# Water-Sorption Properties of Poly(ε-Lysine): Carboxymethyl Cellulose (CMC) Dietary Complex Films

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## SYNOPSIS

To investigate the application of supersorbing materials in the food industry, the water sorption of polyion complex films, prepared by solvent-casting from poly(ε-lysine) (PEL) and carboxymethyl cellulose (CMC), were examined and some physical properties of the hydrogels were studied. Three CMC samples having various degrees of substitution (DS) and molecular weights (MW) were applied to show a variety of water-sorption behavior and physical properties. The ratio of PEL/CMC was changed from 10/90 to 50/50. Good film swelling in water was achieved when the complex was made from PEL and CMC with a low DS and an appropriate molecular weight, and when the PEL/CMC ratio was low. This indicated that the carboxyl groups in the CMC are not readily dissociated, and that the strength of cationic and anionic interactions affects the film swelling. In line with this view, the addition of electrolytes markedly lowered the film swelling. Nonelectrolytes such as sucrose or urea had no effect. IR and NMR spectra showed that a fraction of the ionic groups in the polyelectrolytes interact. Tensile moduli of complex dry films decreased linearly as the PEL content increased. Compressive moduli of swollen films were affected by neither the degree of swelling nor the molecular weights of CMC. The lower the DS values of CMC, the higher the compressive moduli were. We also examined the relation between the rigidity of polymer component and the ratio of nonfreezable bound water in complex films. On swelling in acetone–water or ethanol–water mixed solvent, expansion–shrinkage transition of the polymer gels occurred at about 20 mol % acetone or ethanol concentrations for every complex film. The overall characteristics of PEL/CMC consisting of a linear polyanionic polypeptide and a chain of cationic rings are reminiscent of polycationic chitosan and polyanionic polyglutamic acid. © 1994 John Wiley & Sons, Inc.

## INTRODUCTION

Superabsorbent polymers swell to hydrogel structures with absorbed water which can amount to 1,000 times the weight of the polymer. Such properties can be applied to the food industry for various purposes, but natural polymer derivatives (such as polysaccharides and polypeptides) must be used and chemical crosslinks that prevent their dissolution must be avoided. Ionic interactions between two polymers of natural origin having opposite ionic

properties may be the answer. A number of studies have been performed on polyionic polymer complexes,[1-5] but most of those studies concentrated on the conditions for complex-forming reactions, and few have focused on their water-absorption behavior.[6,7]

The water-sorption capacity of polyelectrolytes is strongly influenced by polymer–water affinity. Polymer chains tend to extend in water as a result of repulsion between charges that are the same as those of the polymer. On the other hand, ionic interactions with other polymers having opposite charges tend to restrain the extension of polymer chains, which suppresses water sorption. Thus, the mobility of a polymer, or the rigidity or flexibility of the chain as well as the ionic nature, are important

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factors that affect the water-sorption properties of polyionic complexes.

In this study, we chose poly-$\epsilon$-lysine (PEL), which is a linear flexible chain, as the cationic component, and carboxymethyl cellulose (CMC), which is a chain of ring structures, as the anionic component. CMCs with various degrees of substitution and molecular weights were mixed with PEL in solution to form complex films, and the water-sorption behavior, as well as other physical properties, were investigated. The results showed that mixed polymers of natural origin having opposite ionic charges can behave as appropriate water-sorbing agent, and that they can retain "entrapped" water molecules.

## EXPERIMENTAL

### Materials

PEL [weight: average molecular weight (MW) = 4.7 $\times$ 10$^3$] was obtained from Chisso Co. Ltd. (Japan). CMC-A [degree of substitution (DS) = 0.8, MW = 4.9 $\times$ 10$^4$, and viscosity in 2% aqueous solution at 25°C, $\eta$ (cp) = 145], CMC-B [DS = 1.4, MW = 5.0 $\times$ 10$^4$, and $\eta$ (cp) = 150], CMC-C [DS = 1.4, MW = 1.0 $\times$ 10$^5$, and $\eta$ (cp) = 1,700] were obtained from Daiichi Kogyo Seiyaku Co. Ltd. (Japan). Formic acid (special grade reagent) used as a solvent was purchased from Wako Pure Chemicals Co. Ltd. (Japan).

### Preparation of Films

PEL and CMC were separately dissolved in aqueous 20% formic acid (solvent) and mixed at different ratios by agitation on a magnetic stirrer for about 30 min at room temperature. The concentration of the total polymer was adjusted to 3% by adding the solvent, and each solution was then poured onto Teflon petri dishes. After evaporating the solvent in a draught chamber, the films were washed with ethanol and dried under vacuum. The thickness of all prepared films was about 60–80 $\mu$. Films containing more than 50% PEL were not obtained due to deliquescence.

### Analyses

The dried 6-mm $\phi$ film sample was dipped in a 5,000-fold amount of solution at 20°C for an appropriate period. The extent of swelling was estimated with the value of W/W$_0$, where W$_0$ and W are the weights of dry and swollen samples, respectively.

Infrared spectra patterns of dry films were measured using a Japan Spectroscopic Co. Ltd. FT. IR-7300 spectrometer, and the $^{13}$C-NMR spectra of powdered samples from the dry films were analyzed using an XL-300 spectrometer (Varian Instruments Ltd.).

Differential scanning calorimetry (DSC) of the thermal properties of water absorbed in the complex film was performed using a Seiko Electric DSC-100. About 1 mg of film and 3 $\sim$ 4 mg of pure water were mixed and sealed in an aluminum pan and cooled in a DSC cell from 30 to $-100$°C in liquid nitrogen. The cell was then slowly heated to 50°C at a rate of 10°C/min.

Tensile strength was measured with 2-mm $\times$ 10-mm films under a periodic load of 100 g $\pm$ 40 g/1.5 min of sine–wave function using a Seiko Electric TMA/SS-100. Elastic modulus was calculated from the stress–strain curves. Compressive modulus was measured with films dipped in water for defined periods, under a load increasing from 5 to 100 g at a rate of 10 g/min.

## RESULTS AND DISCUSSION

### Swelling Characteristics of PEL/CMC Complex Films

To see how mixtures of polypeptide cations and polysaccharide anions behave, complex films with various PEL/CMC molar ratios were prepared and the rate of swelling in water was measured. The results are shown in Table I. The rate and degree of film swelling was significantly affected by the ratio of cations and anions in the complex, as has been shown with other types of complexes.[8-10] PEL/CMC-A and PEL/CMC-B complexes swelled less when the PEL content was high, probably because of the stronger ionic interactions between the carboxyl group in CMC and the amino groups in PEL at a high PEL concentration. Stable complexes were not made when the PEL content was increased to above 50%. The complex PEL/CMC-C behaved differently: when the PEL content was about 10–20%, the water absorption was low, but at about 30–40%, the complex behaved as a highly sorptive agent. This indicates that high molecular weight CMC-C with high viscosity in solution during film preparation cannot form stable ion complexes with PEL when the content of the latter is too low.

Dipping films in water for a long time (5–18 h) caused considerable swelling of the PEL/CMC-A complex containing 10–20% PEL, or gel collapse of

**Table I  Effects of PEL/CMC Composition and Dipping Time on the Degree of Film Swelling in Water**

| Sample | PEL (%) | Dipping (h) | | |
| --- | --- | --- | --- | --- |
| | | 1 | 5 | 18 |
| | | $(W/W_0)$ | $(W/W_0)$ | $(W/W_0)$ |
| PEL/CMC-A | 10 | 277.5 | 303.8 | 429.4 |
| | 20 | 91.1 | 150.5 | 179.4 |
| | 30 | 60.5 | 101.5 | 120.6 |
| | 40 | 5.5 | 5.9 | 4.6 |
| | 50 | 3.0 | 2.5 | 2.5 |
| PEL/CMC-B | 10 | 259.1 | 220.8 | 262.7 |
| | 20 | 176.5 | 95.0 | 105.1 |
| | 30 | 66.2 | 53.0 | —* |
| | 40 | 32.6 | —* | —* |
| | 50 | 10.3 | 10.1 | 7.1 |
| PEL/CMC-C | 10 | 20.5 | 19.1 | 19.6 |
| | 20 | 38.9 | 35.5 | 58.7 |
| | 30 | 182.0 | 166.8 | 162.5 |
| | 40 | 163.1 | 153.9 | 149.9 |
| | 50 | 34.0 | 34.1 | 48.4 |

\* Hydrogel collapsed.

the PEL/CMC-B complex containing of 30–40% PEL. The PEL/CMC-C complex showed no time-dependent behavior. Thus, the rate and degree of swelling are affected by the amount of ionic groups in the complex, as well as by the molecular weight of the polymer.

The addition of low molecular weight ions lowers the water-sorption capacity, the effect being especially serious with $CaCl_2$ (Fig. 1). The effects of electrolytes which interact with ionic groups in the polyion complexes have been discussed.[8–10] On the other hand, nonelectrolytes such as sucrose or urea had little effect on swelling (Fig. 2).

The water-sorbing capacity of the PEL/CMC complex under favorable conditions is comparable to the best combination of chitosan/CMC or chitosan/polyglutamic acid complexes that showed a $W/W_0$ value of about 500.[8,9] These values are considerably better than those of the most effective combination of polylysine/polyglutamic acid that has a $W/W_0$ value of about 80.[10] In the presence of NaCl, the water-sorbing capacity of PEL/CMC was not seriously affected when the $W/W_0$ ratio was high, in contrast to chitosan/CMC or polyglutamic acid complexes.[8,9] Thus, we concluded that PEL/CMC in an appropriate composition can serve as an ideal water-sorbing material.
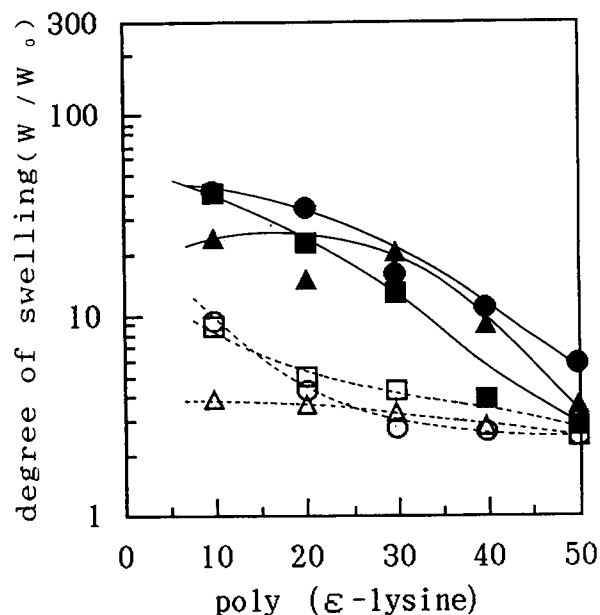


**Figure 1**  Degree of swelling of PEL/CMC complex films in 0.31 mol % NaCl and 0.16 mol % $CaCl_2$ for 0.5 h: (filled symbols) NaCl; (unfilled symbols) $CaCl_2$; (squares) PEL/CMC-A; (circles) PEL/CMC-B; (triangles) PEL/CMC-C.

## Infrared and NMR Analyses

Infrared spectra showed that intermolecular and intramolecular interactions occurred in the complex
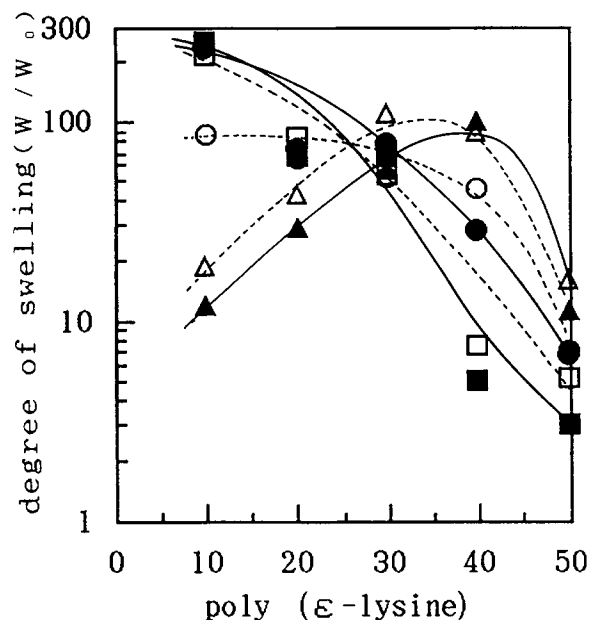


**Figure 2**  Degree of swelling of PEL/CMC complex films in 1% sucrose and urea for 0.5 h: (filled symbols) sucrose; (unfilled symbols) urea; (squares) PEL/CMC-A; (circles) PEL/CMC-B; (triangles) PEL/CMC-C.
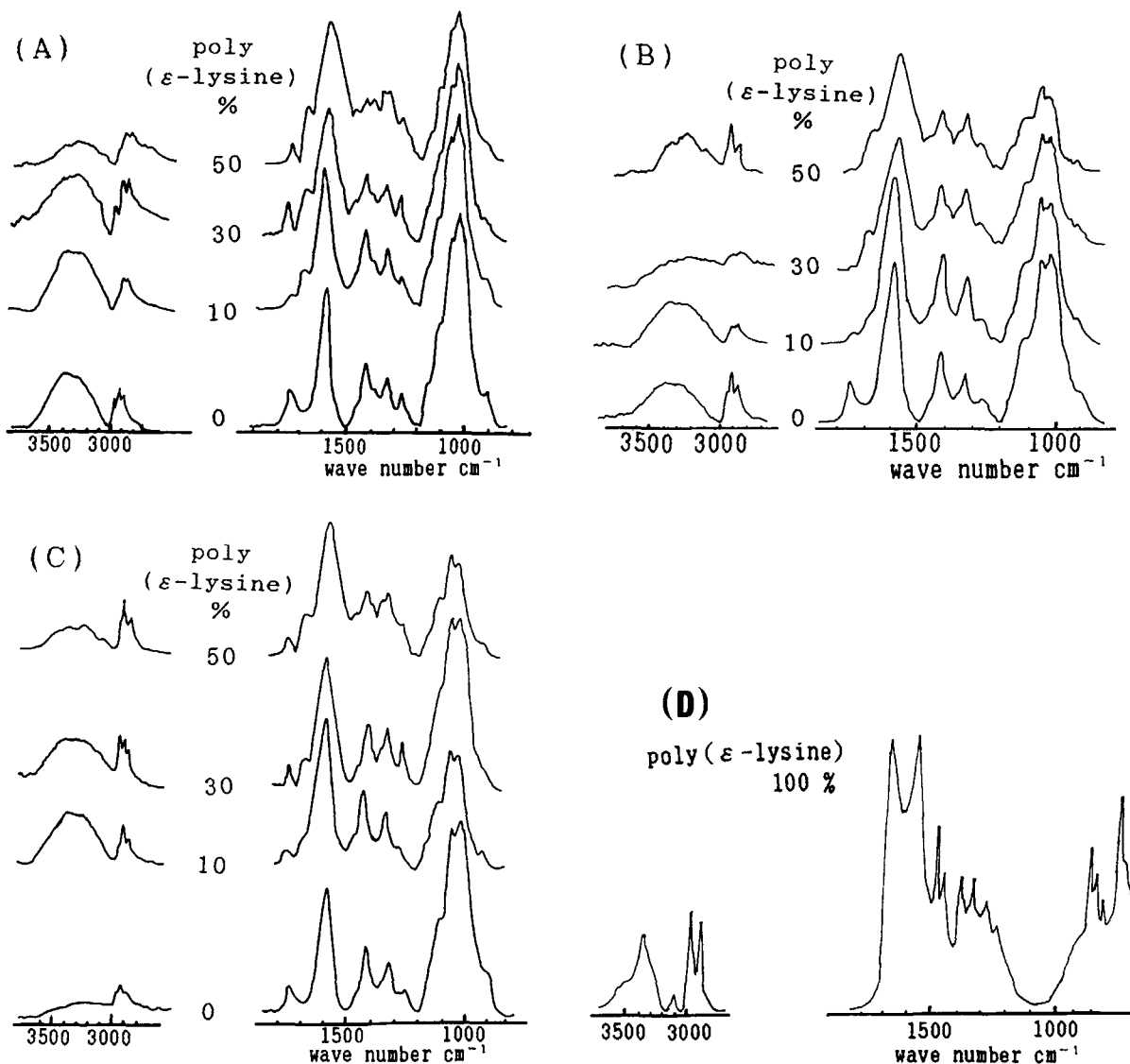
**Figure 3**    Infrared spectra of PEL/CMC complex films: (a) PEL/CMC-A; (b) PEL/CMC-B; (c) PEL/CMC-C; (d) PEL alone.

films. Kim et al.[7] tested a chitosan–poly(vinyl alcohol) membrane and found that the most intense band from each component disappeared in the blended membrane, indicating intermolecular interactions within the membrane. We performed similar tests on PEL/CMC complexes. Characteristic absorption patterns for PEL/CMC complex dry films and PEL sample are shown in Figure 3. No substantial decrease in the unique bands of CMC was detected by blending with PEL, but a new band at 1,670 cm$^{-1}$ was readily detected in complex films containing PEL 30–50%.[6] This band is characteristic of an amino group, but differs from that of PEL alone at 1,639 cm$^{-1}$, indicating a change in frequency

by a strong ionic interaction between the two oppositely-charged compounds.

Figure 4 shows high-resolution solid $^{13}$C-NMR spectra of powdered PEL/CMC complexes from dry films. Intensities of signal peaks at 160–180 ppm (assigned to carboxyl group carbons) and those at 60–100 ppm (assigned to pyranose ring carbons) changed remarkably in PEL/CMC-B and PEL/CMC-C, on increasing the PEL concentration. Kotz et al. reported that position and intensities of the $^{13}$C-NMR signals of polyanion–polycation complexes changed most significantly for the C atoms adjacent to the charge centers at a high charge density of the components.[11] This observation is true

in the case of carboxyl group carbons of CMC-B and CMC-C with high DS values in our study. Kotz et al.[11] reported that conformational changes of the polymer chains resulted in complicated spectra, together with line broadening due to declination of the mobility. Figure 4 shows the similar behavior of the CMC molecules by ionic interaction with the PEL.

## Modulus of the PEL/CMC Films

Elasticity is an important parameter in the application of a supersorbent. Figures 5 and 6 show the tensile modulus of dry films and the compressive modulus of swollen films. The type of CMC and the PEL/CMC ratios affected these values. The tensile modulus decreased as the amount of PEL in the
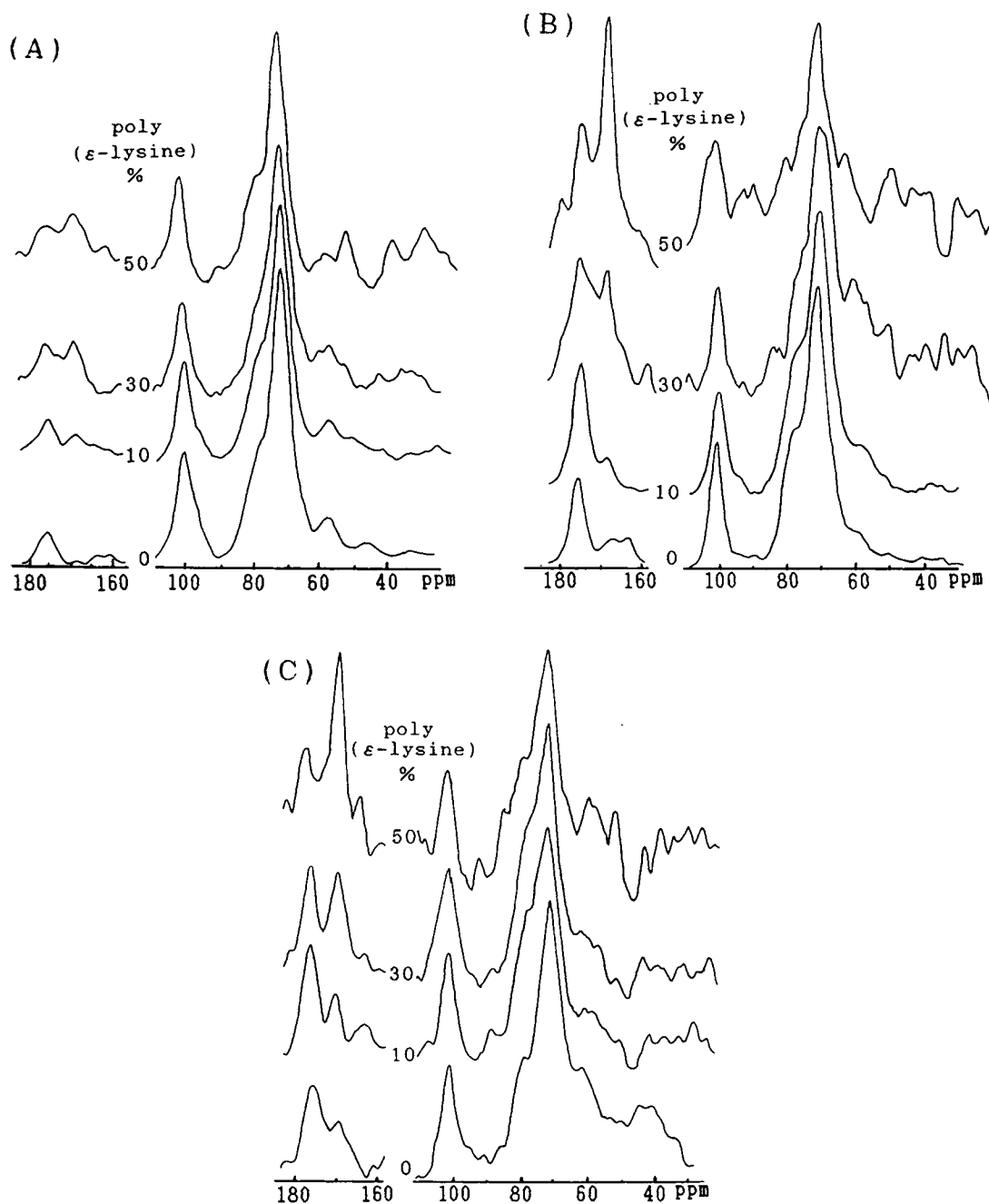
**Figure 4**   ¹³C-NMR spectra of PEL/CMC complex films: (a) PEL/CMC-A; (b) PEL/CMC-B: (c) PEL/CMC-C.
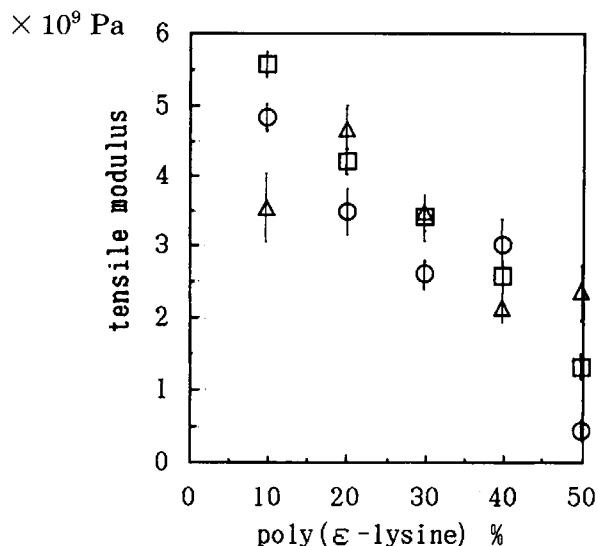
**Figure 5**   Relationship between the tensile moduli (dry film) and the composition of PEL/CMC complex films: (□) PEL/CMC-A; (O) PEL/CMC-B; (△) PEL/CMC-C. Vertical lines through symbols show standard deviations.

blend increased in PEL/CMC-A and PEL/CMC-B (Fig. 5). Qurashi et al.[12] showed that the modulus of chitosan/polyvinylpyrrolidone (PVP) membranes lowered as the PVP content in the blends increased. They proposed that this behavior was caused by the amorphous and hydrophilic properties of the PVP, and hydrogen bonds formed between the tough cyclic polyelectrolyte and the flexible chain polyelectrolyte. The PEL and CMC in our system played roles similar to those of PVP and chitosan, respectively. Compared with our previous observation,[8] the high water-sorbable PEL/CMC-A or PEL/CMC-B complex in the dry state (tensile modulus $\approx 4 \sim 5 \times 10^4$ Pa) is nearly as rigid as the high water-sorbable chitosan/CMC whose tensile modulus value was about $4.5 \times 10^4$ Pa.

The compressive moduli observed with swollen complexes are shown in Figure 6. The type of CMC and the PEL/CMC ratio affected the compressive modulus. Compared with PEL/CMC-B containing the similar molecular weight CMC with higher DS, PEL/CMC-A had a higher compressive modulus. On the other hand, PEL/CMC-C containing the higher molecular weight CMC with the equal DS to that of CMC-B, had as low compressive moduli as PEL/CMC-B except in one case. In the case of PEL/CMC-C with 10% PEL content, the significantly higher compressive modulus was obtained, whereas the water-sorbing capacity was lower. Perhaps this is because with film preparation, the high

molecular weight CMC-C was dissolved to form high viscous solution and to bring about the inhomogeneity of PEL distribution in the complex, as mentioned above. These observations demonstrate that compressive modulus depends on the nature of the polymer component in the complex, and that the water-sorbing capacity does not necessarily parallel the compressive modulus of the swollen gel. In summary, to prepare PEL/CMC complex films having high compressive modulus, low molecular weight CMC and a low proportion of PEL are recommended.

Ionic interactions between amino groups in PEL and carboxyl groups in CMC are rather weak under dry conditions, as represented by the IR and NMR spectra. When water is added, ionic interactions help to form firm network structures in the gel. For this to occur, the optimal ratios of polyanion and polycations must be calculated. PEL/CMC containing a high proportion of PEL does not swell well, is not rigid, and is not compressive. On the other hand, with a low proportion of PEL, effective swelling can occur and the swollen complex has high compressive values. The maximal water-sorbing capacity (W/$W_0$ value) observed with PEL/CMC-A (10% PEL) was 429, and the swollen membrane had a compressive modulus value of $4.79 \times 10^4$ Pa. These values are roughly comparable to chitosan/polyglutamic acid (PGA) complex (10% PGA), of which the maximal sorbing capacity was 499 and the compressive modulus of the swollen gel was $4.42 \times 10^4$ Pa.[9] On the other hand, poly-L-lysine/PGA com-
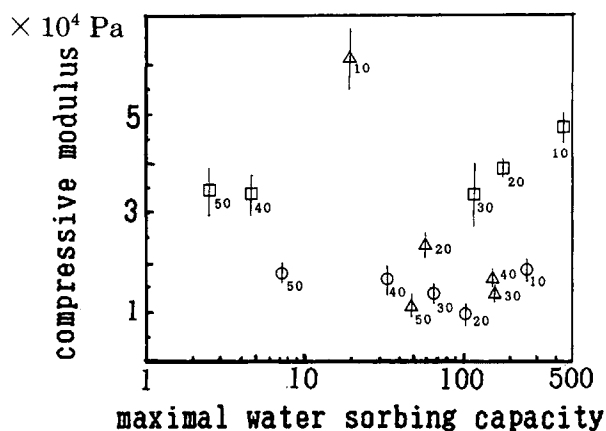


**Figure 6**   Relationship between the compressive moduli (swollen films) and the water-sorbing capacity of the PEL/CMC complex films. The small numbers with data points indicate the PEL concentration: (□) PEL/CMC-A; (O) PEL/CMC-B; (△) PEL/CMC-C. Vertical lines through symbols show standard deviations.

plex,[10] consisting of anionic and cationic polypeptide, did not show such sorption and modulus. Thus, we propose that to produce a polyion complex having a high water sorption capacity and high modulus upon swelling, one component should consist of polymerized rings such as CMC or chitosan to provide rigidity to the complex, and the other component should be a polymerized flexible linear molecule such as PEL or polyglutamic acid.

## Analyses of Water Absorbed in Films by Differential Scanning Calorimetry

Water absorbed in polyionic complex films should exist in one of three different forms: free, freezable bound, and nonfreezable water that is completely bound to the polyions.[8] Among these, free and freezable bound water can be monitored by measuring the peak areas of DSC curves obtained while melting the frozen samples. These areas are expressed as enthalpy values in Table II.[13] Completely bound water shows no DSC peak because it does not freeze.

The content of bound water was assayed as Wb = (Wt − Ws)/Wt, where Wb is the proportion of nonfreezable water in complex films, Wt is the enthalpy value of pure water, and Ws is the enthalpy value of water absorbed in the complex films, representing the sum of free and freezable bound water. By comparing the DSC peak patterns and the enthalpy values, whether or not the water absorbed in complex films takes part in forming the film network by interacting with polyionic polymer components can be understood.

Table II shows the content of free, freezable bound water in CMC, PEL, and PEL/CMC complex films using differential scanning calorimetry (DSC). Distilled ion exchanged water was used as a control.

The results show the presence of a large peak representing free water. In addition, another peak was detected in the melting profiles. This characteristic is remarkable in a complex having a larger PEL content. These peaks represent the freezable bound water in the ionic network in the film. The calculated Wb value shows that the ratio of non-

**Table II   Differential Scanning Calorimetry of Water Absorbed in CMC, Complex Films and PEL, and of Pure Free Water**

| CMC | PEL (%) | Melting (°C) | Peak I (°C) | Peak II (°C) | Enthalpy (mJ/mg) | F.W. (%) |
|---|---|---|---|---|---|---|
| A | 0 | −31.5 ~ 29.0 | | 1.3 | 284.4 | 85.1 |
| | 10 | −30.7 ~ 28.3 | * | 2.1 | 289.0 | 86.5 |
| | 20 | −30.4 ~ 29.0 | * | 1.6 | 275.3 | 82.4 |
| | 30 | −30.4 ~ 28.6 | −15.9 | 1.3 | 283.8 | 84.9 |
| | 40 | −33.2 ~ 25.2 | −14.7 | 1.5 | 281.4 | 84.3 |
| | 50 | −30.8 ~ 33.8 | −14.4 | 2.0 | 296.1 | 88.6 |
| B | 0 | −39.9 ~ 28.4 | | 0.7 | 275.5 | 82.5 |
| | 10 | −36.4 ~ 27.8 | * | 1.2 | 285.2 | 85.4 |
| | 20 | −32.1 ~ 23.7 | * | 0.9 | 275.5 | 82.5 |
| | 30 | −37.8 ~ 28.6 | * | 1.2 | 275.9 | 82.6 |
| | 40 | −33.5 ~ 31.8 | −18.9 | 1.8 | 282.7 | 84.6 |
| | 50 | −32.1 ~ 25.5 | −17.0 | 0.5 | 278.4 | 83.4 |
| C | 0 | −37.0 ~ 31.6 | | 1.4 | 290.6 | 87.0 |
| | 10 | −35.5 ~ 27.4 | * | 1.0 | 289.0 | 86.5 |
| | 20 | −31.1 ~ 25.1 | * | 1.0 | 273.7 | 81.9 |
| | 30 | −29.7 ~ 29.2 | * | 1.6 | 286.6 | 85.8 |
| | 40 | −30.2 ~ 20.6 | −17.8 | 1.1 | 267.1 | 80.0 |
| | 50 | −27.2 ~ 33.5 | −15.5 | 2.5 | 291.3 | 87.2 |
| | 100 | −35.4 ~ 38.1 | | 4.7 | 286.0 | 85.6 |
| H₂O | | − 0.1 ~ 31.9 | | 5.4 | 334.0 | 100.0 |

\* Overlap in peak II.
F.W., freezable water (free water + freezable bound water).
For details, see the Experimental section.

freezable bound to whole water absorbed in the complex films is 15–25%. In our studies of other polyion complexes with the same cation/anion ratio, similar Wb values were obtained as follows: 25–35% in chitosan/CMC, 15–25% in chitosan/polyglutamic acid (PGA), and 10–20% in poly-L-lysine/PGA. These values demonstrate the characteristics of polyionic polymer structures categorized as (A) polysaccharides with a ring structure, such as CMC or chitosan, and (B) linear chain polypeptides, such as PEL, PGA, or poly-L-lysine. The order of Wb values of different combinations of polyions was: A – A > A – B > B – B. Therefore, polyions with a ring structure in complex films can absorb larger

amounts of water in the nonfreezable polymer-bound form, and form a firm network structure.

## Expansion–Shrinkage Transitions in Organic Solvent/Water Mixtures

Some polymeric hydrogels drastically expand or shrink in solution at critical temperatures, pH or solute concentrations.[14–16] The swelling of PEL/CMC complex films was examined in acetone–water and ethanol–water mixtures. Figure 7 shows the degree of swelling of PEL/CMC-A complex films in mixed solvents with actone–water or ethanol–water systems. Expansion–shrinkage transition occurred at about 20 mol % acetone or ethanol concentrations. When water contents were more than 20 mol % in solvent, good film swelling occurred, but it didn't occur when water content was less than 20 mol %.

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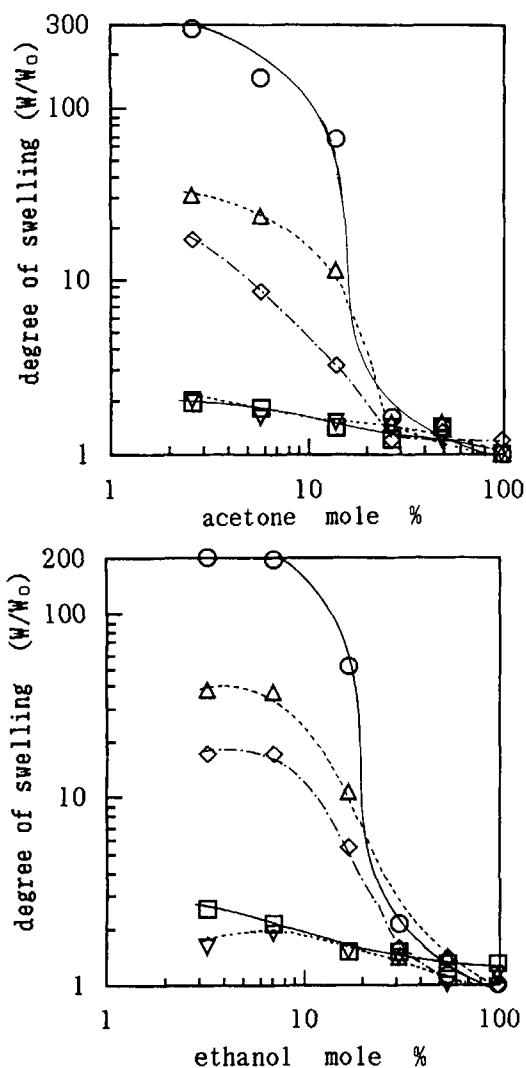16. S. Katayama and A. Ohta, *Macromolecules*, **18**, 2782 (1985).

**Figure 7** Degree of swelling of PEL/CMC-A complex films in (a) acetone or (b) ethanol. (O) 10% PEL; (△) 20% PEL; (◇) 30% PEL; (□) 40% PEL; (▽) 50% PEL. The results obtained were not affected by the time of dipping (0.5, 1.0, and 24 h) and the CMC samples.