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Preparation of Poly(D,L-lactide) and Copoly(lactide-glycolide) Microspheres of Uniform Size

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Abstract

In an attempt to prepare monodisperse poly(D,L-lactide) and copoly(lactide-glycolide) microspheres, a novel emulsification technique (membrane emulsification) was employed and the preparation conditions which might affect the monodispersity were evaluated.

With this technique nearly monodisperse poly(D,L-lactide) and copoly(lactide-glycolide) microspheres were successfully prepared and their sizes were controllable only by making use of microporous glass membranes of different pore sizes. However, in the present system of emulsion (methylene chloride/water) the surfactant used was limited to ionic ones and the amount of polymers available for the formation of microspheres was inevitably too small in concentration to entrap sufficient amounts of drug. As for the drug release, the effect of particle size was not appreciable but the method of solvent removal gave a great influence; the solvent extraction method showed a more drug-sustaining effect than did the solvent evaporation method.

The present results suggest the possibility of making drug-loaded and biodegradable monodisperse microspheres.

Many studies have been reported on the materials, shape and size of drug carriers, the route of their administration, kinetics and mechanisms of drug release in developing sustained or controlled drug-delivery systems. Spherical and biodegradable particles have drawn much attention as drug carriers since they have a larger surface area available for drug release, are easily formulated as injectable dosage forms, and do not usually need to be removed after releasing the entrapped drugs.

Biodegradability and biocompatibility of carrier materials are now becoming the essential factors for designing drug-delivery systems. Many materials have been proposed as candidates for biodegradable carriers, among which poly(D,L-lactide) and copoly(lactide-co-glycolide) are preferentially used since they are biologically decomposed into endogenous metabolites. Although microspheres prepared from these polymers can have a perfectly round shape and a smooth surface morphology, the size distribution is usually wide because it is difficult to produce uniform droplets using the conventional preparation methods which utilize mechanical agitation. Uniform particle size is important as distribution within the body and interaction with biological cells are greatly affected by particle size. Furthermore, if monodisperse microspheres are available, their degradation rate and drug release kinetics can be manipulated, thereby making it easier to formulate more sophisticated systems.

This paper describes an experimental trial to prepare monodisperse poly(D,L-lactide) and copoly(lactide-glycolide) microspheres containing a model drug, progesterone, with a membrane emulsification technique which allows the formation of uniform sized spheres.

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Materials and Methods

Materials

Glass membranes of defined pore size were purchased from Ise Chemical Co. (Tokyo,Japan). Poly(D,L-lactide) (mol. wt = 20 000 Da) and copoly(lactide-glycolide) (mol. wt = 20 000 Da, 50 : 50 M/M, random copolymer) were purchased from Wako Pure Chemical Industries (Osaka, Japan), and were designated as PLA and PLGA, respectively. Progesterone and sodium lauryl sulphate (SLS) were from Sigma Chemical Co. (St Louis, USA) and all other chemicals were of reagent grade.

Method

Oil/water type emulsion, PLA and PLGA microspheres, and progesterone-loaded microspheres were prepared using a membrane emulsification apparatus, the operative method of which was described in detail elsewhere (Muramatsu et al 1994). Briefly, an organic solvent, methylene chloride or cyclohexane, was forced by nitrogen gas pressure through a microporous glass membrane slowly into a circulating continuous water phase containing 7 mM SLS as a surfactant. Emulsification was continued until the volume of the disperse phase reached approximately 5%. For preparing the microspheres and the drug-loaded microspheres, the emulsification was carried out in a bath surrounded by ice, because of the low glass transition temperature of PLA and PLGA. The emulsion thus obtained was gently stirred with a magnetic stirrer in a cold room until the solvent completely evaporated (solvent evaporation method), or was mixed with the same volume of cold methanol to precipitate PLA or PLGA (solvent extraction method). The microspheres were collected and washed twice with cold water on the centrifuge.

The emulsion droplets were observed and photographed under an optical microscope, and the microspheres were also observed by scanning electron micrography (JSM T-20, JEOL)

after ion-sputtering with gold. The size distribution of the microspheres was measured with a laser diffraction size analyser (Malvern model 3601).

Progesterone in the microspheres was evaluated according to the procedure of Izumikawa et al (1991))with some modifications. A weighed amount of the progesterone-loaded microspheres (1 mg) was dissolved in 1 mL acetonitrile. To the solution was added the same volume of water to precipitate the polymer, and the mixture was filtered through a nylon membrane (pore size = 0·20 μm). The filtrate was injected into an HPLC system consisting of a column (TSK-ODS80TM, Tosoh), a pump (Tosoh CCPM) and a UV detector (Tosoh UV-8010) equipped with a data analyser (Chromatocorder 12, SIC). The mobile phase was a mixture of acetonitrile and water (65 : 35), the flow rate was set at 1 mL min^{-1}, and the eluant was monitored and recorded at 240 nm.

To evaluate in-vitro release of progesterone, a weighed amount of microspheres (20 mg) was placed into 100 mL phosphate-buffered solution (pH 7·4). The suspension was gently shaken at 37°C and a portion (5–10 mL) was withdrawn at appropriate time intervals. The microspheres were immediately washed with the buffer solution on the centrifuge and the content of progesterone remaining in the microspheres was determined by HPLC.

Results and Discussion

Drug-loaded PLA or PLGA microspheres are routinely prepared by the solvent evaporation or extraction method, in which an organic polymer solution is emulsified into water to give an oil/water emulsion and then the solvent is removed to form PLA or PLGA microspheres. To obtain monodisperse PLA or PLGA microspheres the emulsion droplets should be uniform in size and kept stable during solvent removal. We first tried to prepare a monodisperse emulsion of the solvent, methylene chloride, in water with the membrane emulsification apparatus. Fig. 1 shows optical microphotographs of the emulsion droplets obtained. Although they appeared to have a far narrower size distribution than those prepared by a conventional mechanical agitation technique, the emulsion droplets were not uniform. When we used cyclohexane as the solvent, the emulsion droplets were more uniform, although exactly the same preparation conditions as those for methylene chloride were employed. This difference would be caused by the physicochemical property of the solvent. Since membrane emulsification proceeds by forcing the solvent into water as small amounts at a time, polar solvents such as methylene chloride can be transferred into and mixed with water until the water phase is saturated with the solvent. The emulsion droplets formed at the earlier stage could be smaller than those formed later, thereby causing the emulsion to have a wider size distribution. This is not the case for cyclohexane since it is immiscible with water. Unfortunately, since nonpolar solvents are in general poor solvents for polymers such as PLA or PLGA, we were obliged to use a polar solvent, methylene chloride. To circumvent the problem we used as the continuous phase methylene chloride-saturated water which was expected to prevent the solvent from moving into the water phase. With this treatment the uniformity in size of the emulsion droplets was found considerably improved.

For stabilizing the emulsion we employed sodium lauryl sulphate as a surfactant. Preliminary experiments revealed that protective colloids such as polyvinylalcohol, although widely used in these cases, were not efficient presumably because polymer colloids were not supplied to the interface when the droplets were formed at the glass membrane surface. Nonionic surfactants were also unsuccessful because typical nonionic surfactants (Tweens or Pluronics) were soluble both in water and in methylene chloride, thereby causing less effects as surfactants at the interface. Cationic surfactants were inadequate in this system since they interacted electrically with negatively charged glass membranes.

Preparation of monodisperse PLA and PLGA microspheres

In principle, monodisperse emulsion droplets of polymer solutions can lead to the formation of microspheres of uniform size after removal of the solvent. Thus, PLA or PLGA was dissolved in methylene chloride, which was pre-saturated with water, since water is partly miscible with the solvent. The polymer solution was emulsified into the solvent-saturated water with the aid of SLS, and the emulsion was gently stirred

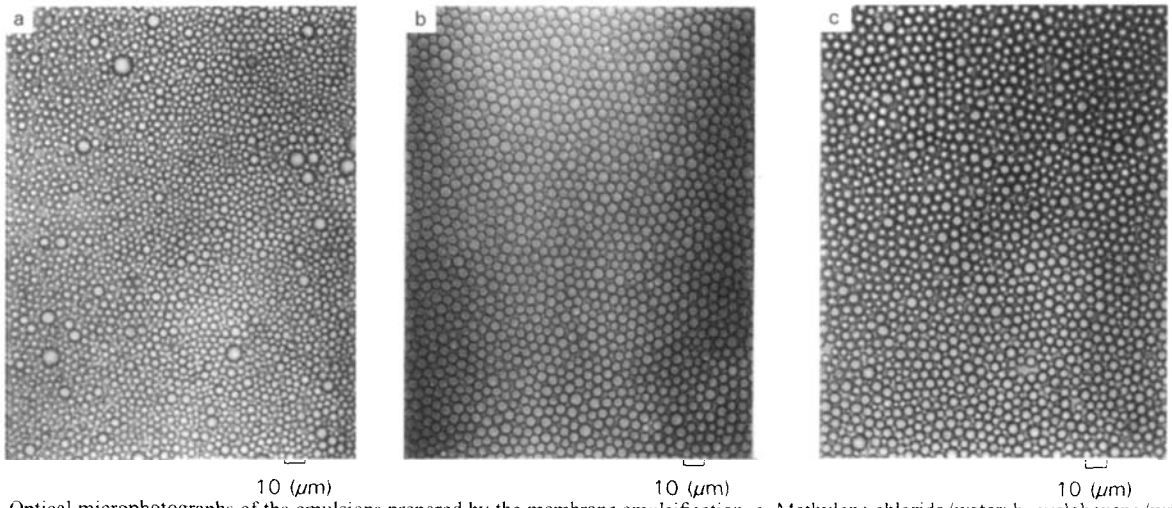

FIG. 1. Optical microphotographs of the emulsions prepared by the membrane emulsification. a. Methylene chloride/water; b. cyclohexane/water; c. methylene chloride/methylene chloride-saturated water. The pore size of the membrane was 1120 nm.

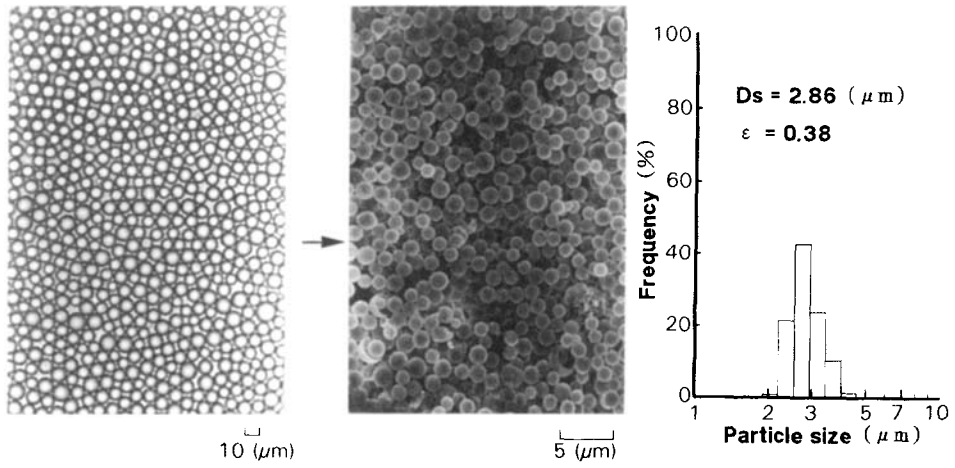

FIG. 2. Optical microphotograph of emulsion and SEM photograph of PLGA microspheres and its size distribution profile. The pore size of the membrane was 2430 nm.

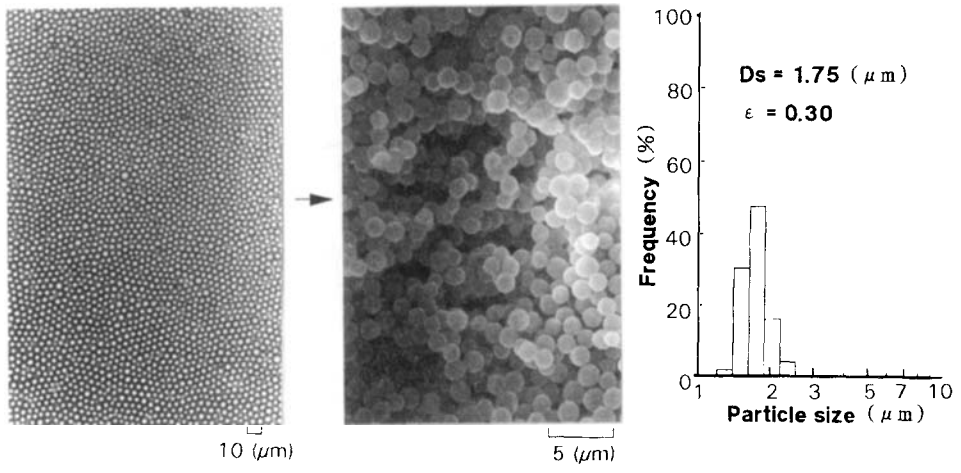

FIG. 3. Optical microphotograph of emulsion and SEM photograph of PLGA microspheres and its size distribution profile. The pore size of the membrane was 1120 nm.

with a magnetic stirrer until the solvent evaporated. Figs 2–4 show an optical microphotograph of the emulsion droplets, a scanning electron micrograph and a size distribution profile of the PLGA microspheres prepared by employing a glass membrane of three different pore sizes; 2430, 1120 and 730 nm respectively. The Figs show the mean diameter (Ds) and the index of monodispersity (ε) which is defined as the difference in undersize volume distribution between those at 90 and 10% divided by the median value. The microspheres looked spherical and fairly uniform in size, and it was possible to prepare almost monodisperse microspheres of different sizes only by using glass membranes of different defined pore sizes. In addition, we could obtain PLGA particles of submicron and uniform size simply with a membrane of smaller pore size (Fig. 5). Only the data for PLGA spheres are given here, but those for PLA were similar.

Preparation of progesterone-loaded microspheres

Progesterone, a model drug, was dissolved in water-saturated methylene chloride containing 2% PLA or PLGA, and the solution was emulsified into methylene chloride-saturated water with SLS by the membrane emulsification method. Increase in progesterone payload up to 50% did not appreciably change the mean diameter and the uniformity in size of either emulsion droplets or microspheres, but its content remaining in the microspheres was less than 10% (Fig. 6), for either PLA or PLGA microspheres. Since there is a small amount of methylene chloride in the continuous water phase, the progesterone was easily partitioned to the water phase during emulsification and solvent evaporation, which inevitably required a longer time. The conventional preparation for this kind of microsphere is to disperse the solvent containing drug into water, subsequently removing the solvent to precipitate the polymer. Since it is possible to increase the polymer content almost up to its saturation amount, precipitation of the polymer occurs immediately after the disperse phase is mixed with water, thereby preventing the drug from diffusing out of the precipitated polymer bulk. However, the membrane emulsification method cannot utilize this advantage since PLA or PLGA would not dissolve in water-saturated methylene chloride to a concentration of more than 5%. Even if higher concentrations could be obtained, viscous solutions pass

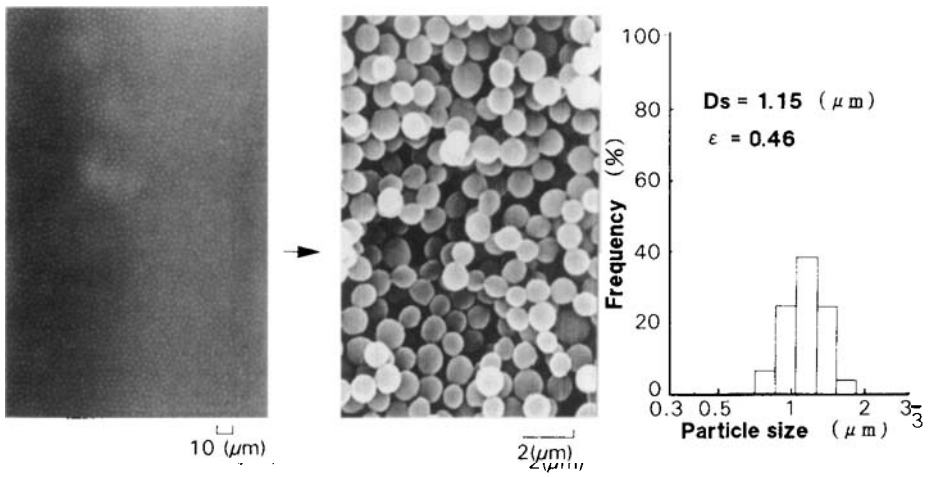

FIG. 4. Optical microphotograph of emulsion and SEM photograph of PLGA microspheres and its size distribution profile. The pore size of the membrane was 730 nm.

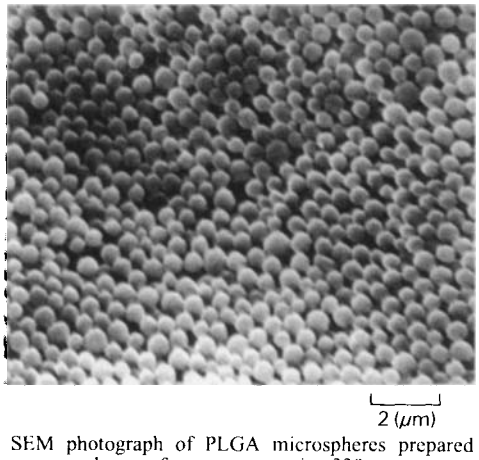

FIG. 5. SEM photograph of PLGA microspheres prepared with a microporous membrane of average pore size 330 nm.

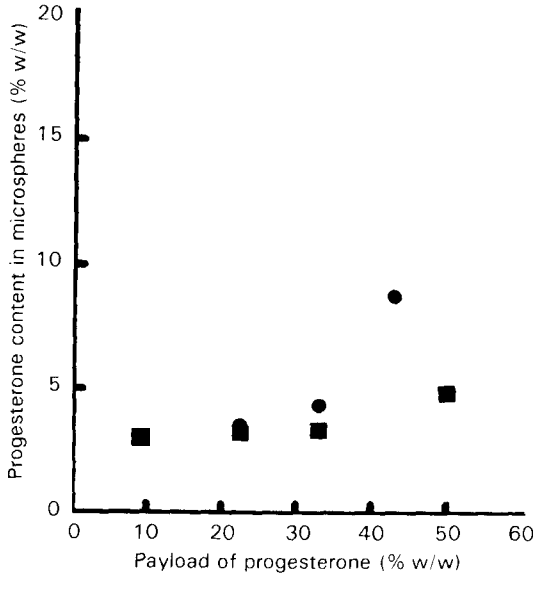

FIG. 6. Entrapment efficiency of progesterone with PLA (■) and PLGA (●) microspheres.

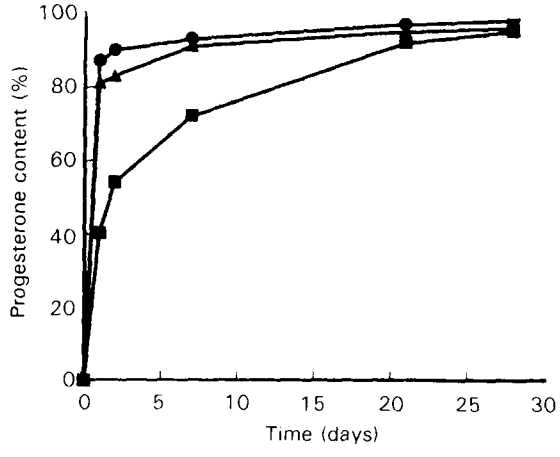

FIG. 7. In-vitro release profile of progesterone from PLA microspheres. ■ 730 nm extraction; ▲ 1120 nm; ● 730 nm.

through the pores of the glass membrane with difficulty. To minimize the drug loss the solvent, methylene chloride, was extracted with methanol instead of being evaporated. This procedure reduced the time for preparing microspheres but caused no improvement of drug entrapment; the transfer of progesterone into the outer phase was assumed to occur during the membrane emulsification step.

In-vitro release of progesterone from microspheres

Fig. 7 shows the progesterone release from PLA microspheres. More than 80% progesterone was released within one day from the microspheres prepared by the solvent evaporation method. The effect of the size of microspheres on release profile was not appreciable, although a slight delay was observed for larger microspheres. A considerable decrease in drug release was seen for the microspheres prepared by solvent extraction, probably due to the difference in drug distribution within the microspheres. During solvent evaporation the entrapped drug would diffuse to the oil/water interface concomitantly with diffusion of methylene chloride and tend to accumulate at the interface since the outer phase has already reached saturation

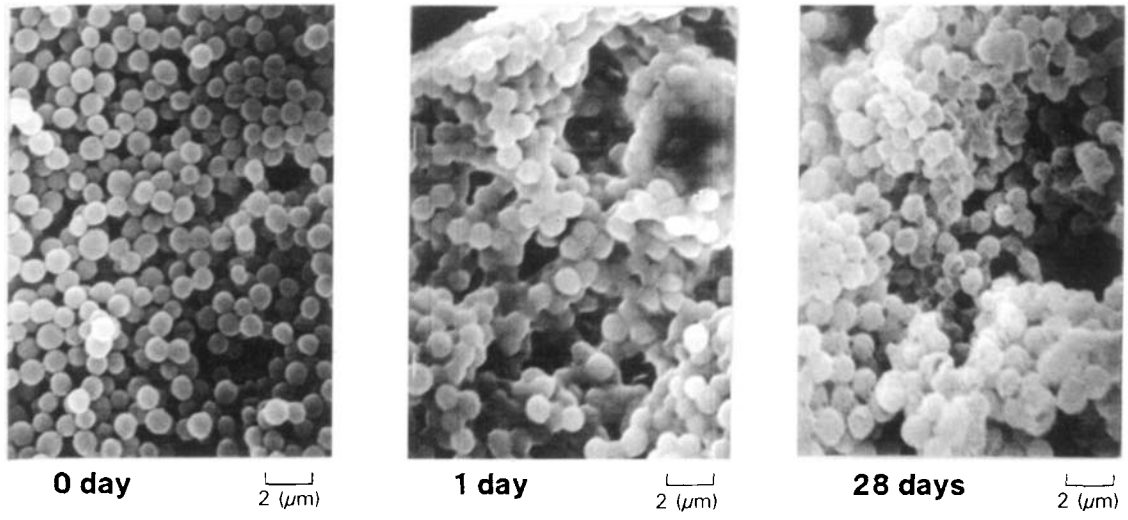

FIG. 8. SEM photographs of progesterone-loaded PLA microspheres incubated in-vitro for 28 days.

with progesterone, thereby causing larger concentrations there than in the bulk. While solvent extraction would give insufficient time for the drug to diffuse to the interface, uniform distribution of progesterone within the microspheres could be expected. Therefore, the initial burst effect was more remarkable for the microspheres prepared by the evaporation method than for those by extraction. In addition, since the size and morphology of the PLA microspheres were not appreciably changed after 28 days incubation (Fig. 8), the release of progesterone was assumed mainly to be diffusion-controlled.

Although further improvements are needed, this membrane emulsification technique could be applied to prepare biodegradable microspheres of uniform size which contain therapeutically important medicines.

References

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