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RESEARCH PAPER

Preparation and Physical Characterization of Alginate Microparticles Using Air Atomization Method

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ABSTRACT

Alginate microparticles were prepared using an air atomization method and varying formulation and processing conditions. Thereafter, the size and surface morphology of alginate microparticles were characterized. The trapping efficiencies of the ketoconazole, acetaminophen, vitamin C, and Bifidobacteria bifidum *as model core materials were then determined. The air atomization process produced free-flowing and small-size microparticles after the freeze-drying process. The size distribution and surface morphology varied depending on the concentration of wall-forming materials and processing conditions. Generally, the geometric mean size increased as the concentration of alginate and poly-*l*-lysine and the delivery rate increased, but the air pressure decreased. Most of all, the ratio of delivery rate of alginate solution and air pressure could affect the size and surface morphology of alginate microparticles. However, the geometric mean size of alginate poly-*l*-lysine microparticles reproducibly ranged from about 80 to 130 μm. The microparticles were irregularly spherical or elliptical. The trapping efficiencies of ketoconazole, acetaminophen, vitamin C, and bifidobacteria were determined to be 71.5%, 60.1%, 1.6%, and 31%, respectively, when alginate concentration (1.5%), poly-*l*-lysine concentration (0.02%), air pressure (0.75 bar), delivery rate (8 ml/min), and spraying*

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distance (45 cm) were applied. The current microencapsulation process using the air atomization method provides an alternative to entrapping small molecules and macromolecules without using harmful organic solvents. In addition, the small-size and free-flowing alginate microparticles containing active substances can be used as an intermediate in pharmaceutical applications.
KEY WORDS: *Alginate microparticles; Air atomization; Size distribution; Surface morphology; Trapping efficiency.*

INTRODUCTION

Microencapsulation methods have been widely utilized to control drug release rate, avoid unpleasant tastes, separate incompatible drugs, reduce gastrointestinal toxicity, and protect microorganisms or cells from environmental and physiological degradation (1–4). Various microencapsulation methods, such as coating, hot melt coating, emulsion stabilization, coacervation, emulsion solvent evaporation, spray-drying, and interfacial complexation have been widely established (1). However, the choice of methods depends on the physicochemical properties of the entrapping materials and polymers without using harmful organic solvents, heat, high shear, and complicated processing conditions (2,5).

Alginate microparticles were previously developed to entrap microorganism using an air atomization device known as the Turbotak® (4–6). The device is an air-assisted nebulizer that produces a high-velocity spray and fine mist of alginate mixtures. Alginate gels when it comes in contact with calcium and multivalent cations. Alginate microparticles are stable in low pH conditions, but swell in weak basic solution, followed by disintegration and erosion (7,8).

Based on these principles, alginate microparticles containing various trapping materials were produced by spraying alginate solution into $CaCl_2$ solution using an air atomization device with air as an atomizing gas. The formed alginate gel structure of the microparticles can be further strengthened for increased stability by adding oppositely charged cationic poly-*l*-lysine, chitosan, or other polymeric solutions, resulting in a condensed and complexed surface (3,4,6,7,9).

These atomization processes are useful for producing smaller size microparticles, ranging from 5 to 200 μm, by adjusting liquid flow and operating air pressure. Free-flowing alginate microparticles also can be obtained after freeze-drying of the wet slurry of the gelled alginate structure. However, size distribution and surface morphology are important criteria to evaluate physical characteristics of alginate microparticles in pharmaceutical applications. They may be affected by various formulation compositions and processing parameters.

The purpose of the present study was to prepare alginate microparticles using an air atomization method. The effect of concentration of alginate, gelatin, polyethylene glycol 6000 (PEG6000), and poly-*l*-lysine treatment were examined as formulation parameters. The processing conditions, such as air pressure, solution delivery rate, and spraying distance were also varied. Thereafter, the size distribution and surface morphology of the alginate microparticles were extensively characterized by sieve analysis and scanning electron microscopy (SEM), respectively. Finally, the trapping efficiencies of the ketoconazole, acetaminophen, vitamin C, and *Bifidobacteria bifidum* as model core materials were investigated under the optimal formulation and processing conditions.

EXPERIMENTAL

Materials

Sodium alginate was purchased from Junsei (Tokyo, Japan). Calcium chloride was purchased from Shinyo (Osaka, Japan). Poly-*l*-lysine (molecular weight [MW] 38,500), *p*-hydroxy-benzoic acid *n*-butyl ester (butyl paraben), and L-cysteine-HCl were purchased from Sigma (St. Louis, MO). Ketoconazole was obtained from Choong-Wae Pharmacy Company (Seoul, Korea). Acetaminophen and vitamin C were donated courtesy of Chong Kun Dang (Seoul, Korea).

Freeze-dried single-strain *Bifidobacteria bifidum* culture (Bb-11), as a model, was purchased from Christian Hansen's Laboratory (Horsholm, Denmark). The culture is primarily applied in the production of probiotic milk products or yogurt cultures. The manufacturer claimed a minimum cell concentration of 5×10^{10} colony-forming units (cfu)/g when incubated in Lactobacilli MRS agar at 37°C for 3 days. Lactobacilli MRS broth and yeast extract were purchased from Difco Laboratories (Detroit, MI). Anaerobic BBL GasPak® jar, disposable H_2/CO_2 generator envelope, catalyst, and indicator strip were purchased from Becton Dickinson and Microbiology Systems (Cockeysvile, MD). All other chemicals were reagent grade and were used without further purification.

Preparation of Alginate Microparticles

Sodium alginate (1.5%) and entrapping substances as shown in Table 1 were mixed and suspended in distilled water. The mixtures were sprayed into a bath containing 500 ml of 0.2 M $CaCl_2$ solution using an air atomizing device (Turbotak®, Inc., Waterloo, Ontario, Canada) with a 1.0-mm orifice diameter. The device is an air-assisted nebulizer that produces a high-velocity spray and fine mist of alginate mixtures. Air and solution enter the side and top tube connected to the device simultaneously (6). A piece of dry sterilized cotton was placed inside the air tube to remove any insoluble particles. Using a peristaltic pump, sodium alginate solution was fed through the air atomizer from the top, and pressurized air was fed into the side. The pressurized air mixed with solution was forced into the device, resulting in tiny liquid droplets through the orifice of the atomizing nozzle (1.0 mm in diameter). Sodium alginate was gelled to form microgel droplets when in contact with divalent calcium ions. The resulting microgel droplets were cured for 15 min and filtered through two layers of filter paper in a Buchner funnel under vacuum. Thereafter, the filtered alginate microparticles were washed twice with distilled water.

Finally, the alginate microparticles were then optionally dipped into 0.02% poly-*l*-lysine solution to cross-link formed microparticles for 5 min. The resulting poly-*l*-lysine–treated microparticles were then separated by filtration under vacuum on a Buchner funnel. The filtered microparticles were washed twice again with distilled water to remove the additional $CaCl_2$ solution and then frozen at −37°C for 2 h. The final free-flowing bifidobacteria-loaded alginate poly-*l*-lysine microparticles were obtained after freeze-drying at −52°C under the pressure of 8 mmHg for 14–16 h.

The detailed formulation compositions and processing conditions for the preparation of alginate microparticles are given in Table 1. These formulation and processing parameters were reasonably chosen based on our previous preliminary experiments. The extremely high or low conditions resulted in failed formation of microparticles using air atomization methods.

After optimizing these formulation and processing conditions, the microparticles containing drugs or bifidobacteria were prepared as follows. First, 1 g of ketoconazole, acetaminophen, and vitamin C as model drugs were added to the 1.5% alginate solution and homogeneously mixed for 2 h. In the case of bifidobacteria cultures (0.5 g), nutrients containing 0.5% yeast extract, 0.5 $NaHSO_3$, 5% glycerol, and 1% $Mg_3(PO_4)_2$ were added based on our preliminary study. The other formulation and processing conditions were followed according to the parameters for sample 2 in Table 1.

Table 1

*Formulation and Processing Conditions for the Preparation of Alginate Poly-*l*-lysine Microparticles Using Air Atomization Method*

	Formulation Parameter[a]				Processing Parameter		
No.	Alginate (%)	Poly-*l*-lysine (%)	Gelatin (%)	PEG6000 (%)	Pressure (bar)	Delivery Rate (ml/min)	Spraying Distance (cm)
1	1.2	0.02	—	—	0.75	8	45
2[b]	1.5	0.02	—	—	0.75	8	45
3	2.0	0.02	—	—	0.75	8	45
4	1.5	0	—	—	0.75	8	45
5	1.5	0.04	—	—	0.75	8	45
6	1.5	0.02	1.5	—	0.75	8	45
7	1.5	0.02	—	0.75	0.75	8	45
8	1.5	0.02	—	—	0.50	8	45
9	1.5	0.02	—	—	1.0	8	45
10	1.5	0.02	—	—	0.75	4	45
11	1.5	0.02	—	—	0.75	12	45
12	1.5	0.02	—	—	0.75	8	15
13	1.5	0.02	—	—	0.75	8	30

[a] The concentration is given as percentage based on total weight.

[b] No. 2 was compared as a reference condition.

Size Characterization of Alginate Microparticles

About 10 g of alginate microparticles were exactly weighed. The size was analyzed in duplicate using the sieve method and based on weight gains in the range 45–212 μm. Geometric mean diameter and standard deviation of the microparticles were calculated from the curve of the logarithm of the particle size plotted against the cumulative percentage frequency of probability scale (10). However, the totals of the cumulative amount that passed through the sieves of the given opening size were slightly less than 100% (i.e., mostly 90% because of loss of some samples, discussed below).

Surface Morphology of Alginate Microparticles

The surface morphology of alginate microparticles was visualized using a scanning electron microscope (Jeol, Tokyo, Japan). An appropriate amount of freeze-dried microparticles were dispersed in ethanol and air dried onto metal stubs. The dried samples were coated with gold using a sputter coater (SPI, Westchester, PA). Micrographs were taken at an accelerating voltage of 20 kV with a 120-mm roll film camera system (Jeol).

Trapping Efficiency in Alginate Poly-*l*-lysine Microparticles

About 1 g of alginate poly-*l*-lysine microparticles containing ketoconazole were exactly weighed and suspended in 200 ml of phosphate buffer (pH 7.4) for 2 h. The chloroform (100 ml) was added to this turbid solution and stirred for 3 h. After centrifugation for 20 min at 3000*g*, the supernatant was carefully drawn and filtered through a Millipore nylon membrane filter (pore size 0.45 μm, diameter 13 mm). The concentration of ketoconazole was determined using a reverse-phase high-performance liquid chromatography (HPLC) apparatus consisting of an ultraviolet-visible (UV-Vis) detector at a wavelength of 254 nm and an Inertsil® ODS column (5 μm, 4.6 × 150 mm). The mobile phase consisted of 20% ethanol in a phosphate buffer (pH 6.8). The butyl paraben was used as an internal standard. The flow rate was 1 ml/min.

Also, about 1 g of alginate poly-*l*-lysine microparticles containing acetaminophen were exactly weighed and dissolved in 200 ml of phosphate buffer (pH 7.4) for 2 h. The 50 ml of methanol was then added to the resulting turbid solution and mixed for 1 h. Thereafter, 4 ml of this solution was drawn and vigorously mixed with methylene chloride (4 ml). After 1 h, 2 ml of the lower phase were carefully taken. The concentration of acetaminophen was determined at a wavelength of 254 nm.

In the case of vitamin C, the microparticles (1 g) were exactly weighed and completely dissolved in 200 ml of phosphate buffer (pH 7.4) for 12 h. The resulting solution was then filtered through a Millipore polycarbonate membrane filter (pore size 0.45 μm, diameter 13 mm). The filtered solution (1 ml) was then mixed with 2 ml of water-HCl buffer (pH 3). The concentration of vitamin C was determined at a wavelength of 244 nm.

For the determination of survival of bifidobacteria, the bifidobacteria-loaded alginate poly-*l*-lysine microparticles (1 g) were incubated at 37°C for 4 h in simulated intestinal fluid (pH 6.8 without pancreatin) containing 0.5% yeast extract and 0.05% L-cysteine HCl. Of the samples, 1 ml was withdrawn and aseptically diluted by

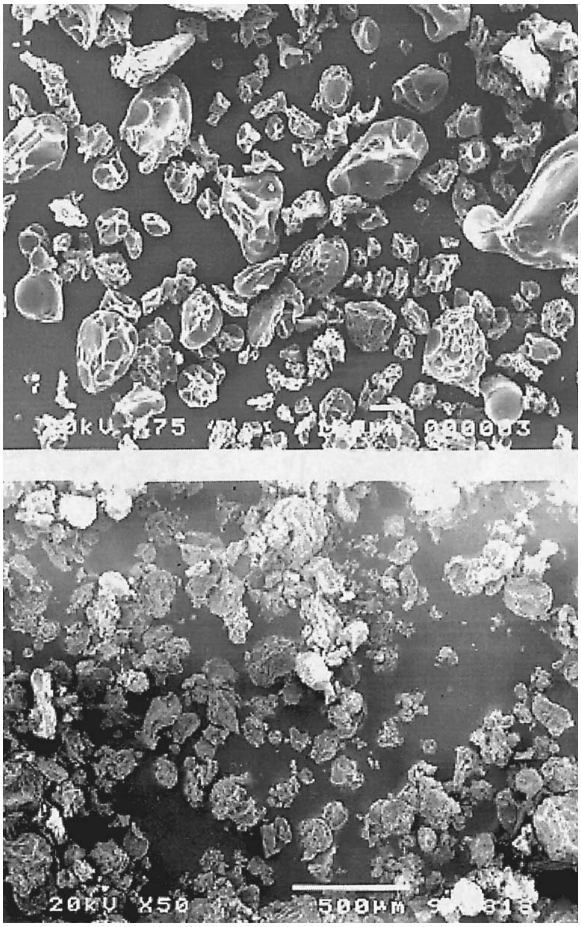

Figure 1. Overall surface morphology of acetaminophen- (top) and bifidobacteria-loaded (bottom) microparticles.

serial dilutions. The serial dilute solutions were composed of 5.5% MRS broth and 0.05% L-cysteine HCl. The diluted samples were plated in triplicate in MRS agar and incubated at 37°C in an anaerobic GasPak® jar containing generator envelope, indicator strip, and catalyst for 48–72 h. Colony-forming units were counted to assess the survival of bifidobacteria.

Based on the contents of core materials in the microparticles (mg/g), the trapping efficiency (%) was calculated as follows:

$$\text{Trapping efficiency (\%)} = \frac{\text{Contents} \times \text{Amount of microparticles recovered}}{\text{Amount of core materials added}} \times 100$$

RESULTS AND DISCUSSION

The air atomization method using a Turbotak device is known to be useful to entrap various materials, from small molecules to macromolecules, for taste and enhanced stability in some cases (4–6). In this study, the ketoconazole, acetaminophen, and vitamin C, as small model drugs with different solubilities, were selected. The bifidobacteria, as a macromolecule, was also selected. Most of all, the air atomization process produced free-flowing and small-size microparticles (about 80–130 μm) after the freeze-drying process without using harmful organic solvents. The overall surface morphology of acetaminophen- and bifidobacteria-loaded microparticles is given in Fig. 1. Generally, the microparticles had an irregular spherical or elliptical shape. The surface appeared variable, depending on formulation and processing parameters (Table 1). Effect of concentrations

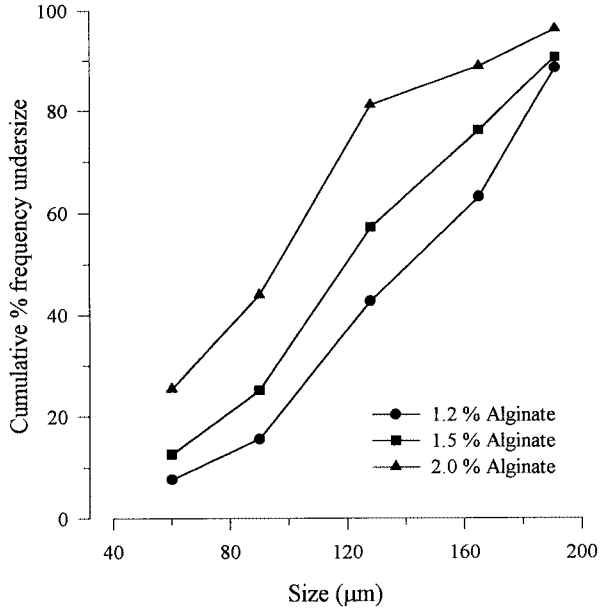

Figure 2. Effect of alginate concentration on the size distribution of alginate poly-*l*-lysine microparticles.

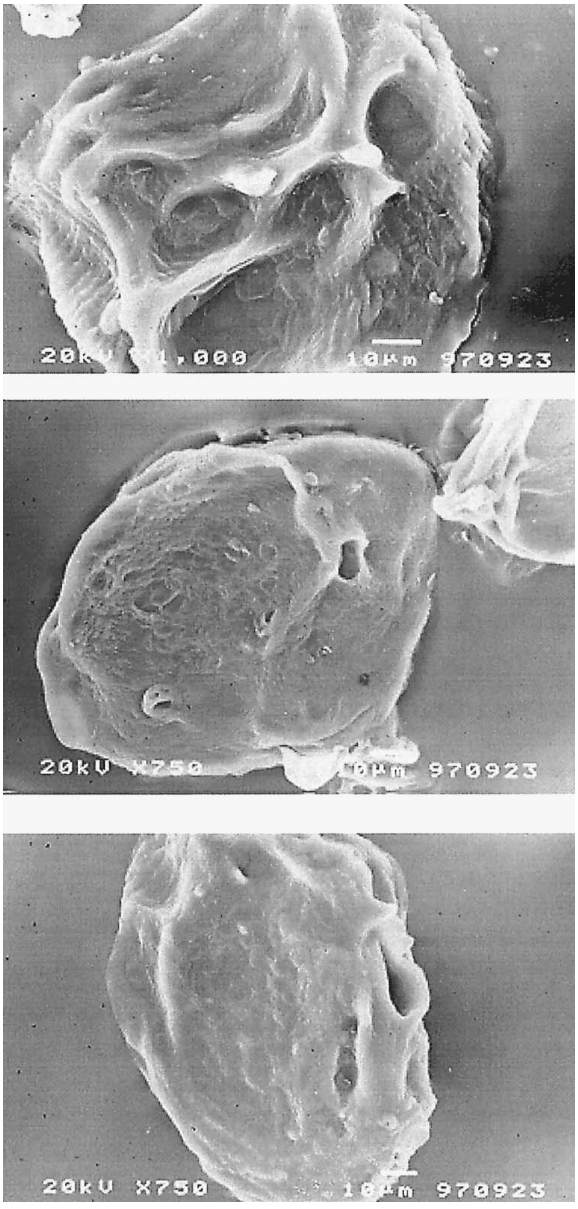

Figure 3. Effect of alginate concentration on the surface morphology of alginate poly-*l*-lysine microparticles; top, sample 1 (1.2%); middle, sample 2 (1.5%); bottom, sample 3 (2.0%).

and compositions of wall-forming materials and processing parameters on size distribution and surface morphology were considered to produce small-size microparticles with reliability. The sample 2 in Table 1 was compared as a reference condition: alginate concentration 1.5%, poly-*l*-lysine concentration 0.02%, air pressure 0.75 bar, delivery rate 8 ml/min, and spraying distance 45 cm.

Effect of Alginate Concentration

Effect of alginate concentration on the size distribution of alginate poly-*l*-lysine microparticles is shown in Fig. 2. As the sodium alginate concentration increased, the size of the alginate poly-*l*-lysine microparticles was significantly reduced. Geometric mean diameters were 128.5, 112.9, and 89.6 μm for 1.2%, 1.5%, and 2.0% concentrations of alginate, respectively. An inverse correlation between alginate concentration and size of microparticles was observed as described previously (6).

Effect of alginate concentration on the surface morphology of alginate poly-*l*-lysine microparticles is shown in Fig. 3. The shape of the microparticles was irregular spherical or elliptical. The surface structure was wrinkled and concave at the low alginate concentration. However, the surface of the microparticles was became dense and tight as the concentration of alginate solution increased. The wrinkled surface and irregular shape of the alginate microparticles resulted from evaporation of water during the freeze-drying process.

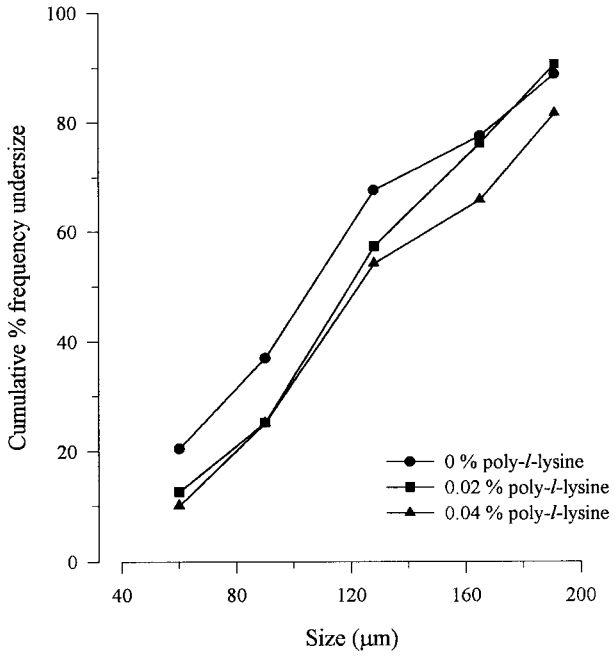

Figure 4. Effect of poly-*l*-lysine concentration on the size distribution of alginate microparticles.

Effect of Poly-*l*-lysine Concentration

It is known that the formed alginate gel structure of the microparticles can be strengthened further by oppositely charged cationic poly-*l*-lysine, resulting in a layer of condensed and insoluble complex (3,4,6,9). Effect of poly-*l*-lysine concentration on the size distribution of alginate poly-*l*-lysine microparticles is shown in Fig. 4. The size of alginate poly-*l*-lysine microparticles had a tendency to increase as the concentration of poly-*l*-lysine increased, maybe due to ionic complexation. The differences in the

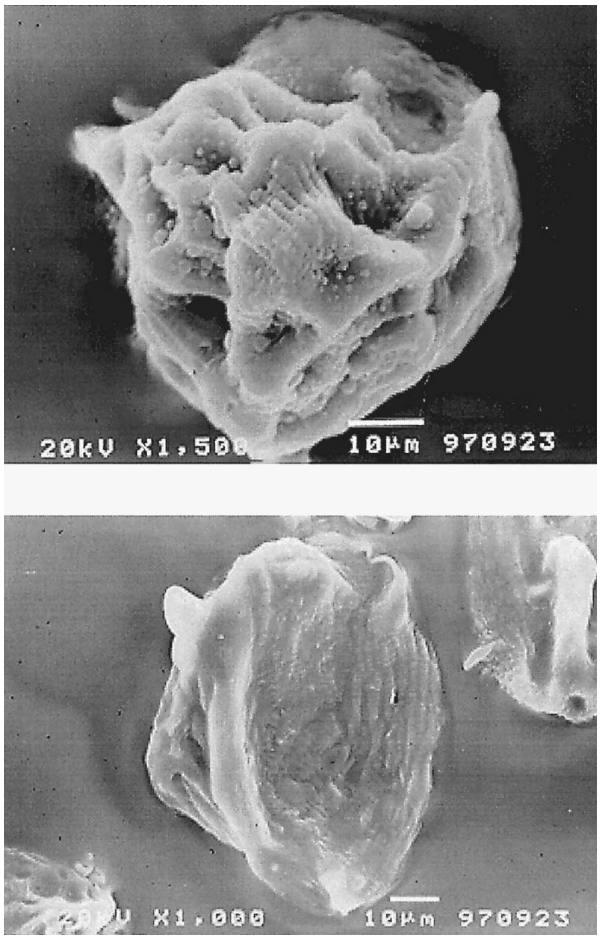

Figure 5. Effect of poly-*l*-lysine concentration on the surface morphology of alginate microparticles: top, sample 4 (0%); bottom, sample 5 (0.04%).

size distributions of microparticles between 0.02% and 0.04% poly-*l*-lysine concentrations were seen only at the larger sieve fractions. The geometric mean diameters were 101.9, 112.9, and 121.9 μm at 0%, 0.02% and 0.04% concentrations of poly-*l*-lysine, respectively.

Effect of poly-*l*-lysine concentration on the surface morphology of alginate microparticles is shown in Fig. 5. The surface of alginate microparticles became dense and tight when poly-*l*-lysine was used. Even though the surface shrank when 0.04% poly-*l*-lysine was used, the overall spherical shape of the alginate microparticles was almost unchanged.

Effect of Gelatin and Polyethylene Glycol 6000

When alginate is gelled in $CaCl_2$ solution, the compositions of alginate mixtures affect the size and structure of the microparticles. The gelatin and PEG6000 were added in alginate solution. The sizes of the alginate poly-*l*-lysine microparticles were 104.8 and 102.2 μm, respectively, when gelatin and PEG6000 were added. The size was reduced compared to alginate solution alone. The microparticles were almost spherical, but were wrinkled and loose in surface morphology (not shown).

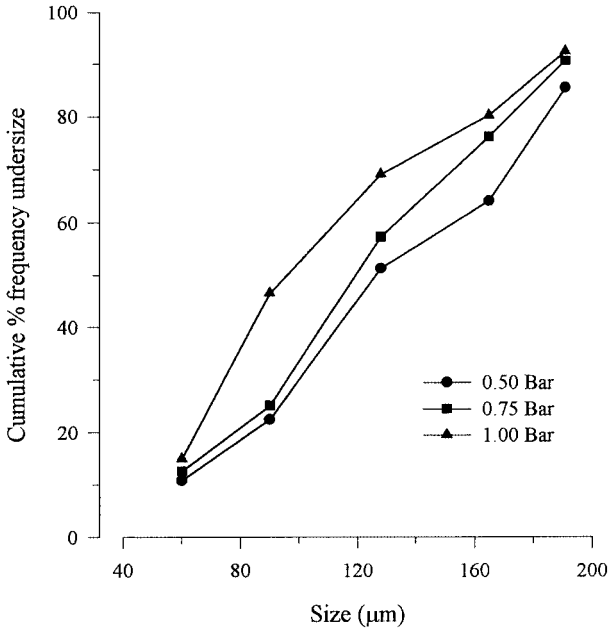

Figure 6. Effect of air pressure on the size distribution of alginate poly-*l*-lysine microparticles.

Effect of Air Pressure

The effect of air pressure on the size distribution of alginate poly-*l*-lysine microparticles is shown in Fig. 6. The geometric mean diameters of the microparticles were 122.9, 112.9, and 104.1 μm when the air pressure was 0.5, 0.75, and 1.0 bar, respectively. While the air pressure increased, the size of microparticles had a tendency become smaller because the droplets of alginate solution were becoming smaller, but no statistical significance was observed. Smaller particles would be produced as the atomization air pressure increased, as also was evident in a previous study, in which alginate poly-*l*-lysine microcapsules 5–15 μm in diameter containing bacillus Calmette Gurin (BCG) were produced when the air pressure was set higher, at 2.8 bar (6).

The effect of air pressure on the surface morphology

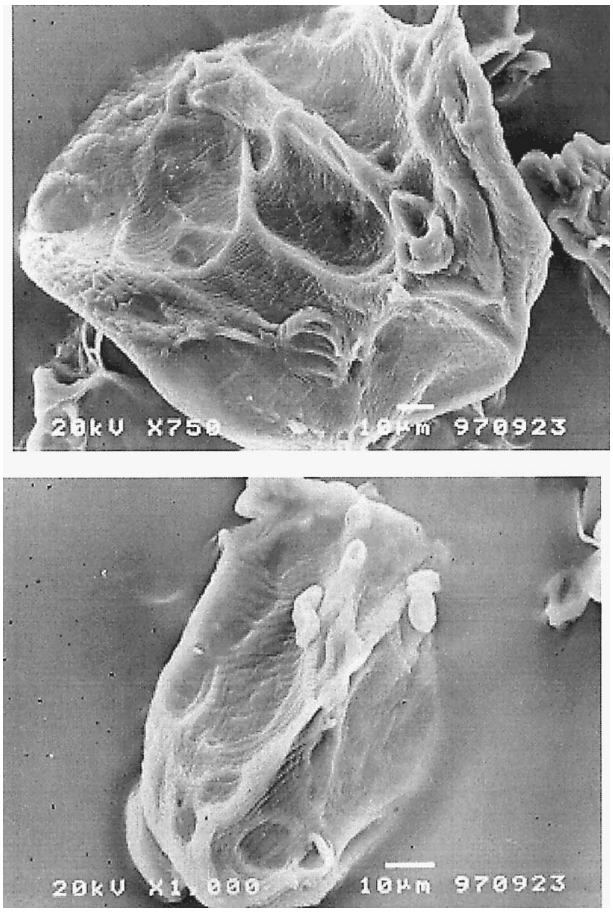

Figure 7. Effect of air pressure on the surface morphology of alginate poly-*l*-lysine microparticles; top, sample 8 (0.5 bar); bottom, sample 9 (1.0 bar).

of alginate poly-*l*-lysine microparticles is shown in Fig. 7. The structure and shape of alginate poly-*l*-lysine microparticles were irregular, loose, and wrinkled at low air pressure. Flakes or irregular lumps were also observed at high air pressure. When air pressure was set at 0.75 bar, the shape of alginate poly-*l*-lysine microparticles was relatively smooth and regular.

Effect of Delivery Rate

The effect of delivery rate of alginate mixtures on the size distribution of alginate poly-*l*-lysine microparticles is given in Fig. 8. The size of alginate poly-*l*-lysine microparticles became larger as the delivery rate of alginate solution increased due to the increased amounts of alginate mixtures. The geometric mean diameters of the alginate poly-*l*-lysine microparticles were 98.3, 112.9, and 117.6 μm when the delivery rates were 4, 8, and 12 ml/min, respectively. However, no statistical significance was observed.

The effect of delivery rate of alginate solution on the surface morphology of alginate poly-*l*-lysine microparticles is shown in Fig. 9. The shape of the microparticles was irregularly elliptical. At low (4 ml/min) or high (12 ml/min) delivery rates, bulging spots were observed. It

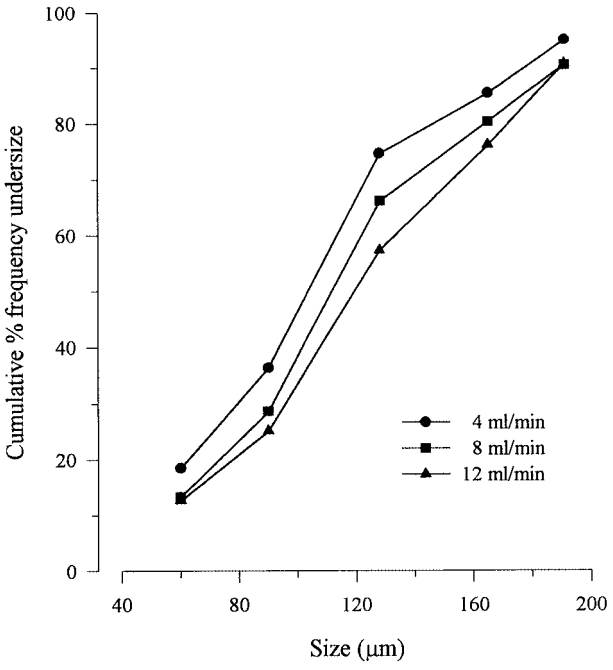

Figure 8. Effect of delivery rate of alginate solution on the size distribution morphology of alginate poly-*l*-lysine microparticles.

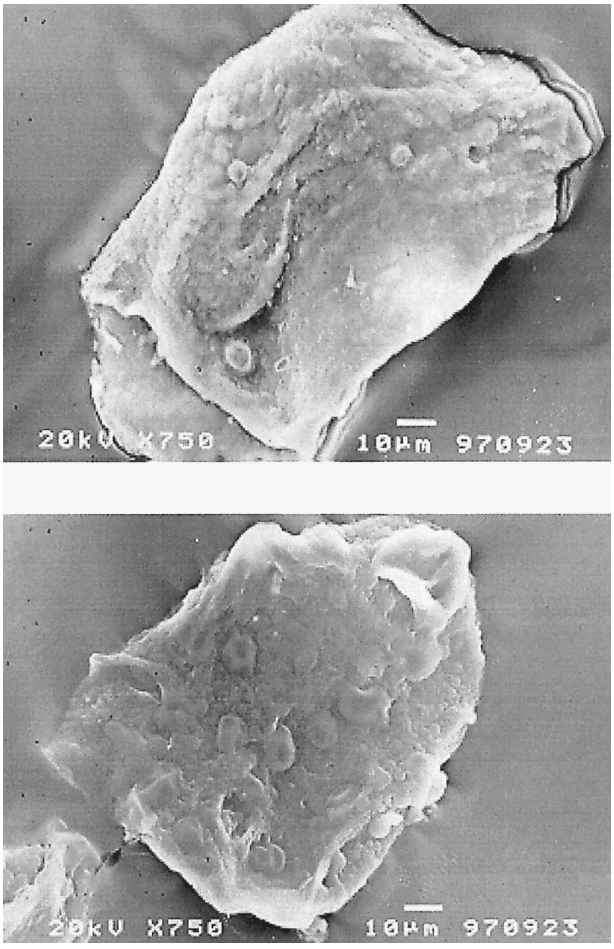

Figure 9. Effect of delivery rate of alginate solution on the surface morphology of alginate poly-*l*-lysine microparticles: top, sample 10 (4 ml/min); bottom, sample 11 (12 ml/min).

seems that the ratio of delivery rate of alginate solution and air pressure could affect the size and surface morphology of alginate poly-*l*-lysine microparticles. Relatively smooth and regular shapes were observed at a delivery rate of 8 ml/min when air pressure was fixed at 0.75 bar.

Effect of Spraying Distance

The spraying distance between the atomization device and reaction bath containing $CaCl_2$ solution is also an important factor for the preparation of alginate poly-*l*-lysine microparticles using the air atomization method (5). The effect of spraying distance on the size distribution of alginate poly-*l*-lysine microparticles is shown in

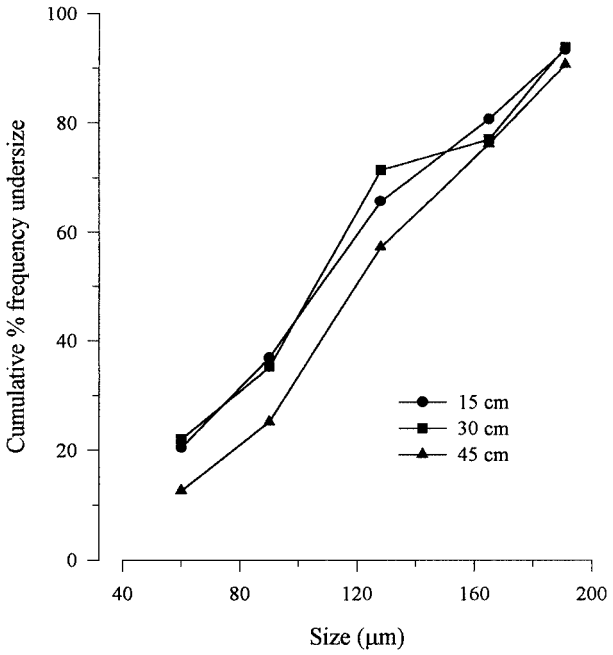

Figure 10. Effect of spraying distance on the size distribution of alginate poly-*l*-lysine microparticles.

Fig. 10. Although no direct correlation between spraying distance and size was observed, the size was slightly smaller when sprayed at too short or too long a distance. The aggregation phenomenon happened in the bath of $CaCl_2$ solution when the distance was too short (15 cm) because of the lack of proper atomization. In contrast, alginate droplets could be lost if the spraying distance were beyond the appropriate range or at a high air pressure. Some drying of the particles might also occur before the contact with the $CaCl_2$ solution, but it was not validated.

The effect of spraying distance on surface morphology of alginate poly-*l*-lysine microparticles is shown in Fig. 11. The clusters were clearly found on the surface of the alginate poly-*l*-lysine microparticles at the 15 cm spraying distance. The air-trapping phenomenon in alginate droplets was also observed when air pressure was relatively higher compared to spraying distance. The shape of the alginate microparticles was spherical or became smoother when sprayed at a 45 cm spraying distance. The 30 cm spraying distance was also reasonable, but flakes and irregular particles were found. The surface was shown to be rough. Therefore, a 45 cm spraying distance was selected in the preparation of alginate microparticles.

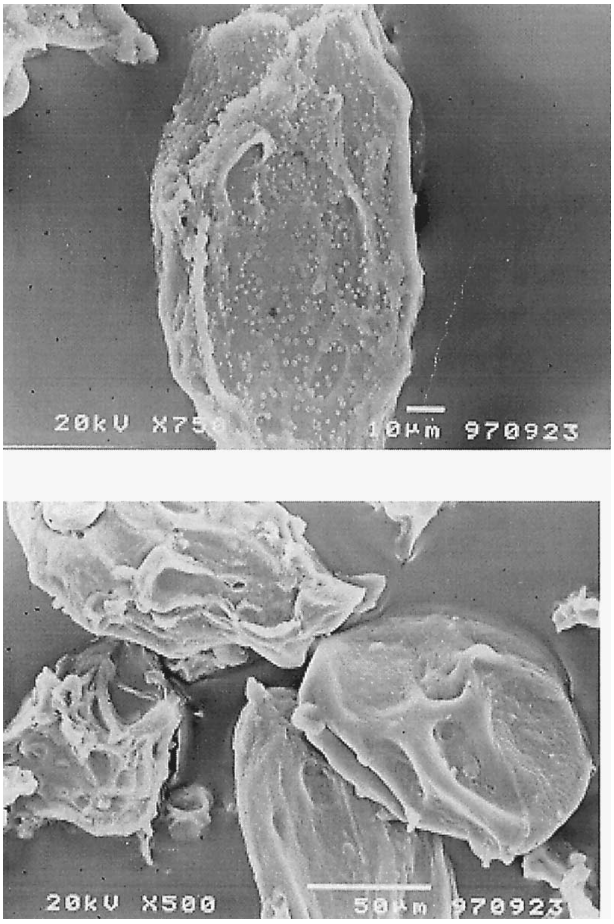

Figure 11. Effect of spraying distance on the surface morphology of alginate poly-*l*-lysine microparticles: top, sample 12 (15 cm); bottom, sample 13 (30 cm).

From these results, the size distribution and surface morphology of alginate microparticles using the air atomization method at various formulation and processing conditions are summarized in Table 2. It was evident that size, density (or weight of particles), and surface morphology could be optimized by properly combining processing parameters such as spraying distance, air pressure, and delivery rate.

Trapping Efficiency of Alginate Poly-*l*-lysine Microparticles

Based on the analysis of size and surface morphology of alginate poly-*l*-lysine microparticles, the formulation and processing parameters were optimally decided at the following conditions: alginate concentration 1.5%, air pressure 0.75 bar, delivery rate 8 ml/min, spraying dis-

Table 2

Size Distribution and Surface Morphology of Alginate Microparticles Using Air Atomization Method at Various Formulation and Processing Conditions

No.	Size (μm) (Mean ± SD)	Characterization	Surface Morphology
1	128.5 ± 44.7	Alginate concentration	Irregularly spherical, wrinkled, and loose
2[a]	112.9 ± 42.7	Alginate concentration	Almost spherical, dense/tight
3	89.6 ± 48.9	Alginate concentration	Elliptical, dense/tight
4	101.9 ± 50.2	Poly-*l*-lysine concentration	Irregularly spherical, wrinkled, and loose
5	121.9 ± 52.4	Poly-*l*-lysine concentration	Elliptical, tightly shrunk
6	104.8 ± 24.0	Gelatin addition	Almost spherical, wrinkled, and loose
7	102.2 ± 25.6	PEG6000 addition	Almost spherical, wrinkled, and loose
8	122.9 ± 51.3	Air pressure	Almost spherical, wrinkled, and loose
9	104.1 ± 40.7	Air pressure	Elliptical, dense/tight surface with flakes/lumps
10	98.3 ± 42.7	Delivery rate	Irregularly elliptical, bulged spots
11	117.6 ± 41.7	Delivery rate	Irregularly elliptical, bulged spots
12	100.9 ± 57.0	Spraying distance	Irregularly elliptical surface with aggregation, clusters and air trapping
13	99.8 ± 53.7	Spraying distance	Irregularly elliptical and rough surface with aggregation and lumps

[a] No. 2 in Table 1 was compared as a reference condition.

tance 45 cm, and poly-*l*-lysine concentration 0.02%. Thereafter, four different types of core trapping materials (ketoconazole, acetaminophen, vitamin C, and bifidobacteria) were selected to entrap in the alginate poly-*l*-lysine microparticles.

The trapping efficiencies of various core materials in alginate poly-*l*-lysine microparticles are given in Table 3. The trapping efficiencies of ketoconazole, acetaminophen, vitamin C, and bifidobacteria were estimated as 71.5%, 60.1%, 1.6%, and 12.3%, respectively. It seemed that the trapping efficiencies of core materials have a strong correlation with solubility. The drug content and trapping efficiency were inversely proportional to solubility, except for bifidobacteria. This suggested that the drug with high solubility in the exposing media could be readily released from the alginate droplets during the gelling process, resulting in low trapping efficiency. In addition, because the process is operated in the aqueous condition, spraying and gelling time also must be considered (8,12). It was also known that the trapping efficiency of drugs in alginate systems was dependent on curing time, $CaCl_2$ concentration, and drug solubility (11,12). In

Table 3

Trapping Efficiency of Various Materials in Alginate Microparticles

Entrapping Materials	Solubility (mg/ml)[a]	Contents (mg/g)[b]	Trapping Efficiency (%)
Ketoconazole	Practically insoluble (water), 0.5–1.2 (gastric), 0.05–0.08 (intestinal)	102.2 ± 17. 8	71.5 ± 13.1
Acetaminophen	12.7–21.4 (water, gastric)[c]	40.0 ± 0.81	60.1 ± 1.51
Vitamin C	Highly soluble (water)	9.63 ± 5.98	1.61 ± 0.54
Bifidobacteria	NA[d]	$(3.2 \pm 2.4) \times 10^9$ [e]	12.3 ± 3.1

[a] Solubility was given in water or simulated gastric or intestinal fluid, as indicated.
[b] Contents are given as milligram or colony-forming unit (cfu) per gram of alginate microparticles.
[c] From Ref. 11.
[d] Not available, but expressed as survival.
[e] cfu/g.

the case of bifidobacteria, the viability of the cell was more important than the solubility. The survival was rather affected by the microencapsulation process, culturing conditions, and exposing media (13).

CONCLUSIONS

Freeze-dried alginate microparticles with 80–130 μm of geometric mean size could be reproducibly produced in mild conditions using the air atomization method. The size distribution and surface morphology of alginate microparticles were varied depending on concentrations and compositions of wall-forming materials and processing parameters. Generally, the mean size increased as the concentration of alginate and poly-*l*-lysine and delivery rate increased, but the air pressure decreased. The current microencapsulation process using the air atomization method provides an alternative to entrapping small molecules and macromolecules without using harmful organic solvents. In addition, the small-size and free-flowing alginate microparticles containing active substances can be used as an intermediate in various pharmaceutical applications.

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