Preparation of Theophylline-Loaded Calcium Alginate Gel Capsules and Evaluation of Their Drug Release Characteristics

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A method for the preparation of theophylline-loaded alginate gel capsules was developed, and their drug release characteristics were investigated. A dispersion containing theophylline and wheat starch suspended in a calcium chloride solution was dropped into a sodium alginate solution. The calcium ions then diffused out of the droplets and reacted with the alginate, resulting in the formation of a water-insoluble calcium alginate gel membrane around each droplet. In subsequent drying, spherical, glossy capsules with a smooth surface were obtained (an average diameter of 3.1 mm). The coat thickness increased with coating time, and the $CaCl_2$ concentration in the core dispersion increased. The efficiency of drug encapsulation (EE) decreased with an increase of the coating time, and increased with an increase of the $CaCl_2$ concentration and the theophylline loading dose in the core dispersion. The coat thickness and EE were almost independent of the sodium alginate concentration in coating fluids (1% and 2%). The theophylline release from the gel capsules followed zero-order kinetics, and the release rates were significantly reduced as the coat thickness increased. Furthermore, the release rates were greatly reduced compared with those of the matrix-type alginate gel beads.

Keywords alginate gel capsule; theophylline; zero-order release; alginate gel bead; controlled release; coat thickness

A major property of naturally occurring polysaccharide alginic acid and its water-soluble salts is the ability to form gels in the presence of calcium and other di- and trivalent metal ions.[1,2] The gelation procedure of alginates can be carried out in a single-step process under very mild conditions. For example, by the dropping of an aqueous solution of sodium alginate into a calcium chloride solution, the rigid spherical gels of calcium alginate (Ca-alginate gel beads) are easily prepared by ionotropic gelation. Recently, the use of Ca-alginate gel beads as a vehicle for controlled drug delivery has attracted considerable attention, because of their nontoxic properties and the ease of bead production. Gel beads loaded with different drugs have been prepared, and various factors which affect the drug release rate from beads have been intensively investigated.[3-9] Also, the useful applications of alginate gel beads in the design of controlled release formulations containing herbicides has been described in several studies.[10-13]

A single-step method similar to alginate gel bead preparation was also applied to prepare calcium alginate gel membrane capsules (Ca-alginate gel capsules).[14,15] In the capsule preparation, the droplets of aqueous solution containing calcium chloride were dropped into a sodium alginate solution. The resulting spherical capsules consist of a liquid core and a coat of calcium alginate gel membrane. However, the drug release properties of gel capsules have not yet been reported. Among the polymeric delivery systems for controlled drug release, Ca-alginate gel capsules are considered to be a reservoir-type diffusion controlled system. This suggests that their drug release character may be different from that of Ca-alginate gel beads, which are a matrix-type diffusion controlled system.[16]

In the present study, we developed a method for the preparation of spherical Ca-alginate gel capsules (about 3.1 mm in diameter in a dried state) containing a model drug, theophylline, and an excipient wheat starch. The objective of this study was to investigate several preparative variables affecting drug release from the dried gel capsules. We also compared the drug release characteristics of the Ca-alginate gel capsules to those of Ca-alginate gel beads.

Experimental

Materials The following chemicals were obtained from commerical suppliers and used without further purification: sodium alginate (Sigma Chemical Co., St. Louis, MO; the viscosity of 2% aqueous solution at 25 °C was 250 cps, and its mannuronate/guluronate molar ratio (M/G ratio) was 1.4[9]), wheat starch (Kishida Chemical Co., Osaka), pullulan (mol wt. *ca.* 200000, Tokyo Kasei Kogyo, Tokyo), theophylline and calcium chloride dihydrate (Wako Pure Chemical Industries, Ltd., Osaka). Theophylline and wheat starch were used after screening with a 120-mesh sieve. The solubility of theophylline in distilled water was 5.8 mg/ml at 25 °C and 11.6 mg/ml at 37 °C. All other chemicals were of reagent grade.

Preparation of Ca-Alginate Gel Capsules Theophylline (0.5—3.0 g) and wheat starch (7.0—9.5 g, for a total amount of theophylline and wheat starch of 10.0 g in each formulation) were added to 10 g of $CaCl_2$ solution (0.2—1.0 M) containing 2% pullulan, and dispersed homogeneously using a homogenizer (model SA, Nippon Rikagaku Kikai Co., Ltd., Tokyo). The dispersions were placed in a 25 ml glass syringe with a 14 gauge needle. Forty droplets of the dispersions were then dropped into 100 g of aqueous solution of sodium alginate (1.0 or 2.0%) during a 2-min period, with air pressure applied to the syringe. After a given reaction time, the resulting capsules were separated, washed in 500 ml of gently stirred distilled water for 1 min, and allowed to incubate for 5 min in 100 ml of 0.1 M $CaCl_2$ solution for hardening the gel membrane. All of these procedures were carried out at 25.0±0.5 °C. After being rinsed with 20 ml of distilled water, the hydrated gel capsules were air-dried for 12 h and then vacuum-dried at 40 °C for 2 h. The diameter of dried capsules was measured using a micrometer. Also, the weights of 10 droplets of the various dispersions were measured to calculate the theoretical theophylline content in the capsules. The droplet weights of the dispersions were found to be almost equal to each other, being 33.5±0.5 mg/drop (mean±SD of the 10 different dispersions (refer to Table I)). In order to examine the water content in the dried capsules, 50 capsules were further dried by heating in an oven at 105 °C for 5 h. The weight loss was less than 6.1%.

Preparation of Ca-Alginate Gel Beads Theophylline (1.0 g) and wheat starch (9.0 g) were dispersed in 10 g of sodium alginate solution (1.0 or 2.0%). The dispersions were dropped using a 14 gauge needle into gently stirred 0.1 M $CaCl_2$ solution (100 ml) so that, over a 2-min period, 40 beads were formed. After 10 min, the gel beads were separated by filtration and briefly rinsed with 20 ml of distilled water. All of these procedures were carried out at 25.0±0.5 °C. The gel beads were dried in the same manner as the gel capsules.

Determination of Theophylline Content and Encapsulation Efficiency (EE) Ten gel capsules or beads were disintegrated completely in 100 ml of 0.05 M phosphate buffer (pH 7.0) containing 5 mM ethylenediamine-

tetraacetic acid. After centrifugation, the supernatant was diluted with distilled water, and the theophylline content was determined spectrophotometrically at 271 nm. The ratio of the actual theophylline content in the gel capsule or bead to the theoretical theophylline content was termed an EE.[17] Here, the product of the weight of one droplet of the dispersion containing theophylline and wheat starch by the weight fraction of the theophylline in the dispersion is the theoretical theophylline content in a capsule or bead.

Estimation of Coat Weight Coat weight of a dried gel capsule was estimated from Eq. 1.

$$W = A - B - C \cdot D \quad (1)$$

where W is the coat weight (mg) of a capsule, A is a dried capsule weight (mg), B is the theophylline content (mg) in a capsule, C is the weight (mg) of one droplet of a core dispersion, and D is the weight fraction of wheat starch in a core dispersion.

Release Studies The theophylline release experiments were performed by the paddle method using a JP XII dissolution test apparatus at a rotating speed of 100 rpm. Forty capsules or beads containing theophylline were placed into 500 ml of distilled water, the temperature of which was maintained at 37±0.5 °C. At appropriate intervals, 5 ml samples were withdrawn and assayed spectrophotometrically at 271 nm, either directly or after appropriate dilution with the dissolution medium. After each sampling, an equal volume of fresh medium was added to the test medium. Release studies were done in duplicate and the average values were obtained.

Scanning Electron Microscopy (SEM) SEM was used to characterize the surface and cross-section of the gel capsules and the gel beads. The samples were coated with gold for 3 min at 15 mA (Hitachi E101 Ion Sputter) and observed with a Hitachi Akashi ALPHA-25A-type scanning electron microscope.

Results and Discussion

Gel Capsule Preparation In this study, wheat starch and pullulan were used as agents to modulate the viscosity and/or the density of the core dispersions to ensure the spherical shape of the hydrated capsules. Wheat starch also served as an excipient to ensure spherical capsules in dried state. As the droplets of core dispersion were dropped into an alginate solution, the calcium ions in the droplets started diffusing out of the droplets, and instantly cross-linked with the interfacial alginate molecules to form a water-insoluble Ca-alginate gel membrane around each droplet. Therefore, the mild agitation of alginate solution using a magnetic stirring bar (100 rpm) was necessary to reduce the possibility of capsules being connected to each other and/or enclosed by a common coat. The constant agitation also resulted in capsules with a uniform and reproducible coating thickness. As the coating progressed, the transparent gel layer grew visibly thicker. For example, after 20 min of coating, spherical, hydrated capsules about 5.2 mm in diameter and 1 mm in coat thickness were produced (Formulation code 5(0.4)/2—20, see Table I). During air drying, glossy capsules with a smooth surface were obtained, without losing their initial sphericity.

The following preparative variables were examined in this study: the alginate concentration, the coating time, the $CaCl_2$ concentration in the core dispersions, and the theophylline loading dose. The formulations and the physical properties of dried Ca-alginate gel capsules prepared are summarized in Table I. A very narrow distribution in size and weight of the capsules was observed in each formulation.

Capsule preparation could not be carried out using sodium alginate concentrations higher than 2% because of the solution viscosity. Moreover, at alginate concentrations below 1%, the capsules were unable to retain their spherical form during the drying process. However, no significant difference in capsule properties was observed between the capsules prepared with 1% and 2% sodium alginate (capsules No. 1, 3 in Table I).

The capsule diameter, capsule weight and coat weight increased as the coating time increased, and they reached a plateau at about 30 min (capsules No. 2—6). Therefore, the coating process appeared to be completed in nearly 30 min. In addition to the calcium ions, a part of the theophylline molecules dissolving in the capsule core was also diffused out of the capsules during the coating process. Thus, a consistent decrease in EE value was observed as the coating time increased. Meanwhile, an increase in $CaCl_2$ concentration in the core dispersions led to a marked increase in capsule diameter and weight (capsules No. 4, 7—10). It is of interest to note that the coat weight showed a linear incremental increase with $CaCl_2$ concentration, as exhibited in Fig. 1. Also, a higher $CaCl_2$ concentration resulted in a higher EE. This suggests that the outward diffusion of theophylline was gradually restricted by the coat of the Ca-alginate gel membrane. An increase in EE was further observed in capsules with an increasing

TABLE I. Formulations and Properties of Ca-Alginate Gel Capsules

Capsule No.	Formulation code[a]	Diameter[b] (mm)	Weight[b] (mg)	Content[c] (mg)	EE[d] (%)	Coat weight (mg)	Coat thickness (μm)
1	5(0.4)/1-20	3.07±0.17	18.9±0.2	1.40	83.1	2.3	50
2	5(0.4)/2-10	2.99±0.06	17.7±0.2	1.48	89.0	1.1	10
3	5(0.4)/2-20	3.10±0.06	19.2±0.1	1.42	83.7	2.7	65
4	5(0.4)/2-30	3.12±0.05	19.8±0.1	1.40	83.9	3.3	75
5	5(0.4)/2-45	3.12±0.04	20.0±0.1	1.29	78.0	3.6	75
6	5(0.4)/2-60	3.10±0.04	20.0±0.1	1.25	75.2	3.7	65
7	5(0.2)/2-30	3.03±0.02	17.8±0.2	1.33	81.3	1.4	30
8	5(0.6)/2-30	3.17±0.06	21.5±0.1	1.44	83.6	5.0	100
9	5(0.8)/2-30	3.23±0.06	23.1±0.5	1.49	89.3	6.4	130
10	5(1.0)/2-30	3.30±0.05	24.1±0.1	1.53	90.5	7.5	165
11	2.5(0.4)/2-20	3.08±0.05	19.2±0.1	0.60	72.7	2.7	55
12	7.5(0.4)/2-20	3.14±0.04	19.6±0.1	2.30	91.4	3.1	85
13	10(0.4)/2-20	3.14±0.01	19.2±0.1	3.19	95.0	2.6	85
14	15(0.4)/2-20	3.15±0.03	19.4±0.1	4.62	93.8	3.1	90

a) Formulation code nomenclature: the first number and the number in parentheses refer to theophylline weight percentage and molar concentration of $CaCl_2$ in core dispersion, respectively; the first number after the slash indicates Na alginate weight percentage in coating solution, and the second number, coating time in min. *b*) Data were the average±standard deviation of a minimum of 40 capsules. *c*) Theophylline content in a gel capsule. *d*) The efficiency of theophylline encapsulation.

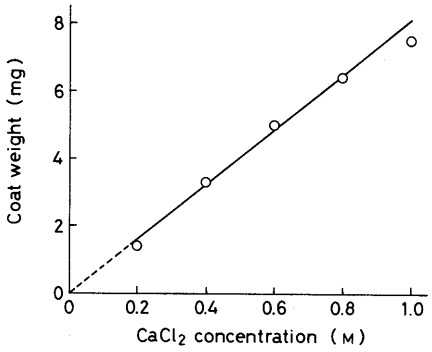

Fig. 1. Coat Weight of Ca-Alginate Gel Capsule as a Function of $CaCl_2$ Concentration in Core Dispersion

theophylline loading dose (capsules No. 3, 11—14).

Using the data obtained, we attempted to estimate the coat thickness of the Ca-alginate gel membrane. The coat weight (mg) of a capsule, W, is given by

$$W = W_t - W_c = \frac{\pi d_m}{6}(D^3 - D_c^3) \quad (2)$$

where W_t and W_c are the weights (mg) of capsule and capsule core, respectively, d_m is the density (g/cm^3) of the coating membrane, and D and D_c are the diameters (mm) of the capsule and capsule core, respectively. Upon re-arrangement, the following relationship is obtained.

$$D^3 = \frac{6}{\pi d_m} W + D_c^3 \quad (3)$$

Equation 3 indicates that the third power of capsule diameter is linearly correlated with the coat weight under the condition of a constant D_c. In this study, capsules No. 1 through 10 were prepared with fixed amounts of theophylline and wheat starch in the core dispersions (1 and 9 g, respectively), and thus, these capsules are conceivable of having the same D_c value. Capsules No. 11 through 14 were prepared by keeping the total amounts of theophylline and wheat starch constant, at 10 g, in the core dispersions. This also results in the same D_c, provided that the apparent specific volumes of theophylline and wheat starch do not differ widely. The plots according to Eq. 3 showed a good linearity ($r=0.944$, $n=14$), as depicted in Fig. 2. From the values of slope and intercept of the linear plot, d_m and D_c were found to be 1.55 g/cm^3 and 2.97 mm, respectively. Therefore, the coat thickness (in mm) of Ca-alginate gel capsules in each formulation could be calculated by subtracting 2.97 from a capsule diameter and then dividing by 2. The values thus estimated ranged from 10 to 165 μm (Table I).

Drug Release Profiles The theophylline release profiles of capsules prepared in varying coating times are shown in Fig. 3. The capsules exhibited a zero-order drug release up to the theophylline release percentage of 70—80. Also, as the coating time increased, the drug release rate decreased. However, in capsules coated for more than 30 min, no further pronounced decrease in release rate was observed. These results are entirely relevant to the increase in coat weight as a function of coating time. The capsule membrane neither dissolved nor swelled in the release medium after the drug was completely released, and the shape of the

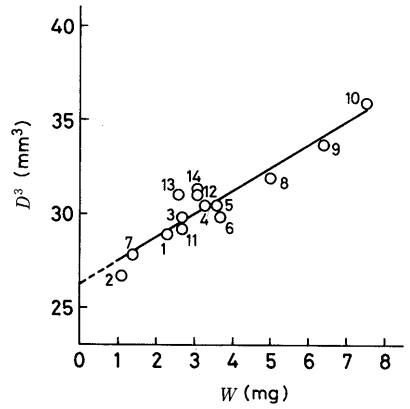

Fig. 2. Relationship between D^3 and W of the Ca-Alginate Gel Capsules

The numbers in the figure refer to the capsule No. in Table I.

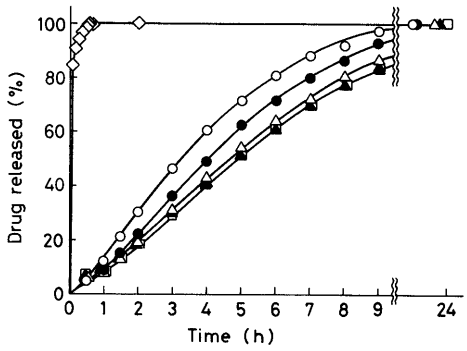

Fig. 3. Effect of Coating Time on the Theophylline Release from Ca-Alginate Gel Capsules in Distilled Water

Capsule No. (see Table I): ○, 2; ●, 3; △, 4; ▲, 5; □, 6; ◇, theophylline powder.

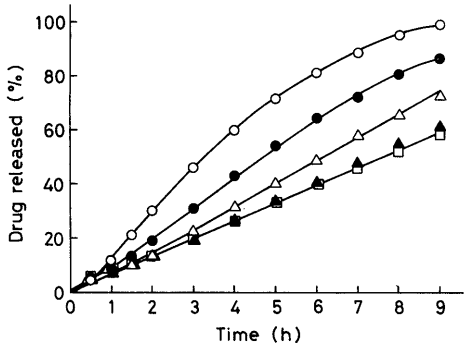

Fig. 4. Effect of $CaCl_2$ Concentration in the Core Dispersion on the Theophylline Release from Ca-Alginate Gel Capsules in Distilled Water

Capsule No. (see Table I): ○, 7; ●, 4; △, 8; ▲, 9; □, 10.

capsules did not change in appearance. It is therefore very likely that the rate of theophylline release from the gel capsules depends on the rate of penetration of the release medium into the capsules, and also on the rate of dissolution and subsequent diffusion of the drug through the dense Ca-alginate membrane. Thus, the Ca-alginate gel capsules can be categorized as a capsule-type drug delivery system with a rate-controlling polymer membrane.[16] On the other hand, the dissolution of pure theophylline powder was rapid, and was completed within 20 min (Fig. 3).

Figure 4 shows the release profiles of capsules prepared with varying $CaCl_2$ concentrations. The increase of $CaCl_2$ concentration in the core dispersions resulted in a more

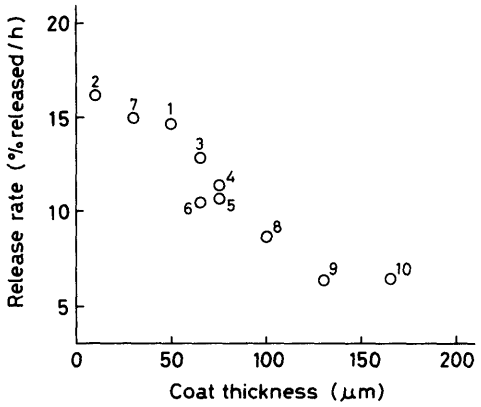

Fig. 5. Relationship between Theophylline Release Rate and Coat Thickness of Ca-Alginate Gel Capsules

The numbers in the figure refer to the capsule No. in Table I.

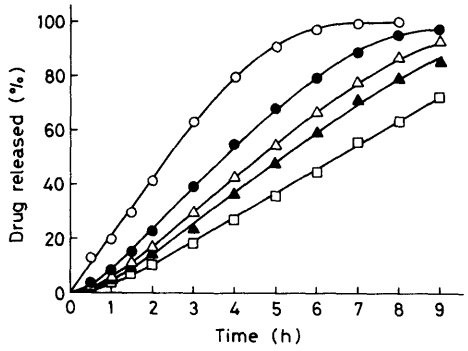

Fig. 6. Effect of Theophylline Content on the Theophylline Release from Ca-Alginate Gel Capsules in Distilled Water

Capsule No. (see Table I): ○, 11; ●, 3; △, 12; ▲, 13; □, 14.

reduced release rate. For example, the half-life ($t_{1/2}$, the time required to release half the drug in the capsules) increased from 3.4 h (capsule No. 7) to 7.5 h (capsule No. 10). In contrast, the theophylline release rate was not greatly influenced by a sodium alginate concentration (Fig. 7). These findings further confirm that the membrane thickness of gel capsules is an important factor in determining the drug release rate from the capsules. In Fig. 5, the theophylline release rate, given by the slope of the straight line in the drug release profiles, is plotted against the coat thickness. It is clear that the theophylline release rate can be controlled by the coat thickness of the gel capsules.

The effect of theophylline content in the capsules on the release rate is shown in Fig. 6. The greater the drug content, the slower the release rate. The $t_{1/2}$ value varied from 2.5 h (capsule No. 11) to 6.5 h (capsule No. 14). The same trend in theophylline release rate was observed when the release experiments were carried out using the calculated numbers of capsules equivalent to 60 mg theophylline (13—100 capsules, depending on the drug content in a capsule). These findings indicate that the theophylline release rate can also be controlled by the drug content in the capsules.

Comparison of the Gel Capsules to the Gel Beads In order to evaluate the gel capsules, the theophylline release profiles were compared to those of Ca-alginate gel beads. The gel beads were prepared with an initial alginate concentration of 1% and 2% (see Experimental for details). Their diameter, weight and theophylline content per one

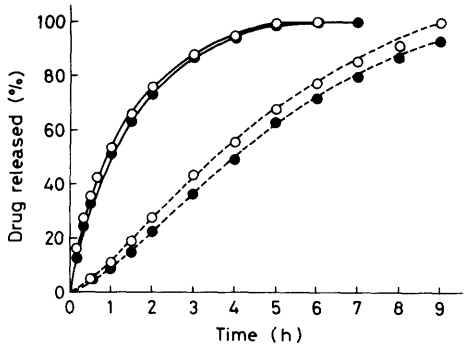

Fig. 7. Release Profiles of Theophylline from Ca-Alginate Gel Capsules and Ca-Alginate Gel Beads in Distilled Water

The dotted lines refer to the Ca-alginate gel capsules prepared with 1% (○, capsule No. 1 in Table I) and 2% (●, capsule No. 3) sodium alginate solutions. The solid lines refer to the Ca-alginate gel beads prepared with 1% (○) and 2% (●) sodium alginate solutions (see Experimental for the conditions of gel bead preparation).

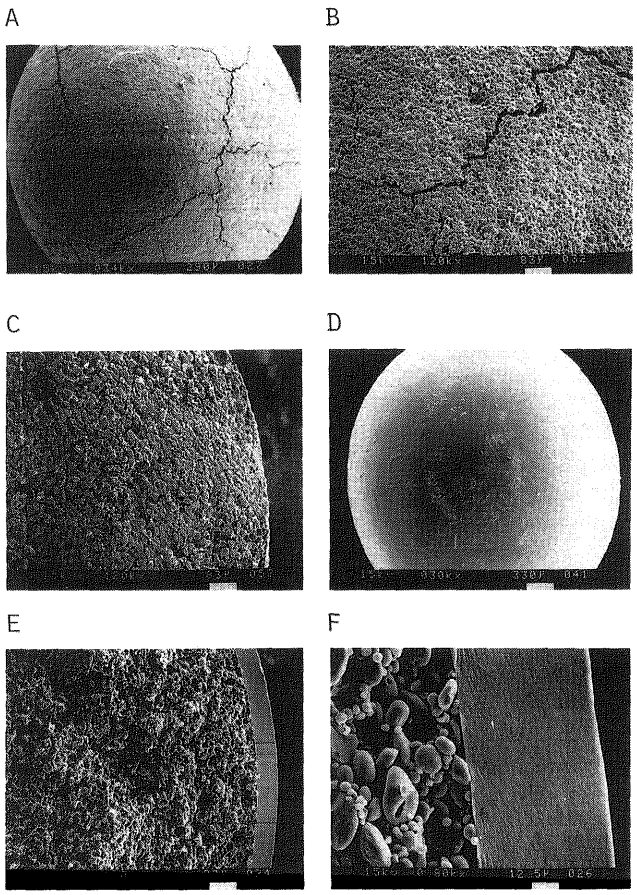

Fig. 8. Scanning Electron Micrographs of Ca-Alginate Gel Bead and Ca-Alginate Gel Capsule Containing Theophylline and Wheat Starch

A, bead surface (scale bar: 290 μm); B, bead surface (83 μm); C, bead cross section (83 μm); D, capsule surface (330 μm); E, capsule cross section (83 μm); F, capsule cross section (12.5 μm).

bead were 3.07±0.13 mm, 15.20±0.07 mg and 1.46 mg for the beads prepared with 1% alginate, and 3.01±0.14 mm, 15.35±0.05 mg and 1.44 mg for the beads prepared with 2% alginate, respectively. Thus, the gel beads and the gel capsules did not differ greatly in physical dimensions and theophylline content. Like the gel capsules, the gel beads did not disintegrate or swell in the release medium. However, theophylline was released more rapidly from the gel beads than from the gel capsules, as shown in Fig. 7. In addition,

the theophylline release from the gel beads did not follow zero-order, and a linear relationship was found between the amount of drug released and the square root of release time (figure not shown). A similar trend in the drug release profiles of Ca-alginate gel beads has been reported.[4]

To explain the distinct difference in theophylline release rate, the morphology of the gel beads (2% alginate gel beads in Fig. 7) and gel capsules (capsule No. 3) was investigated by SEM. SEM photographs of the surface and a cross section of these particles are shown in Fig. 8. On the surface of the gel bead, not only the crystals of theophylline and wheat starch, but also some cracks, are observed (Fig. 8A, B). Similar cracks have been reported on alginate gel beads which contain drugs.[4,6] These are probably formed during the drying of the wet beads. A cross section of the gel bead shows that the drug and wheat starch crystals were embedded in the alginate matrix (Fig. 8C). On the other hand, the gel capsule had a smooth surface, and neither cracks nor the crystals were observed on the surface (Fig. 8D). It is clear that the capsule core was coated with a dense Ca-alginate gel layer (Fig. 8E, F). Note that the coat is perfectly uniform in thickness. Also note that the estimated coat thickness of capsule No. 3, 65 μm, is in excellent agreement with the coat thickness found on the SEM photograph, 62 μm (Fig. 8F).

Undoubtedly, all the results obtained in this study indicate that Ca-alginate gel capsules are a capsule-type drug delivery system with a rate-controlling polymer membrane, whereas the Ca-alginate gel beads are a matrix-type drug delivery system in which the drug crystals are homogeneously dispersed.[16] The following are likely to be responsible for the fast theophylline release rate of the gel beads compared to that of the gel capsules. First is the existence of cracks in the gel beads. This factor is also supported by the fact that the release rate of blue dextran from the dried alginate gel beads is faster than that from the wet gel beads.[5] Second is the increase in the number and size of pores on the gel beads as the dissolution of the drug crystals on the surface of the gel beads proceeds. Third is the low concentration of Ca-alginate gel; the net weight of gel matrix for the gel beads prepared with 2% alginate is 0.328 mg/one bead ($=2/100 \times 10 \times 1/20 \times 32.81$, where 32.81 is the weight (mg) of an initial droplet), which is much lower than the coat weight of a capsule (Table I). Thus, the matrix density of the gel beads is much lower than that of the gel capsules in which the gel is concentrated in the surface of the capsules.

In conclusion, drug-containing Ca-alginate gel capsules were successfully prepared through ionotropic gelation. The gel capsules may offer several potential advantages over conventional Ca-alginate gel beads as a polymeric delivery system for controlled drug release. First, the zero-order drug release usually required for polymeric devices can be easily achieved using gel capsules. Second, the drug release rate from the gel capsules can be regulated by the coat thickness of the Ca-alginate gel membrane. Further work is being undertaken to prepare gel capsules with pH-independent and zero-order drug release characteristics.

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