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Influence of Physicochemical Properties of Polylactic Acid on the Characteristics and *in Vitro* Release Patterns of Polylactic Acid Microspheres containing Local Anesthetics

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DL-Polylactic acid (PLA) microspheres were prepared from three lots of PLA by a solvent-evaporation process and release patterns of butamben, tetracaine and dibucaine from these microspheres were examined. As a first step, the physicochemical characteristics of three lots of PLA were examined. The release mechanism was examined by scanning electron microscopy. Microsphere characteristics and release patterns from the microspheres were dependent on both PLA characteristics and the local anesthetics used for the preparation.

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

Polymer membranes and matrices act as a barrier which controls the rate of delivery. Several reports on sustained drug delivery using several polymers have recently been published.[1-4]

However, the application of such polymers as silicone rubber and ethylene vinyl acetate copolymer is of limited value because of their nonbiodegradability. A significant advance in sustained release formulations requires the development of polymer systems with biodegradability.

Polylactic acid (PLA), polyglycolic acid and their copolymers are known to be biodegradable, and their characteristics, biodegradabilities, and release from these polymers have been investigated.[5-10]

Various applications of these biodegradable systems have been reported, including fertility control,[11,12] narcotic antagonism,[13] and antimalarial chemotherapy.[14] We reported the use of PLA microspheres as a means to achieve sustained release of local anesthetics.[15]

The purpose of the present work was to investigate the physical characteristics of three lots of PLA and the effects of their physical characteristics on the release patterns of local anesthetics from PLA microspheres. PLA microspheres containing local anesthetics may be used 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 Kogyo Co. (Tokyo), Hoei Yakuhin Kogyo Co. (Osaka), and Teikoku Sangyo Co. (Osaka), respectively. Tetracaine hydrochloride and dibucaine hydrochloride were transformed into their bases by treatment with sodium hydroxide solution.

PLA was synthesized from DL-lactic acid[10] purchased from Wako Junyaku Kogyo Co. (Osaka). PLA was characterized morphologically by differential scanning colorimetry (standard type, Rigaku Denki Co., Tokyo) at a heating rate of 10°C/min. Intrinsic viscosity of PLA was determined with an Ostwalt viscometer employing benzene as a polymer solvent at 30°C. Gelatin, alkaline process, 200 bloom, was a gift from Nitta Gelatin Co. (Yao, Osaka). Methylene chloride of reagent grade from Wako Junyaku Kogyo Co. was used without further purification.

Preparation of PLA Microspheres——PLA microspheres were prepared by a solvent-evaporation process similar to that of Beck *et al.*[12] A total of 500 mg of PLA and a local anesthetic was dissolved in 5 ml of methylene chloride. The solution was added dropwise from a syringe into a round-bottomed flask containing 100 ml of 1% gelatin. The stirring rate was kept at 800 rpm. Reduced pressure was applied to the suspension to evaporate off the methylene chloride at room temperature, and then the microspheres were collected by filtration. The collected microspheres were sized through a standard sieve.

Observation of PLA Microspheres——The dried microspheres were observed with a scanning electron microscope (model S-430, Hitachi Manufacturing Co., Tokyo) to examine their shapes and surface characteristics. Moreover, 200 dried microspheres were observed with an optical microscope (model BH-2, Olympus Optic Industrial Co., Tokyo). Observations were made twice, and the mean of 2 experiments was recorded as an approximate percentage.

Solubility of Local Anesthetic——About 100 mg of butamben, tetracaine, or dibucaine was suspended in 10 ml of isotonic citrate-phosphate buffer solution, pH 7.4, in a flask. The flask was placed in a shaker bath (model R-100, Taiyo Scientific Industrial Co., Tokyo) maintained at 37°C and was shaken horizontally at a rate of 90 cpm for 24 h. Solubilities were obtained by measuring the concentrations of drugs spectrophotometrically after appropriate dilution; butamben at 285 nm, tetracaine at 310 nm, and dibucaine at 326 nm.

Release Studies——About 10 mg of microspheres was suspended in 25 ml of isotonic citrate-phosphate buffer solution, pH 7.4, in a flask. The flask was placed in a shaker bath (model R-100, Taiyo Scientic Industrial Co., Tokyo) maintained at 37°C and was shaken horizontally at a rate of 90 cpm. Release patterns were obtained by measuring the concentrations of drugs released from the microspheres spectrophotometrically as described for the solubility measurement.

Results and Discussion

Physicochemical Characteristics of PLA

The physicochemical characteristics of PLA are shown in Table I. Glass transition temperature, melting point and degradation point of PLA 3 were higher than those of PLA's 1 and 2. PLA's 1, 2, and 3 showed intrinsic viscosity values in benzene at 30°C of 0.32, 0.54, and 0.70 dl/g, respectively. Their molecular weights, which were calculated from the intrinsic viscosity data were 9100, 17000, and 25000, respectively.[10] The high glass transition temperature, melting point and degradation point of PLA 3 may be related to its high molecular weight. Moreover, the colors of PLA's 1, 2, and 3 were light brown, light cream, and white, respectively, and only PLA 1 did not form a membrane in a melt press.

Table I. Physicochemical Characteristics of Three Lots of Polylactic Acid

Polymer	Glass transition temperature, °C	Melting point, °C	Degradation point, °C	Intrinsic viscosity, dl/g	Molecular weight
PLA 1	55	270—280	280	0.32	9100
PLA 2	58	266	274	0.54	17000
PLA 3	58	294	324	0.70	25000

Physical Characteristics and Drug Contents of Microspheres

Table II shows the physical characteristics and butamben contents in microspheres prepared from three lots of PLA. The yield, diameter, shape, and drug content of preparations

Table II. Characteristics and Content of Butamben in Microspheres prepared by *in Vacuo* Evaporation employing Methylene Chloride as a Polymer Solvent and 1% Gelatin as a Nonsolvent with a Drug/polymer Ratio at Preparation of 10/90

Preparation	Polymer used	Yield, %	Diameter, μm	Drug content, %	Shape
A	PLA 1	79	50.6±3.4	8.1±0.25	Sphere
B	PLA 2	79	62.5±4.7	7.8±0.43	Sphere
C	PLA 3	70	60.2±5.5	7.4±0.07	Sphere

Table III. Characteristics and Content of Tetracaine in Microspheres prepared by *in Vacuo* Evaporation employing Methylene Chloride as a Polymer Solvent and 1% Gelatin as a Nonsolvent with a Drug/polymer Ratio at Preparation of 30/70

Preparation	Polymer used	Yield, %	Percent of size below 149 μm	Diameter, μm	Drug content, %	Shape
D	PLA 1	51	56	50.4±3.1	16.5±1.64	Sphere
E	PLA 2	66	28	——	18.1±1.13	10% sphere
F	PLA 3	61	96	48.8±2.7	13.2±0.75	Sphere

Table IV. Characteristics and Content of Dibucaine in Microspheres prepared by *in Vacuo* Evaporation employing Methylene Chloride as a Polymer Solvent and 1% Gelatin as a Nonsolvent with a Drug/polymer Ratio at Preparation of 30/70

Preparation	Polymer used	Yield, %	Percent of size below 149 μm	Diameter, μm	Drug content, %	Shape
G	PLA 1	57	94	56.4±2.6	24.2±0.20	Sphere
H	PLA 2	78	94	55.1±3.5	23.8±0.35	50% sphere
I	PLA 3	74	89	57.4±4.4	20.9±0.01	Sphere

A, B, and C were only slightly different. The physicochemical characteristics of PLA did not very much influence the physical characteristics and butamben contents of microspheres.

Tables III and IV show the physical characteristics and tetracaine or dibucaine content, respectively, in microspheres. When PLA 2 was used, the proportion of small microspheres (size below 149 μm) was decreased in tetracaine microspheres and mixtures of spheres and nonspherical forms were obtained in tetracaine and dibucaine microspheres. PLA 2 tended to aggregate easily in 1% gelatin solution.

Release Patterns

The effect of the characteristics of PLA on the relase patterns of butamben *in vitro* is shown in Fig. 1. It is evident that the drug release from preparation A (PLA 1) was more rapid than that from preparation B (PLA 2) or C (PLA 3). Scanning electron microscopic observation revealed that the surface of PLA 1 microspheres after release was very porous, whereas PLA 2 and 3 microspheres after release were mixtures of round and very smooth spheres and disintegrated forms (Fig. 2). The above observation may explain the rapidity of drug release from preparation A (PLA 1).

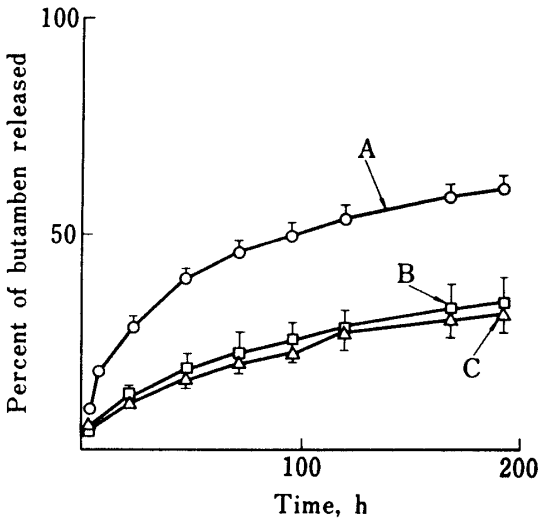


Fig. 1. Release Patterns of Butamben from Preparations A (○, PLA 1), B (□, PLA 2), and C (△, PLA 3)

Each point represents the mean ± S.E. of 3 experiments.

The effect of the characteristics of PLA on tetracaine release patterns *in vitro* is shown in Fig. 3. It is evident that the drug release from preparation F (PLA 3) was much slower than that from preparation D (PLA 1) or E (PLA 2). We reported that the release of tetracaine from microspheres was a result of disintegration of microspheres.[15] Moreover, scanning

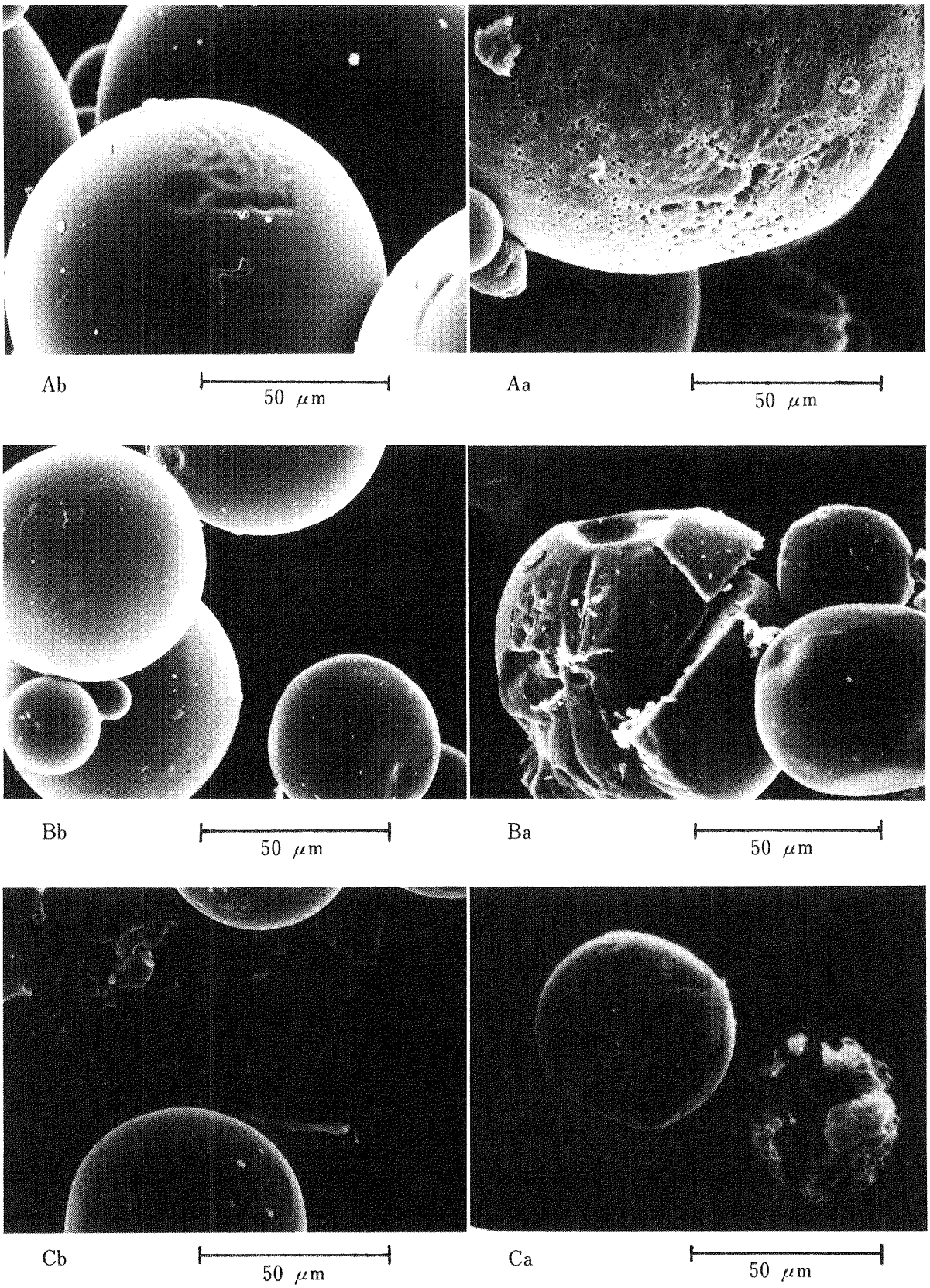


Fig. 2. Scanning Electron Photomicrographs of PLA Microspheres containing Butamben before and after Drug Release

Key: Ab, preparation A before release; Aa, preparation A after release; Bb, preparation B before release; Ba, preparation B after release; Cb, preparation C before release; Ca, preparation C after release.

electron microscopic observation revealed that three kinds of PLA microspheres containing tetracaine had disintegrated after drug release (Fig. 4). PLA 3 seems to be the most stable among the three lots of PLA; therefore, its disintegration rate was smaller than those of PLA's 1 and 2, so that tetracaine release from PLA 3 microspheres was slow.

The effect of the characteristics of PLA on dibucaine release patterns *in vitro* is shown in Fig. 5. Release patterns varied somewhat among the three. It seems likely that the disintegration of dibucaine microspheres was slower than that of tetracaine microspheres. Therefore, the disintegration of dibucaine microspheres may be relatively little influenced by the characteristics of PLA. It is evident that three kinds of PLA microspheres containing dibu-

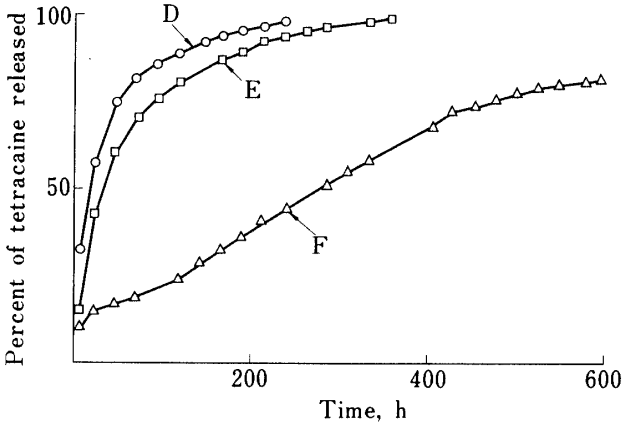
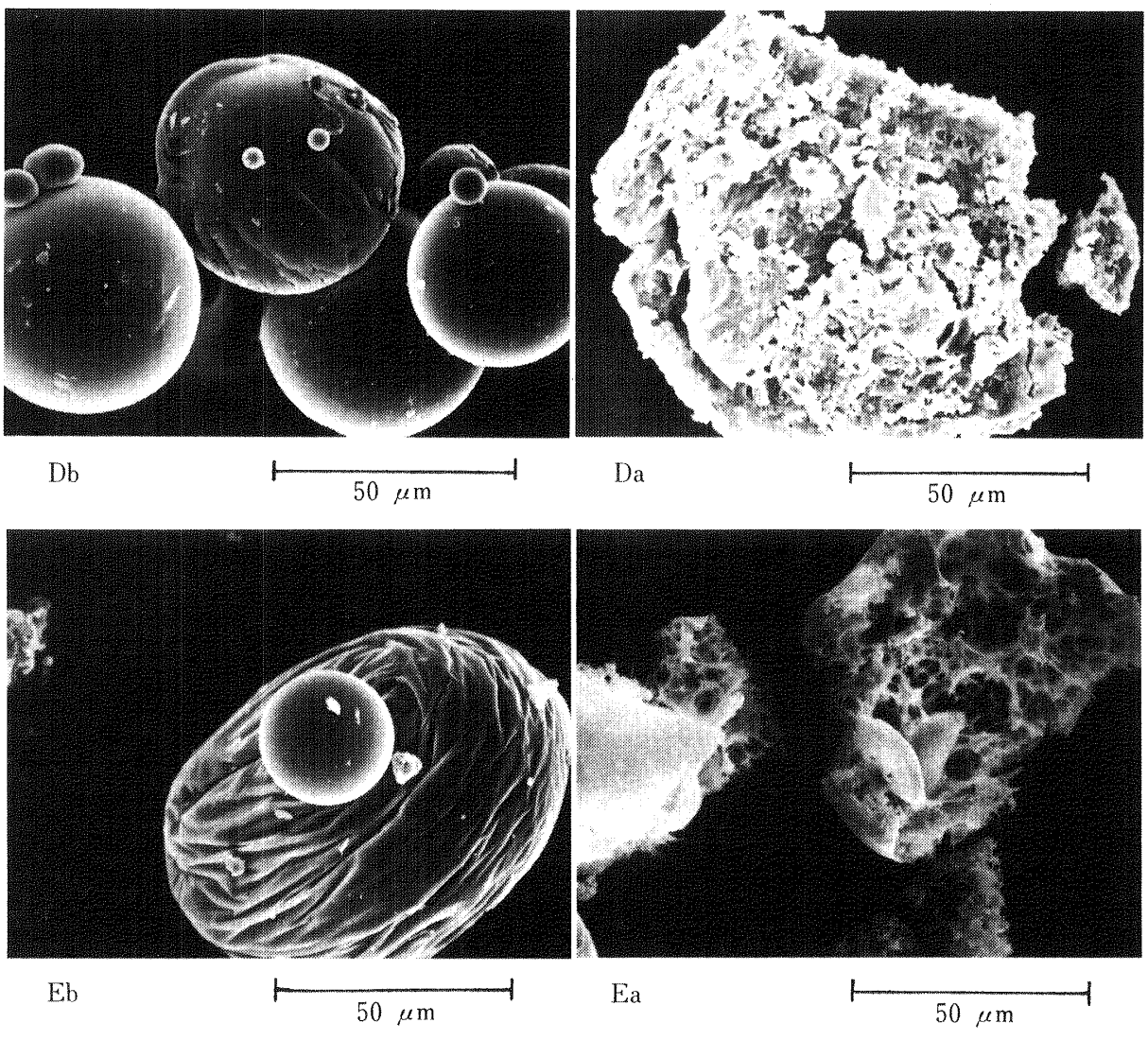


Fig. 3. Release Patterns of Tetracaine from Preparations D (○, PLA 1), E (□, PLA 2), and F (△, PLA 3)

Each point represents the mean of 2 experiments.



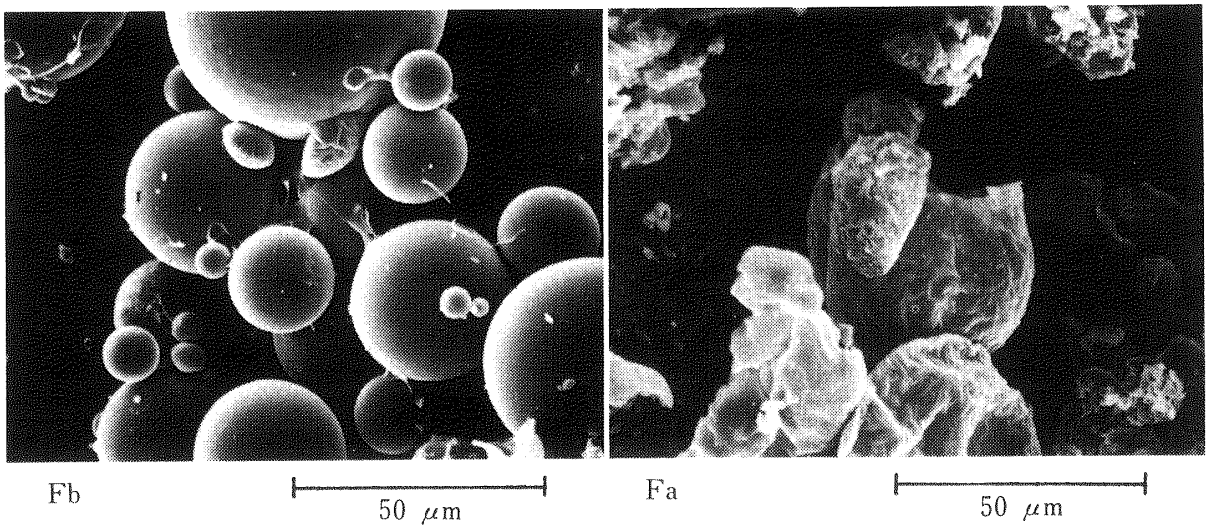


Fig. 4. Scanning Electron Photomicrographs of PLA Microspheres containing Tetracaine before and after Drug Release

Key: Db, preparation D before release; Da, preparation D after release; Eb, preparation E before release; Ea, preparation E after release; Fb, preparation F before release; Fa, preparation F after release.

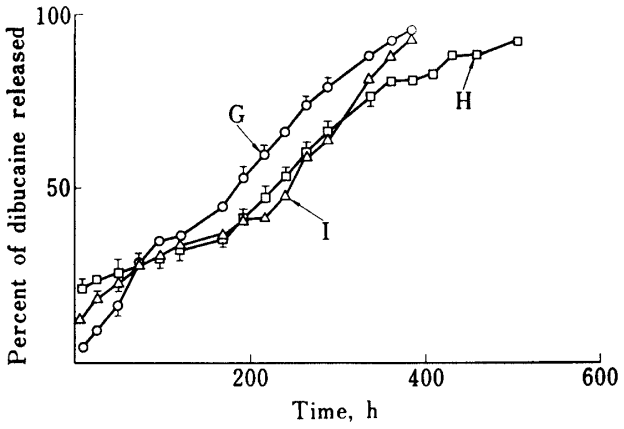


Fig. 5. Release Patterns of Dibucaine from Preparations G (○, PLA 1), H (□, PLA 2), and I (△, PLA 3)

Each point represents the mean ± S.E. of 3 experiments.

caine (preparations G, H, and I) had disintegrated much less after drug release (Fig. 6) than tetracaine microspheres (Fig. 4).

The mechanism of release of drugs from microspheres may be either permeation through matrices or permeation through canals. The latter is related to the solubility of drugs in the release medium.

Solubility values of butamben, tetracaine, and dibucaine in an isotonic citrate-phosphate buffer solution, pH 7.4, at 37°C were 8.9×10^{-4}, 1.0×10^{-2}, and 1.3×10^{-3} M, respectively. Because of the large solubility of tetracaine in the release medium, osmotic pressure may build up within microspheres, since the effective osmotic pressure π_e may be related to solubility by the following equation.[16]

$$\pi_e = \sigma RTC$$

where σ is ratio of effective osmotic pressure to the theoretical value, R is the gas constant, T is the absolute temperture, and C is the solubility of the drug in the medium. If a large osmotic pressure builds up, microspheres may disintegrate, and this may have occurred with the tetracaine microspheres shown in Fig. 4.

The relationship between disintegration and release patterns may be summarized in the following way.

When little disintegration takes place, the release patterns (Fig. 1) are those predicted by the Higuchi square-root-of-time equation.[17] When extensive disintegration takes place, most of the drugs are released in the initial stage (D and E in Fig. 2). When gradual disintegration takes place, release patterns can approach those of zero-order release (F in Fig. 3 and Fig. 5).

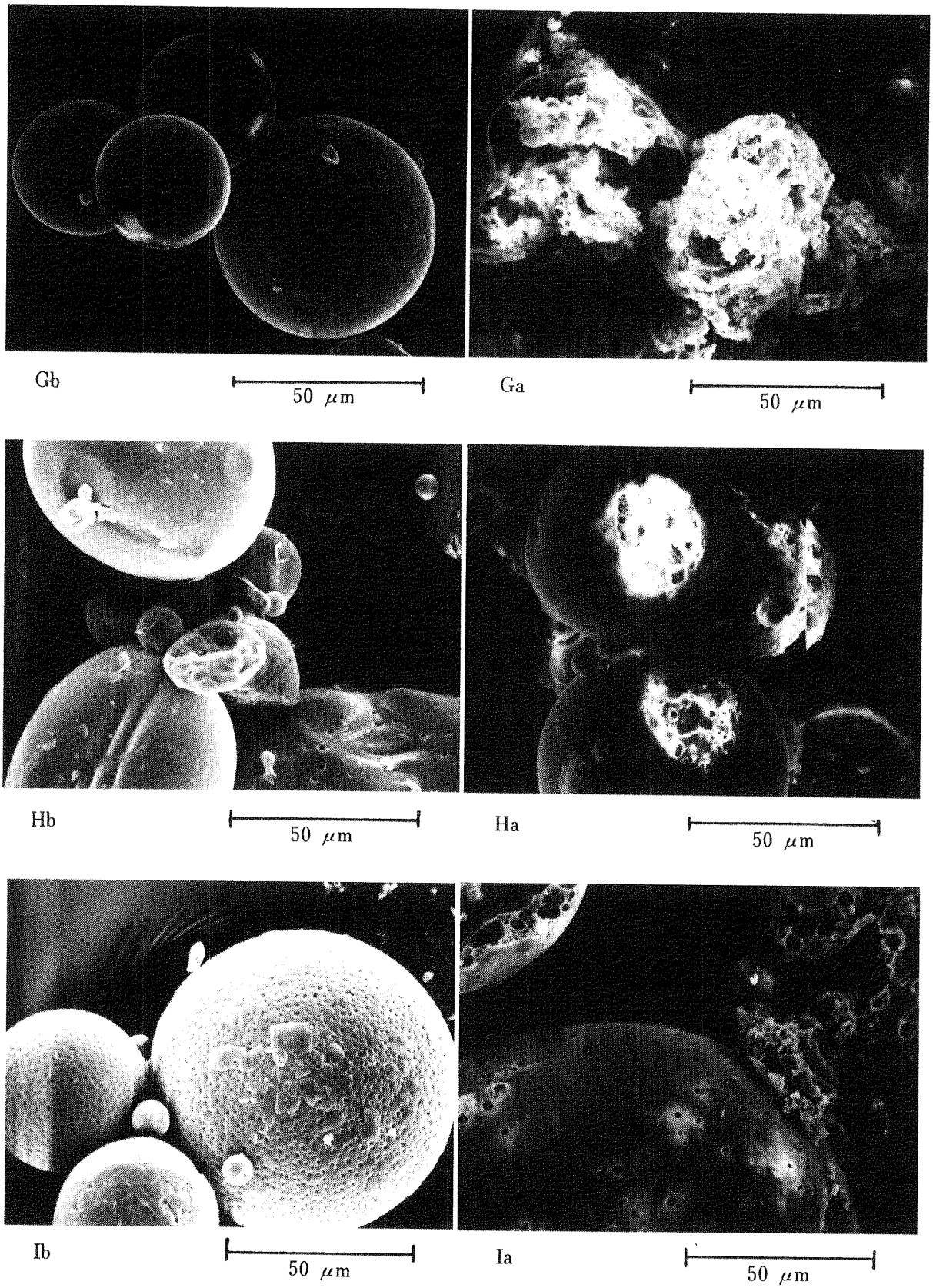


Fig. 6. Scanning Electron Photomicrographs of PLA Microspheres containing Dibucaine before and after Drug Release

Key: Gb, preparation G before release; Ga, preparation G after release; Hb, preparation H before release; Ha, preparation H after release; Ib, preparation I before release; Ia, preparation I after release.

The results obtained in the present study indicate that the release rate can be controlled by choice of PLA's with different characteristics. These sustained release formulations may be applicable to the control of pain in clinics, though extensive preliminary examinations in experimental animals will be required.

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