Preparation of Egg Albumin Microcapsules and Microspheres

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Abstract □ Protein microspheres and microcapsules containing fine hydrophilic particles (spherical silica gel beads, iron hydrous oxide-modified nylon 12, or phenacetin) were prepared by using water-soluble egg albumin, which is heat-coagulable at 50–80°. The size and size distribution of the microspheres and microcapsules formed and the microencapsulation percentage of fine particles were determined, and the factors affecting them were studied. Favorable conditions are suggested to obtain phenacetin-containing microcapsules in high yield.

Keyphrases □ Albumin, egg—preparation of microcapsules and microspheres containing fine hydrophilic particles of silica gel beads, iron hydrous oxide-modified nylon 12, and phenacetin, heat coagulation method □ Silica gel, spherical beads—preparation of microcapsules and microspheres of silica gel beads using egg albumin, heat coagulation method □ Phenacetin—preparation of microcapsules and microspheres of phenacetin using egg albumin, heat coagulation method □ Microencapsulation—symposium, preparation of microcapsules and microspheres containing fine hydrophilic particles of silica gel beads, modified nylon 12, and phenacetin using water-soluble egg albumin, heat coagulation method, evaluation of factors affecting size, size distribution, and percent of microencapsulated particles

Interest in the application of microcapsules to many industrial fields is increasing, and application to the modification of drugs has been studied, but many problems remain. Nixon and Walker (1) reported the release of sulfadiazine from microcapsules prepared by simple gelatin coacervation, and Luzzi *et al.* (2) investigated the prolonged release of nylon microcapsules prepared by emulsion polymerization. Zolle *et al.* (3) prepared radiolabeled albumin microspheres to study blood circulation in humans and animals.

Sugibayashi *et al.* (4) studied the drug-carrier property of albumin microspheres in chemotherapy and the preparation and tissue distribution of microsphere-entrapped [6-^{3}H]fluorouracil in mice. Kramer (5) investigated albumin microspheres containing an anticancer agent as vehicles for achieving specificity in drug delivery.

Widder *et al.* (6) reported the preparation and characterization of a novel delivery system for water-soluble drugs. The system consisted of albumin microspheres (average of 1 μm in diameter) containing ultrafine magnetic particles, magnetite (ferrosoferric oxide), and a prototype drug, doxorubicin hydrochloride.

This paper reports the microencapsulation of fine hydrophilic particles and drug using a metabolizable protein, albumin, as the membrane material.

EXPERIMENTAL

Materials—Isooctane, dibasic potassium phosphate, monobasic sodium phosphate dodecahydrate, acetic acid, and sodium acetate were reagent grade. Phenacetin JP[1] (*p*-acetophenetidide) was ground with an agate mortar and then classified with stainless steel sieves. Powder, 250–300 mesh (specific gravity 1.275), was used for microencapsulation.

Egg albumin[2] was used following some purification. Albumin was dissolved in pH 8.0, 0.1 *N* KH_2PO_4–0.1 *N* Na_2HPO_4 (1:16 v/v). The solution was filtered[3] after centrifugation at 16,000×*g* for 30 min to remove undissolved material, and the solution concentrations were ~5, 10, and 20% (w/w).

Spherical porous silica gel beads[4] [Brunauer, Emmett, and Teller (BET) specific surface area, 420 m^2/g; mean pore size, 80 Å; specific gravity, 2.127; and mean diameter, 52.5 ± 12.7 μm] and nylon 12 spheres[5] (BET specific surface area, 1.0 m^2/g; specific gravity, 1.02; and mean diameter, 3 μm) modified with iron hydrous oxide also were used as core materials. Iron hydrous oxide (iron hydrosol) was used to modify the surface of the nylon 12 spheres to enhance their wettability by aqueous albumin solution. Iron hydrous oxide settles from solution onto such hydrophobic surfaces as those of polyethylene, polytef, and paraffin, rendering the surfaces hydrophilic (7). Once deposited and dried, these colloid particles have a high degree of adherence to the substrate surface.

Preparation of Iron Hydrous Oxide Solution—Twenty-five milliliters of an aqueous 10% (w/w) ferric chloride solution was added to 200 ml of boiling water, and boiling was continued for 3 min. After cooling to room temperature, the solution was treated with 40 ml of an anion-exchange resin[6] and stirred for 5 min. The iron hydrous oxide solution obtained was used for surface modification of nylon 12 spheres.

Preparation of Microcapsules and Microspheres—Albumin microcapsules and microspheres were prepared by a modification of the method used by Farhadieh (8).

To 100 ml of isooctane containing 0.1, 0.5, 1.0, or 5.0% (v/v) sorbitan trioleate as an emulsifier in a three-necked flask was added, with stirring, 15 ml of 5, 10, or 20% (w/w) egg albumin solution or the albumin solution containing solid powders [silica gel beads (1.063, 2.126, or 3.190 g)], iron hydrous oxide-modified nylon 12 (2.250 g), and phenacetin (1.912, 2.854, or 3.825 g) as the core materials. After 10 min with continuous stirring, the flask was immersed in a water bath at 50° for 5 min and then at 75–85° for 10 min to denature the egg albumin. The resultant dispersion was cooled to room temperature.

The particles separated by decantation were mixed with 100 ml of isooctane, and this mixture was agitated gently for 1 hr. The separated particles again were dispersed into 10 ml of pH 4.7 acetate buffer solution,

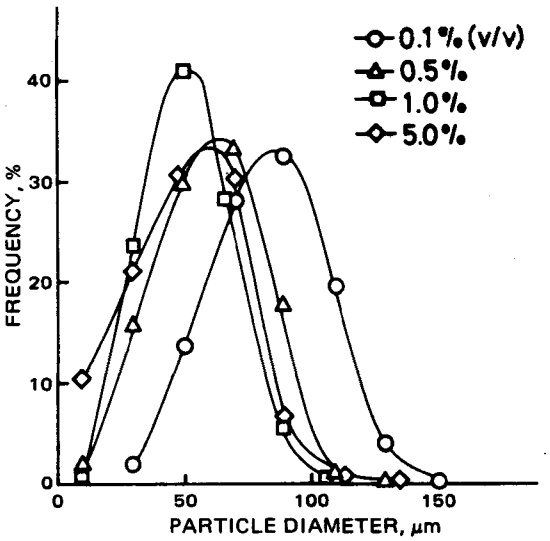

Figure 1—*Effect of sorbitan trioleate concentration on size distribution of albumin microspheres dispersed in isooctane. The albumin concentration was 10% (w/w) at a speed setting of 512 rpm.*

[1] Hoei Pharmaceutical Co., Tokyo, Japan.
[2] Tokyo Kasei Kogyo Co., Tokyo, Japan.
[3] No. 7 paper filter, Toyo Roshi Kaisha, Tokyo, Japan.
[4] Iatrobeads, Iatron Laboratories, Tokyo, Japan.
[5] Toyo Rayon Co., Tokyo, Japan.
[6] Amberlite IRA-410, Japan Organo Co., Tokyo, Japan.

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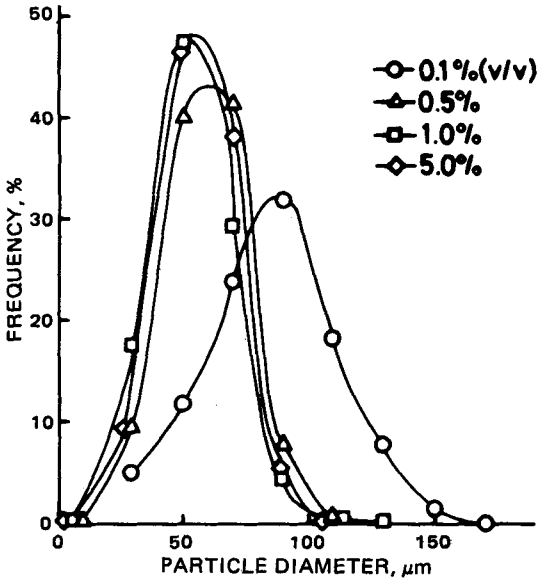

Figure 2—*Effect of sorbitan trioleate concentration on size distribution of albumin microspheres dispersed in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The albumin concentration was 10% (w/w) at a speed setting of 512 rpm.*

consisting of 0.1 *N* acetic acid–0.1 *N* sodium acetate (1:1 v/v) and 10% (v/v) polyoxyethylene sorbitan monolaurate, with vigorous agitation. Then the dispersion was diluted with the same buffer solution to make the concentration of polyoxyethylene sorbitan monolaurate 2.0% (v/v).

The total numbers of microcapsules and microspheres ranging from 1000 to 2500 were measured by projecting their photomicrographs on a screen. Size measurements were performed in isooctane and then in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution after treating the capsules with acetate buffer. Each film strip was projected onto a large section of paper by a slide projector[7], and the enlarged images of the microcapsules and microspheres were measured to the nearest 1.0 μm. The scales in the micrometer were used for calibration. If the contour of projected images was of a complex or indistinct shape, these images were not measured. Then calculations were made on the mean length diameter, size distribution, and standard deviation.

Measurements of Viscosity and Interfacial Tension—Relative viscosity of albumin solutions was measured at 25° with an Ubbelohde viscosimeter. The pendant drop method (9) was used for measuring the interfacial tension between albumin solution and 5.0% (v/v) sorbitan trioleate solution in isooctane (10).

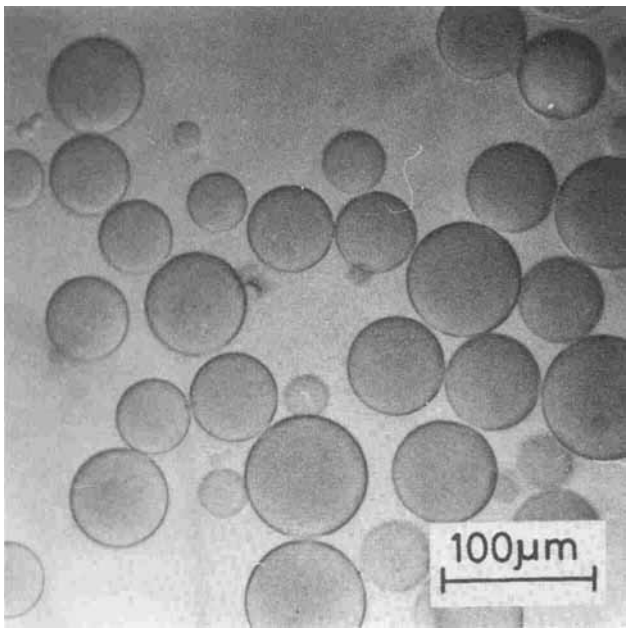

Figure 3—*Photomicrograph of albumin microspheres in isooctane.*

[7] Color Cabin, Cabin Industry Co., Tokyo, Japan.

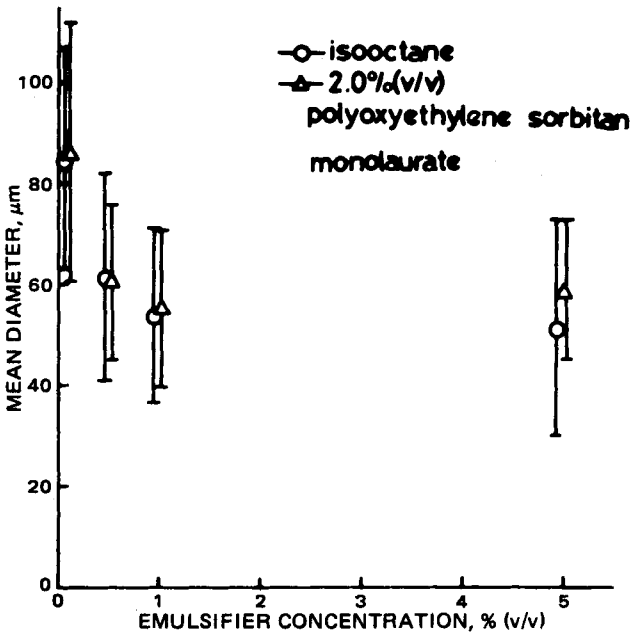

Figure 4—*Effect of sorbitan trioleate concentration on the mean diameter of albumin microspheres dispersed in isooctane and in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The albumin concentration was 10% (w/w) at a speed setting of 512 rpm.*

RESULTS AND DISCUSSION

Size Distribution and Mean Size—The size distribution of the albumin microspheres was strongly affected by the sorbitan trioleate concentration, mechanical agitation, and albumin concentration in the first step of the preparation.

Figures 1 and 2 show the effect of the sorbitan trioleate concentration

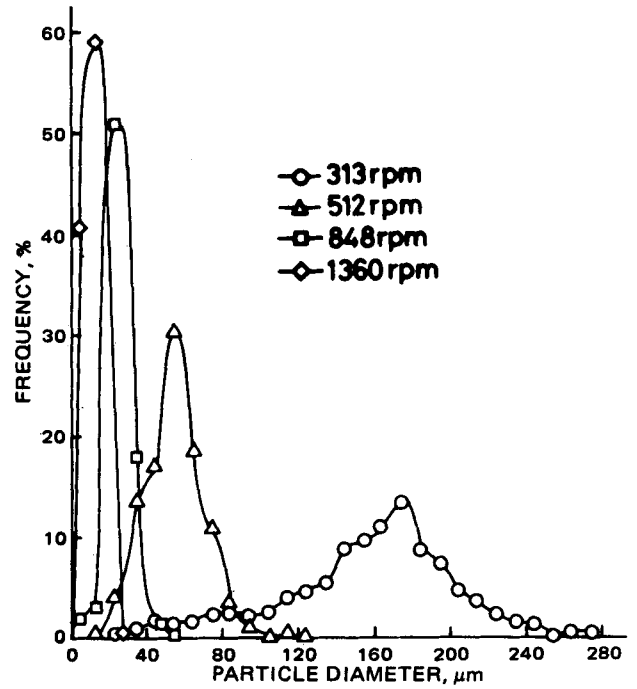

Figure 5—*Effect of stirring speed on size distribution of albumin microspheres dispersed in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The albumin concentration was 10% (w/w), and the emulsifier was 1.0% (v/v) sorbitan trioleate.*

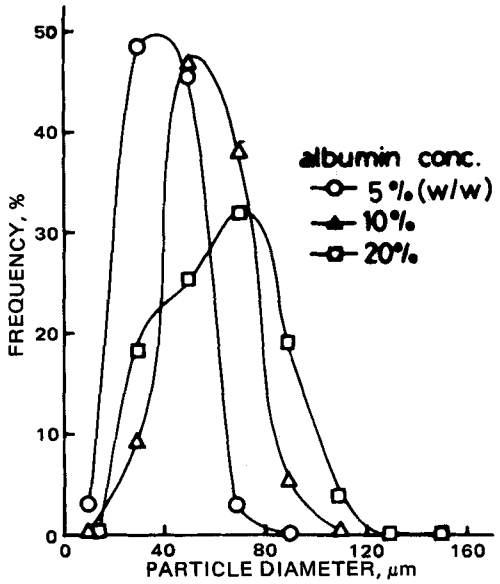

Figure 6—*Effect of albumin concentration on size distribution of microspheres dispersed in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The emulsifier was 1.0% (v/v) sorbitan trioleate at a speed setting of 512 rpm.*

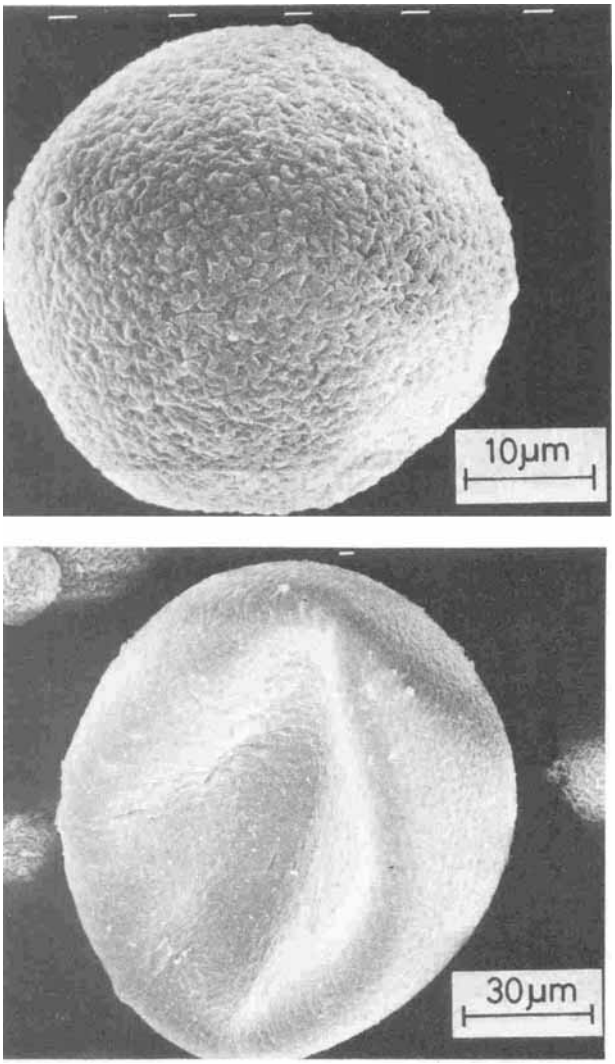

Figure 7—*Scanning electron micrographs of albumin microcapsules containing phenacetin.*

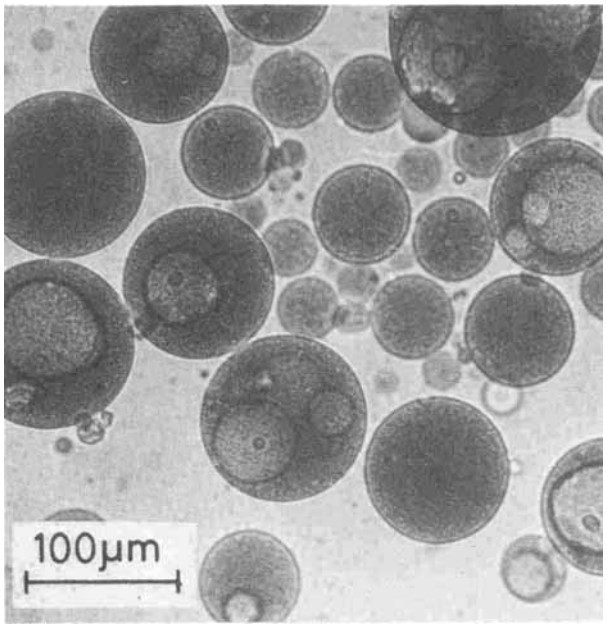

Figure 8—*Photomicrograph of albumin microcapsules containing silica gel beads in isooctane.*

on the size distribution of the microspheres prepared from 10% (w/w) albumin solution at 512 rpm. The size distribution curve, obtained from the photographs taken in isooctane and in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution, distinctly shifted toward the lesser particle diameter with increasing sorbitan trioleate concentration up to 0.5%, above which no appreciable shift was observed. Typical albumin microspheres dispersed in isooctane are shown in Fig. 3.

Figure 4 shows the relationship of the mean length diameter and its standard deviation of albumin microspheres prepared at different sorbitan trioleate concentrations. A decrease in the mean diameter was apparent with increasing sorbitan trioleate concentration. There are no observable differences in diameter between those measured in isooctane and in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The results indicate that albumin microspheres were formed tightly by heat denaturation and, thus, the spheres did not swell in the surfactant solution.

Figure 5 shows the size distributions of the microspheres in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution prepared at various stirring speeds. The size distribution curve clearly became narrower and

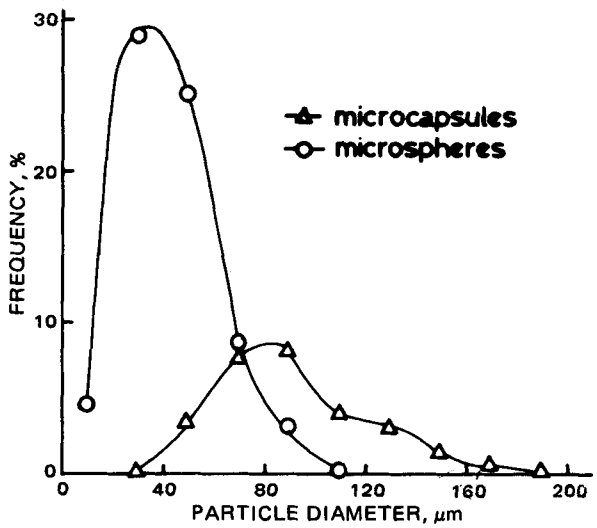

Figure 9—*Size distribution of albumin microcapsules containing silica gel beads dispersed in isooctane. The volume ratio of silica gel beads to albumin solution was 1:9, and the weight of beads was 3.190 g. The albumin concentration was 10% (w/w), and the emulsifier was 1.0% (v/v) sorbitan trioleate. The speed setting was at 512 rpm. The microspheres contained no silica gel beads.*

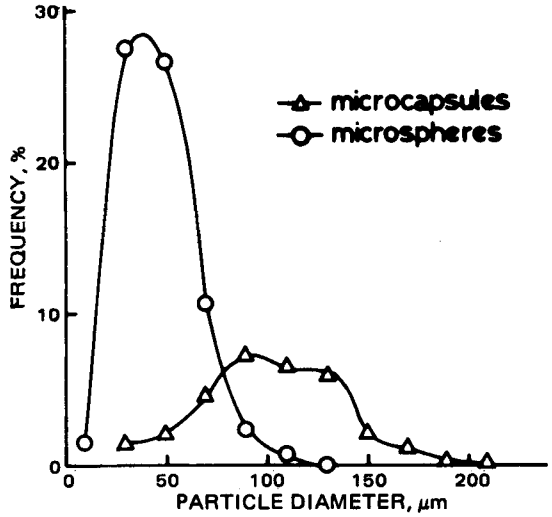

Figure 10—*Size distribution of albumin microcapsules containing silica gel beads dispersed in isooctane. The volume ratio of silica gel beads to albumin solution was 1:14, and the weight of beads was 2.126 g. The albumin concentration was 20% (w/w), and the emulsifier was 1.0% (v/v) sorbitan trioleate. The speed setting was at 512 rpm. The microspheres contained no silica gel beads.*

sharper with increased stirring speed. Similar results were reported by Koishi *et al.* (11) on the size of polyphthalamide microcapsules prepared by the interfacial polycondensation method. The size distribution curve of albumin microspheres in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution prepared using 5,10, and 20% (w/w) albumin solutions clearly became broader with increasing albumin concentration (Fig. 6).

These results indicate that the size distribution of the microspheres depends mainly on the size distribution of the droplets of albumin dispersed in isooctane during preparation. The changes in the dispersed phase caused by an increase in albumin concentration, interfacial tension between dispersed phase (albumin solution) and dispersing medium (sorbitan trioleate solution in isooctane), and viscosity of albumin solution seem to influence the size distribution of the albumin droplets. The results on the interfacial tension and relative viscosity of the albumin solution (Table I) show that the increase in viscosity of the dispersed phase is certainly a major influence on the size distribution. Albumin microspheres prepared using 20% (w/w) albumin solution (Fig. 6) had an unsymmetrical size distribution. Namely, subdivision of globules of 20% (w/w) albumin, dispersed in isooctane, into many smaller sizes is prevented by the large increase in the resistance to deformation of the globules brought about by high interfacial viscosity due to the high relative viscosity of the protein solution (Table I).

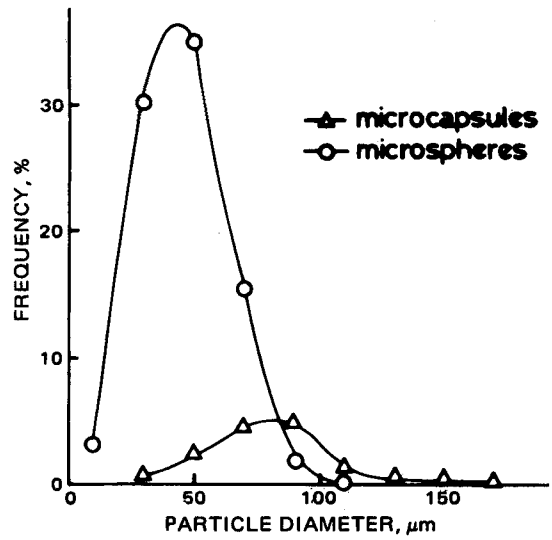

Figure 11—*Size distribution of albumin microcapsules containing silica gel beads dispersed in isooctane. The volume ratio of silica gel beads to albumin solution was 1:14, and the weight of beads was 2.126 g. The albumin concentration was 20% (w/w), and the emulsifier was 1.0% (v/v) sorbitan trioleate. The speed setting was at 848 rpm. The microspheres contained no silica gel beads.*

Table I—Interfacial Tensions between Albumin Solutions and 5.0% (v/v) Sorbitan Trioleate Solution in Isooctane and the Viscosities of Albumin Solutions

Albumin, % (w/w)	Relative Interfacial Tension[a]	Relative Viscosity
0	0.051	1.02
5	0.024	1.43
10	0.026	2.07
20	0.027	5.00

[a] The ratio of the interfacial tension between two phases to the surface tension of water.

Microencapsulation of Powder Particles—Figure 7 show scanning-electron micrographs of the surface appearances of albumin mi-

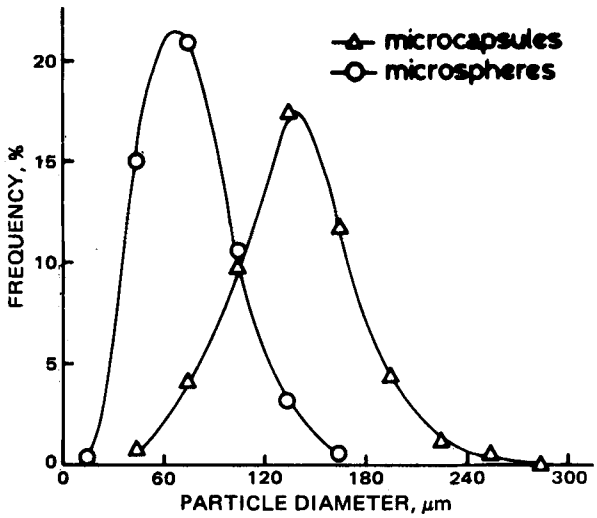

Figure 12—*Size distribution of albumin microcapsules containing silica gel beads dispersed in isooctane, prepared at the volume ratio of silica gel beads to albumin solution of 1:29, and the weight of beads was 1.063 g. The albumin concentration was 20% (w/w), and the emulsifier was 1.0% (v/v) sorbitan trioleate. The speed setting was at 512 rpm. The microspheres contained no silica gel beads.*

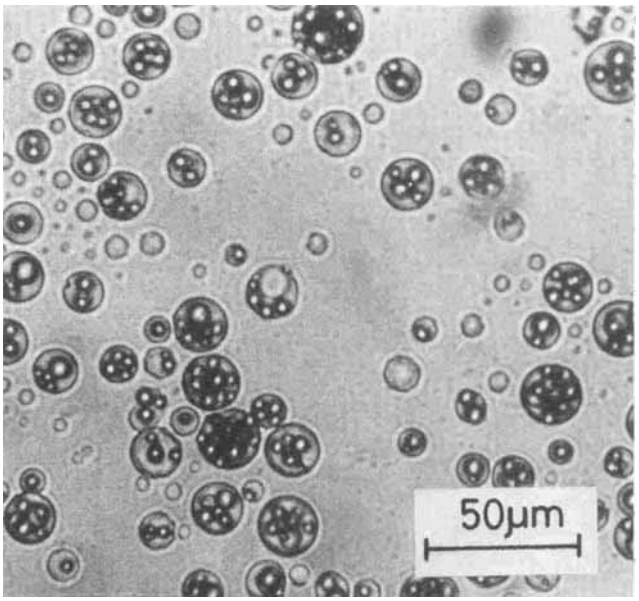

Figure 13—*Photomicrograph of albumin microcapsules containing iron hydrous oxide-modified nylon 12 particles in isooctane.*

Table II—Mean Diameter (d), Standard Deviation (δ), and Microencapsulation Percentage (P_m) of Albumin Microcapsules Dispersed in Isooctane, Prepared by Using Silica Gel Beads as a Core Material

Albumin, % (w/w)	Volume Ratio[a]	Weight of Silica Gel Beads Used, g	Speed Setting, rpm	Sorbitan Trioleate, % (v/v)	Microcapsule Containing Silica Gel Beads			Microcapsule Containing No Silica Gel Beads			Mean Value[b]		P_m[c], %
					d, μm	δ, μm	Number, M_1	d, μm	δ, μm	Number, M_2	d, μm	δ, μm	
10	1:9	3.190	512	1.0	92.1	30.2	288	43.3	17.8	705	57.5	31.2	29.0
20	1:14	2.126	512	1.0	101.6	33.6	329	46.3	17.6	749	63.2	34.8	30.5
20	1:14	2.126	848	1.0	79.1	24.4	353	46.0	16.5	2146	50.7	21.2	14.1
20	1:29	1.063	512	1.0	138.7	37.2	950	76.2	26.8	967	107.2	45.0	49.6

[a] Silica gel beads to albumin solution. [b] Calculated from total size distribution. [c] Microencapsulation percentage: $M_1/(M_1 + M_2) \times 100$.

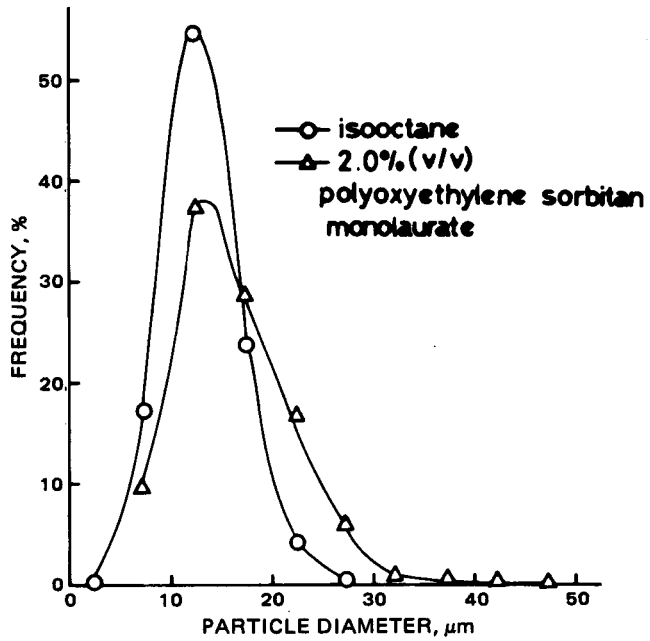

Figure 14—*Size distribution of albumin microcapsules containing iron hydrous oxide-modified nylon 12 particles prepared at the volume ratio of iron hydrous oxide-modified nylon 12 to albumin solution of 1:5.6, and the weight of beads was 2.250 g. Microcapsules were dispersed in isooctane or 2.0% (v/v) polyoxyethylene sorbitan monolaurate. The albumin concentration was 10% (w/w), and the emulsifier was 5.0% (v/v) sorbitan trioleate. The speed setting was at 1360 rpm.*

crocapsules containing fine phenacetin particles. After the microcapsules were suspended in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution, the suspension was poured slowly, stepwise, into sufficient ethanol. The suspension then was dried under reduced pressure at room temperature. Finally, the dried microcapsules were vacuum coated with gold in an ion coater[8].

Silica gel beads were microencapsulated as fine hydrophilic particles. Typical examples of the microcapsules dispersed in isooctane are shown in Fig. 8. Uncovered beads and racemose aggregates of albumin and beads together with microcapsules were found. Figures 9–12 show the size distributions of the microcapsules containing silica gel beads dispersed

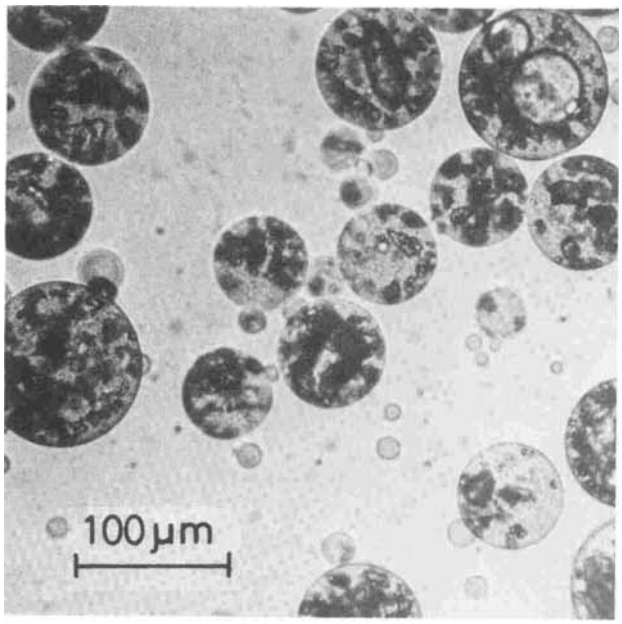

Figure 15—*Photomicrograph of albumin microcapsules containing phenacetin in isooctane.*

[8] JEC 1100, JEOL Ltd., Tokyo, Japan.

Table III—Mean Diameter (d) and Standard Deviation (δ) of Microcapsules Containing Phenacetin, Prepared by Using 10% (w/w) Albumin and 5.0% (v/v) Sorbitan Trioleate at a Speed Setting of 512 rpm

Volume Ratio[a]	Weight of Phenacetin Used, g	In Isooctane		In 2.0% (v/v) Polyoxyethylene Sorbitan Monolaurate Solution		
		d, μm	δ, μm	d, μm	δ, μm	P_m[b], %
1:9	1.912	50.4	25.6	59.3	26.4	100
1:5.7	2.854	48.1	25.8	38.8	19.1	100
1:4	3.825	57.7	33.4	53.1	26.9	100

[a] Phenacetin to albumin solution. [b] Microencapsulation percentage.

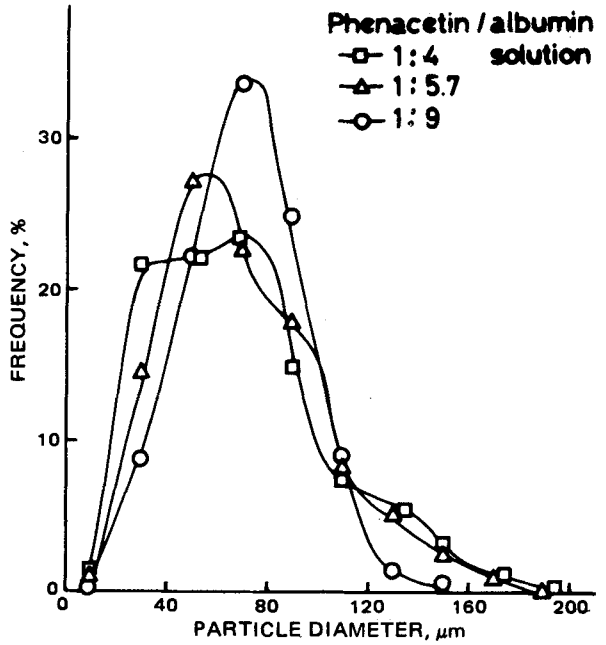

Figure 16—*Size distribution of albumin microcapsules containing phenacetin dispersed in isooctane, prepared at volume ratios of phenacetin to albumin solution of 1:9, 1:5.7, and 1:4 (weight of nylon 12 was 1.912, 2.854, and 3.825 g, respectively). The albumin concentration was 10% (w/w), and the emulsifier was 5.0% (v/v) sorbitan trioleate. The speed setting was at 512 rpm.*

in isooctane. Table II summarizes the mean diameter, standard deviation, and microencapsulation percentage (percentage of the number of microcapsules containing beads and not containing beads).

The mean diameters of albumin microcapsules containing silica gel beads were always about two times larger than those of the microcapsules containing no beads. The size distribution of the microcapsules containing silica gel beads shifted toward the larger particle diameter and broadened over a wide range as compared with that of the capsules containing no beads (Figs. 9–12). Broadening of the size distribution was caused by the size of silica gel beads (mean diameter of 52.5 ± 12.7 μm) and the viscosity increase of the albumin solution containing beads. However, the number of beads in each microcapsule also depended on the factors described previously (Fig. 8).

Typical microspheres and microcapsules containing iron hydrous oxide-modified nylon particles dispersed in isooctane are shown in Fig. 13. The percentage of modified nylon particles that were microencapsulated was ~70%.

Figure 14 shows the size distribution curves of the albumin microcapsules containing modified nylon particles in isooctane and in 2.0% (v/v) polyoxyethylene sorbitan monolaurate solution. The mean diameter and its standard deviation of the microcapsules in isooctane were 13.3 and 3.7 μm, respectively. Furthermore, the mean diameter was slightly larger for the microcapsules than for the microspheres (~10.0 μm).

Microencapsulation of Phenacetin—Phenacetin particles, 49–63 μm in diameter, were encapsulated. Typical albumin microcapsules containing phenacetin dispersed in isooctane are shown in Fig. 15. The size distribution of these albumin microcapsules prepared at three volume ratios of albumin solution to phenacetin is shown in Fig. 16. Table III summarizes the mean diameter and its standard deviation of the albumin microcapsules.

The mean diameter did not vary appreciably with the volume ratio of albumin to phenacetin, and the size distribution curves of the capsules were similar to each other. Furthermore, phenacetin was encapsulated completely in contrast with the silica gel beads, as seen from the P_m values in Table III. The marked difference in the percentage of microencapsulation between silica gel beads having a high porous surface and crystalline phenacetin are due to differences in: (*a*) the surface chemical properties such as hydrophilicity, porosity, surface area, and specific gravity, and (*b*) the adsorption amount of albumin or the adhesiveness of albumin thin film, formed by denaturation, onto the surface of solid particles. These problems will be discussed in a future paper.

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