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Preparation and Evaluation *in Vitro* of Polylactic Acid Microspheres Containing Local Anesthetics

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The use of DL-polylactic acid (PLA) microspheres as a means to achieve sustained release of butamben, tetracaine, and dibucaine was examined *in vitro*. As a first step, PLA solutions in an organic solvent containing the drug were dispersed in viscous aqueous solutions, then microspheres were formed by a solvent-evaporation process. The release mechanism was examined by scanning electron microscopy. The effects of polymer solvents and nonsolvents on the microsphere characteristics and release patterns from the microspheres were examined. The effects of drug contents and microsphere size on release patterns were also investigated.

Keywords——microsphere; sustained release; butamben; tetracaine; dibucaine; solvent-evaporation process; scanning electron microscopy

During recent years there has been an upsurge of research into means of providing prolonged action formulations. A considerable amount of work has been reported in this area, with synthetic polymers as a major component on the formulation.[1–8]

In pioneering research work, non-degradable polymers were employed; however, biodegradable polymers can be used to advantage in many applications. The applications for which these biodegradable systems were employed included fertility control,[9,10] narcotic antagonism,[11] and antimalarial chemotherapy.[12–14] DL-Polylatic acid (PLA), polyglycolic acid and their copolymers are known to be biodegradable polymers.[15–19]

The purpose of the present work was to evaluate PLA microspheres as an injectable sustained release delivery system for local anesthetics. Sustained action forms of local anesthetics have been sought in pain clinics for the control of intractable pain caused by cancer and trigeminal neuralgia.

Experimental

Materials——*n*-Butyl *p*-aminobenzoate (butamben), tetracaine hydrochloride and dibucaine hydrochloride were purchased from Tokyo Kasei Co. (Tokyo), Hoei Yakuhin Kogyo Co. (Osaka), and Teikoku Kagaku Sangyo Co. (Osaka), respectively. Tetracaine hydrochloride and dibucaine hydrochloride were transformed into their bases by treatment with sodium hydroxide solution.

PLA was prepared from DL-lactic acid[15] purchased from Wako Junyaku Kogyo Co. (Osaka). The mean molecular weight was determined viscometrically in an Ostwald viscometer to be 9100.[20]

Gelatin alkaline process, 200 bloom, was a gift from Nitta Gelatin Co. (Yao, Osaka), whereas sodium alginate was purchased from Tokyo Kasei Kogyo Co. (Tokyo).

Methylene chloride (bp 39.8°C), methyl acetate (bp 56.9°C), and ethyl acetate (bp 77.1°C) of reagent grade from Wako Junyaku Kogyo Co. (Osaka) were used without further purification.

Preparation of PLA Microspheres——Microspheres made of PLA were prepared by a solvent-evaporation process similar to that of Beck *et al.*[10] PLA and a local anesthetic were dissolved in an organic solvent with a low boiling point. The solution was then added dropwise into a round bottom flask containing 100 ml of 1% gelatin or 1% sodium alginate. The stirring rate was kept constant. Reduced pressure or heat was applied to the suspension to evaporate off the organic solvent, and then the microspheres were collected by filtration or centrifugation. The collected microspheres were dried at room temperature under a vacuum, and were sized through a standard sieve.

Observation of PLA Microspheres by Scanning Election Microscopy——The dried microspheres were observed with a scanning electron microscope (model S-430, Hitachi Manufacturing Co., Tokyo) to examine their shapes and surface characteristics.

Release Studies——The microspheres were placed inside a flask and suspended in a flask containing 25 ml of isotonic citrate–phosphate buffer solution, pH 7.4. The flask was placed in a shaker bath (model R-100, Taiyo Scientific Industrial Co., Tokyo) maintained at 37°C and was shaken horizontally. Release patterns were obtained by measuring the concentrations of drugs released from the microspheres spectrophotometrically; butamben at 285 nm, tetracaine at 310 nm, and dibucaine at 326 nm.

Results and Discussion

Physical Characteristics and Drug Contents of Microspheres

The physical characteristics and drug contents of microspheres are shown in Table I. With increase in the drug contents at preparation, the drug contents in the microspheres were increased. Butamben and dibucaine contents were larger than the tetracaine content, because butamben and dibucaine are in unionized forms whereas tetracaine is in an ionic form in 1% gelatin solution at pH 7.5. The average diameter of the microspheres was about 50 μm as measured in photomicrographs. The shape of the microspheres was essentially spherical.

Table I. Characteristics and Contents of Microspheres Prepared by Evaporation *in vacuo* Employing Methylene Chloride as a Polymer Solvent and 1% Gelatin as a Nonsolvent

Preparation	Drug	Drug/polymer ratio at preparation	Yield, %	Diameter, μm	Drug content, %
A	Butamben	10/90	79	50.6±3.4	8.1
B	Butamben	20/80	63	54.8±2.9	15.3
C	Butamben	30/70	51	54.6±2.3	25.5
D	Tetracaine	10/90	56	59.4±3.7	3.0
E	Tetracaine	20/80	75	55.3±3.3	8.9
F	Tetracaine	30/70	52	50.0±3.0	18.9
G	Dibucaine	30/70	57	56.5±2.6	24.2

Table II. Effects of Polymer Solvents and Nonsolvents on the Characteristics of the Microspheres Containing Butamben with a Drug/polymer Ratio of 10/90 at Preparation

Preparation	Method of evaporation	Polymer solvent	Nonsolvent	Stirring rate, rpm	Yield, %	Diameter, μm	Drug content, %
A	*In vacuo*	CH_2Cl_2	1% gelatin	800	79[a]	50.6±3.4	8.1
H	Warming at 40°C	CH_2Cl_2	1% gelatin	800	72[a]	84.7±6.0	6.2
I	*In vacuo*	CH_2Cl_2	1% sodium alginate	1000	10[b]	10.5±0.9	5.2
J	*In vacuo*	Methyl acetate	1% sodium alginate	1400	62[a]	35.5±1.9	6.8

a) Collected by filtration.
b) Collected by centrifugation.

The effects of polymer solvents and nonsolvents on the physical characteristics of the microspheres are shown in Table II. Preparation H, which was prepared by evaporation at 40°C under atmospheric pressure, had greater diameter than Preparation A, possibly because the viscosity of 1% gelatin solution at 40°C was smaller than that at room temperature. Preparation I, for which 1% sodium alginate was used as a nonsolvent, had very small diameter, 10 μm, possibly because the viscosity of 1% sodium alginate solution was higher than that of 1% gelatin solution. The very small microspheres could not be collected by filtration, and therefore they were collected by centrifugation. However, the yield was only about 10%.

Since methylene chloride is not a good solvent from a toxicological point of view, methyl acetate and ethyl acetate were examined as polymer solvents. Microspheres prepared by employing methyl acetate as a polymer solvent were prepared only under the following conditions; sodium alginate was used as a nonsolvent, and the stirring rate was 1400 rpm. In this case, the microspheres were larger than those with methylene chloride (Table II).

Table III. Effects of Nonsolvent Compositions on Tetracaine Contents of Microspheres Prepared by Using Tetracaine with a Drug/polymer Ratio of 20/80 at Preparation, Methylene Chloride as a Polymer Solvent and Evaporation *in vacuo*

Preparation	Nonsolvent	Yield, %	Diameter, μm	Drug content, %	Shape
E	1% gelatin	75	55.3±3.3	8.9	Sphere
L	0.27 M Na_2HPO_4 in 1% gelaitn	55	58.9±2.5	16.4	80—90% Sphere
M	0.75% tetracaine in 1% gelatin	73	54.7±2.8	19.8	80—90% Sphere
N	0.50% tetracaine in 1% gelatin	66	56.8±3.1	16.3	Sphere

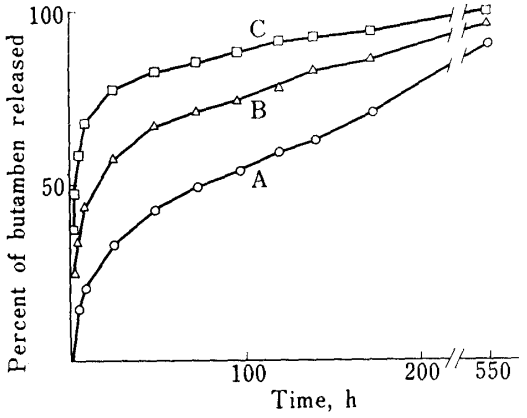


Fig. 1. Release Patterns of Butamben from PLA Microspheres Containing 8.1% (A: ○), 15.3% (B: △), and 25.5% (C: □) Butamben

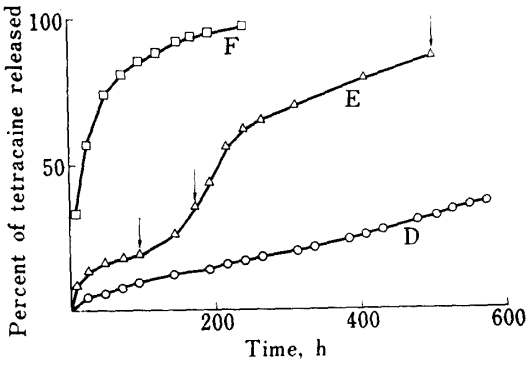
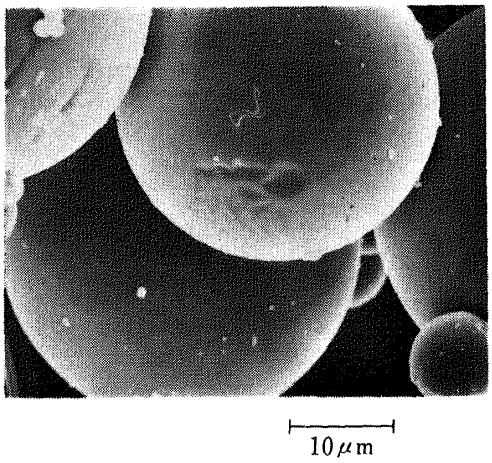


Fig. 3. Release Patterns of Tetracaine from PLA Microspheres Containing 3.0% (D: ○), 8.9% (E: △), and 18.9% (F: □) Tetracaine



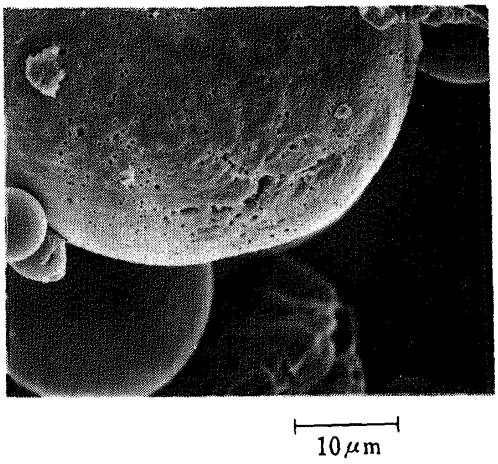


Fig. 2. Scanning Electron Photomicrographs of PLA Microspheres Containing 8.1% Butamben before Release (up) and after Release for 550 h (down)

Attempted preparation of microspheres by employing ethyl acetate as a polymer solvent failed.

In order to improve the tetracaine content (Table I), a salt or the drug itself was added to the nonsolvent. The results are shown in Table III. Preparation L, which was prepared in the presence of 0.27 M sodium phosphate (dibasic) in 1% gelatin solution, had a greater tetracaine content in the microspheres. Because tetracaine changed from an ionic form to an unionized form due to the change in pH from 7.5 in 1% gelatin solution to 9.0 in the presence of sodium phosphate (dibasic), its solubility was decreased. In this case, however, the particles were mixtures of spheres and nonspherical forms and the percentage of the particles in spherical form was decreased probably because 0.27 M sodium phosphate (dibasic) affected the viscosity of 1% gelatin solution. Preparations M and N prepared in the presence of tetracaine in gelatin solution had greater tetracaine contents. Preparation N had a high tetracaine content and a spherical form.

Release Patterns

The effect of butamben content on release patterns *in vitro* is shown in Fig. 1. It is evident that the greater the butamben content, the more rapidly was the drug released. Scanning electron microscopic observation revealed that the surface of the microspheres before release was round and very smooth, but it became very porous after release (Fig. 2). It seems likely that the release mechanism of butamben from microspheres is diffusion.

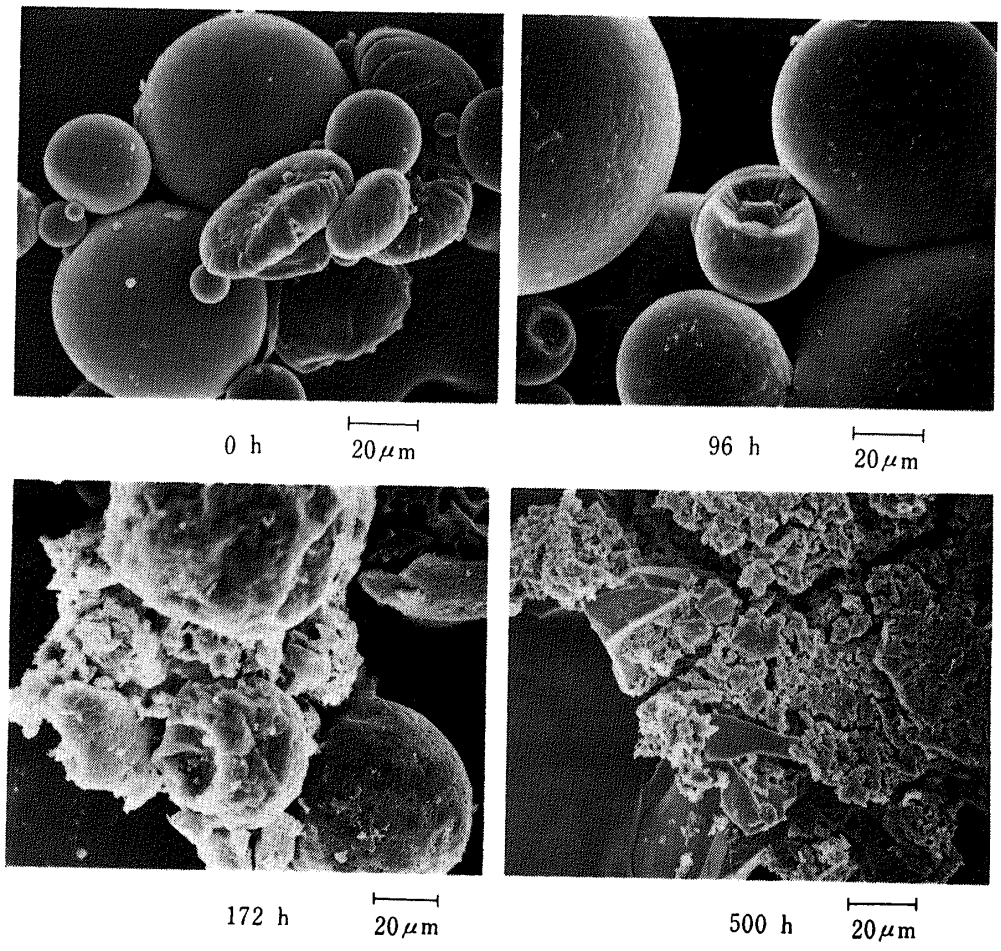


Fig. 4. Scanning Electron Photomicrographs of PLA Microspheres Containing 8.9% tetracaine (Preparation E) before Release (0 h) and after Release for 96, 172, and 500 h

The effect of tetracaine content on release patterns *in vitro* is shown in Fig. 3. The release pattern of the microspheres containing 18.9% tetracaine, Preparation F, was similar to those of the microspheres containing butamben (Fig. 1). The release patterns of the microspheres containing smaller amounts of tetracaine were different, however. For example, in Preparation E, the release rate was fast in the initial period, then it decreased, and it increased again after about 120 h, and finally it decreased after about 250 h. In Preparation D also, the release rate increased after about 400 h. Scanning electron photomicrographs showing changes in the surface characteristics of microspheres of Preparation E with time (0, 96, 172, and 500 h) are shown in Fig. 4. It is evident that second rise in the rate was a result of disintegration of the microspheres . The release pattern of microspheres containing 24.2% dibucaine (Preparation G) is shown in Fig. 5. The release pattern is similar to that of Preparation E in Fig. 3. Scanning electron microscopic observation revealed that the microspheres had disintegrated (Fig. 6). It is evident that a part of the release of the drug from the microspheres is a result of disintegration.

The effect of microsphere size on butamben release patterns *in vitro* is shown in Fig. 7. It is evident that the smaller the microsphere size, the more rapidly was the drug released, because the smaller the microsphere size, the greater was the surface area. Sustained release

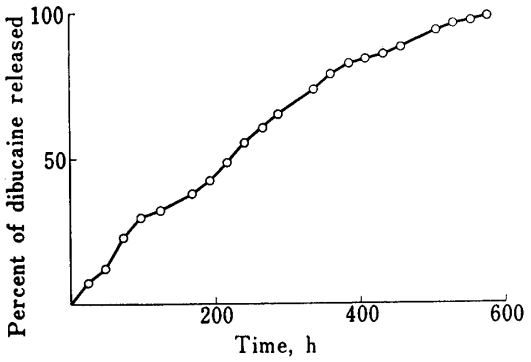
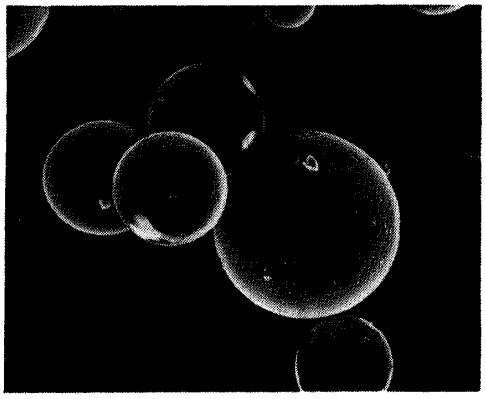


Fig. 5. Release Pattern of Dibucaine from PLA Microspheres Containing 24.2% Dibucaine, Preparation G



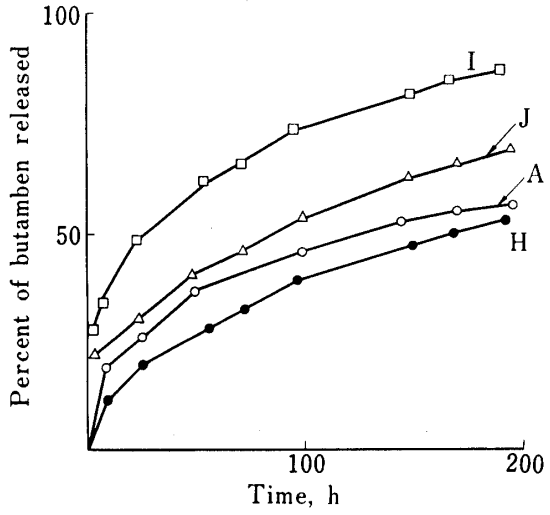


Fig. 7. Release Patterns of Butamben from PLA Microspheres of Different Sizes; 10.5± 0.9 (I: □), 35.3 ± 1.9 (J: △), 50.6±3.4 (A: ○), and 84.7±6.0 μm (H: ●)

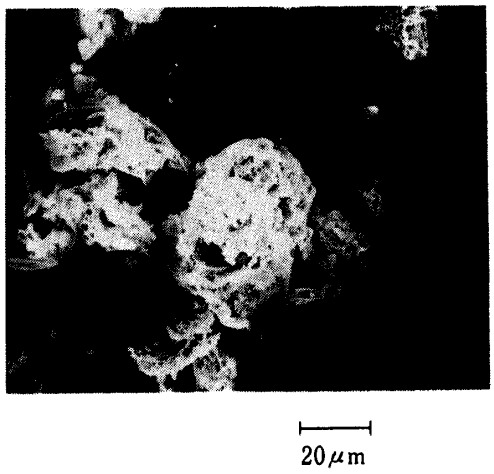


Fig. 6. Scanning Electron Photomicrographs of PLA Microspheres Containing 24.2% Dibucaine (Preparation G) before Release (up) and after Release for 600 h (down)

of local anethetics from microspheres was thus demonstrated *in vitro*. The results obtained in the present study may be applicable to the sustained control of pain in pain clinics. Sustained pharmacological effects observed after implantation of the microspheres in guinea pigs will be described in a separate paper.

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