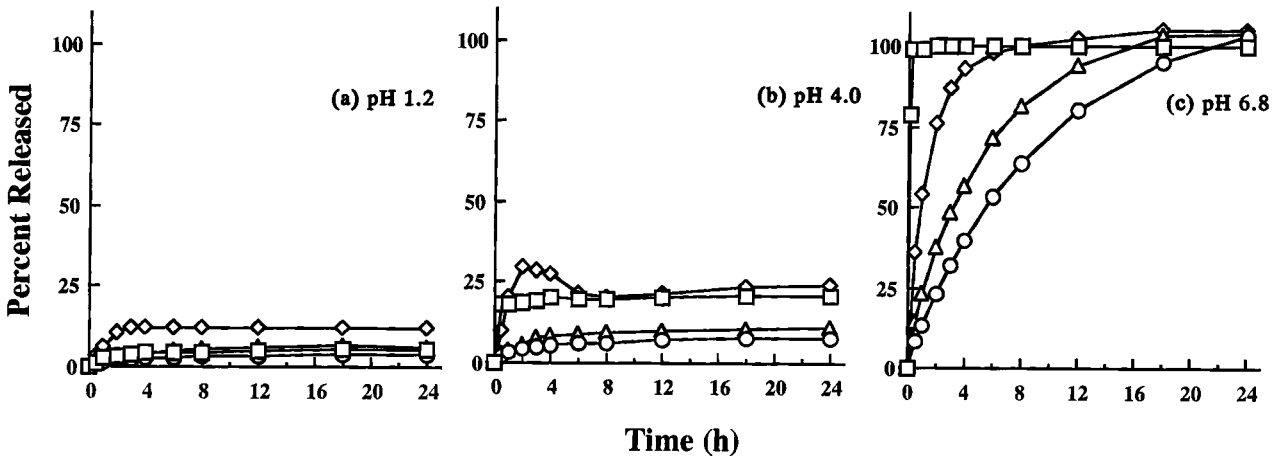

Figure 1. Dissolution profiles of DS from Voltaren and WMGs: (a) pH 1.2; (b) pH 4.0; (c) pH 6.8. Voltaren, □; WMG-A, ○;WMG-B, △;WMG-C, ◇.

paper (12), an increase of HPC produces a new dissolution surface when cracks are formed by the swelling of HPC, which then results in an increase in DS release.

The values of MDT in pH 6.8 were calculated as 0.3, 2.2, 5.8, and 8.0 hr for Voltaren, WMG-C, WMG-B, and WMG-A, respectively. These results indicate that the release of DS in the stomach would be depressed by its intrinsic solubility and accelerated in the small intestine, depending on the release rate of the product. Accordingly, the onset of drug absorption is determined by the GETs of the samples, and their bioavailabilities are controlled by the release rate of DS in the small intestine.

An in vitro evaluation of the influence of physical factors such as the mechanical destructive force of GI motility on drug release was reported using a modified dissolution method. For example, Aoki et al. (16) investigated the correlation between drug release in vivo and in vitro using the paddle-beads method in which the mechanical impact force between the beads and the sensor was employed as an index to estimate the destructive force in the GI tract. According to their results, the in vivo release using the paddle-beads method with the rotation speed at 25 rpm 250 ml of medium containing 2500 beads was similar to that of in vivo release in fasted dogs.

On the other hand, Katori, Aoyagi, and Terao (17) examined the effect of hydrodynamic flow around the dosage form in the GI tract on drug release using USP flow-through cell methods and the disintegration apparatus in the Japanese Pharmacopoeia XIII. They found that the in vivo release rate of controlled-release acetaminophen tablets in dogs was estimated to be equivalent to the release rate determined by the paddle methods at 100 rpm^{-1}.

With this in mind, the effect of destructive force in the GI tract on the release of DS from WMG was evaluated at three different paddle rotation rates (50, 100, 200 rpm) in this study. As clearly shown in Fig. 2, release rates of

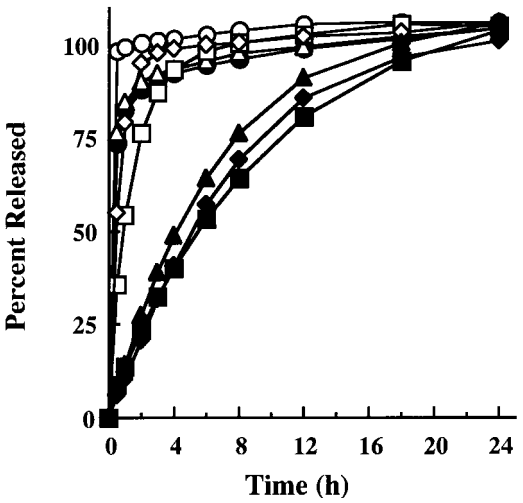

Figure 2. Influence of paddle rotation rate and milled WMGs on dissolution profiles of DS. WMG-A 50 rpm, ◆; WMG-A 100 rpm, ■; WMG-A 200 rpm, ▲; WMG-C 50 rpm, ◇; WMG-C 100 rpm, □; WMG-C 200 rpm, △; milled WMG-A, △; milled WMG-C, ○.

DS were almost independent of paddle rotation speed and increased with an increase in HPC content in the WMG. On the other hand, DS was quickly released (within 5 min) when the WMGs were pulverized beforehand with a pestle and mortar.

These results suggest that in vivo release of DS from WMGs would not be changed by GI motilities. Ogura (18) reported that mechanical strength of wetted granules should be more than 10 g to maintain their original shape in the GI tract. If WMGs were disintegrated when the destructive force by GI motility is far stronger than that anticipated from the results of the mechanical strength of WMGs and the present dissolution study, DS is supposed to be released immediately in the GI tract. The occurrence of WMG disintegration could be estimated by the evaluation of bioavailability. In addition, the strength of GI motility in humans is reported to be weak compared with that in dogs (17).

Mean plasma concentration–time curves of DS after oral administration of the samples are shown in Fig. 3, and pharmacokinetic parameters obtained are summarized in Table 2. The values of C_{max} (7.7 μg/ml), AUC_{12} (16.8 μg hr/ml), and MRT (3.1 hr) in Voltaren were found to be comparable with those reported by Sagara et al. (14) (C_{max}, 8.1 μg/ml; AUC_{12}, 23.6 μg hr/ml; MRT, 3.2 hr). This result suggests that the pharmacokinetic parameters of Voltaren obtained in this study may serve as a reference for the evaluation of sustained action of WMGs in the study. The values of C_{max} obtained in WMG-A, WMG-B, and WMG-C were clearly lower than in the Voltaren tablet and decreased as HPC content in the WMGs decreased. The differences in T_{max} between Voltaren and the three WMGs were relatively small, although T_{max}, in general, tends to be prolonged in sustained-release dosages.

Release of DS in the stomach is considered to be low due to its low solubility at pH 1.2, and further GET of small pelletlike WMGs is relatively fast as discussed above. The time required for DS to arrive at the absorption site in the small intestine did not differ much among four samples. On the other hand, the MRT values of WMG-A and WMG-B were almost the same and significantly longer (about 1.5 times) than those of the other two samples.

Furthermore, the relationships between MDT and AUC and between MDT and MRT in Fig. 4 indicated that the difference of dissolution profiles of DS from the dosage form was reflected in the results of pharmacokinetic parameters. Dose corrections were made on AUC

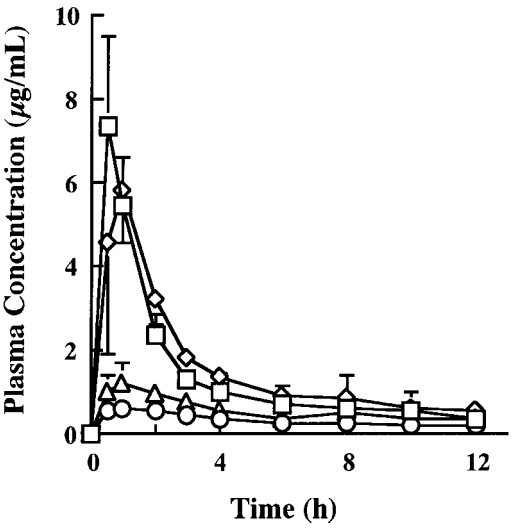

Figure 3. Mean plasma concentration of DS after administration of Voltaren or WMGs. Voltaren, □; WMG-A, ○; WMG-B, △; WMG-C, ◇. Data are mean ± SD ($n = 4$).

Table 2

Pharmacokinetic Parameters After Administration of Voltaren or Wax Matrix Granules

	Content of HPC (% w/w)	C_{max} (μg/ml)	T_{max} (hr)	AUC (μg · hr/ml)	MRT (hr)
Voltaren	—	7.7 ± 1.7	0.6 ± 0.3	16.8 ± 3.3	3.1 ± 0.7
WMG-A	30	0.6 ± 0.3**	1.3 ± 0.5	3.6 ± 1.6**	4.6 ± 0.7*
WMG-B	40	1.4 ± 0.4**	1.8 ± 1.0	6.5 ± 0.9**	4.7 ± 0.7*
WMG-C	60	6.5 ± 1.8*	1.1 ± 0.6	18.6 ± 3.2	3.6 ± 0.2

Results are expressed as the mean ± SD of four dogs.
*Statistically significant ($p < .05$) versus Voltaren.
**$p < .01$ versus Voltaren.

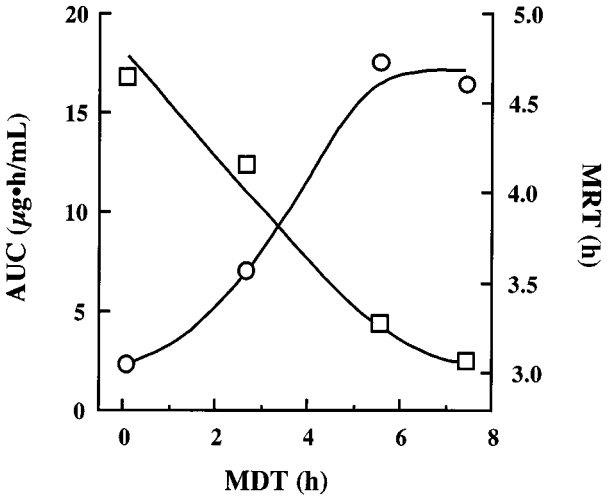

Figure 4. Relationship between MDT and AUC (□) or MRT (○).

values in Fig. 4 because amounts of DS administrated as WMGs (37.5 mg/capsule) and Voltaren (25 mg/tablet) were different. Like most drugs, DS is thought to be absorbed in the small intestine, and SITT of the dosage form in beagle dogs is estimated to be around 2 hr (19). If the release rate of DS from WMGs is slow enough to release all of the drug within the SITT, WMGs may pass through the absorption site without adequately releasing DS. From this viewpoint, the values of AUC_{12} in WMG-B and WMG-C are considered to be the result of incomplete release of DS in the small intestine due to short SITT. This idea is supported by the findings of Sagara et al. (14) obtained using GI physiology–regulated dogs. According to their report, the AUC value with DS from the sustained-release capsule Voltaren sustained-release capsule was almost the same as for the Voltaren tablet when sustained-release capsules were administered to beagle dogs with prolonged GET and SITT.

The SITT of pharmaceuticals in humans is reported to be around 2–3 hr, which is longer than that of beagle dogs (19,20). In addition, food intake before drug administration further prolongs GET and SITT, so pharmacokinetic parameters of WMG-B and WMG-C are expected to be improved in humans. On the other hand, as mentioned, the intensity of GI motility in beagle dogs is estimated to be stronger than that in humans. Consequently, WMGs are expected to be used as a sustained-release dosage form in humans, although the release rate of DS still needs to be adjusted to the SITT in humans.

CONCLUSION

The in vivo performance of WMGs prepared by the twin-screw compounding extruder was evaluated in fasted beagle dogs. Good correlation between in vitro dissolution parameter (MDT) and in vivo parameters (MRT and AUC) indicated that the design of WMGs with a desired in vivo performance would be possible by controlling HPC content in the formulation. However, the transit time of WMGs through the small intestine is strongly suggested to control total bioavailability of DS.

REFERENCES

1. C. J. Kenyon, G. Hooper, D. Tierney, J. Butler, J. Devane, and I. R. Wilding, The effect of food on the gastrointestinal transit and systemic absorption of naproxen from a novel sustained release formulation, J. Controlled Release, 34, 31–36 (1995).
2. I. R. Wilding, R. A. Sparrow, S. S. Davis, and R. J. Horton, The role of gastric emptying in the absorption and metabolism of nifedipine given in a modified release pellet formation, Int. J. Pharm., 84, 59–67 (1992).
3. M. Sournac, E. Beyssac, J. Maublant, J. Aiache, A. Veyre, and J. Bougare, Scintigraphic study of the gastrointestinal transit of a sustained release theophylline tablet. II. Administration in non-fasting state, J. Controlled Release, 15, 113–120 (1991).
4. B. Abrahamsson, M. Alpsten, M. Hugosson, U. E. Jonsson, M. Sundgren, A. Svenheden, and J. Tolli, Absorption, gastrointestinal transit, and tablet erosion of felodipine extended-release tablet, Pharm. Res., 10, 709–714 (1993).
5. S. S. Davis, J. G. Hardy, and J. W. Fara, Transit of pharmaceutical dosage forms through the small intestine, Gut, 27, 886–892 (1986).
6. S. S. Davis, R. Khosla, C. G. Wilson, N. Washington, and S. T. Leslie, The gastrointestinal transit of controlled release formulation of indomethacin, Int. J. Pharm., 60, 191–196 (1990).
7. R. Khosla and S. S. Davis, Gastric emptying and small and large bowel transit of non-disintegrating tablets in fasted subjects, Int. J. Pharm., 52, 1–10 (1989).
8. H. Bechgaard and G. H. Nielsen, Controlled-release multiple-units and single-units doses. A literature review, Drug Dev. Ind. Pharm., 4, 53–67 (1978).
9. M. Shameen, N. Katori, N. Aoyagi, and S. Kojima, Oral solid controlled release dosage forms: role of GI-mechanical destructive forces and colonic release in drug absorption under fasted and fed conditions in humans, Pharm. Res., 12, 1049–1054 (1995).
10. N. Katori, W. Ma, N. Aoyagi, and S. Kojima, Effect of destructive force on drug release from multiple unit con-

trolled release dosage forms in humans, Pharm. Res., 13, 1541–1546 (1996).
11. Y. Miyagawa, T. Okabe, Y. Yamaguchi, M. Miyajima, H. Sato, and H. Sunada, Controlled-release of diclofenac sodium from wax matrix granule, Int. J. Pharm., 138, 215–224 (1996).
12. H. Sato, Y. Miyagawa, T. Okabe, M. Miyajima, and H. Sunada, Dissolution mechanism of diclofenac sodium from wax matrix granule, J. Pharm. Sci., 86, 929–934 (1997).
13. K. Nakamichi, H. Yasuura, H. Fukui, M. Oka, S. Izumi, T. Andou, N. Shimizu, and K. Ushimaru, Preparation of nifedipine-hydroxypropylmethylcellulose phthalate solid dispersion by twin screw extruder and its evaluation, Yakuzaigaku, 56, 15–22 (1996).
14. K. Sagara, Y. Nagamatsu, I. Yamada, M. Kawata, H. Mizuta, and K. Ogawa, Bioavailability study of commercial sustained-released preparation of diclofenac sodium in gastrointestinal physiology regulated-dogs, Chem. Pharm. Bull., 40, 3303–3306 (1992).
15. D. F. Evans, G. Pye, R. Bramley, A. G. Clark, T. J. Dyson, and J. D. Hardcastle, Measurement of gastrointestinal pH profiles in normal ambulant human subjects, Gut, 29, 1035–1041 (1988).
16. S. Aoki, K. Uesugi, K. Tatsuishi, H. Ozawa, and M. Kayano, Evaluation of the correlation between in vivo and in vitro release of phenyl propanolamine HCl from controlled-release tablets, Int. J. Pharm., 85, 65–73 (1992).
17. N. Katori, N. Aoyagi, and T. Terao, Estimation of agitation intensity in the GI tract in humans and dogs based on in vitro/in vivo correlation, Pharm. Res., 12, 237–243 (1995).
18. T. Ogura, Design and evaluation of sustained-release dosage form for rhinitis, Proceedings of Dai 14 kai Seizai Seminar [in Japanese], 132–137 (1989).
19. J. B. Dressman, Comparison of canine and human gastrointestinal physiology, Pharm. Res., 3, 123–131 (1986).
20. K. Yamada, A. Furuta, M. Akimoto, T. Maki, T. Suwa, and H. Ogata, Evaluation of gastrointestinal transit controlled-beagle dog as a suitable animal model for bioavailability testing of sustained-release acetaminophen dosage form, Int. J. Pharm., 119, 1–10 (1995).