Preparation and Characterization of Alginate–Carrageenan Complex Films

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ABSTRACT: Alginate–carrageenan (Al–Ca) complex films were synthesized and characterized. The Al–Ca ratio and the crosslinking agent type were important factors in determining the pore size of the complex film. The pore size decreased with an increasing carrageenan content and was reduced further by a crosslinking reaction with $CaCl_2$. The most uniform and flexibile film was formed at an Al–Ca ratio of 6:4. The degree of swelling of crosslinked films increased with an increasing carrageenan content. With a combination of $CaCl_2$ and $ZnSO_4$, the water content of the film due to swelling was smallest, which was more suitable than with $CaCl_2$ alone. The permeabilities of glucose and dextrans for Al–Ca complex films increased as the alginate content increased, because complex films with a high alginate content had large pores. The partition coefficients of glucose and dextrans for films were in a range of 0.2 to 1.0 depending on the contents of alginate and carrageenan. A 6:4 Al–Ca complex film was most stable in a phosphate buffer solution, indicating that the 6:4 content ratio was suitable to maintain the mechanical strength of alginate–carrageenan chains. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 99: 3483–3490, 2006

Key words: alginate–carrageenan; films; gelation; crosslinking; permeability

INTRODUCTION

Various biopolymers have been developed from marine sources.[1–3] Carrageenans, water-soluble galactose polymers extracted from red seaweed, are extensively used in the food and pharmaceutical industries as gelling and stabilizing agents. κ-Carrageenan can form excellent gels or films because of a negative charge per disaccharide. Carrageenans play a structural role in cellular matrix materials in numerous seaweed species of the class *Rhodophyta*. Their amorphous structures provide the flexibility required for adaptation to varying amounts of tidal and wave motion stress. A wide variety of structures exist that differ in both number and location of substituents in the polysaccharide chain. The main substituents consist of hemi-ester sulfate groups.[4] Alginic acid is a copolysaccharide extracted from brown algae consisting of D-mannuronic and L-guluronic acid monomers. Na-alginate is a water-soluble salt of alginic acid and is a naturally occurring nontoxic polysaccharide.[5]

Hydrogel is a polymer with a three-dimensional network that swells when immersed in water. Hydrogels having the film-forming properties of Na-alginate and κ-carrageenan have been applied in the engineering and biotechnology fields for antimicrobial films,[5] cell culture substrates,[6,7] food additives,[8] materials for controlled drug delivery,[9] and membranes for separation processes.[10,11] These delivery gels are formed when monovalent and water-soluble alginate salts undergo an aqueous sol–gel transformation into water-insoluble salts by addition of divalent ions such as calcium, strontium, or barium.[12] Although strontium and barium alginate form strong insoluble matrices, commonly used calcium alginate can form a matrix for various delivery systems, including gels, films, beads, microparticles, and sponges.[13–17] There is a report[18] that a gel consisting of κ-carrageenan and gelatin as gelling agents was used for the oral delivery of paracetamol in rabbits with high bioavailability. Kubo et al.[19] reported the potential of a formulation with gellan gum or Na-alginate as a vehicle for the sustained delivery of paracetamol.

There have been reports of films consisting of either alginate or carrageenan, but there are few reports concerning complexes of both materials. In this study, various preparation conditions for alginate–carrageenan (Al–Ca) complex films were optimized and the physical properties of complex membrane films were characterized. Permeabilities of glucose and dextrans in the membrane films and partition coefficients of the solutes between the membrane and a water solution were measured.

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EXPERIMENTAL

Materials

Na-alginate was obtained from the Yakuri Pure Chemicals Co. (Japan). κ-Carrageenan, dextrans (average MW: 500,000, 150,000 and 10,000), L-lysine, L-arginine, and glutaraldehyde were purchased from Sigma Chemical Co. All other chemicals were products of Duksan Pure Chemical Co. (Korea).

Procedures of film preparation and crosslinking reactions

Al–Ca complex films were prepared from powder forms of Na-alginate and κ-carrageenan dissolved in distilled water at 80°C for 5 h to prepare 2% (w/v) solutions. The notation Al:Ca indicates the ratio mixture of alginate and carrageenan. Various mixtures of alginate and carrageenan (10:0, 8:2, 6:4, 4:6, 2:8, and 0:10) were homogenized at 5000 rpm for 10 min. Six milliliters of the mixed solution was poured into a polystyrene petri-dish (diameter 5cm, depth 0.8 cm), and frozen at −70°C overnight, followed by lyophilization at −48°C for 48 h. The soluble membrane films were immersed directly in 30 mL of an ethanol–water mixture (6/4) containing 3% (w/v) of a calcium chloride and/or a zinc sulfate solution at room temperature for 12 h.

Scanning electron microscopy

The overall surfaces and cross sections of alginate–carrageenan complex films were examined under a scanning electron microscope (SEM; JSM-5410LV, Jeol Co., Japan) at 15 kV. Samples were mounted on metal stubs using double-sided adhesive tape and coated with a 10 mA gold sputter (JFC-1100E, Jeol Co., Japan) for 4 min under an argon atmosphere. Samples were examined in a high vacuum mode.

Measurement of film swelling

Membrane films were immersed in water or different compositions of an ethanol-water mixture for 24 h at 25°C. Swollen films were then placed between two dry filter papers to remove residual liquids from the film surface, weighed, dried under a vacuum, and reweighed. The water contents in complex films were calculated using eq. (1).

$$\text{Water content (\%)} = (Ws - Wd)/Ws \times 100 \quad (1)$$

where Ws and Wd indicate the weights of swollen and vacuum-dried films, respectively.

Measurement of total carbohydrate

The total carbohydrate contents of filtrates obtained from permeability tests were determined by the phenol–sulfuric acid method. A microfuge tube containing 150 μL of sample was treated with 150 μL of 5% phenol. The tube was mixed by vortexing gently for 30 s, then put on ice. An amount of 750 μL of sulfuric acid was added, and the tube was again vortexed gently for 30 s, then incubated at 80°C for 30 min, followed by cooling to room temperature. The absorbance was measured at 492 nm and converted into either a glucose or dextran content.

Permeability measurement

Membranes for permeability experiments were maintained in fresh water for at least 3 days to reach a complete swelling equilibrium. A circular piece of the film was then fixed between equal volume compartments on both sides of a permeation cell. The upstream side was filled with either a glucose or a dextran solution, and the downstream side was filled with pure water. The solution in the downstream compartment was magnetically stirred at a constant speed. Increases in the glucose and dextran concentrations on the downstream side were monitored by measuring the absorbance at 492 nm, using a UV–Vis spectrophotometer. The permeability (P) was calculated using eq. (2).[20]

$$P = (dC/dt)\,(V\,d/C\,A) \quad (2)$$

The term dC/dt represents the slope of a straight line where V is the volume crossing the film layer, C is the initial glucose or dextran concentration, and A and d represent the area and thickness of film, respectively. The d value of the film was measured under an equilibrium condition in the swollen state.

Measurement of partition coefficient

To obtain the partition coefficients of glucose and dextrans between hydrogel films and the water solution, a piece of membrane film was soaked in either a glucose or dextran aqueous solution for 24 h. The concentration of either glucose or dextran remaining in the aqueous solution was then measured. The solute solubility in a film hydrogel was calculated from the difference between the solute concentrations before and after soaking. The partition coefficients of glucose and dextrans at various alginate–carrageenan contents were determined using eq. (3).

$$\text{Partition coefficient (K)} = \text{Membrane solubility}/\text{Water solubility} \quad (3)$$

Stability test of membrane films

Alginate gels can be completely redissolved by phosphate, whereas carrageenan gels require phosphate to stabilize the network. Crosslinked films were weighed after vacuum-drying, then immersed in a phosphate

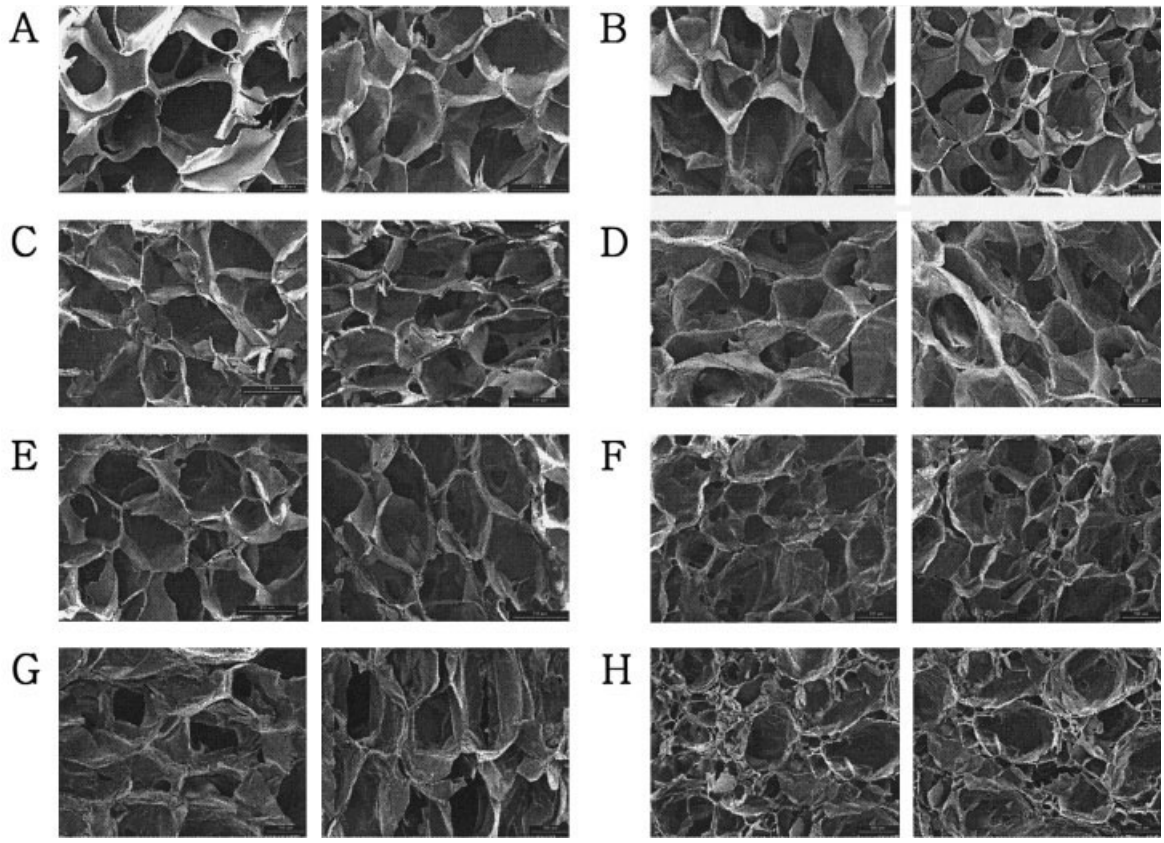

Figure 1 Representative SEM images (×200) of alginate-carrageenan (Al–Ca) films noncrosslinked (A,C,E,G) and crosslinked (B,D,F,H) with $CaCl_2$. Al–Ca ratio: (A) and (B), 10:0; (C) and (D), 8:2; (E) and (F), 6:4; (G) and (H), 4:6.

buffer for 24 h. After that, the films were rinsed with ethanol, vacuum-dried, and reweighed. Weight loss was used as an indication of film dissolution.

Statistical analysis

All results were analyzed by Student's t-test (MS Office XP, Microsoft, WA), which was used to make a statistical comparison among the groups with results expressed as the mean ± the standard deviation. A value of $P < 0.05$ was interpreted as statistically significant.

RESULTS AND DISCUSSION

Morphologic characteristics of alginate–carrageenan complex films

Various complex films were prepared from different ratios of an alginate–carrageenan (Al–Ca) mixture by the ionotropic gelation method.[21] The films were then crosslinked by reaction with $CaCl_2$. SEM images of crosslinked and noncrosslinked complex films were compared (Fig. 1). Figures 1(A), 1(C), 1(E), and 1(G) and 1(B), 1(D), 1(E), and 1(H) show the cross-sectional morphologies of noncrosslinked and crosslinked complex films, respectively.

Noncrosslinked films were complex with thinner strands and a smaller pore size than a pure alginate film. The film pore size was distributed in a range of 30–150 μm and decreased with an increasing carrageenan content up to 40%. At 60% or greater, the cavities were considerably deformed, indicating that the carrageenan content is a key factor for controlling the size of film pores.

Complex films were crosslinked by reaction with $CaCl_2$ (Figs. 1(B), 1(D), 1(E), and 1(H)). Regardless of film composition, the pore size of crosslinked films was significantly smaller than the pore size of noncrosslinked films, and many pores in noncrosslinked films were deformed by crosslinking. The best crosslinked film was prepared with a 6:4 Al–Ca mixture, followed by reaction with $CaCl_2$. This film was uniform and flexible. The 6:4 ratio provided a good miscibility suitable for film construction.

Xu et al.[22] reported that the morphology of complex films depended on the Al–Ca ratio of a mixture rather than the degree of crosslinking with ions. However, our results showed that the Al–Ca ratio and the degree of crosslinking are both important for size control of film pores.

Crosslinking of alginate–carrageenan complex films

Crosslinking agents are usually dissolved in ethanol–water mixtures. However, the ethanol concentration

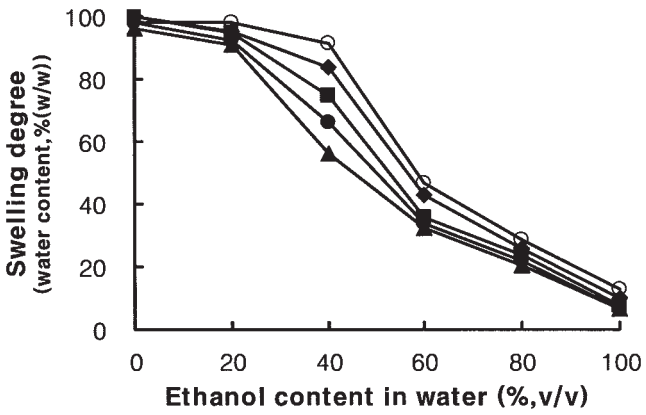

Figure 2 The degree of swelling of noncrosslinked complex films in different concentrations of an ethanol–water solution. Al–Ca ratio: (○), 0:10; (♦), 2:8; (■), 4:6; (●), 6:4; (▲), 8:2. Experiments were repeated three times and average values are presented.

should be chosen depending on the types of polymers and crosslinking agents.[23] Since alginate and carrageenan are highly water-soluble, alginate–carrageenan complex films can be dissolved by water during the crosslinking process. The degree of dissolution of a film can be regulated by the ethanol or water content of the solution in which the crosslinking agent is dissolved.

We measured the degree of swelling of noncrosslinked complex films in 0–100% (v/v) ethanol solutions (Fig. 2). Swelling decreased with an increasing ethanol concentration from 100% in pure water to less than 30% in 60% or greater ethanol solutions. On the other hand, the composition of films affected the degree of swelling. A film made of pure carrageenan showed the greatest swelling, and swelling increased gradually with an increasing carrageenan content in complex films. Results were strikingly different in a 40% ethanol solution in which 40% swelling of a 20% carrageenan film was shifted to 95% swelling of a pure carrageenan film. These results indicate that the water sorption ability of carrageenan is much larger than the sorption ability of alginate. It was expected that a high carrageenan content in complex films would cause severe collapse. However, the increase in the alginate content can result in mechanical strengthening of the carrageenan chains in a complex film. The water sorption ability of carrageenan is probably related to highly polar sulfate groups in the structure. The hydrophilicity of complex films can be enhanced by increasing the carrageenan content, leading to an increased degree of swelling.

The swelling of complex films can be controlled by crosslinking. Both the solubility of the crosslinking agent in ethanol and the penetration of water into film should be considered. The optimum $CaCl_2$ concentration was found to be 3% (w/v), based on preliminary analyses, and complex films were crosslinked by reaction with 3% $CaCl_2$. The swelling of crosslinked films was measured. Figure 3 shows the water-sorption patterns over time of films crosslinked with $CaCl_2$. The swelling of crosslinked complex films decreased with an increasing time of crosslinking up to 3 h and thereafter remained constant. Similar to patterns for noncrosslinked complex films, as the alginate content of crosslinked complex films increased, swelling decreased. The least swelling was obtained for the film composed of 80% alginate and 20% carrageenan. Since crosslinking agents are poorly dissolved in ethanol, the ethanol solution should have a sufficiently high content of water or a low content of ethanol for efficient crosslinking.[23]

Crosslinking of a film can enhance resistance to chemical and long-term biological degradation and can prevent alginate and carrageenan moieties from being dissolved out of the film. Various crosslinking agents, such as formaldehyde, glutaraldehyde, ammonium ions, metal ions, amine groups, and amino acids and their derivatives, were used. However, the well-known crosslinking agents, glutaraldehyde and hexamethylenediamine, were excluded because of serious toxicities.[24] Only nontoxic and biocompatible crosslinking agents were used. After complex films were treated with different crosslinking agents, the degree of swelling in water was measured. As shown in Figure 4, films treated with either $CaCl_2$ or $ZnSO_4$ showed swelling decreasing proportionally with either an increasing alginate content or a decreasing carrageenan content. However, with ethanol, NH_4Cl, KCl, $MgCl_2$, L-lysine, or L-arginine, the degree of swelling of crosslinked films was little affected by changing the carrageenan content.

Various crosslinking agents were combined with $CaCl_2$. Complex films were first treated with $CaCl_2$ for

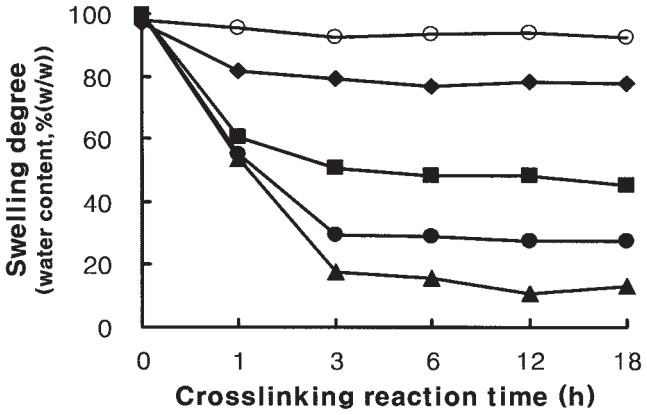

Figure 3 The degree of swelling of complex films crosslinked with $CaCl_2$ for different lengths of time. Al–Ca ratio: (○), 0:10; (♦), 2:8; (■), 4:6; (●), 6:4; (▲), 8:2. Experiments were repeated three times and average values are presented.

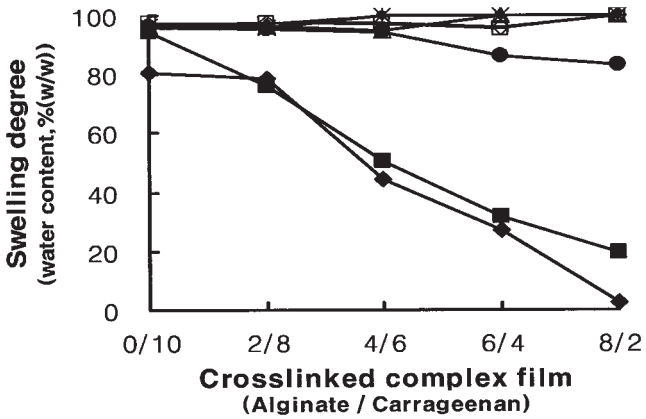

Figure 4 The degree of swelling of complex films treated with different crosslinking agents. Crosslinking agents: (○), none; (△), ethanol; (□), NH_4Cl; (◇), KCl; (●), $MgCl_2$; (▲), L-Lysine; (■), $ZnSO_4$; (♦), L-Arginine; (×), $CaCl_2$. Experiments were repeated three times and average values are presented.

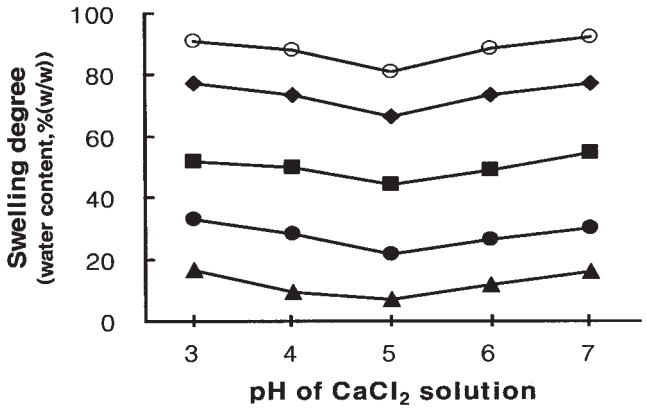

Figure 6 The degree of swelling of Al–Ca complex films crosslinked with $CaCl_2$ at different pH values. Al–Ca ratio: (○), 0:10; (♦), 2:8; (■), 4:6; (●), 6:4; (▲), 8:2. Experiments were repeated three times and average values are presented.

6 h, followed by 6 h of incubation with another crosslinking agent. Figure 5 shows that films crosslinked with $CaCl_2$ and $ZnSO_4$ exhibited the least swelling, but these results were not significantly different from results with $CaCl_2$ alone. However, complex films crosslinked with a combination of $CaCl_2$ and $ZnSO_4$ appeared to have a smaller pore size than films crosslinked with $CaCl_2$ alone (data not shown). The degree of crosslinking for all crosslinked films showed a decreasing trend with a decreasing carrageenan content. It is known that zinc and calcium cations bind with different sites on Al–Ca complex films.[25] Alginic acid is a linear polymer containing various proportions and sequential arrangements of α-L-guluronic acid and β-D-mannuronic acid units.

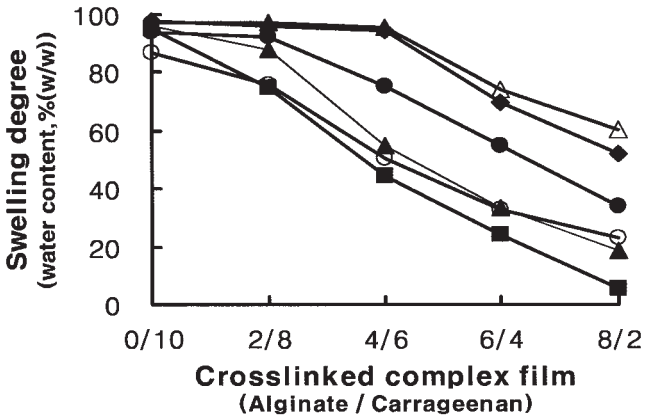

Figure 5 The degree of swelling of complex films cocrosslinked with $CaCl_2$ and additional agents. Crosslinking agents: (○), $CaCl_2$; (△), $CaCl_2$ + L-Arginine; (●), $CaCl_2$ + $MgCl_2$; (▲), $CaCl_2$ + KCl; (■), $CaCl_2$ + $ZnSO_4$; (♦), $CaCl_2$ + NH_4Cl. Experiments were repeated three times and average values are presented.

Soluble sodium alginate can be crosslinked with divalent or polyvalent cations to form insoluble alginate gels. A uniform region of guluronic acid is the main site of cation interaction.

Since zinc and calcium cations can facilitate the formation of ester linkages between the carboxylic groups of alginate and the sulfate groups of carrageenan, the pH of a crosslinking agent solution is important. Below pH 3.0, sodium alginate is known to be rapidly converted to alginic acid, which swells but is insoluble and is very resistant to erosion. However, at a neutral pH, viscous gels are formed that are much less resistant to attrition.[26] The degree of swelling of films crosslinked at different pH values in a $CaCl_2$ solution was measured (Fig. 6). The pH value was maintained in a range from 3.0–7.0. The least swelling of complex films was observed at a pH of 5.0. Similar to previous results, swelling decreased with a decreasing carrageenan content. Figure 7 shows the degree of swelling of films treated with $ZnSO_4$ instead of $CaCl_2$. Swelling decreased with an increasing pH of the $ZnSO_4$ solution, unlike results with $CaCl_2$.

Ionotropic binding of Al–Ca complex films with Ca or Zn cations was expected. In a strong acidic solution, the carboxylic groups of the alginate moiety and the sulfate groups of the carrageenan moiety in complex films are nonionized by binding with H^+, weakening the binding of films with Ca^{2+} or Zn^{2+}. Al–Ca complex films apparently maintain randomly coiled conformations because of weak charge repulsions between the sulfate and carboxylic groups. However, at acidic or neutral pH values, Ca^{2+} and Zn^{2+} can increase interlinkages between the carboxylic groups of alginate and the sulfate groups of carrageenan with negative charges.[26] The films can maintain more extended forms because of strong repulsions between the negative charges of dissociated sulfate and carboxylic groups. The crosslinking of complex films is ap-

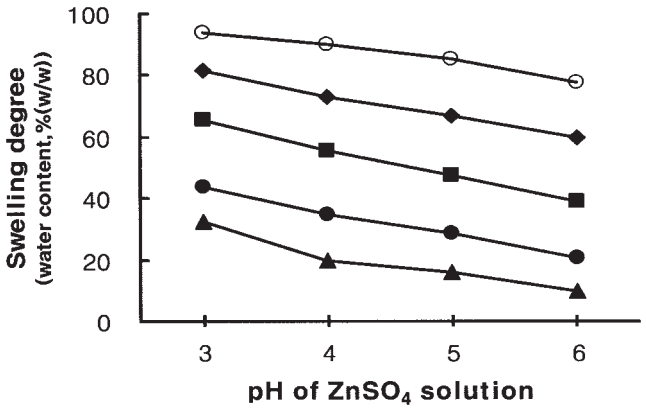

Figure 7 The degree of swelling of Al–Ca complex films crosslinked with $ZnSO_4$ at different pH values. Al–Ca ratio: (○), 0:10; (♦), 2:8; (■), 4:6; ●, 6:4; (▲), 8:2. Experiments were repeated three times and average values are presented.

parently controlled by the number of ionized groups present at a given pH.

Permeabilities of glucose and dextrans through Al–Ca complex films

The porosity and physical stability of films affect the permeability (P) of a solute molecule through membrane pores. Films consisting of only alginate are very porous and can be dissolved. Solute molecules can easily diffuse out of the film. We investigated a means to limit solute material loss from the film. Complex films with varying alginate and carrageenan contents were prepared for the purpose of reducing the pore size.

The permeabilities of glucose and dextrans through complex films were measured. A concentration gradient of either glucose or dextrans was maintained between two compartments divided by a membrane film. The amounts of solute diffused through the membrane film were measured over time. As shown in Figure 8, the concentrations of glucose and dextrans in the downstream compartment increased linearly for approximately the first 30 min. Film permeabilities were calculated using eq. (3) and the experimental data are summarized in Table I. The glucose permeability decreased with an increasing carrageenan content in the film (Fig. 9). This trend was also observed with the large solutes of 10 kD and 150 kD dextrans. However, the permeability of 500 kD dextran was not varied on the carrageenan content of film. The permeability decreased remarkably with an increasing molecular mass of the solute, with similar results for all membrane films. Our results indicate that the permeability is apparently directly related to the pore size of complex films, which can be controlled by the Al–Ca ratio. The film pore size can be reduced by increasing the carrageenan content. However, when the carrageenan content was 80% or greater, the films were structurally unstable during permeability tests. On the other hand, since the water sorption of a film is stimulated by increasing the carrageenan content, the permeability was expected to be enhanced. Our results, however, showed that the film permeability was reduced by increasing the carrageenan content, indicating that the water sorption ability does not affect the pore size.

Partition coefficients of glucose and dextrans in complex films

Solute permeability can be altered by the Al–Ca ratio, which affects the pore size and the internal morphology of complex films. In addition, an interaction of solute with film is considered to affect the permeability. As a means to evaluate the affinity of a solute for a film, the partition coefficients (K) of solutes (solute concentration in solution/solute concentration in film) were measured. The coefficient can be used to evaluate the degree of hydrophobicity/hydrophilicity of a film.[27,28]

Figure 10 shows the partition coefficients of glucose and dextrans for various complex films. The maximum solute partition coefficients were obtained at a 40–60% carrageenan content (6:4 or 4:6 Al–Ca film).

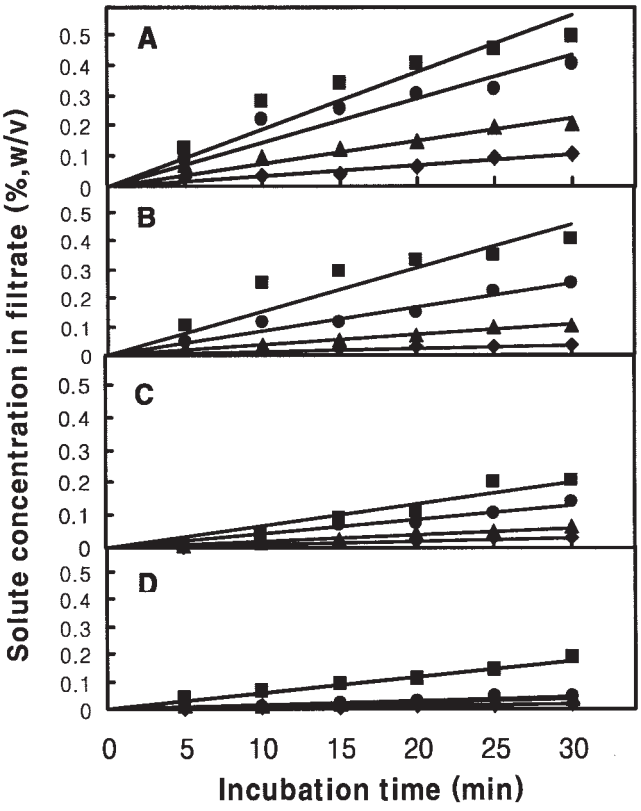

Figure 8 The diffusion rates of glucose through Al–Ca complex films. (A): glucose; (B): dextran (M_w 10,000); (C): dextran (M_w 150,000); (D): dextran (M_w 500,000). Al–Ca ratio: (■), 10:0; (●), 8:2; (▲), 6:4; (♦), 4:6. Experiments were repeated three times and average values are presented.

TABLE I
Permeabilities of Glucose and Dextrans through Alginate–Carrageenan Membrane Films

Penetrator	Membrane film	Before-swelling thickness (mm)	After-swelling thickness (mm)	dC/dt[a]	Initial penetrator concentration % (w/v)	Permeability (10^5 mm^2 min^{-1})
Glucose	Al–Ca (8:2)	1.87	1.92	0.0156	1.28	86.07
	Al–Ca (6:4)	1.40	1.51	0.0068	1.52	34.95
	Al–Ca (4:6)	1.09	1.19	0.0059	1.41	28.24
Dextran (M_w 10,000)	Al–Ca (8:2)	1.87	1.92	0.0083	1.53	38.79
	Al–Ca (6:4)	1.40	1.51	0.0043	1.59	19.99
	Al–Ca (4:6)	1.09	1.19	0.0015	1.57	9.18
Dextran (M_w 150,000)	Al–Ca (8:2)	1.87	1.92	0.0036	1.55	16.40
	Al–Ca (6:4)	1.40	1.51	0.0021	1.44	8.09
	Al–Ca (4:6)	1.09	1.19	0.0012	1.51	3.46
Dextran (M_w 500,000)	Al–Ca (8:2)	1.87	1.92	0.0012	1.74	4.87
	Al–Ca (6:4)	1.40	1.51	0.0011	1.45	4.23
	Al–Ca (4:6)	1.09	1.19	0.0006	1.24	2.11

[a] The term dC/dt represents the slope of the straight line.
Volume of the upstream compartment, 50 mL; area of the membrane film, 1361 mm^2.

The partition coefficient of glucose increased gradually with an increasing carrageenan content, resulting from an increased hydrophilicity. A maximum value of 1.0 was obtained for a 4:6 Al–Ca film. However, when the carrageenan content was increased further, the film was deformed. On the other hand, the partition coefficient for dextrans decreased from 0.8 to 0.2 with an increasing solute molecular mass, probably caused by a reduced diffusion rate of the large solute molecule through the film pores and the increased hydrophobicity of the solute molecule.

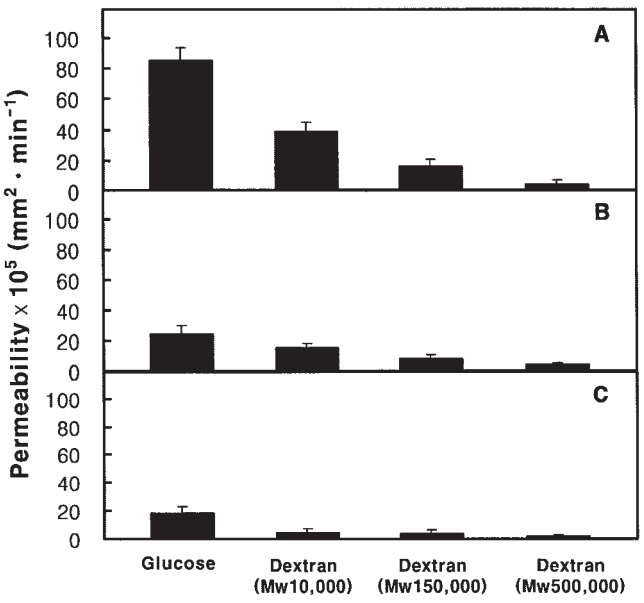

Figure 9 The permeabilities of glucose and dextrans through Al–Ca complex films. (A): Al–Ca, 8:2; (B): Al–Ca, 6:4; (C): Al–Ca, 4:6.

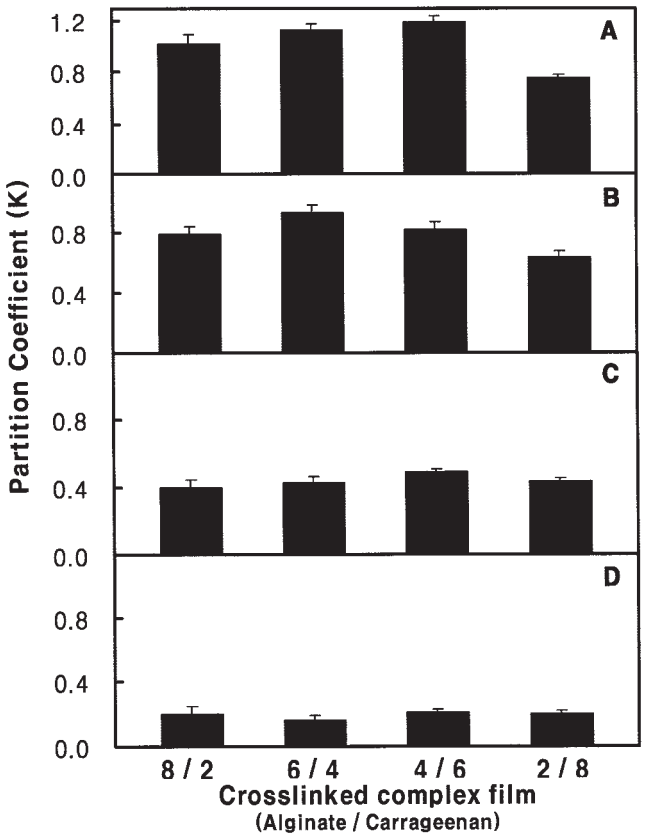

Figure 10 The partition coefficients of glucose and dextrans in Al–Ca complex films. (A): glucose; (B): dextran (M_w 10,000); (C): dextran (M_w 150,000); (D): dextran (M_w 500,000). Experiments were repeated three times and average values are presented.

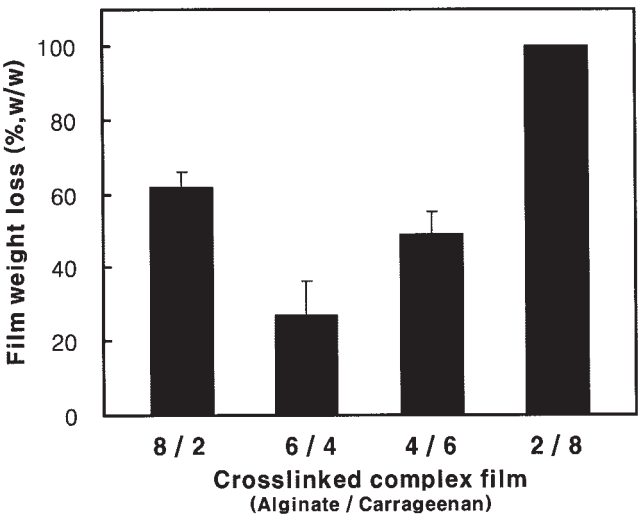

Figure 11 The stabilities of Al–Ca complex films in a phosphate buffer (pH 7.0). Solutions were incubated for 24 h at 37°C. Experiments were repeated three times and average values are presented.

Stabilities of complex films in a phosphate buffer

The stability of membrane films should be maintained during oral delivery. In general, aqueous fluids were used as a test medium. The pH of the test medium should be neutral to mimic the pH of human saliva.[29,30] It is often useful to use a buffer solution as a test medium.

Figure 11 shows the stabilities of complex films in a phosphate buffer. A complex film containing 40% carrageenan (6:4 Al–Ca complex film) was the most stable in a phosphate buffer (pH 7.0), but the film became unstable with both an increasing and decreasing carrageenan content. The good stability of a 6:4 Al–Ca complex film is probably caused by strong Ca-bindings between the alginate and carrageenan moieties. This phenomenon can be explained by the uniform and compact pores shown in the TEM photograph of Figure 1. Films with a high alginate content (10:0 and 8:2) experience alginate moiety dissolution out of the film because of phosphate ions, thus enhancing the water-swelling property. On the other hand, films with a high carrageenan content (2:8 and 0:10) suffer structural degradation of the film due to swelling. Alginate dissolution and water-swelling both stimulate the disintegration of complex films.

CONCLUSIONS

The pore size of complex films consisting of alginate and carrageenan was significantly affected by the Al–Ca ratio. The pore size was reduced by increasing the Al–Ca ratio, resulting in decreased film permeability. Use of a combination of $CaCl_2$ and $ZnSO_4$ as crosslinking agents reduced the pore size further and enhanced the film stability, probably because of enhanced interchain linkages between the alginate and carrageenan moieties of the film. Films with a high alginate content were severely degraded by dissolution with phosphate ions, whereas films with a high carrageenan content suffered from severe water sorption degradation. The most stable and compact film contained an Al–Ca ratio of 6:4 with crosslinking.

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