A New Technique to Efficiently Entrap Leuprolide Acetate into Microcapsules of Polylactic Acid or Copoly(Lactic/Glycolic) Acid

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The aim of this work was to develop injectable microcapsules containing leuprolide acetate, that would deliver the drug at a zero-order rate over a period of about one month. A large amount of leuprolide acetate, a hydrophilic drug, was entrapped into polylactic acid (PLA) and copoly-(lactic/glycolic) acid (PLGA) microcapsules prepared by an in-water drying method using a (w/o)/w emulsion. The basic techniques were designed to increase the viscosities of the inner water phase and w/o emulsion. Under the conditions used, it was possible for the drug to be completely entrapped by the microcapsules in the range of 10 to 20%, on the basis of the polymer. However, the release profiles of the drug *in vitro* were inadequate for controlled release for one month.

Keywords——injectable controlled-release formulation; leuprolide acetate; microencapsulation; polylactic acid; copoly(lactic/glycolic) acid; in-water drying process; entrapment

Leuprolide acetate, a highly potent analogue of luteinizing hormone–releasing hormone (LH–RH), when administered repeatedly to animals, produces the so-called "paradoxical effect," that is, the suppression of gonadal steroidogenesis, leading to a weight depression of the accessory sex organ.[1] Consequently, leuprolide acetate is indicated as an agent to treat steroid hormone-dependent tumors, such as advanced prostatic cancer. However, this treatment requires once-daily repeated injection of the drug over a long therapeutic period. To overcome this practical disadvantage, a prolonged-release dosage form would be preferable. Biodegradable and biocompatible polymers such as polylactic acid (PLA) and copoly(lactic/glycolic) acid (PLGA) might be suitable for this purpose. Since these polymers have a long history of use in sutures and bone plates,[2] many investigators have studied them as carriers for controlled-release dosage forms. The major studies have been of the prolonged release of lipophilic drugs such as naltrexone.[3] Sanders *et al.*,[4] and Redding *et al.*,[5] reported controlled release of a hydrophilic drug such as an LH–RH agonist by using a microcapsule system prepared by means of a phase separation technique. However, particles prepared by this method generally tend to be large and transformed.[6]

The purpose of this study was to develop a new method of obtaining an injectable and fine spherical microcapsule dosage form containing leuprolide acetate with a high entrapment ratios, with the aim of delivering the drug at a zero-order rate over a period of about one month.

Experimental

Materials——Leuprolide acetate synthesized in the Chemical Development Laboratories of Takeda Chemical Industries, Ltd. (Osaka) was used. PLA of average molecular weight 22500 (abbreviated as PLA-22500 hereafter)

and PLGA of average molecular weight 14000 (abbreviated as PLGA(75/25)-14000, where (75/25) refers to the copolymer ratio by molar ratio), were supplied by Wako Pure Chemical Ind. (Osaka). These compounds were synthesized without catalysts, and the average molecular weight was determined by gel permeation chromatography (GPC) by the supplier. Purified gelatin (Nitta Gelatin Co., Osaka), and Gosenol EG-40 (polyvinyl alcohol, Nihon Synthetic Chemical Ind., Ltd., Osaka) were used. Other chemicals were of reagent grade.

PLA with an average molecular weight of more than 20000 was synthesized by the method of Woodland et al.[7] involving the ring-opening polymerization of DL-lactide using a zinc catalyst. Chart 1 summarizes the synthetic process. Approximately 70 g of DL-lactide was placed in a three-necked flask fitted with a stirrer, a distillation head plus condenser, and a thermometer, and the flask was purged with nitrogen gas. The DL-lactide was melted under a

DL-lactide 70 g
  ←charged under nitrogen
  ←heated under nitrogen over an oil bath at 135–150°C, with stirring, until melting was completed
  ←diethyl zinc/hexane solution (2.8 ml, 25%v/w) rapidly introduced into the stirred melt at 140—150°C
light-yellow solid
  ←dissolved in CH$_2$Cl$_2$
  ←reprecipitated by addition of hexane
  ←evaporation of solvents
polylactic acid

Chart 1. Preparation of Polylactic Acid (Lot 08212-1) (PLA-20000)

DL-lactic acid 168.8 g
glycolic acid   54.3 g
  ←3 g of resin activated by adding 2 N HCl
  ←heated at 130°C for 3 h under low vacuum (by water aspirator)
  ←collected water (66 ml) discarded
  ←heated at 150°C for 5 h under high vacuum (by vacuum pump, 5 mmHg)
  ←3 g of new resin added
  ←heated at 170°C for 7.5 h under high vacuum
  ←heated at 185°C for 7.5 h under high vacuum
light-brown solid
  ←dissolved in CH$_2$Cl$_2$
  ←filtered through paper
  ←reprecipitated by addition of hexane
  ←evaporation of solvents
the copolymer (yield: 130 g)

Chart 2. Preparation of Copoly(lactic/glycolic) Acid (Lot 08303-3) (PLGA(78/22)-10000)

DL-lactic acid 320 g
  ←6 g of resin activated by adding 2 N HCl
  ←heated at 130°C for 3 h under low vacuum
  ←collected water (83 ml) discarded
  ←heated at 150°C for 3 h under high vacuum
  ←6 g of new resin added
  ←heated at 175°C for 24 h under high vacuum
  ←heated at 185°C for 48 h under high vacuum
light-brown solid
  ←dissolved in CH$_2$Cl$_2$
  ←filtered through paper
  ←reprecipitated by addition of hexane
  ←evaporation of solvents
polylactic acid (yield: 139 g)

Chart 3. Preparation of Polylactic Acid (Lot 08304-5) (PLA-15000)

DL-lactic acid 574 g
glycolic acid   75 g
  ←9 g of resin added
  ←heated at 130°C for 3.5 h under low vacuum
  ←collected water (150 ml) discarded
  ←heated at 150°C for 3 h under high vacuum
  ←9 g of new resin added
  ←heated at 170°C for 30 h under high vacuum
  ←heated at 180°C for 48 h under high vacuum
light-brown solid
  ←dissolved in CH$_2$Cl$_2$
  ←filtered through paper
  ←reprecipitated by addition of hexane
  ←evaporation of solvents
the copolymer (yield: 385 g)

Chart 4. Preparation of Copoly(lactic/glycolic) Acid (Lot 08305-8) (PLGA(89/11)-19000)

nitrogen stream by heating in an oil bath to 135 to 150 °C. Thereafter the temperature was maintained at between 140 and 150 °C. Addition of 2.8 ml of 25% diethyl zinc solution in hexane, with stirring, caused instantaneous polymerization. The polymer obtained was purified by dissolving it in dichloromethane and subsequently reprecipitating it by adding n-hexane. This was repeated three times. The polymer containing the solvent was hardened into a foam under vacuum and pulverized. The remaining solvent was removed from the polymer flakes under vacuum drying. PLGA was synthesized by the method described by Nevin,[8] involving the polycondensation of lactic and glycolic acids using an acid ion-exchange resin catalyst. A PLA of average molecular weight less than 20000 was also synthesized by the same method. Charts 2, 3, and 4 summarize the syntheses of PLGA(78/22)-10000, PLA-15000, and PLGA(89/11)-19000, respectively. For example, the synthesis of PLGA(78/22)-10000 was carried out as follows: 168.8 g of DL-lactic acid, 54.3 g of glycolic acid, and 3 g of Dowex 50W × 4H (Dow Chemical Co., U.S.A.) as a preactivated acid ion-exchanger were placed in a three-necked flask and heated to 130 °C for 3 h in an oil bath under slightly reduced (water aspirator) pressure. Approximately 66 ml of water was removed by this process. The temperature was then raised to 150 °C and, after a 5-h dehydration reaction, 3 g of new resin was added. Polycondensation was executed at 185 °C for 7.5 h under high vacuum. The polymer obtained was dissolved in dichloromethane and filtered to remove resin. The polymer was purified by reprecipitation with dichloromethane and n-hexane as described above, and hardened into a foam, followed by pulverization. About 130 g of the polymer (Lot 08303-3) was obtained.

Preparation of PLA and PLGA Microcapsules——PLA and PLGA microcapsules were prepared by an in-water drying method similar to that of Vrancken *et al.*[9] The microcapsules which are prepared by this method are the monolithic form. Such microcapsules are sometimes called microspheres,[10] but the term microcapsule is used in this paper. Their compositions are shown in Tables II and III. The inner water phase consisted of leuprolide acetate in an aqueous solution or in a mixture of water plus gelatin at about 60 °C. The oil phase consisted of PLA or PLGA dissolved in dichloromethane solution. The oil phase was gradually poured into the inner water phase under vigorous stirring with a homogenizer or ultrasonicator (Ohtake Manufacturing Co., Tokyo) over a few minutes to make a microfine w/o emulsion. The emulsion obtained was cooled to about 15 °C to increase the viscosities of the inner water phase and the w/o emulsion itself, and then poured into an aqueous 0.5% polyvinyl alcohol solution through a nozzle of about 1.5 mm i.d. under stirring with Auto-Homomixer (Tokushu Kika Kogyo Co., Osaka) at 5000 rpm. The fluid was stirred for 2 min to make a (w/o)/w emulsion. To evaporate dichloromethane, the (w/o)/w emulsion was stirred gently with a propeller mixer, or placed in a rotary evaporator for approximately 2 h while the emulsion was warmed to approximately 30 °C. The hardened microcapsules in the wet state were sized using sieves having apertures of 125, 88, 74, 44, and 37 μm, rinsed with water three times and lyophilized into a powder, which was dried under reduced pressure over a few days.

Determination of the Leuprolide Acetate Content in Microcapsules——The leuprolide acetate in the microcapsules was determined by a high performance liquid chromatography (HPLC) procedure using Shimadzu LC-3A equipment (Kyoto). Microcapsules (50 mg) were dissolved in a mixture of 10 ml of dichloromethane and 20 ml of 1/30 M phosphate buffer, pH 6.0, and leuprolide acetate extracted into the buffer was assayed by an HPLC procedure with an ultra violet (UV) detector under the following conditions: column, Lichrosorb RP-18 250 mm in length with 4 mm i.d.; column temperature, 30 °C; mobile phase, a mixture of 100 ml of 0.25 M acetonitrile and 150 ml of methyl alcohol; flow rate, 0.7 ml/min; wavelength, 280 nm.

Determination of the Average Molecular Weight and the Copolymer Ratio of Polymers——The average molecular weight of each polymer was determined by a gel permeation chromatography (GPC) procedure using a polystyrene reference standard. The polymer (100 mg) was dissolved in 10 ml of tetrahydrofuran (THF), and 200 μl of the solution was injected into the GPC equipment. The molecular weight distribution was determined under the following conditions: column, Shim-pack HSG-40s, HSG-30s, HSG-20s, and 2 columns of HSG-15s; mobile phase, THF; flow rate, 1 ml/min; column temperature, 50 °C; polystyrene standard, Mw 92600, 50000, 19000, 9000, 4000, 2100, 800, and 500 (purchased from Du Pont, U.S.A.); detection, refractive index (Showdex RI SE-31, Showa Denko KK, Japan). The molecular weight was calculated by microcomputer software developed by Shimadzu. The copolymer ratio was calculated from the integration values of the nuclear magnetic resonance (NMR) signals of the methyl group of lactic acid and the methylene group of glycolic acid.

Leuprolide Acetate Release Studies——The leuprolide acetate released from microcapsules was determined by the rotating bottle procedure using a RT-50 rotator (Taiyo Scientific Industrial Co., Tokyo) at 37 ± 1 °C. The microcapsules (50 mg) were suspended in 10 ml of the release medium consisting of 1/30 M phosphate buffer, pH 7.0, containing 0.05% Tween-80 (Kao-Atlas, Tokyo). Since leuprolide acetate was poorly recovered from the release medium because of its instability in this buffer solution, the residual leuprolide acetate in the microcapsules was periodically determined after filtering the microcapsules through a 0.8 μm Millipore filter by the analytical method mentioned above.

Measurement of Viscosity——Viscosities were measured with an Ubbelohde viscometer at various temperatures following the procedure specified in JP X.

Observation of Microcapsules——The shapes and surface characteristics of the dried microcapsules were examined by a scanning electron microscope (model JSM T-300, JEOL-Technics Co., Ltd., Tokyo).

Results and Discussion

Synthesis of Polylactic Acid and Copoly(Lactic/Glycolic) Acid

The average molecular weights of PLA and PLGA and the copolymer ratios of PLGA prepared by two different synthetic routes are shown in Table I. In the ring-opening method, the reaction proceeded rapidly and various PLAs were obtained by changing the reaction temperature. For instance, reaction temperatures of 135 to 140, and 160 °C favored the production of PLAs with average molecular weights of 20000 and 73000, respectively. The zinc catalyst could not be removed from the polymer.

In the polycondensation procedure using the acid-ion exchanger resin, copolymer of lactic and glycolic acid of any copolymer ratio could be synthesized, and the catalyst could be

TABLE I.   Average Molecular Weight and Copolymer Ratio
of Synthesized PLA and PLGA

Lot	Synthesis method	Copolymer ratio (lactic acid/glycolic acid)	Average molecular weight
0818-2	RO	100/0	20000
08109-3	RO	100/0	50000
0812-2	RO	100/0	73000
08304-5	RES	100/0	15000
0803-1	RES	100/0	6800
08303-2	RES	55/45	20000
0803-3	RES	78/22	10000
08305-8	RES	89/11	19000
W.P.	PC	100/0	22500
W.P.	PC	75/25	14000

RO, ring-opening method; RES, resin catalyzing method; PC, polycondensation method without catalyst; .W.P., polymer synthesized by Wako Pure Chemical Ind.

TABLE II.   Entrapment Ratio of Leuprolide Acetate into Microcapsules Prepared with or without Gelatin

No.	Polymer	Gelatin (%)[a]	Entrapment ratio (%)	Viscosity of inner water phase (cP)[b]
21	PLA-50000	—	6.7	1.6
22	PLA-50000	—	5.5	1.6
23	PLA-50000	—	1.9	1.6
31	PLA-73000	200	70.4	5000 <
32	PLA-50000	200	70.7	5000 <
34	PLA-15000	200	54.8	5000 <
35	PLA-6000	200	55.8	5000 <
36	PLGA(89/11)-19000	200	44.0	5000 <
37	PLGA(78/22)-10000	200	58.3	5000 <
38	PLGA(55/45)-20000	200	53.1	5000 <
101	PLGA(75/25)-14000	320	16.0	26
102	PLGA(75/25)-14000	250	32.0	6000

The formulation of Nos. 21 to 23 was as follows: leuprolide acetate, 200 mg; water, 2.5 ml; polymer, 2 g; CH_2Cl_2, 10 ml; 0.5% polyvinyl alcohol solution, 100 ml.   The formulation of Nos. 31 to 38 was the same as that of No. 21 but with the addition of gelatin in the inner water phase.   The formulation of Nos. 101 and 102 was as follows: leuprolide acetate, 200 mg; gelatin, 640 and 500 mg, respectively; water, 8 and 2.5 ml, respectively; polymer, 4 g; CH_2Cl_2, 10 ml; 0.5% polyvinyl alcohol solution, 320 ml.   a) Percent with respect to loaded amount of leuprolide acetate.   b) Viscosity was measured under the conditions employed for making the (w/o)/w emulsion.

TABLE III. Formulas of Microcapsules and Entrapment Ratio of Leuprolide Acetate

| No. | Inner water phase | | | Oil phase | | Outer water phase | Trap ratio (%) |
	Leuprolide acetate (g)	Water (g)	Gelatin (g)	PLA (g)	CH_2Cl_2 (ml)	PVA soln.[a] (ml)	
Y-1	0.4	2.0	0.4	4	5.0	200	57.8
Y-2	0.4	1.0	0.2	4	7.5	400	70.0
Y-3	0.4	0.5	0.1	4	10.0	400	61.8
Y-4	0.4	0.5	0.1	4	20.0	400	23.6
Y-5	0.4	0.5	0.1	4	5.0	400	111.0

a) Polyvinyl alcohol. PLA used was PLA-22500.

easily removed. However, polymers of average molecular weight exceeding 20000 could not be synthesized.

Effect of the Viscosity of the Inner Water Phase on the Entrapment of Leuprolide Acetate by Microcapsules

Table II shows various entrapment ratios of the drug (defined as the value of the actual leuprolide acetate content in microcapsules relative to the expected content calculated from the loaded amounts of the drug and the polymer) in microcapsules prepared using an inner water phase of high viscosity (more than 5000 cP) by incorporating gelatin, and of low viscosity (1.6 cP), without gelatin. The entrapment ratios into microcapsules without gelatin were from 1.9 to 6.7%, whereas those into microcapsules with gelatin were from 32.0 to 70.7%, when the microcapsules were made by an in-water drying method. However, as shown in Nos. 101 and 102 in Table II, unless the viscosity of the inner water phase was increased by means of cooling or some other technique, the entrapment ratio of leuprolide acetate was relatively low even in the case of an inner water phase containing gelatin. It was concluded that an increase in the viscosity of the inner water phase was related to an increase in the entrapment ratio for hydrophilic drugs when microcapsules were prepared by an in-water drying method using (w/o)/w emulsion, as described in the patent.[11]

Effect of the Viscosity of the w/o Emulsion on Entrapment of Leuprolide Acetate by Microcapsules

Table III shows the entrapment ratios of leuprolide acetate with respect to different formulas of microcapsules prepared with PLA-22500. The entrapment ratio of the drug decreased as the amount of dichloromethane increased, i.e. it decreased as the viscosity of the w/o emulsion decreased. A relatively high entrapment ratio of leuprolide acetate was attained by decreasing the volume of dichloromethane with respect to the polymer in the oil phase, i.e. by increasing the viscosity of the w/o emulsion. Since increasing the volume of the inner water phase with respect to the drug decreased the viscosity of the inner water phase, the entrapment ratio was low, as shown in the results for Nos. Y-1 and Y-5. It was concluded that an increase of the viscosities of the inner water phase and the w/o emulsion brought about a higher entrapment ratio of leuprolide acetate in the microcapsules. The relationship between temperature and the viscosity of the w/o emulsion is shown in Fig. 1. The viscosity of the w/o emulsion of the formulation with a high entrapment ratio at 15 °C, at which the w/o emulsion was emulsified into (w/o)/w, was more than 1000 cP, about 5 times higher than the viscosity of the formulation with a low entrapment ratio. When microcapsules were prepared by emulsifying a w/o emulsion with a viscosity exceeding 8000 cP into a (w/o)/w type, the average particle size of the microcapsules was relatively large and the appearance was not spherical (Fig. 2). It is thought that the higher viscosity of the w/o emulsion produced the higher
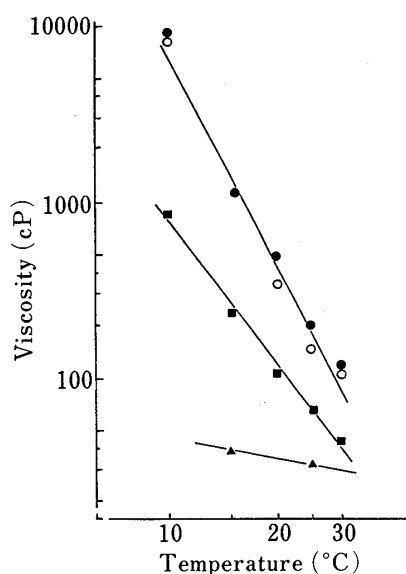
Fig. 1. Influence of Temperature on the Viscosity of w/o Emulsion

○, ●, entrapment ratio, more than 95% (formulas were the same as Y-5); ■, entrapment ratio, 70.0% (formula was the same as Y-2); ▲, entrapment ratio, 23.6% (formula was the same as Y-4).
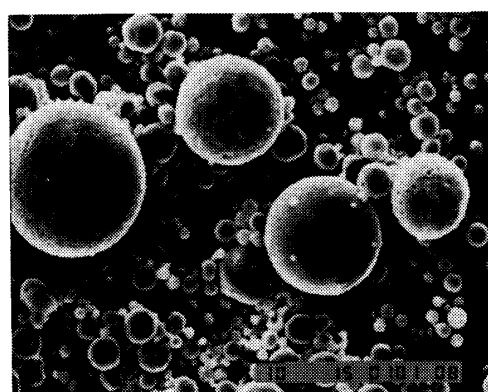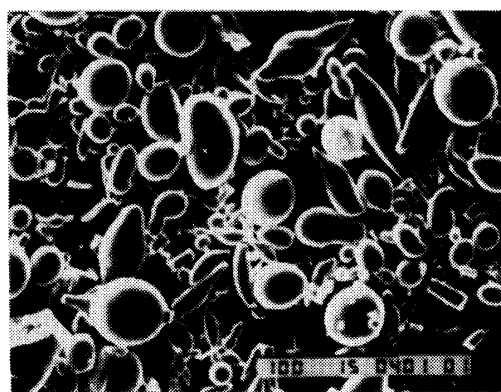

——————— 100 μm   — 10 μm

Fig. 2. Scanning Electron Photomicrographs of Microcapsules (PLA-22500) Containing 10% Leuprolide Acetate

Left side, transformed microcapsules; right side, spherical.

entrapment ratio of leuprolide acetate because the mechanical strength produced by the relatively high viscosity prevented the inner water phase from migrating to the outer water phase due to local demulsification produced by the vigorous stirring.

The reason why microcapsules were transformed may be as follows: when w/o emulsion having a viscosity of more than 8000 cP is poured into the outer water phase through a fine nozzle, dichloromethane in the surface of w/o emulsion immediately dissolves in the outer water phase. Therefore, the surface of the w/o phase shows increasingly high viscosity and the surface tension becomes so high that the w/o phase hardens to form a microcapsule before it can acquire a spherical shape.

Leuprolide acetate when prepared by the in-water drying method was completely entrapped in microcapsules with high reproducibility by means of increasing the viscosities of the inner water phase and w/o emulsion. Another significant advantage of the in-water drying process is the ability to produce microcapsules easily on a large scale. This is a new technique to efficiently entrap a hydrophilic drug into PLA or PLGA microcapsules.

Effect of Loading Amount of Leuprolide Acetate and Particle Size of Microcapsules on Entrapment Ratio

Microcapsules (PLA-22500) prepared with various loadings (percent) of leuprolide acetate were separated by sieving into groups with the particle size ranges listed in Table IV, and the entrapment ratio of each group was determined. As shown in Table IV, the entrapment ratio (total) decreased as the loading (percent) of leuprolide acetate increased and it decreased as the particle size decreased. The latter tendency may be understood as follows: in smaller particles, the frequency of contact between the outer water phase and the inner water phase increased because of the extended surface area of the w/o emulsion. The former tendency may be understood as follows: at higher loading of leuprolide acetate, migration of the inner water phase to the outer water phase increased because the particles of the inner water phase easily aggregated owing to changes in the surface properties, and their inner water phases were connected with each other from the surface into the core as described by Siegel and Langer.[12] To explain the relationship between the entrapment ratio of leuprolide acetate and particle size, the following assumption was made: a perturbation such as a local phase inversion is generated in a thickness, d, of a microcapsule with diameter, D, regardless of particle size (see Fig. 3), and the leuprolide acetate in the thickness, d, of the microcapsule is removed to the outer water phase by the perturbation. The entrapment ratio, C, in this case is represented by the following Eq. 1:

$$C = (1 - 2d/D)^3 \cdot 100(\%) \tag{1}$$

The results shown in Table IV (relationships of entrapment ratio to particle dimensions (d and D) of a microcapsules) are plotted in Fig. 4, where the means of the ranges of particle size, 88—125, 53—88, 37—53, and less than 37 μm, were taken as 106.5, 70.5, 45, 20 μm, respectively, and the dimension d was chosen arbitrarily. The entrapment ratio for particles exceeding 125 μm was taken as 100% because these had the maximum entrapment ratio for this formulation. If the dimension d of the microcapsules prepared with formulations

TABLE IV. Effects of Loaded Amount of Leuprolide Acetate on the Entrapment Ratio and Particle Size of Microcapsules

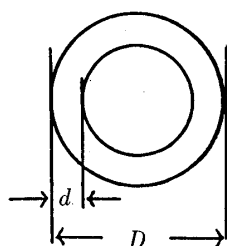| Loading (%)[a]	Entrapment ratio (total) (%)	Entrapment ratio for each particle size				
		125 μm < (%)	88—125 μm (%)	53—88 μm (%)	37—53 μm (%)	37 μm > (%)
10	111	114	114	119	113	101
10	101	105	101	101	103	99
20	96	113	107	96	90	68
20	95	112	117	109	84	63
20	87	115	105	93	80	73
30	71	100	86	65	53	46

a) Relative to the amount of polymer.

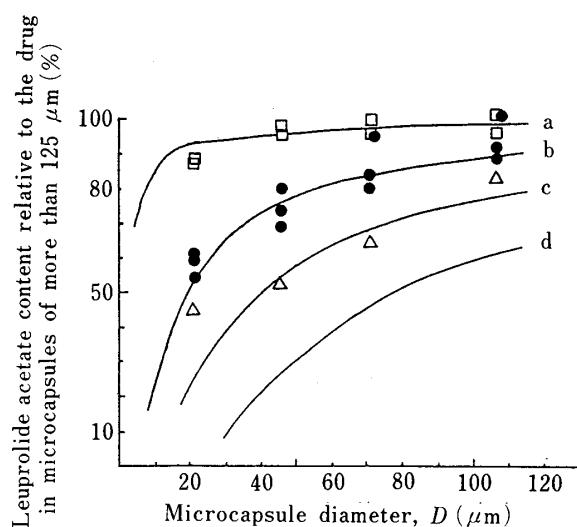Fig. 3. Diameter, D, and Thickness, d, of Microcapsules

Fig. 4. Relative Entrapment Ratios of Leupro-
lide Acetate into Microcapsules (PLA-22500)
of Various Particle Sizes

Loadings (percent) of leuprolide acetate with re-
spect to amount of polymer were 10% (□), 20% (●),
and 30% (△).
Solid lines represent the value calculated by using
Eq. 1.
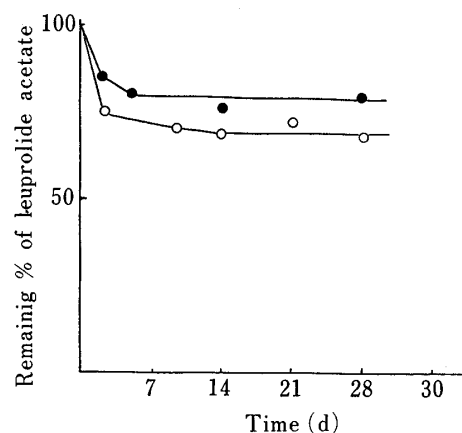a, $d = 0.25\,\mu$m; b, $d = 2.0\,\mu$m; c, $d = 4.0\,\mu$m; d, $d = 8.0\,\mu$m.

Fig. 5. Release Patterns of Leuprolide Acetate
from Microcapsules (PLA-22500) in Vitro

Loadings (percent) of leuprolide acetate with re-
spect to amount of polymer were 10% (●), and 20%
(○)

TABLE V. Particle Size Distribution of Microcapsules under
Different Production Conditions

	C-1	C-2	C-3	C-4	C-5
PLA (g)	4	4	4	4	4
CH_2Cl_2 (ml)	10	10	10	5	5
Outer phase (ml)	400	400	400	400	200
Mixing speed (rpm)	3500	3750	4000	3500	3500
$125\,\mu$m < (%)	0	0	0	38.9	76.9
88—125 μm (%)	0	0	0	31.2	13.1
53—88 μm (%)	61.5	55.0	39.2	14.0	10.0
37—53 μm (%)	38.5	45.0	60.8	15.9	0

PLA used was PLA-22500.

containing loadings of the drug of 10, 20, and 30% are 0.25, 2.0, and 4.0 μm, respectively, the
relationship between the observed entrapment ratio and the one calculated by Eq. 1 is good. If
the model with respect to entrapping a hydrophilic drug into microcapsules prepared by the
in-water drying method is appropriate, increasing the loading amount of the drug and
decreasing the viscosity of the w/o phase would lead to an increased value of d, which readily
induces perturbation.

Factors Influencing the Particle Size of Microcapsules

Table V shows the particle size distribution of the microcapsules (PLA-22500) prepared
as a function of the mixing speed of the turbine mixer as the w/o was emulsified into the
(w/o)/w, the volume ratio of dichloromethane to PLA, and the outer water phase volume. A
small particle was produced when the mixing speed and the outer water phase volume were

high and the volume ratio of dichloromethane was low. The mixing speed and the volume ratio markedly influence particle size. Particle sizes of microcapsules prepared with 200 ml of outer water phase volume were larger than those produced with 400 ml. This can be explained if we assume that particles readily coalesced in the (w/o)/w phase since the particle concentration was high before the w/o phase became hard owing to evaporation of dichloromethane.

Release of Leuprolide Acetate from Microcapsules *in Vitro*

Figure 5 shows the release patterns *in vitro* of leuprolide acetate from microcapsules composed of PLA-22500 and two different loadings of the drug. Initially, there was a sharp release of leuprolide acetate, but subsequent to this, almost no more drug was released; this pattern is inadequate for providing controlled release for one month. It was found that the microcapsules loaded with a greater amount of the drug give a greater initial release. It was also found that leuprolide acetate in the PLA-microcapsules exhibits sufficient stability at 37°C for 30 d.

Appearance of the Microcapsules

As shown in Fig. 2, the microcapsules (PLA-22500) prepared with a mean diameter of about 20 μm were fairly spherical but the surface was slightly uneven. The many micropores, which were observed on the surfaces, were probably generated by evaporation of dichloromethane or water, but there is no evidence for this as yet.

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