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Preparation and Evaluation *in Vitro* of Polycarbonate Microspheres Containing Local Anesthetics

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To achieve sustained release of local anesthetics from injectable formulations, polycarbonate microspheres were prepared by a solvent-evaporation process. Release patterns of benzocaine, lidocaine, and dibucaine from polycarbonate microspheres *in vitro* were investigated. Drug contents and sizes of microspheres affected the release patterns of the local anesthetics. The release rate of drugs from smaller microspheres was greater than that from larger microspheres, and with increase in the drug contents, the release rate increased. Microspheres before and after release studies were observed by scanning electron microscopy. It was confirmed that the use of polycarbonate microspheres resulted in sustained release of local anesthetics.

Keywords——poly(propylene carbonate); poly(ethylene carbonate); biodegradable polymer; microsphere; release rate; sustained release; local anesthetics; benzocaine; lidocaine; dibucaine

Biodegradable polymers such as polylactic acid and polyglycolic acid have been examined as materials for sustained release formulations.[1–4] When a polymer system is to be used as a drug carrier, biodegradability of the polymer is a significant advantage.

Applications of biodegradable polymer systems to fertility control,[5] narcotic antagonism,[1] anticancer chemotherapy,[6,7] and local anesthesia[8] have been reported. Poly(propylene carbonate) and poly(ethylene carbonate) have been synthesized from carbon dioxide and the corresponding epoxides,[9] and poly(ethylene carbonate) was found to be biodegradable in animal experiments.[10] Kawaguchi *et al.* prepared polycarbonate pellets containing anticancer drugs which showed sustained release of the drugs over extended periods of time.[11]

In the present work polycarbonate microspheres containing local anesthetics were prepared and their characteristics, including diameter, drug content and drug release-sustaining properties, were examined.

Experimental

Materials——Benzocaine and dibucaine hydrochloride were purchased from Tokyo Kasei Kogyo Co. (Tokyo) and Teikoku Sangyo Co. (Osaka), respectively. Lidocaine was a gift from Fujisawa Yakuhin Kogyo Co. (Osaka). Dibucaine hydrochloride was transformed into its base by treatment with sodium hydroxide solution.

Poly(propylene carbonate) and poly(ethylene carbonate) were prepared according to the procedures reported earlier.[12] Their intrinsic viscosity values were 0.58 and 0.37 dl/g, respectively, in dioxane at 25 °C. Determination of viscosity was carried out by the method of Kawaguchi *et al.*[10] The molecular weights ranged from 50000 to 150000.

Alkaline processed gelatin, 200 bloom, was a gift from Nitta Gelatin Co. (Yao, Osaka). Sodium alginate was purchased from Wako Junyaku Kogyo Co. (Osaka). Methylene chloride, chloroform, and tetrahydrofuran (reagent grade from Wako Junyaku Kogyo Co.) were used without further purification.

Preparation of Polycarbonate Microspheres——Polycarbonate microspheres were prepared by a solvent-evaporation process similar to that of Wakiyama *et al.*[13] The polycarbonate and local anesthetic (total of 500 mg) were dissolved in 5 ml of methylene chloride. The solution was then added to a round-bottomed flask containing

100 ml of 2% gelatin or 1% sodium alginate solution as a nonsolvent by using a syringe. The stirring rates of 2% gelatin and 1% sodium alginate solutions were 850 and 1350 rpm, respectively. Methylene chloride was evaporated off *in vacuo* at room temperature or by warming at 40 °C, and the polycarbonate microspheres were collected by centrifugation and filtration. The microspheres were then dried *in vacuo* in a desiccator for at least 24 h and sized through a sieve (100 mesh).

Observation of Polycarbonate Microspheres——The obtained microspheres were observed with a scanning electron microscope (model MSM-102, Akashi Manufacturing Co., Tokyo) to check their shapes and surface structures. The diameter of microspheres was determined by using an optical microscope (model BH-2, Olympus Optical Industrial Co., Tokyo) or the scanning electron microscope.

Drug Content of Polycarbonate Microspheres——To determine the drug content of polycarbonate microspheres, weighed amounts of poly(propylene carbonate) microspheres and poly(ethylene carbonate) microspheres were dissolved in tetrahydrofuran and chloroform, respectively. The concentrations of the drugs in the organic solutions were determined spectrophotometrically; benzocaine at 285 nm, lidocaine at 245 nm, and dibucaine at 326 nm.

Release Studies——A weighed amount of microspheres (approximately 10 mg) was placed in 25 ml of isotonic citrate-phosphate buffer solution (pH 7.4) preheated to 37 °C in a flask. The flask was placed in a shaker bath (model Personal H, Taiyo Scientific Industrial Co., Tokyo) maintained at 37 °C and was shaken horizontally at a rate of 100 cpm. An aliquot of the buffer solution was taken out periodically thereafter and the concentration of the drug in the solution was determined spectrophotometrically for benzocaine and dibucaine and gas-liquid chromatographically for lidocaine. The amount of the drug released from the microspheres was calculated.

Gas-Liquid Chromatographic Determination of Lidocaine——Five milliliters of the sample solution containing lidocaine was made alkaline with 0.5 ml of 0.5 M trisodium phosphate solution. Ethyl acetate (3 ml for the first extraction and 2 ml for the second extraction) was added to the solution, and the mixture was shaken for 5 min in each case. The ethyl acetate layer was obtained by centrifugation at 2500 rpm for 5 min. Then 4 ml (2 ml plus 2 ml) of the combined ethyl acetate extract was evaporated *in vacuo* at 50 °C. The residue was dissolved in 1 ml of chloroform containing *p*-dimethylaminobenzaldehyde (20 μg/ml) as an internal standard, and 2 μl of the solution was injected into the column. A Shimadzu GC-3BF gas chromatograph equipped with a flame ionization detector was used under the following conditions: 0.3 cm × 2.1 m glass coil column packed with 1% Carbowax-20M on 100—200 mesh Gaschrom P; carrier gas, N_2 (100 ml/min); column temperature, 190 °C; injection port temperature, 215 °C.

Results and Discussion

Benzocaine–Poly(propylene carbonate) Microspheres

The physical characteristics and benzocaine contents of poly(propylene carbonate) microspheres are shown in Table I. With increase in the benzocaine/polymer ratio at preparation, the benzocaine content of microspheres increased, while the yield of microspheres decreased. When the drug/polymer ratio at preparation was higher, more drug dissolved in the nonsolvent during the preparation. The decrease in the yield of microspheres therefore resulted from escape of the drug into the nonsolvent.

The diameter of Preparation D was smaller than those of Preparations A, B, and C. The formation of smaller microspheres might be attributed to the higher viscosity of 1% sodium

Table I. Characteristics of Benzocaine–Poly(propylene carbonate) Microspheres Prepared by *in Vacuo* Evaporation of Methylene Chloride

Preparation	Drug/polymer ratio at prep.	Nonsolvent	Stirring rate (rpm)	Yield[a] (%)	Diameter[b] (μm)	Drug content (%)
A	10/90	2% gelatin	850	88.3	47.9±3.7	3.3
B	30/70	2% gelatin	850	62.4	37.7±2.8	9.9
C	50/50	2% gelatin	850	52.9	38.5±2.6	17.9
D	10/90	1% sodium alginate	1350	32.4	9.8±0.9	3.0

a) $\text{Yield (\%)} = \dfrac{\text{total weight of microspheres obtained}}{\text{total weight of polymer and drug used}} \times 100.$

b) Mean±S.D. ($n=100$).

alginate solution than that of 2% gelatin solution. Such a variation of diameter with change of the nonsolvent has been observed in the preparation of polylactic acid microspheres by Wakiyama *et al.*[13]

In the preparation of microspheres, a longer evaporating time for methylene chloride was needed in 1% sodium alginate solution than in 2% gelatin solution. The microspheres prepared in 1% sodium alginate solution tended to aggregate. Consequently the yield of Preparation D was much lower than that of Preparation A.

The effect of benzocaine content on release patterns *in vitro* is shown in Fig. 1. The release rate of benzocaine from the microspheres increased with increase in the content of the drug.

The effect of size of microspheres on the release patterns of benzocaine *in vitro* is shown in Fig. 2. It was found that the smaller the size of microspheres was, the more rapid was the release rate of the drug, because smaller microspheres have a greater surface area available for drug release.

Scanning electron photomicrographs of poly(propylene carbonate) microspheres before

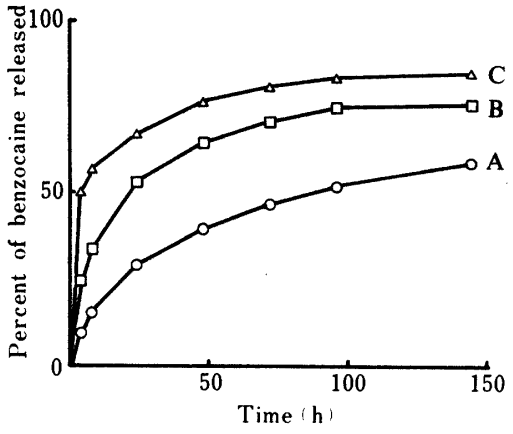

Fig. 1. Release Patterns of Benzocaine from Poly(propylene carbonate) Microspheres Containing 3.3% (A, ○), 9.9% (B, □), and 17.9% (C, △) Benzocaine

Each value represents the mean of two experiments.

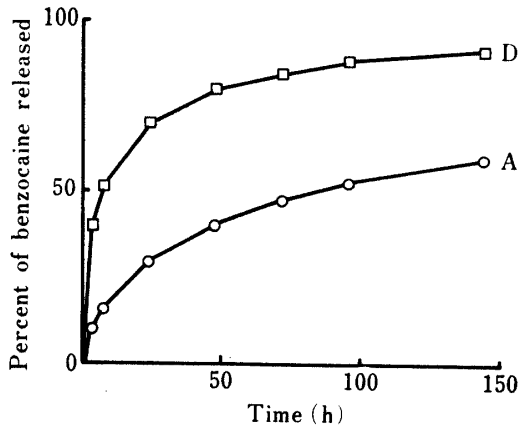

Fig. 2. Release Patterns of Benzocaine from Poly(propylene carbonate) Microspheres of Different Sizes; $47.9 \pm 3.7\,\mu m$ (A, ○), $9.8 \pm 0.9\,\mu m$ (D, □)

Microspheres contained about 3% benzocaine. Each value represents the mean of two experiments.

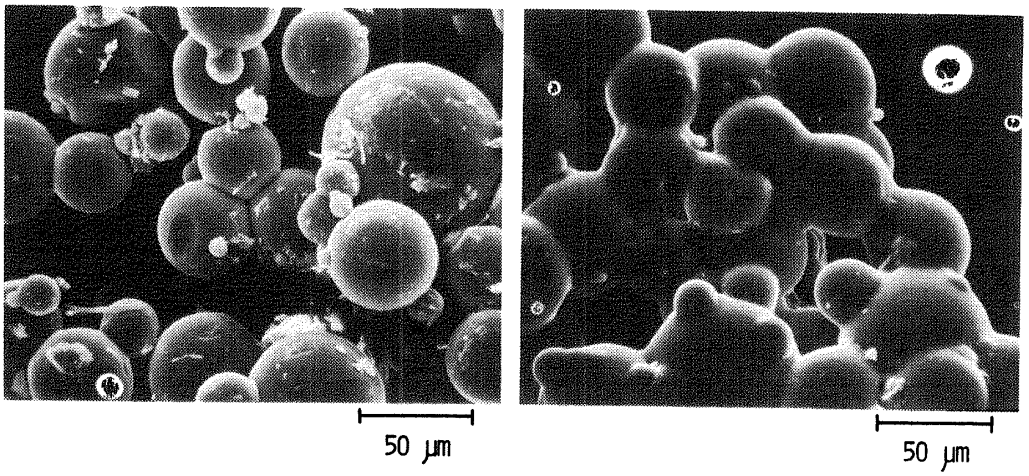

Fig. 3. Scanning Electron Photomicrographs of Poly(propylene carbonate) Microspheres Containing 17.9% Benzocaine before Release (Left) and after Release at 144 h (Right)

and after release experiments are shown in Fig. 3. The microspheres containing 17.9% benzocaine before release were round and possibly contained a small amount of benzocaine crystals attached to the surface. The relatively large initial burst observed in the release pattern of Preparation C (Fig. 1) may be attributable to the drug initially present at or near the surface of the microspheres. Microspheres containing 9.9 and 3.3% benzocaine (Preparations A and B) were round and very smooth-surfaced. The microspheres after release were aggregated; this might be a result of the temperature (37 °C) used in the release experiments.

Lidocaine–Poly(propylene carbonate) Microspheres

Characteristics of poly(propylene carbonate) microspheres containing lidocaine are shown in Table II. Microspheres were prepared under two different evaporating conditions; *in vacuo* and at 40 °C. The diameters of the microspheres when 1% sodium alginate solution was used as a nonsolvent (Preparations G and H) were very small, as in the benzocaine system (Table II and Fig. 4).

The release patterns of lidocaine from poly(propylene carbonate) microspheres are shown in Fig. 5. The size of microspheres affected the release rate of lidocaine; that is, the

TABLE II. Characteristics of Lidocaine–Poly(propylene carbonate) Microspheres

Preparation	Drug/polymer ratio at prep.	Nonsolvent	Stirring rate (rpm)	Method of evaporation	Yield[a] (%)	Diameter[b] (μm)	Drug content (%)
E	10/90	2% gelatin	850	*In vacuo*	58.2	38.5±3.1	2.1
F	30/70	2% gelatin	850	*In vacuo*	32.4	47.9±3.5	5.2
G	30/70	1% sodium alginate	1350	*In vacuo*	25.0	9.3±1.0	4.7
H	30/70	1% sodium alginate	1350	Warming at 40 °C	21.6	9.6±1.0	5.4

a) $\text{Yield}\ (\%)=\dfrac{\text{total weight of microspheres obtained}}{\text{total weight of polymer and drug used}}\times 100.$

b) Mean ± S.D. ($n=100$).

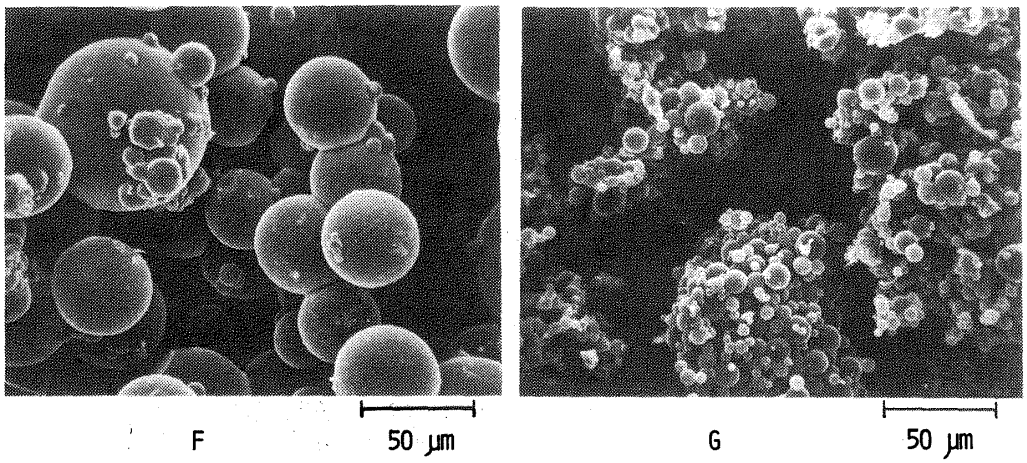

Fig. 4. Scanning Electron Photomicrographs of Poly(propylene carbonate) Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (Left; 47.9±3.5 μm, F) or 1% Sodium Alginate (Right; 9.3±1.0 μm, G) as a Nonsolvent

Microspheres contained 5.2% (left, F) and 4.7% (right, G) lidocaine.

release rate of lidocaine from smaller microspheres (Preparation G) was greater than that from larger microspheres (Preparation F).

The evaporating condition (*in vacuo* or warming) did not affect the size very much (Table II, Preparations G and H) and did not alter the surface structure of the microspheres or the release rate of lidocaine from the microspheres (not shown).

Dibucaine–Poly(propylene carbonate) Microspheres

The characteristics of poly(propylene carbonate) microspheres containing dibucaine are shown in Table III. With increase in the dibucaine/polymer ratio at preparation, the dibucaine content of microsphres was increased. Because of the low solubility of dibucaine in the aqueous solution, the dibucaine content of microspheres was higher than the benzocaine content of microspheres at the same drug/polymer ratio at preparation (Preparations B, C *vs.* J, M).

Scanning electron photomicrographs of Preparations J and M are shown in Fig. 6. The surface of the microspheres was relatively smooth when the dibucaine content was up to 26.3%, but the surface became rough and the shape of microspheres tended to be nonspherical when the dibucaine content exceeded 26.3%.

The effect of the dibucaine content on release patterns from poly(propylene carbonate) microspheres are shown in Fig. 7. The release rates of dibucaine from microspheres containing 15.2 and 26.3% dibucaine (Preparations I and J) were very slow. On the other hand, the release of dibucaine from Preparation M initially containing 47.4% dibucaine was almost

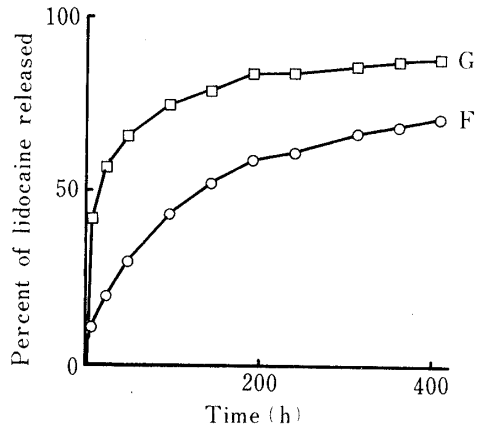

Fig. 5. Release Patterns of Lidocaine from Poly(propylene carbonate) Microspheres of Different Sizes

The microspheres, which had diameters of 47.9±3.5 μm (F, ○) and 9.3±1.0 μm (G, □), contained about 5% lidocaine. Each value represents the mean of two experiments.

TABLE III. Characteristics and Dibucaine Contents of Poly(propylene carbonate) Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (pH 7.5) as a Nonsolvent

Preparation	Drug/polymer ratio at prep.	Yield[a] (%)	Diameter[b] (μm)	Drug content (%)
I	20/80	57.3	41.5±3.6	15.2
J	30/70	69.4	48.7±3.6	26.3
K	40/60	77.1	43.6±3.2	37.4
L	45/55	69.2	49.0±3.7	42.7
M	50/50	79.5	53.1±4.0	47.4

a) $\text{Yield}\ (\%)=\dfrac{\text{total weight of microspheres obtained}}{\text{total weight of polymer and drug used}}\times 100.$

b) Mean±S.D. ($n=100$).

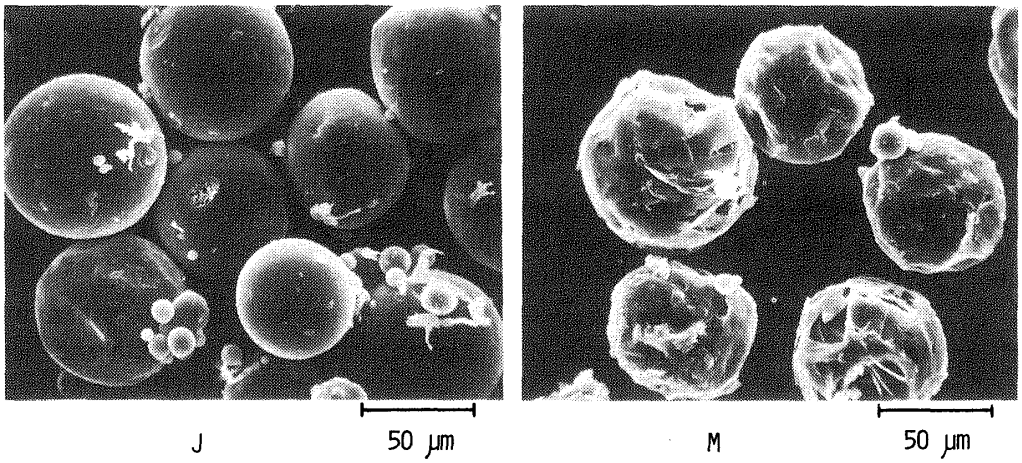

Fig. 6. Scanning Electron Photomicrographs of Poly(propylene carbonate) Microspheres Containing 26.3% (Left, J) and 47.4% (Right, M) Dibucaine before Release

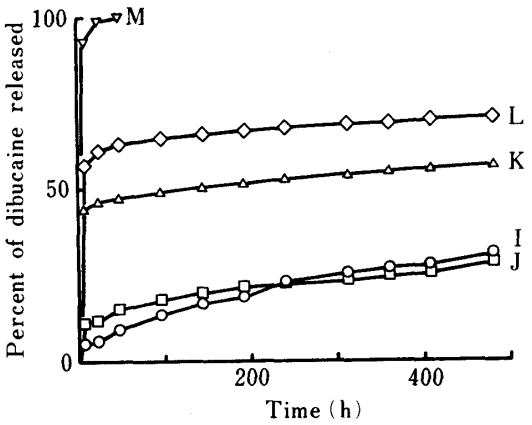

Fig. 7. Release Patterns of Dibucaine from Poly(propylene carbonate) Microspheres Containing 15.2% (I, ○), 26.3% (J, □), 37.4% (K, △), 42.7% (L, ◇), and 47.4% (M, ▽) Dibucaine

Each value represents the mean of two experiments.

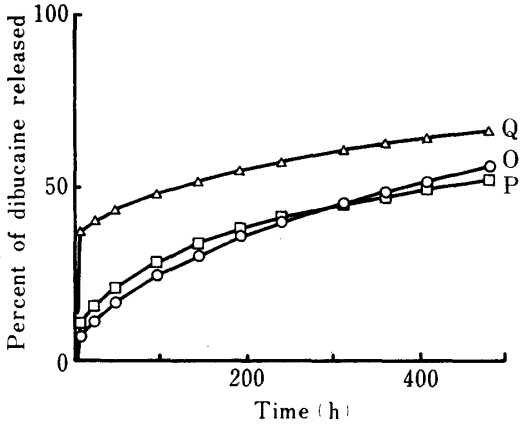

Fig. 8. Release Patterns of Dibucaine from Poly(ethylene carbonate) Microspheres Containing 18.0% (O, ○), 29.0% (P, □), and 41.0% (Q, △) Dibucaine

Each value represents the mean of two (O, Q) or three (P) experiments.

completed in 48 h. The release rates of dibucaine from Preparations K and L (dibucaine contents of 37.4 and 42.7%, respectively) were very fast in the initial period and became very slow thereafter. The microspheres after release were aggregated, as in the benzocaine system.

The large initial burst observed in Preparations K and L was attributable to a relatively large amount of dibucaine initially present at or near the surface of the microspheres. Very slow release after the initial burst may be considered to be a consequence of both the small diffusivity of dibucaine in the polymer matrix and aggregation of the microspheres, which would decrease the surface area available for release.

Dibucaine–Poly(ethylene carbonate) Microspheres

Another polycarbonate, poly(ethylene carbonate), was used to prepare microspheres containing dibucaine. Some characteristics of these microspheres are given in Table IV. The yield and drug content of the microspheres were similar to, though the diameter was larger by approximately 10 μm than, those of corresponding poly(propylene carbonate) microspheres.

TABLE IV. Characteristics and Dibucaine Contents of Poly(ethylene carbonate) Microspheres Prepared by Evaporation *in Vacuo* with Methylene Chloride as a Polymer Solvent and 2% Gelatin (pH 7.5) as a Nonsolvent

Preparation	Drug/polymer ratio at prep.	Yield[a] (%)	Diameter (μm)	Drug content (%)
O	20/80	68.4	53.0±3.7[b]	18.0
P	30/70	64.8	57.2±3.0[c]	29.0
Q	40/60	72.7	61.7±3.0[b]	41.0

a) $\text{Yield (\%)} = \frac{\text{total weight of microspheres obtained}}{\text{total weight of polymer and drug used}} \times 100.$
b) Mean±S.D. ($n=100$).
c) Mean±S.D. ($n=150$).

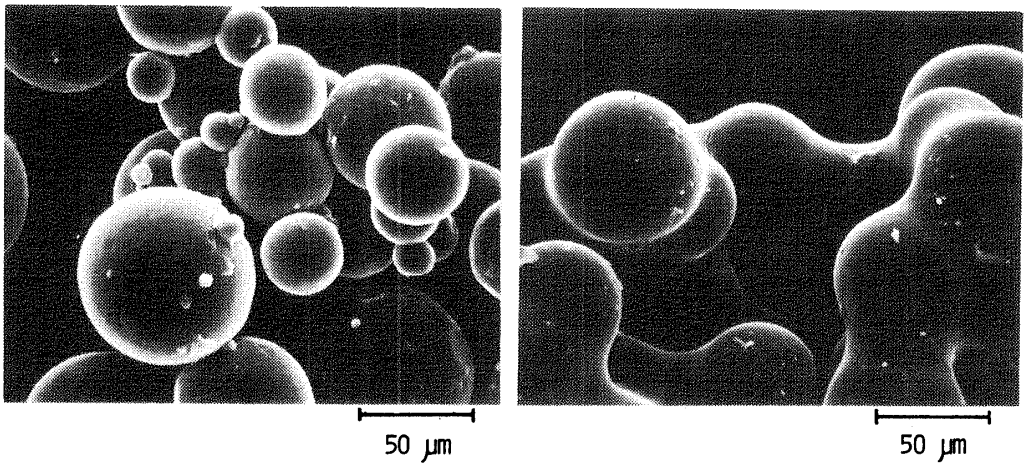

Fig. 9. Scanning Electron Photomicrographs of Poly(ethylene carbonate) Microspheres Containing 29.0% Dibucaine before (Left) and after Release at 480 h (Right)

Figure 8 shows the release patterns of dibucaine from poly(ethylene carbonate) microspheres. In spite of the different drug contents, the release pattern of Preparation O was similar to that of Preparation P. Preparation Q containing 41.0% dibucaine showed a large initial burst (0—8 h) and a slow release rate thereafter. These phenomena were similar to those observed in poly(propylene carbonate) microspheres.

Scanning electron photomicrographs of poly(ethylene carbonate) microspheres containing 29% dibucaine before and after release studies are shown in Fig. 9. There was little difference in shape between poly(ethylene carbonate) microspheres and poly(propylene carbonate) microspheres when the drug contents were approximately the same. Aggregation was observed in the poly(ethylene carbonate) microspheres after drug release, as in the other systems.

General Discussion

In the present study, microspheres containing local anesthetics were prepared by using two polycarbonates; poly(propylene carbonate) and poly(ethylene carbonate). These polycarbonate microspheres prolonged the release of the local anesthetics tested (benzocaine, dibucaine, and lidocaine).

When the drug contents of poly(propylene carbonate) microspheres were compared, dibucaine was contained in a greater proportion than the other two drugs at the same drug/polymer ratio at preparation. As for the effect of size of microspheres on drug release patterns *in vitro*, it is evident that the smaller the size of the microspheres was, the more rapid was the release of the drug, because smaller microspheres have a greater surface area available for the release (Figs. 2 and 4).

The effect of drug content on release patterns was also investigated. With increase in the drug content, the release rate increased, especially in the initial period. The scanning electron microscopic observations showed that polycarbonate microspheres after drug release were aggregated. Such aggregation of the polycarbonate microspheres was considered to be largely a result of the temperature of incubation (37 °C). The local anesthetics were considered to have been released mainly by diffusion through the polymer matrix because the surfaces of the microspheres after the release experiments had not changed significantly.

It was suggested that the diffusivity of the local anesthetics examined is very small through the matrices of poly(propylene carbonate) and poly(ethylene carbonate). The very slow release of each drug from the polycarbonate microspheres after the initial burst may therefore be considered to be a consequence of both the small diffusivity of the drug in the polymer matrix and the aggregation of the microspheres, which resulted in a decrease of surface area available for the release. The degree of aggregation of the microspheres and its contribution to the release rate of the drug should depend greatly on temperature and the medium surrounding the microspheres. Therefore evaluations of the microspheres *in vivo* are essential.

The results obtained in the present study indicated that application of polycarbonate microspheres containing local anesthetics for the sustained control of pain in pain clinics may be feasible. Release patterns of local anesthetics from microspheres prepared by mixing different types of polycarbonates, and the local anesthetic effect of polycarbonate microspheres containing local anesthetics in guinea pigs will be reported shortly.

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