1. Introduction

Temperature-sensitive behaviors, such as the thermo-reversible gelation and the lower critical solution temperature (LCST) type of phase separation, are the typical characteristics of aqueous methylcellulose (MC) [1‒4]. MC attains the water-solubility by reducing the strong hydrogen-bonding among cellulose molecules. The reduction of the hydrogen bonding is achieved by partially substituting hydrophobic methoxy group for hydrophilic hydroxy group in cellulose molecule. The temperature-sensitive behaviors of MC originate from the concert effects of these hydrophilic and hydrophobic interactions [5,6]. The transition temperatures, such as the gelation and phase separation temperatures, are affected by the degree of substitution and also by the distribution pattern of the substituents [5,7‒10]. It is considered that commercially available MC in general has inhomogeneous distribution of the substituents due to the manufacturing process. Eventually, it is natural that the observed transition temperatures differ depending on the sources of MC samples.

There are other factors that make the transition temperatures indefinite, if the employed MC sample is limited to a specific one. Dehydration upon heating is the common origin of both the gelation and the phase separation in aqueous MC [11]. In this respect, there are two concepts whether the gelation and the phase separation are contemporaneous [6,12] or not [13,14]. In practice, the gelation is often accompanied by turbidity. If both the gelation and the turbidity relevant to the phase separation are triggered at the \( \theta \) temperature, the former concept must be plausible.

In contrast, our point in this study is based on the idea that different levels of dehydration are required for the gelation and the phase separation of the commercially available MC [11]. In order to form a hydrophobic aggregation, which is considered to be the primary stage of the gelation, a certain portion of MC segments must be dehydrated. The commercially available MC having inhomogeneous distribution of the substituents, is likely to have a spatial distribution of dehydration ability within a molecule. Therefore, the commercially available MC is an interesting system, in which a thermodynamic property of a
whole molecule on average and that of a local segment are considerably different. It is considered that tri-methyl glucose units, namely, the monomer units in which all three hydroxy groups being substituted by three methoxy groups, are preferentially dehydrated upon heating and promote the gelation [2,15]. In practice, a synthetically prepared diblock MC enriched in tri-methyl glucose units successfully enhanced the gelation ability [16]. In the meantime, the remaining part of the segments i.e. the less substituted segments must still be hydrated to swell in water. On the other hand, phase separation, corresponding to the precipitation in the limiting case [3], requires the higher level of dehydration i.e. the higher temperature, than the gelation, but the phase separation develops rapidly once the dehydration reached the required level. Consequently the judgment of the phase separation i.e. the cloud point, is assumed to be less affected by the kinetic condition compared to the gelation. Although the primary stage of the gelation i.e. dehydration starts in the lower temperature region than the phase separation upon heating, the gelation is a process that takes time because the dehydrated segments must encounter other dehydrated segments by diffusion to form a higher-order structure as described below.

Recent cryogenic transmission electron microscopy (cryo-TEM) and small-angle neutron scattering (SANS) studies demonstrated that the hydrogel of commercially available MC does not consist of the network of single chains, but it consists of the network of fibrils with 14‒15 nm in diameter [17‒22]. The aforementioned tri-methyl glucose units enriched diblock MC forms ribbon-like nanostructures [16]. Formation of such a higher-order structure must cause the slow equilibration [17]. Therefore, the judgment of the gelation should be significantly affected by the kinetic condition, such as, the holding time for gelation in isothermal and the heating rate in non-isothermal processes. Although the kinetic effects on the temperature-sensitive behaviors of aqueous MC have been acknowledged [3,12,17,22‒24] and empirical equations, which predict the gelation temperature at the slow heating rate limit, were proposed [17], we have not yet reached the full understanding of the ideal equilibrium of sol-gel transition in view of the fact that there exists the higher-order structure below the theta temperature [22].

Winter-Chambon criteria for gelation [25,26] are being applied to study the kinetic effects on the gelation of MC [12]. On the other hand, conventional methods, the so-called “falling ball method” [27] and “gradient method” [4] are still used by virtue of the convenience. In this study, we have judged the sol-gel transition within the framework of such a conventional method, however, some refinements were added to take the kinetic effects into consideration by dually extrapolating the holding time for gelation to infinite and the mechanical probing force to zero for a specific MC. Also the lower limit of the cloud points have been obtained by extrapolating the heating rate to zero.

2. Experimental

2.1 Materials

A commercially available methylcellulose (MC), Metolose SM-4, a product of Shin-Etsu Chemical Co., Ltd., was used as the material of present study. The weight-average molecular weight ($M_w$) and the degree of substitution (DS) i.e. the average number of methoxy groups substituted for hydroxy groups per anhydroglucose unit, are $2.5\times10^4$ and 1.77, respectively. The MC powder was dispersed in hot deionized water so as the concentrations become 2, 4, 6, 8 and 10 wt%. Then the sample solutions were cooled by an ice water bath to get transparent solutions and kept in refrigerator until use.

2.2 Modified falling ball method with dual extrapolations

In the conventional falling ball method [27], a probing ball is dropped on the surface of sample solution after applying the prescribed temperature for the prescribed period of time. Then, the gelation is judged based on whether or not the probing ball sinks. This method is convenient and suitable for the judgment of relatively strong gels. This method is, however, not suitable if the gel is fragile, since the fragile gel is easy to be broken by the shock of the falling ball. Since the border region of the gelation is discussed here, the handled gels are very fragile. To address this difficulty, the gelation was judged by the following modified falling ball method in the present study. A probing ball was previously sunk to the bottom of the solution-filled test tube with 10 mm in diameter and then the prescribed temperatures were applied for 1, 6, 12 and 24 h. Subsequently, the gelation was judged based on whether or not the probing ball sinks. This method is convenient and suitable for the judgment of relatively strong gels. This method is, however, not suitable if the gel is fragile, since the fragile gel is easy to be broken by the shock of the falling ball. Since the border region of the gelation is discussed here, the handled gels are very fragile. To address this difficulty, the gelation was judged by the following modified falling ball method in the present study. A probing ball was previously sunk to the bottom of the solution-filled test tube with 10 mm in diameter and then the prescribed temperatures were applied for 1, 6, 12 and 24 h. Subsequently, the gelation was judged based on whether or not the probing ball was driven when the tube was tilted. As for the probing ball, polyacetal, aluminum oxide and stainless-
steel ball with 3 mm in diameter were used. The corresponding bulk densities $\rho_b$ for the polyacetal, aluminum oxide and stainless-steel ball are 1.4, 3.6 and 7.8 g/cm$^3$, respectively. By extrapolating the holding time for gelation to infinite and also extrapolating the density of the probing ball to unity, the lower limit of the gelation temperature was obtained. It is considered that the extrapolation of the density of the probing ball to unity in aqueous solution is equivalent to the extrapolation of the probing force to zero. These dual extrapolation measurements were performed for 2, 6, and 10 wt% aqueous MC. The temperature of the sample solutions was controlled using a thermostatic bath MG-1200, a product of Tokyo Rikakikai Co., Ltd.

2.3 Light transmission measurement

We have ever reported the cloud point for the same MC sample as the present study, which was evaluated at a single heating rate 3 °C/min [28]. As was mentioned in the introduction, it is assumed that the cloud point is less affected by the kinetic condition compared to the gelation. In order to examine the assumption, the light transmission measurements were conducted upon heating with the rates of 3, 5, 10 and 20 °C/min. The sample solutions were sandwiched between two cover glasses with 0.15 mm thick. The optical path i.e. sample thickness, was adjusted to be ca. 0.5 mm by a spacer inserted between two cover glasses. By selecting such a tiny scale for the sample, the retardation in sample heating was minimized and the detailed profile of light transmittance curve was revealed beneficially as will be discussed later. The intensity of the light transmitted through the sample solutions was measured by utilizing the direct beam monitoring optics of a high-resolution small-angle light scattering instrument with a diode laser of wavelength 635 nm as the light source. The details of the instrument and the applications were noted elsewhere [28‒30]. Temperature of the sample solutions were controlled using a hot stage FP 82 HT, a product of Mettler-Toledo International Inc.

3. Results and discussion

3.1 Holding time dependence of gelation temperature

Fig. 1 (a) and (b) show typical examples, which were judged as “sol” and “gel”, respectively, based on the criteria as described in the section 2.2. In Fig. 1 (a), the silvery probing ball (stainless-steel) was driven by tilting and also the surface of solution showed fluidity, whereas the white probing ball (polyacetal) was not driven and the surface of solution showed rigidity in Fig. 1 (b). The applied conditions (temperature and time) to these representative samples are described in the caption for Fig. 1.

Fig. 2 shows the holding time dependence of the gelation temperature for 10 wt% aqueous MC. The gelation temperature $T_{gel}(t)$ decreased with increasing the holding time $t$, besides that, the $T_{gel}(t)$ shifted to the lower temperature when the probing ball with the lower density was used. Thus, the observed gelation temperatures vary significantly depending on the conditions, such as the holding time and the probing force.

As seen from Fig. 2, $T_{gel}(t)$ decreased not linearly
with $t$, but it could fit the following exponential function (eq. 1), where $C_1$, $t_{gel}$ and $T_{gel(\infty)}$ are a fitting constant, a characteristic time of gelation and a gelation temperature after infinite holding time, respectively.

$$T_{gel(t)} = C_1 \exp(-t/t_{gel}) + T_{gel(\infty)}$$ (1)

Fitted lines are shown in Fig. 2. The resulting values of $T_{gel(\infty)}$ are ca. 21, 22 and 24 °C for $\rho_b = 1.4, 3.6$ and 7.8 g/cm$^3$, respectively. Together with the results evaluated for other concentrations, the gelation temperatures after infinite holding time $T_{gel(\infty)}$ as a function of the density of the probing ball $\rho_b$ are displayed in Fig. 3.

$T_{gel(\infty)}$ showed fairly good linearity with $\rho_b$ for all concentrations examined here. The values of $T_{gel(\infty)}$ obtained by extrapolating $\rho_b$ to unity correspond to the gelation temperatures determined at zero in mechanical probing force. These dually extrapolated gelation temperatures correspond to the lower limit of the gelation temperatures. Concentration dependence of the lower limit of the gelation temperature will be shown in Fig. 6 and discussed later together with the cloud point.

It is worth noting that the gelation temperatures without the density extrapolation estimated only using a polyacetal ball ($\rho_b = 1.4$ g/cm$^3$) at 24 h fairly approximates the extrapolated gelation temperatures (Fig. 3). Therefore, it may be convenient to use the polyacetal ball to estimate the approximate value of the lower limit of the gelation temperature without the density extrapolation.

### 3.2 Heating rate dependence of cloud point

Fig. 4 (a) and (b) show the light transmittance curves as a function of temperature and their differential form, respectively, for 10 wt% aqueous MC observed at various heating rates.

At every heating rate, the light transmittance decreased gradually in the beginning upon heating, followed by a sharp fall (Fig. 4 (a)). As will be discussed below, we consider that the former and the latter stage of this two-step behavior link to the gelation and the phase separation, respectively. It should be noted that the light transmittance curve obtained at the rate 20 °C/min shows different trends compared to the curves obtained at other rates. The reason and countermeasure for it will be discussed later.

Formation of the network of fibrils, is accompanied by the spatial heterogeneity, [19] which scatters the visible light in some degree, however, we
are assuming that it is not so significantly compared to the cases of the phase separation [31]. As was demonstrated in Fig. 2 and Fig. 3, the gelation was initiated already at 21 °C and it was a process that takes long time. These factors cause the gradual decrease of light transmittance upon heating. In fact, aqueous hydroxypropyl methylcellulose (HPMC), which has poorer gelation ability than aqueous MC [17,34], does not show such a gradual decrease in the light transmittance curve but shows only a sharp fall [6,11]. Therefore, the gradual decrease and the sharp fall in the light transmittance are assigned to the optical aspects of the gelation and the phase separation, respectively, at least for a specific MC used in the present study.

The cloud points were extracted from light transmittance curves by the differentiation. The temperature at which the differential curve shows a negative peak is considered to be the cloud point [28]. The peak temperature i.e. the cloud point, shifted to the higher temperature with increasing the heating rate (Fig. 4 (b)).

![Fig. 5 Heating rate dependence of cloud point for representative concentrations of MC.](image)

Fig. 5 shows the heating rate dependence of the cloud point for the representative concentrations. The linearity between the cloud points and the heating rates looks fair for the displayed heating rate range (≤ 10 °C/min), however, if the cloud points obtained at the rate 20 °C/min are included in the analysis the linearity becomes poor. It is probably because 20 °C/min is too fast to equilibrate thermally the sample solution with the apparatus. The resulting values of the lower limit of the cloud point obtained by extrapolating the heating rate to zero will be shown in Fig. 6 and discussed later together with the lower limit of the gelation temperature.

Although the kinetic effects on the cloud point is not so serious (Fig. 5) compared to the case of gelation (Fig. 2), the cloud points observed at the smaller heating rates (≤ 3 °C/min) give the better approximation to the lower limit of cloud point if one estimate the cloud point without these extrapolation procedure.

### 3.3 Phase diagram

Fig. 6 shows a phase diagram regarding the concentration dependences of the lower limit of the gelation temperature (diamond marks) and the lower limit of the cloud point (open-circle marks) obtained by the above-mentioned extrapolation methods for a specific MC used in the present study.

![Fig. 6 Lower limit of gelation temperature (diamond marks), lower limit of cloud point (open-circle marks) and conditional gelation temperature estimated by employing a stainless-steel ball after holding 1 h (cross marks). Lines are guide for eyes.](image)

The diagram is divided into three regions, according to the criteria stated in the experimental section and the results of observations. In the region lower than the lower limit of the gelation temperature, the aqueous MC keeps "transparent sol". On the other hand, in the region higher than the lower limit of the cloud point, "turbid gel" is obtained. In the intermediate region between these two curves, "semi-transparent gel" is obtained; naturally the lower the temperature the higher the transparency within the region.

These boundaries have the following characteristics. Firstly the cloud point is located in the higher temperature than the gelation temperature [14,32]. This relative location is reasonably explained...
by that the phase separation requires higher level of dehydration than the gelation. Secondly the cloud point shows weak concentration dependence, whereas the gelation temperature shows stronger concentration dependence than the cloud point. These behaviors indicate that the phase separation is triggered by mainly the level of dehydration i.e. temperature, whereas the gelation is ruled by both the level of dehydration and the probability that the dehydrated segments encounter other dehydrated segments to form the cross-linking point. The probability increases with MC concentration, as a result the gelation temperature decreases with MC concentration. For instance, the lower limit of the gelation temperatures are ca. 41 and 21 °C for 2 and 10 wt% aqueous MC, respectively (diamond marks). For comparison, conditional gelation temperatures estimated by employing a stainless-steel ball after holding 1 h are also plotted in Fig.6 (cross marks). The differences between the gelation temperatures with and without the extrapolation are ca. 8 and 26 °C for 10 and 2 wt%, respectively. Namely, the difference increases drastically with decreasing the concentration. Therefore, from a practical perspective, it should be born in mind that the physical property of aqueous MC changes over time, especially in the lower concentration region.

We are assuming that the two-step behavior i.e. the gradual decrease and the sharp fall in the light transmittance curve, is ascribed to the inhomogeneous distribution of the substituents. In the series of synthetically prepared MCs, the gradual decreasing behavior in the light transmittance was enhanced with increasing the composition of the trimethyl glucose units [9]. To the contrary, MC having homogeneous distribution of the substituents showed only precipitation without gelation [33]. In such a case, the gelation temperature curve does not exist in the phase diagram.

The lower limit of the gelation temperature obtained in this study is a long time limit of the gelation temperature. In this sense, the driving force to form gel is virtually ceased in such a long time limit. On the other hand, the structure of gel is not perfectly homogeneous in general, which means that the gel consists of various levels of local minimums from the energetic perspective. Therefore, the lower limit of the gelation temperature obtained in this study is not the ideal equilibrium temperature, but it should be called “quasi-equilibrium gelation temperature” as was written in the title of present study. The ideal equilibrium gelation temperature, though it cannot be realized in practice, would exist in somewhat lower temperature than the present quasi-equilibrium gelation temperature obtained experimentally on heating.

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