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Sphericity Optimization of Calcium Alginate Gel Beads and the Effects of Processing Conditions on Their Physical Properties

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Abstract In this study, the sphericity of calcium alginate gel beads was optimized using response surface methodology. The optimum conditions for bead sphericity were a concentration of 2.24% sodium alginate, a flow rate of 0.059 mL/sec for the sodium alginate solution, and a 459 rpm rotation for the calcium chloride solution. The predicted and experimental bead sphericities under the optimum conditions were 94.5 and 96.7%, respectively, showing close agreement. We also investigated the processing condition effects for the physical properties of the optimized calcium alginate gel beads. Immersion in hot water slightly decreased bead size and rupture strength. NaCl treatment increased bead size and decreased rupture strength. While the pH of the calcium chloride solution had little effect on bead sphericity, the bead sizes and gel strengths decreased with longer times in each pH solution. The beads coated with pectin and glucomannan showed no significant changes in sphericity, but their sizes decreased with time. The coated beads showed higher rupture strengths than the uncoated beads.

Keywords: sphericity, optimization, response surface methodology, calcium alginate gel bead, physical property

Introduction

Alginate, which naturally occurs in brown algae, is composed of hydrophilic, negative-charged polysaccharides that are connected between D-mannuronic acid and L-guluronic acid by 1,4-glycoside linkages (1, 2). Putative health benefits of alginate include prevention of arteriosclerosis, laxative effects, and chelation of heavy metals in the human body. It also reportedly exerts antimicrobial, anti-obesity, and anti-aging activities. Therefore, alginate has been studied for a wide range of industrial applications, including an ingredient in medicinal products, cosmetics, and foods (3). Grant *et al.* (4) studied the effect of cross-linking on the gelling properties of alginate and how it affects alginate's suitability as a replacement for agar in microbial media. Based on this research, further studies have evaluated its suitability in film processing (5) and capsule manufacturing (6). Lee and Seo (7) studied applications for alginate as an ingredient in antimicrobial products.

Alginate is frequently used in a bead form since its processing is simple and economical, and because the product is not harmful to the human body. Salib *et al.* (8) and Chowdary and Suresh Babu (9) studied the use of alginate beads as a coating material in the microencapsulation of drugs by using the gelling property enhanced by the incorporation of divalent cations. Yoo *et al.* (10) studied acetic acid production by using *Acetobacter aceti* fixed to calcium alginate. Bang and Seo (11) elucidated the NaCl absorbing properties of calcium alginate gel beads; and Bang (12) studied the lead absorption of alginate gel beads that were fixed with *Aspergillus niger*. They also conducted several other experiments based on the hypothesis that completely spherical calcium alginate gel beads could be obtained (11, 12). Sphericity is a requisite for the effective use of calcium alginate gel beads in some industrial applications, including the food industry. Numerous studies on the utilization of calcium alginate gel beads have assumed that almost perfect spherical shapes were obtained, but few studies have investigated the processing conditions required for producing high quality spherical beads.

Capsule manufacturing, which is similar to bead manufacturing, was studied by Cheong and Park (13) for preparing material suitable for the fixation of whole cells. They reported that when the encapsulated cells had a tailing shape, but not an almost perfect spherical shape, breakage, and cracking of the capsules occurred, resulting in the release of the microorganisms. Therefore, they mentioned that studying the mechanisms and factors required for sphericity is important.

This study investigated the sphericity of calcium alginate gel beads at different concentrations and flow rates of sodium alginate solution, and different agitation speeds (rpm) of calcium chloride solution, which were determined to be important independent variables by pre-experiments. Furthermore, the optimum processing conditions for obtaining highly spherical calcium alginate gel beads was investigated using response surface methodology (RSM, 14).

RSM shows the interrelationship between a dependent variable and independent variables under various conditions (15), and it's known to be very effective for process optimization (16, 17). The physical properties of the beads, as affected by processing conditions such as pH, heat, salt, and polysaccharide coating, were also measured in order to investigate the processing usefulness of the beads prepared using the optimized conditions. Lastly, this research will facilitate the use of optimized calcium alginate gel beads in medical and cosmetic products, enzymatic and microbial applications, and functional nutraceuticals.

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Materials and Methods

Materials The sodium alginate was purchased from Katayama Chemical Co., Ltd. (Osaka, Japan) and the anhydrous calcium chloride from Yakuri Pure Chemicals Co., Ltd. (Osaka, Japan) for preparing the calcium alginate beads. All other chemicals used in this study were analytical grade.

Bead manufacturing process from sodium alginate The beads were made with the device shown in Fig. 1. The operating conditions were 100 mL of solution, a height of 4.8 cm, a vessel area of 5.6 cm^2, a length of 43.9 cm, a magnetic bar height of 0.8 cm, and ambient temperature. The bead sphericities were optimized based on the following manufacturing conditions: the sodium alginate concentration, flow rate of the peristaltic pump (Cassette tube pump SMP-23; Eyela, Tokyo, Japan), and the agitation rate of the reactor. At a fixed concentration of 2.0%(w/v) calcium chloride, which did not have a large effect on the processing of the calcium alginate gel beads, we evaluated the sodium alginate concentrations of 1.0, 1.6, and 2.2%(w/v). The flow rates of the mixed solutions were 0.03, 0.06, and 0.09 mL/sec through tubes connected to the nozzle of the peristaltic pump. The inside and outside diameters of the nozzle were 1.94 and 2.40 mm, respectively. The mixed solutions were dropped into the stabilization solution of 2%(w/v) calcium chloride. The dropping height from the nozzle to the surface of the calcium chloride solution was fixed at 8 cm (Fig. 1). The agitation rates of the reactor containing the stabilization solution were 300, 400, and 500 rpm. The drop time of the mixed solution was 2 min and the reaction time was 20 min for stabilization. The beads prepared under the above conditions were separated with a mesh sieve. The separated beads were washed with distilled water and stored at an ambient temperature until use.

pH, heating, sodium chloride treatment, and coating of the beads The physical properties of the beads made under the optimized conditions were measured from 20 to 120 min at 20 min intervals, at pH 3, 5, 7, 9, and 11 at an ambient temperature. The pH of the solution was adjusted with 0.1 N HCl and 0.1 N NaOH. To determine their stability within industrial applications, the physical property changes of the beads were investigated after immersion in 90°C hot water, and after NaCl treatment at 30 min intervals from 30 min to 3 hr. The coating materials evaluated for the processed beads were pectin and glucomannan. The coating was applied by placing the beads into 1.0%(w/v) pectin or glucomannan solution, and drying. The beads made under the conditions for optimum sphericity were washed through sieves and the moisture removed. Then, the adequately dried beads were immersed for 1 min into the pectin or glucomannan solutions at temperatures of 90°C or higher, and then dried, leaving the polysaccharide coating. The finished beads were evaluated for size, sphericity, and rupture strength.

Determination of bead diameter, sphericity, and rupture strength Bead size was analyzed by optical microscopy at 40× magnification (BX-50; Olympus, Tokyo, Japan) with the microscope connected to a computer for analysis with Image-Pro software. Five beads made under each condition were randomly selected, and the averages of the longest and shortest lengths were calculated for comparisons. The bead sphericity, which is defined as how close the bead is to a perfectly spherical shape, was calculated by the ratio of the longest length of the bead to the shortest length of the bead. The bead sphericity value was used to fit the following equation (1).

$$\text{Sphericity (\%)} = \frac{\text{Shortest length}}{\text{Longest length}} \times 100 \quad (1)$$

To determine rupture strength, 5 beads for each condition were randomly chosen, and their rupture strengths were measured using a rheometer (model CR-100D; Sun Scientific Co., Ltd., Tokyo, Japan) with a 10 mm circular disk plunger.

Response surface methodology The beads made from sodium alginate and calcium chloride were evaluated using a central composite design, and the process was optimized for bead sphericity by statistical response surface methodology (RSM). The sodium alginate concentration (X_1, %), flow rate of the sodium alginate solution (X_2, mL/sec), and rotation speed of the calcium chloride solution (X_3, rpm) were selected as the independent variables. The range and center point values of the 3 independent variables were based on the results of preliminary experiments (Table 1). Experimental runs were randomized in order to minimize the effects of unexpected variability in the observed responses. The response surface regression (RSREG) procedure of SAS (Version 8.01, SAS Institute Inc., Cary, NC, USA.) was used to fit the following quadratic polynomial equation (2):

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (2)$$

where Y is the dependent variable (sphericity, %), β_0 is constant, β_i, β_{ii}, β_{ij} are regression coefficients, and X_i, X_j are levels of the independent variables.

The response surface plots were developed using Maple software (Maple 7, Waterloo Maple Inc., Waterloo, OT, Canada). The relationships as a function of 2 independent variables were shown at the fixed value of the optimum condition for the other independent variable.

Statistical treatment The data were subjected to analysis of variance (ANOVA) ($p<0.05$), and the means were separated using Duncan's multiple range tests (α= 0.05). The analysis was performed using the REG procedure of SAS.

Results and Discussion

Optimization of processing for maximal sphericity by response surface methodology The factors that were determined to be important for bead sphericity were the concentration, flow rate (mL/sec), and dropping height (cm) of the sodium alginate solution, as well as the concentration and agitation speed (rpm) of the calcium

chloride solution. Preliminary data indicated that the concentration and flow rate of the sodium alginate solution, and the agitation speed (rpm) of the calcium chloride solution, significantly affected bead sphericity, but the dropping height (cm) of the sodium alginate solution and concentration of the calcium chloride solution did not affect sphericity.

The study results of the 3 important factors for producing nearly perfect spherical-shaped beads are described as follows. At low concentrations of sodium alginate, the gel strength of the calcium alginate gel beads was weak, so tailing occurred. Even if the correct shape was formed, the maintenance of the shape was difficult. At high concentrations, tailing also occurred, as mentioned by Hwang *et al.* (18). Low flow rates of sodium alginate did not produce any problems for bead sphericity, but the yield of the beads was poor, showing low efficiency. At high flow rates of sodium alginate, the solution was not cut, so spherical shapes were difficult to produce. Finally, low agitation speeds did not significantly affect bead sphericity, but high speeds made oval-shaped beads due to the strong centrifugal force of the solution.

The calcium alginate gel beads were made within the conditions of the central composite design using sodium alginate. Their central points and ranges were previously decided for the central composite design (18). The central points of the independent variables for the bead processing experimental design were 1.6%(w/v) for the sodium alginate concentration (X_1, %), 0.06 mL/sec for the flow rate of the sodium alginate solution (X_2, mL/sec), and 400 rpm for the rotation speed of the calcium chloride solution (X_3, rpm) (Table 1). Fifteen experimental points were evaluated and their bead sphericities are shown in Table 2. Based on the results in Table 2, the RSREG procedure of SAS for response surface methodology was carried out. Their significance on linear (X_1, X_2, X_3), quadratic (X_{11}, X_{22}, X_{33}), and interaction terms was analyzed using the *t*-statistic (Table 3). The statistical *p* values for the linear coefficients was p=0.0001 for X_1, p=0.0111 for X_2, and p=0.0275 for X_3, showing that all the linear coefficients were significant at the level of $p<0.05$. The quadratic coefficients of X_{11} (p=0.0086) and X_{22} (p=0.0246) were significant at the level of $p<0.05$, but X_{33} (p=0.3621) was not. The interaction coefficients X_1X_3 (p=0.1929) and X_2X_3 (p=0.6892) were not significant ($p<0.05$), whereas X_2X_3 (p=0.0343) was significant ($p<0.05$). After removing 3 terms that were not significant at the 95% probability level, the resulting response surface model equation was formed:

$$Y = 89.3000 + 11.5000\ X_1 - 4.2625\ X_2 - 3.3375\ X_3 - 6.6750\ X_1^2 + 4.4250\ X_1X_2 + 4.8750\ X_1X_3 \quad (2)$$

Table 2. Central composite design and responses of the dependent variables for bead processing to the independent variables[1)]

Run No.	Coded level of variable			Response
	X_1	X_2	X_3	Y
1	-1	-1	0	84.0
2	+1	-1	0	94.2
3	-1	+1	0	67.0
4	+1	+1	0	94.9
5	-1	0	-1	77.1
6	+1	0	-1	94.3
7	-1	0	+1	58.0
8	+1	0	+1	94.7
9	0	-1	-1	95.9
10	0	+1	-1	88.3
11	0	-1	+1	93.2
12	0	+1	+1	83.0
13	0	0	0	88.8
14	0	0	0	90.2
15	0	0	0	88.9

[1)] Y (sphericity, %), X_1 (sodium alginate concentration, %), X_2 (flow rate of alginate solution, mL/sec), X_3 (rotation speed of calcium chloride solution, rpm).

The coefficient of determination (R^2) on the fitted quadratic polynomial equation was 0.9726, and the *p* value of the model equation was p=0.0022. Because the experimental design utilized preliminary data, the R^2 value was high and the model equation significant. By statistical analysis of the response surface methodology, the coded and uncoded values for the optimal conditions were determined, and are shown in Table 4. According to the results of the RSREG procedure, the eigen values were positive and negative, and the stationary point was shown in a saddle point. For the predicted optimum conditions for calcium alginate gel bead processing, the coded values were 1.07 for the sodium alginate concentration (X_1, %), -0.02 for the flow rate of the sodium alginate solution (X_2, mL/sec), and 0.59 for the rotation speed of the calcium chloride solution (X_3, rpm). Their uncoded values were obtained by calculating the above coded values that were used for the experimental design, so the uncoded values of the optimum conditions are represented as the concentration and agitation speed of the sodium alginate, and the

Table 1. Experimental ranges and values of the independent variables in the central composite design for the bead manufacturing process

Independent variable	Symbol	Range and level		
		-1	0	+1
Sodium alginate concentration (%, w/v)	X_1	1.0	1.6	2.2
Flow rate of alginate solution (mL/sec)	X_2	0.03	0.06	0.09
Rotation speed of calcium chloride solution (rpm)	X_3	300	400	500

Table 3. Estimated coefficients of the fitted quadratic polynomial equation for different responses based on *t*-statistics[1)]

	Y (Sphericity, %)	
	Coefficient	*p*-Value
Intercept	89.3000	<.0001
X_1	11.5000	0.0001
X_2	-4.2625	0.0111
X_3	-3.3375	0.0275
X_1X_1	-6.6750	0.0086
X_1X_2	4.4250	0.0343
X_1X_3	4.8750	0.0246
X_2X_2	2.4000	0.1929
X_2X_3	-0.6500	0.6892
X_3X_3	-1.6000	0.3621

[1)]X_1 (sodium alginate concentration, %), X_2 (flow rate of alginate solution, mL/sec), X_3 (rotation speed of calcium chloride solution, rpm).

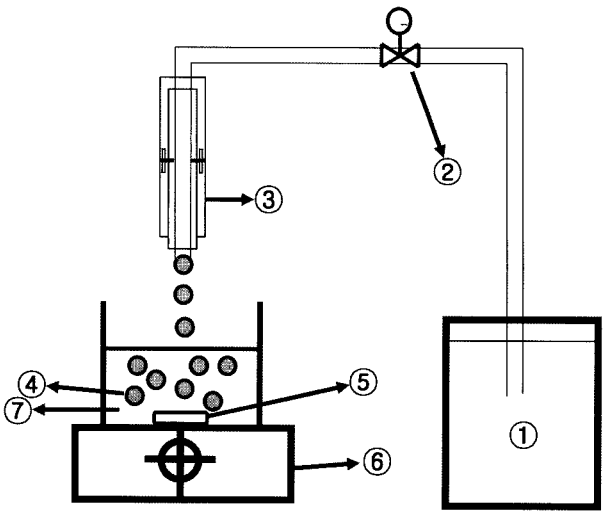

Fig. 1. Simple schematic diagram for preparing calcium alginate gel beads with a single nozzle.
① sodium alginate solution, ② peristaltic pump, ③ nozzle, ④ calcium alginate gel beads, ⑤ magnetic bar, ⑥ magnetic stirrer, ⑦ calcium chloride solution.

concentration of the calcium chloride of the real values in the optimum conditions for sphericity. Their uncoded values at the optimum conditions for bead sphericity were a 2.24% concentration of sodium alginate (X_1, %), a flow rate of 0.059 mL/sec for the sodium alginate solution (X_2, mL/sec), and a 459 rpm rotation speed for the calcium chloride solution (X_3, rpm). The predicted value for bead sphericity in the optimum conditions was 94.5%. The experimental value obtained using the optimum conditions, which was calculated using the SAS program, was 96.7%, showing no large difference (Table 4).

Response surface plots The effects of the independent variables (X_1, X_2, X_3) on the value of the dependent variable (Y) are shown in a 3 dimensional graph in Fig. 1. Even though several factors in bead processing can affect bead sphericity, the 3 factors we believed to have the greatest affect on bead sphericity in this study were the sodium alginate concentration (X_1, %), the flow rate of the sodium alginate solution (X_2, mL/sec), and the rotation speed of the calcium chloride solution (X_3, rpm). The effects of these 3 factors on bead sphericity (Y, %) are shown in Fig. 2, which illustrates the interrelationship of each independent variable. At low concentrations of sodium alginate, bead sphericity had a low value of 75%, which was not related to the other 2 variables. The bead sphericity increased with the concentration of sodium alginate, and the effects of the 2 other variables also increased. The agitation rate and flow rate similarly affected the bead sphericity, so the sphericity increased as they decreased.

In the response surface plot, as the sodium alginate concentration (X_1, %) increased and the flow rate of the sodium alginate solution (X_2, mL/sec) and rotation speed of the calcium chloride solution (X_3, rpm) decreased, bead sphericity (Y, %) increased. The sodium alginate concentration (X_1, %) was the most important factor affecting bead sphericity (Y, %) during bead processing. In this study, sodium alginate concentrations over 2.4% produced tailing phenomena, reducing the sphericity of the beads. A similar result was shown by Hwang *et al.* (18) who reported that a high sodium alginate concentration of 3.0% produced tailing phenomena during bead processing.

Effect of pH on the physical properties of the beads The physical properties of the calcium alginate gel beads made under the optimal conditions were measured from 20 to 120 min at 20 min intervals, at pH 3, 5, 7, 9, and 11. The results are shown in Fig. 3. Bead sphericity was more than 95% and was not affected by treatment time within the pH range (Fig. 3A). The bead size decreased with time for all pH treatments (Fig. 3B). In the initial 20 min of pH

Table 4. Optimal conditions for the bead manufacturing process from sodium alginate

Dependent variable	Independent variable[1)]	Critical value		Stationary point	Predicted value	Experimental value
		Coded	Uncoded			
Y (Sphericity, %)	X_1	1.07	2.24	Saddle point	94.5	96.7
	X_2	-0.02	0.059			
	X_3	0.59	459			

[1)]X_1 (sodium alginate concentration, %), X_2 (flow rate of sodium alginate solution, mL/sec), X_3 (rotation speed of calcium chloride solution, rpm).

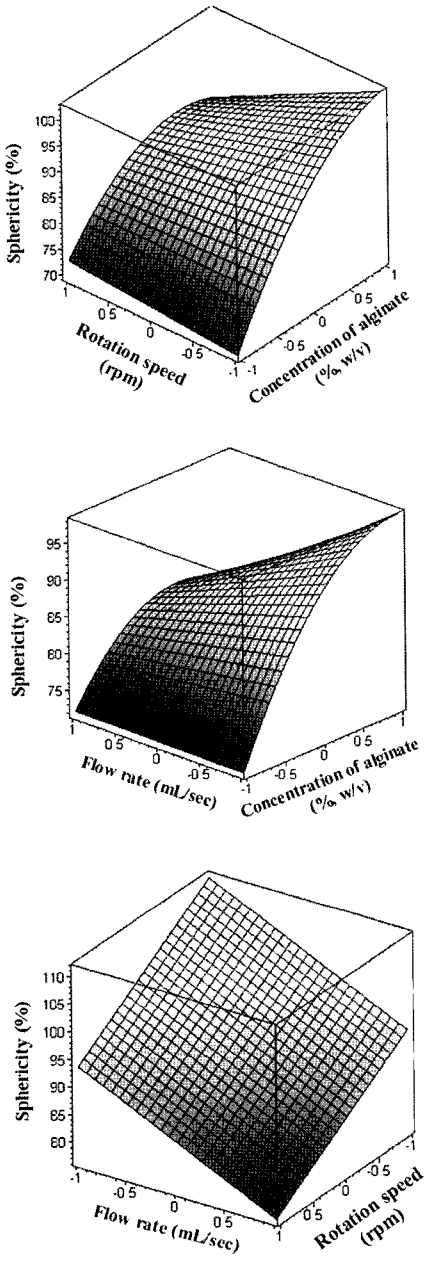

Fig. 2. Response surface plots for bead manufacturing process from sodium alginate. X_1 (concentration of alginate, %), X_2 (flow rate of alginate solution, mL/sec), X_3 (rotation speed of calcium chloride solution), Y (sphericity, %).

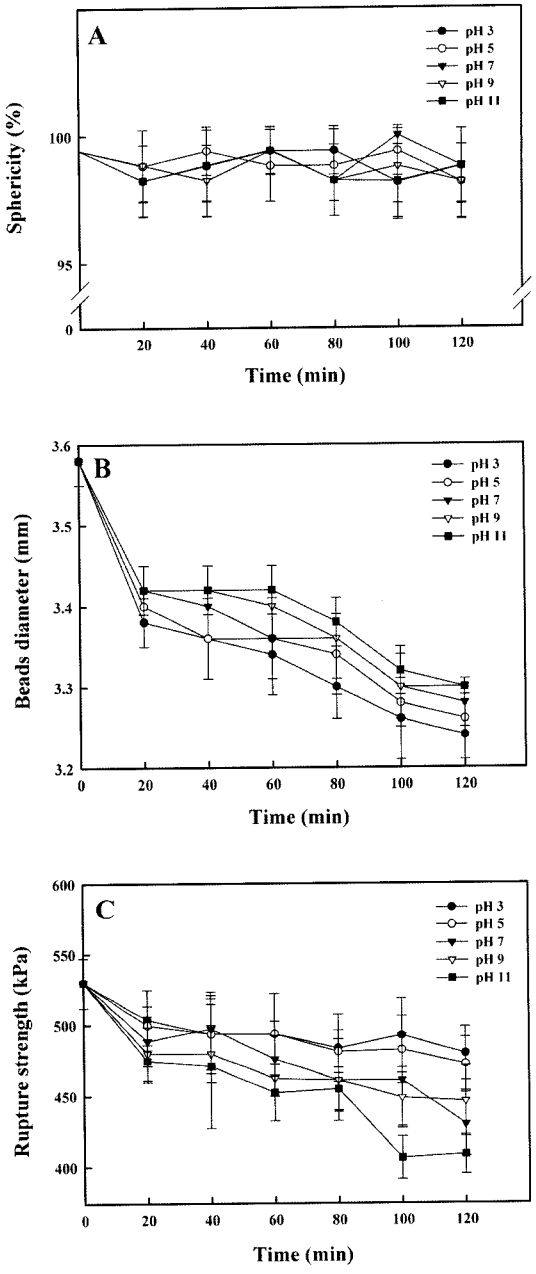

Fig. 3. Effect of pH on physical properties of the beads according to different treatment time.

treatment, the bead size decreased sharply, and then slowly. The rate of decrease was less in the alkali solution, so the bead size at pH 11 was larger than at the other pHs, and at pH 3 it was the smallest. Finally, the rupture strength of the beads is shown in Fig. 3C. In all pH ranges the rupture strength tended to decrease with increasing treatment time, exhibiting a similar tendency as the bead size. The rate of decrease in rupture strength was lower at lower pH, showing a reverse tendency of the bead size. Go *et al.* (2) reported that sodium alginate formed a gel in acidic pH. The reason was thought to be due to an excellent gelling ability by the exchange between calcium ions and sodium ions in the low pH sodium alginate. The fact that sodium alginate gel is better formed at acidic pH explains why greater gel strength was formed at acidic pH than at alkali pH. While the pH of the solution had little effect on bead sphericity, size, and gel strength decreased with increasing soaking time at each pH level. The bead sizes were smaller at acidic pH, as demonstrated by bead sizes of 3.24 and 3.30 mm when treated with pH 3 and 11 solutions for 2 hr, respectively. However, the gel strengths of the beads were reduced more by alkali pH, as shown by gel strengths of 479.73 and 408.52 kPa, when the beads were treated with pH 3 and 11 solutions for 2 hr, respectively.

Effects of heating and sodium chloride treatment on the physical properties of the beads The effects of heating and NaCl treatment on the physical properties of the calcium alginate gel beads are shown in Fig. 4. The sphericity of the beads before heat treatment was 96.7±2.7 %, showing near spherical shapes (Table 4). The range of bead sphericities after heat treatment was 92-96%, showing a loss of sphericity (Fig. 4A). The bead sphericities after 1 and 3 hr treatment times were significantly lower, but exhibited no consistent trend. The bead size before heat treatment was 3.6±0.14 mm, for beads that had been processed under optimum conditions (Table 5). The average bead size after 30 min of heat treatment was 3.32 mm, showing 0.3 mm of shrinkage (Fig. 4B). Heat treatment beyond 30 min did not affect the bead sizes. The change in bead size with heat treatment was thought be due to moisture loss within the beads during the first 30 min of heat treatment, and further heat treatment did not result in additional moisture loss. For the beads treated with 2% NaCl solution for 30 min, the bead size was 4.17 mm, which was approximately 0.5 mm larger than the untreated beads. Bang and Seo (11) reported that alginate bead size increased with time as a result of salt and water adsorption. Finally, the rupture strength of the optimally processed beads was 520.96±29.98 kPa (Table 5).

The changes in rupture strength over the 30 min intervals of 90°C heat and NaCl treatment are shown in Fig. 4C. The rupture strength decreased to 302.33 kPa after 30 min of heat treatment, but it was not further affected by longer heat treatment. In contrast, the rupture strength of the beads decreased sharply with NaCl treatment. The rupture strength of the beads after 1 hr of heat treatment was 191.14 kPa; but in many cases it had decreased sharply and could not be measured, such as when the beads were salt treated for more than 2 hr. The stability of the beads made under the optimal condition was affected by NaCl and by heat.

Effects of polysaccharide coatings on the physical properties of the beads by heating and sodium chloride treatments As shown in Fig. 4, the rupture strength of the beads was remarkably reduced by NaCl treatment, so it was almost impossible to form a bead. Therefore, we attempted the use of polysaccharide coating materials in order to improve the physical properties of the beads. The bead size was measured at constant time intervals after the coated beads were treated with 2.0%(w/v) NaCl, and underwent heat treatment. The results are shown in Fig 5. Lee *et al.* (19) studied microencapsulation using polysaccharide-coating materials. The polysaccharides used in this study were pectin and glucomannan. The beads made at 90°C were placed into solutions containing 1%(w/v) of each polysaccharide, and then the beads were taken out and dried. The sphericities of the coated beads are shown in

Table 5. Physical properties of beads manufactured using the optimal processing condition

	Sphericity (%)	Diameter (mm)	Rupture strength (kPa)
Bead	96.7±2.7	3.60±0.14	520.96±29.98

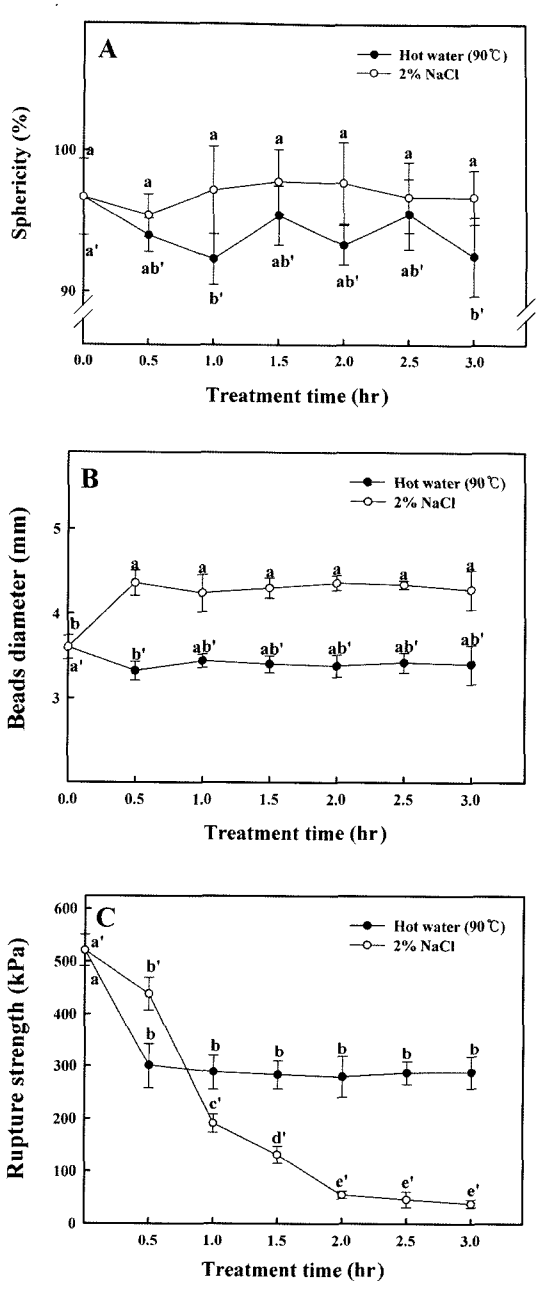

Fig. 4. Effect of hot water and NaCl treatments on physical properties of the beads according to treatment times.

Fig. 5A. There was little change in sphericity for the coated beads after treatment with 2%(w/v) NaCl solution and heat, with all beads remaining at no less than 90% after all time intervals, and showing no significant differences. The sizes of the beads coated with the 1%(w/v) pectin were reduced from 3.75 to 3.34 mm by NaCl treatment, and the sizes of the glucomannan-coated beads decreased from 3.84 to 3.60 mm with NaCl treatment. As shown in Fig. 4B, the sizes of the coated beads were reduced when the beads were immersed in 90°C hot water. The coating materials made little difference in bead size; however, the sizes of the glucomannan-coated beads were slightly larger. Comparatively, the sizes of the coated beads were similar and bigger in all experimental ranges

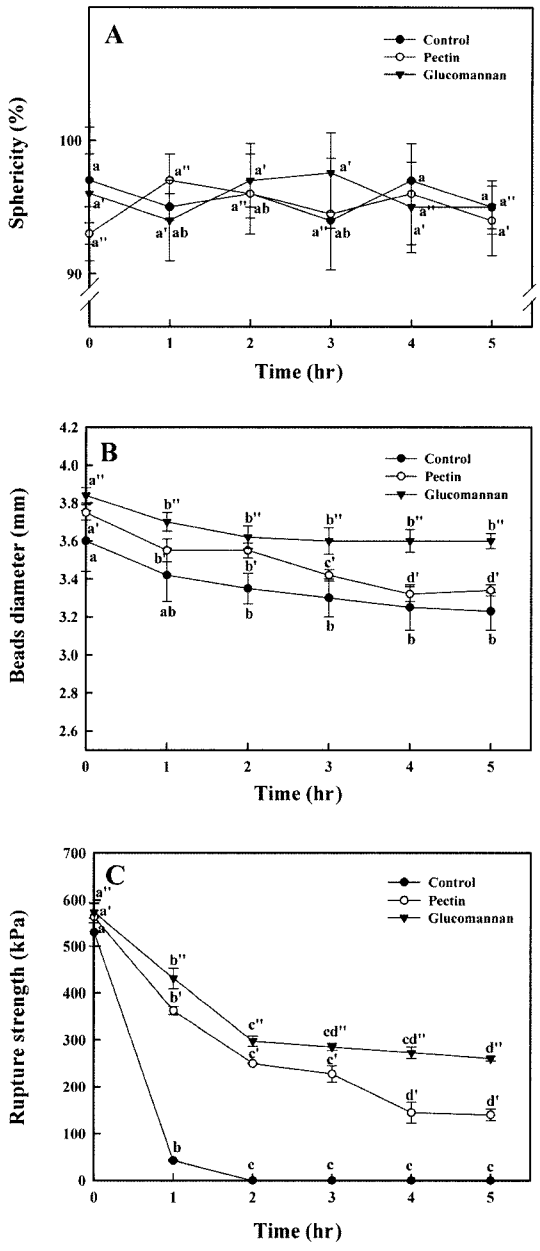

Fig. 5. Effect of coating treatment with polysaccharides on physical properties of the beads treated in 2% NaCl of 90°C hot water according to treatment time.

than the uncoated beads.

The rupture strength of the coated beads is shown in Fig. 5C. The rupture strength of the uncoated beads was very low after 30 min of heat and NaCl treatment, and was too weak to measure after 2 hr of treatment. Like the uncoated beads, the rupture strength of the coated beads was constantly and significantly reduced by the heat treatment time, and the time in the NaCl solution. Although the rupture strength of the coated beads was low, the strength could still be measured after 2 hr of treatment. The rupture strength of the glucomannan-coated beads was higher than that of the pectin-coated beads. Therefore, the rupture strength of the polysaccharide-coated beads was thought to be higher than that of the uncoated beads. This method of coating beads is useful in several industrial applications, including food additives.

Acknowledgments

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