

Light-Scattering and Size-Exclusion Chromatographic Characterization of Hydroxyethyl Cellulose Acetate

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SYNOPSIS

A recently developed analytical method of combining off-line laser light scattering (LLS) and size exclusion chromatography (SEC) was used to investigate a set of moderately distributed hydroxyethyl cellulose acetate (HECA) samples in tetrahydrofuran (THF) at room temperature. Our results have shown that this new LLS + SEC method is suitable for the characterization of molecular weight distribution of HECA. By using this method, we have simultaneously determined two calibrations of $V \text{ (cm}^3\text{)} = 45.3 - 1.89 \log(M)$ and $D \text{ (cm}^2\text{/s)} = 2.45 \times 10^{-4} M^{-0.80}$, where M is the molecular weight of HECA; V , the elution volume in SEC; and D , the translational diffusion coefficient in dynamic LLS. In addition, our results have also indicated that the chain conformation of HECA in THF at room temperature is a slightly extended linear coil. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Cellulose constitutes a ubiquitous and renewable natural resource that has served human needs for thousands of years. Since the discovery of guncotton by Schonbein in 1845, it has been clear that various cellulose derivatives are also of great industrial importance. Nowadays, the applications of various esters and ethers are in many areas that are far from those traditionally associated with cellulose itself. They are widely used in plastics, fibers, microporous membranes, food additives, antiredeposition agents, and oil-well drilling applications.¹⁻³

Cellulose and its derivatives can form liquid crystals in appropriate solvents.⁴ Thermotropic cellulose derivatives have been prepared by introducing some appropriate flexible substituents. The spinning of these mesophases is of particular interest as it gives ready access to fibers with much improved strength, modulus, and lower moisture sensitivity.⁵ In this aspect, the thermotropic behaviors of hydroxyethyl cellulose acetate (HECA) have been studied in de-

tail.⁶ However, there is only a limited correlation between its thermotropic behaviors and its molecular weight and molecular weight distribution.⁷

Similar to other polymer material, the performance of the cellulose and its derivatives greatly depends on their molecular weight and molecular weight distribution. A conventional size exclusion chromatography (SEC) is a convenient and established method. In general, the molecular weight is calibrated with the standard polystyrene with the narrow distributed molecular weight. However, cellulose and its derivatives are rigid or semirigid polymers, they have different conformation with polystyrene, and a big error is normally introduced when SEC is calibrated with polystyrene standards for the characterization of cellulose and its derivatives. There is a constant search for a proper method to calibrate SEC so that it can be routinely used for the characterization of the true molecular weight and molecular weight distribution of cellulose and its derivatives.

Our objective in this study is to show that a newly developed method of combining off-line static and dynamic light scattering (SLS and DLS) and SEC can be used to investigate HECA in tetrahydrofuran (THF) at room temperature.

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BASIC PRINCIPLES

Recently, a combination of off-line laser light scattering (LLS) and SEC was successfully utilized to characterize the molecular weight distribution of gelatin.⁸ For the convenience of discussion, we outline the basic principles of this novel analytical method.

In SEC and LLS, an elution volume distribution $C(V)$ and a translational diffusion coefficient distribution $G(D)$ can be respectively measured. We can convert $C(V)$ or $G(D)$ into the molecular weight distribution if we have the calibrations of

$$V = A + B \cdot \log(M) \quad (1)$$

or

$$D = k_D \cdot M^{-\alpha_D} \quad \text{i.e.,}$$

$$\log(D) = \log(k_D) - \alpha_D \log(M) \quad (2)$$

where A , B , k_D and α_D are the calibration constants. It should be noted that in eqs. (1) and (2) we have assumed that both V and $\log(D)$ are linear functions of $\log(M)$, that is, the first-order approximation, because this will simplify, but not affect, our following discussions. However, if the sample has a special molecular weight distribution or V and $\log(D)$ cannot be linearly scaled by $\log(M)$, eqs. (1) and (2) have to be properly modified. In that case, additional information about the molecular weight distribution and the dependence of V and $\log(D)$ on $\log(M)$ is required. The main task of the calibration is to find A and B in SEC or k_D and α_D in LLS.

A combination of eqs. (1) and (2) leads to

$$V = A + B \cdot \log(D) \quad (3)$$

where $A = A + B \cdot \log(k_D)/\alpha_D$ and $B = -B/\alpha_D$. Further, by taking the square of both sides of eq. (3), we obtain

$$V^2 = A^2 + 2 \cdot A \cdot B \cdot \log(D) + B^2 \cdot \log^2(D). \quad (4)$$

After integrating both sides of eqs. (3) and (4), we have

$$\langle V \rangle = A + B \cdot \langle \log(D) \rangle \quad (5)$$

and

$$\langle V^2 \rangle = A^2 + 2 \cdot A \cdot B \cdot \langle \log(D) \rangle + B^2 \cdot \langle \log^2(D) \rangle \quad (6)$$

where

$$\langle V \rangle = \frac{\int_0^\infty VC(V) dV}{\int_0^\infty C(V) dV} \quad (7)$$

$$\langle V^2 \rangle = \frac{\int_0^\infty V^2 C(V) dV}{\int_0^\infty C(V) dV} \quad (8)$$

$$\langle \log(D) \rangle = \frac{\int_0^\infty \log(D)C(V) dV}{\int_0^\infty C(V) dV} \quad (9)$$

and

$$\langle \log^2(D) \rangle = \frac{\int_0^\infty \log^2(D)C(V) dV}{\int_0^\infty C(V) dV} \quad (10)$$

On the one hand, because $C(V)$ is a weight (or concentration) distribution of the elution volume, we have⁹

$$\begin{aligned} \int_0^\infty C(V) dV &\propto \int_0^\infty F_w(M) dM \\ &\propto \int_0^\infty F_w(M)M d(\log(M)) \quad (11) \end{aligned}$$

where $F_w(M)$ is a differential weight distribution. According to eq. (1), dV is proportional to $d(\log(M))$. Then, it follows eq. (11) that in the space of $\log(M)$,

$$C(V) \propto F_w(M)M. \quad (12)$$

On the other hand, because $G(D)$ is an intensity distribution of the translational diffusion coefficient, we have¹⁰

$$\int_0^\infty G(D) dD \propto \int_0^\infty F_w(M)M dM \quad (13)$$

or

$$\begin{aligned} \int_0^\infty G(D)D d(\log(D)) \\ \propto \int_0^\infty F_w(M)M^2 d(\log(M)). \quad (14) \end{aligned}$$

According to eq. (2), $d(\log(D)) \propto d(\log(M))$. Thus,

$$G(D)D \propto F_w(M)M^2. \quad (15)$$

Using eqs. (2), (12), and (15), we rewrite eqs. (9) and (10) as

$$\langle \log(D) \rangle = \frac{\int_0^\infty \log(D)G(D)D^{1/\alpha_D} dD}{\int_0^\infty G(D)D^{1/\alpha_D} dD} \quad (16)$$

and

$$\langle \log^2(D) \rangle = \frac{\int_0^\infty \log^2(D)G(D)D^{1/\alpha_D} dD}{\int_0^\infty G(D)D^{1/\alpha_D} dD}. \quad (17)$$

We are able to calculate **A** and **B** in eqs. (5) and (6) if we have $\langle V \rangle$, $\langle V^2 \rangle$, $\langle \log(D) \rangle$, and $\langle \log^2(D) \rangle$. Furthermore, a combination of eqs. (2), (3), (11), (13), and the definition of the weight average molecular weight,

$$M_w = \frac{\int_0^\infty MF_w(M) dM}{\int_0^\infty F_w(M) dM} \quad (18)$$

leads to

$$\begin{aligned} M_{w,SEC} &= \frac{\int_0^\infty MC(V) dV}{\int_0^\infty C(V) dV} \\ &= \frac{k_B^{1/\alpha_D} \int_0^\infty 10^{(A-V)/(\alpha_D B)} C(V) dV}{\int_0^\infty C(V) dV} \end{aligned} \quad (19)$$

and

$$\begin{aligned} M_{w,DLS} &= \frac{\int_0^\infty G(D) dD}{\int_0^\infty G(D)/M dD} \\ &= \frac{k_B^{1/\alpha_D} \int_0^\infty G(D) dD}{\int_0^\infty G(D)D^{1/\alpha_D} dD}. \end{aligned} \quad (20)$$

For a given sample, $M_{w,SEC}$ should be equal to $M_{w,DLS}$. Therefore, on the basis of eqs. (19) and (20), we have

$$\frac{\int_0^\infty 10^{(A-V)/(\alpha_D B)} C(V) dV}{\int_0^\infty C(V) dV} = \frac{\int_0^\infty G(D) dD}{\int_0^\infty G(D)D^{1/\alpha_D} dD}. \quad (21)$$

There is only one unknown parameter α_D in eq. (21). For a chosen α_D , we can *first* calculate $\langle \log(D) \rangle$ and $\langle \log^2(D) \rangle$ on the basis of eqs. (9) and (10); *then* solve **A** and **B** by using eqs. (5) and (6); and *finally* calculate and compare the left and right sides of eq. (21). An iteration of α_D will enable us to find a proper α_D value to minimize the difference between the left and right sides of eq. (21). With this α_D , we can calculate k_D from either eq. (19) or (20) by using the measured M_w in static LLS and $C(V)$ in SEC or $G(D)$ in DLS. Finally, the constants **A** and **B** in eq. (1) can be calculated from **A**, **B**, k_D , and α_D . In this way, we are able to calibrate not only V with M in SEC but also D with M in DLS in one single process with only one polymer sample.

EXPERIMENTAL

Sample Preparation

HECA was prepared through the esterification of a commercial hydroxyethyl cellulose with acetic anhydride, where the degree of ether substitution of hydroxyethyl cellulose was 1.5–1.8, and the degree of esterification of HECA was 2.8. Three HECA samples with different molar masses denoted hereafter as HECA-1, HECA-2, and HECA-3 were obtained by using a conventional fractionation/precipitation method, wherein acetone and petroleum ether were respectively used as solvent and precipitant. The details of the sample preparation can be found elsewhere.⁷

LLS

THF (analytical grade) was used as solvent in this LLS study. The HECA solutions of five different concentrations ranging from 1 to 5 mg/mL were prepared by dissolving a certain amount of HECA in THF at room temperature for at least 1 day. After complete dissolution, all solutions were filtered by using a 0.22- μ m Millipore filter to remove the dust.

The light intensities scattered from the clarified solutions at different scattering angles (15° – 150°) were measured with a commercial LLS spectrometer (ALV/SP-150 equipped with ALV-5000 multi-tau digital correlator). An argon-ion laser (Coherent INNOVA 90, operated at $\lambda_0 = 488$ nm with an output power 400 mW) was used as the light source. The incident beam was vertically polarized with respect to the scattering plane. All measurements were done at $T = 25.0 \pm 0.1^\circ\text{C}$. The details of LLS principles and instrumentation can be found elsewhere.¹¹

SEC

A commercial SEC (Waters, ALC/244) was used with a combination of μ -Styragel columns (designated as 10^2 , 10^3 , and 10^4 Å and connected in series). THF was used as an eluting solvent. Different flow rates were used in this study. The flow rate for HECA-1 was 1.2 mL/min; and for both HECA-2 and HECA-3, 1.5 mL/min. All SEC measurements were done at $T = 25^\circ\text{C}$. A differential refractometer (Waters, Model 410) was used as the detector. The resulted elution volume distributions $C(V)$ were digitalized into a PC.

RESULTS AND DISCUSSION

Figure 1 shows a plot of the refractive index increment (Δn) versus concentration (C) for HECA in THF at $T = 25^\circ\text{C}$ and $\lambda_0 = 488$ nm, where Δn was measured by using a novel differential refractometer recently designed and constructed in our labora-

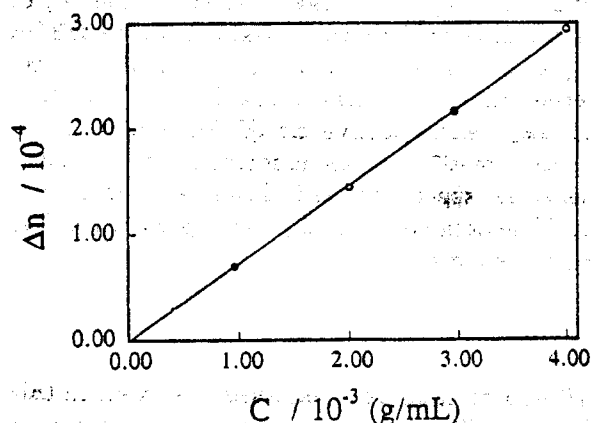


Figure 1 Plot of the refractive index increment (Δn) versus concentration (C) for hydroxyethyl cellulose acetate in tetrahydrofuran at $T = 25^\circ\text{C}$ and $\lambda_0 = 488$ nm, where the slope is the specific refractive index increment ($dn/dC = 7.34 \times 10^{-2} \text{ cm}^3/\text{g}$).

tory.¹² The relative error associated with the specific refractive index increment (dn/dC) of $7.34 \times 10^{-2} \text{ cm}^3/\text{g}$ calculated from Figure 1 is less than $\pm 1\%$. The accuracy of dn/dC is vital for the precise characterization of the M_w in static LLS. On the basis of the light scattering theory,¹¹ it is known that for a dilute macromolecular solution at concentration C (g/mL) and scattering angle θ , the angular dependence of the excess absolute time-averaged scattered intensity, known as the excess Rayleigh ratio [$R_{vv}(\theta)$], can be approximately expressed as

$$\frac{KC}{R_{vv}(\theta)} \cong \frac{1}{M_w} \cdot \left(1 + \frac{1}{3} \langle R_g^2 \rangle q^2\right) + 2A_2C \quad (22)$$

where $K = 4\pi^2 n^2 (\partial n / \partial C)^2 / (N_A \lambda_0^4)$ with N_A , n , and λ_0 being Avogadro's number, the solvent refractive index, and the wavelength of light *in vacuo*, respectively, and $q = (4\pi n / \lambda_0) \sin(\theta/2)$. By measuring $R_{vv}(\theta)$ at different C and θ , we are able to determine the M_w , the z-average radius of gyration ($\langle R_g^2 \rangle_z^{1/2}$ or simply as R_g), and the second virial coefficient (A_2) from the Zimm plot that incorporates θ and C extrapolations on a single grid. The static LLS results are summarized in Table I. The positive A_2 values show that THF at $T = 25^\circ\text{C}$ is a good solvent for HECA. The radii of gyration of 20–28 nm for polymers with M_w of 45,000–66,000 g/mol confirm that the polymer chain of HECA in THF forms an extended random coil.

Figure 2 shows typical measured SEC elution curves of HECA-1, HECA-2, and HECA-3, respectively, where $C(V)$ is the normalized differential weight distribution of the elution volume. All SEC experimental conditions were stated in the previous section. It can be seen that all HECA samples are moderately distributed. The values of the average elution volume ($\langle V \rangle$) are listed in Table I. A comparison of $\langle V \rangle$ with the corresponding M_w in Table I indicates that the SEC columns used in this study were fairly effective for the separation of HECA in terms of molecular weight.

Figure 3 shows typical translational diffusion coefficient distributions ($G(D)$) of HECA-1, HECA-2, and HECA-3 at $\theta \rightarrow 0$ and $C \rightarrow 0$, respectively, that were calculated from a Laplace inversion of the time correlation functions measured in DLS. The CONTIN¹³ program equipped with the ALV-5000 time correlator was used in the calculation. The average values of the translational diffusion coefficient ($\langle D \rangle$) are also listed in Table I. $\langle D \rangle$ can be further related to the hydrodynamic radius (R_h) by using the Stokes–Einstein equation: $R_h = k_B T / (6\pi\eta \langle D \rangle)$ with k_B , T , and η being the Boltzmann constant, the

Table I LLS and SEC Results of Hydroxyethyl Cellulose Acetates (HECA) in Tetrahydrofuran at $T = 25^\circ\text{C}$

Sample	$10^{-4} M_w$ (mol^{-1}g)	$\langle R_g^2 \rangle^{1/2}$ (nm)	$10^3 A_2$ ($\text{g}^{-2} \text{mol mL}$)	$\langle V \rangle$ (cm^3)	$10^7 \langle D \rangle$ ($\text{cm}^2/\text{s}^{-1}$)	R_g/R_h
HECA-1	4.54	20	2.9	25.4	4.00	1.74
HECA-2	6.15	24	1.8	24.5	3.40	1.78
HECA-3	6.56	28	1.1	24.3	3.16	1.69

Note: The relative errors of the listed parameters are: M_w , $\pm 5\%$; $\langle R_g^2 \rangle^{1/2}$, $\pm 10\%$; A_2 , $\pm 20\%$; $\langle V \rangle$, $\pm 1\%$; and $\langle D \rangle$, $\pm 1\%$.

absolute temperature (K), and solvent viscosity, respectively. The ratio of R_g/R_h can be used to indicate the polymer chain conformation.¹⁴ For a flexible coil in good solvent, $R_g/R_h \sim 1.5$ and for a rodlike polymer chain, $R_g/R_h \geq 2$. The R_g/R_h values in Table I show that the polymer chain of HECA in THF at room temperature behaves like a slightly extended random coil.

The distributions in Figure 3, especially $\langle D \rangle$, are quite stable even though some uncertainties existed at the lower and higher ends of the distributions. It should be stated that it is the advantage of using this LLS + SEC method wherein the average values instead of the individual fraction in $C(V)$ and $G(D)$ are used. With each pair of $C(V)$ and $G(D)$ obtained from a given HECA sample, we are able to calculate the calibration constants of A and B in eq. (1) and k_D and α_D in eq. (2) by adopting the analytical procedure described in the Basic Principles section. The calculated values of A , B , k_D , and α_D are summarized in Table II. In comparison with the α_D value (~ 0.57) listed in the literature for a similar cellulose system (cellulose diacetate in THF),¹⁵ the obtained α_D values of the present cellulose system are slightly higher. This higher α_D value further indicates that

HECA in THF at room temperature behaves like a slightly extended coil. With these calibration constants, we are ready to convert both $C(V)$ and $G(D)$ into molecular weight distributions.

Figures 4 and 5, respectively, show the differential weight distributions ($F_w(M)$) calculated from the elution volume distributions ($C(V)$) in Figure 2 and the translational diffusion coefficient distributions ($G(D)$) in Figure 3 for HECA-1, HECA-2, and HECA-3. According to the definitions of the M_w in eq. (18) and the number-average molecular weight (M_n) in the following,

$$M_n = \frac{\int_0^\infty MF_n(M) dM}{\int_0^\infty F_n(M) dM} = \frac{\int_0^\infty MF_w(M)/M dM}{\int_0^\infty F_w(M)/M dM} \quad (23)$$

we can calculate the values of M_w and M_n for each HECA from $F_w(M)$ in Figures 4 and 5. All results are also summarized in Table II. The ratios of M_w/M_n in Table II show that all HECA samples used

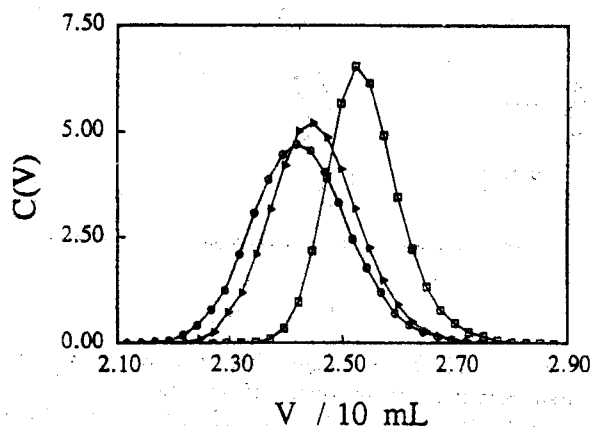


Figure 2 Typical measured SEC elution curves of (□) HECA-1, (Δ) HECA-2, and (○) HECA-3.

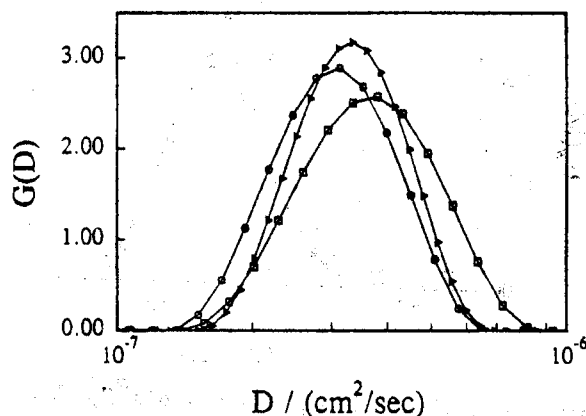


Figure 3 Typical translational diffusion coefficient distributions ($G(D)$) of (□) HECA-1, (Δ) HECA-2, and (○) HECA-3 at $\theta \rightarrow 0$ and $C \rightarrow 0$.

Table II Summarization of Calibration Constants and M_w and M_w/M_n

Sample	A	B	$10^4 k_D$	α_D	From $C(V)$		From $G(D)$	
					$10^{-4} M_w$	M_w/M_n	$10^{-4} M_w$	M_w/M_n
HECA-1	45.8	-1.91	2.51	0.598	4.61	1.13	4.54	1.30
HECA-2	44.8	-1.85	2.43	0.594	6.11	1.21	6.15	1.20
HECA-3	45.3	-1.92	2.66	0.605	6.52	1.23	6.56	1.23

Calibration constants A, B, k_D , and α_D determined in the LLS + SEC method; and M_w and M_w/M_n calculated from $F_w(M)$ in Figures 4 and 5.

Note. Relative errors of the listed parameters are: A, $\pm 3\%$; B, $\pm 5\%$; k_D , $\pm 5\%$; α_D , $\pm 1\%$; and M_w , $\pm 5\%$.

in this study are moderately distributed. Both the values of M_w and M_w/M_n obtained from $C(V)$ in SEC are in agreement with that from $G(D)$ in dynamic LLS, except an unexpected difference in the distribution width of HECA-1. This difference might be attributed to the flow rate difference. It should be noted that in comparison with the results reported in the previous SEC study,⁷ both the M_w and M_w/M_n values obtained in SEC where polystyrene standards were used in the SEC calibration could contain an error as high as 100%. This clearly shows that the polystyrene calibration of SEC for the characterization of cellulose and its derivatives should be avoided if we are serious about the absolute values of M_w and M_w/M_n .

Figure 6 shows a direct comparison of two cumulative weight distributions ($I_w(M) = \int_M^\infty F_w(M) dM$) calculated respectively from $C(V)$ by using eq. (1) and from $G(D)$ by using eq. (2) for the same HECA-2 sample. Figure 6 clearly demonstrates that the cumulative weight distributions obtained from SEC and DLS are basically identical except for a

small difference in the lower molecular weight tail. This difference is understandable because the scattered light intensity is proportional to $F_n(M)M^2$, where $F_n(M)$ is differential number distribution, and small molecules in the distribution cannot be "seen" by the light scattering detector. On the other hand, a small baseline uncertainty normally affects the lower molecular weight end in the elution volume distribution. Therefore, for a very broadly distributed sample, there could be some difference in the widths of the molecular weight distributions obtained from $C(V)$ in SEC and $G(D)$ in dynamic LLS.

CONCLUSIONS

This study has shown that the recently developed analytical procedure of simultaneously calibrating dynamic LLS and SEC is useful in the characterization of HECA in THF at room temperature. By using this procedure, we have determined both the calibration constants in SEC and in dynamic LLS.

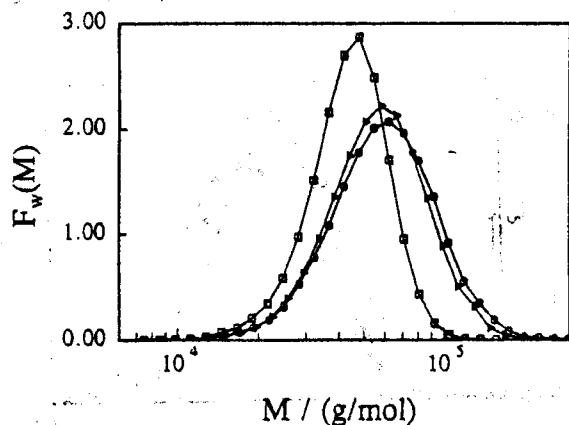


Figure 4 Differential weight distributions ($F_w(M)$) calculated from the elution volume distributions ($C(V)$) in Figure 2 for (\square) HECA-1, (Δ) HECA-2, and (\circ) HECA-3 by using A and B in Table II.

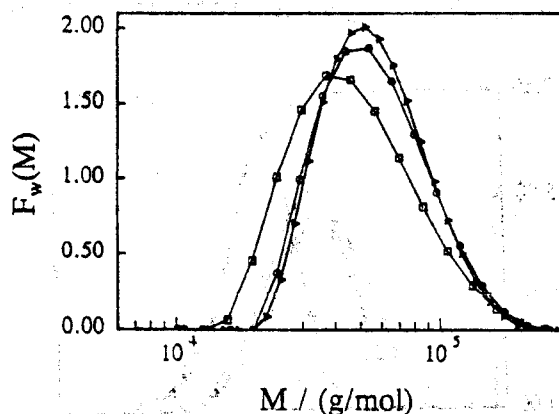


Figure 5 Differential weight distributions ($F_w(M)$) calculated from the translational diffusion coefficient distributions ($G(D)$) in Figure 3 for (\square) HECA-1, (Δ) HECA-2, and (\circ) HECA-3 by using k_D and α_D in Table II.

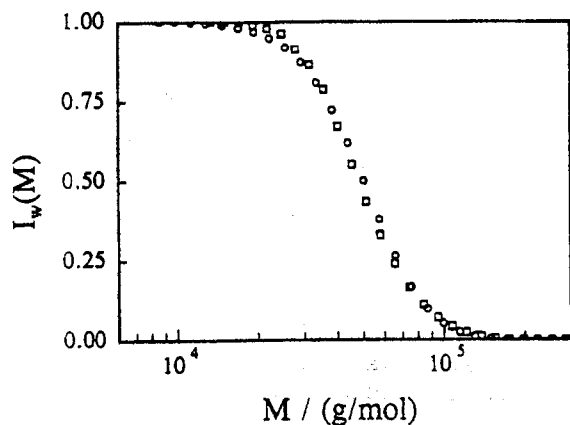


Figure 6 Comparison of two cumulative weight distributions ($I_w(M) = \int_M^\infty F_w(M) dM$) calculated, respectively, from (O) $C(V)$ by using eq. (1) and from (□) $G(D)$ by using eq. (2) for the same HECA-2 sample.

With these constants, we have accomplished the molecular weight characterization of the HECA samples. It should be noted that the constants obtained in this study from dynamic LLS, that is, k_D and α_D in eq. (2), are independent on our particular LLS spectrometer, which means that these two constants can be used in any other LLS spectrometer as long as the same solvent and temperature are used. In addition, our results indicate that the chain of HECA in THF at room temperature behaves like a slightly extended coil.

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