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Flow Irregularities due to Polymer Elution in Size Exclusion Chromatography and its Effect on Viscometric Detection

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A new experimental setup for detecting flow-rate fluctuations caused by the passage of polymer through a separation system, including a differential refractometer and a single-capillary on-line viscometric detector in size exclusion chromatography (SEC) is demonstrated. Numerical methods for correction of elution volume and the baseline of the viscometric output, including the “Lesec effect”, are presented on SEC data of (2-hydroxyethyl) cellulose in 50% methanol/50% water 10 mM NaCl (mobile phase). The effect of flow-rate fluctuations on experimental molecular weight averages is discussed.

Keywords: Size exclusion chromatography; Viscometric detection; Flow-rate fluctuations; Molecular weight distribution

INTRODUCTION

Based on indirect evidence, it was proposed that in size exclusion chromatography (SEC) of polymers, a regular flow of mobile phase through the SEC separation system is disturbed by fluctuations when the polymer starts to elute from the separation system.^[1–5] The disturbances may have deleterious effect on the experimental data,

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especially on those obtained by single-capillary viscometric detection.^[2] This study is focused on the flow-rate fluctuations due to polymer and its effects on the molecular weight averages obtained using a single-capillary viscometer for the determination of polymer molecular weight.

The differences in the construction of various parts of the SEC separation system (injection valve, columns, frits, connecting capillaries, detectors) lead necessarily to differences in their resistance to flow. When the mobile phase containing polymer passes from one of these parts to another, pressure- and, consequently, flow-rate fluctuations may be expected. The description of the flow-rate fluctuations can be simplified by dividing it into 3 phases according to the passage of the polymer through three important sites in the separation system: In the first phase, after the injection of the polymer sample, the resistance of the separation system is increased by the injected polymer (and, of course, by short-time closure of the injection valve). The equilibrium flow is disturbed, but after some time, some solvent accumulates in the separation system and a new equilibrium is reached. In the second phase, when the polymer starts to elute from the separation system and enters the connecting tubing between and inside the detectors, the resistance of the separation system increases again and some flow-rate disturbance may also result. In the third phase, the polymer starts eluting from the detection system. The departure of the polymer from the detection system decreases the resistance of the system and, consequently, a small increase in the flow rate is assumed to result, thus compensating for the two previous phases. Phases 2 and 3 were proposed as the "Lesec effect"^[3] which correspond, respectively, to phases 1 and 2 in Ref. [3].

The flow-rate fluctuations may result in disturbances of the viscometric tracing, in particular that obtained using a single-capillary viscometer. This effect can be expressed as a deformation of the baseline^[3] and can be corrected if the actual flow rate is known. The fluctuations may also cause some shift of both (viscometric and differential refractometer) tracings along the elution volume axis.

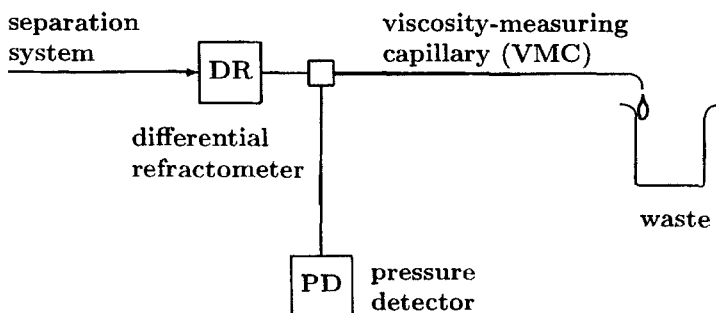
In this paper, a new experimental setup for detecting the flow-rate fluctuations and a new computational procedure for correcting flow-rate fluctuations of elution volume and the baseline of the viscometric tracing are presented. The effect of the corrections on the

molecular-weight averages obtained by SEC with single-capillary viscometric detection is demonstrated.

EXPERIMENTAL

Two arrangements of viscometric detection were used for the SEC experiments (Figure 1). In the first, called "viscometric" arrangement,

(a) "Viscometric" detection



(b) "Flow-rate" detection

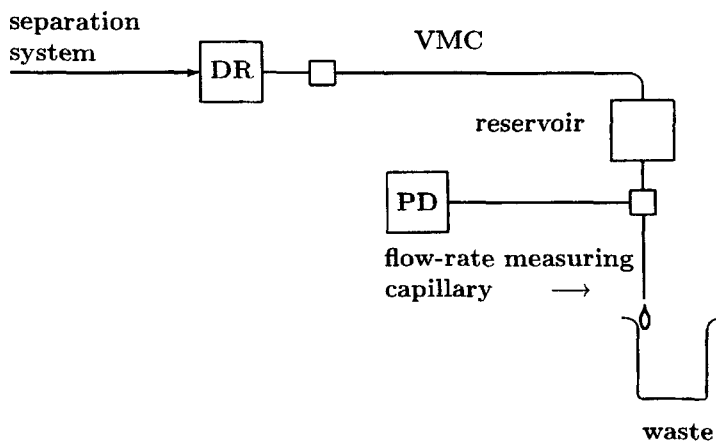


FIGURE 1 The viscometric (a) and flow-rate (b) arrangements used in the experiments (for details, see Experimental section).

a differential refractometer (DR) (Laboratory Instruments, Prague, RIDK 101 on-line with a Blackstar 2308 A/D interface) was connected using a T-piece with a metal measuring capillary (length 70 cm, 0.31-mm i.d.) and to a stainless steel metal housing with a silicon-membrane pressure detector model DPI-280, Druck Ltd, obtained from AMTELE MÅTINSTRUMENT AB, Sweden, with an AMTELE RS 232 A/D interface. The inner volume of the housing was 0.5 μL . The housing was provided with inlet and outlet fittings enabling air bubbles to be washed out. The outlet fitting was closed during the experiments. Both signals were stored in an IBM-compatible PC via COM1 and COM2 ports and processed using custom-made software.

In the second, "flow-rate" arrangement, a reservoir (made of an empty column of volume 26.9 mL) filled with mobile phase was connected to the outlet of the measuring capillary (without its measuring function) ensuring that the liquid phase entering the (second) measuring capillary (12.5-cm long PEEK tubing, 0.12-mm i.d.) has a constant (negligible) polymer concentration because the eluted polymer is diluted in the reservoir volume. This ensures that the response of the pressure detector is proportional to the flow through the system. Compared with the arrangement proposed in Ref. [3], where the flow-rate detector is separated from the measuring capillary by the separation system with a pressure drop typically about 11 MPa (in our experiments), the advantage of our arrangement is that it works in a comparable pressure regime that makes it possible to obtain an exact record of the flow-rate proportional signal.

The SEC system consisted of the LDC Analytical Constametric 3200 P/F solvent delivery system (dual-piston reciprocating pump), degasser DG-12100, a pressure-fluctuation dampener Tillquist of a total volume of about 40 mL with two chambers separated with rubber diaphragm (one of them filled with inert medium), injection valve Rheodyne 7125 with a 200- μL injection loop, and three diol modified Lichrospher columns^[9] (length 250 mm, 8-mm i.d.) packed with Lichrospher Si (Merck, Darmstadt, Germany), particle size 10 μm , pore size 30, 100 and 400 nm.

The SEC apparatus was placed into a temperature-controlled chamber. The temperature was 25°C during the experiments.

A characterized^[9,10] sample of (2-hydroxyethyl)cellulose (HEC) (Aldrich-Chemie, Steinham, Germany) was used in the experiments:

intrinsic viscosity was $[\eta] = 273 \text{ mL/g}$ ($k_H = 1.22$) in water and $[\eta] = 285 \text{ mL/g}$ ($k_H = 0.88$) in 50% methanol (55.9 wt%)/50% 10 mM NaCl (44.1 wt%), which was the mobile phase in the SEC experiments.

The coefficients of Equation (18) for the HEC/water system are^[12] $K = 9.483 \times 10^{-3} \text{ mL/g}$ and $\alpha = 0.87$. Since the coefficients of Equation (18) in the mobile phase are not known and that the values of intrinsic viscosity in water and mobile phase are close one to another, we employed these K and α values also for the evaluation of the influence of flow fluctuations on experimental molecular weights in the SEC experiments. The corresponding viscosity-average molecular weight was $M_\eta = 1.40 \times 10^5$ and the molecular weight obtained by SEC with the dual light scattering/DR detection was^[9,10] $M_w = 1.89 \times 10^5$ ($M_w/M_n = 4.8$), while the molecular weight determined by off-line light scattering was somewhat higher,^[9,10] $M_w = 2.28 \times 10^5$.

Stock solutions of samples in water were prepared according to the method described elsewhere.^[9,11] Solutions for SEC measurement were prepared by mixing a water solution of appropriate NaCl concentration and methanol with the stock solution in water so that the composition of the mobile phase was reached. Various concentrations of these solutions were obtained by dilution with the mobile phase.

THEORETICAL

Determination of the Instant Flow Rate and Elution Volume

The determination of the instant flow rate is performed in the following way: According to the Poiseuille law, the instant flow rate, $\dot{V}(t)$, expressed as the time change of the solvent volume $V(t)$, is proportional to the instantaneous pressure drop $p(t)$ on the capillary, that is

$$\dot{V}(t) = kp(t) \quad (1)$$

where the proportionality constant is

$$k = \left(\frac{8l\eta}{\pi r^4} \right)^{-1} \quad (2)$$

where l is the length of the capillary, r its diameter, η the mobile-phase viscosity and π has its usual meaning. We can assume that mean flow

rate $\langle \dot{V} \rangle$, which is identical with the macroscopic flow rate of the SEC pump, is proportional in the same way to the mean value of the pressure drop

$$\langle p \rangle = \frac{1}{t} \int_0^t p(t) dt \quad (3)$$

where the integration is performed over a sufficient span of time t (duration of the experiment), which gives for the proportionality constant

$$k = \langle \dot{V} \rangle / \langle p \rangle. \quad (4)$$

Knowing k , the instant flow rate can be calculated according to Equation (1) and the real elution volume (corrected for the fluctuations) is calculated as

$$V(t) = \int_0^t \dot{V}(t) dt. \quad (5)$$

The integration constant in Equation (5) was set to zero, which physically means that the calculation of $V(t)$ starts at the instant of injection ($V(0) = 0$).

Determination of the Flow-Rate during Polymer Exclusion and Correction of the Viscometric Baseline

In order to determine the corrected baseline of the viscometric tracing, it is necessary to combine the data obtained from the viscosity detection arrangement with the data obtained in the flow-rate arrangement (Figure 1).

According to the Poiseuille law (1), the pressure drop $p(t)$ in the flow-rate measuring capillary is given by

$$p(t) = k_f^{-1} \dot{V}(t) \quad (6)$$

where subscript f refers the constant k_f defined by Equation (1) to the flow-rate measuring capillary.

In the absence of the polymer (denoted by the subscript 0), the pressure drop should be approximately constant and can be expressed as

$$p_0(t) = k_r^{-1} \dot{V}_0(t). \quad (7)$$

In the real experiment, $p_0(t)$ defined by Equation (7) can be approximated by a straight line, connecting the points $p(t_1)$ and $p(t_2)$, respectively referring to elution times t_1 and t_2 before and after the chromatographic wave

$$p_0(t) = a + bt \quad (8)$$

where a and b are constants. (This assumption is not quite correct because it does not consider the flow fluctuations caused by random effects or a periodic action of the pump. A numerical method of solving this problem will be presented in following sections.) The slope a of Equation (8) may compensate for a possible drift of the pressure detector signal, caused, for example, by temperature changes.

The pressure drop $P_0(t)$ in the viscosity measuring capillary (denoted by the subscript m in k_m) (Figure 1), in the absence of the polymer

$$P_0(t) = k_m \dot{V}(t) \quad (9)$$

can be fitted between the points $P(t_1)$ and $P(t_2)$ in the real experiment (in the presence of the polymer) in a similar way

$$P_0(t) = A + Bt \quad (10)$$

where A and B are constants with a similar meaning as a and b in Equation (8).

The corrected pressure drop $P_{0,c}(t)$ (denoted by the subscript c), in the measuring capillary hypothetically caused by pure solvent (baseline of the viscosity-measuring pressure-detector tracing) during the analysis at the instant flow rate $\dot{V}(t)$, can be expressed as

$$P_{0,c}(t) = k_m \dot{V}(t) \quad (\text{pure solvent}). \quad (11)$$

Combining Equations (6) and (7) with Equations (9) and (11), we have

$$\frac{P_{0,c}(t)}{P_0(t)} = \frac{p(t)}{p_0(t)}. \quad (12)$$

Inserting for $p_0(t)$ and $P_0(t)$ from Equations (8) and (10), respectively, the corrected baseline defined by Equation (11) is computed by

$$P_{0,c}(t) = \frac{A + BV}{a + bV} p(t). \quad (13)$$

Note that for $p(t)$ identical with $p_0(t)$ (given by Equation (8)), $P_{0,c}(t)$ calculated according to Equation (13) is identical with $P_0(t)$ given by Equation (10) (a linear baseline without corrections).

Calculation of Intrinsic Viscosity and Molecular Weight

Intrinsic viscosity at elution time t , $[\eta](t)$, is defined by the Huggins equation

$$\frac{\eta_{sp}(t)}{c(t)} = [\eta](t) + k_H [\eta]^2(t) c(t) \quad (14)$$

where $c(t)$ is concentration, k_H is the Huggins constant, and η_{sp} is specific viscosity, calculated as

$$\eta_{sp}(t) = \frac{P(t) - P_0(t)}{P_0(t)} \quad (15)$$

where $P(t)$ is the pressure drop in the viscosity-measuring capillary in the viscometric arrangement and $P_0(t)$ is the viscometric baseline either linear or corrected ($P_0(t) \equiv P_{0,c}(t)$) according to Equation (13). Solving Equation (14) for intrinsic viscosity, we have (for $k_H > 0$)

$$[\eta](t) = \frac{-1 + \sqrt{1 + 4 \times k_H \eta_{sp}(t)}}{2 \times k_H c(t)} \quad (16)$$

where concentration $c(t)$ is calculated by

$$c(t) = h(t) \frac{60 \times c_{inj} V_{inj} f_d}{\langle \bar{V} \rangle \int h_{DR}(t) dt} \quad (17)$$

where $h(t)$ is the height (intensity) of the differential detector tracing, c_{inj} is the concentration of injected sample, V_{inj} is the volume of the injection loop, f_d is the frequency of data logging (in s^{-1}) and $\langle \dot{V} \rangle$ is flow rate (in mL/min), the integration is performed over the range of the chromatographic wave.

Molecular weight was calculated using the Mark–Houwink–Kuhn–Sakurada equation^[8]

$$[\eta] = KM^\alpha \quad (18)$$

where K and α are constants.

Calculation of Molecular-Weight Averages

Molecular-weight averages, M_n , M_w and M_z are calculated from the heights $h(V)$ of the DR tracing as

$$M_j = \frac{\int h(V)M^j(V) dV}{\int h(V)M^{j-1}(V) dV} \quad (19)$$

for $j=1, 2$ and 3 , respectively, where the integrations are performed within the limits of the chromatogram wave. If the number of points is sufficient, the integrals can be replaced by sums

$$M_j = \frac{\sum_i h(V_i)M^j(V_i)\Delta V_i}{\sum_i h(V_i)M^{j-1}(V_i)\Delta V_i} \quad (20)$$

where i refers to experimental points and the term ΔV_i was calculated as

$$\Delta V_i = V_i - V_{i-1}. \quad (21)$$

Accordingly, the viscosity-molecular weight average is computed as

$$M_\eta = \left(\frac{\sum_i h(V_i)M^\alpha(V_i)\Delta V_i}{\sum_i h(V_i)\Delta V_i} \right)^{1/\alpha}. \quad (22)$$

RESULTS

The dual SEC analyses of HEC were performed in the flow-rate, as well as in the viscometric arrangement (Figure 1) at several concentrations (Tables I and II).

A typical example of the dual DR/VIS SEC tracing is depicted in Figure 2. The flow-rate responses are depicted in Figure 3 with the viscosity response, for comparison, and its baseline (which is discussed hereinafter).

TABLE I Parameters of Equation (23) for the calculation of $\log M$ vs V for (2-hydroxyethyl)cellulose for the SEC analyses at various concentrations of injected sample c_{inj}

$c_{inj} \times 10^3$ (g/mL)	Correction*	c_0	c_1	c_2	$c_3 \times 10^3$
6.06	-	14.8540	-2.1085	0.16507	-4.8978
	+	12.9207	-1.5218	0.05967	-2.8966
4.55	-	26.1137	-5.1994	0.44504	-13.333
	+	24.2758	-4.6517	0.38849	-0.0113
3.06	-	25.7331	-4.9286	0.40461	-11.622
	+	28.7001	-5.6001	0.45476	-12.465

*"Correction" denotes whether the correction of the baseline for flow rate, Equation (13), and of elution volume, Equation (5), were applied.

TABLE II Molecular-weight averages of (2-hydroxyethyl)cellulose calculated from the DR tracings using parameters in Table I determined at various concentrations of injected sample c_{inj}

$c_{inj} \times 10^3$ (g/mL)	Correction*		$M \times 10^{-3}$				
	M	V	M_n	M_η	M_w	M_z	M_w/M_n
6.06	-	-	37.8	112.0	118.8	265.7	3.14
	+	-	58.3	122.4	128.8	267.4	2.21
4.55	+	+	63.5	131.8	138.7	289.0	2.18
	-	-	27.2	105.5	113.1	327.0	4.16
3.06	+	-	41.0	103.3	109.5	280.1	2.67
	+	+	45.1	110.5	117.4	311.5	2.61
1.61 [†]	-	-	43.7	119.0	128.5	406.1	2.94
	+	-	62.4	129.0	138.7	477.5	2.15
1.61 [†]	+	+	64.9	135.0	144.6	512.4	2.23
	-	+	39.9	119.3	129.0	462.0	3.23
	+	+	42.1	124.2	134.5	454.3	3.20

*"Correction M " denotes whether the values of q_i corresponding to corrected or uncorrected data from Table I were used. "Correction V " denotes whether the correction of V for the flow-rate, Equation (5), was applied.

[†]The set of coefficients q_i from Table I, determined for $c_{inj} = 3.06 \times 10^{-3}$ g/mL was used for the evaluation of the DR signal.

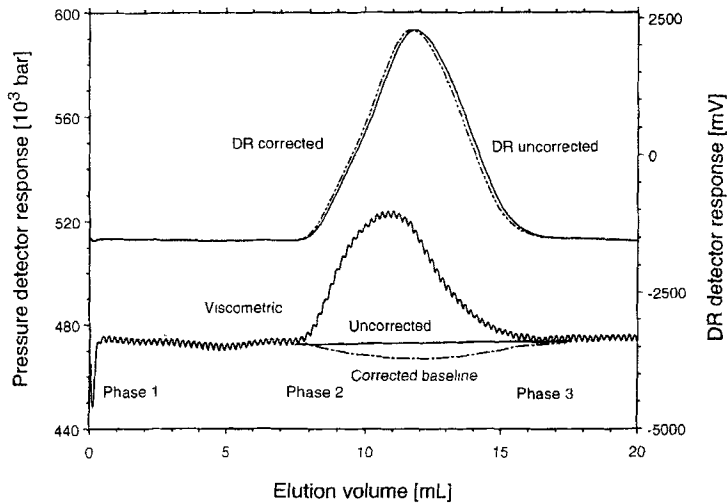


FIGURE 2 Typical dual tracing of SEC analysis of (2-hydroxyethyl)cellulose. The DR tracing (upper curves) is uncorrected (left curve) or corrected (right curve, dashed) for the error in V according to Equation (5). The baseline of the viscometric tracing (lower curves, left scale), corrected for the flow-rate fluctuation (dashed curve) and compared with the uncorrected one (solid line), was calculated according to Equation (13). The phases of the flow-rate fluctuation are explained in the text.

For further calculations, the flow-rate tracings characterized by the short-time pulsations (pump noise) were tentatively fitted by polynomials varying in degree from linear to cubic and the particular range of the curve fitted (Figure 3). This procedure produces smooth curves, which ensures that the elution volume and flow-rate corrections do not produce additional noise of the calculated curves.

The crude experimental data (dual tracing), for all injections (differing in concentrations) treated in the same way, were used for the calculations thus demonstrating the effect of polymer concentration on the flow fluctuations. In all cases, the DR baselines were set between elution volumes of 7.22 and 15.9 mL. The baselines of the viscometric tracings were shifted by the effective instrument delay^[6,7] (interdetector volume of 0.38 mL), which gave consistent results for all concentrations of the injected samples.

Intrinsic viscosity and molecular weights of HEC dependent on V were calculated from dual tracings by Equations (16) and (18), respectively, using the uncorrected values of V as well as those corrected according to Equation (5).

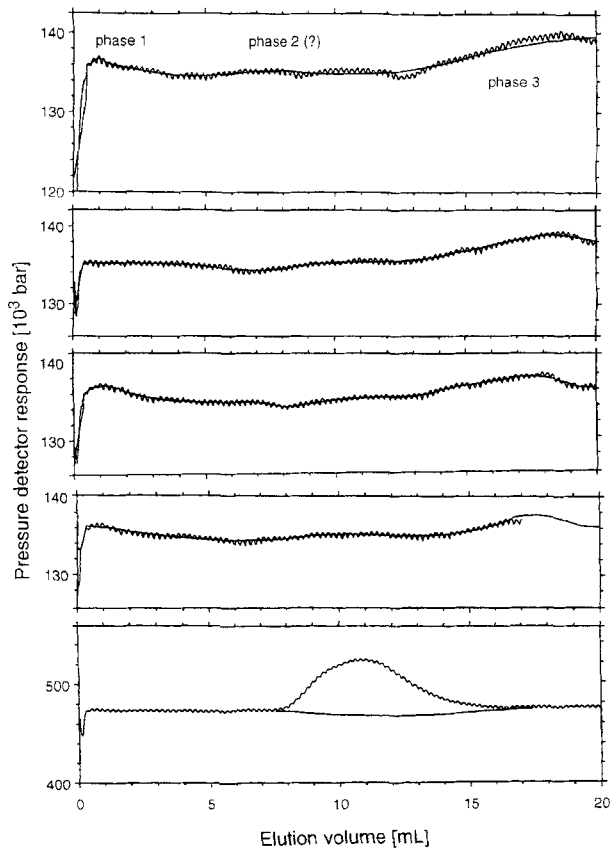


FIGURE 3 The pressure detector response in the flow-rate detection arrangement in the SEC of (2-hydroxyethyl)cellulose compared with the pressure detector response in the viscometric arrangement. The hypothetical phases of the flow-rate fluctuation, denoted on the curves are described in the text. The injection concentrations were, from top to bottom, respectively, $c_{inj} \times 10^3 / (\text{g/mL}) = 6.06, 4.55, 3.06, 1.61$ and (the viscometric tracing) 6.06. The solid lines represent a polynomial fit of the tracing $p_0(t)$ from the flow-rate measuring capillary used for calculation of corrected baselines according to Equation (13).

The resulting dependences of $\log M$ vs V were fitted between $V = 8.09$ and 13.48 mL by

$$\log M = \sum_{i=0}^3 q_i V^i \quad (23)$$

where q_i are coefficients summarized in Table I.

The number-, viscosity-, weight- and z-average molecular weights, M_n , M_η , M_w and M_z , respectively, calculated from the DR tracings using q_i from Table I are summarized in Table II. The lowest concentration ($c_{inj} = 3.061 \times 10^{-3}$ g/mL) was too low to produce signals satisfactory for constructing $\log M$ vs V dependences. For this reason, q_i obtained at $c_{inj} = 4.548 \times 10^{-3}$ g/mL were used for the calculation.

DISCUSSION

Since the purpose of our experimental setup was to detect flow fluctuations in the separation system, no extensive pressure fluctuation dampening was used. Owing to this, the setup was also sensitive to fluctuations of duration about 40 s (Figure 4), originating from the pump piston movement (pump noise), which is a frequent source of trouble when using a single-capillary viscometer.^[13] The shape of the pump-noise curves and the length of fluctuations is given by the fact that a dual-piston reciprocating pump was used.

The response of the flow rate due to the polymer (Figure 3) is visible both on the experimental and on the fitted curves. The initial drop due to the instant cessation of flow and injection of the polymer (proposed phase 1) can be seen at the beginning of each curve. The magnitude of the drop varied in the experiments, which may depend on the speed of the manual injection. Since it is assumed that a new equilibrium between pressure in the system and the flow rate is established before the polymer starts to elute, the magnitude of the initial drop is unimportant.

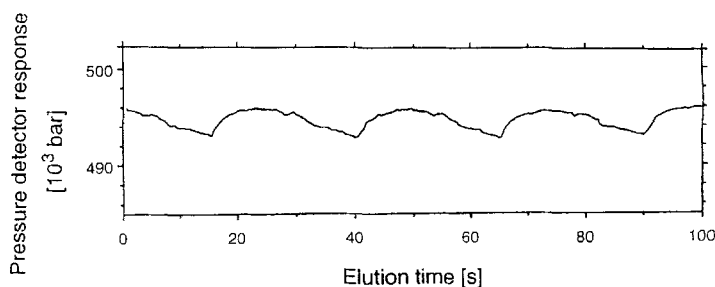


FIGURE 4 The pressure detector tracing of neat solvent passage (in the viscometric arrangement) obtained using a data logging frequency of 5 s^{-1} .

Contrary to expectation,^[3] the flow-rate drop due to the entry of the polymer into the separation system (proposed phase 2) cannot be clearly detected or is very flat on the curves, although some fluctuations seem to be present on some curves at the corresponding elution volumes (Figure 3). The flow-rate increase starting at the tail of the chromatogram (proposed phase 3) is, however, clearly observable. Nevertheless, the flow-rate equilibrium according to assumptions^[3] is not reached within the range of the chromatographic wave or immediately after the exit of polymer from the system (the wave-tail) but in a period extending beyond the observable length of the tracing. The consequence of this fact is the depression observable in Figures 2 and 5 on the baselines corrected according to Equation (13). The curvature of the baseline decreases with decreasing concentration (Figure 5) roughly proportionally to the decrease of the viscometric signal. At lower concentrations, however, the curvature of the baseline (Figure 3) may be more influenced by the long term flow-rate fluctuations which may originate, e.g., from small temperature fluctuations, and become more complex (Figure 5).

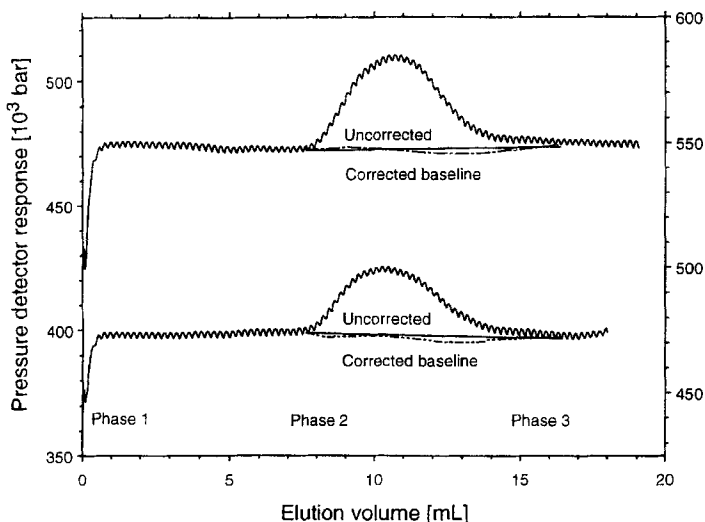


FIGURE 5 Viscometric tracings of (2-hydroxyethyl)cellulose with baselines uncorrected (solid lines) and corrected according to Equation (13) (dashed curves). The concentration of injected sample was $c_{inj} = 4.55 \times 10^{-3}$ g/mL (upper curves, left scale) and 3.06×10^{-3} g/mL (lower curves, right scale). The phases of the flow-rate fluctuation are explained in the text.

The correction of the elution volume according to Equation (5) is demonstrated in Figure 2, depicting the uncorrected, as well as corrected DR tracings and the viscometric tracing (with the baseline corrected according to Equation (13), discussed later). It follows roughly the pattern proposed in Figure 4 of Ref. [3] The difference is that both uncorrected and corrected curves begin and end approximately at the same V because the main flow-rate disturbance is within the range of the wave.

The observed differences can be explained by the fact that the flow rate increase at the tail of the chromatographic wave (proposed phase 3), caused by the exit of the polymer from the separation system, compensates for the increase in pressure following the injection of the polymer (proposed phase 1). For this reason, the expected depression at the beginning of the wave (phase 2, if present) is not of the same magnitude as the increase at the end.

Owing to the "Lescq effect", the peaks are shifted along the V axis (Figure 2), as envisaged theoretically by Yau *et al.*,^[2] in the same direction as they are shifted because of the concentration effect.^[16-21] (With increasing c_{inj} , the value of V increases.) This brings a theoretical possibility of combining both effects. Classical studies of the concentration effect,^[16-19] however, were made by measuring V using a siphon. This method eliminates the effect of the shift. (The use of gravity-force suspended-level viscometers periodically filled from a siphon^[22,23] eliminates completely the "Lescq effect", including both the peak shift and the baseline curvature. These viscometers are, however, incompatible with modern high-performance columns.)

Figure 6 presents a comparison of the corrected and uncorrected raw and fitted dependences of $\log M$ vs V for $c_{inj} = 6.06 \times 10^{-3}$ g/mL. The scattered open circles at both ends of the tracings were excluded from the calculations (the ranges of calculations were mentioned in the Results section). It can be seen that the slopes of the corrected dependences are lower, which suggests a lower polydispersity. This is also illustrated by the molecular weight averages (Table II). Polydispersities expressed as M_w/M_n and calculated using corrected baselines are lower than those calculated using the uncorrected ones. This can be explained by the consideration that the depression in the middle of the baselines (Figures 2 and 5) has the same effect as an increase in the middle of the viscometric tracings which results in having narrow curves.

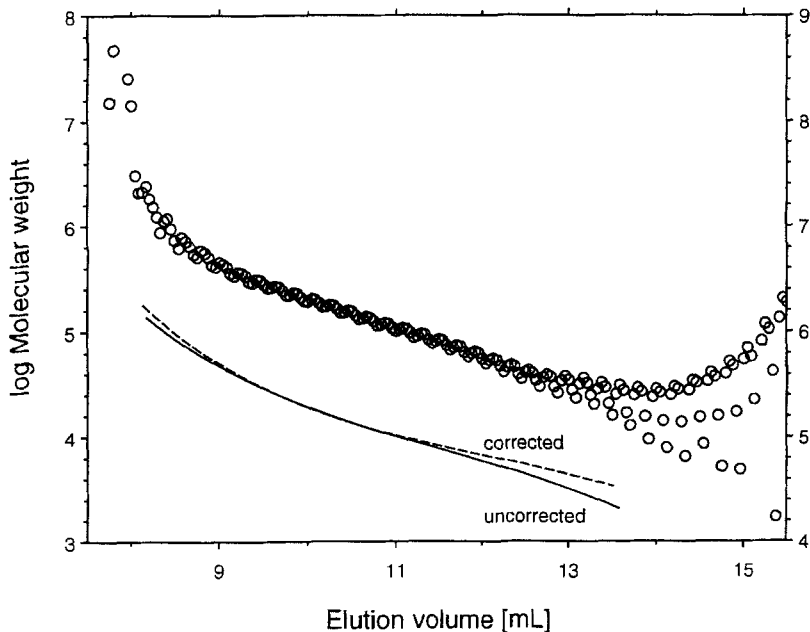


FIGURE 6 An example of the dependence of $\log M$ vs V in SEC of (2-hydroxyethyl)cellulose for concentration $c_{inj} = 4.55 \times 10^{-3}$ g/mL: the uncorrected experimental data (open circles, left scale), the corrected (dashed curves) and uncorrected (solid curves) dependences fitted by a polynomial with coefficients from Table I (lower curves, right scale).

The effect manifested as the change in slope (rotation) of the $\log M$ vs V dependences (Figure 6) may lead to erroneous calculation of the Mark–Houwink–Kuhn–Sakurada coefficients for example, by combining the universal calibration^[14] with the experimental dependences $\log[\eta]$ vs V as pointed out by Yau *et al.*^[2] This procedure yields the so-called secondary $\log M$ vs V correlation and the effect of rotation of the $\log[\eta]$ vs V dependence on the determination of the Mark–Houwink–Kuhn–Sakurada coefficients was theoretically investigated.^[15]

In general, the values of molecular-weight averages of HEC, obtained using different techniques, vary considerably,^[9,10] which reflects difficulties in fractionating and characterizing, including the determination of the Mark–Houwink–Kuhn–Sakurada parameters by light scattering.^[11] The values of M_η and M_w fluctuate around $M_\eta = 1.403 \times 10^5$ for an unfractionated sample, which is apparently due to the use of the viscometric detection.

The correction of V , however, leads to broadening of the polydispersity, although it does not exceed that calculated from the uncorrected data.

The above discussed results demonstrate problems expected in attempts to improve the detection based on the single-capillary viscometry. The undampened flow tends to fluctuation caused by the strokes of the pistons. Their duration is too long to be dampened out satisfactorily (Figure 4) even by using large columns with a high pressure drop. An attempt to dampen the fluctuations leads necessarily to an increased capacity of the system to accumulate the solvent and, consequently, to an increased "Lescq effect". A similar effect can be expected from using single-stroke large-volume piston pumps. (The increase in c_{inj} does not bring much improvement. At low concentrations, the signal-to-noise ratio is unfavorable; with increasing concentrations, the "Lescq effect" is increased.)

The solution to the problem of pressure fluctuations was the introduction of the four-capillary-bridge on-line viscometer.^[24,25] The pressure fluctuations are compensated because they are approximately the same on both sides of the differential pressure sensor which results in smoothing the viscometric signal. This makes it possible to decrease the concentration and thus to decrease the elution volume effect. The necessary condition, however, is good balancing of the resistance to flow (of a neat solvent) on both sides of the bridge system.

CONCLUSIONS

The results obtained in this study can be summarized as follows: The flow fluctuation due to the flow of the injected polymer through the separation-system outlet has been experimentally demonstrated by a newly constructed apparatus. Flow-rate disturbances resulting in the deformation of the baseline of the viscometric tracing is similar to the proposed "Lescq effect"^[3] but differ in the extents of deformations within the range of the observable chromatographic wave.

Correction methods for the viscometric-tracing baseline as well as for elution volume were demonstrated. It has been found that the effect of the correction of the baseline deformation is in a way compensated by the effect of the elution volume correction.

The effect of disturbances on elution volume decreases with decreasing concentration. However, the effect on baseline is not likely to completely disappear at $c_{inj} \rightarrow 0$ because both the signal of the viscometric detector and the disturbance of the viscometric-signal baseline are roughly proportional to c_{inj} . For this reason, care must be taken while evaluating single-capillary viscometer data (if the pressure noise is reduced, which is a very difficult task for the single-capillary setup.^[13])

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