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Analysis of Water State and Gelation of Methylcellulose Thermo-reversible Hydrogels by Thermal Analysis and NMR

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The gelation of aqueous methylcellulose (MC) solutions containing polyethylene glycol (PEG) was studied by the combination of differential scanning calorimetry (DSC) and Raman spectrometry. The gelation of MC hydrogels containing PEG occurred in two-steps. First, the gel network was formed by the hydrophobic interaction between MC and PEG at 310 – 313 K, and then, the gel network was formed between MC chains at 323 K. On the other hand, in the MC hydrogels containing PEG and NaCl, sodium ion assumed to be enclosed by PEG, forming a helix with the hydrophobic groups outward. The sodium ion in the gel was expected to be surrounded by the ether oxygen of PEG as crown ether.

Keywords Methylcellulose, polyethylene glycol, hydrogel, eutectics, DSC-Raman, water state analysis, gelation

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Introduction

Methylcellulose (MC) is a water-soluble cellulose derivative, where the hydroxyl groups of cellulose are substituted with methoxy groups. The degree of substitution (DS) of MC is defined as the number of hydroxyl groups substituted by the methoxy groups in a glucose unit. MC exhibits unique characteristics; on heating, the aqueous MC solutions can form hydrogels, and the rheological gel point is closely correlated to the appearance of optical turbidity. The MC gel is a completely thermo-reversible gel. The MC gel has been reported to be formed through hydrophobic interaction between adjacent molecular chains containing methoxy groups.[1–10] The gelation temperature of MC can be altered by adding different additives such as synthetic polymers and salts.[11–14] The salt reduces the gelation temperature of MC because of the salting-out effect.[15] NaCl is used for decreasing the gelation temperature of MC for the formation of drug delivery.[16,17] On the other hand, polyethylene glycol (PEG) belongs to the group of polyols; it can alter the physical properties of MC hydrogels. PEG is nontoxic and biocompatible. It is used to enhance viscosity and decrease gelation temperature. The drug release time has been reported to increase with the increase in the molecular weight of PEG in an MC-PEG-salt system.[16] The gelation mechanism of MC hydrogels is not sufficiently clear. The gel state of MC hydrogels exhibits a fibrillary diameter of 14 – 15 nm. The development of a fibrillar structure with increasing temperature correlates with the rheological and turbidity behavior.[18,19] The MC-PEG-water system forms a thermo-reversible gel during heating. We have investigated the water state and dynamic mechanical property of an MC-PEG-water system and reported that the memory of the gel state was maintained for three days after gelation at 277 K. The period of maintaining the memory of the gel state after gelation is in agreement with the strength of the PEG-water interaction. The interaction was observed by differential scanning calorimetry (DSC).[20,21] The PEG-water system has been reported to form a eutectic of PEG and water at low temperature.[22] In the case of ethanol or 1-propanol aqueous solutions, the water–alcohol interaction can be detected by the examination of the melting enthalpy of a eutectic of alcohol and water.[23,24] With heating, the MC-alkali chloride-water system forms a thermo-reversible gel. Alkali chloride aqueous solutions form a eutectic of alkali chloride and water, and amino acids, sugars, and some organic compounds dissolved in eutectics of salt and water.[25–27] The melting behavior of these eutectics can be investigated by DSC, and the results obtained from DSC are in good agreement with those obtained by near infrared spectroscopy (NIR) or ^{17}O nuclear magnetic resonance (NMR).[20,23,27] In this study, we focused on the gelation and water structure of MC hydrogels containing PEG and salt. As compared to the anions, the cations have been reported to exhibit lesser effects on MC solutions.[14] In this study, we examined the effect of alkali chloride as compared to that of alkali bromide.

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Experimental

Reagents and chemicals

MC400 (M_w = 84000, DS = 26 – 33%), MC4000 (M_w = 140000, DS = 26 – 33%), LiCl, NaCl, KCl, RbCl, CsCl, LiBr, NaBr, KBr, RbBr, and CsBr were purchased from Wako Pure Chemical Industries, PEG6000 was purchased from MERCK, and used without further purification. PEG (molar fraction of

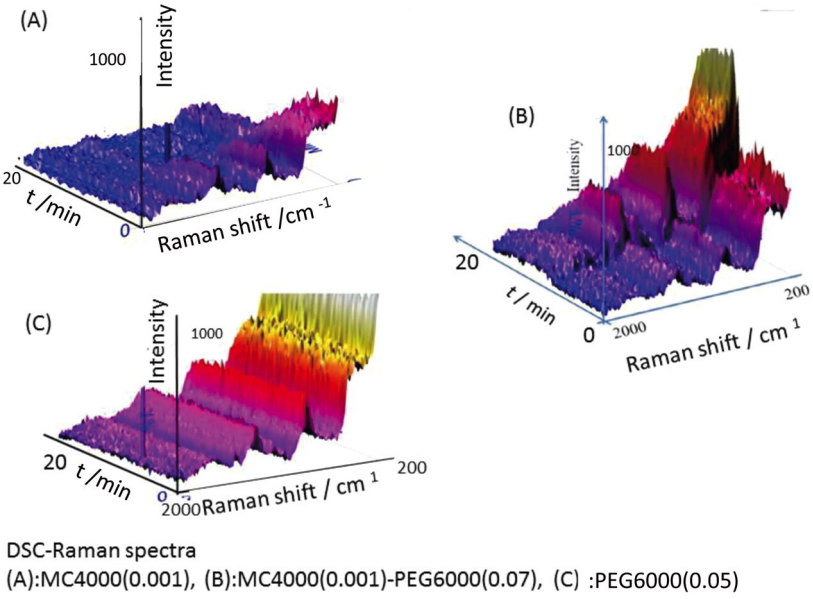

Fig. 1 Raman spectra of MC4000 (molar fraction of MC: 0.001) (A), MC4000 (molar fraction of MC: 1×10^{-3})-PEG6000 (molar fraction of EOX: 0.07) (B), PEG6000 (molar fraction of EOX: 0.05) (C) obtained during heating by DSC-Raman spectroscopy.

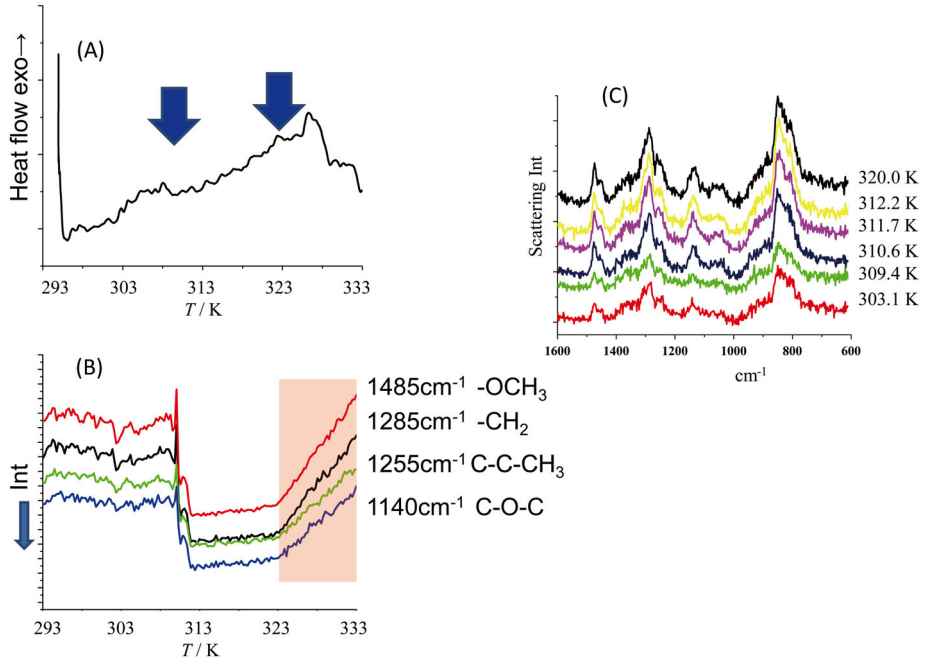

Fig. 2 DSC-Raman spectroscopy of MC4000 (0.001)-PEG6000 (0.07). (A) DSC data, (B) Peak intensities of 1140, 1255, 1285, 1485 cm^{-1}, (C) Raman spectra.

ethylene oxide (EOX) unit: 0.05), MC (molar fraction of MC unit: 1×10^{-3}) , and alkali halide (molar fraction of alkali chloride or alkali bromide : 1.8×10^{-3}, 0.1 mol/L), were used in this study.

Apparatus

Measurements were carried out using a Hitachi High-Tech Science X-DSC7000, a JEOL NMR JNM ECA400, a JASCO UV/Vis/NIR V570, and AND Sine Wave Vibro Viscometer SV-10. DSC-Raman measurements were carried out using a Perkin Elmer Raman Station 400F, DSC 8500.

Measurement conditions

DSC measurements were carried out using aqueous MC solutions containing PEG and an alkali halide. The solution was sealed in an Al hermetically sealed sample vessel. First, the sample was cooled to 123 K at 4 K min^{-1}, then, it was heated to 293 K at 4 K min^{-1}. α-Al_2O_3 was used as the reference. NIR

measurements were carried out at 298 K in the range of 900 – 1300 nm. Pure water was used as the reference. ^{17}O NMR measurements were carried out at 303, 313, 323, 333, and 343 K, with the spectrometer operating at 54.10 MHz for ^{17}O. ^{17}O spectra were recorded at a natural abundance of the ^{17}O isotope. D_2O was used as the external reference. ^{23}Na NMR measurements were carried out at 303, 313, 323, 333, and 343 K with the spectrometer operating at 104.72 MHz for ^{23}Na. ^{23}Na spectra were recorded at a natural abundance of the ^{23}Na isotope. A NaCl D_2O solution was used as the external reference. DSC-Raman measurements were carried out using an aqueous MC solution, a PEG solution, and an MC-PEG solution. Each of these solutions was added to an Al hermetically sealed sample vessel fitted with a SiO_2 cover. The sample was heated from 293 to 333 K at 2 K min^{-1}. Laser irradiation conditions are as follows: 100 mW power, 6 s irradiation, and 10 s interval. Viscosity measurements were using aqueous MC solutions containing PEG and an alkali halide. The sample was heated from 293 to 343 K at 0.5 K min^{-1}.

Results and Discussion

Gelation mechanism of methylcellulose hydrogels containing PEG by DSC-Raman

Because the gelation of MC hydrogels is a rate-dependent process,[18,19] simultaneous measurement is essential. Raman spectroscopy is a non-contact technique. As Raman scattering occurs in all directions, access is required only from one side of the sample. Hence, it is significantly easier to interface with a transmission technique such as FT-IR. Laser energy is readily coupled to an optical fiber. The sol-gel transition of the MC-PEG-water system exhibits a small heat change.[1,2] It is difficult to examine the gelation mechanism of MC-PEG-water system in detail by DSC. Hence, we applied DSC-Raman spectroscopy. Figure 1 shows the temperature dependence of the Raman spectra. For the MC solution, Raman intensity of –CH_3, –OCH_3, –C–CH_3, and –COC decreased with gelation. On the other hand, for the MC-PEG solution, Raman intensity of –CH_2– and –COC first increased, and then decreased after maintaining a constant value. Moreover, for the PEG solution, the peak intensities of these functional groups were constant. Changes in peak intensities in the MC-PEG solutions were expected to correspond to the interaction between MC and PEG. Figure 2 shows the temperature dependence of peak intensities of 1485 cm^{-1} (–OCH_3), 1285 cm^{-1} (–CH_2–), 1255 cm^{-1} (C–C–CH_3), and 1140 cm^{-1} (C–O–C). The intensities of these peaks rapidly increased at approximately 310 – 313 K, and gradually decreased at greater than or equal to 323 K. The increase of the intensity of the peaks at 310 – 313 K is attributed to the hydrophobic interaction of PEG and MC. The decrease in the peak intensity greater than 323 K is attributed to gelation by the hydrophobic interaction of MC. The gelation of MC hydrogels containing PEG proceeded in two-steps. These results were consistent with those obtained from viscosity measurements.

Water structure of the sol state

The water state in the MC thermo-reversible hydrogel containing PEG or an alkali halide was detected using the melting enthalpy of the eutectic of water and PEG or an alkali halide. In this study, we investigated the MC hydrogels containing PEG and salt.

In the case of the MC-PEG-water system, the strength of the PEG-water interaction was detected by the melting behavior of the eutectic of PEG and water at 260 K.[20] Figure 3 shows the

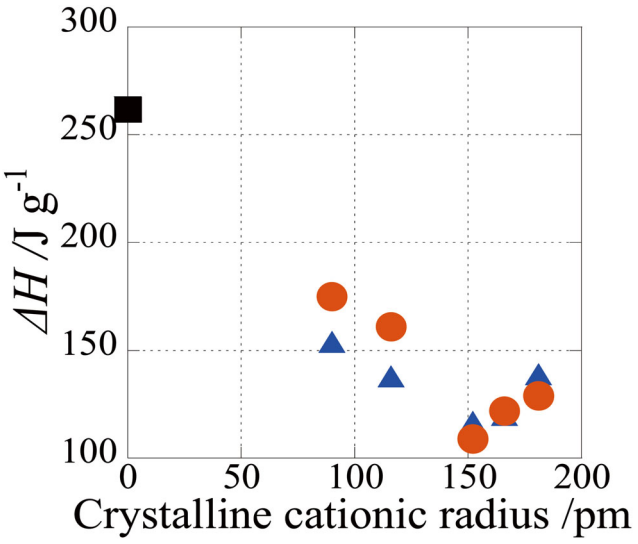

Fig. 3 Melting enthalpy of eutectics of MC4000 (0.001)-PEG6000 (0.05)-salt (0.0018).
The vertical axis shows melting enthalpy per gram of PEG. ●: alkali chloride, ▲: alkali bromide, ■: without salt.

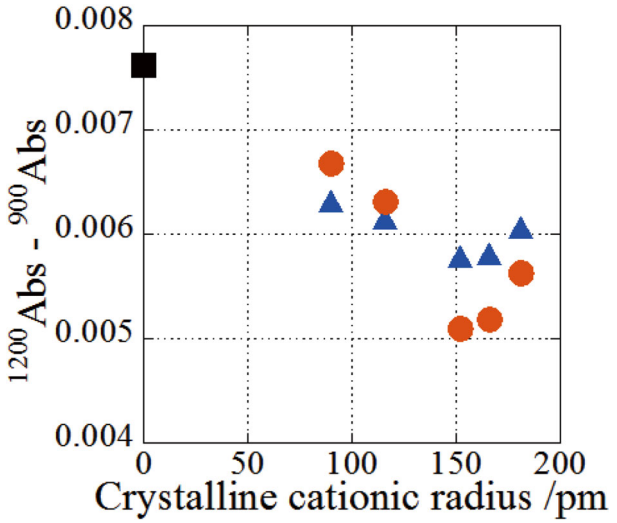

Fig. 4 Absorbance of hydrogen-bonded water at 1200 nm of MC4000 (0.001)-PEG6000 (0.05)-salt (0.0018). ●: alkali chloride, ▲: alkali bromide, ■: without salt.

melting enthalpy of eutectics of PEG and water of MC4000 (0.001)-PEG6000 (0.05)-alkali chloride or alkali bromide solutions. The vertical axis shows in the melting enthalpy per gram of PEG. First, the melting enthalpy decreased with the radius of the alkali metal ions from Li to K, and after a minimum at K, it increased again until Cs. Alkali bromides and alkali chlorides exhibited the same tendency. The melting temperature and melting enthalpy of the eutectics exhibits the same tendency. The overtone and combination tones of the fundamental vibration of water were observed at approximately 1000 nm and 1160 – 1200 nm, respectively. The absorption near 1160 – 1200 nm can be attributed to $\nu_1 + \nu_2 + \nu_3$, and it shifted to long wavelength by hydrogen bonding.[28] Figure 4 shows the absorbance of hydrogen-bonded water at 1200 nm. First, the

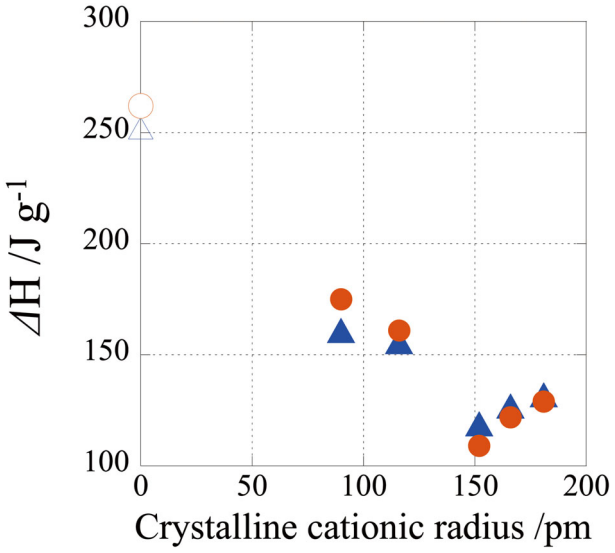

Fig. 5 Melting enthalpy of eutectics of MC (0.001)-PEG6000 (0.05)-alkali chloride(0.0018). The vertical axis shows melting enthalpy per gram of PEG. ●: MC4000, ○: MC4000 without salt, ▲: MC400, △: MC400 without salt.

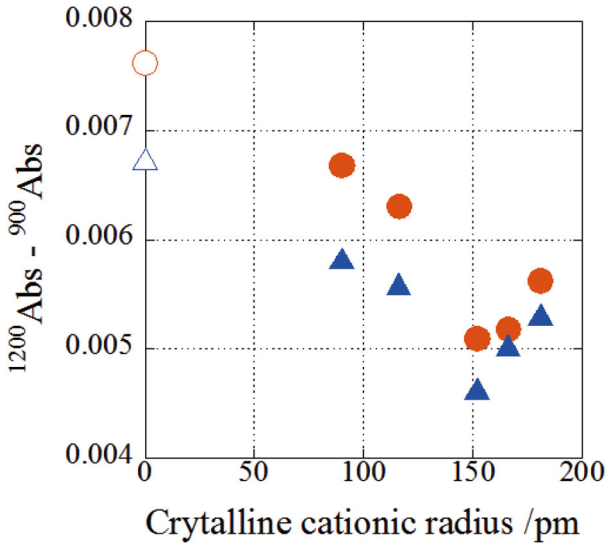

Fig. 6 Absorbance of hydrogen-bonded water at 1200 nm of MC (0.001)-PEG6000 (0.05)-alkali chloride (0.0018). ●: MC4000, ○: MC4000 without salt, ▲: MC400, △: MC400 without salt.

amount of hydrogen-bonded water decreased with the radius of the alkali metal ions from Li to K, and after a minimum at K, it increased until Cs. Alkali bromides and alkali chlorides exhibited the same tendency. The effect of cations was considered to be greater than that of anions. In the case of MC hydrogels, cations were proven to exhibit effects weaker than anions.[14] Our results were considered to be caused by the inclusion of PEG. A detailed study will be conducted in the future.

Figure 5 shows the melting enthalpy of eutectics of PEG and water of MC400 or MC4000 (0.001)-PEG6000 (0.05)-alkali chloride solutions. First, the melting enthalpy decreased with the radius of the alkali metal ions from Li to K, and after a

Table 1 Gelation onset temperature of MC hydrogels

Composition			Gelation onset
MC400 molar fraction of MC unit	PEG6000 molar fraction of EOX unit	Molar fraction (salt)	Temperature/ K
0.001	—	—	329.5
0.001	0.05	—	323.1
0.001	—	0.0018 (NaCl)	326.7
0.001	—	0.0018 (KCl)	327.0
0.001	0.05	0.0018 (LiCl)	321.4
0.001	0.05	0.0018 (NaCl)	321.0
0.001	0.05	0.0018 (KCl)	321.0
0.001	0.05	0.0018 (RbCl)	320.6
0.001	0.05	0.0018 (CsCl)	320.4

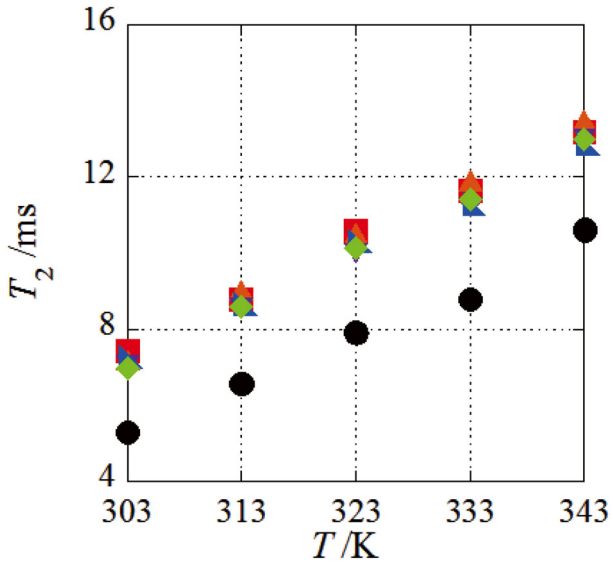

Fig. 7 Temperature dependence of the relaxation time (T_2) of ^{17}O NMR of MC400 (0.001)-PEG6000 (0.05)-alkali chloride (0.0018). ●: without salt, □: LiCl, △: NaCl, ▽: KCl, ◿: RbCl, ◇: CsCl.

minimum at K, it increased again until Cs. MC400 and MC4000 exhibited the same tendency. Figure 6 shows the absorbance of hydrogen-bonded water at 1200 nm. First, the amount of hydrogen-bonded water decreased with the radius of the alkali metal ions from Li to K, and after a minimum at K, it increased until Cs. MC400 and MC4000 exhibited the same tendency. The difference between the MC4000 and MC400 was considered to be marginal. The structure making ions like Li^+ or Na^+, gave different effects from the structure breaking ions like K^+, Rb^+ and Cs^+ into the solution structure.

Gelation process

Table 1 lists the gelation onset temperature of MC hydrogels by viscosity measurements. In this experiment, the gelation onset temperature of MC hydrogels was 329.5 K; however, it decreased with the addition of PEG and salt. The gelation of MC hydrogels under the current conditions was not clearly observed by DSC measurements.

Figure 7 shows the change in the T_2 of ^{17}O NMR during gelation. The T_2 of the MC-PEG system was shorter than that of the MC-PEG system containing salt. The difference caused

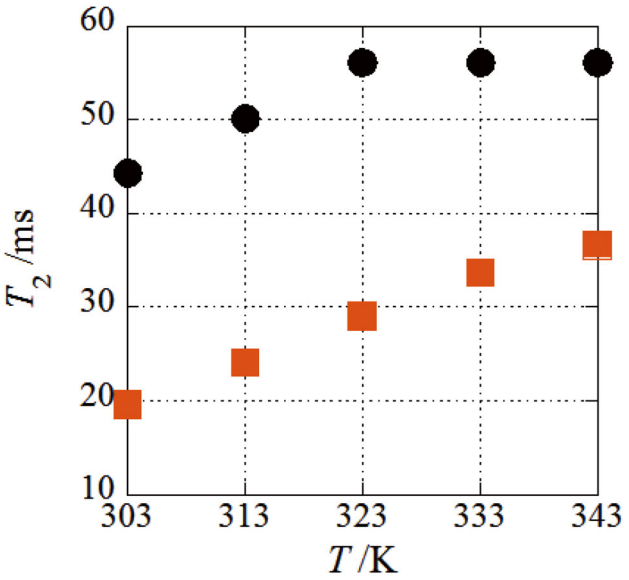

Fig. 8 Temperature dependence of relaxation time (T_2) of ^{23}Na NMR of MC400 (0.001)-[PEG6000 (0.05)]-NaCl (0.0018). ●: without PEG, ■: with PEG.

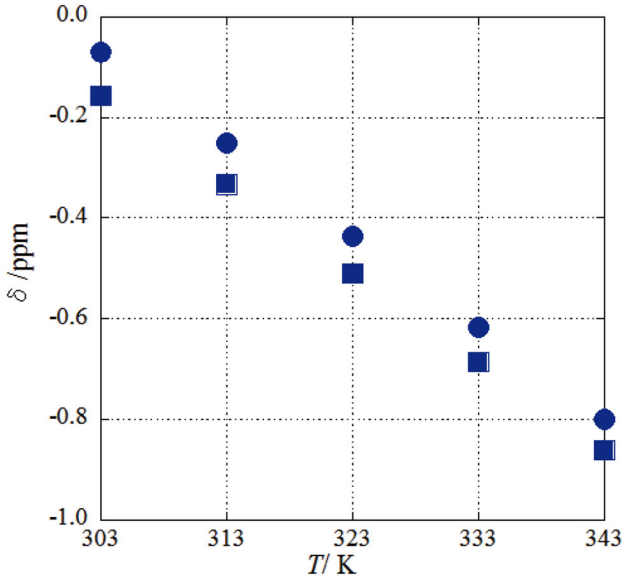

Fig. 9 Temperature dependence of chemical shift of ^{23}Na NMR of MC400 (0.001)-[PEG6000 (0.05)]-NaCl (0.0018). ●: without PEG, ■: with PEG.

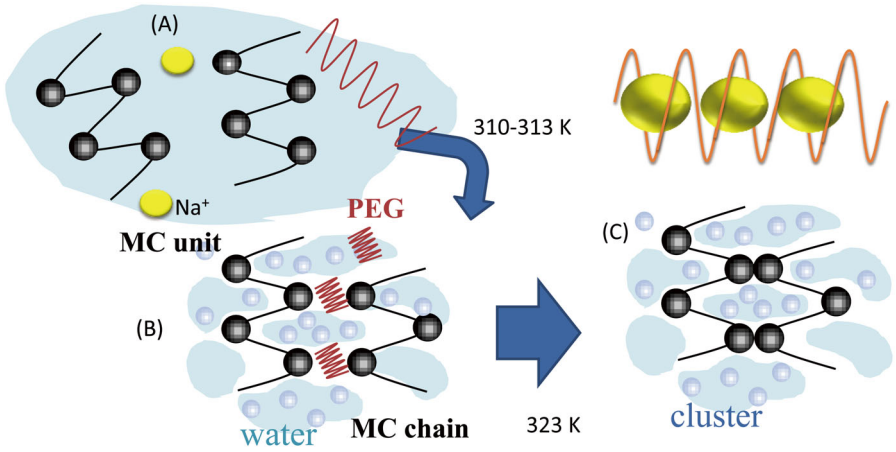

Fig. 10 Schematic illustration of the gelation of MC hydrogels containing PEG and NaCl. (A) sol state, (B) gel formed by the hydrophobic interaction of MC and PEG at 310 – 313 K, (C) gel formed by the hydrophobic interactions between the MC chains at 323 K.

by the type of cation was not observed. Figure 8 shows the change in the T_2 of ^{23}Na during gelation. The T_2 of the MC-NaCl system was longer than that of the MC-NaCl system containing PEG. Figure 9 shows the change in the chemical shift of ^{23}Na NMR during gelation. The chemical shifts of the sample containing PEG were shifted to low field. On the other hand, for the sample without PEG, T_2 remained constant after gelation as the diffusion constant of water changed by gelation. However, for the MC-NaCl system containing PEG, T_2 increased with temperature, possibly attributed to the interaction of sodium ion and PEG. Sodium ions were assumed to be enclosed by PEG, forming a helix with the hydrophobic groups toward the outside. Sodium ion in the gel was expected to be surrounded by ether oxygen in PEG as crown ether. Approximately 20 of sodium ions were expected to be enclosed in one molecule of PEG. Figure 10 shows a schematic of gelation of MC hydrogels containing PEG and salt. The gelation of MC hydrogels containing PEG proceeded in two steps. First, the gel network was formed by the hydrophobic interaction between MC and PEG at 310 – 313 K, and then the gel network was formed between MC chains at 323 K.

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