Gelatin-Acacia Microcapsules for Trapping Micro Oil Droplets Containing Lipophilic Drugs and Ready Disintegration in the Gastrointestinal Tract

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Nonhardened gelatin-acacia microcapsules were studied for encapsulation of microdroplets of oil solution containing a lipophilic drug as core material and ready disintegration with release of micro oil droplets in the gastrointestinal tract. Probucol and S-312-d, a Ca-channel blocker, were employed as model lipophilic drugs. Glyceryl tricaprylate and tricaprate mixture solutions containing these drugs were encapsulated according to the complex coacervation method and were recovered as free-flowing powders without any hardening (cross-linking) step. The microcapsules obtained were disintegrated, and the emulsion was reproduced within 3 min at 37°C in the first or second test solution defined in the Japanese Pharmacopeia XII. When the microcapsules were stored as a powder at room temperature in a closed bottle, no significant change in their appearance or disintegration time upon rehydration was observed even after 1 year. Oral bioavailabilities of model drugs from the microcapsules were tested in rats and dogs and compared with those from other conventional formulations. Gastrointestinal absorption of both probucol and S-312-d from the microcapsules was remarkably more efficient than that from other formulations such as powders, granules, or oil solution. The proposed method for microencapsulation could be useful for powdering drug-containing oil solutions or O/W emulsions while maintaining excellent bioavailability.

KEY WORDS: nonhardened gelatin–acacia microcapsules; complex coacervation; bioavailability; lipophilic drugs; O/W emulsions.

INTRODUCTION

Pharmaceutical microencapsulation has been used for controlled (sustained) drug release (1–9), but it may also be useful for improving the bioavailability of lipophilic drugs. We prepared microcapsules capable of readily disintegrating and regenerating micro oil droplets containing lipophilic drugs in the gastrointestinal tract and then evaluated their usefulness.

The gelatin–acacia complex coacervation method employed in the present study can serve for microencapsulating oily materials. Typical examples of application are noncarbon paper (pressure-sensitive duplication paper) and liquid crystal thermometers. In the process of manufacturing these products, microcapsules are usually hardened with formaldehyde, glutaraldehyde, or tannin and used without any powdering steps. However, few pharmaceutical applications of microcapsules exist on a commercial basis, because of technical problems. In order to allow practical pharmaceutical application, the following requirements must be met: (i) removal of residual reagents in microcapsules, such as hardening agents (formaldehyde or glutaraldehyde) or organic solvents; (ii) reproducible control of disintegration or permeability; and (iii) recovery or collection of microcapsules as discrete, free-flowing powders.

In this study, we first produced gelatin–acacia microcapsules, capable of trapping micro oil droplets containing a lipophilic drug, as a powder without any hardening process, and then evaluated the usefulness of these microcapsules as a new oral dosage form designed for ready disintegration and reproduction of an emulsion in the gastrointestinal tract.

MATERIALS AND METHODS

Materials

The selected gelatin (from Miyagi Chemical Industry Co., Japan) had the following specifications: bloom, 300; viscosity, 60.9 mP; pH, 4.2; moisture, 9.9%; and isoelectric point, 9.0. Acacia was obtained from Gokyo Sangyo Co. Glyceryl tricaprylate and tricaprate mixture (ODO) were obtained from Toshin Chemical Co., Japan. Methyl *S*-(+)-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno[2,3-*b*]pyridine-5-carboxylate (S-312-d) and Carplex No. 80 were obtained from Shionogi & Co., Ltd. Probucol was purchased from Sigma Chemical Co., USA.

Microencapsulation

Probucol (4.0 g) or S-312-d (300 mg) was dissolved in ODO (30 g), then this solution was added to an aqueous solution of gelatin (10 g in 340 mL purified water) previously warmed to about 50°C in a 1-L beaker. The mixture was subjected to homogenization with a Silverson homogenizer (Silverson Machines Ltd., England). To the resultant emulsion was added an aqueous solution of acacia (10 g in 340 mL purified water) previously warmed to about 50°C. The mixture was adjusted to about pH 4.0 by adding 5% aqueous acetic acid solution with stirring. Next, the mixture was slowly cooled with stirring in an ice-water bath, during which 4.0 g of colloidal silica (Carplex No. 80) was added to obtain free-flowing, discrete microcapsules. When it had cooled to below 10°C, the stirring was stopped and the upper liquid was removed by decantation. The slurry of microcapsules obtained was dehydrated with 1 L of isopropanol, then the dehydrated microcapsules were dried on filter paper at room temperature for 2 days.

In Vitro Studies

Rehydration. The powdery microcapsules were dispersed in the first test solution (artificial gastric juice, pH 1.2) or the second test solution (artificial intestinal juice, pH 6.8) defined in the Japanese Pharmacopeia XII and observed with an optical microscope (Nikon AFM, Nihon Kogaku Ltd., Japan). The first test solution was prepared by mixing NaCl (2.0 g), diluted hydrochloride (24.0 mL), and water to

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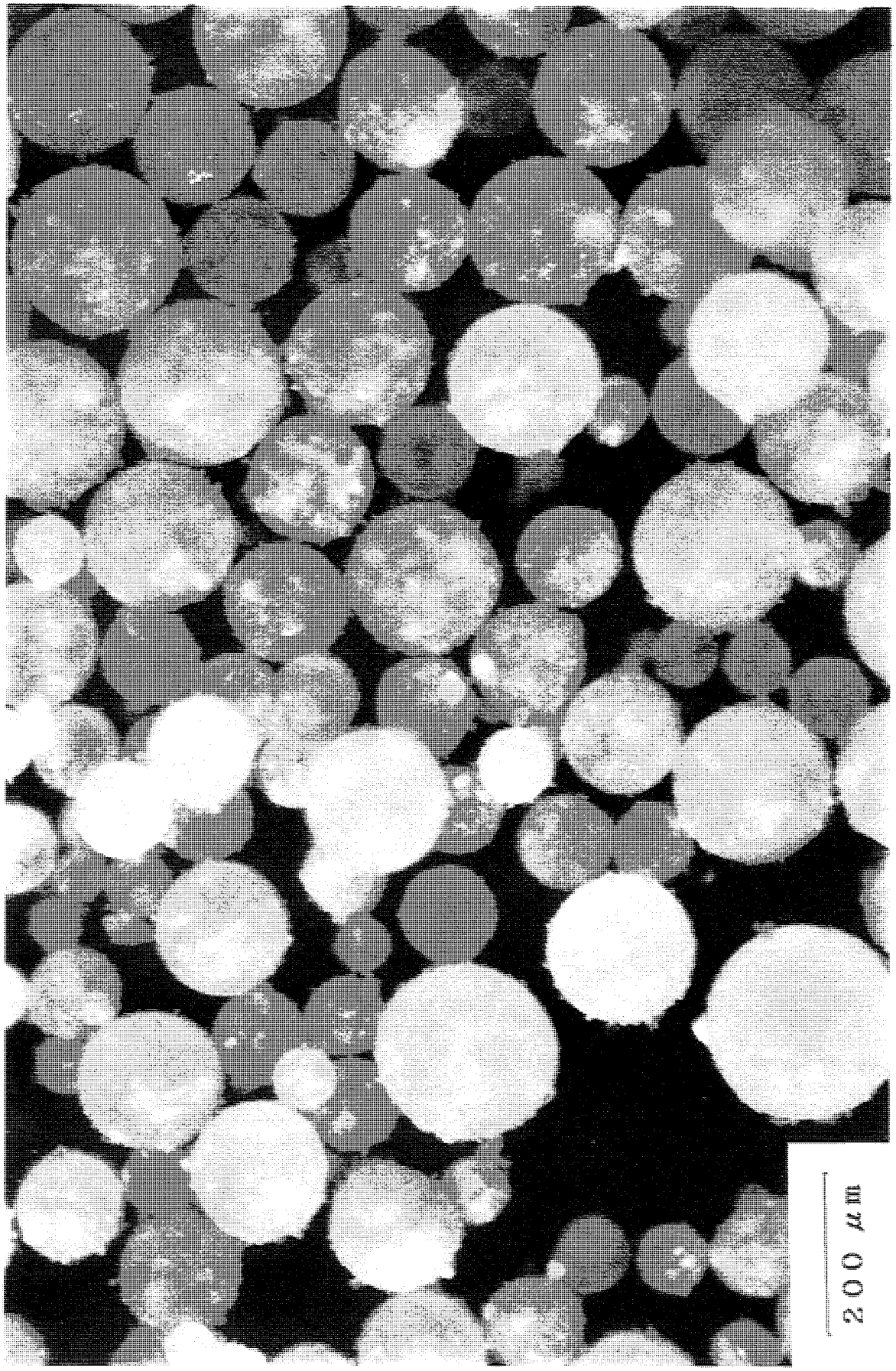

Fig. 1a. Microscopic photograph of powdered gelatin–acacia microcapsules.

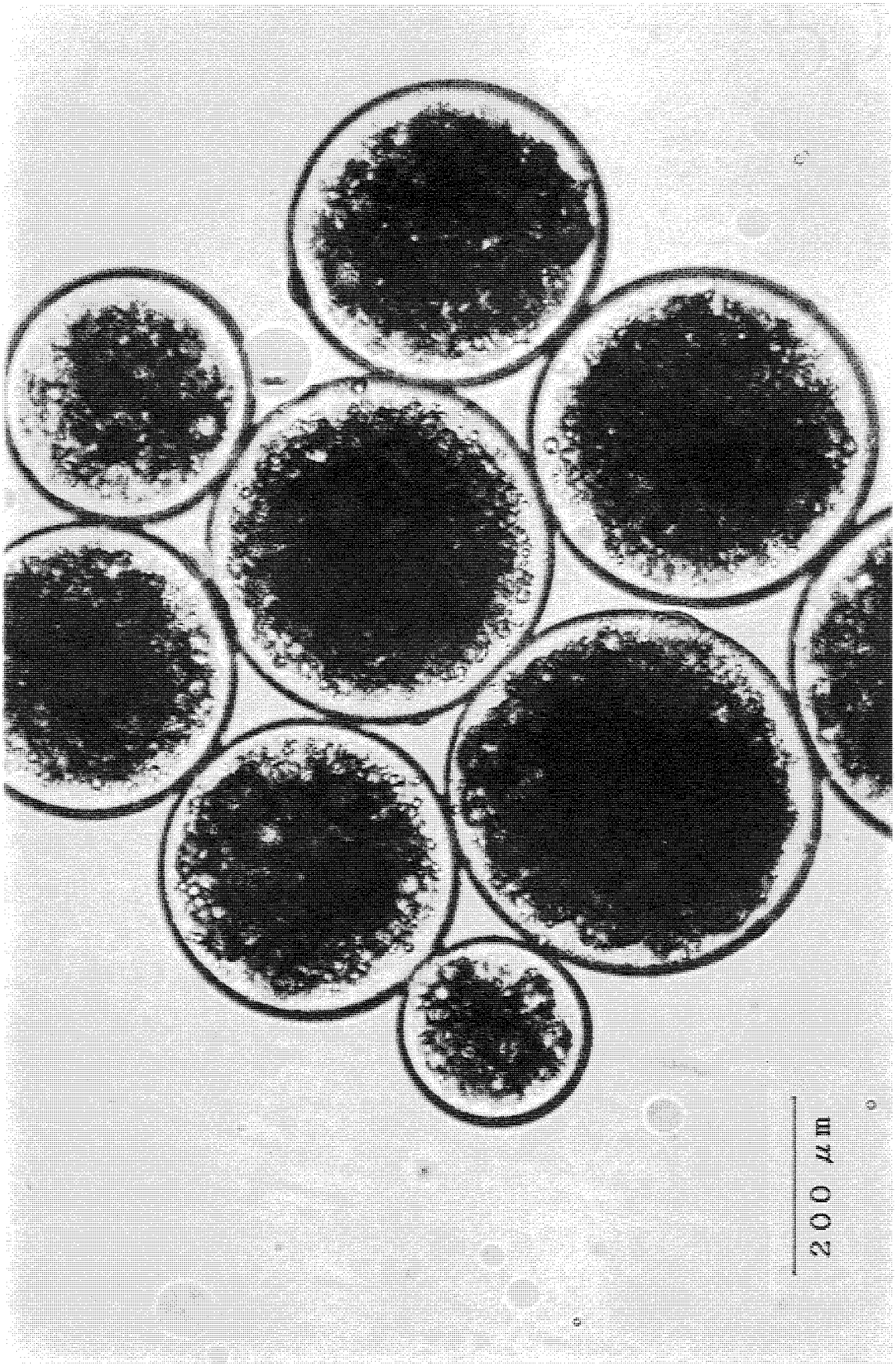

Fig. 1b. Microscopic photograph of microcapsules before dehydration or drying.

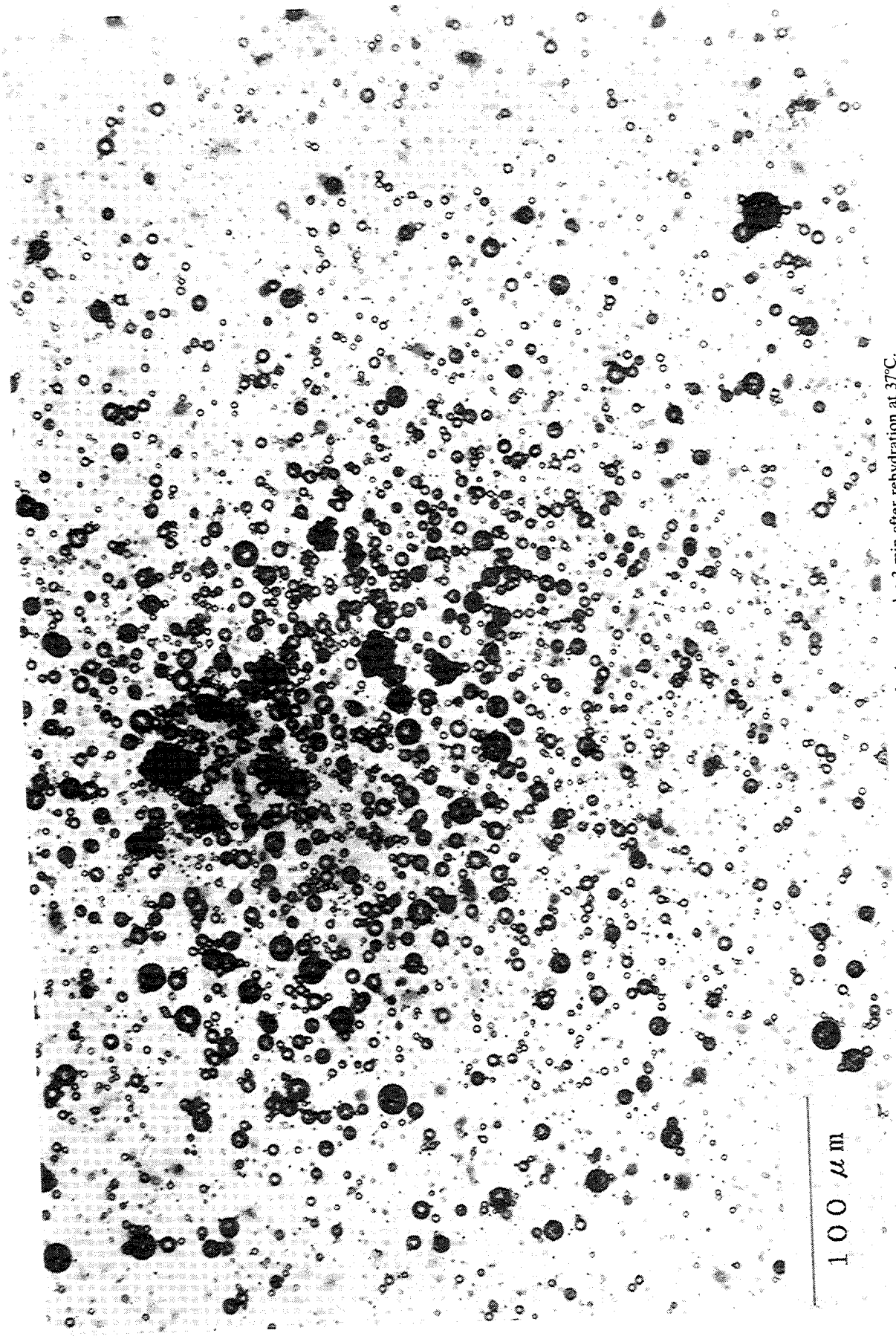

Fig. 1c. Microscopic photograph of microcapsules 3 min after rehydration at 37°C.

Table I. Disintegration Time and Appearance of Powdered Gelatin–Acacia Microcapsules (MC) After Preservation[a]

Conditions	1 month			1 year		
	Disintegration time upon rehydration (37°C)		Alteration in appearance of MC	Disintegration time upon rehydration (37°C)		Alteration in appearance of MC
	JP-1	JP-2		JP-1	JP-2	
40°C, RH = 0%	<3 min	<3 min	–	3–5 min	3–4 min	–
40°C, RH = 51%	<3 min	<3 min	–	4–5 min	4–6 min	–
40°C, RH = 75%	10 min	10 min	–	>4 hr	>4 hr	±
40°C, RH = 89%	1 hr	1 hr	–	>4 hr	>4 hr	+
40°C, closed	<3 min	<3 min	–	3–5 min	4–5 min	–
25°C, closed	<3 min	<3 min	–	<3 min	<3 min	–
4°C, closed	<3 min	<3 min	–	<3 min	<3 min	–
−20°C, closed	<3 min	<3 min	–	<3 min	<3 min	–

[a] JP-1 and JP-2 are the first and second solutions defined in the Japanese Pharmacopeia XII, respectively. (±) Very slight coloring; (+) faintly brown but more coloring than the class ±.

give a total volume of 1000 mL, and the second test solution by mixing 0.2 *M* dipotassium hydrogen phosphate (118 mL) and water to give a total volume of 1000 mL.

Preservation Test. The microcapsules in powder form were kept under various humidities (RH = 0, 51, 75, 89% at 40°C) and temperatures (−20, 4, 25, and 40°C in a closed container) in the dark and observed in terms of alteration in their appearance and disintegration time upon rehydration after storage.

In Vivo Studies

Microcapsules Encapsulating Probucol. Male Slc-Wistar rats (220–250 g) which had been kept overnight fasting or nonfasting were used (n = 4 or 5). The microcapsules were given orally, and absorption of probucol from the gastrointestinal tract was compared with that of other dosage forms including an ODO solution containing probucol (42 mg/g), an O/W emulsion [probucol, 5.0% (w/w); ODO, 30% (w/w); purified yolk lecithin, 3.6% (w/w)] prepared by microfluidization (Nanomizer LA-10H, Sayama Trading Co., Ltd., Tokyo), and granules containing 50% (w/w) probucol prepared from starch, lactose, and polyoxyethylenesorbitan fatty acid ester in the usual manner. Each formulation was administered orally at a dose of 20 mg/kg as probucol under light anesthesia with ether. The microcapsules and the granules were given orally via a soft polyethylene tubing with 1 mL of water, and the emulsion and the ODO solution were administered via a metallic gastric tube with 1 mL of water as described above. At indicated time intervals after administration, blood samples were collected from the tail veins of the rats under light anesthesia with ether.

Microcapsules Encapsulating S-312-d. The same group of male beagle dogs (Shionogi Aburahi Farm; mean body weight, 10.2 kg; n = 6) which had been kept fasting for 24 hr were used at 1-week intervals for the experiments. The absorption from the gastrointestinal tract was compared with that of other dosage forms, i.e., an ODO solution containing S-312-d (0.30 g/30 g) and powders diluted 10 times with lactose. All these preparations were placed in hard capsules (Torpac Ltd., size No. 13) and administered at a dose of 10 mg/dog as S-312-d. Blood samples were collected from the vein of foreleg.

Analytical Method

The plasma concentration of probucol (10) was determined by HPLC with a UV detector (at 242 nm). To 200 μL of plasma, 2 mL of ethyl alcohol containing probucol analogue [2-pentanone-bis(3,5-di-t-butyl-4-hydroxyphenyl)-mercaptol] was added as an internal standard. After centrifugation (10 min, 3000*g*), the upper layer was decanted into fresh tubes. The residue was redissolved in 200 μL of methanol and 100 μL of the solution was injected into an HPLC apparatus (Shimadzu LC-6A, Kyoto, Japan) equipped with an ultraviolet detector (Shimadzu SPD-6A), integrator (Shimadzu C-R 6A), and reversed-phase octadecyl silica column (Cosmosil $5C_{18}$, 150 × 4.6 mm). The mobile phase employed was acetonitrile–water (92:8).

The plasma concentration of S-312-d was determined by HPLC with electrochemical detection according to the method of Ueno *et al.* (11). The content of isopropanol in microcapsules was determined using *n*-propanol as an internal standard by gas chromatography after rehydration and disintegration of the microcapsules at 50°C and addition of an equal volume of acetone to the resultant aqueous solution. The gas chromatograph apparatus was a Shimazu GA-7A (Kyoto, Japan) equipped with a column (Chromosorb M with 20% 20 *M* PEG; 3 mmϕ × 2.1 m; 60–80 mesh), the carrier gas was He at 40 mL/min, and the column temperature was 60°C.

RESULTS AND DISCUSSION

Gelatin–acacia microcapsules trapping microdroplets of oil solution containing a lipophilic drug, probucol or S-312-d,

Table II. Stability of Probucol Trapped in Microcapsules

Preservation period[a]	Content (mg/g)	% of initial
Initial	72.5	100
3 months	70.6	97
11 months	71.4	99

[a] Microcapsules were preserved at room temperature in a closed container in the dark.

Table III. Characteristics of Microcapsules (MC) Evaluated *in Vivo*

	Drug content (mg/g MC)	Yield (%)	Particle size (μm)	Bulk density (g/cm^3)	Repose angle (deg)
Probucol	71.8	98	130–200	0.53	19
S-312-d	5.1	90	100–250	0.51	27

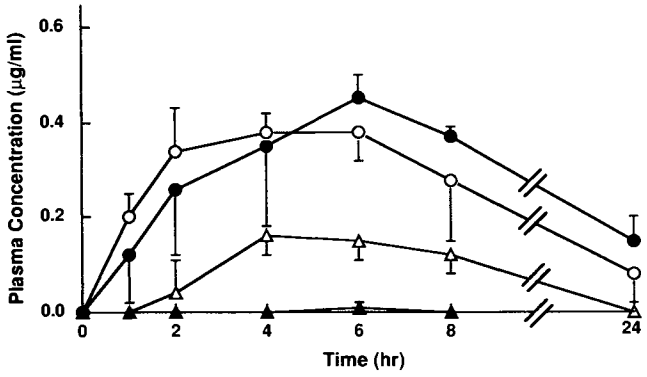

Fig. 2. Plasma probucol concentrations after oral administration of the microcapsules and other dosage forms containing probucol to fasting rats. (○) Microcapsules; (●) emulsion (O/W); (△) ODO solution; (▲) granules.

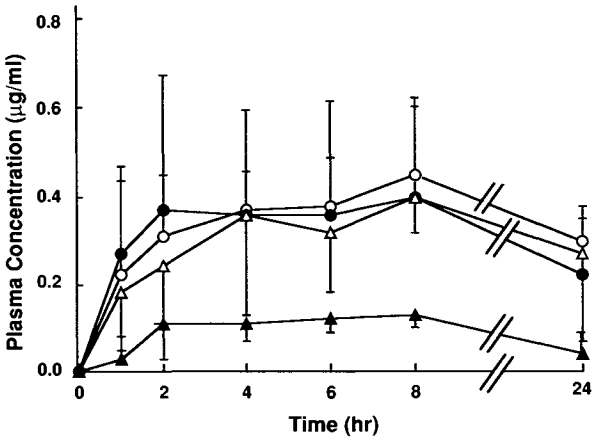

Fig. 3. Plasma probucol concentrations following oral administration of the microcapsules and other dosage forms containing probucol to nonfasting rats. (○) Microcapsules; (●) emulsion (O/W); (△) ODO solution; (▲) granules

Table IV. Comparison of Bioavailability Parameters Among Microcapsules and Other Dosage Forms of Probucol in Fasting Rats (Dose: 20 mg/kg)[a]

Dosage form	*N*	C_{max} (μg/mL)	T_{max} (hr)	$AUC_{0-24\ hr}$ (μg · hr/mL)
Microcapsules	4	0.41 ± 0.03	5.5 ± 1.9	5.40 ± 1.46
Emulsion (o/w)	4	0.46 ± 0.06	5.5 ± 1.0	6.64 ± 0.49
ODO solution	4	0.17 ± 0.04	4.5 ± 1.0	1.79 ± 0.58
Granules	4	0.01 ± 0.01	6.0	0.04 ± 0.03

[a] Means ± SD.

Table V. Comparison of Bioavailability Parameters Among Microcapsules and Other Dosage Forms of Probucol in Nonfasting Rats (Dose: 20 mg/kg)[a]

Dosage form	*N*	C_{max} (μg/mL)	T_{max} (hr)	$AUC_{0-24\ hr}$ (μg · hr/mL)
Microcapsules	5	0.53 ± 0.11	5.4 ± 3.0	8.66 ± 1.14
Emulsion (o/w)	4	0.44 ± 0.28	6.5 ± 3.0	7.68 ± 4.80
ODO solution	5	0.48 ± 0.08	5.6 ± 2.2	7.66 ± 2.14
Granules	4	0.16 ± 0.02	5.0 ± 3.5	2.21 ± 0.79

[a] Means ± SD.

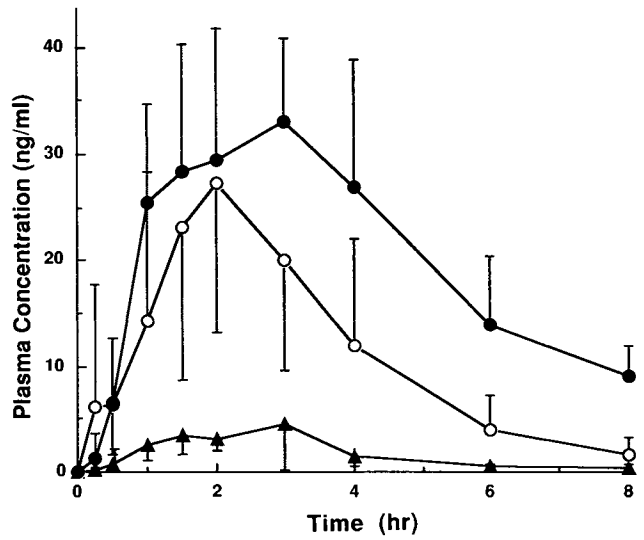

Fig. 4. Plasma S-312-d concentrations after oral administration of the microcapsules and other dosage forms containing S-312-d to fasting dogs. (●) Microcapsules; (○) ODO solution; (▲) powders diluted 10 times with lactose.

as core materials were prepared according to the complex coacervation method without the hardening step to allow ready disintegration in the gastrointestinal tract. They were collected as discrete, free-flowing powders by a dehydration procedure using isopropanol after the addition of colloidal silica (Carplex No. 80). The obtained microcapsules were evaluated by *in vitro* and *in vivo* studies as described below.

In Vitro Studies

Rehydration of the Powdered Microcapsules. When the powdered microcapsules (Fig. 1a) were added to the first or the second test solution under stirring, optical microscopy at room temperature revealed that the microcapsules swelled with hydration and returned almost to the initial state, that is, the microcapsule suspension before dehydration or drying (Fig. 1b). At 37°C, the coating layer disintegrated within 3 min and the micro oil droplets were regenerated (Fig. 1c). This observation suggests that the microencapsulation method proposed in this study allows conversion of the emulsion to a powdered form followed by ready regeneration upon rehydration at 37°C.

Preservation Test. The powdered microcapsules were preserved for 1 year under various humidity and temperature conditions, and their stabilities were evaluated in terms of appearance and time required for disintegration of the microcapsular wall upon rehydration (Table I). Under high humidity at high temperature (RH = 75 or 89% at 40°C), the disintegration time was markedly prolonged and slight coloration was observed on the microcapsular wall, but under the other conditions tested, no significant alteration was observed. This is interpreted by speculation that the high moisture content in microcapsule shells induces denaturation or cross-linking and transforms the gelatin into an insoluble gel structure at high temperatures (12). These results indicate that the microcapsules remain stable as a powder for at least 1 year if they are stored at room temperature in a closed container. Although the microcapsules shown in Fig. 1 and Table I contained no drug, similar characteristics of disintegration or stabilities were observed in the case of microcapsules containing a drug. Table II indicates that the stability of probucol is not affected by microencapsulation and it can be preserved for a long time in this form.

In Vivo Studies

The characteristics of the microcapsules used in *in vivo* evaluations are shown in Table III. This table shows that both microcapsules have similar properties except for drug content and are free-flowing powders. The small amount of S-312-d in microcapsules is due to its poor solubility in ODO. The content of isopropanol in these microcapsules was less than 0.002%.

Microcapsules of Probucol. The microcapsules were orally given to rats, and the gastrointestinal absorption of probucol was compared with that of the other dosage forms (Figs. 2 and 3, Tables IV and V). These results indicate that the microcapsules have dramatically more effective bioavailability than the granules and are more effective than the ODO solution under fasting conditions. Also, the bioavailability for the microcapsules is almost equal to that for the emulsion. The latter results mean that the microcapsules readily disintegrate in the gastrointestinal tract to regenerate the O/W emulsion.

Under nonfasting conditions, the $AUC_{0-24\ hr}$ of the ODO solution was close to those for the O/W emulsion and microcapsules. This is considered to be due to emulsification of the ODO solution by food components. However, the $AUC_{0-24\ hr}$ for the granules was still lower than that for the microcapsules.

Microcapsules of S-312-d. The microcapsules were orally administered to beagle dogs and the absorption of S-312-d was compared with the other dosage forms. The results are shown in Fig. 4 and Table VI. For S-312-d, it was also confirmed that the microcapsules have a bioavailability

Table VI. Comparison of Bioavailability Parameters Among Microcapsules and Other Dosage Forms of S-312-d in Fasting Beagle Dogs (Dose: 10 mg/Dog)[a]

Dosage form	*N*	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0-8\ hr}$ (ng · hr/mL)
Microcapsules	6	40.1 ± 7.3	2.4 ± 0.9	162.1 ± 27.8
ODO solution	6	32.0 ± 10.1	1.6 ± 0.7	90.7 ± 45.8
Powders (diluted with lactose)	6	6.0 ± 3.8	2.2 ± 0.9	13.6 ± 4.3

[a] Means ± SD.

that is remarkably higher than that of the powders diluted 10 times with lactose and relatively higher than that of the ODO solution.

CONCLUSION

From these results, we concluded the following.

1. Microencapsulation by the gelatin–acacia complex coacervation method without the hardening (cross-linking) step permits conversion of an O/W emulsion or oily drug (solution) into a nonsticky powder.

2. The present microcapsules readily disintegrate and regenerate O/W emulsion (in other words, release micro oil droplets) in the gastrointestinal tract.

3. The proposed microcapsules can offer a higher bioavailability than conventional dosage forms, such as powders, granules, tablets, and oil solution, not only for the model drugs used in this study but also for other water-insoluble drugs.

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