Influence of Indomethacin and Isopropanol on Hydrophobic Interaction through Long-Chain Alkyl Groups of Hydrophobically-Modified Hydroxypropyl Methylcellulose[1)]

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The hydrophobic interaction between long-chain alkyl groups of hydrophobically-modified hydroxypropyl methylcellulose (HM-HPMC) and indomethacin (IM) was studied using a fluorescence probe method in which pyrene molecules are allowed to associate with long-chain alkyl groups. In an HM-HPMC solution, the hydrophobicity of the pyrene environment markedly increased as the concentration of HM-HPMC was increased, indicating an increase of hydrophobic interaction between the long-chain alkyl groups. When IM was added to this solution, the hydrophobicity of the pyrene environment decreased as the IM concentration increased. Thus, it was suggested that the hydrophobic interaction between long-chain alkyl groups and IM caused a weakening of the hydrophobic interaction between HM-HPMC molecules themselves.

When isopropanol (IPA) was added to an HM-HPMC solution, IPA weakened the hydrophobic interaction through long-chain alkyl groups. Thus, IPA caused an expansion of the intermolecular hydrophobic regions. For this reason, the amount of IM entrapped in these regions increased when the IPA concentration was increased. However, when the IPA concentration was further increased, the hydrophobic interaction finally disappeared. Thus, as IM molecules could no longer be trapped in the hydrophobic regions, the activity of HM-HPMC suppressing the growth of IM crystals disappeared.

Key words hydrophobically-modified; hydroxypropyl methylcellulose; long-chain alkyl group; indomethacin; pyrene; isopropanol

We have conducted various studies for applying hydrophobically-modified hydroxypropyl methylcellulose (HM-HPMC) to drug manufacturing as a new gel matrix.[2-4)] In the previous paper,[4)] we reported that in a supersaturated solution of indomethacin (IM), a slightly soluble drug, HM-HPMC suppresses the growth of IM crystals, thereby inhibiting the deposition of IM crystals and stabilizing the supersaturated IM solution. HPMC, the original polymer of HM-HPMC, already has the ability to suppress crystal growth, but the introduction of long-chain alkyl groups into HPMC increases this activity. Thus, it was suggested that the crystal-growth-suppressing activity of HM-HPMC was due to a hydrophobic interaction between long-chain alkyl groups and IM molecules. In the present study, the hydrophobic interaction between long-chain alkyl groups of HM-HPMC and IM was investigated using a fluorescence probe method in which pyrene molecules were allowed to associate with the long-chain alkyl groups.

Alcohol is thought to weaken the hydrophobic interaction between long-chain alkyl groups of HM-HPMC.[2)] In order to confirm this, the effects of isopropanol (IPA) on the viscosity of an HM-HPMC solution were investigated in detail. In addition, the effects of IPA on the crystal deposition-suppressing activity of HM-HPMC was examined.

Experimental

Materials HM-HPMC and HPMC were HM-90SH100000 containing 0.6% and 1.1% of long-chain alkyl groups and 90SH100000, respectively, and were obtained from Shin-Etsu Chemical Co., Ltd. (Niigata, Japan). IM (Sigma, U.S.A.), pyrene (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and IPA (Kanto Chemical Co., Inc., Tokyo, Japan) were of guaranteed special grade.

Preparation of Pyrene-Polymer Solution The method of Winnik *et al.*[5)] was applied. That is, HM-HPMC containing 0.6% of long-chain alkyl groups was dissolved in a 0.02 M phosphate buffer solution (pH 4.0) to an HM-HPMC concentration of 0.05 to 0.3%. To 5.0 g of this solution, 5 μl of an acetone solution of pyrene (25 μg/ml) was added (final pyrene concentration of test solution: 25 ng/g), and the mixture was stirred and sonicated for 10 min in an ultrasonic bath (J.M. Ney Company, U.S.A). After equilibration by standing overnight at room temperature, the fluorescence of the mixture was measured. An aqueous solution of HPMC and solvents containing no polymers were similarly treated to prepare solutions containing pyrene, and they were used as the controls.

Hydrophobic Interaction between Long-Chain Alkyl Groups of HM-HPMC and IM One milliliter of a methanol solution containing IM at a concentration of 0, 50 or 100 μg/ml was added to 5.0 g of an aqueous HM-HPMC containing 0.6% of long-chain alkyl groups solution or an HPMC solution or solvent, each containing pyrene (thus, the final IM concentration of the test solution was 0, 8.3 or 16.6 μg/g). The solution was shaken for 30 min, allowed to stand overnight at room temperature to equilibrate the solution, and then the fluorescence was measured.

Fluorescence Measurements Fluorescence spectra were recorded on a model RF-540 spectrofluorophotometer (Shimadzu Co., Kyoto, Japan). A constant-temperature 4-pc. mounting cell holder (Shimadzu Corp., Kyoto, Japan) with a jacket was installed in RF-540. The temperature of the cell holder was maintained at 20 °C by circulating water through the jacket with a model LTP-110 thermoregulator (Tabai Espec Corp., Osaka, Japan). Each sample was placed in a standard quartz cell, and the cell was set to the cell holder. The excitation wavelength was 336 nm, and band paths were 5 nm (excitation) and 2 nm (emission). A ratio of the emission intensity of the pyrene monomer at 383 nm to that at 372 nm was calculated as the $[I_3/I_1]$ ratio.

Effects of IPA on Viscosity of Polymer Solutions The solutions of 0.05 to 0.6% HM-HPMC containing 0.6% of long-chain alkyl groups and HPMC were prepared using a 0.02 M phosphate buffer solution (pH 4.0) containing IPA at a concentration of 0 to 30% (w/w) as the solvent. The viscosity of these solutions was measured at room temperature with a Brookfield rotational viscometer (Tokyo Keiki, Tokyo, Japan).

Effects of IPA on Crystal Deposition-Suppressing Activity Using the 0.02 M phosphate buffer solution (pH 4.0) containing IPA at a concen-

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tration of 0 to 25% (w/w) as the solvent, 0.2% solutions of HM-HPMC containing 0.6 and 1.1% of long-chain alkyl groups were prepared. To 16 g of each solution or solvent, 2 ml of an IPA solution of IM (IM concentration: 20 mg/ml) was added and stirred. After standing for a predetermined time, about 2 ml of the solution was withdrawn, and this was centrifuged at 20000 rpm at 25 °C for 10 min with a model J2-MC (Beckman, U.S.A), followed by measurement of the IM concentration in the supernatant. The above sampling procedure was performed at intervals to monitor the IM concentration until no further decrease in IM concentration could be observed (*i.e.*, until stabilization of the supersaturated solution was achieved).

IM Concentration Determination About 800 μl of a test solution was accurately weighed. Parahydroxybenzoic acid *n*-butyl ester dissolved in methanol was added as the internal standard. This mixture was diluted with methanol and analyzed by HPLC for IM. The HPLC system (Waters, U.S.A.) comprised a Model 486 variable-wavelength UV detector, model 600E high pressure pump, model 717 autosampler, and a Novapak C_{18} column, 4 mm i.d. × 15 cm. The detection wavelength was set at 254 nm. The mobile phase was a 52 : 48 mixture of aqueous RCSS reagent (Waters, U.S.A.) and acetonitrile. The flow rate was 1 ml/min.

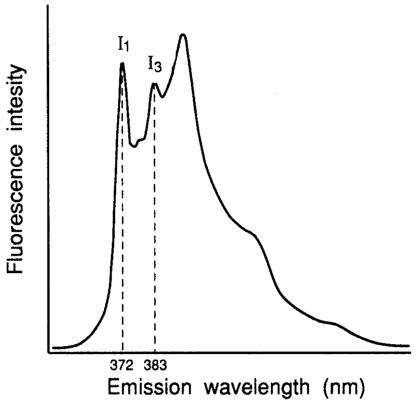

Fig. 1. Fluorescence Spectrum of Pyrene Monomer in HM-HPMC Solution

Excitation wavelength is 336 nm. I_1 is the fluorescence intensity at 372 nm and I_3 is that at 383 nm. HM-HPMC concentration is 0.25%.

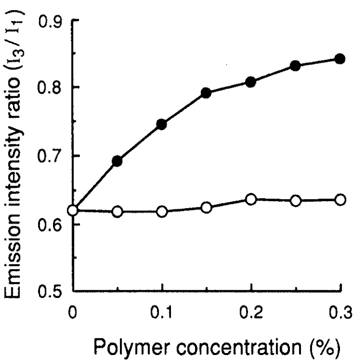

Fig. 2. Polarity of Pyrene Environment as a Function of Polymer Concentration

●, HM-HPMC; ○, HPMC.

Results and Discussion

Polarity of the Pyrene Environment in HM-HPMC Solution Pyrene is a hydrophobic fluorescent probe with very slight solubility in water, and the fluorescence spectrum of the pyrene monomer at a low concentration possesses a vibrational band structure which exhibits a strong sensitivity to the polarity of the pyrene environment.[6] This fluorescence spectrum has five predominant peaks. Peak I (I_1; fluorescence intensity of the (0, 0) band) increases in a polar solvent, while peak III (I_3; fluorescence intensity of the (0, 2) band) is essentially unaffected. Hence, the fluorescence ratio of the two peaks, I_3/I_1, can be used as an index of the polarity of the solvent, that is, the larger the I_3/I_1 ratio, the higher the hydrophobicity.[6] Utilizing this fact, pyrene has also been used as a probe to determine the critical micelle concentration of surfactants.[7] Winnik *et al.*[5] used the fluorescence probe method using pyrene in their study on the interaction between the hydrophobic groups of hydrophobically-modified polyisopropyl acrylamide (PNIPAM) and surfactants. With the objective of applying their method to our study on the interaction between long-chain alkyl groups of HM-HPMC and IM, the polarity of the pyrene environment in an aqueous HM-HPMC solution containing pyrene was measured. The fluorescence spectrum of pyrene monomer in HM-HPMC solution (Fig. 1) exhibited peak I (I_1; $\lambda = 372$ nm) and peak III (I_3; $\lambda = 383$ nm). The ratio of the fluorescence intensity, I_3/I_1, greatly increased as the concentration of HM-HPMC was increased, while almost no change was observed in HPMC solution; the value of I_3/I_1 was equal to that in the solvent (Fig. 2). Winnik *et al.* state that in water, hydrophobically-modified PNIPAM provides pyrene with a hydrophobic domain into which it is entirely solubilized, and that the range of I_3/I_1 values reflects a partition of pyrene between the polymeric microphases and water.[5] Therefore, in HM-HPMC solution, it is considered that pyrene molecules are entrapped in the hydrophobic regions formed by long-chain alkyl groups of HM-HPMC as the concentration of HM-HPMC increases.

Hydrophobic Interaction between Long-Chain Alkyl Groups of HM-HPMC and IM When IM was added to a polymer solution of pyrene, the values of I_3/I_1 in HM-HPMC solution decreased as the concentration of IM was increased, whereas those in the HPMC solution remained almost the same (Fig. 3). This result indicates that the hydrophobic regions formed by long-chain alkyl groups of HM-HPMC decrease as the concentration of IM increases. Consequently, it is considered that IM molecules are entrapped in hydrophobic regions and interact with long-chain alkyl groups of HM-HPMC. As the result of this interaction, the hydrophobic interaction between the long-chain alkyl groups themselves weakened or disappeared. It is thought that the fact that the addition of IM to a gel drug preparation using HM-HPMC caused some decrease in its viscosity (Fig. 4) can also be explained by this result. As similar phenomena, it has been reported that in the interaction between an amphiphilic polymer with long-chain alkyl groups as the hydrophobic groups and a surfactant,[8] or in the interaction between hydrophobically-modified hydroxyethylcellulose (HM-HEC) and a surfactant,[9] the polymer network structure formed by the association of long-chain alkyl groups was loosened as a result of the association of the surfactant with the long-chain alkyl groups of the polymer.

We reported previously[4] that HM-HPMC suppressed the deposition of IM crystals from supersaturated IM solution and stabilized the supersaturated solution by suppressing the growth of IM crystals, that this activity

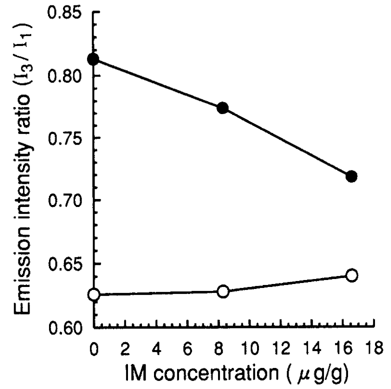

Fig. 3. Influence of IM on the Polarity of Pyrene Environment in Polymer Solutions

●, HM-HPMC; ○, HPMC. Polymer concentration is 0.3%.

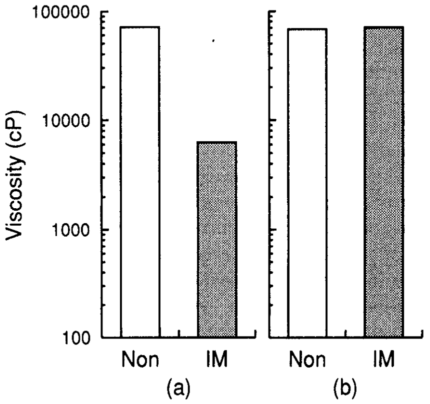

Fig. 4. Effect of IM on Viscosity of Gel Preparation with (a) HM-HPMC or (b) HPMC

Non: gel preparation without IM. IM: gel preparation with IM. IM concentration: 1.0%. Polymer concentration: HM-HPMC 1.5%, HPMC 2.0%. Solvent composition: 0.02 M Phosphate buffer solution (pH 4.0)/IPA = 5/1 (w/w).

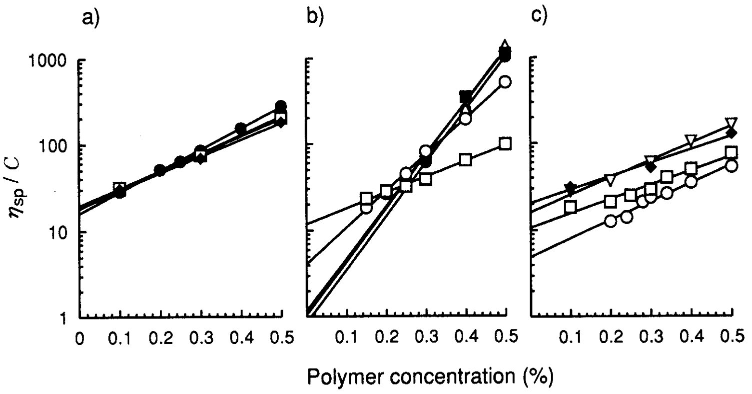

Fig. 5. Reduced Viscosity as a Function of Concentration of a) HPMC, b) HM-HPMC (Alkyl Group Content 0.6%) and c) HM-HPMC (Alkyl Group Content 1.1%)

IPA concentration: ●, 0%; △, 5%; ■, 10%; ○, 15%; □, 20%; ▽, 25%; ◆, 30%.

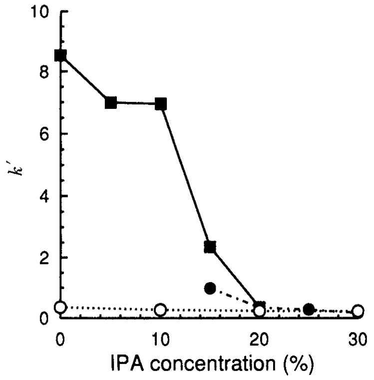

Fig. 6. Influence of IPA Concentration on Huggins Constant (k')

—■—, HM-HPMC (alkyl group content 0.6%); --●--, HM-HPMC (alkyl group content 1.1%); ···○···, HPMC.

of HM-HPMC was stronger than that of HPMC, and that the activity was stronger when the degree of substitution with long-chain alkyl groups was higher. From the results of the present study, the reason for the above phenomena is inferred to be as follows. Namely, in HM-HPMC solution, IM molecules are trapped in hydrophobic regions formed by long-chain alkyl groups causing a hydrophobic interaction between the IM molecules and the long-chain alkyl groups, and this strongly suppresses the growth of IM crystals.

Influence of IPA on Hydrophobic Interaction between Long-Chain Alkyl Groups: Effects on Viscosity of Polymer Solution HM-HPMC solution exhibits a high viscosity due to the intermolecular hydrophobic interaction through long-chain alkyl groups. Thus, in order to investigate the influence on this hydrophobic interaction, IPA was added to a polymer solution, and its effects on the viscosity of the solution were studied. Figures. 5a, b and c plot the dependency of the reduced viscosity (η_{sp}: specific viscosity/C: polymer concentration) of HM-HPMC or HPMC solutions on polymer concentration as a function of the IPA concentration. From these results, Huggins constant k' and intrinsic viscosity $[\eta]$ were calculated using Martin's equation[10]: $\ln(\eta_{sp}/C) = \ln[\eta] + k'[\eta]C$, and the results are shown in Figs. 6 and 7. Changes in the reduced viscosity of the HPMC solution as a function of concentration of HPMC are in good agreement with Martin's equation, and the change in the IPA concentration exerted almost no influence on either the intrinsic viscosity or Huggins constant (Fig. 5a). On the other hand, in the case of a solution of HM-HPMC containing 0.6% or 1.1% of long-chain alkyl groups, the IPA concentration has great influence on reduced viscosity (Figs. 5b, c). In a solution

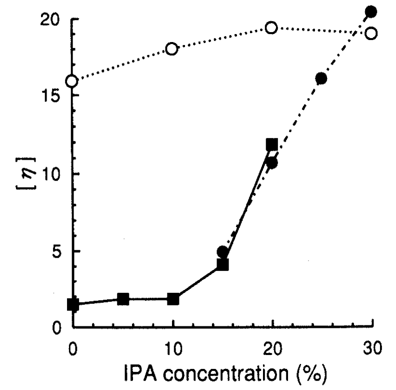

Fig. 7. Influence of IPA Concentration on Intrinsic Viscosity ([η])

—■—, HM-HPMC (alkyl group content 0.6%); --●--, HM-HPMC (alkyl group content 1.1%); ···○···, HPMC.

of HM-HPMC containing 0.6% of long-chain alkyl groups, the Huggins constant became smaller, from 8.535 (IPA: 0%) to 0.351 (IPA: 20%), and it approached that of the HPMC solution. In a solution of HM-HPMC containing 1.1% of long-chain alkyl groups, again the Huggins constant decreased from 0.979 (IPA: 0%) to 0.173 (IPA: 30%). Generally, the Huggins constant ranges from about 0.3 to about 0.7, and the larger value indicates the occurrence of a polymer association.[11] Therefore, the fact that the Huggins constant became smaller as the IPA concentration was increased (Fig. 6) indicates that IPA weakens the intermolecular hydrophobic interaction through long-chain alkyl groups of HM-HPMC. The intrinsic viscosity was very small at a low IPA concentration in HM-HPMC solution containing 0.6% of long-chain alkyl groups, and increased significantly above about 15% of IPA, whereas that in the HPMC solution did not change (Fig. 7). In the HM-HPMC solution containing 1.1% of long-chain alkyl groups as well, intrinsic viscosity increased as the IPA concentration was increased. The same phenomenon has been reported when methanol was added to an HM-HEC solution.[12] Hence, these results indicate that IPA causes an expansion of the intermolecular distance of HM-HPMC since there are fewer intermolecular hydrophobic interactions.

Influence of IPA on Hydrophobic Interaction between Long-Chain Alkyl Groups: Activity to Suppress Deposition of Crystals The present study revealed that IPA weakens intermolecular hydrophobic interaction through long-chain alkyl groups of HM-HPMC. Thus, the effects of IPA on the crystal deposition-suppressing activity of HM-HPMC were investigated. If a high-concentration IM solution is added to HM-HPMC solution or its solvent, the solution becomes supersaturated with respect to IM, but IM crystallizes gradually and the IM concentration of the solution decreases. When IM concentration is monitored with time, it is found that the IM concentration of each solution eventually reaches a certain stabilized level. However, due to the activity of HM-HPMC in suppressing the growth of IM crystals, the IM concentration in HM-HPMC solution is maintained at a higher level than that in the solvent.[4] Assuming the IM concentration in the solvent is A and that in HM-HPMC solution is B, the difference, B−A, is defined as the "excess solubility of IM" (Fig. 8) at the time when supersaturated solutions become stabilized. Thus, the values of the "excess solubility of IM" indicate the ability of HM-HPMC to suppress the growth of IM crystals. First, the IM concentration in the HM-HPMC solution containing 0.6% of long-chain alkyl groups and that in the solvent were plotted against the IPA concentration (Fig. 9a). For both the solution and solvent, the IM concentration increased as the IPA concentration was increased, and a straight regression line was obtained on the semi-logarithmic scale. The two lines meet when the IPA concentration reaches a certain value. The same relationship is observed for a solution of HM-HPMC, with a higher degree of substitution (Fig. 9b). The IPA concentration at which two lines (HM-HPMC solution and solvent) meet is higher for a solution of HM-HPMC with a higher degree of substitution (1.1%). The meeting of the two lines means that the HM-HPMC's ability to stabilize a supersaturated solution, or its crystal deposition-suppressing activity, is no longer effective. To elucidate this phenomenon, the value of "excess solubility of IM" was plotted against the

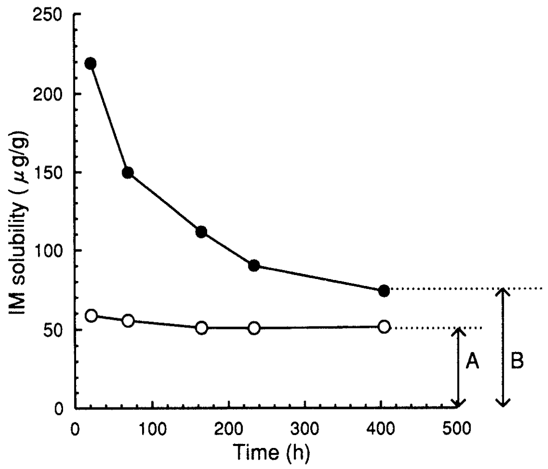

Fig. 8. Example of Measuring Excess Solubility of IM in HM-HPMC Solution

—●—, HM-HPMC; —○—, HPMC. B−A: excess solubility of IM, A: solubility of IM in solvent, B: solubility of IM in HM-HPMC solution at the time when supersaturated solution of IM became stable. Solvent composition: 18.1% isopropanol aqueous solution.

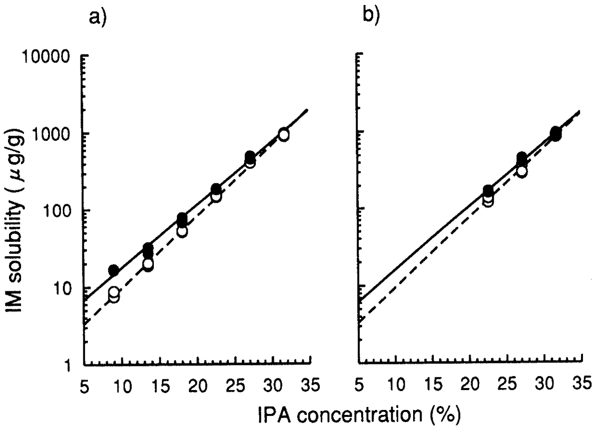

Fig. 9. Effect of a) HM-HPMC (Alkyl Group Content 0.6%) and b) HM-HPMC (Alkyl Group Content 1.1%) on IM Solubility

—●—, HM-HPMC; --○--, solvent. HM-HPMC concentration is 0.2%.

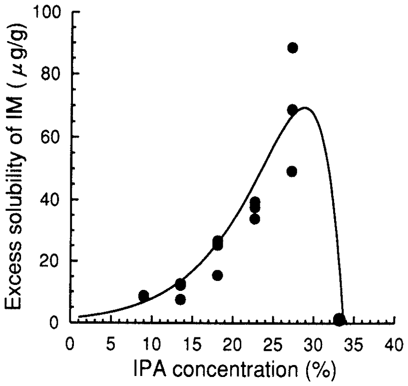

Fig. 10. Influence of IPA on the Activity of HM-HPMC to Stabilize Supersaturated Solutions of IM

HM-HPMC; alkyl group content 0.6%. HM-HPMC concentration is 0.2%. ●, experimental value; ——, calculated curve.

concentration of IPA (Fig. 10). Figure 10 shows the calculated curve of $Y=G_1(x)-G_2(x)$, the function of the differences between $Y=G_1(x)$; the regression line for the IM concentration in the HM-HPMC solution containing 0.6% of long-chain alkyl groups and $Y=G_2(x)$; and that in the solvent shown in Fig. 9a. This calculated curve is in good agreement with the measured values of "excess solubility of IM" presented with closed circles. The value of "excess solubility of IM," which means the crystal deposition-suppressing activity, increased as the IPA concentration was increased, but it reached a maximum at an IPA concentration of about 29%, and then it decreased rapidly, reaching 0 at an IPA concentration of about 34%. These results were supported by the fact that the Huggins constant and intrinsic viscosity of HM-HPMC reached the same value as HPMC at an IPA concentration of about 30%. Consequently, as an increase in the IPA concentration causes a weakening of the intermolecular hydrophobic interaction through long-chain alkyl groups of HM-HPMC and expansion of intermolecular hydrophobic regions, many IM molecules are likely entrapped in these regions as long as the intermolecular hydrophobic interaction is present; however, further addition of IPA breaks the hydrophobic interaction, resulting in the disappearance of the hydrophobic regions which suppress the deposition of IM crystals.

Conclusion

IM molecules are entrapped in hydrophobic regions formed as a result of the hydrophobic interaction of long-chain alkyl groups of HM-HPMC, and thus the growth of IM crystals is suppressed. This incorporation of IM molecules weakens the HM-HPMC intermolecular hydrophobic interaction. Although IPA also weakens the HM-HPMC intermolecular hydrophobic interaction, the amount of IM that can be entrapped increases, since the HM-HPMC intermolecular hydrophobic regions expand as long as the hydrophobic interaction is present. However, further addition of IPA causes an elimination of the hydrophobic interaction and disappearance of the hydrophobic regions. Thus, because HM-HPMC can no longer entrap IM molecules, its activity to suppress the growth of IM crystals disappears.

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