DSC studies on bound water in carboxymethylcellulose–polylysine complexes[☆]

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Abstract

Sodium carboxymethylcellulose (NaCMC) with various degrees of substitution (carboxymethylation, DS = 0.72–2.38) was mixed with polylysine (PLys) in aqueous media and water insoluble sheets of carboxymethylcellulose–polylysine (CMC–PLys) molecular complexes were prepared. CMC–PLys molecular complexes form three-dimensional network structures. Non-freezing water content (W_{nf}) of the complexes was measured by differential scanning calorimetry (DSC). W_{nf} of NaCMC increased with increasing DS, since hydrophilic groups restrict water molecules. W_{nf} of CMC–PLys complexes decreased with increasing PLys/CMC ratio due to cross-linking formation. It was found that the amounts of W_{nf} calculated from melting enthalpies (W_{nfm}) were larger than those calculated from crystallization enthalpies (W_{nfc}). The difference between W_{nfm} and W_{nfc} ($\Delta W_{nf} = W_{nfc} - W_{nfm}$) of CMC–PLys increased with increasing DS and PLys/CMC ratio. It was concluded that W_{nf} is markedly affected by DS and cross-linking density. The amount of super-cooled water in CMC–PLys complexes is the same as that in ΔW_{nf}.
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1. Introduction

Sodium carboxymethylcellulose (NaCMC) is a representative polyelectrolyte used widely in various fields, such as food and textile industries. Although NaCMC is readily dissolved in water, water insoluble CMC can be prepared when sodium ions of NaCMC are replaced by di- and trivalent metal cations and other polycations. Among the molecular complexes prepared by ionic complex formation, synthetic polyelectrolytes have extensively been studied [1–5]. In contrast, polysaccharide electrolytes have received little attention [6]. In our previous studies, we reported that sodium carboxymethylcellulose (NaCMC) can be converted into water insoluble solids when Na ions are replaced by di- and trivalent cations [7]. At the same time, CMCs with various types of mono- and di-valent cations form a liquid crystal in

the presence of a characteristic amount of water at around room temperature [8,9]. The above facts suggest that CMC is a suitable polyanion for preparation molecular complexes.

Recently, ε-polylysine (PLys) in linear form has been industrially synthesized and used as an antiseptic agent in food. It is considered that linear PLys can be used as a counter ionic polymer to CMC. Both polyelectrolytes are known to be biocompatible and a complex formation is considered to be carried out in mild conditions. It is also expected that physical properties can be changed by both preparative method and controlling the amount of water in the systems.

We have studied thermal properties of NaCMC and water–NaCMC systems [10–12] and found that the functional properties of NaCMC are affected by the presence of bound water. We have also estimated the bound water content in carboxymethylcellulose and cellulose sulfate substituted by various valences of cations and suggested that the bound water content of polyelectrolyte systems depends strongly on the kind of cations [10,13,14].

In this study, CMC–PLys molecular complexes (CMC–PLys) with controlled PLys/CMC ratio were prepared. NaCMC having various degrees of substitution (DS) and PLys with molecular weight of about 5000 having random

coil structure were used. Thermal properties of the complexes were measured by differential scanning calorimetry (DSC) in order to investigate the higher order structure of CMC–PLys and its gelation mechanism.

2. Experimental

2.1. Sample preparation

NaCMC in the powder form was obtained from Daicel Chemical Industries Ltd. Degree of substitution (DS) of calboxymethyl group of NaCMC was varied in the range of 0.72, 0.93, 1.28, 1.53 and 2.38. DS of NaCMC was defined as the number of substituted hydroxyl groups in a pyranose ring of cellulose. ε-Type PLys obtained from Tisso Co Ltd. was supplied as 25% aqueous solution. Chemical structures of NaCMC and PLys used in this experiment are shown in Fig. 1.

Fig. 2 shows a preparative scheme of the samples: (1) NaCMC and PLys 1 wt.% aqueous solutions were prepared; (2) after maintaining each solution at room temperature for 60 min, both solutions were slowly mixed using a homogenizer; (3) mixed solution was poured into a frame on a glass plate and dried in air at room temperature; (4)
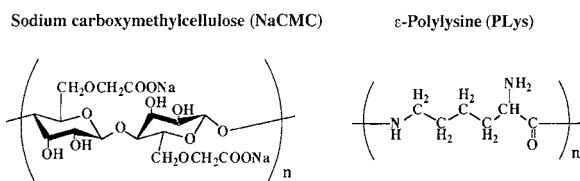

Fig. 1. Chemical structures of sodium carboxymethylcellulose and ε-polylysine.
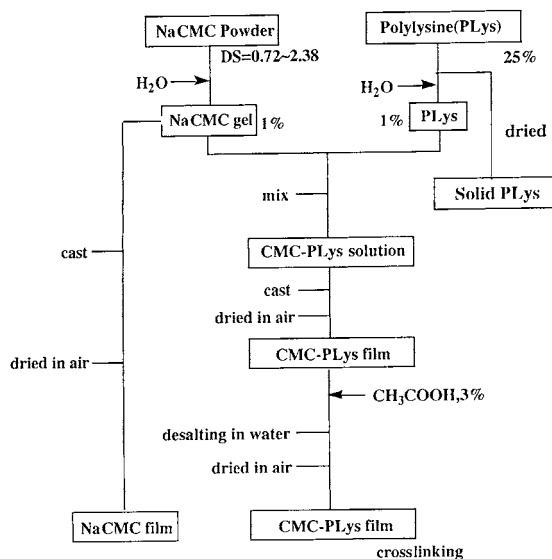

Fig. 2. Preparation scheme of CMC–PLys complexes and NaCMC.

CMC–PLys films were immersed in 3% acetic acid aqueous solution for about 10 h. The film was washed in running water for 60 min; (5) obtained films with thickness = ca. 0.1 mm were dried in air for 3 h and then successively dried in a vacuum oven. CMC–PLys complex ratio (PLys/CMC) was varied at stage (2). In this study, PLys/CMC ratio was changed from 0 to 1.0 mol/mol. PLys/CMC = 0 corresponds to CMC film. CMC and PLys films were also prepared in a similar manner as shown in Fig. 2.

2.2. Measurements

2.2.1. Infrared spectroscopy (FT-IR)

CMC–PLys complex formation was confirmed by infrared spectroscopic measurement using a Fourier transform infrared spectrophotometer (FT-IR-700, Japan Spectroscopy Co. Ltd.). The resolving power of FT-IR was $8\,cm^{-1}$, the number of integration was a 100 times. The thickness of sample films was $80\,\mu m$. Measurements were carried out in dry atmosphere at $25\,°C$.

2.2.2. Differential scanning calorimetry (DSC)

Phase transition behavior of NaCMC, PLys and CMC–PLys complex films in the presence of various amounts of water was measured by a differential scanning calorimeter (DSC, Seiko Instruments Inc., DSC EXSTER6000). Cooling and heating rates were $10\,°C/min$. Sample weight was about 3–5 mg in a sealed type aluminum vessel. For the measurement of samples containing sorbed water, a small amount of pure water was added using a microsyringe. The water content (W_c, g/g) was calculated from the following equation (1):

$$\text{water content, } W_c \text{ (g/g)} = \frac{\text{mass of sorbed water}}{\text{mass of dry sample}} \quad (1)$$

The sample having a certain amount of water content was cooled from room temperature to $-150\,°C$ and then heated to about $60\,°C$.

After measurement, phase transition temperatures in DSC curves were determined. The starting temperatures of crystallization peak and sub-peak at lower temperature side were defined as T_c and T_c', respectively. Glass transition temperature (T_g) was defined at the cross-point of baseline and contact line of transition region. Cold crystallization temperature (T_{cc}) was defined as the starting temperature, and melting temperature (T_m) was defined as the peak temperature.

Crystallization (ΔH_c), melting (ΔH_m) and cold crystallization enthalpies (ΔH_{cc}) of water sorbed on the samples were calculated. Crystallization enthalpy (ΔH_c) of water at the temperature T_c was calibrated by Eq. (2):

$$\Delta H_c = \Delta H_{c0} - (C_{pw} - C_{pi})(T_0 - T_c) \quad (2)$$

where ΔH_{c0} ($334\,J\,g^{-1}$) is crystallization enthalpy of water at $0\,°C$, C_{pw} ($4.217\,J\,g^{-1}\,K^{-1}$) is heat capacity of water at

$0 \, ^{\circ}C$, C_{pi} is C_p of ice at T_c ($^{\circ}C$). Non-freezing water content (W_{nf}) was calculated by Eq. (3):

$$W_{nf} \, (g/g) = W_c - W_f - W_{fb} \tag{3}$$

where W_f is free water content calculated by ΔH_c or ΔH_m, W_{fb} is freezing bound water calculated by enthalpy of crystallization at lower temperature side. W_{nfc} and W_{nfm} are defined as non-freezing water content calculated from crystallization and melting enthalpies, respectively. The water content of cold crystallization peak (W_{cc}) is calculated from the enthalpy of cold crystallization.

3. Results and discussion

Colorless and transparent NaCMC and CMC–PLys complex films were obtained. NaCMC films were water soluble, in contrast, CMC–PLys complex films were water insoluble. DSC heating curves of dry NaCMC–PLys complex films showed single glass transition depending on PLys/CMC ratio. The above evidences suggest that no phase separation occur in the complex films. Formation of the complex was confirmed by infrared spectroscopy. The intensity of C=O stretching absorption band decreased with increasing PLys/CMC ratio. Moreover, C=O stretching peak shifted to the low wave number side.

Fig. 3(a) shows DSC cooling curves of NaCMC–water systems. Three kinds of sorbed water were observed in NaCMC–water systems as reported previously [10–13]. When W_c was more than 1.43 g/g, a sharp crystallization peak was observed at about $-25 \, ^{\circ}C$. A small sub-peak (T_c') was observed at about $-45 \, ^{\circ}C$. The shape of this sharp peak was almost similar to that of pure water. When water content was more than 0.51 g/g, small broad crystallization peak was observed at about $-40 \, ^{\circ}C$. This water is categorized as freezing bound water [15,16]. When water
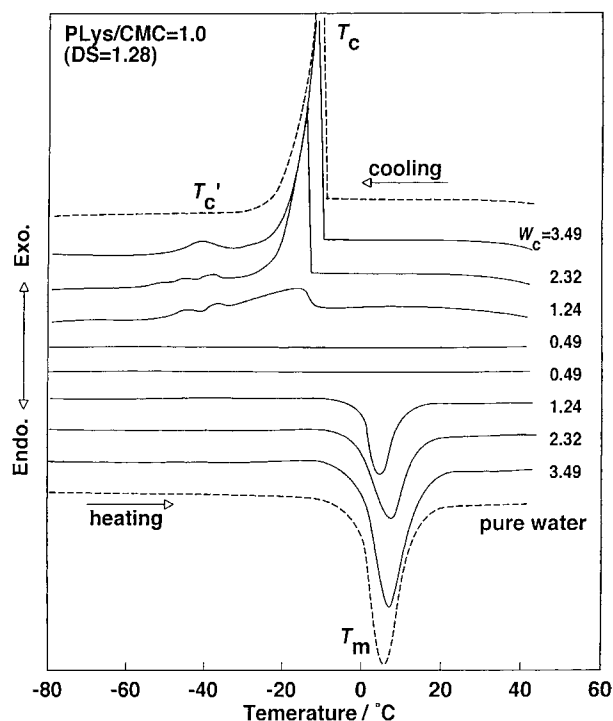

Fig. 4. DSC curves of CMC–PLys–water systems containing various water contents.

content was less than 0.51 g/g, no phase transition of water sorbed on NaCMC was observed. It is considered that the amount of water lower than this critical value is frozen as non-freezing water. Fig. 3(b) shows DSC heating curves of NaCMC–water systems. In the heating curves, glass transition (T_g) at about $-80 \, ^{\circ}C$, cold crystallization (T_{cc}) at about $-40 \, ^{\circ}C$ and melting (T_m) at about $0 \, ^{\circ}C$ were observed. T_{cc} was hardly observed with increasing W_c.

Fig. 4 shows DSC curves of CMC–Plys–water systems containing PLys/CMC = 0.5 mol/mol. Almost similar DSC
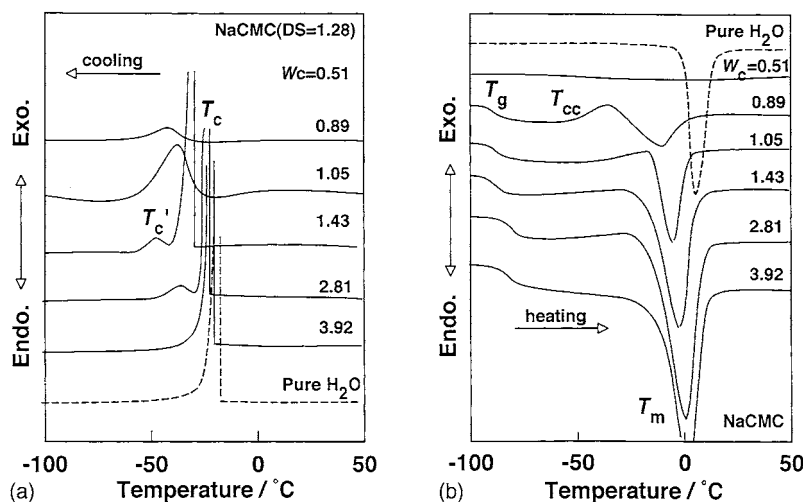

Fig. 3. (a) DSC cooling curves of NaCMC–water systems containing various water contents. (b) DSC heating curves of NaCMC–water systems containing various water contents.
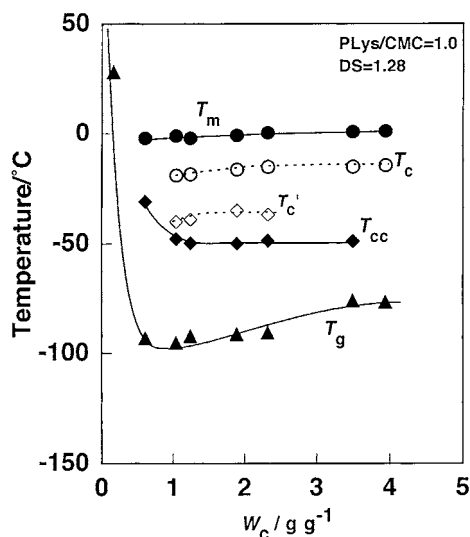
Fig. 5. Phase diagrams of CMC–PLys–water systems. T_c and T_c' were observed in cooling process.

Fig. 6. Relationship between phase transition enthalpies (ΔH_m, ΔH_c, ΔH_{cc} and $\Delta H_c'$) and water content (W_c).

curves compared with those of NaCMC–water systems were observed. Although neither T_g nor T_{cc} were recognized in Fig. 4, if DSC heating curves are magnified at a temperature range from −100 to 0 °C, T_g was observed for samples with W_c 0.52 to 3.45 g/g and T_{cc} was found for the samples with W_c range from 0.52 to 2.89 g/g. From these DSC curves, phase transition temperatures and enthalpies of transition were calculated.

Fig. 5 shows the phase diagrams of CMC–PLys–water systems with PLys/CMC ratio 1.0 mol/mol and DS of CMC 1.28. In the phase diagram obtained from the cooling process, T_c and T_c' were observed at about −20 and −40 °C, respectively. In the phase diagram obtained from the heating process, T_g steeply decreased in a small W_c range, reached a minimum at about −100 °C and then became saturated at around −80 °C. T_{cc} was observed in W_c of 0.5–3.5 g/g. T_m was observed at about 0 °C. Similar phase diagrams of CMC–water systems with different DS were obtained. However, T_g shifted to the higher temperature side with increasing PLys/CMC ratio and shifted to the lower temperature side with increasing DS. T_{cc} could not be observed in PLys–water system.

Fig. 6 shows the relationship between phase transition enthalpies and water content in CMC–PLys–water systems with PLys/CMC ratio 0.6 mol/mol and DS of CMC 0.91. Both enthalpies of crystallization (ΔH_c) and melting (ΔH_m) increased linearly with increasing W_c. However, the enthalpies of crystallization at the low temperature side ($\Delta H_c'$) and cold crystallization (ΔH_{cc}) are small and saturated at certain values. From the results of Fig. 6, it is found that a large difference between the values of ΔH_{nfc} and ΔH_{nfm} calculated from cooling and heating process can be observed. As clearly seen in Fig. 6, ΔH_m is larger than ΔH_c. Similar results can be observed not only in the complex systems but also in the other polysaccharide–water systems. In
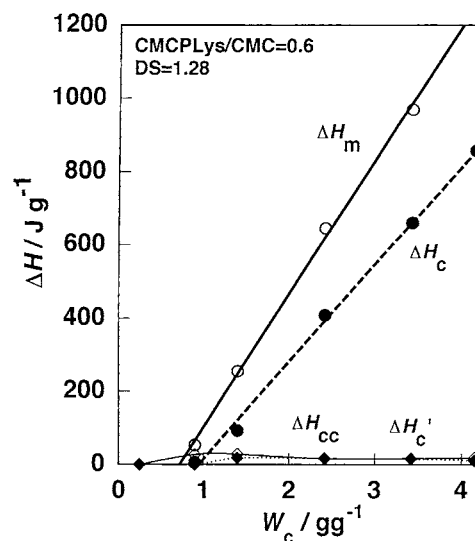
our previous studies, we suggested this enthalpy difference ($\Delta H_0 = \Delta H_m - \Delta H_c$) relates to the amount of W_{nf} which is frozen in the form of non-crystalline structure. In this study, the amount of ΔH_0 is discussed in relation to the amount of W_{nf}.

Fig. 7 shows the relationship between non-freezing water content (W_{nf}) and water content (W_c) of CMC–PLys–water systems with PLys/CMC ratio 0.3 mol/mol and DS of CMC 1.28. Non-freezing water content calculated from the enthalpy of crystallization (W_{nfc}) linearly increased until water content about 1.0 g/g, and then became saturated at 1.1 g/g when W_c exceeded 4.5 g/g. In contrast, W_{nf} calculated from the enthalpy of melting (W_{nfm}) was saturated at around
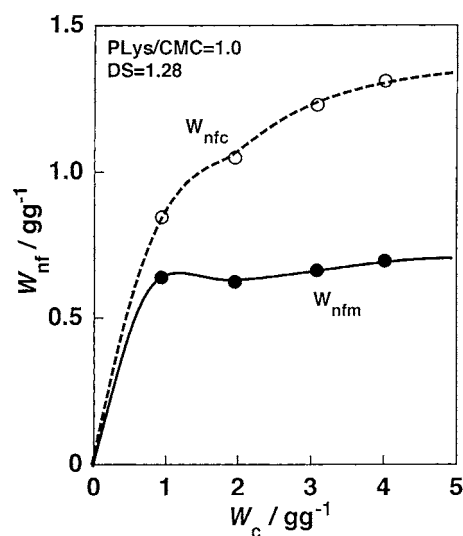

Fig. 7. Relationship between non-freezing water contents (W_{nfc} and W_{nfm}, which were calculated from the enthalpies of crystallization and melting, respectively) and water content (W_c).
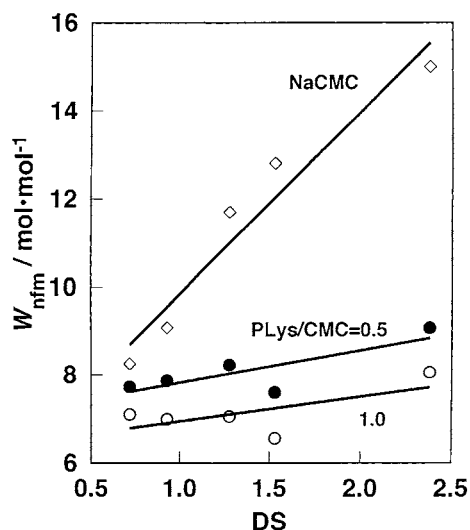
Fig. 8. Relationship between non-freezing water content calculated from melting (W_{nfm}) and DS of CMC.

Fig. 9. Relationship between W_{nfm} and PLys/CMC ratio.

0.7 g/g. From the results of Fig. 7, it is found that a large difference between the values of W_{nfc} and W_{nfm} calculated from cooling and heating process can be observed. It was found that W_{nfc}, calculated from the enthalpy of crystallization is markedly larger than W_{nfm} calculated from melting enthalpy. The above difference obviously increases when PLys/CMC ratio increases.

In order to investigate the effect of DS of CMC, W_{nf} values of complexes with the same PLys/CMC ratio with different DS were calculated. The difference between non-freezing water contents calculated from the enthalpies of crystallization (W_{nfc}) and melting (W_{nfm}) increased with increasing DS.

Fig. 8 shows the relationship between W_{nfm} and DS. W_{nfm} linearly increased from 8 to 15 mol/mol for NaCMC, from 8 to 9 mol/mol for CMC–PLys complexes having PLys/CMC ratio = 0.5 and from 7 to 8 mol/mol for PLys/CMC = 1, respectively. In the case of NaCMC, W_{nfm} increases with increasing DS due to the increase of sodium calboxymethyl groups. In the case of CMC–PLys complexes, having PLys/CMC = 1.0, W_{nfm} increases slightly due to the unreacted hydroxyl groups. It is known that W_{nf} increased with decreasing cross-linking density [7].

Fig. 9 shows the relationship between W_{nfm} and PLys/CMC ratio. When PLys/CMC ratio increases, W_{nfm} maintained until about PLys/CMC = 0.3, then decreases with increasing PLys/CMC ratio. This means that CMC–PLys complexes form bulky three-dimensional network structures in low cross-linking density (PLys/CMC = 0.3) and then forms closely packed structures in high cross-linking density (PLys/CMC > 0.3). It is thought that bulky network chains retain a large amount of W_{nf}, however, when molecular chains align regularly, water molecules are excluded from the well-arranged portion, and the number of restrained water molecules (W_{nf}) diminishes.
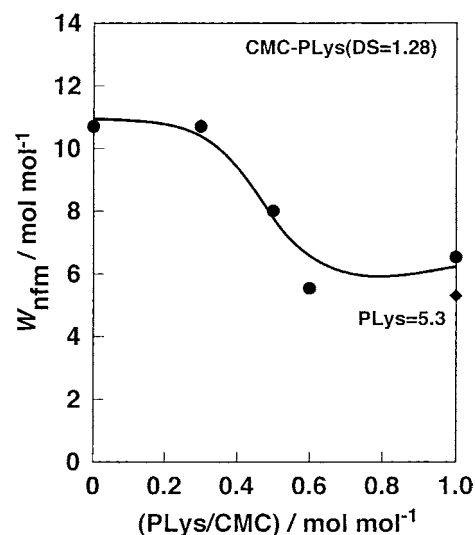
Fig. 10 shows the difference between non-freezing water contents calculated from the enthalpies of crystallization and melting ($\Delta W_{nf} = W_{nfc} - W_{nfm}$) plotted against DS. ΔW_{nf} increased with increasing DS and PLys/CMC. This suggests that the amount of super-cooled water formed during cooling process increased with increasing DS and PLys/CMC ratio. That is, the amount of super-cooled water increases when the complexes form three-dimensional bulky networks having hydrophilic groups.

As stated above, the super-cooled water is restrained by complexes network structures and frozen as W_{nf}. When W_{nf} is heated, it starts to be mobile at T_g and is transformed into more stable structures at T_{cc}. The crystalline part, either normal or irregular ice, melts at T_m. In order to create the enthalpy balance of the system, it is considered that the
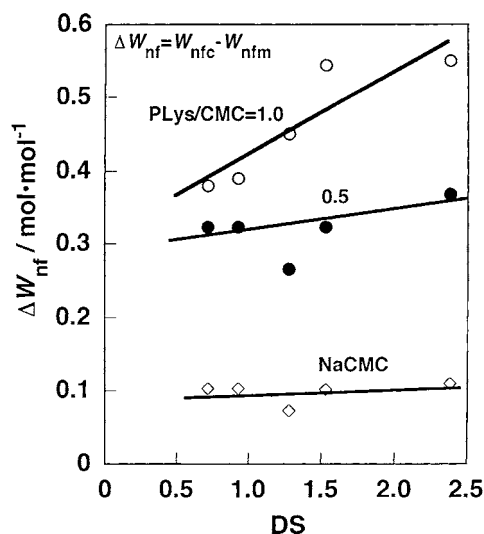

Fig. 10. Relationship between ΔW_{nf} (= $W_{nfc} - W_{nfm}$, which is the difference between non-freezing water contents calculated from the enthalpies of crystallization and melting) and DS of CMC.
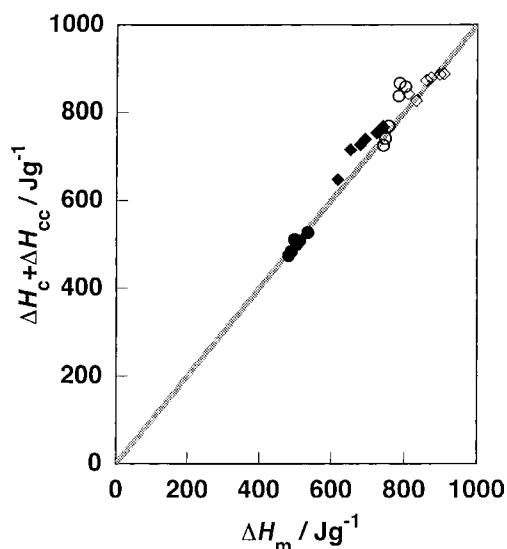
Fig. 11. Relationship between summation of the enthalpies of ΔH_c and ΔH_{cc}, and ΔH_m.

Fig. 12. Relationship between ΔW_{nf} and ΔW_{cc} (which is calculated from ΔH_{cc}).

summation of ΔH_c and ΔH_{cc} correspond to ΔH_m. Fig. 11 shows the relationship between summation of the enthalpies of ΔH_c and ΔH_{cc}, and ΔH_m. The values of $\Delta H_c + \Delta H_{cc}$ agreed well with ΔH_m. This indicates that the calculated ΔW_{nf} accords with the amount of water calculated from ΔH_{cc}, that is, the water content corresponded to cold crystallization peak (W_{cc}).

Fig. 12 shows the relationship between W_{cc} and ΔW_{nf} represented by mol/mol. The amount of water of W_{cc} also agreed well with ΔW_{nf}. These results suggest that the amounts of super-cooled water corresponded to that of water crystallized at cold crystallization temperature (T_{cc}).

From the above results, it is summarized that no phase transition of CMC–PLys–water systems is observed when W_c is less than 0.5 g/g. It is considered that non-freezing water is restricted by hydrophilic groups and three-dimensional networks of carboxymethylcellulose–polylysine molecular complexes. W_{nf} is markedly affected by DS and cross-linking density. It was found that the amounts of W_{nf} calculated from melting enthalpies (W_{nfm}) were larger than those calculated from crystallization enthalpies (W_{nfc}). The difference between W_{nfm} and W_{nfc} ($\Delta W_{nf} = W_{nfc} - W_{nfm}$) of CMC–PLys increased with increasing DS and PLys/CMC ratio. It is concluded that super-cooled water is formed during the cooling process and that the amount of super-cooled water is equal to a ΔW_{nf}.
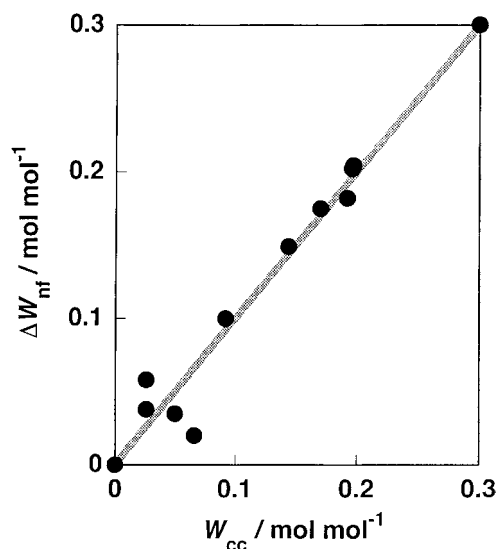
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