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Physical gelation process for cellulose whose hydroxyl groups are regioselectively substituted by fluorescent groups

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Five cellulose derivatives whose hydroxyl groups are regioselectively substituted by benzyl ether and methyl ether groups were prepared, i.e. 6-*O*-benzylcellulose, 2,3-di-*O*-methyl-6-*O*-benzylcellulose, 2,3-di-*O*-benzylcellulose (23B6O), 2,3-di-*O*-benzyl-6-*O*-methylcellulose and 2,3,6-tri-*O*-benzylcellulose. The gelation did not take place in tetrahydrofuran solutions of the cellulose derivatives whose hydroxyl group at the 6-position was substituted to methyl ether or benzyl ether groups, but only the cellulose derivative having the 6-position hydroxyl group, i.e. 23B6O, was found to form gels. All the samples except 23B6O showed only usual fluorescence of benzyl group over the temperature range 200–310 K. In contrast, the fluorescence of 23B6O shifted to the red and the excimer fluorescence increased with an increase of interactions between the cellulose molecules. Thus, our fluorescent probe method could elucidate the gelation process with a change of temperature in terms of the molecular association involving a hydrogen bond. In addition, the new absorption corresponding to the red-shifted fluorescence peak was also confirmed. The new species were concluded to be a ground state dimer formed intermolecularly between benzyl groups, meaning that there exists a hydrophobic interaction between them. In conclusion, (1) the main cause for the gel formation in our system is the hydrogen bonding by means of the 6-position hydroxyl group, and (2) the hydrophobic interaction between benzyl groups also keeps 23B6O molecules associated with one another, as well as the hydrogen bonds, after it is aggregated. © 1997 Elsevier Science Ltd.

(Keywords: thermoreversible gel; hydrogen bond; hydrophobic interaction; ground state dimer)

INTRODUCTION

The characteristic features of cellulose molecules such as the rigidity of the chain and the insolubility in many solvents are attributed to three hydroxyl groups (2-, 3- and 6-positions) in an anhydroglucose unit of cellulose forming both inter- and intra-molecular hydrogen bonds. The substitution of the hydroxyl groups by functional groups is known to induce gelation[1] or liquid crystallization[2–4], where the intermolecular hydrogen bonds may play an important role. Thus, we aimed at elucidating the function of each hydroxyl group of cellulose in aggregated states such as gels.

We have already succeeded in studying the gelation process of isotactic polystyrene in decalin by means of the fluorescent probe method[5,6], since luminescent probe techniques can be effective tools for investigating microstructures and motions of polymer molecules, especially because of their having the advantage of high sensitivity[7]. However, fluorescence measurements have not been so effectively employed in investigating microstructures and motions of cellulose molecules thus far except for the work of Winnik and co-workers[8–12], who introduced pyrenyl groups into hydroxypropylcellulose as a fluorescent probe and monitored their emission behaviour in relation to polymer aggregation. Although their trial should be highly valued, there were some problems in their samples: (i) the pyrenyl moiety was too bulky to neglect its hydrophobic interaction with another pyrenyl group for the polymer–polymer association in water, (ii) as the pyrenyl group of Winnik and co-workers' samples was far from the cellulose main chain (12 atoms spaced between the pyrenyl group and the anhydroglucose unit), it was difficult to represent the motion of the molecule, and (iii) as the fluorescence probes were randomly introduced into the cellulose polymer, it was hard to observe a very local change in a molecule.

In order to obtain more microscopic information on the aggregated state formed by the hydrogen bonds of cellulose, we chose the benzyl group as one of the smallest fluorescent probes. We prepared cellulose samples whose hydroxyl groups are regioselectively substituted by them and reported that the hydroxyl groups at the 6-position of the cellulose repeating unit plays the most important role in their gelation process[13]. The benzyl fluorescence in a gel form was found to be different from that in a solution form, indicating that the

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fluorescence was influenced by the polymer–polymer association, since the fluorescence probes were situated in the vicinity of the anhydroglucose unit. In the present paper, we prepared five kinds of cellulose samples, whose regioselective substitution patterns by benzyl ether and methyl ether groups were different from one another, and tried to clarify (1) the function of each hydroxyl group of cellulose having different preferences for forming a hydrogen bond, and (2) what causes the aggregation among the polymers. With the fluorescent-labelled cellulose, we report the temperature dependence of fluorescence behaviour accompanying the gelation process.

EXPERIMENTAL

Materials

The cellulose samples, whose hydroxyl groups were regioselectively substituted by benzyl ether and methyl ether groups, used in the present study were 6-*O*-benzylcellulose (23O6B), 2,3-di-*O*-methyl-6-*O*-benzyl-cellulose (23M6B), 2,3-di-*O*-benzylcellulose (23B6O), 2,3-di-*O*-benzyl-6-*O*-methylcellulose (23B6M) and 2,3,6-tri-*O*-benzylcellulose (236B) (*Table 1*). The details of the method for preparing 23O6B and 23B6O have already been reported[13]. The synthesis of the other cellulose derivatives was carried out as follows. Through the preparation of the samples, they were all arranged to have almost the same degree of polymerization: M_w of 4×10^4. All the products were dried under vacuum at 65°C and characterized by Fourier-transform infra-red (*FT*i.r.) and ^1H and ^{13}C nuclear magnetic resonance measurements.

2,3-Di-O-methyl-6-O-benzylcellulose (23M6B). First, 2,3-di-*O*-methylcellulose (23M6O) was prepared by the method reported previously[14]. The free hydroxyl groups at the 6-position were then benzylated in a dimethyl sulfoxide (DMSO) solution of the 23M6O, and the product was isolated and purified in the same manner as before[15]. The degree of substitution (DS) by benzyl groups was 1.0, while DS by methyl groups was 2.0.

2,3-Di-O-benzyl-6-O-methylcellulose (23B6M). Following the preparation of 23B6O[15], the latter was dissolved completely at 50°C in DMSO containing a trace amount of water. Methylation of the 23B6O was performed and then the 23B6M produced was isolated and purified according to a previous method[14,16,17]. The DS by benzyl groups was 2.0, while DS by methyl groups was 1.0.

2,3,6-Tri-O-benzylcellulose (236B). To prepare 236B, we used 23B6O prepared above as a starting material to obtain the polymer with the same DP. It was dissolved completely in DMSO at 50°C and the same benzylation and isolation procedures for the above 23M6B were followed. The DS by benzyl groups was 3.0.

Fluorescence measurements

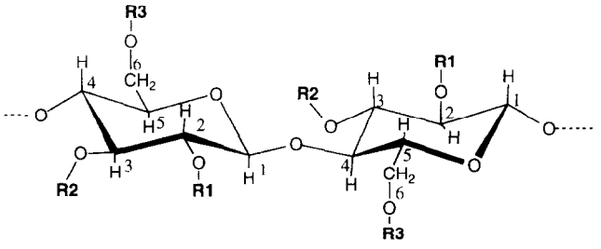
Fluorescence spectra and fluorescence excitation spectra were measured on a Hitachi F-3000 spectro-fluorometer. The emission signal was digitized and transferred into an NEC personal computer system. All the measurements were performed for aerated solutions of tetrahydrofuran (THF), which was purchased from Wako Co. (spectrograde). Fluorescence measurements for the concentrated THF solution of all the cellulose samples were carried out in a quartz cell with an optical pathlength of 1 mm. A cell was set at 45° to the exciting beam. The excitation wavelength was chosen to be 257 nm. The sample temperature was controlled by an Oxford DN1704 cryostat with an ITC-4 digital temperature controller. The temperature regulation was easily better than ±0.1 K; independent temperature measurements were carried out by means of a second thermocouple and a potentiometer. All samples were kept at each set temperature, and spectra were run repeatedly for quite a long time (~ 100 h) even after perfect duplication was obtained, since one of the main aims of the present work is to determine the time required for the equilibrium.

RESULTS AND DISCUSSION

Gelation of the cellulose derivatives in THF

Concentrated THF solutions of the cellulose samples were quenched to several low temperatures in order to

Table 1 Structure of the cellulose derivatives used for the measurements



Sample		R_1	R_2	R_3
Cellulose		H	H	H
6-*O*-Benzylcellulose	(23O6B)	H	H	Benzyl
2,3-Di-*O*-methyl-6-*O*-benzylcellulose	(23M6B)	Methyl	Methyl	Benzyl
2,3-Di-*O*-benzylcellulose	(23B6O)	Benzyl	Benzyl	H
2,3-Di-*O*-benzyl-6-*O*-methylcellulose	(23B6M)	Benzyl	Benzyl	Methyl
2,3,6-Tri-*O*-benzylcellulose	(236B)	Benzyl	Benzyl	Benzyl

examine occurrence of the gelation after preparing isotropic solutions above 313 K. Even the saturated (~ 3%) solutions of 23O6B, 23M6B, 23B6M and 236B were found to remain in solution state below 258 K. In contrast, the gelation of 23B6O in THF was ensured by tilting the test-tube containing the solution[18]: the solution of 23B6O with a concentration higher than 1% (w/w) formed gel below 270 K. These results give two important suggestions: (1) the intermolecular relationship between our cellulose molecules requires the hydrogen bond engaged in the 6-position hydroxyl group, while the 2- and 3-position hydroxyl groups do not contribute to making aggregation, and (2) as is distinct from the case of aqueous solutions, the hydrophobic interaction between the bulky phenyl groups may not be attributed to the polymer–polymer association in a relatively polar organic solvent such as THF. The 6-position hydroxyl group is thought to be favourable for engaging the hydrogen bond with other repeating anhydroglucose units or other molecules, because it is a primary hydroxyl and the farthest group from the rigid polymer main chain and may be more mobile than other hydroxyls. Kondo and Sawatari[19] showed by *FT*i.r. analysis of amorphous cellulose solid that the hydroxyl group at the 6-position plays a crucial role in determining the crystallization state for cellulose due to its high ability to form interchain hydrogen bonds.

Fluorescence behaviour of 23M6B, 23O6B, 23B6M and 236B in THF

Next, we measure the fluorescence behaviour of concentrated THF solutions of 23O6B, 23M6B, 23B6O, 23B6M and 236B in order to study the information on the microenvironment around phenyl groups.

Figure 1 shows the temperature dependence (from 300 to 210 K) of fluorescence spectra of 1.5% 23M6B in THF. All the spectra are normalized at the peak. The fluorescence spectra almost completely agree with one another and no other emission such as an excimer one appears. Each fluorescence spectrum is reproducible and identical at each temperature for both heating and cooling processes. We also measured the time change of the fluorescence spectra of 23M6B at 210 K for 30 h; however, all the spectra perfectly coincide with one another. Moreover, it was ascertained that the temperature

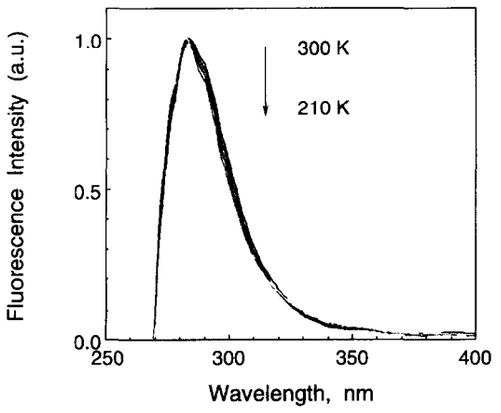


Figure 1 Temperature dependence of fluorescence spectra of 1.5% 23M6B in aerated THF in the cooling process from 300 to 210 K: the spectra are normalized at the peak (excitation wavelength 257 nm)

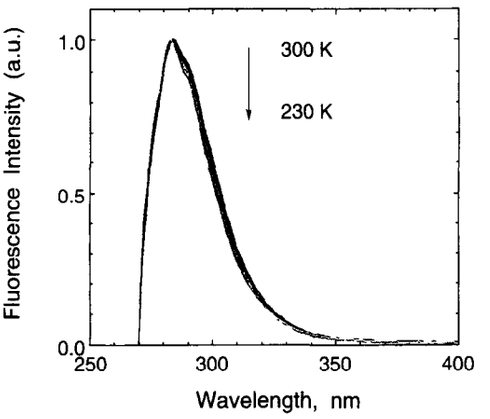


Figure 2 Temperature dependence of fluorescence spectra of 1.5% 23B6M in aerated THF in the cooling process from 300 to 230 K: the spectra are normalized at the peak (excitation wavelength 257 nm)

dependence of fluorescence spectra of 23M6B at a diluted concentration of 5×10^{-3} M for benzyl moiety in THF completely agrees with those of concentrated solutions. As a matter of course, the gelation did not take place in 1.5% 23M6B in THF. Thus, *Figure 1* shows the fluorescence behaviour of an isolated benzyl group at the 6-position even at such higher concentrations.

Figure 2 shows the temperature dependence (from 300 to 230 K) of fluorescence spectra of 1.5% 23B6M in THF. All the spectra normalized at the peak are identical with one another and almost agree with the spectra of 23M6B. It suggests that (1) there is no special interaction such as forming an excimer between the benzyl groups at the 2- and 3-positions and (2) the fluorescence behaviour is almost the same among the benzyl ether groups at the 2-, 3- and 6-positions in the solution state.

The same results are obtained for the temperature dependence of 23O6B and 236B: no excimer fluorescence is detectable. In conclusion, no bimolecular process such as excimer or dimer formation is observed in concentrated THF solutions of the cellulose derivatives that do not form gels.

Fluorescence behaviour of concentrated 23B6O in THF

Figure 3A shows the temperature dependence (from 300 to 200 K) of fluorescence spectra of 2.3% 23B6O in THF. The spectra are found to change, apparently depending on temperature. In order to clarify the temperature dependence of 23B6O fluorescence, we compared the shape of the fluorescence spectra normalized at each peak. The change for the cooling process can be divided into two temperature regions, i.e. (I) 305–270 K (*Figure 3B*) and (II) 270–210 K (*Figure 3C*). The change in region (I) was due to the red-shift of the fluorescence, while the change in region (II) was due to the formation of excimer. When the temperature of 23B6O in THF was raised to 300 K, the spectral shape that was shifted to the red at low temperatures returns to the shape before cooling.

The gelation of 2.3% 23B6O in THF was ensured when the solution was cooled to 290 K; thus, both the fluorescence changes (I) and (II) should reflect the change accompanied by the gelation process and/or the aggregation process.

As a matter of fact, it took about 2 weeks to obtain *Figure 3* because we ascertained whether equilibrium was

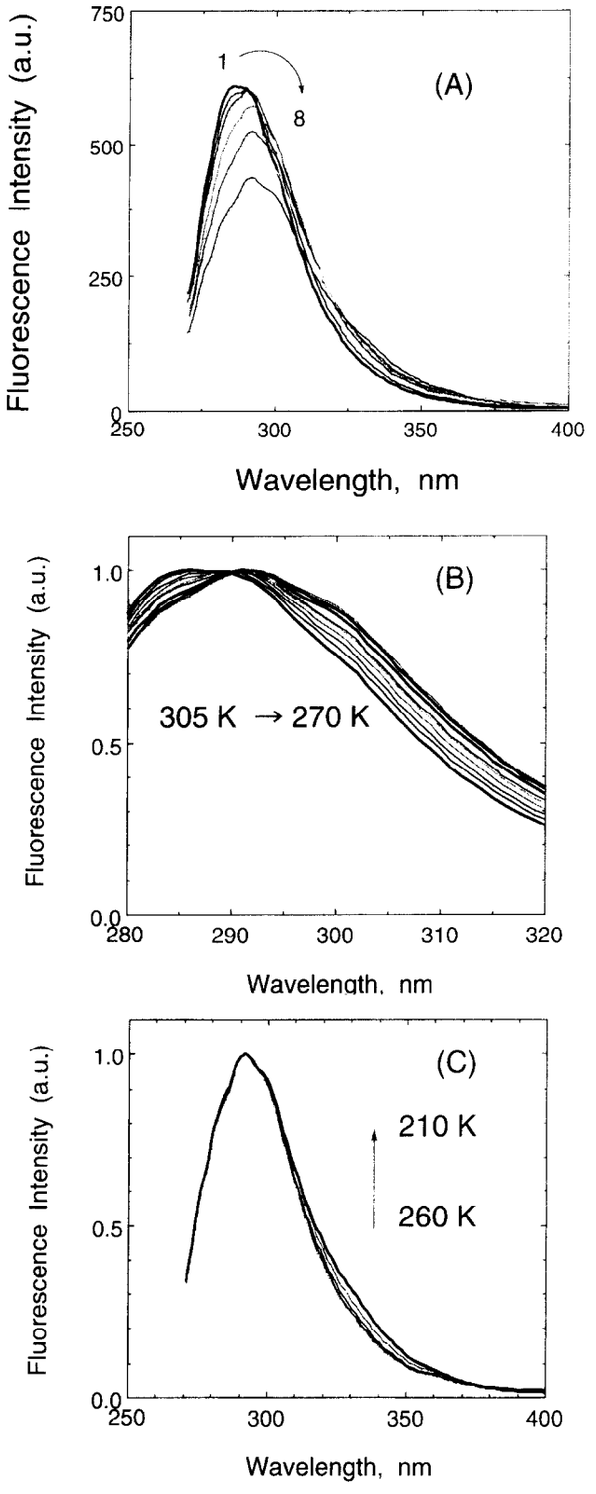


Figure 3 Temperature dependence of fluorescence spectra of 2.3% 23B6O in aerated THF in the cooling process from 300 to 200 K: (A) 1, 305 K; 2, 295 K; 3, 283 K; 4, 275 K; 5, 270 K; 6, 250 K; 7, 230 K; 8, 210 K (excitation wavelength 257 nm). The spectra normalized at the peak are also shown separately in two figures: (B) 305–270 K; (C) 270–210 K

reached at each temperature. In the case of a gradual cooling process like this, the time required for the equilibrium at each temperature is not so long as the rapid quenching process: the equilibrium is reached within 10 h. However, the shape of the fluorescence spectra changes with time for 4 or 5 days in the case of

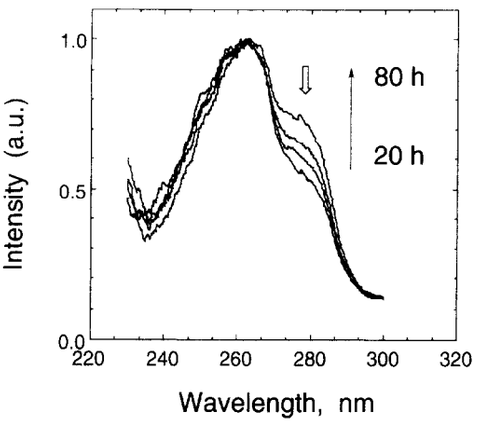


Figure 4 Fluorescence excitation spectra at 320 nm of 1% 23B6O in THF, when it is rapidly cooled from 315 to 273 K. The arrow shows the new absorption band corresponding to ground state dimer

rapid cooling from ~315 K to lower temperatures such as 270 K. The spectra change following the rapid quenching is identical with that shown in *Figure 3*, i.e. the red-shift of the fluorescence peak and the formation of excimer.

Figure 4 shows the excitation spectra for 320 nm fluorescence of 1% 23B6O in THF, when it is rapidly cooled from 315 to 273 K. It shows that it takes nearly 4 days to attain the equilibrium state. The most important point shown in *Figure 4* is that the red-shift of benzyl fluorescence is due to the formation of the new complex that is supposed to be a ground state dimer: the absorption near 280 nm appears and its intensity increases as time proceeds. This change coincides with the red-shift of the fluorescence shown in *Figure 3B*. The temperature dependence of excitation spectra corresponding to the fluorescence change shown in *Figure 3* is identical with the change shown in *Figure 4*. The excitation wavelength was fixed at 257 nm; thus, the change in the fraction of the free benzyl group and the ground state dimer of the benzyl groups would give rise to the apparent red-shift of the fluorescence peak. Since new emission was not observed in the concentrated solutions of 23B6M and 236B, the ground state dimer is concluded to be formed intermolecularly.

The above results suggest that the extent of the aggregation can be estimated by the change in the fluorescence spectra. The fluorescence change can be expressed by the intensity ratio of the emission at 330 nm, I_{330}, to that at the peak, I_{peak}. When the degree of the polymer–polymer interaction increases, the I_{330}/I_{peak} increases because both the red-shift and the excimer formation make it larger. *Figure 5* shows the temperature dependence of I_{330}/I_{peak} of 2.3% 23B6O in THF, which is obtained by continuous measurements for 3 weeks from 305 to 200 K and again from 200 to 310 K. All the I_{330}/I_{peak} values are of the equilibrium state. *Figure 5* demonstrates that a hysteresis loop is observed in the temperature dependence of fluorescence behaviour of 23B6O. When the measurements were finished at 310 K, 23B6O in THF was of the gel form and it was found to be melted to a transparent solution at 320 K. Thus, all the data except a few intial points in the cooling process shown in *Figure 5* were found to give information on a gel form of 23B6O in THF.

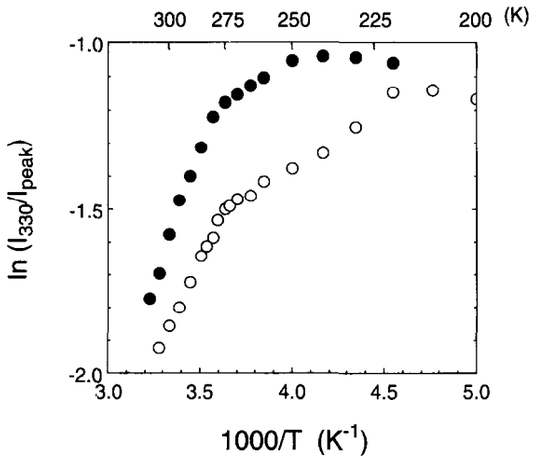


Figure 5 Temperature dependence of I_{330}/I_{peak} for 2.3% 23B6O in THF: (○) cooling from 305 to 200 K; (●) heating from 200 to 310 K. All the I_{330}/I_{peak} values are the equilibrium ones

The hysteresis loop observed in *Figure 5* suggests that the cooling to 200 K makes the intermolecular interaction stronger at lower temperatures. The formation of the polymer network in 23B6O–THF attains equilibrium very quickly at each temperature; however, the number of cross-linking points of the gel network is shown to increase with lowering of the temperature. Our fluorescence probe method first clarified that the change in the cellulose gel structure still proceeds at low temperatures. In general, the intermolecular hydrogen bond is not necessarily fixed at high temperatures where molecular motion is sufficiently fast. However, once the hydrogen bond is formed, it does not dissociate until the temperature is high enough to exceed the binding energy of the bond. Since lowering the temperature restricts the motion of groups such as hydroxyls at the 6-position, the formation of a cross-linking point due to a hydrogen bond is considered to be very much encouraged at low temperatures.

Moreover, we would like to point out one important conclusion from our experimental results. It is the formation of the bimolecular complex supposed to be a ground state dimer, as shown in *Figure 3B*. The formation of an intermolecular dimer between the benzyl groups indicates the presence of a hydrophobic bond connecting two cellulose units. This dimer is not very stable, so that its fluorescence and absorption peaks diminish at temperatures higher than 300 K. On the other hand, it is stable at low temperatures once the polymer–polymer interaction is formed by hydrogen bonding. Since 23O6B, 23B6M, 23M6B and 236B in THF did not form gels and no dimeric fluorescence was observed, clearly the main cause for the gelation was not the hydrophobic effect between the bulky benzyl groups, but the formation of hydrogen bonds with the hydroxyl group at the 6-position. We can say that the fluorescence probe method has proved that hydrophobic bonding exists in the gel of 23B6O in THF, although its effect is minor in the formation of gels.

SUMMARY

We have examined the occurrence of the gelation in THF solutions of five cellulose derivatives whose hydroxyl groups are regioselectively substituted by benzyl ether and methyl ether groups. Consequently, the gelation did not take place in THF solutions of the cellulose derivatives whose hydroxyl group at the 6-position is substituted by methyl ether or benzyl ether groups: 23M6B, 23O6B, 23B6M and 236B. These samples showed only the usual fluorescence of benzyl groups. However, the cellulose derivative with the 6-position hydroxyl group, i.e. 23B6O, was found to form gels. Accompanying the gelation, the fluorescence of 23B6O shifted to the red and the excimer fluorescence increased. The new absorption corresponding to the red-shifted fluorescence peak appeared. This new species was concluded to be a ground state dimer formed intermolecularly between benzyl groups.

In conclusion, the hydrogen bond engaged in the hydroxyl group at the 6-position of the repeating unit of cellulose is the major interaction forming a cross-linking point of the cellulose gel network in THF. Our fluorescence probe method first clarified that the change in the cellulose gel structure still proceeded at low temperatures. At lower temperatures, the molecular motion of the hydroxyl group at the 6-position was restricted very much; thus, the number of hydrogen bonds is concluded to increase with lowering of the temperature.

Although the main cause of gel formation in 23B6O–THF system is the formation of an intermolecular hydrogen bond, the fluorescence behaviour has also elucidated that the hydrophobic interaction between benzyl groups keeps 23B6O molecules associated with one another.

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