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Preparation and evaluation of enteric granules of aspirin prepared by acylglycerols *

Yoshiteru Watanabe, Toru Kogoshi, Youichi Amagai and Mitsuo Matsumoto

Department of Pharmaceutics, Showa College of Pharmaceutical Sciences, Machida, Tokyo 194 (Japan)

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Summary

The pH-independent enteric granule containing aspirin prepared using a monoacylglycerol preparation (Poemu S100V®) commercially available as glyceryl monostearate and a triacylglycerol, glyceryl trilaurate (GTL), digested by lipase and bile salts in intestinal juice was investigated. The preparation processes consisted of a two-step dry mixing method using a centrifugal rotating mixer (Mechano-mill®). When aspirin was in powder form (80–100 mesh), it was aggregated by melted Poemu S100V; the aspirin-Poemu S100V mass was formed in the first step of the preparation process. During the subsequent step, the crushed aspirin-Poemu S100V mass was coated with melted GTL. Finally, an enteric granule (5.5–12 mesh) was obtained. In the dissolution test in vitro, the dissolution percentages of aspirin from the granule over a 2 h period at pH 1.2 were very low (~ 10%), while aspirin from the granule in pH 6.4 phosphate buffer solution (PBS) containing lipase and cholic acid was well dissolved (~ 90%). Aspirin was insufficiently dissolved (~ 40%) from the crushed aspirin-Poemu S100V mass without the GTL coating in PBS containing lipase and cholic acid. When aspirin was in crystalline form (12–42 mesh), the surfaces of individual crystals of aspirin were coated with Poemu S100V in the first step of the preparation process. However, a low dissolution percentage of aspirin from this product was not obtained at pH 1.2. The results obtained in human subjects suggested that the prepared granule of aspirin had the property of pH-independent dissolution.

Introduction

Synthetic polymers which remain undissociated in the region of low pH but ionize (dissolve) at pH 4 or 5 (pH-dependent dissolution) provide a useful means of coating enteric tablets, capsules or granules (Porter et al., 1982; Porter, 1985). In recent years, extensively employed polymers such as methacrylic acid copolymer (Eudragit LD) and hydroxypropylmethylcellulose acetate succinate (HPMCAS) have been used in the enteric coating process with an aqueous system instead of organic solvents. However, it has been reported (Meldrum et al., 1972; Fordtran et al., 1973; Ogata et al., 1984) that the gastric pH can be changed from 1 to 7 by the physiological conditions. Furthermore, Maekawa et al. (1970c) and Kaniwa et al. (1985a,b)

Correspondence: Y. Watanabe, Showa College of Pharmaceutical Sciences, Department of Pharmaceutics, 3165, Higashitamagawagakuen 3-Chome, Machida, Tokyo 194, Japan.

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found that the pH-dependent enteric preparations coated with these materials were often ineffective in those human subjects with low gastric acidity (e.g., anacidity, achylia (Landau, 1986)).

In these cases, a dosage form prepared using long-chain acylglycerols, edible and safe pharmaceutical ingredients, which are digested by lipase and bile salts in intestinal juice (pH-independent dissolution), should be made available. Drugs could not be released from this dosage form in the stomach at low pH values and are thus dissolved in the small intestine.

In the present paper, formulations comprising two types of pH-independent (pancreatic lipase sensitive) enteric granules containing drugs were prepared by surface modification of the drug crystal coated with the melted monoacylglycerol preparation, Poemu S100V® (a commercially available glyceryl monostearate) or by formation of a drug and Poemu S100V mass (drug-Poemu S100V mass) coated with melted glyceryl trilaurate (GTL) using a reported mixing procedure (Nakagawa et al., 1984; Ishizaka et al., 1988). The enteric-coated granule of aspirin has previously been reported (Maekawa et al., 1970a,b). We chose aspirin as a model drug for the enteric preparation in our study. The dissolution behavior of aspirin from the prepared granules in vitro and the bioavailability of the aspirin granule in human subjects were investigated.

Materials and Methods

Materials

Aspirin (JP XI) was purchased from Iwaki Seiyaku (Tokyo, Japan). Glyceryl monostearate (Poemu S100V®, Riken Vitamin, Tokyo, Japan) was used after the removal of free fatty acids and glycerol by reprecipitation of monoacylglycerol in ethanol. Purified Poemu S100V was dried in vacuo overnight and was analyzed (glyceryl monostearate, 65%; glyceryl monopalmitate, 29%; total monoacylglycerol, 94% or above; m.p. approx. 80 °C) using the gas-liquid chromatographic method described by Watts and Dils (1969) with a GC instrument (GC-15A system, Shimadzu Seisakusho, Kyoto, Japan). Glyceryl trilaurate (GTL, content 98% or above, m.p. approx. 45 °C; Tokyo Kasei Kogyo, Tokyo, Japan) was used. Lipase (triacylglycerol lipase; EC 3.1.1.3) and cholic acid (sodium salt) were obtained from Sigma (St. Louis, MO, U.S.A.). Other reagents used were of analytical grade.

Preparation of granules

The process of granule preparation is shown in Scheme 1. Fig. 1 illustrates the centrifugal rotating mixer (Mechano-mill®, Model MM-10, Okada Seiko, Tokyo, Japan) used for dry mixing of the drug and Poemu S100V with or without GTL under heating by infrared irradiation. Using this method, only Poemu S100V or GTL melt at the lower temperature as compared with the melting points of the drugs.

The preparation process consists of two steps: In the first, the mixer was charged with 20 g of aspirin powder and 10–30 g of Poemu S100V. Then 20 g of the crushed aspirin-Poemu S100V mass and 1–10 g of GTL (100–200 mesh) were added to the mixer in the second step.

Aspirin and Poemu S100V mass were prepared in the first step. In the second step, the crushed aspirin-Poemu S100V mass for the dry mixing experiments was obtained by sieving the products through a 5.5–12 or 12–42 mesh. GTL was used for the surface modification of the crushed aspirin-Poemu S100V mass. After GTL coating, free GTL which did not adhere to the surface of the crushed aspirin-Poemu S100V mass was removed by sieving with a 12-mesh sieve; finally, granules I and II were obtained.

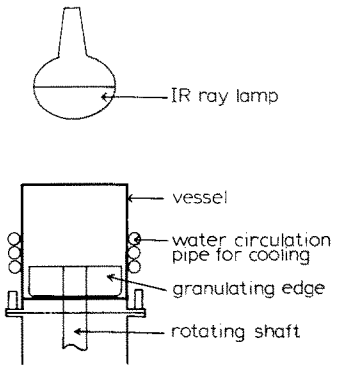
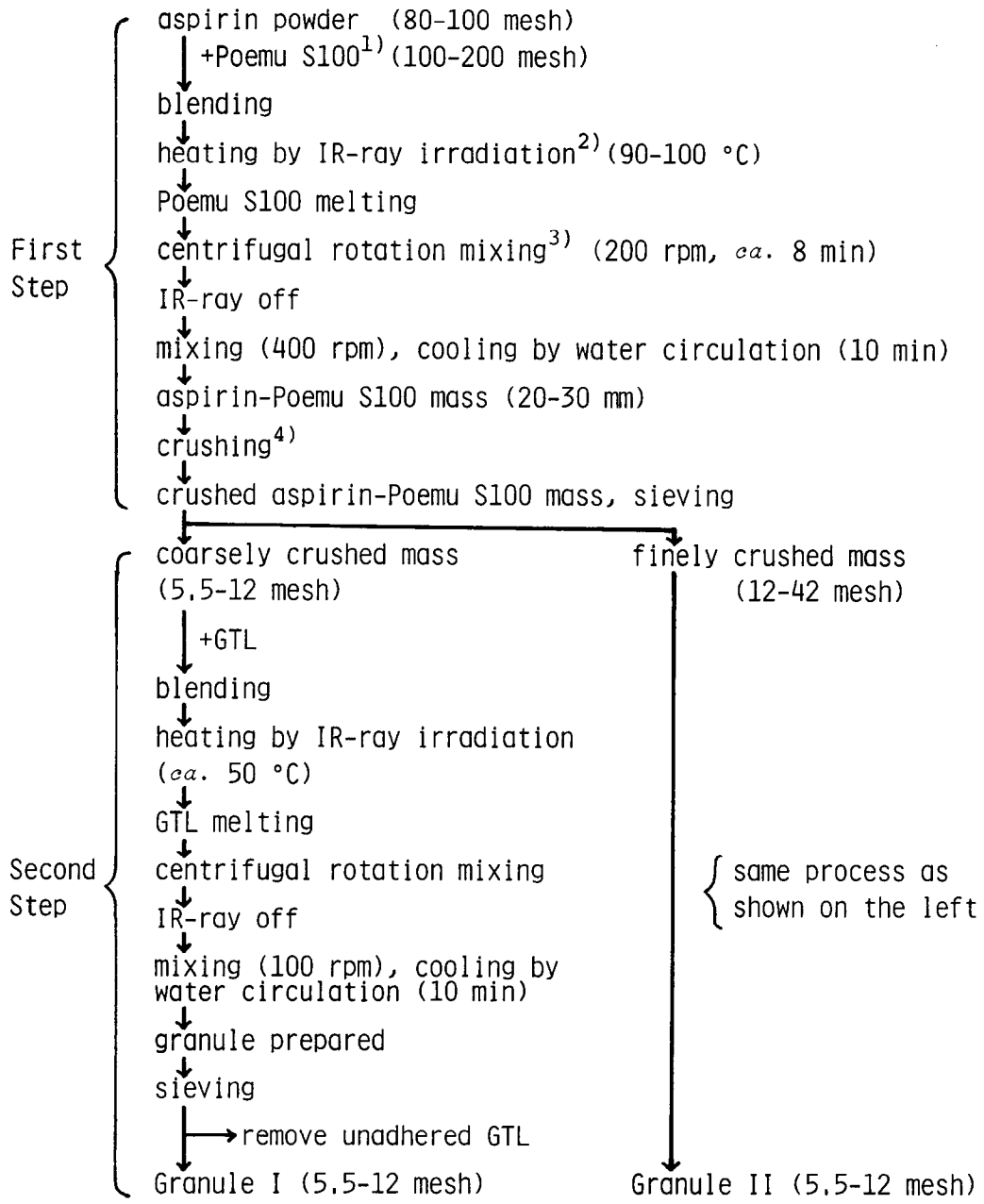


Fig. 1. Schematic illustration of centrifugal rotating mixer and IR ray lamp for preparation of the enteric granule using acylglycerols. The volume of the vessel is approx. 1000 cm^3.

Determination of aspirin content

The aspirin content of the products was determined spectrophotometrically using a UV-visible recording spectrophotometer (Model UV-240, Shimadzu Seisakusho, Kyoto, Japan). The crushed aspirin-Poemu S100V mass was dissolved in ethanol and the absorbance of the diluted solution of aspirin was measured at 275 nm.



Scheme 1. Preparation process of the enteric aspirin granule using Poemu S100V and GTL. (1) A commercially available glyceryl monostearate (Riken Vitamin, Tokyo, Japan). (2) An IR-ray lamp (500 W) was set approx. 15 cm above the materials. (3) A centrifugal rotating mixer (Fig. 1) was used. (4) An electric mill (Model HCM-501, Toshiba, Tokyo, Japan) was used.

TABLE 1

Experimental conditions of dissolution test

Mode	Paddle method
Apparatus	Model NTS-VS, (Toyama Sangyo, Osaka, Japan)
Sample amount	200 mg calculated as drug
Temperature	37 ± 0.1 °C
Dissolution medium	500 ml
	(1) pH 1.2 aqueous solution (the first fluid for disintegration test, JP XI)
	(2) pH 6.4 phosphate buffer solution [a] (PBS)
	(3) PBS containing lipase (0.6%(w/v)) and cholic acid (0.1%(w/v))
Sampling time	10, 20, 30, 45, 60 and 120 min

[a] The salt component described by Koizumi et al. (1964) was used.

The Poemu S100V content was calculated via Eqn 1. The total content of Poemu S100V and GTL was calculated based on Eqn 2.

$$\text{Poemu S100V (\%)} = 100 - \text{aspirin content(\%)} \quad (1)$$

$$\text{Poemu S100V + GTL (\%)} = 100 - \text{aspirin content(\%)} \quad (2)$$

In vitro dissolution test

The rate of dissolution of the drug from the products was studied in a manner similar to the paddle method described in JP XI, using a dissolution test apparatus. The experimental conditions employed for the dissolution test are listed in Table 1.

The dispersion was immediately filtered through a 0.2 μm cellulose nitrate membrane filter (Dismic-25, Toyoroshi, Tokyo, Japan) to remove the particles. The concentration of salicylic acid was determined by measuring the absorbance at 300 nm on a UV-visible spectrophotometer after the hydrolysis of aspirin with 1 N NaOH.

Bioavailability study in human subjects

Healthy male volunteers (23–25 years old; 65–70 kg weight), who were fully informed of the nature of the study, fasted for at least 10 h before and 6 h after oral administration of the drug. Each volunteer was given orally granule I or II containing 500 mg of aspirin together with 200 ml of water. For the control experiments, volunteers received 500 mg of aspirin (12–42 mesh) orally with 200 ml of water.

Urine samples were collected at predetermined time intervals; the amount of salicylic acid excreted in the urine for 24 h after oral administration was determined by the colorimetric method of Brodie et al. (1944). The mean residence time (MRT) of the drug in the body was determined by the statistical moments theory (Gibaldi and Perrier, 1982).

For statistical analysis of the results, one-way ANOVA and Dunnett's test were used. A P value less than 0.05 was considered a significant difference.

Results and Discussion

Dissolution of aspirin from the products obtained in the first step of the preparation

In this investigation, the expected levels of percentages of drug dissolved from the enteric preparation within 2 h were predetermined below 20% in pH 1.2 aqueous solution and above 80% in PBS (pH 6.4) containing lipase and cholic acid.

When the 12–42 mesh-size aspirin crystal was used in the first step of the process, the surface of the individual crystal of aspirin was coated with melted Poemu S100V. Although the content of Poemu S100V in contact with the aspirin crystal increased with increasing amount of Poemu S100V used, the Poemu S100V content in the product was no more than 20%. The Poemu S100V content in the product was slightly increased (by approx. 35%) on repeating the first-step treatment, and about 40% of the aspirin was dissolved from this

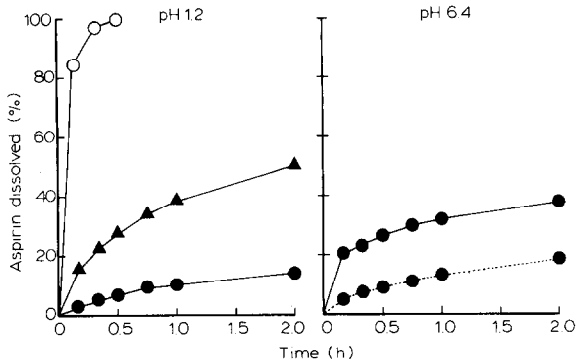


Fig. 2. Dissolution of aspirin from the crushed aspirin-Poemu S100V mass prepared in the first step of the preparation process. Aspirin content in the crushed mass was 50%. Results are expressed as the mean of three experiments. (○) Fine powder (80–100 mesh); (▲) finely crushed aspirin-Poemu S100V mass (12–42 mesh); (●) coarsely crushed aspirin-Poemu S100V mass (5.5–12 mesh). Dissolution medium at pH 6.4; (- - - -) PBS, (———) PBS containing lipase (0.6% (w/v)) and cholic acid (0.1% (w/v)).

product at pH 1.2. The expected dissolution percentage (below 20%) of aspirin was not obtained by the aspirin crystal coated with Poemu S100V.

In the case of fine powder (80–100 mesh) aspirin, Poemu S100V acted as a binding agent and aggregated the aspirin powder. Consequently, the masses (20–30 mm) of aspirin and Poemu S100V (aspirin-Poemu S100V mass) were formed in the first step of the process. Because this product contained all of the aspirin and Poemu S100V used, an aspirin-Poemu S100V mass with a large amount of Poemu S100V (above 50%) could be prepared. After crushing the mass with an electric coffee mill, two sizes of crushed aspirin-Poemu S100V masses were obtained by sieving the product through a 5.5–12 and 12–42 mesh. The dissolution profiles of aspirin from the crushed masses at pH 1.2 and pH 6.4 are presented by filled symbols in Fig. 2.

The dissolution behavior of aspirin was changed by the difference in particle size of the crushed aspirin-Poemu S100V mass. Even though the Poemu S100V content in the aspirin-Poemu S100V mass exceeded 50%, in the case of the finely crushed mass (12–42 mesh), shown as filled triangles in Fig. 2, approx. 50% of the aspirin was dissolved within 2 h at pH 1.2. When the coarsely crushed mass (5.5–12 mesh) was used, the dissolution percentage of aspirin from the mass containing 50% Poemu S100V at pH 1.2 (shown as filled circles) was approx. 15% at 2 h.

The dissolution of aspirin from the coarsely crushed mass in pH 6.4 PBS was examined. The dissolution percentage of aspirin in pH 6.4 PBS (broken line in Fig. 2, right-hand panel), approx. 20% at 2 h, did not increase compared to the value obtained at pH 1.2; in contrast, aspirin was more efficiently dissolved from the crushed aspirin-Poemu S100V in PBS containing lipase and cholic acid (represented by the straight line in Fig. 2, right). However, the maximum dissolution percentage of aspirin was approx. 40% within 2 h.

The dissolution of aspirin from the crushed aspirin-Poemu S100V mass obtained in the first step was not sufficiently increased by lipase and cholic acid at pH 6.4. This result is probably related to the nature of Poemu S100V. To accelerate the dissolution of aspirin from the crushed aspirin-Poemu S100V mass, the combined use of Poemu S100V and triacylglycerols that are readily digested by lipase was investigated.

Dissolution of aspirin from granules I or II prepared in the second step of preparation

When the crushed mass of aspirin and Poemu S100V with a triacylglycerol GTL was prepared in the first step, the dissolution percentage was increased (about 80% at 2 h) by lipase and cholic acid at pH 6.4. However, the expected low level of dissolution percentage (below 20%) of aspirin in pH 1.2 aqueous solution could not be achieved. Therefore, we carried out the second step of the preparation process which involved coating the surface of the crushed aspirin-Poemu S100V mass with triacylglycerol. To prevent melting of Poemu S100V (m.p. ~ 80 °C) in the crushed aspirin-Poemu S100V mass during the heating procedure of the second step, GTL was used instead due to its lower melting temperature (m.p. ~ 45 °C).

Granules I and II were prepared by coating the crushed aspirin-Poemu S100V masses of 5.5–12 and 12–42 mesh sizes with GTL (Scheme 1). When the content of Poemu S100V in the crushed aspirin-Poemu S100V mass was below 20%, it was impossible to coat the crushed mass with GTL in

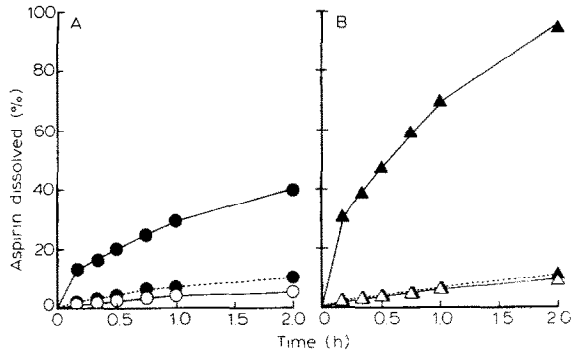


Fig. 3. Dissolution of aspirin from granules I (A) and II (B) prepared in the second step of the preparation process at pH 1.2 and 6.4. The aspirin content in the granule was approx. 50%. Results are expressed as the mean of three experiments. Dissolution medium: (○———○) and (△———△), pH 1.2 aqueous solution; (●------●) and (▲------▲), PBS (pH 6.4); (●———●) and (▲———▲), PBS containing lipase (0.6% (w/v)) and cholic acid (0.1% w/v)).

the second step of the process due to the centrifugal mixing procedure being insufficiently effective. Fig. 3 shows the time courses of aspirin dissolution from granules I and II (aspirin, 50%; Poemu S100V, 25%; GTL, 25%) at pH 1.2 (empty symbols) and 6.4 (filled symbols). The dissolution rates of aspirin from granules I and II at pH 1.2 decreased as the total content of Poemu S100V and GTL increased. Consequently, a very low dissolution rate (below 10%) was attained for granules containing 50% of Poemu S100V + GTL. These results may be due to an increase in hydrophobicity caused by GTL present on the surface of the granules. Without lipase and cholic acid in PBS at pH 6.4 (●---●, Fig. 3), the dissolution percentage (about 10% at 2 h) of aspirin was similar to that obtained at pH 1.2 (unfilled symbols). On the other hand, dissolution of aspirin (continuous curves) was significantly increased in PBS containing lipase and cholic acid. Interestingly, the dissolution percentage (above 90% at 2 h) from granule II was higher than that (approx. 40% at 2 h) of granule I.

These results suggest that the dissolution of aspirin from both granules is influenced by lipase and cholic acid in the dissolution medium and the particle size of the crushed aspirin-Poemu S100V mass used in the second step of the preparation process. Furthermore, it was found that aspirin in these granules was more efficiently dissolved by lipase than by cholic acid. The effect of lipase on the dissolution of aspirin increased with increasing concentration in the dissolution medium and was maximal at approx. 0.6% (w/v).

Bioavailability of aspirin from the granules in human subjects

Fig. 4 illustrates the mean cumulative amount of salicylic acid excreted in urine following oral administration of granules I, II or aspirin crystal in adult male volunteers according to the method described in Materials and Methods.

In the case of aspirin crystal (12–42 mesh) (indicated: ○), salicylic acid was excreted (19 ± 3 mg) 1 h after administration. The cumulative amount of salicylic acid excreted was increased; ultimately, the mean value within 24 h after administration was 430 ± 9 mg. The mean value of the MRT was determined as 6.8 ± 0.9 h.

After the administration of granule I having a low dissolution of aspirin observed in vitro, no

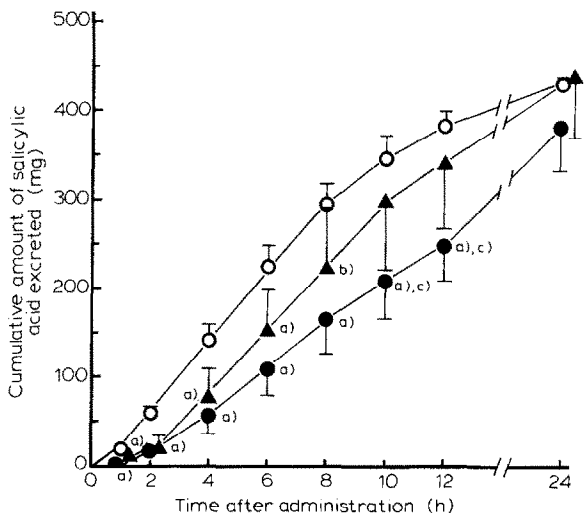


Fig. 4. Mean cumulative amount of salicylic acid excreted in urine following oral administration of aspirin, granules I, II (5.5–12 mesh) or crystal (12–42 mesh) in adult male volunteers. Each point represents the mean ± S.D. (vertical bar) of six experiments. (○———○) Crystal, (●———●) granule I, (▲———▲) granule II. Statistically significant differences: (a) $p < 0.01$ in granules vs crystal; (b) $p < 0.05$ in granules vs crystal; (c) $p < 0.05$ in granule I vs granule II.

salicylic acid was excreted in urine (below 1 mg) within 1 h and the mean cumulative amount excreted (248 ± 39 mg) at 12 h was statistically lower ($p < 0.01$) than that (383 ± 16 mg) for the aspirin crystal. Granule I had a significantly ($p < 0.01$) increased MRT (13.9 ± 2.0 h). However, the mean value of the cumulative amount excreted (382 ± 50 mg) within 24 h was not significantly different from the value resulting from the use of aspirin crystal (430 ± 9 mg).

When granule II was administered, the mean value of the cumulative amount excreted (225 ± 64 mg) within 8 h of administration was lower ($p < 0.05$) than that of aspirin powder (295 ± 24 mg). The MRT increased significantly ($p < 0.05$) to 9.6 ± 1.8 h. However, the cumulative amount excreted-time curve of granule II was not significantly different from that for aspirin crystal between 10 and 24 h.

The differences in the cumulative amount of salicylic acid excreted and MRT (I, 13.9 ± 2.0 h: II, 9.6 ± 1.8 h) between granules I and II were in general agreement with the difference in the dissolution rates of aspirin in vitro. The results obtained in vivo suggested that aspirin dissolution from these enteric granules was retarded in the stomach.

Conclusions

We have shown that the pH-independent controlled-release granule can be prepared by using Poemu S100V and GTL which are digested by lipase and bile salts in the intestinal juice. Poemu S100V (glyceryl monostearate) and GTL are useful pharmaceutical ingredients for pH-independent (pancreatic lipase sensitive) enteric preparations.

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