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A Study on the Preparation of Antibacterial Biopolymer Film

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Abstract Preparation of antibacterial biopolymer film which is suitable for food packaging film was investigated using κ-carrageenan as a base material. κ-Carrageenan showed good biodegradability and film-forming characteristic but poor mechanical properties under humid condition. Also, various bacteria grew well on its surface. The poor mechanical properties could be improved by mixing with alginate at a 1:1 ratio and crosslinking with $CaCl_2$ solution. Antibacterial property could be provided by modifying the κ-carrageenan film surface with acrylic acid plasma followed by ion-exchange with Ag^+ ions. Such prepared film still showed good biodegradability by various fungi.

Key words: Biopolymer, κ-carrageenan, antibacterial property, improvement of mechanical properties, plasma modification

As polymer products occupy a large portion of solid wastes, it is necessary to gradually replace the traditional polymers which are chemically and biologically inert with degradable polymers. In fact, biopolymers may be the best candidate for such replacement, because they are environmentally friendly and easily degradable by various microorganisms or enzymes [2].

Among various biopolymers, polysaccharides produced from microorganisms have high potential as a material for food packaging film. They are compatible with foods, have excellent barrier characteristic against gas (oxygen) permeation, and can easily be processed in the form of film. However, it will not be easy to be commercialized unless two major problems are solved. One is that they are very hydrophillic, and thus vulnerable to moisture. As a result, they show very poor mechanical properties under humid conditions. The other is that they can be easily attacked by various bacteria.

A tremendous amount of work has been done to overcome the first problem. In particular, modification through chemical derivatization has long been examined [3, 6, 9]. Depending on the nature of substituents and the degree of substitution, mechanical properties of polysaccharide films could be improved to some extent [6]. However, they still possessed brittleness. Unfortunately, there was little effort, if any, to provide biopolymer films with antibacterial property. In the case of conventional polymer packaging films, methods such as the addition of antibacterial chemicals or fillers and surface modification have been tried [4, 5].

In this study, the preparation of antibacterial biopolymer film was tried with κ-carrageenan to investigate the potential application of polysaccharide as a material for food packaging film. Improvement of mechanical properties was tried by mixing κ-carrageenan with other kinds of polysaccharide, sodium alginate, and crosslinking with salt solution. Improvement of antibacterial property was tried by modifying the surface of the film to have Ag^+ ions, using plasma polymerization and ion-exchange. Interestingly, Ag^+ ions are known to have antibacterial property [4].

Materials and Methods

Materials

The polysaccharide used for the preparation of biopolymer films was κ-carrageenan. It is more water-resistant compared to other polysaccharides, easy to fabricate in the form of film, and crosslinkable with salts such as $CaCl_2$.

Bacteria used for the measurement of the antibacterial property of the films were *Rahnella aquatilis*, *Escherichia*

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coli, and *Salmonella typhimurium*. The microorganism used for measurement of biodegradability of the films was *Aspergillus niger* (KCTC 1374), a microorganism designated in American Standard Test Method (ASTM G21-90).

Preparation of Biopolymer Films

Biopolymer films were prepared by pouring 40 ml of 1.5% (w/v) aqueous κ-carrageenan solution or a mixed solution of κ-carrageenan and sodium alginate at a 1:1 ratio into a plastic petri dish followed by drying at 30°C for 24 h. The dried films were soaked in 2% $CaCl_2$ solution for a certain period of time to induce crosslinking between polymer molecules.

Measurement of Tensile Strength and Elongation

Tensile strength and elongation were measured after aging the prepared films under the condition of 20°C and 65% R.H. using the Universal Testing Machine (United Calibration Corporation, STM-5) according to KSM 3006. The maximum capacity of the load cell was 2.0 lb and the grip distance was 30 mm.

Surface Modification for Antibacterial Property

Antibacterial property was provided by depositing the ultra thin polymeric layer containing carboxyl groups on the film surface through plasma polymerization of acrylic acid. This was followed by ion exchange in 0.01 N AgCl solution for 1, 3, or 5 min. For the surface modification, a cylindrical Radio Frequency (F.R.) plasma reactor was used, shown in Fig. 1. The plasma polymerization was carried out at a discharge power of 20 W for 10 min.

Measurement of Antibacterial Property and Biodegradability

Antibacterial property was measured by placing the prepared film with size of 5.0×5.0 cm on a petri dish with a diameter of 9 cm containing LB agar medium and then adding 0.5 ml of bacteria medium (colony number: 5.0×10^8/ml). The number of surviving bacteria was counted after incubation for 2 days at 37°C.

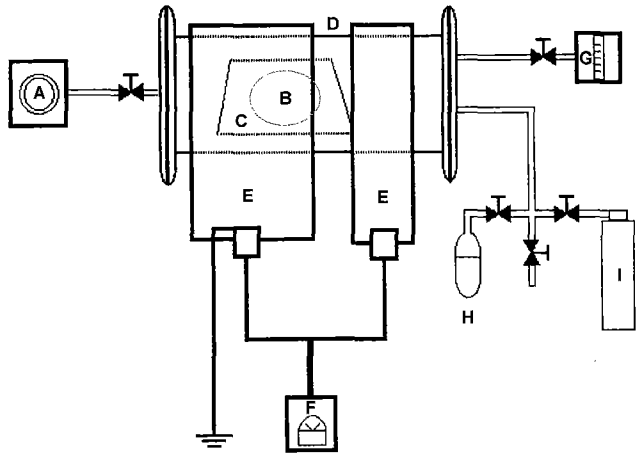

Fig. 1. Schematic diagram of the tubular plasma reactor.
A: vacuum pump; B: sample; C: sample holder; D: reaction chamber; E: electrodes; F: R.F. power supply; G: pressure gauge; H: liquid monomer reservoir; I: gas reservoir.

Table 1. Composition of nutrient-salt agar medium used for the biodegradability test.

Component	Concentration (g/l)
KH_2PO_4	0.7
$MgSO_4 \cdot 7H_2O$	0.7
NH_4NO_3	1.0
NaCl	0.005
$FeSO_4 \cdot 7H_2O$	0.002
$ZnSO_4 \cdot 7H_2O$	0.002
$MnSO_4 \cdot 7H_2O$	0.001
Agar	1.5
Carbon source	-

*pH of medium: 6.0–6.5.

Biodegradability of films was measured by using the soft agar overlay method of a modified ASTM G21-90. The prepared film with size of 5.0×5.0 cm was placed on a petri dish with a diameter of 9 cm containing the nutrient-salt agar medium (see Table 1). One ml of *Aspergillus niger* suspension (spore number: 2.0×10^5/ml) was added to a 7-ml sterilized bottle containing 1 ml of nutrient-salt agar. After proper mixing, the mixture was poured onto the film in a petri dish. When the mixture was uniformly distributed on the film, agar was solidified. The sample prepared in such manner was cultured at 28°C for 4 to 5 weeks and then a degree of fungi growth was measured every 5 days. The results were graded in 5 steps according to ASTM G21-70.

Results and Discussion

Influence of Crosslinking with $CaCl_2$ Solution and Mixing with Alginate on Mechanical Properties of κ-Carrageenan Film

Table 2 shows that κ-carrageenan film possesses poor mechanical properties under humid conditions, which results from its poor water-resistance. Water-resistance of the film was improved by crosslinking molecules with Ca^{++} ions in 2% $CaCl_2$ solution. Tensile strength and elongation increased up to 3.17 and 1.87 times, respectively, after crosslinking for 7 min. The water-resistances of the crosslinked κ-carrageenan film in terms of swelling capacity in water (weight after soaking/weight before soaking) are shown in Fig. 2. Swelling capacity of the uncrosslinked κ-carrageenan film gradually increased up to 36 times as the soaking time increased and started to dissolve after 20 min. The maximum swelling capacities of crosslinked films ranged between 22 and 29 depending on the crosslinking time. Therefore, it

Table 2. Mechanical properties of crosslinked κ-carrageenan films with $CaCl_2$ solution*.

Crosslinking time (min)	Thickness (mm)	Tensile strength at yield (kg/mm^2)	Tensile strength at break (kg/mm^2)	Elongation at yield (%)	Elongation at break (%)
0	0.060	1.50	1.50	2.71	2.72
1	0.060	2.64	2.55	2.55	5.69
3	0.060	3.54	3.26	3.54	3.55
5	0.055	4.56	4.56	2.17	2.18
7	0.060	4.76	3.96	5.08	5.09
Conventional Plastic films [11]					
PVC		4.9	5.1	3	30
HDPE		3.06	3.06	9	600
Polypropylene		3.26	3.37	12	400

*These values are mean values obtained from five experiments.

was clear that crosslinking was very helpful for reduction of swelling capacity. Also, the reduced swelling capacity resulted in the improvement of mechanical properties.

Swelling capacity of the film was also reduced by mixing with sodium alginate at a 1:1 ratio. The maximum swelling capacity of the mixed film decreased to 19.5, as shown in Fig. 3. Figure 3 also shows that the crosslinked κ-carrageenan/alginate films had very low swelling capacities ranging between 1.62 and 2.3, which indicates that water-resistance was remarkably improved.

The film became tough as crosslinking time increased. Figure 4 shows tensile strengths and elongations of the films. The film crosslinked for 7 min had tensile strength at a yield as high as 6.9 kg/mm^2 with the same elongation at break as uncrosslinked κ-carrageenan/alginate film. Such improvement may be explained by the "egg-box" model. That is, crosslinked κ-carrageenan molecules with single or double spiral structure may be hard and brittle (eggs) but will be reinforced if they are encapsulated by softer alginate molecules (box). This concept is further supported by the fact that pure alginate film is easily dissolved in water and shows large shrinkage after crosslinking with $CaCl_2$ solution.

Influence of Surface Modification on Antibacterial Property, Biodegradability, and Mechanical Properties

The crosslinked κ-carrageenan/alginate film was surface-modified with acrylic acid plasma to have -COOH groups on the surface and ion-exchanged with Ag^+ ions to have anti-bacterial property. When analyzed with ESCA after the ion-exchange for 5 min, the film was found to contain 2.47 atomic % of silver ions on the surface layer.

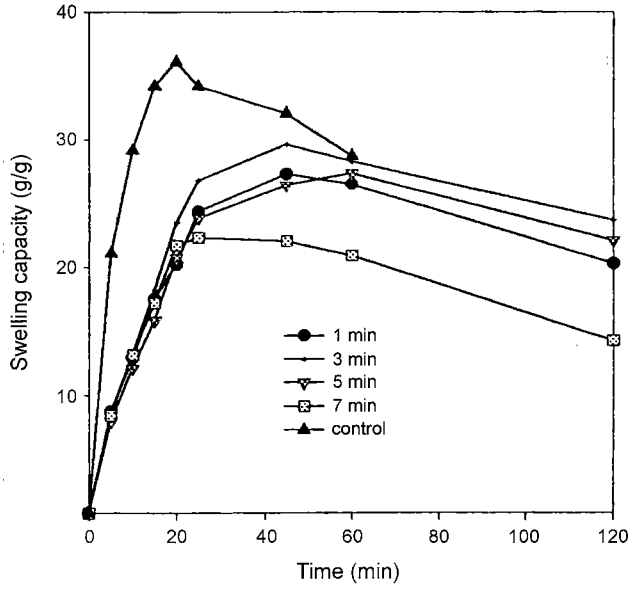

Fig. 2. Swelling capacities of κ-carrageenan films in water as a function of soaking time for various crosslinking times with $CaCl_2$ solution.

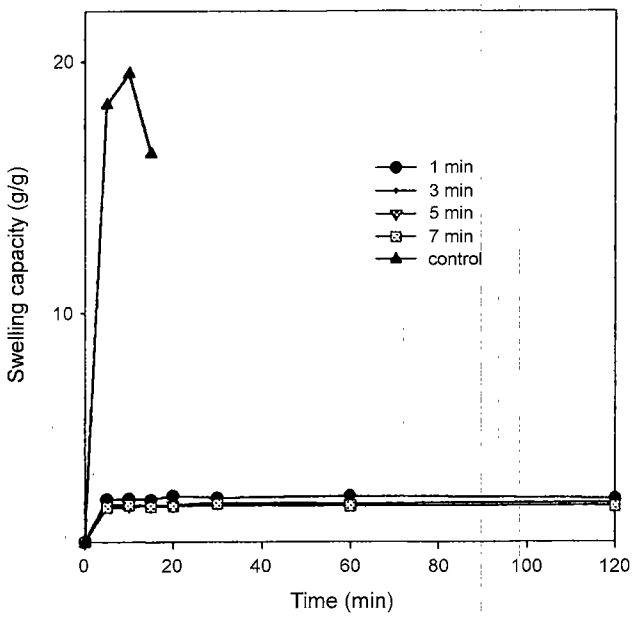

Fig. 3. Swelling capacities of κ-carrageenan/alginate films in water as a function of soaking time for various crosslinking times with $CaCl_2$ solution.

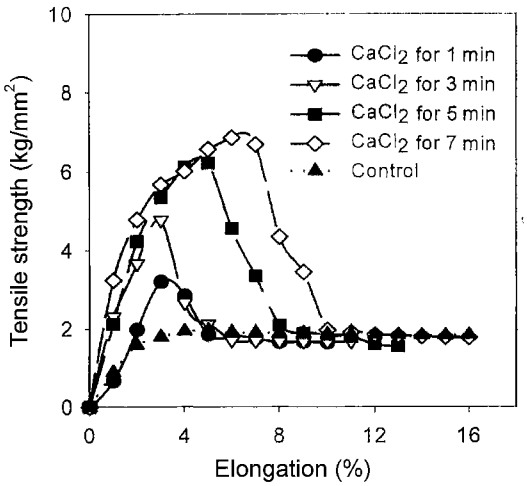

Fig. 4. Tensile strength versus elongation of κ-carrageenan/alginate films for various crosslinking times with $CaCl_2$ solution.

The inhibition function of silver ions on the growth of bacteria was examined. Table 3 and Fig. 5 show antibacterial properties of various films. When tested for *E. coli*, *R. aquatilis*, and *S. typhimurium*, there was no symptom of growth for 48 h on ion-exchanged films,

Table 3. Growing status of bacteria in 2 days on κ-carrageenan/alginate films modified with acrylic acid plasma and ion-exchanged with Ag^+ ions.

Ion-exchange time (min)	Degree of coverage by bacteria*		
	E. coli	*S. typhimurium*	*R. aquatilis*
control	4	4	4
0	4	4	4
1	0	0	0
3	0	0	0
5	0	0	0

*0: no growth; 1: less than 10%; 2: 10–30%; 3: 30–60%; 4: more than 60%.

independent of ion-exchange time, while the bacteria began to grow after 12 h on both unmodified film and modified film without ion-exchange. Therefore, it is clear that silver ions have strong antibacterial property.

The antibacterial property of silver ions results from its interaction with the bacteria cell. The silver ions penetrate into the bacteria cells, where they can bind with dithioketal moieties of the cellular proteins and enzymes [1, 12]. Accordingly, they replace silver ions already present in enzyme prosthetic groups, which results in disruption of the enzyme structure and proper functions [10]. The silver

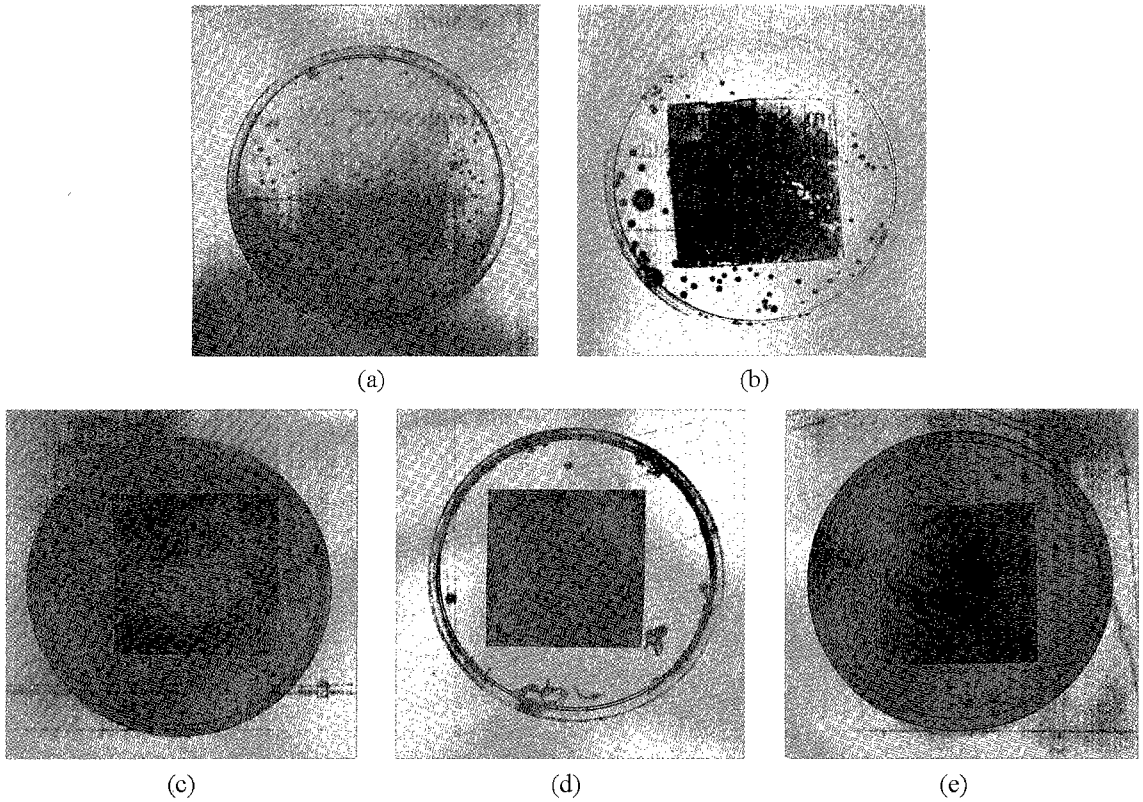

Fig. 5. Microscopic pictures of *E. coli* growth on various κ-carrageenan/alginate films after 2 days.
(a): Unmodified; (b): modified with acrylic acid; (c): modified with acrylic acid and ion-exchanged for 1 min; (d) modified with acrylic acid and ion-exchanged for 3 minutes; (e): modified with acrylic acid and ion-exchanged for 5 min.

Table 4. Growing status of *Aspergilus niger* on κ-carrageenan/alginate films modified with acrylic acid plasma and ion-exchanged with Ag^+ ions.

Ion-exchange time (min)	Degree of coverage by *Aspergilus niger**					
	5	10	15	20	25	30
control	1	2	3	4	4	4
0	1	2	4	4	4	4
1	0	0	1	1	2	3
3	0	0	0	0	0	0
5	0	0	0	0	0	0

*0: no growth; 1: less than 10%; 2: 10- 30%; 3: 30- 60%; 4: more than 60%.

ions are also reported to have an ability to bind and disrupt native DNA [7, 8]. Silver-DNA complexes occur at bases, which causes denaturation by displacing hydrogen bonds between adjacent nitrogen of purines and pyrimidines, thereby preventing replication.

In contrast with bacteria, the fungus *Aspergilus niger* could grow on the modified film unless the surface contained excess amount of silver ions despite the fact that growth was retarded. Table 4 and Fig. 6 show the growing status of *Aspergilus niger* on various films. Growth of the fungus was observed in 5 days on unmodified film and modified film without ion-exchange, in 15 days on modified film ion-exchanged for 1 min, in 30 days on modified film ion-exchanged for 3 min, but not until 30 days on modified film ion-exchanged for 5 min. This indicates that the biopolymer film still possesses biodegradability even after the modification.

The growth of the fungus on the modified films may be explained by the difference in the cellular structure between bacteria and fungi. While bacteria are prokaryotic organisms whose cell wall consists of the muco-complex, fungi are eukaryotic organisms whose cell wall consists of cellulose, hemicellulose, and chitin. Since cellulose, hemicellulose, and chitin can complex with metal, the silver ions cannot enter into the fungi to bind with dithioketal moieties. When excess number of silver ions do exist, however, some silver ions may quite well pass the cell wall of fungi. This postulation is supported by the fact that metal ions such as silver, copper, cobalt, nickel, and zinc inhibited pathogenic bacterial growth but only zinc and its organic or inorganic derivatives showed antifungal activity [12].

The modification did not give any negative influence on the water resistance and mechanical properties of the film. Figure 7 shows the swelling capacities of the films. Compared to unmodified film, the modified film had a slightly higher swelling capacity before ion-exchange but lower swelling capacity after ion-exchange. Table 5 shows the mechanical

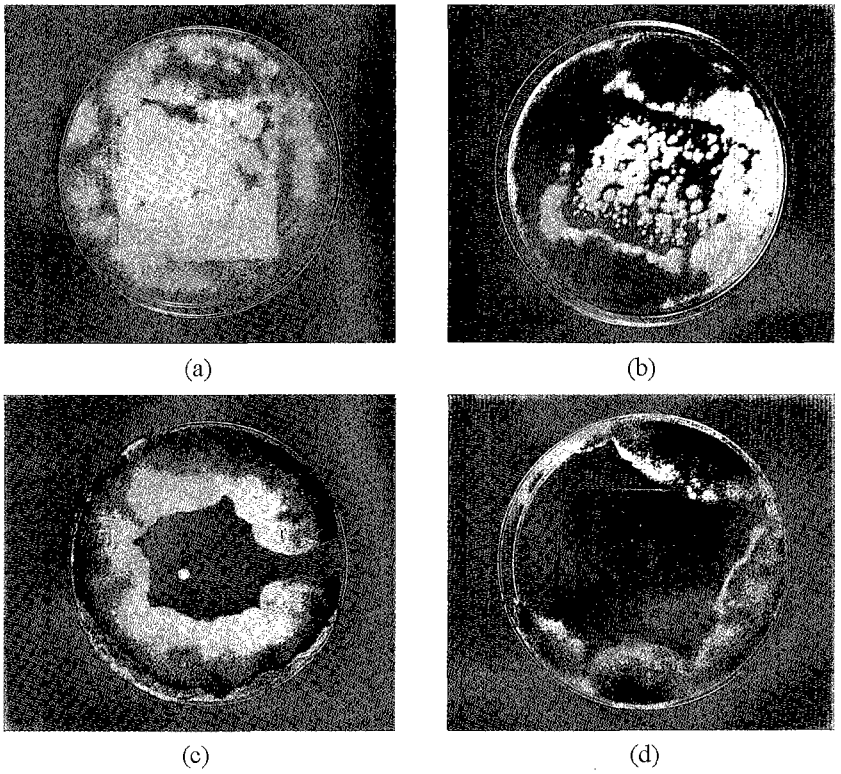

Fig. 6. Microscopic pictures of *Aspergilus niger* growth on various κ-carrageenan/alginate films.
(a): Unmodified (after 15 days); (b): modified with acrylic acid and ion-exchanged for 1 min (after 30 days); (c) modified with acrylic acid and ion-exchanged for 3 min (after 30 days); (d): modified with acrylic acid and ion-exchanged for 5 min (after 30 days).

Table 5. Mechanical properties of *k*-carrageenan/alginate films modified with acrylic acid plasma and ion-exchanged with Ag^+ ions.

Ion-exchange time (min)	Thickness (mm)	Tensile strength at yield (kg/mm^2)	Tensile strength at break (kg/mm^2)	Elongation at yield (%)	Elongation at break (%)
control	0.056	6.97	1.86	6.45	6.58
0	0.055	6.53	1.66	3.69	9.63
1	0.056	6.65	2.12	4.05	11.06
3	0.068	6.86	1.86	9.15	20.58
5	0.066	6.29	1.79	6.12	13.17

*These values are mean values obtained from five experiments.
**κ-Carrageenan/alginate films were crosslinked with $CaCl_2$ solution for 7 min before the modification.

properties of the films. The modified film had slightly lower tensile strength but much higher elongation at break. Therefore, the modification may be an alternate way to solve the problem of brittleness which is encountered with polysaccharides.

From the results described above, it is suggested that κ-carrageenan is a promising material for food packaging film. If properly modified, it can show strong mechanical properties with antibacterial property and biodegradability. Besides, the time for degradation can be controlled by adjusting the amount of silver ions on the film surface.

Acknowledgment

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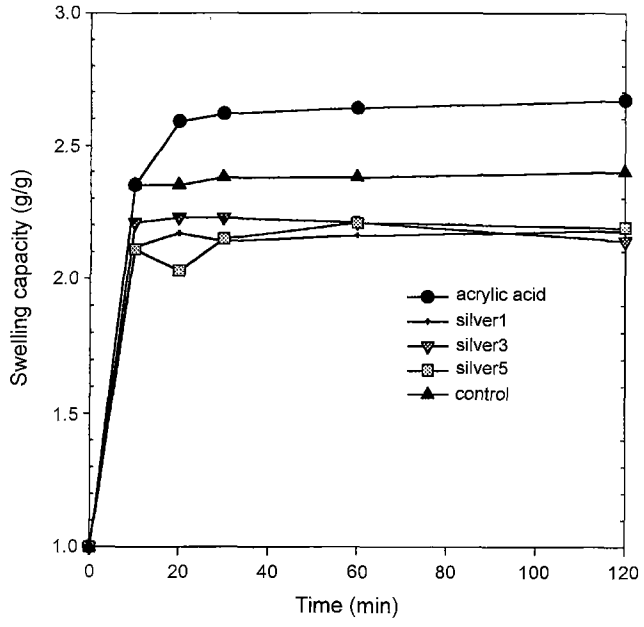

Fig. 7. Swelling capacities of κ-carrageenan/alginate films modified with acrylic acid plasma in water as a function of soaking time for various ion-exchange times. The films were crosslinked with $CaCl_2$ solution for 7 min.

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