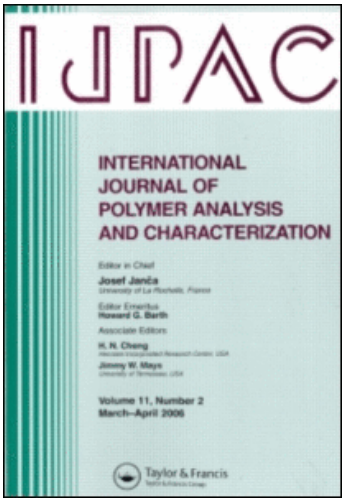

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Characterization of Hypromellose Acetate Succinate by Size Exclusion Chromatography (SEC) Using Viscotek Triple Detector

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Abstract: Size exclusion chromatography (SEC) coupled with Viscotek triple detection technique is used to characterize the molecular weight distributions of hypromellose acetate succinate polymers. The experimental results demonstrate that both conventional and universal calibrations failed due to solution phase conformation difference between HPMCAS and the calibration standard (polyethylene oxide). SEC with triple detection in TriCal mode is able to detect not only the absolute molecular weight distribution difference, but also the difference in molecular size and conformation changes of HPMCAS polymers. The decrease in Mark–Houwink–Sakurada constants with increasing molecular weight for the HPMCAS polymers indicates that both acetyl and succinoyl groups may not be evenly substituted along the HPMC polymer chains during the esterification process. This nonhomogeneity in the substitution pattern would cause HPMCAS polymer chains acting like a block co-polymer in terms of solution behavior.

Keywords: Cellulose derivative; Hypromellose acetate succinate; Pharmaceutical excipient characterization; Size exclusion chromatorgraphy

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INTRODUCTION

Hypromellose acetate succinate (also called hydroxypropyl methylcellulose acetate succinate, abbreviated as HPMCAS, CAS 71138-97-1) is a mixed ester cellulose derivative from the esterification of hypromellose (also called hydroxypropyl methylcellulose, abbreviated as HPMC, CAS 9004-65-3).[1–3] In Figure 1, two anhydrous glucose units (AGU) in HPMCAS polymer are shown, which are the basic repeat unit along the polymer chain with a variety of substitutions represented by R. Hypromellose and hypromellose acetate succinate are important polymeric excipients that are used in pharmaceutical solid tablet dosage form manufacturing. Hypromellose is used as a binder in immediate-release tablets, in film coating, and as a matrix in extended-release tablets. Hypromellose acetate succinate is used in enteric-coatings for delayed-release tablets.[4] Characterizing excipients in drug formulations is important during the drug development process in order to ensure bioavailability, performance, quality, and stability of the drug product.

Size exclusion chromatography (SEC) is often used to characterize molecular weight distributions of many cellulose derivatives.[5] SEC combined with a Viscotek triple detector is a very useful tool to study solution behaviors of cellulose derivatives.[6–8] The triple detection mode represents a combination of three detectors: a right-angle light scattering detector, a capillary viscometer,[9] and a differential refractometer. When the chromatographic process (SEC) separates the polymer molecules according to their hydrodynamic volume (the molecular size), the light-scattering detector gives the absolute molecular weight distribution and in the meantime the viscometer detector yields a response inversely proportional to molecular density. Together, triple detection presents

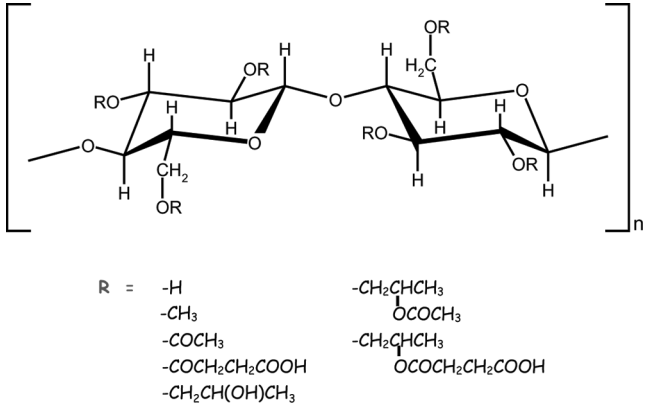

Figure 1. Structure of hypromellose acetate succinate.

a more complete picture of the polymer molecular chain structure under study: we simultaneously get information about molecular size, molecular weight, and characteristics of chain conformation, branching, and aggregation. This mode of operation is referred to as the TriCal mode (triple calculation mode in TriSEC software by Viscotek).[7] In this article, the information obtained from the TriCal mode for the characterization of HPMC and HPMCAS will be compared to results from conventional calibration and universal calibration modes.

INSTRUMENTATION AND EXPERIMENTAL CONDITIONS

SEC System

The SEC system used was an HP1100 from Hewlett Packard, which was coupled with a T60A Dual Detector and a LR40 differential refractometer from Viscotek Inc. The dual detector was placed in parallel with the refractometer via a Valco tee fitting connecting the outlet of the column to the inlets of both detectors. A pneumatic pulse dampener was also connected to the tee to further dampen the high-performance liquid chromatography (HPLC) pump flow pulsation. The waste line outlets from both detectors were connected to another Valco tee to form a single waste outlet in order to maintain consistent back pressure on both detectors.

Sample and Solvent Preparation

In all experimental work, HPLC-grade solvents were used. The HPMC and HPMCAS polymers were from Shin Etsu Chemical Ltd. (Tokyo, Japan). The following lots were used in this experiment: HPMC lot # R10595-3730, HPMCAS-LF lot # R10600-4300, HPMCAS-MF lot # R11758-3736, and HPMCAS-HF lot # R09583-3929. Polyethylene oxide standards were purchased from Polymer Standard Inc. The aqueous buffer/acetonitrile mixed solvent was filtered through a 0.2 μm Gelman Sciences 47 mm nylon membrane filter prior to use. The polymer solutions were prepared by dissolving a weighed amount (20 mg) of polymer in a vial. The dry polymer powder was stirred inside the vial and 10 mL of the eluent was added to dissolve the samples. The solution was stirred for 24 h. All polymer solutions were centrifuged (Jouan C4-12 centrifuge at 3200 rpm for 10 min) prior to analysis. The standard solution (PEO-polyethylene oxide with M_p in the range of 20 to 25 K) for normalization was prepared by dissolving a weighed amount (15 mg) of polymer into a vial. Addition of 3 mL of the eluent allows the sample to

dissolve (by shaking occasionally for 24 h) at ambient temperature. The standard solutions were all filtered through a 0.02 μm Whatman Anotop 25 mm syringe filter prior to use.

Experimental Conditions

The column used was a TosoHaas TSK-GEL GMPW$_{XL}$ column (300 × 7.8 mm) with a TSK-GEL guard PW$_{XL}$ column (40 × 6.0 mm). Both the column and the detectors were at ambient temperature. The mobile phase was 55% 50 mM NaH_2PO_4, 0.1 M $NaNO_3$, pH = 7.0, and 45% acetonitrile and was degassed with the Hewlett Packard in-line degasser. The flow rate was 0.5 mL/min. The ratio of the split flow rates to the dual detector and the refractometer was 55%:45%. The injection volume was 100 μL. The experimental data were processed by the Viscotek TriSEC software (Version 3.0).

Calculation Parameters and Calibrations

The viscosity of the mobile phase and its temperature coefficient were estimated from the corresponding values of the buffer and the acetonitrile at 20°C. These values were needed for the inlet pressure calculation. The values of dn/dc for the polyethylene oxide standards, HPMC, and HPMCAS polymers were taken from our SEC/multiple angle laser light scattering (MALLS) (Wyatt Technology Inc.) measurement in the same solvent system. The mass constant for the refractometer and the light scattering constant for the right-angle light scattering detector were calibrated by using a polyethylene oxide standard (PEOX70K) with a known concentration and measured molecular weight (M_w) in our laboratory by SEC/MALLS. As the intrinsic viscosity of the polyethylene oxide standard (PEOX70K in our solvent system of 55% 50 mM NaH_2PO_4, 0.1 M $NaNO_3$, pH = 7.0, and 45% acetonitrile) is not known, the calibration constant for the viscometer was taken from the calibration done in tetrahydrofurane (THF) using polystyrene standards.

RESULTS AND DISCUSSION

The experimental data were analyzed by the three analysis modes offered by the TriSEC software and the results were compared. The conventional calibration mode is the traditional polymer analysis method, utilizing only the refractometer signal.[10] It relies on the information of the molecular weights and retention volumes of a set of calibration standards

to construct a calibration curve. This calibration curve is then used for the determination of molecular weight distribution of unknown polymers relative to that of the standards by comparing the retention volumes. The overlay of the 12-point calibration curve with the chromatograms for HPMC and HPMCAS polymers are shown in Figure 2. The analysis results of HPMC and HPMCAS polymers based on the conventional calibration are shown in Table I in the column labeled ConvCal results.

Universal calibration utilizes two detection signals from a differential viscometer and a differential refractometer.[10] The combination of a differential viscometer and a differential refractometer enables the on-line determination of intrinsic viscosity, [η]. Combining this information with the known molecular weights of the calibration standards, Log [η]M can be plotted against the retention volume. For many polymer chains in solution, it is found that such a plot is usually independent of polymer structure and is the basis for a "universally" applicable calibration. Furthermore, by analyzing the molecular weight and the intrinsic viscosity distributions together, the radius of gyration can also be determined. Finally, the ratio of the intrinsic viscosity to molecular weight of branched and unbranched samples can lead to valuable information on branching, conformation, and structure. We applied the universal calibration by constructing a calibration curve based on 12

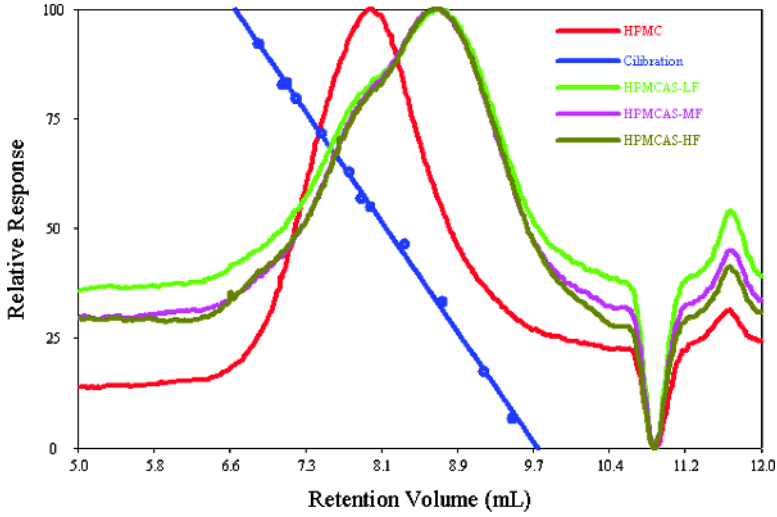

Figure 2. Conventional calibration curve using polyethylene oxide standards and overlay of RI signals for HPMC and HPMCAS polymers.

Table I. Summary of analytical results for molecular weight distributions and related properties

Sample	ConvCal results	UniCal results				TriCal results				
	M_w (Da)	M_w (Da)	$\langle R_g \rangle_w$ (nm)	$[\eta]_w$ (dL/g)	a	M_w (Da)	$\langle R_g \rangle_w$ (nm)	$\langle R_h \rangle_w$ (nm)	$[\eta]_w$ (dL/g)	a
HPMC	98,100	74,500	16.70	2.489	0.647	88,100	18.54	14.24	2.493	1.016
HPMCAS-LF	83,400	160,200	13.03	0.859	0.663	162,300	13.49	10.36	0.870	0.689
HPMCAS-MF	74,700	128,800	12.47	0.871	0.690	167,500	13.50	10.36	0.882	0.688
HPMCAS-HF	72,500	141,400	12.52	0.788	0.597	360,900	15.95	12.25	0.792	0.667

For definition of the terms, see the main text. The subscript w designates weight-averaged values, and a is the Mark–Houwink–Sakurada constant, the exponent in the Mark–Houwink–Sakurada equation.

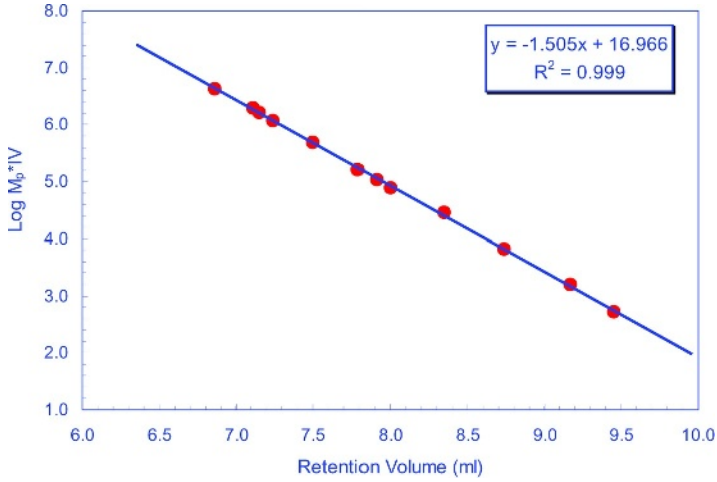

Figure 3. Universal calibration curve for polyethylene oxide standards.

polyethylene oxide standards (shown in Figure 3). The analysis results of HPMC and HPMCAS polymers based on the universal calibration are shown in Table I in the columns labeled UniCal results.

In the TriCal mode, the molecular weight distribution is calculated from the right-angle light scattering signal combined with the concentration information provided by the refractometer. The intrinsic viscosity distribution is calculated from the viscometer signal combined with the concentration information provided by the refractometer. With SEC/light scattering coupled with an on-line viscometer, the molecular size at every elution volume can be calculated, and thus meaningful information of polymer branching and conformational differences can be obtained.

The basic static light scattering equation is shown below:[11]

$$\frac{K^* c}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c \tag{1}$$

where K^* is a constant, c is the concentration of the polymer solution, $R(\theta)$ is the scattered light intensity from the sample, M_w is the weight-average molecular weight, and A_2 is the second virial coefficient, which is a thermodynamic term indicative of the solvent-solute interaction. K^* is proportional to $(dn/dc)^2$, where, dn/dc is the differential refractive index increment of the solvent-solute solution with respect to a change in solute concentration. $P(\theta)$ is the form factor or "scattering function," which relates the scattering intensity to the scattering angle. In a highly diluted solution, as is the case for SEC separation, the second term on the

right-hand side of Equation (1) can be neglected. Therefore, the amount of light scattered is directly proportional to the product of the weight-average molar mass and the concentration of the polymer.

The coupling of a viscometer and a light-scattering detector makes it possible to calculate the molecular size at every elution volume in addition to the molecular weight value. For the hydrodynamic radius (R_h) of the molecule, we have:

$$R_h = \left[\frac{3}{4P(\theta)} \times \frac{[\eta]M}{0.025}\right]^{1/3} \tag{2}$$

where $P(\theta)$ is the form factor or "scattering function"; $[\eta]$ is the intrinsic viscosity that is a fundamental parameter of the polymer solution; and M is the molecular weight.

For linear flexible chain polymers, the root-mean-square radius, or the radius of gyration (R_g), can be calculated using the Flory–Fox and Ptitsyn–Eisner equations:[12]

$$R_g = \frac{1}{\sqrt{6}} \times ([\eta]MF)^{1/3} \tag{3}$$

where:

$$F = 2.86 \times 10^{21} \times (1 - 2.63\varepsilon + 2.86\varepsilon^2)$$

$$\varepsilon = \frac{2a - 1}{3}$$

where a is the exponent in the Mark–Houwink–Sakurada equation and is often cited as the Mark–Houwink–Sakurada constant (or Mark–Houwink constant) in the literature:[10]

$$[\eta] = K \times M^a$$

where K is another constant in the Mark–Houwink–Sakurada equation.

The experimental results from the TriCal mode are shown in Figures 4 and 5 for HPMC and HPMCAS (MF grade) polymers, respectively. For HPMC, the peak is relatively symmetrical, whereas for the HPMCAS polymers, the peak shape is very asymmetrical. The apex of the light-scattering peak is skewed towards the high molecular weight range. In contrast, the peak from the refractive index signal has the opposite shape of the peak from the light-scattering signal. This is due to the fact that the differential refractive index signal is directly proportional only to concentration, while the light-scattering signal is proportional to concentration times the molecular weight. Furthermore, the differential pressure signal from the viscometer has the most sensitivity in the middle molecular weight range. The different sensitivities of the three detectors in respect to molecular weight distribution from low

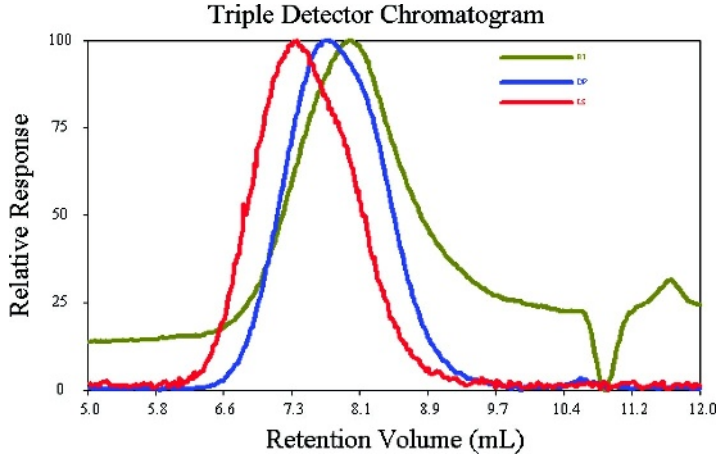

Figure 4. Chromatograms for HPMC polymer using triple detection.

to high molecular weights indicate that this triple detection technique is a powerful method for characterizing polymers such as HPMCAS that have a wide range of molecular weight distributions. The calculated results from the triple detection mode are summarized in Table I in the columns labeled TriCal results. The comparison of the differential molecular weight distributions for these polymers is shown in Figure 6. In this solvent system, HPMCAS-LF and HPMCAS-MF

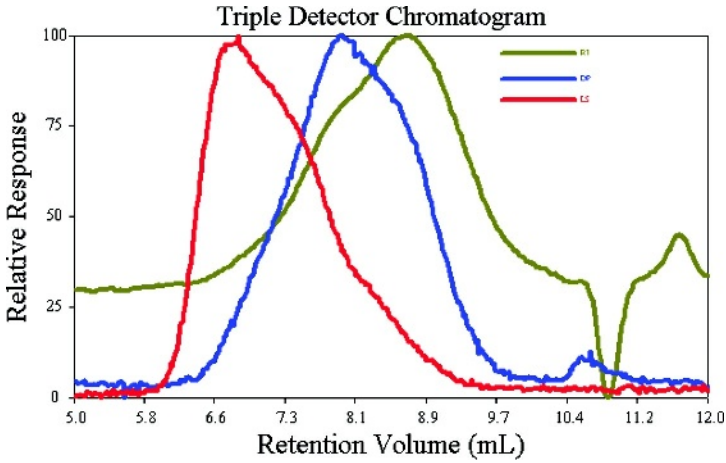

Figure 5. Chromatograms for HPMCAS polymer (MF grade) using triple detection.

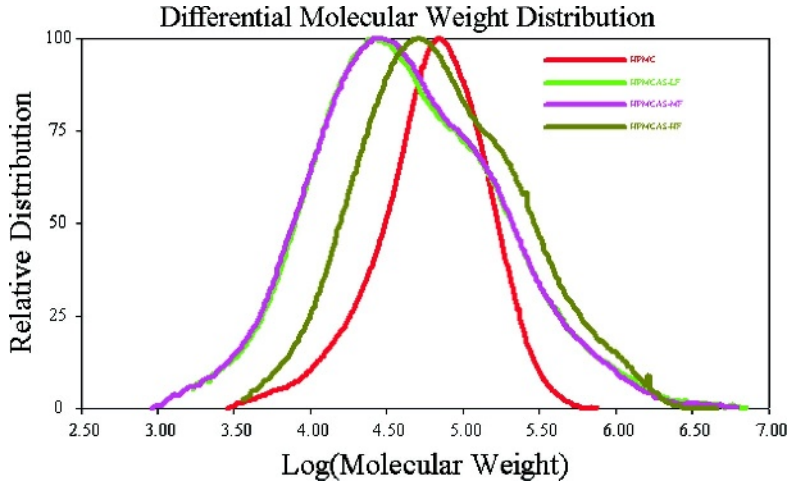

Figure 6. Overlay of molecular weight distributions of HPMC and HPMCAS (LF, MF, and HF grades) polymers.

have very similar molecular weight distributions, whereas the molecular weight distribution for HPMCAS-HF shifts significantly toward the high molecular weight range.

As shown in Table I, the trend of M_w change from the conventional calibration (decrease of M_w from LF $\rightarrow$ MF $\rightarrow$ HF) is off in the wrong direction of M_w change (increase of M_w from LF $\rightarrow$ MF $\rightarrow$ HF) from triple calculation based on the light-scattering method. Conventional calibration fails to truly characterize the molecular weight distribution of these polymers because it is invalid to assume that the linear relationship of molecular weights (in log scale) versus hydrodynamic volumes of the standards can be applied to the HPMCAS polymers.

The universal calibration method gives a better prediction than that from the conventional calibration method (see Table I) as it incorporates the density information included in the intrinsic viscosity of the polyethylene oxide standard solutions. However, it still fails to truly characterize the molecular weight distributions of HPMCAS (especially for the MF and HF grades) polymers due to the fundamental conformation difference in solution between the polyethylene oxide and the HPMCAS polymers, as discussed below.

Triple detection has the ability to detect not only the absolute molecular weight distribution difference, but also the difference in molecular size and conformation change. This is shown in Figure 7 with the comparison of Mark–Houwink–Sakurada plots. The Mark–Houwink–Sakurada constant is a polymer conformation parameter. By definition,

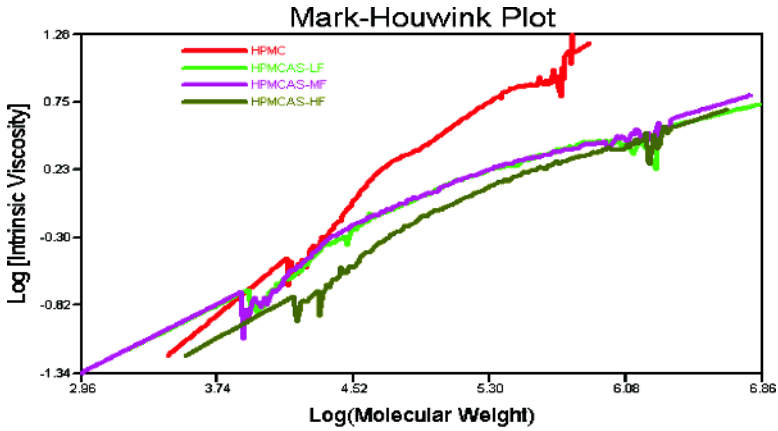

Figure 7. Overlay of Mark–Houwink–Sakurada plots for HPMC and HPMCAS (LF, MF, and HF grades) polymers.

the Mark–Houwink–Sakurada constant should be constant if the polymer chains in solution have a consistent conformation across the whole molecular weight range. Such is the case for the polyethylene oxide standards, as the Mark–Houwink–Sakurada plot for the polyethylene oxide standards in the comparable molecular weight range fits a linear line (Log [Intrinsic Viscosity] = 0.7532 × Log [Molecular Weight] − 3.6374; $R2 = 0.995$; the Mark–Houwink–Sakurada constant = 0.753). The Mark–Houwink–Sakurada constant is smaller for polymer chains with a more compact structure in a relatively "bad" solvent. Looking at the Mark–Houwink–Sakurada constant (*a* values) in Table I, it is evident that the HPMC polymer chains have a more extended conformation than the HPMCAS polymers in this solvent system (see the lower molecular weight and higher radius of gyration for HPMC, compared to those obtained for HPMCAS). An even more important observation is the fact that *the Mark–Houwink–Sakurada constants for HPMCAS polymers in the solvent are not constant across the whole molecular weight range and decrease significantly from low to high molecular weight, indicating that there is a conformation change for these polymers across the molecular weight range observed here*.

Hypromellose acetate succinate is a mixture of acetic acid and monosuccinic acid esters of hypromellose. The extent of substitutions (acetyl and succinoyl groups) varies from grades L to M to H, with L grade low in acetyl (5.0–9.0% w/w) but high in succinoyl (14.0–18.0% w/w) and H grade high in acetyl (10.0–14.0% w/w) but low in succinoyl (4.0–8.0% w/w).[2] High acetyl content will make the HPMCAS polymer

chain more hydrophobic, while high succinoyl content will make the chain more hydrophilic. This is because succinoyl group can be ionized but acetyl group cannot. Therefore, L grade HPMCAS polymer starts to dissolve in an aqueous buffer at a pH of 5.5, while H grade dissolves only in an aqueous buffer of pH 6.5 or higher. If both acetyl and succinoyl groups are evenly substituted along the polymer chain of HPMC, one would expect that the average molecular weight of the resulting HPMCAS polymer is the sum of the average molecular weight of HPMC polymer used plus the average weight percentages of the acetyl and succinoyl groups on a molar substitution equivalence basis.

Previously we have developed a procedure to hydrolyze both acetyl and succinoyl groups of HPMCAS polymers so the contents of acetyl and succinoyl groups can be accurately analyzed by a HPLC method.[13] When both acetyl and succinoyl groups are completely hydrolyzed, HPMCAS polymer reverses back to the starting HPMC prior to the esterification. We have analyzed molecular weight distributions of the hydrolyzed HPMCAS polymers. The M_w results are 20150, 21170, and 20130 g/mol, respectively, for the LF, MF, and HF lots used in this study. These values are 8 to 18 times smaller than the M_w values (see Table I) measured for the respective HPMCAS polymers. As shown in Figure 1, for one anhydrous glucose unit in the cellulose backbone, there are only three hydroxyl positions that can be substituted, so the total degree of substitution can only be as high as three. Typical degrees of substitution in HPMC are 1.4 to 1.9 for methoxyl and 0.1 to 0.2 for hydroxypropyl.[14] Therefore, the total degree of substitution of acetyl and succinoyl groups in a HPMCAS polymer could not exceed 1.6 (there is a hydroxyl position in hydroxypropyl that could be substituted). One assumes that all these available hydroxyl positions remaining in the anhydrous glucose unit (molar weight of 162 g/mol without consideration of the methoxyl and hydroxypropyl groups) of the HPMC polymer chains are substituted by the heavier succinoyl group (molar weight of 108.01 g/mol). The molecular weight of the resulting HPMCAS polymer from esterification of an HPMC polymer could only be doubled at most, should the acetyl and succinoyl groups be evenly substituted along the polymer chain. The fact that the M_w values of HPMCAS polymers are at least several multiples higher than the values of corresponding hydrolyzed HPMCAS polymers indicates that both acetyl and succinoyl groups may not be evenly substituted along the HPMC polymer chains during the esterification process. This nonhomogeneity in the substitution pattern would cause the polymer chains to act like a block co-polymer in terms of solution behavior. While a "homogeneous" polymer chain such as HPMC in a good solvent would have an extended random coil conformation, an unevenly substituted

"heterogeneous" HPMCAS polymer chain would behave like block co-polymer. A particular solvent may be good for dissolving one block, but it may not be good for dissolving another block. Thus, the "heterogeneous" HPMCAS polymer chains in solution would form a "shell-core" type aggregation and have the characteristic conformation change from low to high molecular weights shown in Figure 6. In the low molecular weight range, the HPMCAS polymer chains are short and have less degree of "heterogeneity." As a result, the polymer chains are less entangled, have a relatively larger hydrodynamic volume, and have a lower density in solution (larger hydrodynamic volume for a given molecular weight). As the molecular weight increases, the polymer chains will become longer and have progressively higher degree of "heterogeneity." As a consequence, the polymer chains become increasingly entangled, have a relatively smaller hydrodynamic volume, and have a higher density in solution (smaller hydrodynamic volume for a given molecular weight).

CONCLUSIONS

The triple detection technique is able to detect not only the absolute molecular weight distribution difference, but also the difference in molecular size and conformation changes of HPMCAS polymers. The decrease in Mark–Houwink–Sakurada constants with increasing molecular weight for the HPMCAS polymers indicates that both acetyl and succinoyl groups may not be evenly substituted along the HPMC polymer chains during the esterification process. This nonhomogeneity in the substitution pattern would cause the polymer chains to act like a block co-polymer in terms of solution behavior.

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