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Biopharmaceutical Evaluation of Gelatin Microcapsules of Sulfonamides[1)]

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To evaluate the efficacy of gelatin microcapsules containing sulfonamides (sulfanilamide, sulfisomidine and sulfamethizole), blood concentration–time curves were obtained using gastric-emptying-controlled rabbits after intravenous or oral administration of powder and microcapsule dosage forms. Using the pharmacokinetic parameters derived from the intravenous study, the oral administration rate constant for sulfanilamide was calculated and the extent of bioavailability of the microcapsules was compared with that of the powder. It was very hard to obtain a significant advantage with regard to the prolongation effect in the gelatin microcapsules containing sulfanilamide. Similar result was also obtained in the case of sulfisomidine. However, prolonged and sustained release properties were clearly obtained with the microencapsulated sulfamethizole prepared by this method.

Keywords——gelatin microcapsule; sulfonamide; blood concentration; gastric-emptying controlled rabbit; microencapsulated sulfamethizole

The use of drugs in sustained release formulations may be advantageous because the reduced frequency of administration is more convenient for the patients and may possibly improve compliance.[2-4)] Maintaining active blood levels of drug for much longer periods between doses is also of benefit to the patient. If zero-order drug release is realized as the rate-determining step of drug absorption from microcapsule formulations, constant blood concentrations of drug may be readily achieved and maintained.[2-4)] However, most microcapsule formulations release drug with first-order rather than zero-order kinetics. Sustained release formulations may be classified into the following three categories:[2,3)] (1) ideal sustained-action type with zero-order release (2) repeated-action type (3) general prolonged-action type.

The present investigation deals with the *in vivo* evaluation of microcapsules containing sulfonamides, which may be classified as "prolonged-action type" according to the above classification, with respect to absorption, distribution and elimination in rabbits with controlled gastric-emptying.

Experimental

Materials——The sulfonamides(sulfanilamide(SA), sulfisomidine (SI) and sulfamethizole(SM)) were purchased on the open market from three manufacturers (Nakarai, Hoei and Ebisu Co.) Gelatin (Gelatin Weiß, Merck) used in this experiment was acid-cured materials; its isoelectric point and viscosity were 4.63 (20 °C) and 13.0 cs (30% aqueous solution, 40 °C), respectively. Liquid paraffin (Nakarai Co.) was of J.P. IX grade with a viscosity of 37.6 cs (37.8 °C). Other reagents were all of specific reagent grade.

Microencapsulation——The procedure was the same as described previously.[1)]

Procedure for Gastric-emptying-controlled Rabbits——White male rabbits, 2.6—3.8 kg, were used and the gastric emptying rate was controlled by the method established by Maeda *et al.*[5,6)] The procedure is outlined in Chart 1.

The rabbits were fed SD-1, which is a special solid diet prepared by removing alfalfa from commercial solid food (Nihon Clea Co.) for one week prior to the absorption experiment. During the experiment, coprophagy was prevented by placing a yoke around the neck so that it was impossible for the mouth of the rabbit to touch the anus. Drinking water was freely available. The rabbits were fasted overnight, with free access to water, and anesthetized by intravenous injection of 0.4 ml/kg of 5% pentobarbital-Na 3 d before the administration of sulfonamides. A rubber stomach tube, 25 cm in length and 5 mm in external diameter with a large hole on the side of the tip, was inserted into the stomach, and 50—100 ml of warm saline was instilled. The fluid in the

	day	
	−9	feed 100 g of SD-1[a]
	−3	fast overnight
place in a yoke[d] with free access to water	−2	wash inside of stomach under Na-pentobarbital anesthesia (gastrolavage)
	−1	feed 200 g of SD-2[b]
	0	administer sulfonamides, 30ml water and 22 g/kg SD-3[c]
	1	feed 100 g of SD-1 every 12h after administration
	2	free access to diet

Chart 1. Standard procedure for Gastric-emptying-controlled Rabbit

a) The special solid diet (SD-1) was prepared by removing alfalfa from commercial solid diet.
b) SD-2 was prepared by adding 60 parts of water to 40 parts of SD-1.
c) SD-3 was prepared by adding 70 parts of water to 30 parts of SD-1.
d) A yoke consisting of two boomerang-like plastic plates (1mm in thickness and 100 g in weight) was placed around the rabbit neck to prevent coprophagy.

stomach was then withdrawn by suction with a syringe. This procedure was repeated until the fluid withdrawn contained hardly any solid material. Two hundred grams of SD-2 which had been prepared by adding 60 parts of water to 40 parts of SD-1, were given to the rabbit on the day preceding the absorption experiment.

Design of Absorption Experiment——Single Intravenous or Oral Administration: Prior to the absorption study of microcapsules in rabbits, control experiments were performed by administering a single intravenous infusion for 3min through the left ear vein. The injections were specially prepared by dissolution of sulfonamides (135 mg/kg for SA, 126 mg/kg for SI and 102 mg/kg for SM) in 5 ml of 0.5 or 1 N hydrochloric acid. Blood samples were withdrawn from the right marginal ear vein for 15 h after administration and assayed for intact drug by analysis based on diazotization with the use of 0.1% Tsuda reagent as stated in the previous paper.[1] Single oral administration of powder or microcapsules of sulfonamides (250 mg/kg) was done with 30 ml water and 22 g/kg of SD-3 which had been prepared by adding 70 parts of water to 30 parts of SD-1. Blood sampling and assay were done as described above.

Repeated Oral Administration: The gastric-emptying-controlled rabbits were used in this study. They received for one day or three days 250 mg/kg sulfonamides every 12 h as powder (less than 149 μm in particle diameter) or 500 mg/kg sulfonamides every 24 h as microcapsules. Blood samples were collected on the first and third days. The rabbits were utilized for additional experiments after their ear vein had fully healed.

Assay Method——The concentrations of unchanged and total sulfonamides in whole blood were determined by a spectrophotometric method. One ml of blood was diluted with 2 ml of water and then 1 ml of 35% trichloroacetic acid aqueous solution was added. The mixture was shaken for 5 min, followed by centrifugation (2500 rpm) for 15 min. The aqueous phase (1 ml) was separated and unchanged sulfonamides were determined as stated in the previous paper.[1] To 1 ml of aqueous phase, of 5% hydrochloric acid was added and the mixture was incubated at 100°C for 60 min. After cooling, the sample solution was used for determination of total SA concentration including acetylated and glucuronic acid metabolites.

Results and Discussion

Administraion Studies of Sulfanilamide (SA)

Representative blood levels of unchanged SA following a single intravenous infusion of 134.6 mg/kg SA and a single oral administration of 250 mg/kg SA are shown in Fig. 1. The blood profile of unchanged SA can be described by a pharmacokinetic one-compartment open model, and close agreement between the elimination patterns after intravenous and oral admin-

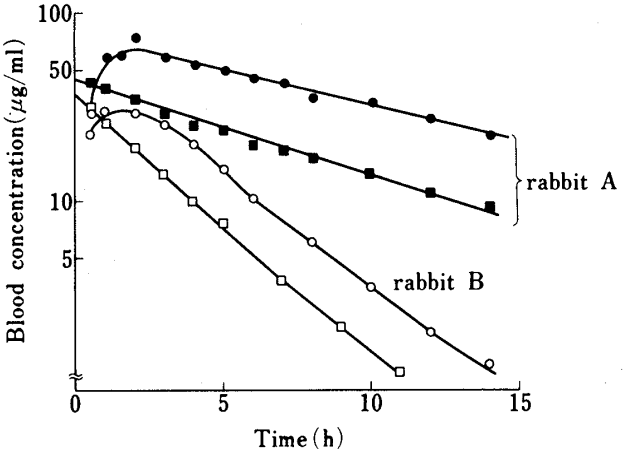


Fig. 1. Blood Concentration of Unchanged Sulfanilamide following Oral and Intravenous Administration to Rabbits with Slow (●, ■) and Rapid (○, □) Elimination Characteristics

Oral administration (●, ○); 250 mg/kg.
Intravenous administration (■, □); 134.6 mg/kg.

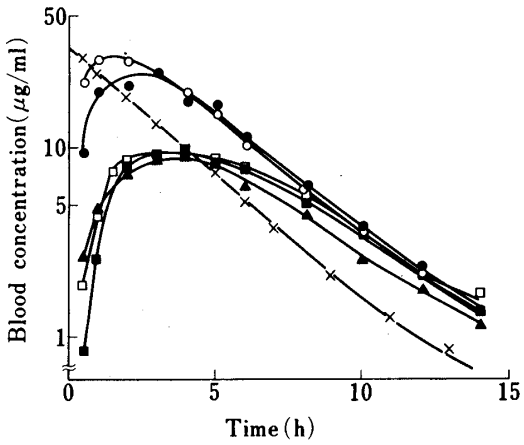


Fig. 2. Mean Blood Concentration of Unchanged Sulfanilamide (SA) following a Single Oral Administration of Powder or Microcapsules (250 mg/kgSA) to Gastric-emptying-controlled Rabbits

—○—, SA powder (n=6); —●—, MC-SA-40 (n=2); —■—, MC-SA-30 (n=2); —▲—, MC-SA-20 (n=3); —□—, MC-SA-10 (n=2); —×—, *i.v.* of 134.6 mg/kg SA.

TABLE I. Pharmacokinetic Parameters for Oral Administration of Sulfanilamde (SA) to Rabbits

Dosage form	$AUC^{0\to14\,h}$ [a] ($\mu g \cdot h \cdot ml^{-1}$)	k_a [b] (h^{-1})	k_e [c] (h^{-1})	c_{max} [d] ($\mu g/ml$)	t_{max} [e] (h)	$t_{0.5}$ [f] (h)
SA powder	156.8±40.4	1.340±0.294	0.291±0.041	26.5±12.6	1.49±0.14	2.43±0.34
MC-SA-10	78.4± 5.8	0.516±0.045	0.247±0.119	12.4± 0.3	2.75±0.19	2.82±0.14
MC-SA-20	65.4± 3.8	0.711±0.300	0.227±0.071	9.0± 4.3	2.52±0.43	3.32±0.85
MC-SA-30	73.0±12.3	0.677±0.176	0.226±0.062	13.5± 1.7	2.50±0.11	3.32±0.92
MC-SA-40	144.0±26.6	0.716±0.250	0.270±0.013	19.9± 9.3	2.33±0.53	2.58±0.13

a) AUC is the area under the blood curve from 0 to 14 h following oral and intravenous administration of sulfanilamide (250 mg/kg SA).
b) Absorption rate constant.
c) Elimination rate constant.
d) Maximum concentration.
e) Time at maximum concentration.
f) Biological half-life.

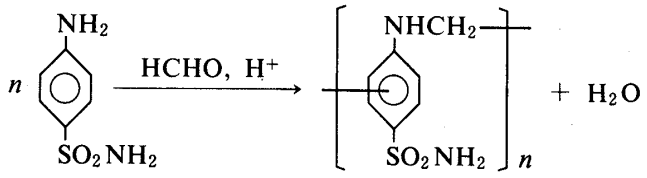
istration was observed in each rabbit (see Fig. 1, rabbits A and B).

However, it became evident that the rabbits used in this experiment could be divided into two different groups in relation to the elimination of SA. One group rapidly eliminated SA, whereas the remaining group slowly eliminated SA. The rate of acetylation to produce the metabolite 4-*N*-acetylsulfanilamide in each rabbit may be correlated with the above experimental finding. Further investigations revealed that most of the rabbits were rapid eliminators of SA in this experiment. Therefore such rabbits were initially selected by following the time course of unchanged SA concentration in the blood after intravenous injection of SA (134.6 mg/kg).

The mean pharmacokinetic parameters for the rapid eliminators following intravenous administration were 4.5 l/kg for the apparent volume of distribution (V_d), 0.288 h^{-1} for the elimination rate constant (k_e) and 209 $\mu g \cdot h \cdot ml^{-1}$ for the area under the blood concentration curve from 0 to 14 h ($AUC^{0\to14\,h}$). The blood levels of unchanged SA following a single oral dose of 250 mg/kg SA in powder and microcapsule preparations are shown in Fig. 2. The

data were corrected for oral administration of SA powder and microcapsules containing various percentages (w/w) of SA according to the Wagner–Nelson method[7] and are summarized in Table I. As shown in Table I, it is clear from the results that the micro-encapsulation of SA induced a large decrease (almost by half) in the extent of bioavailability as represented by $AUC^{0-14\,h}$, except for MC-SA-40.

One reason for the decreased drug concentration may be the condensation of SA with formaldehyde to yield a polymer related to II. However, according to the reports of Donaruma,[8,9] the condensation of sulfonamides with formaldehyde proceeds only under severe conditions (refluxing in aqueous hydrochloric acid for about 6—7 h). No appreciable signs of this reaction were observed during this microencapsulation process. A very small amount of product which did not dissolve in pH 1.2 dissolution medium was often obtained, but the product could not be confirmed to be the copolymer. The other sulfonamides, SI and SM, did not yield such a product under these microencapsulation conditions.



I II

The present study did not provide any indication that SA in microcapsule form provides prolonged or sustained blood levels as compared to those produced when the drug is administered in conventional powder form. Indeed, the bioavailability of the drug after administration of microcapsules appeared to be reduced compared to that of the powder form. SA microcapsules undoubtedly delayed the drug absorption, as shown by a lower absorption rate constant of 0.711 h^{-1} for MC-SA-20 compared with 1.34 h^{-1} for SA powder, but the mean blood levels of SA following microcapsules administration were consistently lower than those from the powder throughout the experiment. Thus, use of the microcapsules appears to be unattractive from a clinical viewpoint.

Administration Studies of Sulfisomidine (SI)

Mean blood unchanged SI levels for 72 h after the first dose of SI are shown in Fig. 3, although blood concentrations of unchanged SI were not measured between 24 and 48 h.

Peak blood levels were obtained within 1 h after oral ingestion of powder, and between 3 and 5 h after that of microcapsules except for microcapsules containing 10% SI (MC-SI-10). The peak blood concentration after microcapsule administration gradually increased on the first day with increasing content of SI in the microcapsules. MC-SI-10 produced lower concentrations throughout the experiment. However, fluctuations of blood concentration were less after administratoin. The area under the blood concentration curve (AUC) was estimated on the third day during a 12 h interval, and no significant difference was observed between the two preparations, powder and microcapsules, except for MC-SI-10. At a few blood concentration measurements, a statistically significant difference was observed between powder and microcapsules administration, as shown in Fig. 3, but no clear advantage of the microcapsules of SI over the powder could be seen.

Administration Studies of Sulfamethizole (SM)

The mean blood SM concentration–time curves for the microcapsules containing 20 and 30% SM (MC-SM-20 and MC-SM-30) are presented in Fig. 4. The results obtained for one-half the dose of SM powder and MC-SM-20 are shown in Fig. 5.

As shown in Figs. 4 and 5, the blood concentration of unchanged SM reached a peak 3—

4 h after oral administration of SM powder. On the other hand, the peak of unchanged SM concentration in blood appeared after 8—10 h for MC-SM-20 and MC-SM-30. Since the blood concentration after microcapsule administration increased and then decreased more gradually than that of SM powder, microencapsulated SM does seem to provide prolonged and sustained drug release. The design of prolonged and sustained-release formulations is based on the desirability of maintaining a relatively flat blood concentration-time curve. The use of a one-compartment model for estimation of single-dose blood concentrations may be generally adequate for the evaluation of microcapsule administration. A multiple dose equilibrium state such as that shown in Fig. 6 seems to indicate that microcapsule administration every 24 h to rabbits would be effective. Moreover, a characteristic of MC-SM-20 is that the

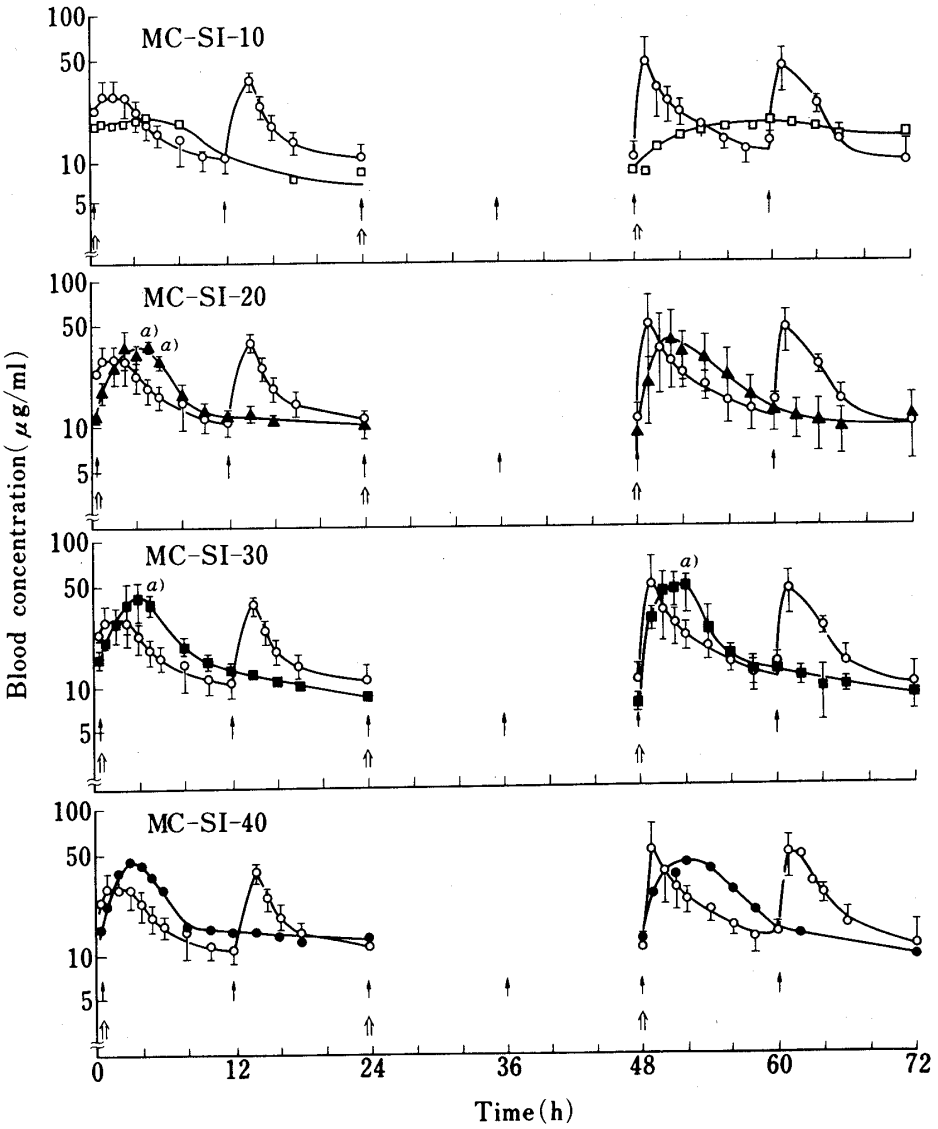


Fig. 3. Blood Concentration of Unchanged Sulfisomidine (SI) following Repeated Oral Administrations of Powder or Microcapsules of Sulfisomidine to Gastric-emptying-controlled Rabbits

—○—, SI powder (250 mg/kg; $n=3$), dosing every 12 h (↑).
—□—, MC-SI-10 (500 mg/kg; $n=1$), dosing every 24 h (⇑).
—▲—, MC-SI-20 (500 mg/kg; $n=3$), dosing every 24 h (⇑).
—■—, MC-SI-30 (500 mg/kg; $n=3$), dosing every 24 h (⇑).
—●—, MC-SI-40 (500 mg/kg; $n=1$), dosing every 24 h (⇑).
Concentrations, given as means±S.D., are significantly higher than the blood concentration after administration of powder *a*) at the 0.05 level.

fluctuations in SM concentration were smaller than with SM powder. Further studies using other drugs are desirable to investigate the applicability of the gelatin microcapsules prepared in this study.

Relation between *in Vitro* and *in Vivo* Studies

A considerable difference in *in vitro* dissolution pattern has been observed between

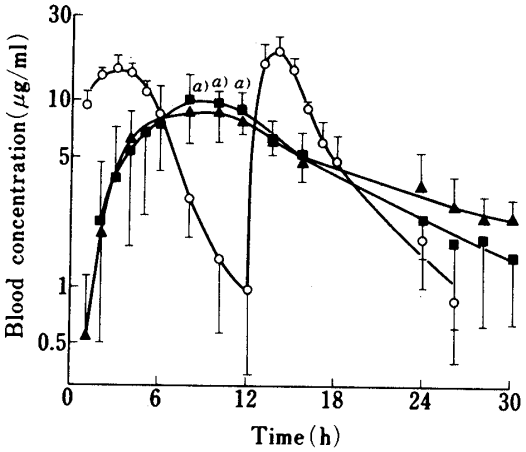


Fig. 4. Blood Concentration of Unchanged Sulfamethizole (SM) following Oral Administration of Powder or Microcapsules of Sulfamethizole to Gastric-emptying-controlled Rabbits

—○—, SM powder (250 mg/kg; $n=3$), dosing at 0 and 12 h.
—▲—, MC-SM-20 (500 mg/kg; $n=5$), dosing at 0 h.
—■—, MC-SM-30 (500 mg/kg; $n=5$), dosing at 0 h.
Concentrations, given as means ± S.D., are significantly higher than the blood concentration after administration of powder *a*) at the 0.05 level.

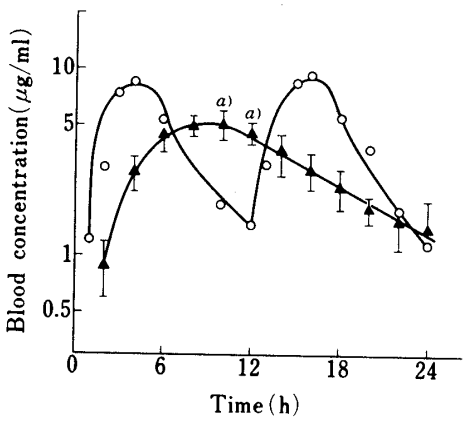


Fig. 5. Blood Concentration of Unchanged Sulfamethizole (SM) following Oral Administration of Powder or Microcapsules of Sulfamethizole to Gastric-emptying-controlled Rabbits

—○—, SM powder (125 mg/kg; $n=2$), dosing at 0 and 12 h.
—▲—, MC-SM-20 (250 mg/kg; $n=3$), dosing at 0 h.
Concentrations, given as means ± S.D., are significantly higher than the blood concentration after administration of powder *a*) at the 0.05 level.

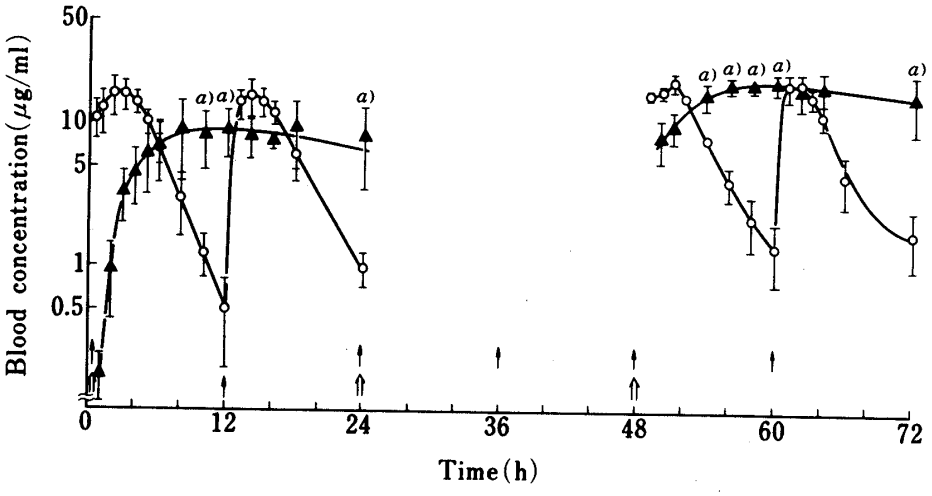


Fig. 6. Blood Concentration of Unchanged Sulfamethizole (SM) following Multiple Dosing of Powder or Microcapsules of Sulfamethizole to Gastric-emptying-controlled Rabbits

—○—, SM powder (250 mg/kg; $n=3$), dosing every 12 h (↑)
—▲—, MC-SM-20 (500 mg/kg; $n=3$), dosing every 24 h (⇧)
Concentrations, given as means ± S.D., are significantly higher than the blood concentration after administration of powder *a*) at the 0.05 level.

powder and microcapsules of sulfamethizole as compared with the cases of other sulfonamides.[16] It is considered that the *in vivo* result obtained after SM administration reflects the *in vitro* dissolution pattern of SM. However, further *in vivo* and *in vitro* studies using many kinds of drugs are necessary.

References and Notes

1) *a*) This paper forms part VII of a series entitled "Evaluation of Microcapsles"; *b*) The preceding paper, part VI: S. Goto, M. Komatsu, K. Tagawa and M. Kawata, *Chem. Pharm. Bull.*, **31**, 256 (1983).
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