JOURNAL OF LIQUID CHROMATOGRAPHY

VOLUME 7 NUMBER 9 1984

Special Issue on
SIZE EXCLUSION CHROMATOGRAPHY

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CODEN: JLCHD8 7(9) i-vi, 1717-1910 (1984)
ISSN: 0148-3919

JOURNAL OF LIQUID CHROMATOGRAPHY

August 1984

Aims and Scope. The journal publishes papers involving the application of liquid chromatography to the solution of problems in all areas of science and technology, both analytical and preparative, as well as papers that deal specifically with liquid chromatography as a science within itself. Included will be thin-layer chromatography and all modes of liquid chromatography.

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Subscription Information. *Journal of Liquid Chromatography* is published in fourteen numbers and two supplements in January, February, March (2 numbers), April, May (2 numbers), June, July (2 numbers), August, September, October (2 numbers), November, and December by Marcel Dekker, Inc., 270 Madison Avenue, New York, New York 10016. The subscription rate for Volume 7 (1984), containing fourteen numbers and two supplements, is $350.00 per volume (prepaid). The special discounted rate for individual professionals and students is $175.00* per volume. To secure this special rate, your order must be prepaid by personal check or may be charged to MasterCard or VISA. Add $40.00 for surface postage outside the United States. For airmail to Europe, add $72.32; to Asia, add $91.52.

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MARCEL DEKKER, INC. New York and Basel

JOURNAL OF LIQUID CHROMATOGRAPHY

Volume 7, Number 9, 1984

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1717-1738 (1984)

A REVIEW OF POLYMER SHEAR DEGRADATION IN SIZE-EXCLUSION CHROMATOGRAPHY

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ABSTRACT

Although there have been only a few studies involving shear degradation of polymers in size-exclusion chromatographic (SEC) columns, it appears that the potential for degradation exists when analyzing high molecular weight polymers. Because of the complex hydrodynamics associated with SEC systems, it is very difficult to arrive at simple correlations between SEC operational parameters and the onset of shear degradation. Also, the dependency of shear degradation on polymer concentration, structure, and the nature of the mobile phase further complicates this issue. Nevertheless, guidelines involving operational parameters are presented based on published data and estimated shear rates produced in various parts of a SEC system.

INTRODUCTION

The introduction of high-performance packings in size exclusion chromatography (SEC) has brought about a significant decrease in analysis time and an increase in resolving power. Like other high-performance liquid chromatographic techniques, the current trend is to use smaller particle size packings to obtain maximum column efficiency. However, most analysts forget

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that high molecular weight polymers, whether synthetic or naturally occurring, are fairly sensitive to shear forces which can lead to chain rupture. As compared with conventional SEC in which mobile phase flow velocities range from about 0.02 to 0.10 cm/sec, velocities used in HPSEC are typically 5 times greater. Also, because of the much smaller particle sizes used in SEC, the shear rates generated in these columns are significantly greater than obtained in conventional columns. As a result, shear degradation of polymers during elution through a column is possible.

Presented are a discussion on the theory of polymer shear degradation, estimates of shear rates which can occur in chromatographic columns, and a review of reports published in this area of study. Guidelines are also given for minimizing the occurrence of polymer shear degradation during SEC.

THEORY

The relationship between shear stress (applied force per unit area) and shear rate of a Newtonian liquid under laminar flow is given by:

$$\tau = \eta\dot{\gamma} \qquad (1)$$

where τ is shear stress (dynes/cm^2), η is viscosity of the solution (poise, g $\cdot$ cm^{-1} $\cdot$ sec^{-1}), and $\dot{\gamma}$ is shear rate (sec^{-1}) or the velocity gradient (dv/dy) formed perpendicular to the direction of flow.

In calculating the shear rate of a packed column, we assume that the column consists of a bundle of capillaries in which the capillary radius can be estimated from the bed hydraulic radius:

$$R_h = D_p \varepsilon / 3(1-\varepsilon) \qquad (2)$$

where D_p is the diameter of the packing and ε is the porosity of a packed column ($\varepsilon = 0.36$) (1). Thus, the shear rate in a packed column, assuming Newtonian behavior, is readily calculated using:

$$\dot{\gamma} = 4\ \bar{v}/R_h = 4Q/\varepsilon A R_h \qquad (3)$$

where $\bar{v}$ is the average linear velocity, Q is the flow rate, and A is the cross-sectional area of the column. For open tubes, ε is unity and the tube radius is equal to R_h.

DEGRADATION MECHANISMS

Because of velocity gradients generated during flow, a polymer becomes extended. Bonds near the middle of the chain become stretched and can rupture if shear rates are great enough. The end segments of a chain maintain their coiled shape; thus, the maximum strain is usually focused near the center of the polymer. To a first approximation, the strain generated in a polymer is proportional to the square of its length (2, 3). The weak points along a polymer chain are determined by its length and structure. According to Bird et al. (4), there is no reliable quantitative theory of

mechanical stability of polymers, and the stability of each polymer must be determined experimentally under strictly defined conditions.

In addition to shear forces, extensional flow components may also play a significant role in polymer degradation. These flow components would cause more stress on the chain than that produced by a laminar flow. Extentional flow occurs at capillary entrances and exits and at convergent-divergent flow paths in packed beds (5-8). Recently, Giddings (25) had shown extentional flow in packed columns multiplies the shear effect by about 10^3 beyond that of tangential shear.

When shear is applied, the most probable sites of stress concentration on the polymer chain are (3):

1. Side chain linkages to main chain, i.e., branch points.
2. Crosslink points in networks.
3. Points of inclusion of heteroatoms and quaternary carbon atoms.
4. Dissymmetry between adjacent atoms which promotes rupture of the chain by stretching forces.

Chain entanglements are also quite sensitive to shear stress. Junction points of entangled chains concentrate energy due to stress and thus can break. The mechanism depends on the time scale of shear deformation, temperature, probability of entanglement, and slippage of junction points (9-11). Thus,

polymer solutions which contain entangled chains are more shear sensitive than more dilute solutions in which entanglements have not occurred. The extent of chain entanglement also depends on the nature of the solvent (4). Recently, Yu et al. (12) have studied the concentration dependency of shear degradation and have postulated that both entanglements and inhibition of molecular extention by surrounding molecules in concentrated solutions are contributing factors. Therefore, although entanglements which occur at high polymer concentrations may favor shear degradation, molecular extensions are inhibited by surrounding molecules.

Polymers that are extended in solution, i.e. dissolved in good solvents, exhibit a higher sensitivity toward shear degradation than more randomly coiled polymers (13-15). For example, the rate of mechanical chain scission of dextran is approximately 100 times lower than for polyacrylamide (13).

With sufficiently high internal stresses, bonds rupture and produce radicals. In the presence of radical acceptors, e.g., oxygen, radicals that are formed are stabilized resulting in a decrease in molecular weight. However, in the absence of radical acceptors, branching and crosslinking are possible (3).

There are two popular theories regarding the mechanism of shear degradation with respect to molecular weight (3). One theory proposes that during degradation a limited molecular weight is approached (16). Once this limited or critical

molecular weight (or more precisely chain length) is reached, no further chain scission can occur (17). This critical chain length is short enough that it can adjust to the applied stress by moving with respect to its sheath of solvent molecules rather than by rupturing primary bonds. The second theory, postulated by Rodriguez and Winding (18), states that there is no critical molecular weight which is resistant to degradation at a given shear rate. There is, however, a decreased probability that degradation will occur as the molecular weight decreases.

POSSIBLE SOURCES OF POLYMER SHEAR DEGRADATION IN SEC

In size-exclusion chromatography, the following are potential sources of polymer shear degradation:

Injection valve
Capillary tubing
Column frits
Packed column

It should be emphasized that shear degradation may also occur during sample preparation. In view of this, it is advisable to use low shear rate conditions such as tumbling and low speed stirring.

The following are estimated shear rates calculated from Equation 3. Because of the many factors which influence polymer degradation, these values are used to demonstrate the magnitude of shear rates which can be developed in various parts of the chromatographic systems.

Injection Valves

Typical sample loop sizes are shown in Table 1 with the corresponding shear rates produced assuming a 1 ml/min flow rate. Also included are shear rates developed in the smallest flow passages of the valve.

Capillary Tubing

Capillary tubes, which are used for column and detector connections as well as for sample loops in injection valves, usually range in ID from 0.01 to 0.04 inches. As shown in Table 2, the shear rates generated in these tubes can be quite high.

Frits

Commonly used stainless steel frits have an average channel radius of about 1 μm. Assuming a flow rate of 1 ml/min through a 6-mm diameter disc (ϵ ~0.3), the estimated shear rate generated is about 7.8×10^3 sec^{-1}.

Shear Rate in Packed Columns

The shear rates generated in columns as a function of particle diameter are shown in Figure 1 for 0.5 and 1.0 ml/min flow rates. Results were calculated assuming a 4-mm ID column. As indicated, columns packed with >40 μm particles exhibit relatively low shear rates of less than 1000 sec^{-1}. For high performance packings of 10 μm, the shear rate ranges from $4\text{-}8 \times 10^3$ sec^{-1}. Packings of 5 μm, which represent about the smallest D_p of commercial SEC packings, will generate shear rates between 0.8 to 1.6×10^4 sec^{-1}.

TABLE 1

Estimated Shear Rates Produced in Injection Valves*

Sample Loop Capacity (µl)	ID, inches	$\dot{\gamma}$, sec^{-1}
10	0.012	6×10^3
20 to 100	0.020	1.3×10^3
Passages in Rotor Seal	0.018	1.8×10^3

*Assume 1 ml/min flow rate. Dimensions are for a Rheodyne 70-10 sample injection valve. Calculated using Equation 3 and setting ϵ to unity.

TABLE 2
SHEAR RATE PRODUCED IN DIFFERENT DIAMETER CAPILLARY TUBING AT 1 ML/MIN

ID, inches	$\dot{\gamma}$, sec^{-1}
0.01	1.0×10^4
0.02	1.3×10^3
0.03	3.8×10^2
0.04	1.6×10^2

The effect of flow rate on shear rate for 4-mm diameter columns packed with 5, 10, and 20 µm particles is shown in Figure 2. For 10-µm packings, the shear rate can be kept below 1×10^4 sec^{-1} by employing flow rates less than 1 ml/min. For flow rates less than 0.1 ml/min, shear rates of $<1 \times 10^3$ sec^{-1} can be achieved for both 5 and 10-µm diameter packings.

As will be discussed, a shear rate below about 1×10^4 sec^{-1} is probably sufficiently low to avoid shear degradation of most polymers of $<1 \times 10^6$ molecular weight. Thus for

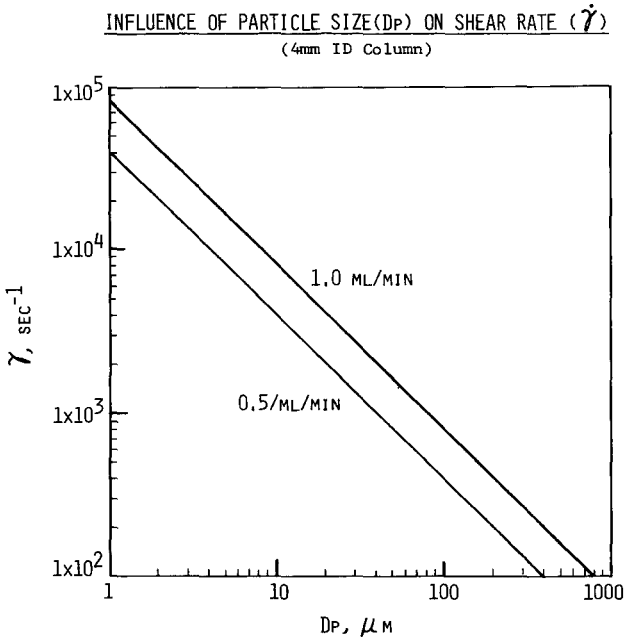

FIGURE 1. Shear rate generated in a 4-mm ID column versus particle diameter at 0.5 and 1.0 ml/min flow rates. Shear rates were calculated using Equation 3.

high performance packings of 10μm, flow rates of less than 1 ml/min are recommended for 4mm-ID columns. For ultrahigh molecular weight (>1 x 10^6) samples, flow rates <0.1 ml/min may be necessary. Because of the inverse logarithmic relationship between D_p and shear rate (Figure 1), the use of SEC packings much lower than 5μm is questionable. These high efficiency packings should be used only for lower molecular weight polymers to avoid shear degradation. Obviously, lower velocities can be easily obtained using wider diameter columns. For decreased analysis times, short columns at low flow rates

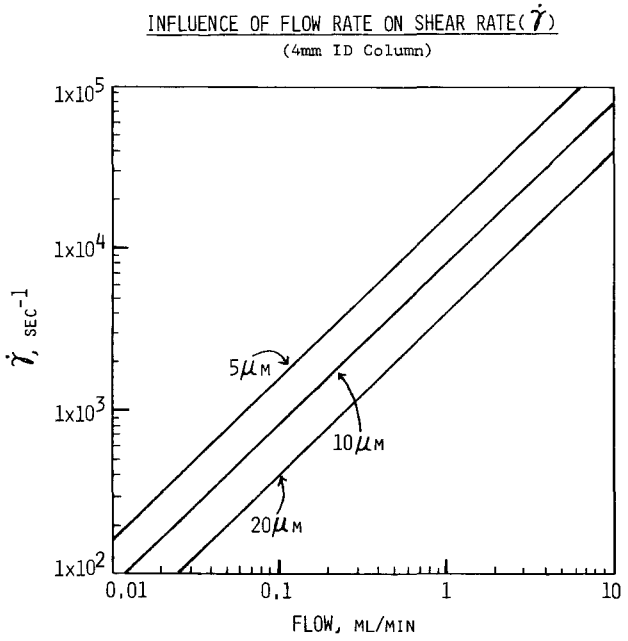

FIGURE 2. Shear rate versus flow rate produced in 4-mm ID columns packed with 5, 10, and 20μm diameter particles. Shear rates were calculated using Equation 3.

may be optimal. For an excellent discussion of the influence of particle size on both critical molecular weight and column efficiency see Giddings (25). Also, as reported by Giddings, elongational strain rates may have a more pronounced effect on polymer chain rupture than shear rate.

The calculated shear rates found in column frits are relatively high. Thus, low porosity frits should be avoided. If possible, 5μm or larger frits should be employed. Capillary tubing appears to be a major potential source of shear

degradation. Since most high performance injectors, detectors, and column connections utilize 0.01 inch ID capillary tubes to reduce band broadening, fairly high shear rates can be produced in these systems. As shown in Table 2, capillary tubes of 0.02 inches ID or greater should be employed when analyzing high molecular weight samples. It should be emphasized that capillary tubing incorporated into detectors can also be a significant source of shear. For example, the Waters 401 refractometer utilizes 0.009 inch ID tubing for the detector inlet. This would generate 1.4×10^4 sec^{-1} at 1 ml/min flow.

A comparison of shear rates in a high performance versus a conventional SEC system is shown in Table 3. As indicated, shear rates generated in high performance systems are one to two orders of magnitude higher.

CRITICAL MOLECULAR WEIGHTS AND SHEAR RATES

It should be emphasized that the shear rates estimated for high performance SEC should serve only as a rough guide when relating these values to critical shear rates (or stress) and critical molecular weights established in other types of shear fields, e.g., capillary tubes, concentric cylinders, or high speed stirring. The hydrodynamics associated with an SEC system, from injector to detector, are highly complex and dependent on the geometry of flow channels, structure of the packed bed, production of local points of turbulence, and

Table 3

Comparison Of Shear Rates Generated In
High Performance Versus Conventional SEC Systems

	$\dot{\gamma}$, sec^{-1}	
Source of Shear	High Performance(1)	Conventional(2)
Capillary Tubing	1×10^4	1.6×10^2
Frits	7.8×10^3	2.2×10^2
Column	8×10^3	3.6×10^2

(1) Flow: 1 ml/min; column: 4 mm ID; D_p: 10 μm; capillary: 0.01 inch ID; frit: 2μm; R_h: 1.86μm (velocity through column: 0.37 cm/sec).

(2) Flow: 1 ml/min; column: 7.9 mm ID; D_p: 56 μm; capillary: 0.04 inch ID; frit: 10μm; R_h: 10.4 μm (velocity through column: 0.095 cm/sec).

inhomogeneity of shear fields. For example, if turbulence were present, different segments of the chain may be caught in eddies moving in various directions thus producing additional strain on the polymer. Thus, the shearing conditions in SEC are very difficult to define.

There appears to be no adequate model to predict shear sensitivity of a given polymer (4). Critical molecular weights and shear rates must be determined experimentally (25). Even when these values are known, they are highly dependent on experimental conditions and the technique used to generate the shear rate or stress (2). Furthermore, it has been proposed that entangled chains rather than individual molecules are more

shear sensitive because of the concentration of energy at junction points (9, 11, 19).

Frenkel (2), who did not take into account entanglements, estimated that the critical shear rate for a polymer of degree of polymerization of 10^3 is 10^5 sec^{-1}. From experimental work and thermodynamic considerations, Morris and Schnurmann (20) reported that shear rates of more than 1.6×10^6 sec^{-1} would be required for shear degradation of hydrocarbon polymers of molecular weights of 2.5×10^4 and less. At 10^5 sec^{-1} under laminar flow conditions, polymers of molecular weights $>10^5$ were degraded.

DEGRADATION OF POLYMERS DURING SEC

Surprisingly, there have been only a relatively small number of papers describing shear degradation of polymers in SEC (19, 21-26). Slagowski et al. (21) have demonstrated that both 4.37×10^7 and 2.73×10^7 molecular weight polystyrene degrades during SEC using a conventional system (Styragel) at flow rates as low as 0.25 ml/min ($\dot{\gamma} = 95$ sec^{-1}). A chromatographed sample of the 4.37×10^7 polystyrene run at 1 ml/min ($\dot{\gamma} = 380$ sec^{-1}) was collected and from viscosity measurements was found to have a reduced viscosity of 25.6 dl/g as compared to 64 dl/g for the initial sample. At 1 ml/min, a 9.6×10^6 molecular weight polystyrene showed no apparent degradation as determined from elution volume measurements.

Using high performance SEC, Kirkland (18) has shown that 7.1×10^6 polystyrene was shear degraded above 0.1 cm/sec ($\dot{\gamma} = 2.7 \times 10^3$ sec^{-1}) using 8μm-silica particles.

Rooney and VerStrate (19) employed an on-line, low-angle laser light-scattering detector (LALLS) to study polymer degradation in SEC columns (μBondagel and Shodex 800 series) and found that polystyrene and several polyolefins of molecular weight $\geq 6.7 \times 10^5$ degraded above 0.5 ml/min flow rate. These authors also undertook a brief study of the effect of polymer concentration on shear degradation. Although they claim to see a small concentration dependency, the scatter about the data points appeared to be too high to warrant any conclusions. Their results suggest that shear rates as low as $2.7\text{-}6.4 \times 10^3$ sec^{-1} (calculated from Equation 3) were sufficient to obtain noticeable shear degradation of polymers $\geq 6.7 \times 10^5$ molecular weight. It should be noted, however, that the $\bar{M}_w$ results obtained at flow rates of 1.0 ml/min and greater may be in error because of excess light scattering presumably caused by thermal and/or flow inhomogeneities in the detector cell. In addition, contributions from peak broadening generated within the column and between the LALLS and differential refractometer may have complicated the results (28).

Huber and Lederer (26) measured the $\bar{M}_w$ of 1×10^6 molecular weight poly(isobutylene) as a function of flow rate employing a LALLS detector and Styragel columns. Polymer

degradation was first noticeable at flow rates greater than 10 ml/hr ($\dot{\gamma}$ = 77 sec^{-1}). At a flow rate of 2 ml/min (930 sec^{-1}), $\bar{M}_w$ decreased by almost a factor of two. It should be noted, as described above, that no band broadening corrections were employed; thus the $\bar{M}_w$ data obtained on-line may be in error.

Using both Shodex and silica high performance columns (8 mm ID), Ye and Shi (23) studied shear degradation effects of polystyrene calibrants of molecular weights of 2.7 x 10^6 to 7 x 10^6 using a flow rate of 2 ml/min. (Equivalent to 0.5 ml/min for a 4-mm ID column.) Measurements were made using an off-line LALLS photometer and intrinsic viscosity measurements of collected samples. No molecular weight decrease was observed for the Shodex column; whereas, for the silica column (particle size not given), some degradation was obvious. The authors claim that since the pressure across the silica column was greater than for the Shodex column, pressure was a contributing factor.

Rand and Mukherji (24) investigated polystyrene shear degradation using two high-performance silica columns, μBondagel (4mm ID) and Zorbax Bimodal columns (6.2 mm ID), and an on-line LALLS detector. Although there was scatter in the data, it appeared that shear degradation had occurred for 3 x 10^6 and 7 x 10^6 molecular weight polystyrene standards at flow rates as low as 0.5 ml/min. Little shear degradation of

polystyrenes in the molecular weight range of 5 x 10^4 to 1.8 x 10^6 was evident at flow rates of 0.5–1.5 ml/min using either of the silica columns.

Giddings (25) developed a relationship between the critical molecular weight of a polymer and shear stress in packed beds and uniform channels. Polyacrylamide ($\bar{M}_w$ ~6.2 x 10^6) was used as the test polymer and shear degradation was determined by measuring the reduction of intrinsic viscosity of the polymer after it passed through a column packed with controlled-pore glass (70μm). The experiment consisted of pumping two column volumes of polymer solution (0.05 and 0.10 wt%) through the column. Substantial viscosity loss was apparent at velocities as low as 0.025 cm/sec. Polymer degradation using nonporous glass particles of the same size as the SEC packing was about 25% lower, suggesting that polymer chains are more prone to rupture as they diffuse into and out of pores. Giddings also presented several useful equations that can be used to establish guidelines for avoiding polymer shear degradation and, at the same time, maximizing column efficiency.

In our laboratory, we have observed shear degradation of polystyrene calibrants using Zorbax PSM silica packings. Figure 3 is a polystyrene calibration curve obtained on a set of Du Pont Zorbax Bimodal columns using THF as the mobile phase. The backward curve of the high-molecular-weight end of the

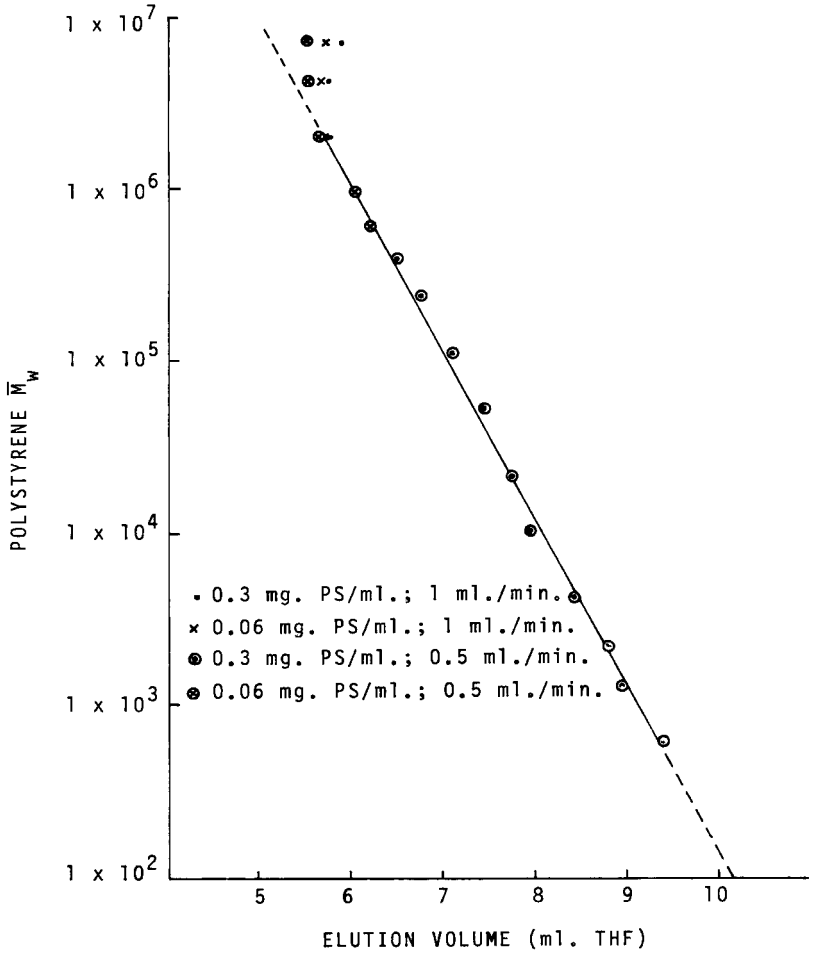

FIGURE 3. Polystyrene calibration curve showing the effect of sample concentration and flow rate on elution volume. A Zorbax PSM 60-S and PSM 1000-S column-set (6.2mm diameter) was used with tetrahydrofuran as the mobile phase and 100-µl injection volumes.

polystyrene calibration curve could be indicative of shear degradation. The 7 x 10^6 molecular weight polystyrene peak elutes near the 9.5 x 10^5 peak, and the 4.1 x 10^6 polystyrene elutes between 1 x 10^6 and 2 x 10^6 molecular weight polystyrene standards. The amount of shear degradation increased with mobile phase flow rate and with polymer concentration. It should be recognized, however, that other flow rate and concentration dependent effects could contribute to these observations.

CONCLUSIONS

Because of the complex hydrodynamics involved in SEC systems, it is very difficult to arrive at a simple correlation between operational parameters, such as flow rate and particle size, and onset of shear degradation. Also, the dependency of shear degradation on polymer concentration, chemical structure, and solvent further complicates this issue. Nevertheless, guidelines can be established, based on published data and estimated shear rates produced in various parts of an SEC system.

Because of the appearance of degradation with conventional columns in which very low $\dot{\gamma}$ are produced within the column, it is obvious that degradation may be occurring in capillary tubing associated with the system. As suggested by Giddings (25), elongational forces within the packed bed may have a more pronounced effect on polymer degradation than do tangential

shear forces. Because of the high shear rates that can be generated in high-performance columns, it is advisable to measure elution profiles of samples as a function of flow rates. If an on-line light scattering photometer is available, the occurrence of degradation can be more readily determined. However, one should be aware of elution volume-flow rate dependence (27) and additional band broadening that can occur between concentration and LALLS detectors used in series (28).

Capillary tubing of large ID should also be employed providing that column efficiency is not sacrificed. Since HPLC detectors contain small (<0.010 inch) ID tubing, alternative detection systems may be required. Because of the inverse relationship between shear rate and particle radius, it is doubtful that small diameter SEC packings (<10μm) would prove useful for the analysis of high molecular weight polymers unless short, wide columns are used to obtain sufficiently low flow rates at acceptable analysis times.

Finally, since shear degradation of polymers may occur more readily with increased polymer concentration, fairly dilute polymer solutions may be required. The use of dilute solutions is also needed to avoid chromatographic effects such as macromolecular crowding and viscous fingering.

As can be seen, a significant amount of work is needed before we can fully understand and predict the extent of polymer degradation in chromatographic systems.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1739-1751 (1984)

MOLECULAR WEIGHT CALIBRATION IN STERIC EXCLUSION CHROMATOGRAPHY OF DIBLOCK COPOLYMERS OF POLYSTYRENE AND POLY(ETHYLENE OXIDE)

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ABSTRACT

The molecular weight calibration curve in steric exclusion chromatography of a diblock copolymer of polystyrene and poly (ethylene oxide) is obtained by a method involving the universal calibration principle. The method is developed from the experimental observation that calibration curves for homopolymers of polystyrene and poly(ethylene oxide) in N,N-dimethylacetamide at 353 K are parallel. It is assumed that the size of the diblock copolymer in solution is linearly related to the sizes of the corresponding homopolymers. The method requires the experimental determination of copolymer composition. Reasonable results for the number average molecular weights of diblock copolymers were obtained with this calibration method.

INTRODUCTION

Grubisic, Rempp and Benoit (1) suggested that hydrodynamic volume can be used for universal calibration in steric exclusion

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chromatography (SEC), showing that a plot of $\log[\eta]M$ against retention volume V_R (in counts) was the same for homopolymers and copolymers having various structures. Here, $[\eta]$ is the intrinsic viscosity (dl g^{-1}) of the polymer in the SEC solvent and M is the molecular weight of the polymer. At a given V_R, it is assumed that all polymers have the same value of $[\eta]M$ so that we can write

$$\log[\eta]_{ps} M_{ps} = \log[\eta]_p M_p \quad (1)$$

where ps refers to a calibration established experimentally with polystyrene standards and p to the calibration for the polymer requiring analysis. It is also assumed that the column combination, solvent and temperature remain constant. Equation (1) therefore permits the determination of M_p from an experimental polystyrene calibration. This may be accomplished with an on-line viscometric detector (2) by establishing the dependence of $[\eta]_{ps}$ and $[\eta]_p$ on V_R as a polymer elutes from the chromatograph. Alternatively, if a viscometric detector is not available, $[\eta]$ and M are related by the Mark-Houwink equation given by

$$[\eta] = KM^{\alpha} \quad (2)$$

in which K and α are constants for a particular homopolymer-solvent-temperature system. Substitution of equation (2) for homopolymers ps and p into equation (1) and rearrangement gives

$$\log M_p - [(1+\alpha_{ps})/(1+\alpha_p)]\log M_{ps} = [1/(1+\alpha_p)]\log(K_{ps}/K_p) \quad (3)$$

In the molecular weight characterisation of block copolymers, a viscometer may be included in a multidetector system comprising one or more concentration detectors selected from refractive index, ultraviolet and infrared detectors (3), so that the

dependence of the intrinsic viscosity $[\eta]_c$ of the block copolymer on V_R may be established. Provided that hydrodynamic volume is a valid universal calibration parameter for a block copolymer (4), then the molecular weight calibration M_c for the block copolymer may be calculated with the equation

$$\log M_c - \log M_{ps} = \log[\eta]_{ps}/[\eta]_c \qquad (4)$$

from an experimental calibration for polystyrene, as in equation (1).

If no viscometer detector is available, then the relation for log M_c against V_R has to be derived from the experimental M_{ps} and M_p calibration curves. Tung and co-workers (5,6) proposed a calibration method which assumed that the size of the copolymer molecule is the sum of the two segments of the molecule considering each block to behave as a separate homopolymer. Their method involved a simple expression for log M_c in terms of the homopolymer calibrations log M_{ps} and log M_p which were weighted according to the copolymer composition. Tung and co-workers (5,6) also reported that their experimental calibration curves M_{ps} and M_p were parallel. Chang (7,8) proposed a molecular weight calibration method for block copolymers, requiring homopolymer calibrations and Mark-Houwink constants as defined in equation (3). Chang (7,8) observed that his method in practice would often involve parallel calibration curves for the homopolymers. This follows because many SEC separations are performed with good solvents for homopolymers (9-11), when $\alpha_{ps} \simeq \alpha_p$ so that the right hand side of equation (3) becomes a shift factor between the parallel M_p and M_{ps} calibration curves. When polymers in good solvents have similar polymer-solvent interactions, then this shift factor for universal calibration may be considered in terms of the unperturbed mean-square end-to-end distance $\langle r^2 \rangle_0$ of a polymer (10-12). In this paper we demon-

strate how universal calibration based on $\langle r^2 \rangle_o$ may be extended to the determination of the calibration M_c for diblock copolymers of polystyrene and poly(ethylene oxide) designated PS-PEO.

CALIBRATION METHOD

The intrinsic viscosity for a polymer solution may be expressed in terms of $\langle r^2 \rangle_o$ for a polymer (9-11) according to the Flory-Fox equation

$$[\eta] = \Phi[\langle r^2 \rangle_o/M]^{3/2} \alpha^3 M^{1/2} \quad (5)$$

where α is the linear expansion factor and Φ is the viscosity constant. Substituting equation (5) into equation (1) and rearranging gives

$$\log M_p - \log M_{ps} = \log[\langle r^2 \rangle_o/M]_{ps}[M/\langle r^2 \rangle_o]_p + \log[\alpha_{ps}^2/\alpha_p^2] \quad (6)$$

assuming that Φ is the same for two polymers when $\alpha_{ps} = \alpha_p$. Results have been presented showing that $\alpha_{ps} \sim \alpha_p$ for homopolymers in good solvents when polymer-solvent interactions are very similar (13). Consequently, equation (6) may be simplified to

$$\log M_p - \log M_{ps} = \log[\langle r^2 \rangle_o/M]_{ps}[M/\langle r^2 \rangle_o]_p \quad (7)$$

Since $\langle r^2 \rangle_o/M$ is a characteristic constant for a homopolymer whose conformation may be represented by a random coil, the shift factor on the right hand side of equation (7) is easily calculated.

Recent studies of diblock copolymers in solution suggest that the conformation may be considered to be that for homopolymers (14,15). Thus, molecular size for an individual block, represented by the mean-square radius of gyration, is the same in the

block copolymer as that in the equivalent homopolymer, i.e. the molecular size of a block in the diblock copolymer is not affected by the presence of the second block. Consequently, the molecular size of a diblock copolymer in solution may be linearly related to the sizes of the homopolymers (16) according to the relation

$$[\langle r^2\rangle_o/M]_c = W_s[\langle r^2\rangle_o/M]_{ps} + (1 - W_s)[\langle r^2\rangle_o/M]_p \qquad (8)$$

where W_s is the weight fraction of styrene in the diblock copolymer. By analogy with the derivation of equation (7) from equation (1), it can be shown that equation (4) for a diblock copolymer may be transformed to

$$\log M_c - \log M_{ps} = \log[\langle r^2\rangle_o/M]_{ps}[M/\langle r^2\rangle_o]_c \qquad (9)$$

where $[\langle r^2\rangle_o/M]_c$ may be calculated from equation (8) as long as the copolymer composition has been determined.

EXPERIMENTAL

Block Copolymers and PEO Homopolymers

All polymers were prepared by anionic polymerisation techniques in order to produce samples with narrow molecular weight distributions. Solvents and monomers were extensively dried and purified, and the polymerisations were performed under conditions of rigorous purity using a high vacuum technique. Ampoules containing the various reactants were equipped with breakseals and were sealed onto an all-glass reactor similar to a reactor described previously (17).

The synthesis of PS-PEO diblock copolymers involving the formation of polystyrylpotassium in tetrahydrofuran followed by the addition of ethylene oxide was performed according to the method described by O'Malley and Marchessault (18). Polymeri-

sations were initiated with cumylpotassium. The synthesis of this initiator involved firstly the preparation of methyl cumyl ether from α-methyl styrene, methanol and hydrochloric acid, and secondly the reaction of this ether with sodium-potassium alloy in tetrahydrofuran. A predetermined volume of a standardised solution of cumyl potassium in tetrahydrofuran was placed into an all-glass ampoule which was evacuated and sealed. Both monomers and methanol were rigorously dried before sealing in ampoules. Tetrahydrofuran which had been dried with calcium hydride, disodium (α-methyl styrene tetramer), and a sodium mirror was distilled into the reactor. Polystyrylpotassium was formed by reaction at 273 K for 30 min. and then initiated the polymerisation of ethylene oxide which was performed at room temperature for 3-4 days before terminating with methanol. Block copolymer was recovered by precipitating the reaction mixture in a five-fold excess of 60/80 petroleum ether. Similar procedures were used to prepare PEO homopolymers by the initiation of the anionic polymerisation of ethylene oxide with cumylpotassium in tetrahydrofuran using an all-glass reactor. Copolymer composition was determined from infrared measurements with a Perkin Elmer 457 spectrometer. Calibration plots of absorbance versus concentration were obtained for absorption peaks at 700 cm^{-1} (PS) and 1105 cm^{-1} (PEO) with solutions of the homopolymers dissolved in trichloroethylene. From the spectrum for each PS-PEO diblock copolymer in trichloroethylene and the calibration plots, the weight fraction of each block was calculated. Trichloroethylene (SLR, Fisons, with 0.2% triethylamine added) was destabilised by shaking with 10% v/v hydrochloric acid, washed three times with distilled water, and dried by stirring with fused calcium chloride for 30 min. before distillation, taking the middle fraction for use.

Steric Exclusion Chromatography

PS-PEO diblock copolymers and PEO homopolymers were characterised by SEC with a Waters Associates model 200 chromatograph

at ICI Organics Division, Blackley, Manchester. A series arrangement of columns containing crosslinked polystyrene gels was used at 353 K with N,N-dimethylacetamide as solvent as described in the paper by Dawkins and Hemming (19). The molecular weight calibration for polystyrene was established with standards supplied by Pressure Chemical Co., Pittsburgh, and Waters Associates. The molecular weight calibration for poly (ethylene oxide) was established with PEO homopolymers prepared by anionic polymerisation and with samples of poly(ethylene glycol) designated PEG assuming values of molecular weight provided by the suppliers (Shell Chemicals, BDH, ICI). The PEO homopolymers were characterised by measuring the solution viscosity of PEO in water with an Ubbelohde-type viscometer at 303 K. Data at several polymer concentrations were extrapolated linearly by the Huggins and Kraemer plots to find $[\eta]$ at infinite dilution. The value of the viscosity average molecular weight $\bar{M}_v$ of PEO for plotting the GPC calibration was calculated from the relation reported by Bailey and co-workers (20)

$$[\eta] = 1.25 \times 10^{-4} \bar{M}_v^{0.78} \qquad (10)$$

The polydispersity, defined as the ratio of weight and number average molecular weights $\bar{M}_w/\bar{M}_n$, was calculated from the chromatograms of the PEO homopolymers without corrections for chromatogram broadening. Values of $\bar{M}_w/\bar{M}_n$ were below 1.3, so that construction of a PEO calibration curve with $\bar{M}_v$ by the peak retention volume procedure should be accurate (10).

RESULTS AND DISCUSSION

Experimental calibration curves for PS standards, PEO homopolymers and PEG samples are shown in Figure 1. Straight line behaviour was assumed for the range of V_R from 16.5 to 22.0

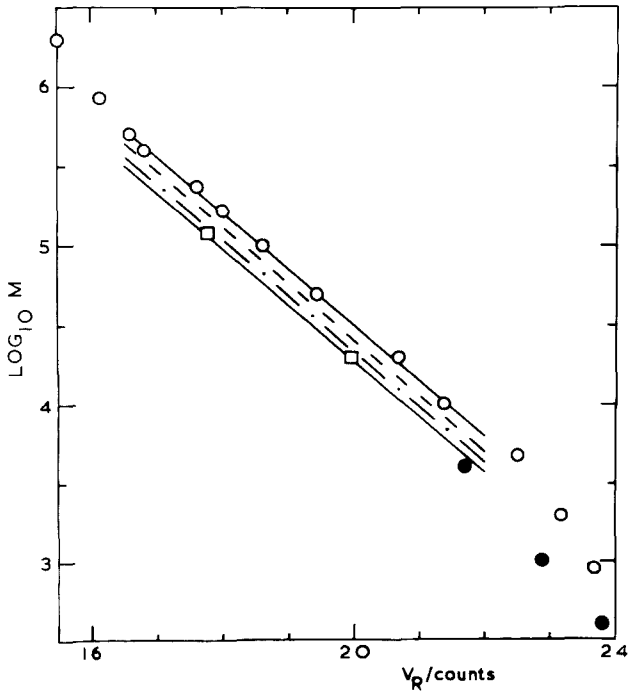

FIGURE 1

Molecular weight calibration plots for N,N-dimethylacetamide at 353 K. ○ polystyrene standards, □ poly(ethylene oxide) homopolymers, ● poly(ethylene glycol) samples. —□—□— predicted M_p calibration for PEO with equation (7) and a shift factor $\log_{10}$ 0.593. --- predicted M_c calibration for PS-PEO (W_s = 0.68) with equation (9) and a shift factor $\log_{10}$ 0.82. ····· predicted M_c calibration for PS-PEO (W_s = 0.37) with equation (9) and a shift factor $\log_{10}$ 0.70.

counts. The calibration curves for PS and PEO homopolymers may be related with equation (7). The value of $[\langle r^2\rangle_o/M]_{ps}^{1/2}$ was assumed to be 0.67 Å, as in previous universal calibration studies (9,12,19). The calibration curve for PEO predicted with equation (7) superimposes with the experimental data for PEO in Figure 1 when the shift factor is $\log_{10}$ 0.593, so that $[\langle r^2\rangle_o/M]_p^{1/2}$ for PEO is then 0.87 Å. This value is very close to the value of 0.84 Å reported by Beech and Booth (21). The difference between these two values of $[\langle r^2\rangle_o/M]_p$ is close to experimental error. The shift factor in equation (7) is presumed to be unaffected by possible small differences in α_{ps} and α_p in equation (6) for PS and PEO in N,N-dimethylacetamide. The results indicate that equation (7) is a satisfactory method for determining the calibration curve for PEO homopolymers. Therefore, it is reasonable to assume that the calibration curves for PS and PEO homopolymers are parallel which is a requirement in the procedure for determining the calibration curve for a PS-PEO diblock copolymer.

In the synthesis of a PS-PEO diblock copolymer, part of the polystyrylpotassium was removed from the polymerisation reactor and deactivated with methanol. A chromatogram for such a sample which corresponds therefore to the PS block is shown in Figure 2. A chromatogram for the PS-PEO diblock copolymer resulting from the same polystyrylpotassium is also shown in Figure 2. The positions of these chromatograms on the V_R axis clearly indicate the success of the sequential polymerisation procedure for forming PS-PEO and also indicate that there is no measurable PS homopolymer in the sample of diblock copolymer. Residual PS homopolymer would have arisen from premature termination of some of the polystyrylpotassium on addition of ethylene oxide monomer, generating a second peak at V_R = 20.15 counts in the chromatogram for the diblock copolymer in Figure 2. If it is assumed that the sample of diblock copolymer does not contain homopolymer, then

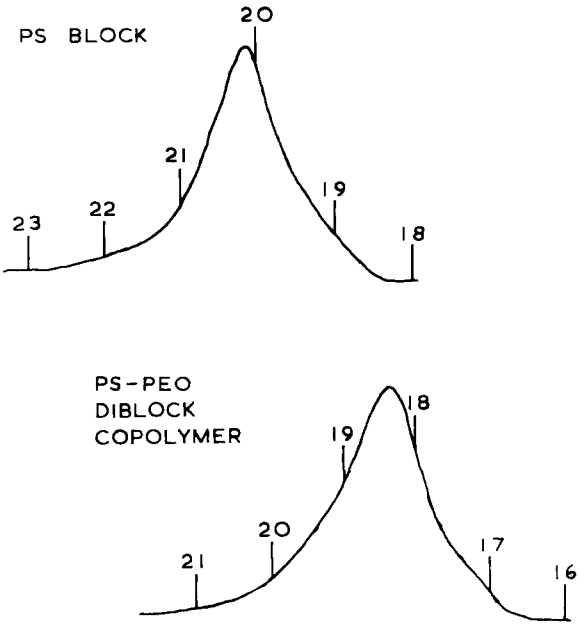

FIGURE 2

Chromatograms for diblock copolymer PS-PEO-4 and the PS block from elution with N,N-dimethylacetamide at 353 K.

values of $\bar{M}_n$ (PS) and $\bar{M}_n$ (PS-PEO) for the polystyrene block and the diblock copolymer respectively are related by

$$W_s = \bar{M}_n \text{ (PS)} / \bar{M}_n \text{ (PS-PEO)} \qquad (11)$$

A value of $\bar{M}_n$ (PS) is determined from the chromatogram for the polystyrene sample in Figure 2 with the PS calibration curve in Figure 1. Since W_s is known from infrared spectroscopy measurements, the value of $\bar{M}_n$ (PS-PEO) may be estimated with equation (11), and results are given in Table 1.

SEC calibration curves for PS-PEO diblock copolymers were determined with equation (9), having calculated $[\langle r^2 \rangle_0 / M]_c$ with

TABLE 1

Characterisation Data for PS-PEO Diblock Copolymers

Copolymer	W_s	$\bar{M}_n$(PS)	$\bar{M}_n$(PS-PEO)	$\bar{M}_n$(SEC)	$\bar{M}_w/\bar{M}_n$
PS-PEO-1	0.72	36200	50300	53900	1.40
PS-PEO-2	0.68	43900	64600	64500	1.50
PS-PEO-3	0.42	36200	86200	76100	1.44
PS-PEO-4	0.37	22100	59700	54900	1.47

equation (8) employing $[\langle r^2\rangle_o/M]_{ps}^{1/2} = 0.67$ Å, $[\langle r^2\rangle_o/M]_p^{1/2} = 0.87$ Å for PEO, and the values of W_s given in Table 1. The copolymer calibrations M_c are parallel to and between the curves for the homopolymers, as shown by the selected copolymer examples in Figure 1. With the calibration curve and chromatogram for a diblock copolymer, values of $\bar{M}_n$(SEC) and the polydispersity were calculated and are listed in Table 1. The SEC method gives values of $\bar{M}_n$ for the copolymers in fair agreement with $\bar{M}_n$(PS-PEO) obtained with equation (11).

In summary, the proposed calibration method is simple to use and provides reasonable values of molecular weight for PS-PEO diblock copolymers. It is likely that many SEC separations of homopolymers and copolymers will be performed with good solvents having similar polymer-solvent interactions because solute-gel interactions are more likely when the eluent becomes less compatible with the gel and when the eluent is a poor or theta solvent for the polymeric solute (22). In this work we have assumed no change in copolymer composition with V_R. It follows from equation (8) that when W_s varies across the chromatogram the M_c calibration calculated with equation (9) must be non-parallel to the calibration curves for the homopolymers. Results for diblock

copolymers having a copolymer composition distribution will be reported in a separate paper.

ACKNOWLEDGEMENTS

The authors thank the Science Research Council for the award of a research studentship.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1753-1767 (1984)

MOLECULAR WEIGHT CALIBRATION OF SEC USING BROAD MWD STANDARDS-APPLICATION FOR POLY (P-METHYL STYRENE)

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ABSTRACT

Broad molecular weight distribution samples of poly (p-methyl styrene) were synthesized using free radical polymerization with thermal initiation over a range of temperatures, 120° - 160°C. The weight-average molecular weights (M_W) of these polymers were measured by low angle laser light scattering photometry (LALLSP) to provide broad MWD standards. Two broad MWD standards were then used to determine the molecular weight calibration curve for poly (p-methyl styrene) using the universal molecular weight calibration curve found using narrow MWD polystyrene standards. SEC was then used to measure the M_W values for the remaining poly (p-methyl styrene) samples. The M_W values by LALLSP and SEC were in excellent agreement confirming the validity of the broad MWD standards calibration method.

INTRODUCTION

Methods of molecular weight calibration using broad MWD standards are of three basic types. Those which employ a broad MWD standard with known molecular weight distribution [1-5]. Those which employ one or more broad MWD standards with known M_N, M_W or $[\eta]$ and assume a linear molecular weight calibration curve [6-9] and finally those which employ one or more broad MWD standards and use the universal molecular weight calibration curve obtained with narrow MWD polystyrene standards [10,11].

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The present investigation makes use of two broad MWD standards and the universal molecular weight calibration curve based on narrow MWD polystyrenes.

THEORY

Let the molecular weight calibration curve for polystyrene be given by

$$M_s = \phi(v) \tag{1}$$

where M_s is the molecular weight of polystyrene and $\phi(v)$ is some arbitrary function of retention volume, v. The universal molecular weight calibration curve can now be given by

$$[\eta]M = K_s \phi(v)^{1+\alpha_s} \tag{2}$$

where $[\eta]$ is the intrinsic viscosity, M molecular weight, K_s and α_s are Mark-Houwink constants for polystyrene. The molecular weight calibration curve for a second polymer such as poly (p-methyl styrene) may be expressed as

$$M_x = A\phi^{\beta}(v) \tag{3}$$

where M_x is the molecular weight of the second polymer and

$$A = (K_s/K_x)^{1/(1+\alpha_x)} \tag{4}$$

$$\beta = (1+\alpha_s)/(1+\alpha_x) \tag{5}$$

where K_x and α_x are Mark-Houwink constants for the second polymer.

We now consider a mass concentration detector and assume that either correction for peak broadening is negligible or that the normalized detector response, F(v) has been suitably corrected for broadening. The weight- average molecular weights of 2 broad MWD standards of the second polymer measured by SEC are given by

$$M_{w_1} = A \int_0^{\infty} F_1(v)\, \phi^{\beta}(v)\, dv \tag{6}$$

$$M_{w_2} = A \int_0^{\infty} F_2(v)\, \phi^{\beta}(v)\, dv \tag{7}$$

Setting M_{w_1} and M_{w_2} equal to those values already measured by light scattering, we now have 2 equations for the 2 unknowns, A and β. Dividing equation (6) by equation (7) gives

$$M_{w_1}/M_{w_2} = \int_0^\infty F_1(v)\phi^{\beta}(v)\,dv \Big/ \int_0^\infty F_2(v)\phi^{\beta}(v)\,dv \tag{8}$$

A single-variable search will provide β and then a direct calculation using either equation (6) or (7) provides A and thus the calibration curve for the second polymer via equation (3). It should be pointed out that the validity of the molecular weight calibration curve for the second polymer does not depend on the validity of the Mark-Houwink constants for polystyrene. However, the validity of the Mark-Houwink constants for the second polymer (found using equations (4) and (5)) does depend on the validity of the polystyrene Mark-Houwink constants. This is inherent in all previous methods of broad MWD standard calibration [11].

Another approach involving broad MWD standards for calibration is to use 2 broad MWD standards with known intrinsic viscosities. The intrinsic viscosities of 2 broad MWD standards measured by SEC are given by

$$[\eta]_1 = K_x \int_0^\infty F_1(v) M_x^{a_x} dv = A^{a_x} K_x \int_0^\infty F_1(v)\phi^{\beta a_x}(v)dv \tag{9}$$

$$[\eta]_2 = A^{a_x} K_x \int_0^\infty F_2(v)\phi^{\beta a_x}(v)dv \tag{10}$$

Given $[\eta]_1$ and $[\eta]_2$ one can solve for $A^{a_x}K_x$ and βa_x. In this case, where whole polymer intrinsic viscosities are used, the validity of the molecular weight calibration curve does depend on the validity of the polystyrene Mark-Houwink constants. The use of this approach to find Mark-Houwink constants K_x and a_x has the same limitations. A procedure which gives both valid molecular weight calibration curve and Mark-Houwink constants for a polymer follows. For this method, 2 broad MWD standards with known M_w are required to find M_x, the molecular weight calibration curve. A knowledge of $[\eta]$ for these two standards or for any other 2 broad MWD standards would then permit one to find valid K_x and a_x using equations (9) and (10). For this method valid Mark- Houwink constants for polystyrene are not required.

In this investigation, the broad MWD standards method involving two M_w, is thoroughly investigated and then applied in the search for the molecular weight calibration curve for poly (p-methyl styrene). The sensitivity of the method was investigated using theoretical distributions and a linear molecular weight calibration curve for polystyrene. This calibration curve for polystyrene is given by

$$M_s = 2.15*10^{10}\exp(-0.357\,v) \tag{11}$$

Using Mark-Houwink constants ($K_s = 1.11*10^{-2}$ and $\alpha_s = 0.723$) for polystyrene provided the following universal molecular weight calibration curve

$$\ell n ([\eta]M) = 36.492 - 0.6151\ v \tag{12}$$

The two broad MWD standards were assumed to have most probable distributions of the form

$$W(M) = M/M^2_N \exp(-M/M_N) \tag{13}$$

Use of the identity

$$W(M)dM = -W(v)dv \tag{14}$$

one can transform this distribution into a SEC mass concentration detector response as follows

$$W(v) = D^2_1 D_2 \exp(-2\ D_2 v) \exp(-(D_1/M_N)\exp(-D_2 v))/M^2_N \tag{15}$$

where

$$M(v) = D_1 \exp(-D_2 v) \tag{16}$$

is the molecular weight calibration curve for the second polymer. After choosing Mark-Houwink constants for the second polymer, one can evaluate D_1 and D_2 using equation (12). To investigate the sensitivity of the method, various Mark-Houwink constants and M_N values were used for the second polymer. It should be noted that $M_w = 2M_N$ for polymers having the most probable distribution. In the computer simulation of sensitivity of the method, two detector responses of the form given by equation (15) were used.

RESULTS AND DISCUSSION

The results of the investigation of sensitivity using computer simulation are shown in Tables 1 and 2.

Figures 1-3 show the chromatograms or detector responses for the cases investigated in Table 1. The recoveries of the Mark-Houwink constants, K_x and α_x for the two methods using pairs of M_w or $[\eta]$ are equivalent and satisfactory when the chromatograms for the standards are not near to overlapping. For the Mw pairs, ($4.0*10^{-5}$, $5.4*10^5$) and ($4.0*10^5$, $4.4*10^5$), the exponent α_x recovered is satisfactory, however, the pre-exponential factor K_x is significantly larger than the true value which is $1.54*10^{-2}$. Errors in the measured detector responses and in M_w and $[\eta]$

TABLE 1

Sensitivity of two broad MWD standards method of calibration found by computer simulation

$K_x = 1.540*10^{-2}$ and $a_x = 0.650$

M_{w_1}	$[\eta]_1$	M_{w_2}	$[\eta]_2$	K_x	a_x
$3.2*10^5$	55.2	$6.8*10^5$	90.1		
X		X		$1.581*10^{-2}$	0.649
	X		X	$1.514*10^{-2}$	0.651
$1.2*10^5$	29.2	$1.20*10^6$	130.0		
X		X		$1.576*10^{-2}$	0.649
	X		X	$1.547*10^{-2}$	0.649
$4.0*10^5$		$5.4*10^5$		$1.614*10^{-2}$	0.647
$4.0*10^5$		$4.4*10^5$		$1.624*10^{-2}$	0.647

TABLE 2

Sensitivity of two broad MWD standards method of calibration found by computer simulation

$K_x = 0.800*10^{-2}$ and $a_x = 0.740$

M_{w_1}	M_{w_2}	K_x	a_x
$3.20*10^5$	$6.80*10^5$	$0.801*10^{-2}$	0.740
$4.0*10^5$	$4.4*10^5$	$0.825*10^{-2}$	0.738
$1.20*10^5$	$1.20*10^6$	$0.799*10^{-2}$	0.740

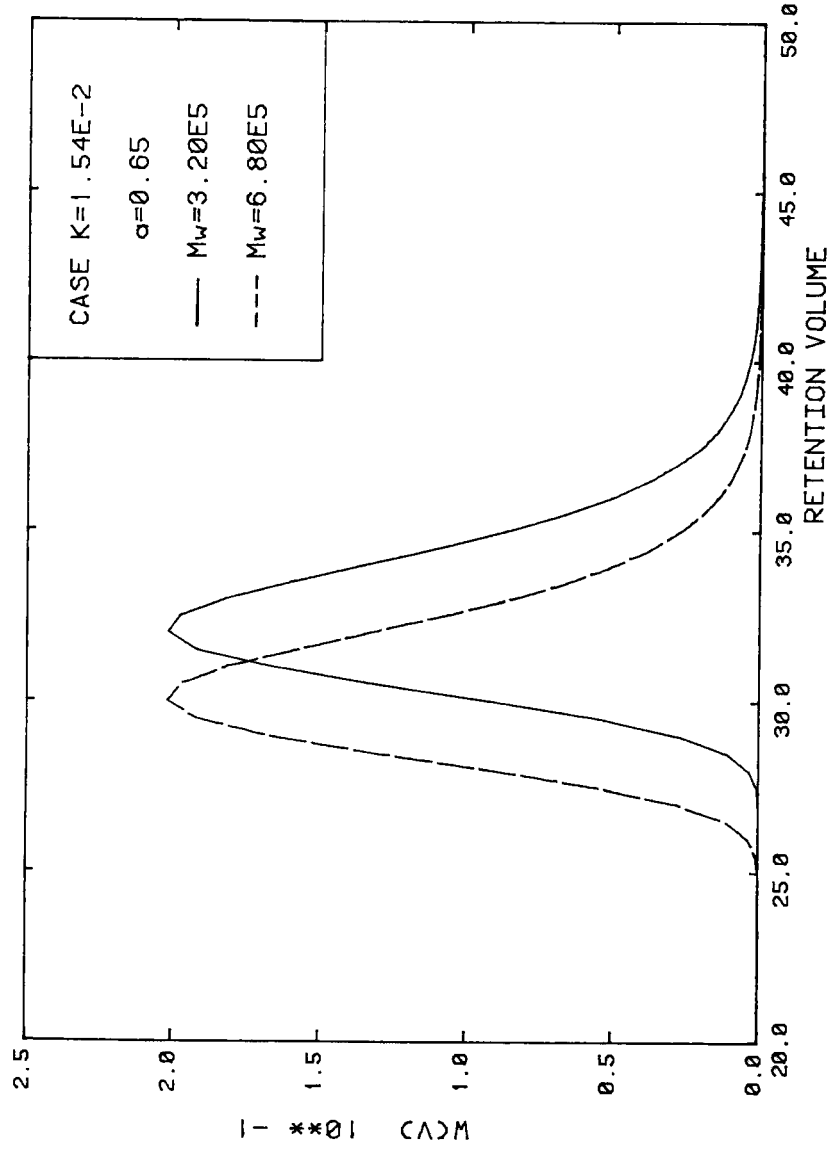
CASE K=1.54E-2
α=0.65
Mw=3.20E5
Mw=6.80E5
W(V) 10** -1
RETENTION VOLUME
0.0
0.5
1.0
1.5
2.0
2.5
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 1

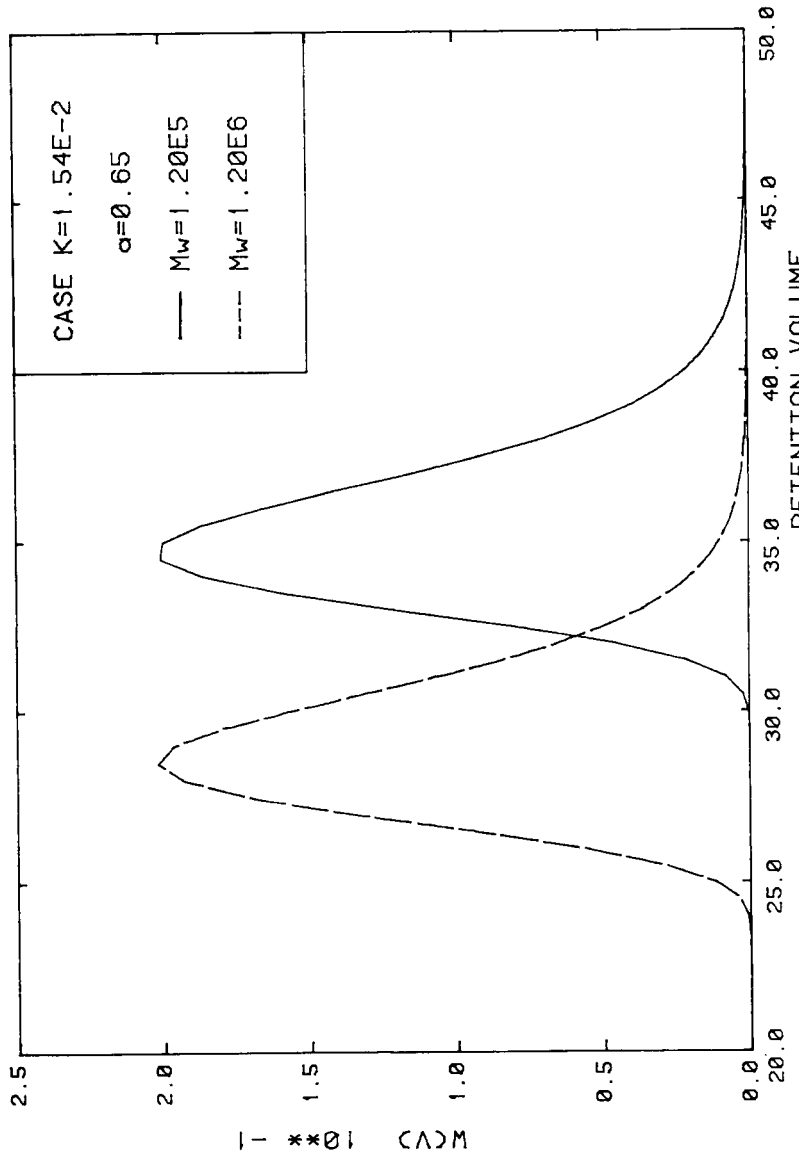
CASE K=1.54E-2
a=0.65
Mw=1.20E5
Mw=1.20E6
W(V) 10** -1
2.5
2.0
1.5
1.0
0.5
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0
RETENTION VOLUME

FIGURE 2

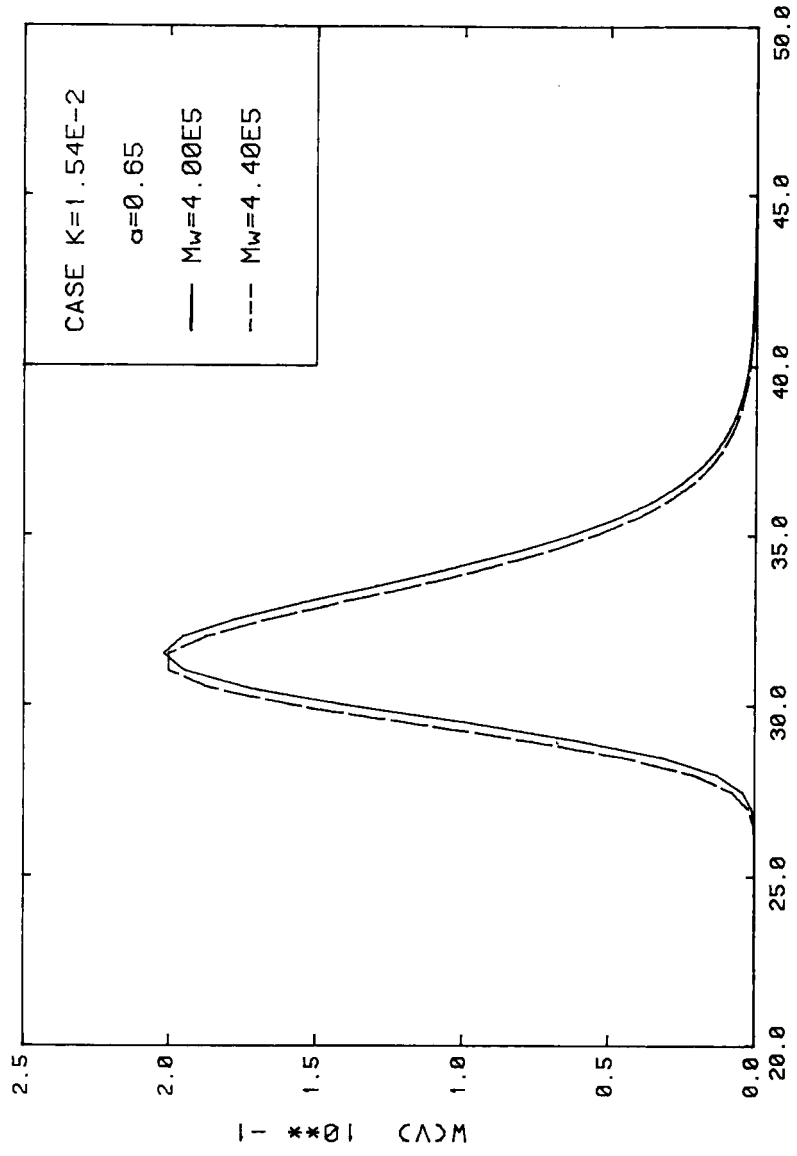
CASE K=1.54E-2
a=0.65
Mw=4.00E5
Mw=4.40E5
W(V) 10** -1
RETENTION VOLUME
2.5
2.0
1.5
1.0
0.5
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 3

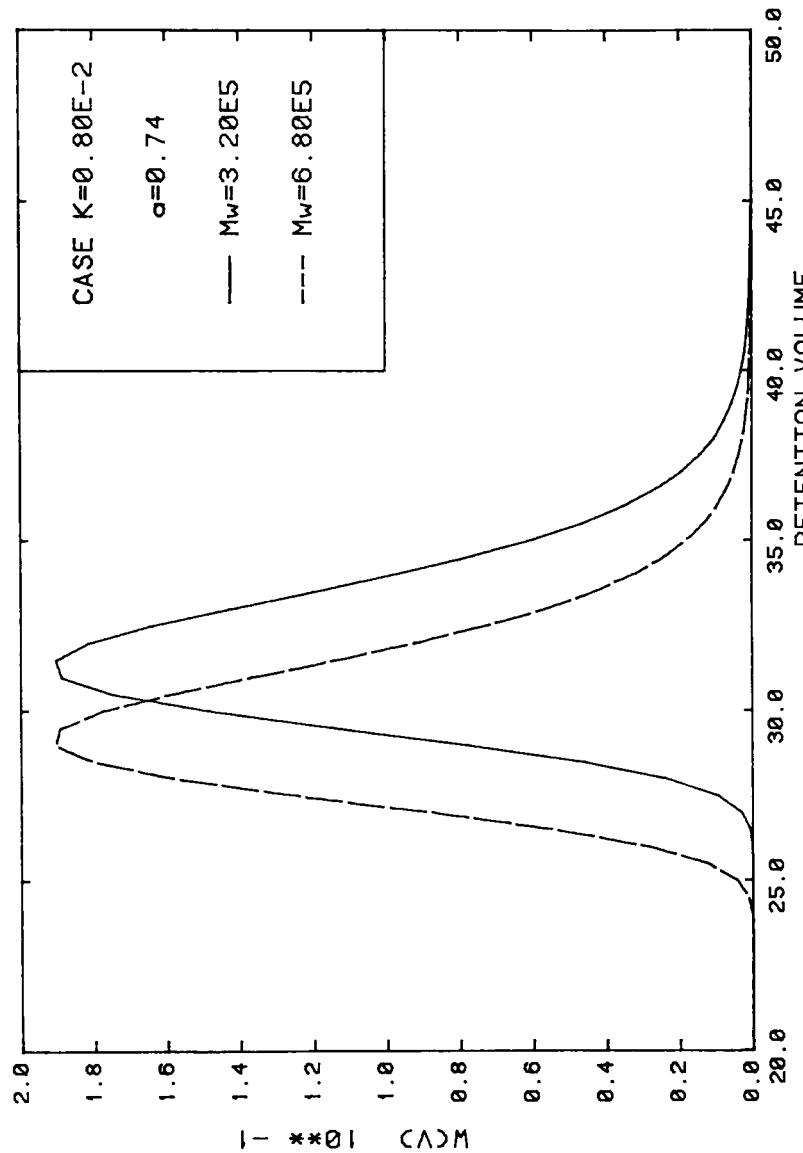
CASE K=0.80E-2
α=0.74
Mw=3.20E5
Mw=6.80E5
W(V) 10** -1
RETENTION VOLUME
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 4

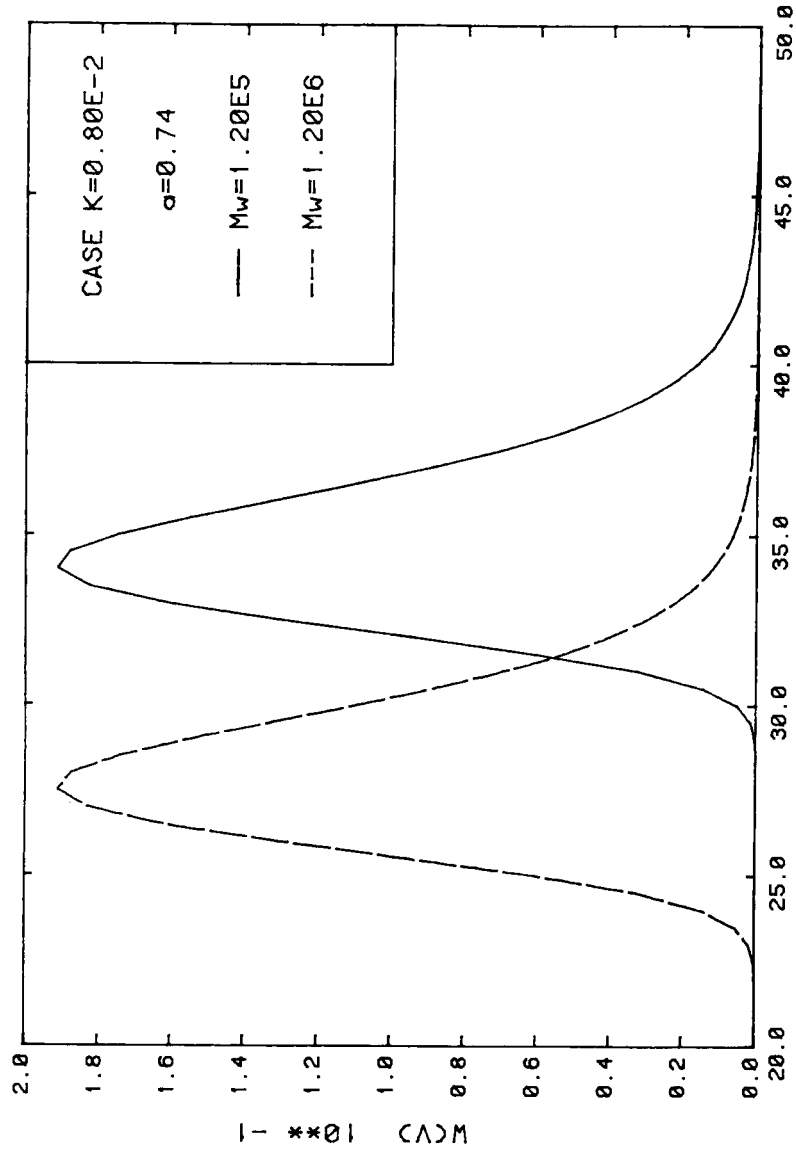
CASE K=0.80E-2
σ=0.74
Mw=1.20E5
Mw=1.20E6
W(V) 10** -1
RETENTION VOLUME
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 5

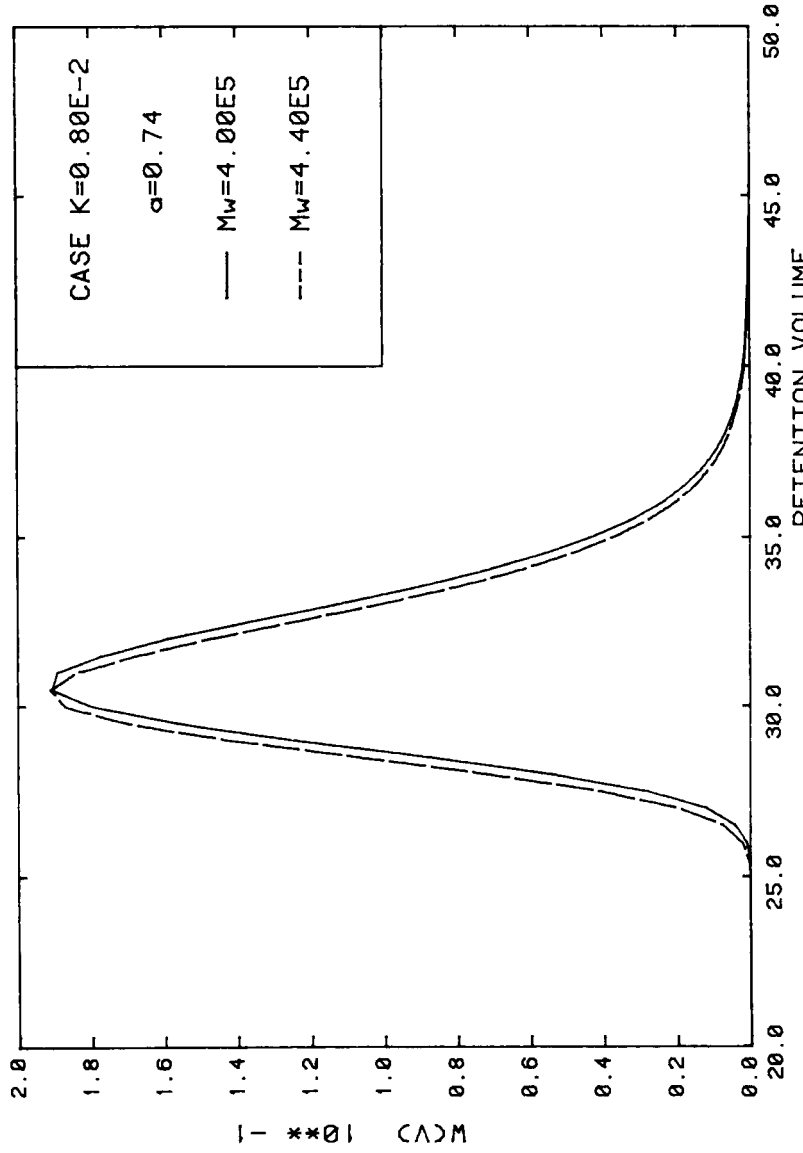
CASE K=0.80E-2
a=0.74
Mw=4.00E5
Mw=4.40E5
W(V) 10** -1
RETENTION VOLUME
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
20.0
25.0
30.0
35.0
40.0
45.0
50.0

FIGURE 6

TABLE 3

Sensitivity of two broad MWD standards method of calibration to peak broadening found by computer simulation - same corrections to both M_w ($M_{w_1} = 3.2*10^5$, $M_{w_2} = 6.8*10^5$).

$K_x = 1.540*10^{-2}$ and $\alpha_x = 0.650$

Peak Broadening Correction (% M_w)	K_x	α_x
0	$1.581*10^{-2}$	0.649
2	$1.640*10^{-2}$	0.649
4	$1.754*10^{-2}$	0.649
10	$2.652*10^{-2}$	0.649

TABLE 4

Sensitivity of two broad MWD standards method of calibration to peak broadening found by computer somulation - different corrections for each M_w ($M_{w_1} = 3.2*10^5$, $M_{w_2} = 6.8*10^5$) 2% correction for M_{w_2})

$K_x = 1.540*10^{-2}$ and $\alpha_x = 0.650$

Peak Broadening Correction (% M_{w_1})	K_x	α_x
2	$1.640*10^{-2}$	0.649
4	$5.568*10^{-2}$	0.559
10	$11.70*10^{-2}$	0.168

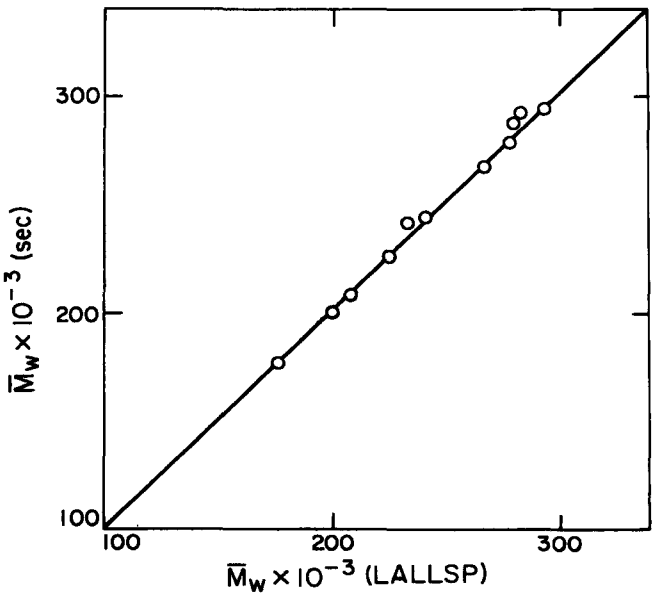

FIGURE 7

values would also be greatly magnified when the two standards are near to overlapping.

In Table 2, results are shown for a polymer whose Mark-Houwink constants are close to those for polystyrene. These results are about the same as those for a polymer whose K_x and α_x are significantly different than those for polystyrene and the same conclusions can be drawn. The chromatograms for these cases are shown in Figures 4-6.

The effect of peak broadening on the recovered Mark-Houwink constants K_x and α_x has also been investigated and the results are given in Tables 3 and 4.

The results in Tables 3 and 4 clearly show the significant effect on recovered K_x and α_x of small corrections for peak broadening. A correction of only 4% to M_w has a large effect on K_x and if the corrections to both M_w are small but different (2% to M_{w_2} and 4% to M_{w_1}) the errors in K_x and α_x are greatly magnified. It is clear that broad MWD standards calibration is very sensitive to peak broadening and if the method is

to be effective careful steps should be taken to minimize peak broadening experimentally or to properly correct detector responses for broadening.

To show that the two broad MWD standards method involving an M_w pair is valid, M_w values for eleven poly (p-methyl styrene) samples synthesized thermally at low conversions were measured by low angle laser light scattering photometry (LALLSP) and by SEC. The molecular weight calibration curve for poly (p-methyl styrene) was found using two of the polymer samples as broad MWD standards with known M_{w_1} and M_{w_2}. This molecular weight calibration curve was then used to measure M_w by SEC for the remaining poly (p-methyl styrene) samples. The M_w values found by SEC and LALLSP are compared in Figure 7. The agreement is excellent confirming the validity of the broad MWD standards calibration method.

ACKNOWLEDGEMENTS

The authors appreciate the financial support for this research provided by Mobil Chemical Co., Edison, N.J. and the Natural Sciences and Engineering Research Council of Canada.

One of the authors (O.C.) acknowledges the Consiglio Nazionale delle Richerche for financial support through a NATO fellowship.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1769-1788 (1984)

STRAIGHTFORWARD PROCEDURE FOR ESTIMATING THE SPREADING FACTOR IN SEC

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ABSTRACT

This contribution compiles SEC plate-height data obtained with various polymer standards and with a low-molecular probe. The latter value is easily measured and commonly given as a test of the apparatus used. Plate-height values h from the low and high-molecular range can be approximated by a straight line when plotted logarithmically:

$$\log h = A + B \log M$$

Knowledge of the slope factor B would enable plate-height data in the high-molecular range to be estimated on the basis of the reliable value from a low-molecular probe. The variance σ^2 and the spreading factor $1/(2\sigma^2)$ can easily be derived from the plate height.

INTRODUCTION

The spreading factor is a quantity which is needed for the evaluation of Tung's integral equation (1):

$$F(v) = \int_0^\infty W(y)\, G(v,y)dy \qquad (1)$$

F(v) is the uncorrected chromatogram, i. e. the detector response at elution volume v. W(y) is the chromatogram corrected

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for peak broadening. G(v,y) is the instrumental spreading function which contains the spreading factor. Graphically, G(v,y) is the detector response due to a single component with elution volume y. G(v,y) is usually assumed to be Gaussian:

$$G(v,y) = \frac{1}{\sigma\sqrt{2\pi}} \exp - \frac{(v - y)^2}{2\sigma^2} \qquad (2)$$

σ^2 is the variance of a Gaussian distribution. The quantity σ is the standard deviation. For a Gaussian curve, it is half the width at the inflection points, i. e. at 60.7 % maximum height of this curve.

Tung (1) called the quantity $1/(2\sigma^2)$ "spreading factor" but there are also papers which use this name for the expressions $1/(2\sigma^2)^{0.5}$ or $1/\sigma^2$. At any rate, the so called spreading factor is related to the reciprocal of the variance σ^2.

Eq. (1) reflects the fact that the chromatogram of a given sample is always broader than its component distribution. The band broadening is due to instrumental spreading. The higher the performance of a chromatographic apparatus, the less dramatically the bands will broaden - but band broadening remains a fundamental problem and especially influences the edges of a chromatogram. Here the uncorrected curve shows constituents which, in reality, are not present.

There are several numerical techniques for the solution of Eq. (1). (For survey, see Ref. 2, e.g.). The methods proposed

by Ishige et al. (3) or by Vozka and Kubin (4) proved to be very effective (5). An analytical solution of Eq. (1) has recently been given by Hamielec et al. (6).

All the correction techniques require precise knowledge of the standard deviation σ . With too small a value the correction will be insufficient, too high a value will yield overcorrection. In SEC of polymers, the quantity σ can be measured by several techniques: (i) by reverse-flow experiments (7), (ii) by chromatographic runs of polymers which are chromatographically monodisperse (8), (iii) by chromatographic runs of samples with precisely known molar mass distribution (MMD), (iv) using samples with exactly known values of average molar mass or statistical moments, or (v) by recycling.

Methods (i) and (v) need special equipment and are rather cumbersome, (ii) requires high effort in fractionating a synthetic polymer to the necessary degree of purity, (iii) and (iv) are strongly dependent on the precission to which the MMD or the average molar mass values of the standard polymers are known.

It is very difficult to obtain the precise data of $\sigma(v)$. In some papers dealing with correction of SEC data the value of σ is assumed to be independent of elution volume, but all experimental work shows a decrease of σ or σ^2 with increasing v. Some results reveal a maximum in the curve of σ^2 vs. v which is located in the vicinity of the exclusion limit of the column. This effect is due to the mass-transfer contribution and will be discussed later.

The purpose of this paper is the presentation of a straightforward procedure for estimating $\sigma^2(v)$. We intend to approach this aim via investigation of plate height as a function of molar mass, $h = f(M)$.

The plate height h (height equivalent to a theoretical plate, HETP) is:

$$h = L/N \tag{3}$$

The plate number N is related to elution volume v and variance σ^2:

$$N = v^2/\sigma^2 \tag{4}$$

Thus the plate height is:

$$h = L\,\sigma^2/v^2 \tag{5}$$

L is the length of column.

The plate height is a measure for the quality of column packing and influences the peak width. The peak width also increases with increasing column diameter and length. In order to get rid of these geometric effects and to approach a more general relation for peak broadening we shall investigate the behaviour of h instead of that of peak width.

According to Eq. (5) and the additivity rule of variances, the quantity h can be treated as the sum of contributions which are, e. g., due to polydispersity of sample (index: P), to diffusion and stream effects (D), and to resistance to mass transfer (index: MT):

$$h_{total} = h_P + h_D + h_{MT} \tag{6}$$

The sum of the second and third term at the right-hand side of Eq. (6) is the instrumental spreading. Only with monodisperse samples h_p is zero, with polydisperse samples the h_{total} should be corrected. This requires the precise knowledge of the distribution of species. If the calibration function of the SEC apparatus is linear,

$$\ln M = \ln D_1 - D_2 v \qquad (7)$$

and the sample distribution is logarithmic-normal, h_p can be calculated from the number and weight averages of molar mass, M_n and M_w, with the help of the equation:

$$\ln (M_w/M_n) = \sigma_p^2 D_2^2 \qquad (8)$$

EXPERIMENTAL

Apparatus: KNAUER Liquid Chromatograph LC/GPC 5050 with high-pressure pump FR 30 and differential refractometer 2025/50, with a home-made siphon of 1.289 cm^3 volume per count.

Column: L = 5 x 0.25 m, d_c = 4.6 mm, packed by supplier (KNAUER KG) with LiChrospher(R) Si 4000, Si 1000, Si 500 (2x) and Si 100, particle diameter d_p = 10 /um.

Solvent: Tetrahydrofuran (THF) "pro analysi", VEB LABORCHEMIE, Apolda, dried with KOH (24 hours), refluxed for 2 hours with Na wire, distilled under nitrogen using a VIGREUX column 0.30 m in length.

Samples: Polystyrene standards for SEC calibration, supplier: KNAUER KG, molar mass values given in column 1 of Table I.

Working conditions: concentration of sample solution c_o = 1.5 g/l, V_o = 538 µl, flow rate $\dot{v}$ = 1 cm^3/min.

RESULTS

The results obtained with this apparatus (9) are compiled in Table I. Column 3 of it shows the observed peak width, which is related to the standard deviation σ_{total} by the expression $W = 4\,\sigma_{total}$. The σ values listed in column 4 are calculated from the peak width after correction for injection volume (0.538 ml).

From the values of elution volume and molar mass (columns 1 and 2), the calibration function was calculated. It reads (for c_o = 1.5 g/l and $\dot{v}$ = 1 cm^3/min):

$$\log M_w = \sum_{i=0}^{7} a_i \, v^i \qquad (9)$$

with a_o = 449.977, a_1 = -144.251, a_2 = 18.0263, a_3 = -1.1768 a_4 = 6.49444-2, a_5 = -4.58180-3, a_6 = 2.25601-4, a_7=-4.3568-6.

The plate height data (column 5) plotted logarithmically vs. log M is shown in Fig. 1. The data are represented by a straight line:

$$\log h = A + B \log M \qquad (10)$$

Values of the slope factor B are compiled in Tab. II.

TABLE I

SEC Data Obtained with Toluene (M = 92) and Several Polystyrene Standards

M g/mol	v ml	W ml	σ ml	h /μm
92	17.04	0.951	0.103	46
600	16.78	1.075	0.134	80
4,000	16.20	1.245	0.177	149
20,400	14.89	1.280	0.186	194
33,000	14.20	1.280	0.186	213
51,000	13.82	1.411	0.218	312
110,000	12.84	1.316	0.195	287
173,000	12.30	1.266	0.182	274
200,000	12.19	1.256	0.180	271
390,000	11.38	1.204	0.167	268
670,000	10.93	1.235	0.174	318
867,000	10.82	1.319	0.195	407
2,000,000	10.27	1.655	0.279	924

DISCUSSION

The result shown in Fig. 1 fully corresponds to previous observation (2). In the course of the present work we used additional data from literature.

Figs. 2 - 4 show results published by Dawkins and Yeadon in 1980 (10). These authors used columns 0.20 m in length and

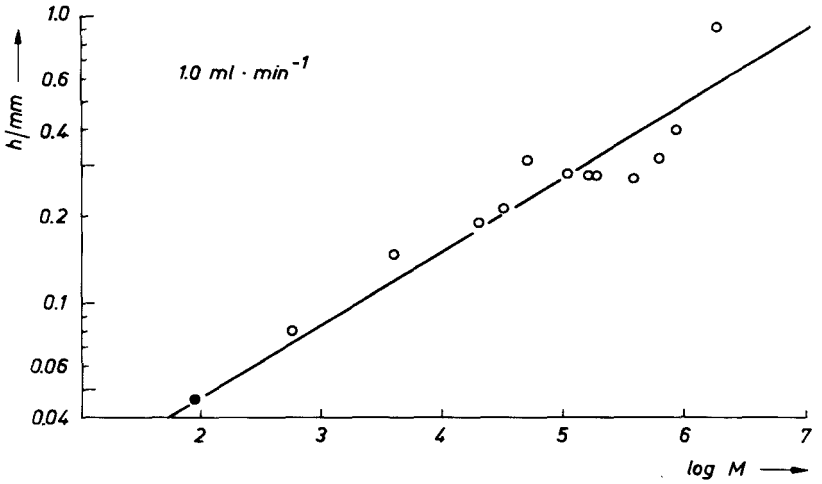

FIGURE 1

Plate height as a function of molar mass, log h vs. log M as measured with toluene (•) and polystyrene standards (o). Column: L = 1.25 m, 4.6 mm I.D., packed with silica microspheres. Eluent tetrahydrofuran, flow rate 1 ml/min, (redrawn from Ref. 9).

3 mm I.D. which had been slurry-packed with silica microspheres. The heterogeneity in particle diameter (weight to number average) was 1.22, 1.30, and 1.67, the number average values d_p = 13.9, 12.8, and 8.5 /um for the packing materials H 2, H 4, and H 6, respectively. The exclusion limits were $>10^6$, 5 x 10^5, and 10^5 g/mol (in the same sequence). The investigations were performed at various flow rates. The straight line for log h vs. log M, which was found at $\dot{v}$ = 1 ml/min, is repeated by a dashed line in the corresponding diagrams for higher (2.0) or lower values of flow rate (0.1 and 0.5 ml/min).

TABLE II

Values of B in Equation (10) as Calculated from Experimental Data by Least-Square Regression

Source	Fig.	Flow rate $\dot{v}$ ml/min	Linear velocity u mm/s	B
this work	1	1.5	2.51	0.24
Dawkins and Yeadon (10) H 2	2	0.1	0.54	0.15
		0.5	2.68	0.29
		1.0	5.36	0.34
		2.0	10.72	0.34
(10) H 4	3	0.1	0.54	0.24
		0.5	2.68	0.33
		1.0	5.36	0.38
		2.0	10.72	0.43
(10) H 6	4	0.1	0.54	0.19
		0.5	2.68	0.29
		1.0	5.36	0.36
		2.0	10.72	0.29
Kirkland (11)	5	0.88	0.76	0.17
		1.4	1.21	0.22
		2.5	2.16	0.26
		5.8	5.01	0.30
Cooper et al. (12)	6	0.055	0.05	0.13
		0.215	0.18	0.17
		1.040	0.90	0.27

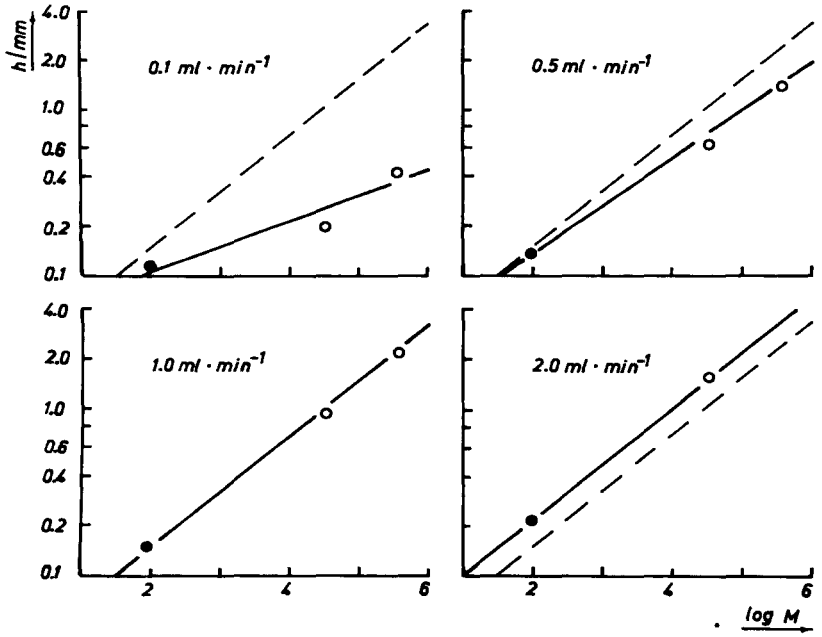

FIGURE 2

Plots of log h vs. log M for polystyrene standard samples (o) and toluene (•) at flow-rate values 0.1 ... 2.0 ml/min. Eluent THF. Column: L = 0.20 m, 3 mm I.D., slurry-packed with silica H 2 (exclusion limit >1,000,000 g/mol). (Data from Ref. 10).

Fig. 5 similarly presents results published by Kirkland in 1976 (11). Fig. 6 gives a corresponding view of data from Cooper et al. (12) which were used by this team again in 1983 (13). The value for a low-molecular probe was not given by the authors. The point indicated at log M = 2 has been estimated from the fact that a WATERS Styragel(R) column 10^5 Å was used which, according to the supplier's warranty, has at least 2100 plates per metre.

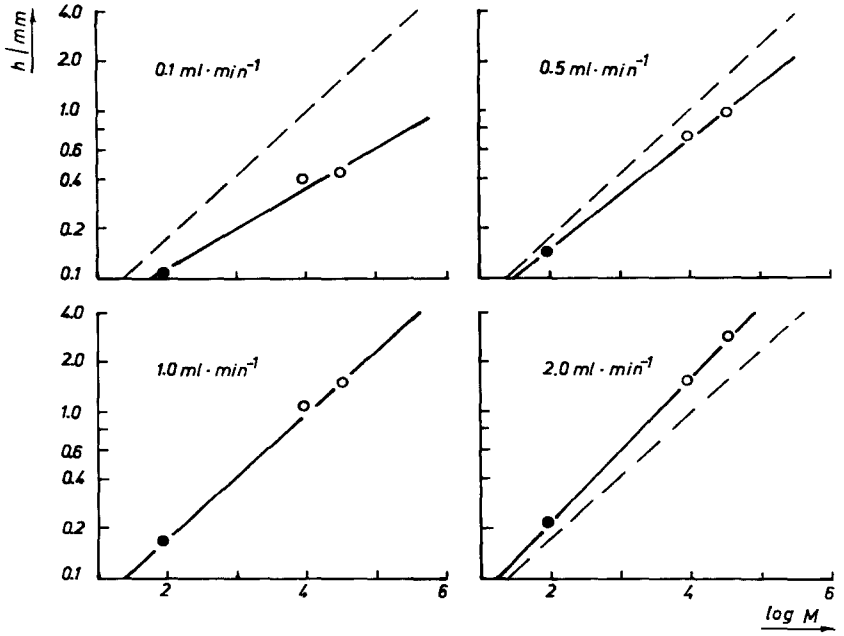

FIGURE 3

Same as Fig. 2, but column packed with silica H 4 (exclusion limit 500,000 g/mol). (Data from Ref. 10).

All the examples presented in Figs. 2 - 6 approximately support a linear relationship as given by Eq. (10). This linear dependence of log h from log M also includes the plate-height value of a low-molecular probe, which is easily measured. It is given as an additional bit of information in most papers. From this plain value and the knowledge of the slope B, the plate height valid for high-molecular samples can be estimated.

It has already been pointed out that there is not a general value of the quantity B. Some of the results presented in Ref.

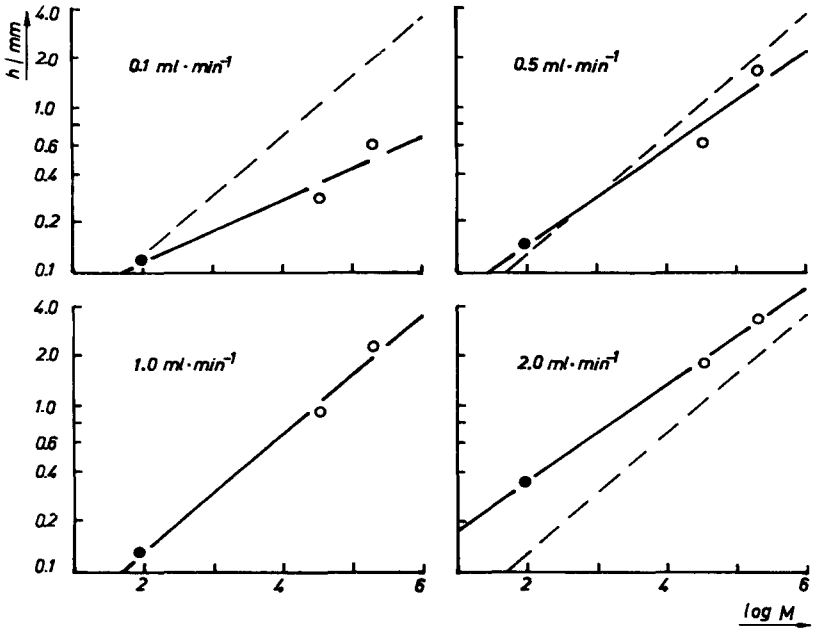

FIGURE 4

Same as Fig. 2, but column packed with silica H 6 (exclusion limit 100,000 g/mol). (Data from Ref. 10).

(2) yielded B = 0.3, but three of the six sets of data investigated led to a smaller value[+].

The B data compiled in Tab. II of this paper obviously show the influence of flow rate. Fig. 7 is a synoptic representation of data measured at different flow rates. In the range of a linear velocity u = 0.05 - 10 mm/s the data given

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[+] Equation (16-34) in Ref. (2) should read:

$$h_M = h_{Bzn}(M/M_{Bzn})^{0.3}$$

Unfortunately, the M_{Bzn} was omitted.

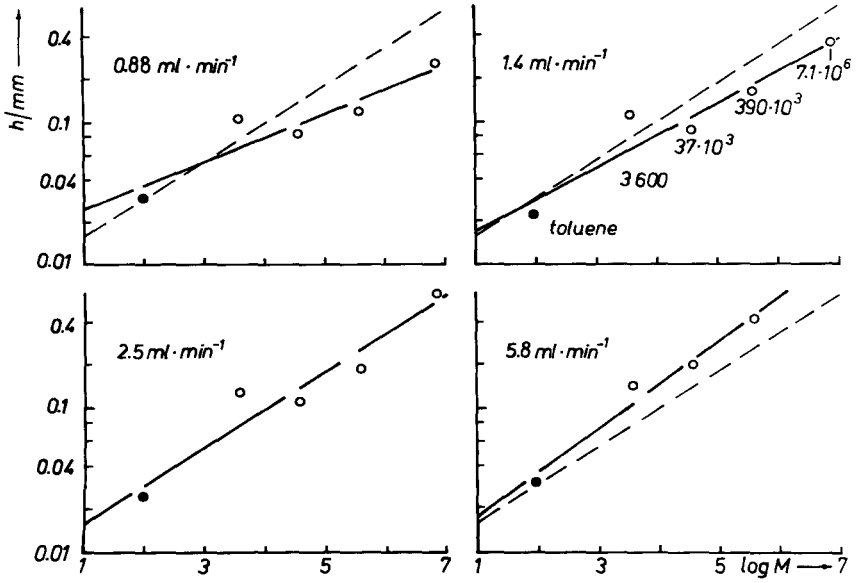

FIGURE 5

Plots of log h vs. log M for polystyrene standards (o) and toluene (•) at flow-rate values 0.88 ... 5.8 ml/min. Eluent THF. Column: L = 0.60 m (concatenation of 2 x 0.15 and 3 x 0.10 m tubes), 7.8 mm I.D., individually packed with 5 species of silanized silica microspheres. (Data from Ref. 11).

by Cooper et al. (12), by Kirkland (11), and by Dawkins et al. (10) yield an almost linear decrease of (d log h / d log M) with log u. The slope of this decrease is about 0.13 and indicated by the thick line in Fig. 7.

Fig. 7 also shows data from Chuang et al. (13) who have recently measured SEC efficiency at very small flow rate. They used two polymer samples with molar mass values within the limits of the separation range of the column. Results for low-molecular probes have not been given. In view of this restric-

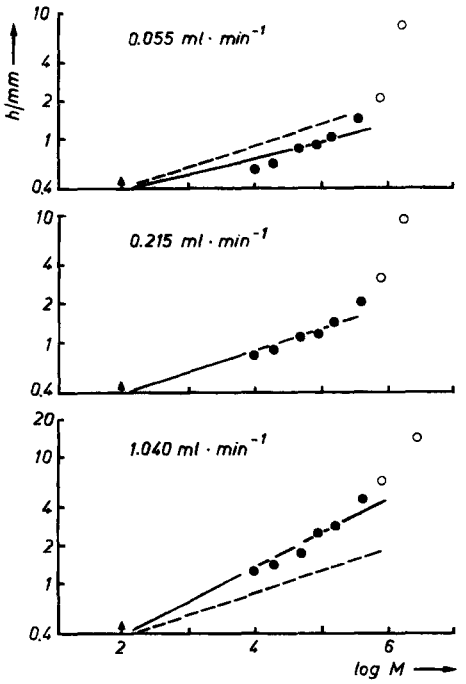

FIGURE 6

Plots of log h vs. log M for polystyrene standards (o, •) at flow-rate values 0.055 ... 1.040 ml/min. Eluent THF. Column: L = 1.22 m, packed with polystyrene gel of nominal porosity of 100,000 Å. (Data from Ref. 12). (The value indicated at M = 100 (▲) is estimated from supplier's column warranty. In calculating the position of the straight line, the open circles were not taken into account.)

tion, the data can only provide approximate information. Nevertheless, they are included in Fig. 7 in order to stress the fact that the thick line must not be extrapolated beyond the range of experimental evidence. Within this range, the data measured by Chuang et al. also support the location of this line.

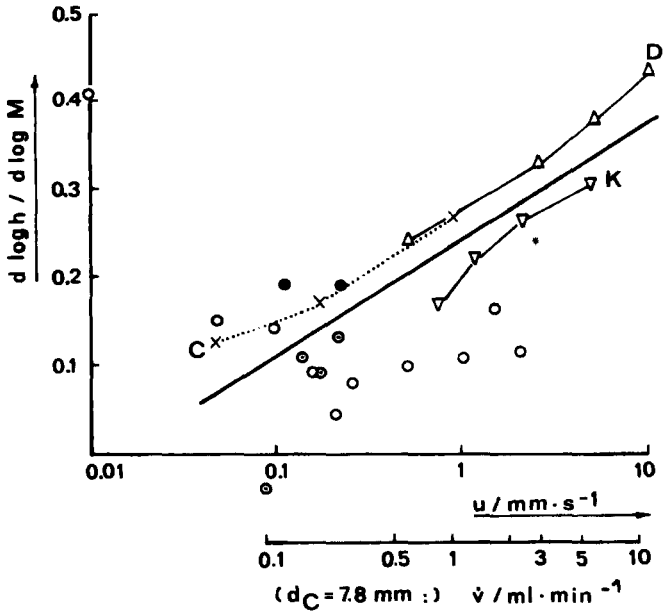

FIGURE 7

Synoptic representation of the flow-rate dependence of dlog h / dlog M. The thick line corresponds to

$$\text{dlog h /dlog M (= B)} = 0.239 + 0.130 \log u$$

and gives a good approximation of experimental data.

* :slope factor from Fig. 1 (PS in THF, silica)
△ —— △ :data from Ref.(10), (PS in THF, silica H4)
▽ —— ▽ :data from Ref.(11), (PS in THF, silica)
x.....x :data from Ref.(12), (PS in THF, Styragel(R) 10^5 Å)
o, ⊙, ● :data from Ref.(13), (PS in trichloromethane, Bio-Glass packings, three different columns)

The presentation of log h vs. log M used here and in Ref. (2) is by no means the only effort to correlate peak broadening and molar mass or SEC elution volume. Eq. (10) obviously works well in most cases, but one should be aware of the fact that the pore-size distribution of the packing mate-

rial might influence the applicability of this equation. We have some experience of this kind with CPG packings.

Bly plotted plate number N as $\sqrt{N}$ vs. elution volume v and found a linear correlation in the high molecular range (14) but the plate number determined with acetone was far aside. Cooper et al. plotted N vs. log M and found correspondence in the high molecular range (12). (Low-molecular values were not given.) Kirkland presented a straight-line correlation between σ and log M which met the value obtained with toluene but was rather badly obeyed by polymers of intermediate molecular weight. This mode of plotting has repeatedly been employed. McCrackin and Wagner (15) found good correlation in the range of 9,000 - 300,000 g/mol. The value for a low-molecular probe was not given, but the extrapolation of the straight line towards M = 100 g/mol would lead to a negative σ which has no physical meaning.

Elution volume and standard deviation are dependent on column diameter and length. Plotting of h vs. M overcomes the shortcomings of other evaluation procedures and enables columns of different size to be compared.

The relationship given by Eq. (10) is in accord with conclusions from general knowledge about polymer solutions and liquid chromatography. The plate height depends on the coefficient of diffusion by:

$$h/d_p = \text{const}(u\, d_p / D')^n \qquad (11)$$

The expression given in brackets on the right-hand side is the reduced velocity ν. For $\nu > 10$, the exponent n in Eq. (11) approximately becomes invariable, $n = 0.4$.

The coefficient of diffusion D' is related to the molar volume V of solute by:

$$D' = 0.00014 / (V^{0.6} \eta') \qquad (12)$$

The viscosity of solvent is indicated by η'.

The combination of Eqs. (11) and (12) yields (for a given solvent and a given velocity)

$$h = \text{const } V^{0.6n} \qquad (13)$$

or

$$\log h = \log \text{const} + 0.6n \log V \qquad (14)$$

If the volume of the solute is proportional to molar mass one obtains Eq. (10) with $B = 0.24$, if it is proportional to unperturbed coil volume one obtains Eq. (10) with $B = 0.36$. Of course, these values are rough approximations only. In pores, the coefficient of diffusion is strongly influenced by the ratio of molecular size to pore diameter.

CONCLUSIONS

The accuracy of plate-height values calculated through the approximation given by Eq. (10) is not less than the precision of most experimental data in the high-molecular range. The advantage of Eq. (10) is the inclusion of the reliable and easily measured value for a low-molecular sample as a base for the estimation of values in the high-molecular range.

On base of this perception, the following procedure for correcting SEC chromatograms can be recommended:

(a) Evaluation of the plate height with a low-molecular probe, e.g. with toluene.

(b) Estimation of another plate-height value using a polymer with a molar-mass value sufficiently smaller than the exclusion limit of the column. This condition is essential because the contribution h_{MT} in Eq. (6) diminishes with excluded samples. Consequently, plate heights measured with excluded samples are smaller than those with penetrating polymers (10, 16). In the vincinity of the exclusion limit, a plot of h (or σ^2) vs. v will show a maximum. Corresponding to this, a plot of $1/(\sigma\sqrt{2})$ will have a minimum. This was demonstrated by Tung and Runyon as early as in 1969 (17).

The distribution of the sample polymer must be either narrow or precisely known. Under favourable circumstances, the contribution of sample heterogeneity can be calculated via Eqs. (5, 6, and 8).

Repetition of this step with another suitable polymer would provide information whether the system really follows the dependence indicated by Eq. (10).

(c) Estimation of the constants A and B in Eq. (10) with the help of the values measured in steps (a) and (b).

(d) Calculation of the M value corresponding to a certain value of elution volume v in the uncorrected chromatogram.

(e) Estimation of the plate-height value at this molar mass via Eq. (10) and calculation of σ^2 or the spreading factor $1/(2\sigma^2)$ via Eq.(5).

(f) Performing the correction of the chromatogram with the help of a suitable algorithm.

(g) Repetition from (d) to (g) for the next value of v.

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DENSIMETRIC DETECTION IN SEC. A SEMI-AUTOMATED METHOD FOR CALCULATION OF MOLECULAR WEIGHT AVERAGES.

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ABSTRACT

It is shown that detection by measurement of density (mass per unit volume) offers some advantages: the signals from such an instrument are inherently digital and integrated over each measuring interval, which makes calculation of molecular weight averages very easy. A BASIC program is described, by which data reduction can be performed with good accuracy by means of a low-cost minicomputer.

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INTRODUCTION

One of the most important advantages of steric exclusion chromatography (SEC) in the characterization of polymers with respect to their molecular weight distribution is the possibility to obtain molecular weight averages (M_n,M_w,M_z,M_η) as well as polydispersity (M_w/M_n) from a single chromatogram. The determination of molecular weight averages by a manual procedure is, however, rather laborious and presents many opportunities for operator errors. Hence, various automated data-handling systems have been developed,[1-10] which save time and improve the accuracy of the results. In general, there are two approaches towards automated data reduction in SEC:

1. Real-time data acquisition and off-line data processing
2. Combined real-time data acquisition and processing

Both of them involve usually the following steps, each of which may be subject to errors, as several authors have pointed out[11-15]:

1. Conversion of the analog signal from the detector (UV, RI etc.) into a digital form.
2. Transformation of elution times into elution volumes.
3. Definition of a baseline

4. Definition of start and end of a peak
5. Division of the peak into small slices (usually of equal elution volume intervals)
6. Assignment of a molecular weight to each slice (from a calibration curve)
7. Determination of the area of each slice (usually by approximation as a rectangle)
8. Calculation of molecular weight averages and polydispersity.

If peak spreading is not negligible, several additional steps may be necessary for the correction of molecular weight averages or even of the whole MWD.[1]

As we have shown in a previous paper,[16] the use of a density measuring device according to the mechanical oscillator method[17,18] as a detector in SEC[19-26] eliminates some of these steps and eliminates consequently some possible sources of error: The signals from such an instrument are inherently digital and integrated over each measuring interval. Data reduction could be performed by means of a programmable pocket calculator with good accuracy; but still many operator manipulations were required in this way.[16] Hence we have developed a much more convenient method, which involves storage of the raw data in the memory of a low-cost minicomputer prior to calculation of molecular weight averages. The main reason for the choice of an off-line method was

that it enables the operator to interact with the computer in the course of the calculations in order to avoid artefacts which might be produced by data reduction "on the fly".

DETECTION BY MEASUREMENT OF DENSITY

The measuring cell of a densimetric detector is an oscillating, u-shaped (glass or metal) tube, the period of which depends on the reduced mass of the oscillator, which itself results from the mass of the empty cell and the mass of the sample. As the sample volume is constant, the period of the cell represents the density of the sample.[17,18,20]

Period measurement is performed by counting the periods of an oven-controlled 5 Mc - quartz oscillator within a predetermined number of periods of the measuring cell.

A small change $\Delta\rho$ in density will cause a change ΔT in the period T_o:

$$\Delta\rho = 2A.T_o.\Delta T$$

wherein A is a constant for each individual cell.

The concentration c_i of a solute is given by

$$c_i = \frac{2A.T_o}{1-\rho_o.\bar{v}_i^*} . \Delta T$$

wherein ρ_o is the density of the pure solvent and $\bar{v}_i^*$ is the (apparent) partial specific volume of the solute.

As the thermal volume expansion coefficient of most organic solvents is in the order of magnitude of $1.10^{-3}K^{-1}$, one will have to keep temperature constant within $\pm 1.10^{-4}K$, if a resolution in density of 1.10^{-7} g/cm^3 shall be achieved, which corresponds to a detection limit of approximately 1 ppm (in the cell) for a usual polymer-solvent system, such as polystyrene in tetrahydrofuran. The more feasible way is, however, the use of a reference cell for compensation of temperature variations.[24,25] By choosing a higher resolution for the reference cell combined with a sliding average a stable baseline can be achieved without an increase of baseline noise.[16]

EXPERIMENTAL

A detailed description of a densimetric detector has been given in a previous communication.[25] It consisted of two cells DMA 602 M of about 100 µl volume (A.PAAR KG, Graz, Austria), and a calculating unit developed in our laboratory. For all measurements, temperature was kept constant at 25±0.01°C using a thermostat Haake F3C. Both cells were arranged parallel in the thermostat circuit; a mixing chamber of about 10 l volume was placed

between thermostat and cells to keep temperature changes slow.

The calculating unit was connected via a VIC 1011B interface to a Commodore VC 64 computer equipped with a monitor, a VC-1541 floppy disk and a matrix printer Epson MX 80.

The chromatographic apparatus consisted of a pump LDC Constametric IIG, a Valco injection valve equipped with a 100 µl loop, a column Microgel M (Polymer Lab.) with an exclusion limit of about 5.10^6, its (mobile phase) volume was about 21 ml, a UV-photometric detector LDC Spectromonitor II and the densimetric detector. Chromatograms were also registered using a 3-channel strip-chart recorder (UV, density with and without temperature compensation).

The solvent (tetrahydrofuran) was distilled over benzophenon-potassium prior to use, polystyrene standards (from Pressure Chem.Co.,Pittsburgh,Pa. and Waters, Framingham,Mass.) were used as received.

All chromatograms were run at a flow rate of 1.00 ml/min, sample concentrations varied from 0.05 to 0.3% (w/v).

THEORETICAL CONSIDERATIONS

Several authors[11-15] have pointed out, that even in a perfect separation system under correct chromatographic

conditions various sources of error have to be taken into account, which might deteriorate the accuracy of molecular weight averages calculated from SEC:

1. Depending on the type of column used errors in the determination of elution volumes of only 0.1 % may cause errors in molecular weight of several per cent.[14,15] Even high quality pumps reproduce flow rates only within 0.3 %, which makes control of flow rates necessary (for example by the use of a low molecular weight internal standard).

2. Finite digitizer resolution as well as noise limit the precision of data, especially at low sample sizes. A sufficiently high sampling frequency (at least 20-30 points per peak) reduces these errors.[12,13,15] With densimetric detection, there are no problems with digitizer resolution; sampling frequency is, however, indirectly proportional to sensitivity, since higher resolution requires longer measuring times. Using 1000 periods of the measuring cell per interval one achieves a resolution in density of 2.8×10^{-7} g/cm^3 at measuring times of $\sim$4 sec (corresponding to $\sim$67 µl at a flow rate of 1.00 ml/min). This proved to be a good compromise: even with standards of very narrow MWD, 20-30 points per peak are obtained.

3. Noise also causes uncertainties in the definition of baseline height as well as of start and end of a

peak,[15] which leads to errors in the area of each slice and of the whole peak.

4. If a drift of the baseline adds to the effects mentioned above, especially the end of the peak may be poorly defined, which leads to serious errors especially in M_n.[15]

5. Additional errors may arise from variation of detector response with concentration or molecular weight. (The response of a differential refractometer for polystyrene in toluene varies up to a molecular weight of approximately 50000)[1].

6. A slight curvature of the calibration curve may also lead to erroneous molecular weights. In this case linear interpolation between standards should be superior to a calibration curve obtained by a least squares linear fit of the same data.[4] Starting from these considerations, our goal was the development of a program which should provide algorithms for minimizing these errors.

SEC - PROGRAM

The basic idea was that the operator should be able to examine the raw data before starting data processing, to eliminate artefacts, and to repeat any step or even the whole calculations. Definition of a baseline and inte-

gration should be performed within the limits entered by the operator, calculation of molecular weights should be possible using a linear calibration or interpolation between standards, alternatively. Flow rate changes should be compensated by the use of an internal standard.

A flow chart of the program is given in Fig.1. To illustrate how the program works, a typical report of a chromatogram and the calculations therefrom are shown in Figures 2 and 3. (The data entered by the operator are underlined.)

Before initializing the program, the expected number NE of values has to be entered (i.e. the number of measuring intervals of the detector within the time required for the whole chromatogram). When data acquisition has been completed (N=NE), the raw data are displayed on the screen for examination: If single values are in error for well understood reasons, they may be corrected. On entering the number of the first and the last value, the interesting part(s) of the chromatogram are plotted on the printer.

To integrate a peak, the operator has to define its start and end as well as a region before and after the peak, respectively, between the averages of which the program establishes a linear baseline. This procedure can be repeated, if there is more than one peak to be integrated.

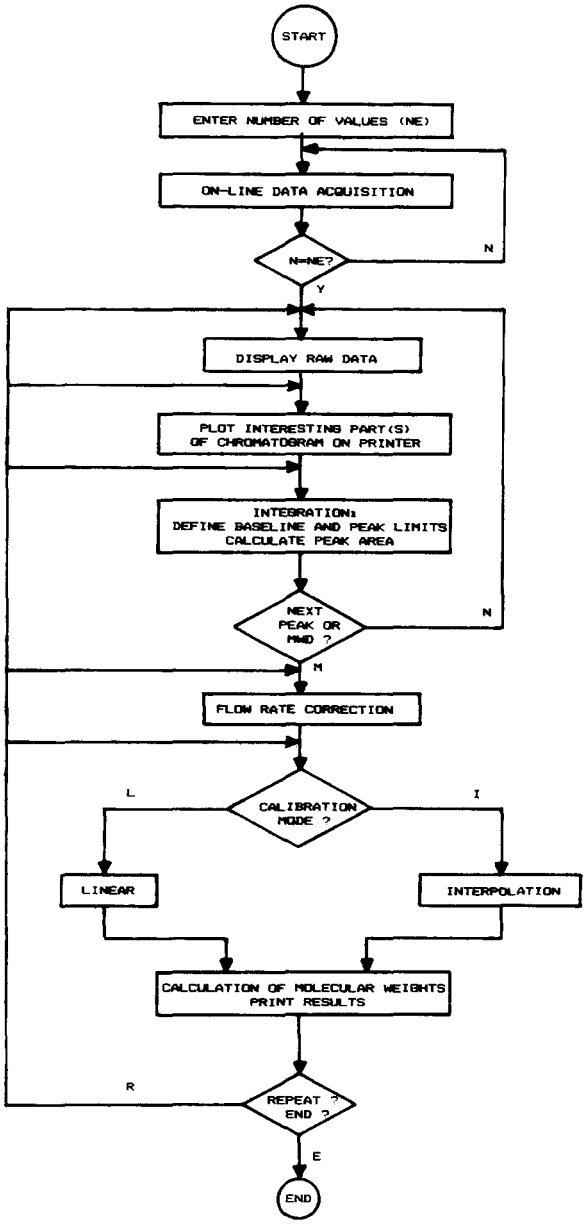

FIGURE 1

Flow chart of the SEC - program

DENSITY DETECTOR DMA 61: CHROM.NR. 7 DATE: 19.12.1983

SAMPLE: POLYSTYRENE 50000

COLUMN SET: MICROGEL M ELUENT: TETRAHYDROFURAN
CONCENTRATION: 0.1 % (W/V) INJECTED VOLUME: 100 MYL
FLOW RATE : 1 ML/MIN BASELINE: 19098910

N	T(MIN)	D(N)	DETECTOR RESPONSE
200	12.73	6	
201	12.80	5	
202	12.86	6	
203	12.92	6	
204	12.99	6	
205	13.05	6	
206	13.11	7	
207	13.18	7	
208	13.24	5	
209	13.31	6	
210	13.37	6	
211	13.43	5	
212	13.50	5	
213	13.56	6	
214	13.62	5	
215	13.69	6	
216	13.75	7	
217	13.81	6	
218	13.88	6	
219	13.94	6	
220	14.01	6	
221	14.07	7	
222	14.13	10	
223	14.20	14	
224	14.26	23	
225	14.32	35	
226	14.39	53	
227	14.45	69	
228	14.52	88	
229	14.58	99	
230	14.64	104	
231	14.71	101	
232	14.77	92	
233	14.83	82	
234	14.90	69	
235	14.96	57	
236	15.02	47	
237	15.09	39	
238	15.15	31	
239	15.22	27	
240	15.28	22	
241	15.34	20	
242	15.41	16	
243	15.47	14	
244	15.53	11	
245	15.60	10	
246	15.66	10	
247	15.72	9	
248	15.79	8	
249	15.85	8	
250	15.92	8	
251	15.98	6	
252	16.04	8	
253	16.11	7	
254	16.17	8	
255	16.23	7	
256	16.30	6	
257	16.36	7	
258	16.43	6	
259	16.49	7	
260	16.55	6	
261	16.62	8	
262	16.68	7	
263	16.74	6	
264	16.81	6	
265	16.87	8	
266	16.93	8	
267	17.00	7	
268	17.06	9	
269	17.13	8	
270	17.19	8	

MAXIMUM AT N = 230 ELUTION TIME: 14.64 MIN
MINIMUM AT N = 201 ELUTION TIME: 12.8 MIN

FIGURE 2

Plot of a chromatogram. Operator responses are underlined.

INTEGRATION

BASELINE BEFORE PEAK : 200 - 221 START OF PEAK: 222
AVERAGE : 5.95 +- .65
BASELINE AFTER PEAK : 248 - 270 END OF PEAK : 247
AVERAGE : 7.26 +- .92

PEAK AREA = 980.02 NUMBER OF VALUES: 26

FLOW RATE CORRECTION

INTERNAL STANDARD : N(MAX) = 324 VE = 20.75 ML
FLOW RATE = 1.006 ML/MIN

*** CALIBRATION ***

DATE: 6.12.1983 ELUENT: THF FLOW RATE: 1 ML/MIN
STANDARDS: POLYSTYRENE CONC.:0.05 - 0.1 % VOLUME:100 MYL

600000	11.84
111000	13.88
50000	14.77
20500	15.66
9000	16.55
4000	17.31
2200	17.76
800	18.71
72	20.75

(LINEAR INTERPOLATION)

TABLE OF MOLECULAR WEIGHTS

N	VI	MI	WI(%)	%CUM
222	14.22	82025	.38	100.00
223	14.28	77450	.79	99.62
224	14.35	73131	1.70	98.83
225	14.41	69052	2.92	97.13
226	14.47	65201	4.76	94.21
227	14.54	61564	6.39	89.46
228	14.60	58131	8.32	83.07
229	14.67	54889	9.44	74.75
230	14.73	51828	9.95	65.30
231	14.79	48813	9.64	55.36
232	14.86	45780	8.72	45.71
233	14.92	42935	7.70	37.00
234	14.99	40267	6.37	29.30
235	15.05	37764	5.14	22.93
236	15.11	35418	4.12	17.79
237	15.18	33217	3.30	13.68
238	15.24	31152	2.48	10.38
239	15.31	29216	2.07	7.90
240	15.37	27401	1.55	5.83
241	15.43	25698	1.35	4.28
242	15.50	24101	.94	2.93
243	15.56	22603	.73	1.99
244	15.63	21199	.42	1.26
245	15.69	19928	.32	.84
246	15.75	18782	.31	.52
247	15.82	17702	.21	.21

MW = 48240 MN = 44719

MW/MN = 1.08

FIGURE 3

Output of calculations from the chromatogram shown in Figure 2. Operator responses are underlined.

Before starting the calculation of molecular weights, the actual maximum of an internal standard and its elution volume in calibration have to be entered, from which the program calculates the actual flow rate.

The calculation of molecular weights can be performed either using a linear calibration or a linear interpolation between standards. The molecular weights and elution volumes of the standards are read from a data file on the floppy disk. After completion of the calculations the results are printed, and the operator can decide whether to repeat any of the steps mentioned above or to finish the calculations.

RESULTS AND DISCUSSION

As has been pointed out in the previous sections, the accuracy of molecular weight averages and polydispersity calculated from SEC is determined by the following criteria:

1. choice of chromatographic conditions
2. quality of the separation system
3. reliability of the calibration curve
4. sensitivity and stability of the detector
5. reliability of data acquisition and data processing

Any deficiency in point 1-3 will result in more or less reproducible, systematic errors, inadequacy in point

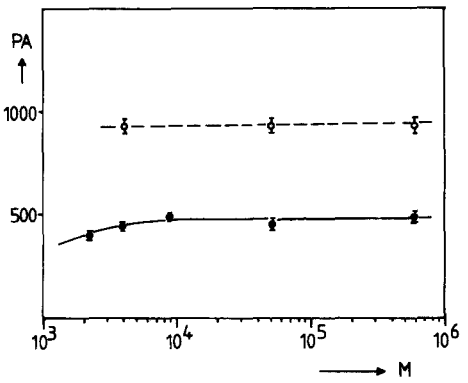

FIGURE 4

Peak areas as a function of molecular weight. Polystyrene standards 600000,50000,9000,4000,2200; Microgel M (60 cm), THF, 1.0 ml/min, injected volume 100 µl, sample concentration: 0.05 % (⊙), 0.1 % (O)

4 and 5 will cause irreproducible, random errors. Since this paper deals mainly with the performance of the detector and data handling, we have tested the accuracy as well as the reproducibility of the results obtained with our system by means of repeated analysis of polystyrene standards.

First of all we had to consider an often neglected source of error, which may occur in the low molecular weight range of the chromatogram, i.e. the variation of detector response with molecular weight. Although we have shown in a previous paper[21] that the response of the densimetric detector is not very sensitive to molecular weight, we have determined the peak areas obtained from

TABLE I

Molecular weight averages and polydispersity from repeated injections of polystyrene standard 60917 (Pressure Chem.Co.) on Microgel M in THF. Flow rate 1.0 ml/min, sample concentration 0.1 %, injected volume 100 µl.

Molecular weights reported by the distributor:

By light scattering	M_w = 53700 ± 6 %
By intrinsic viscosity	M_η = 47400 ± 6 %
By membrane osmometry	M_n = 51150 ± 6 %
Kinetic molecular weight	M_{nk} = 47000 ± 6 %

$M_w/M_n \leq 1{,}06$

M_w	M_n	M_w/M_n
48165	44985	1.07
49894	46820	1.07
48951	45807	1.07
49067	45813	1.07
49489	46307	1.07
49580	46096	1.08
49413	45780	1.08
48551	44911	1.08
49027	45753	1.07
50193	46908	1.07
48813	45289	1.08
48755	45207	1.08
48240	44719	1.08
49063	45652	1.07
49086 ± 1.2 %	45718 ± 1.5 %	1.074 ± 0.05

repeated injections of polystyrene standards (mol.weights from 600000 to 2200; sample volume: 100 µl, concentration: 0.05 and 0.1 %). As can be seen from Figure 4, only below a molecular weight of 4000 a significant decrease of peak areas is observed. The standard deviation of peak areas was typically less than 5 % even at sample sizes of 50 µg (100 µl, 0.05 %).

Baseline stability can be estimated from figures 2 and 3: In general, noise is less than ± 1 digit; after an equilibration period baseline drift within an average chromatogram does not exceed ±5 digits (1 digit corresponds to a density difference $\Delta\rho = 2.8 \times 10^{-7} g/cm^3$!).

Reproducibility and accuracy of molecular weight averages is demonstrated in table 1: even for a narrow MWD standard 25 ± 1 points per peak are obtained, M_w and M_n are determined with a standard deviation of 1.2 % and 1.5 % respectively.

Data reduction and printing of the results can be performed in this manner within less time than the excluded volume requires to pass the column (at 1 ml/min). Hence one may inject the next sample before processing the data from the last one, and start data acquisition thereafter.

CONCLUSIONS

It has been shown that the system described in this paper fulfills the requirements of high performance SEC,

as they have been formulated by Tchir, Rudin, and Fyfe[15]:

1. For an average polymer a sufficient number of points per peak is obtained
2. Noise level is less than 2 % even at sample sizes of 50 µg
3. Baseline level is defined to within 2 % at the same sample size
4. Peak width is defined to within 20 % (typically 5-10 %)

The reproducibility of molecular weight averages is typically better than ± 2 %, if an internal standard is used for flow rate correction.

Hence, the method described in this paper makes rapid and accurate determination of molecular weight averages possible by simple and inexpensive means.

ACKNOWLEDGEMENT

Financial support by the Austrian "Fonds zur Förderung der wissenschaftlichen Forschung" is gratefully acknowledged.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1809-1821 (1984)

Long Chain Branching in Polyethylene

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Abstract

Long chain branching frequency in polyethylene has been measured. Molecular weights determined directly by low angle laser light scattering of eluting species in gel permeation chromatography were compared with those estimated by universal calibration. Erroneous values for long chain branching frequency are produced if care is not taken to disrupt polyethylene aggregates in the GPC solvent.

Ethyl and hexyl side chains do not register as long branches in this analysis but sixteen carbon sidechains are counted.

In low density polyethylene the long chain branching frequency is generally highest at low molecular weights. This is because these polymers are produced in a non-isothermal free radical polymerization. Chain transfer to dead polymer, which produces long branches, occurs most frequently under the reaction conditions that also yield low molecular weight polyethylene.

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Introduction

Gel permeation chromatography (GPC) estimates of long chain branching in polymers start with the structure parameter g' which relates the intrinsic viscosities of branched and linear polymers with the same composition and molecular weight:

$$g' = \frac{[\eta]_b}{[\eta]_\ell} \tag{1}$$

where the $[\eta]_b$ is the intrinsic viscosity of the branched polymer and $[\eta]_\ell$ is that of the linear counterpart, in the GPC solvent. It is necessary to invoke a relationship between g' and g, which is the ratio of the mean radii of gyration $\langle R_G^2 \rangle$ of the same polymers:

$$g = \frac{\langle R_G^2 \rangle_b}{\langle R_G^2 \rangle_\ell} \tag{2}$$

The value of g has been calculated for a number of branched structures such as star-shaped, randomly-branched and comb-type molecules (1,2). Calculation of g' for use in equation (1) is much more difficult than that of g because the degree of draining of the macromolecules is not known exactly and because the degree of expansion of linear and branched molecules in a given solvent may be different (1,3). Various relations have been proposed of the form:

$$g' = g^k \tag{3}$$

where k has been suggested to have magnitudes between 0.5 and 1.5 (4,5).

At equal GPC elution volume and infinite dilution (6) the molecular weights of branched and linear species are related by

$$[\eta]_b M_b = [\eta]^* M^* \tag{4}$$

where the subscript b and superscript * refer to the branched and linear species that elute at the same retention time. In general, $M_b \geq M^*$. Now,

$$[\eta]_b = g'[\eta]_\ell = g'KM_\ell^a = g'KM_b^a \quad (5)$$

since M_ℓ is specified as equal to M_b in the definition of g' (in eq. (1)). In equation (5), K and a are the Mark-Houwink constants for monodisperse versions of the linear polymer in the GPC solvent:

$$[\eta] = KM^a \quad (6)$$

Also,

$$[\eta]^*M^* = K(M^*)^{a+1} \quad (7)$$

Therefore, equation (4) can be written:

$$g'KM_b^{a+1} = K(M^*)^{a+1}$$

and

$$g' = \left(\frac{M^*}{M_b}\right)^{a+1} = g^k \quad (8)$$

M^*, M and g' can be obtained directly. At any given elution volume M_b is measured by low angle laser light scattering (LALLS), while M^* is calculated from the universal calibration curve for linear polymers. The long chain branching frequency is measured implicitly by g'. To estimate the actual number of long branches per molecule it is necessary to assume a value for k and a model for the architecture of the branched species. Following Axelson and Knapp (7) we have assumed the Zimm-Stockmayer relation for a randomly branched macromolecule with trifunctional branch points (2):

$$g = \frac{6}{n_w} \cdot \frac{1}{2}\left(\frac{2 + n_w}{n_w}\right)^{1/2} \ln\left[\frac{(2 + n_w)^{1/2} + n_w^{1/2}}{(2 + n_w)^{1/2} - n_w^{1/2}} - 1\right] \quad (9)$$

Most of our calculations were made with k = 0.5, but other values were also used, as described below.

Data handling procedures used in this study were basically those suggested earlier by Axelson and Knapp (7). However, improvements in analytical and computational methods have produced long chain branching data that are quite different from the cited authors, and, indeed, from all previous analyses, so far as we know.

The results of this investigation provide new insights into the mechanism of the high pressure, free radical polymerization of ethylene.

Experimental

Polyethylene solutions were prepared in trichlorobenzene. All solutions contained 0.1% (w/w) 4,4-thiobis (3-methyl-6-tert-butylphenol) antioxidant. GPC measurements were made at 145°C with a Waters 150 C liquid chromatograph equipped with 500 A°, 10^4 A° and 10^5 A° (nominal porosity) Ultrastyragel columns. A solvent flow rate of 0.5 mL/min was found to give good resolution. In some experiments du Pont Zorbax porous silica columns SE-60, SE-1000 and SE-4000, were used. Both sets of columns gave equivalent results and the molecular weight parameters that were calculated agreed very closely with those of earlier analyses of reference polyethylenes (8).

Polymer concentration in the eluant was monitored with a Waters differential refractive index detector. Molecular weights of the eluting polyethylenes were measured in-line with a Chromatix KMX-6 low angle laser light scattering photometer (LALLS) using light scattered at 6-7° to the incident beam. This photometer incorporates a He-Ne laser source (λ = 6328 A°). The specific refractive index increment (dn/dc) of the various polyethylenes in trichlorobenzene were measured at 145°C with a Chromatix laser differential refractometer. Molecular weights (M_i) of polyethylene species that appeared at elution $(ve)_i$ were calculated

$$\frac{K'c_i}{R_{\theta_i}} = \frac{1}{M_i} + 2A_2c_i \qquad (10)$$

where K' is the appropriate optical constant (related to $(dn/dc)^2$), R_0 is

the excess Rayleigh scattering determined from the LALLS detector response and A_2 is the second virial coefficient of the whole polymer. A_2 was measured from static light scattering analyses of the whole polymer sample. In equation (10) the concentration, c_i, of the eluting species was obtained from the differential refractive index detector response by:

$$c_i = \frac{mX_i}{V_i \Sigma X_i} \qquad (11)$$

where m is the mass of polymer injected, X_i is the detector response and V_i is the increment of solution volume between data points.

Universal calibration for linear species was based on hydrodynamic volumes of anionic polystyrenes (9,10). The Mark-Houwink relation for polystyrene in trichlorobenzene was based on $K = 1.75 \times 10^{-3}$ cm^3g^{-1}, $a = 0.67$ (11). For linear polyethylene, the values of Ram and Miltz (12) ($K = 5.96 \times 10^{-2}$ cm^3g^{-1}, $a = 0.7$) were used.

An analytical solution to equation (9) is not available. An iterative computer program was written to calculate n_w at each value of M_b from equations (8) and (9).

We have previously shown that dissolution of polyethylene in trichlorobenzene at 145° will usually not produce aggregate-free solutions (13). Solutions free of aggregates can be produced, however, by storing the mixtures at 160°C for appropriate times before making molecular weight measurements at 145°C. The effects of aggregation on measurements of long chain branching were examined in this study by analyses of polymer solutions prepared with and without the 160°C treatment. Storage at 160°C for about one hour was sufficient to provide aggregate-free solutions of the polyethylenes studied in this work. Storage at this temperature for longer periods up to several days had no effect on molecular weight or branching results.

NBS 1476 Polyethylene

Figure 1 shows the relation between number of long branches per 1000 carbons and molecular weight for National Bureau of Standards Standard

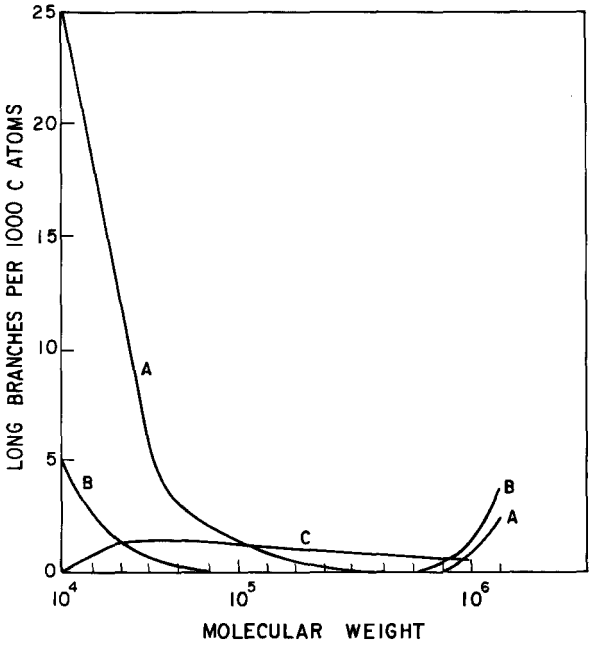

Figure 1

Long branch frequency-molecular weight relation for NBS 1476 polyethylene. Estimate made with k (eq. (3)) = 0.5. A: polymer dissolved and analyzed in trichlorobenzene at 145°C; B: polymer dissolved at 160°C and analyzed at 145°C; C: data of reference (7), measured in alpha-chloronaphthalene at 145°C and recalculated by us from original data.

Reference Material 1476. This is a melt index (14) 1.2, 0.931 g cm^{-3} density polymer that is reported to be a low conversion tubular reactor product (15). Curve A in Figure 1 records long chain branching for samples dissolved and measured at 145°C. Curve B is for the same material after the polyethylene solution was given a 160°C treatment to destroy polymer aggregates. The branching frequency at low molecular weights is seen to have decreased, while that at high molecular weights has increased slightly.

These changes can be explained as follows. The erroneously high values for long chain branching at low molecular weights in aggregate-containing solutions reflects the behavior of smaller agglomerates that appear effectively in the GPC as bigger entities with branches. This will occur if only a portion of a molecule is incorporated into an aggregate with the dangling remainder functioning essentially as a long branch. Large aggregates are presumably composed of large individual macromolecules. These aggregates will appear in the LALLS trace as "spikes" that behave like dust particles. These agglomerates will be rejected in the molecular weight computations (8,16). When the solution is treated to dissolve these aggregates the "spikes" are no longer present and the large branched species produce signals that are registered by the LALLS detector. The frequency of long chain branching at high molecular weights is seen to increase if aggregate-free solutions of NBS 1476 are analyzed.

Curve C records the long chain branching frequencies reported by Axelson and Knapp (7). The discrepancies between their results and ours are, we believe, due to differences in experimental procedures.The cited authors used relatively high concentrations of polyethylene dissolved in alpha-chloronaphthalene, which is a poor solvent for this polymer. No special dissolution time or procedure was measured. All these factors usually lead to aggregation and uncertainties in molecular weight measurements (8). Furthermore, for molecular weights $> 8 \times 10^5$ the calibration procedure used by Axelson and Knapp (7) may not give reliable results.

As mentioned earlier, the value of k for use in equation (8) is uncertain. Figure 2 shows branching frequency calculated with various K's between 0.5 and 1.5, which is the usual range suggested for polyethylenes (17). The choice of this exponent affects the magnitude of the branching frequency calculated, but the form of the branching-molecular weight relation is not altered.

Since the relation observed differs from that reported by earlier workers it may be appropriate here to defend the accuracy of the present

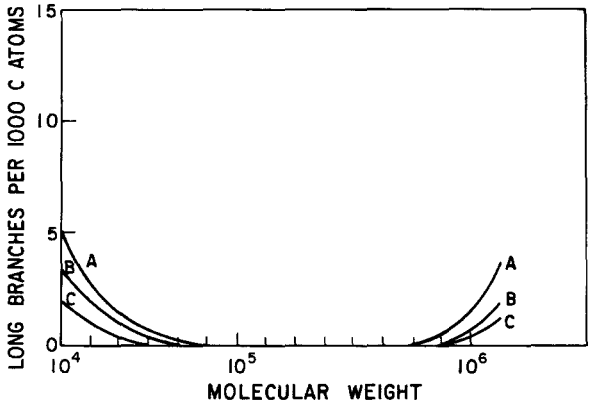

Figure 2

Long chain branch frequency of NBS 1476 polyethylene as a function of molecular weight. Estimates using k (eq. (3)) = 0.5 (A), 0.75 (B) and 1.5 (C).

results by pointing out that our measurements of the molecular weight parameters of this sample agree very well with those from other recent careful studies (8). This agreement holds both for LALLS and universal calibration methods of measurement. The branching frequency is calculated as described above from a combination of these two procedures, each of which is in good agreement with earlier measurements on this polymer.

How Long is a Long Branch?

Short chain branching in polyethylene is believed to have no significant effect on solution or melt rheological behavior, whereas long chain branching is considered to be important in this connection (18). Long branches can be defined generally as having about the same dimensions as the main chain (19). It is obvious, however, that branches much shorter than this length will affect the radius of gyration of solvent-swollen polymer coils. The minimum branch length for long chain behavior has been indeter-

minate, to date. For this reason, the question is not usually addressed in recent reviews of the subject (18,20).

Copolymers of ethylene and 1-olefins that are now available can be used to define long branch length more closely. In this study, linear polyethylene and ethylene copolymers with butene-1, octene-1 and octadecene-1 were examined. Butene and octene comonomers are common bases for current linear low density polyethylenes. Linear polyethylene and ethylene copolymers with butene and octene register as having zero long chain branching with the GPC-LALLS method used here. The octadecene copolymer was counted as long branched, however. Thus, we can conclude that a long branch, as measured by GPC-LALLS, has a minimum length > 6 and < 16 carbons. Details of these measurements follow.

Molecular weight parameters of the 1-olefin copolymers are recorded in Table I. Average molecular weights estimated from universal calibration are compared with those measured with the LALLS detector. $\bar{M}_w$ values agree closely for the butene-1 and octene-1 copolymers that have the same molecular weight-hydrodynamic radius as linear polyethylene. $\bar{M}_n$ and $\bar{M}_z$ are higher for the LALLS data because this detector is more sensitive than the differential refractometer to high molecular weight species and less sensitive to lower molecular weight polymers.

Figure 3 shows the long chain branch frequency estimated for the ethylene-octadecene-1 copolymer. The long branch density is found to decrease with increasing molecular weight. It is known from other measurements that lower molecular weight copolymers are richer in the 1-olefin (22) and these results are consistent with those of fractionation experiments.

Discussion

The dependence of long branch frequency on molecular weight of NBS sample 1476 is not typical of the majority of low density polyethylenes that we have examined. A more general relation is one in which branch frequency decreases monotonically from low to high molecular weights. This is not

Table I

Molecular Weight Parameters of 1-Olefin/Ethylene Copolymers

1-Olefin	Density	$\bar{M}_n$	$\bar{M}_w$	$\bar{M}_z$	SD (N)[a]	SD (W)[a]	SKEW (N)[b]	SKEW (W)[b]
butene-1	0.918							
universal calibration		32,300	132,000	486,500	57,000	216,000	9	12
LALLS		59,500	134,000	669,500	67,000	268,000	15	220
octene-1	0.920							
universal calibration		37,000	132,000	327,000	59,200	160,000	6	3
LALLS		52,600	132,000	530,000	64,500	229,000	11	193
octadecene-1	0.940							
universal calibration		7,100	133,500	1,350,000	30,000	403,000	47	21
LALLS		43,000	158,000	951,000	70,500	354,000	17	125

(a) SD = standard deviation of number (N) and weight (W) distributions (21).

(b) SKEW = skewness of number (N) and weight (W) distributions (21).

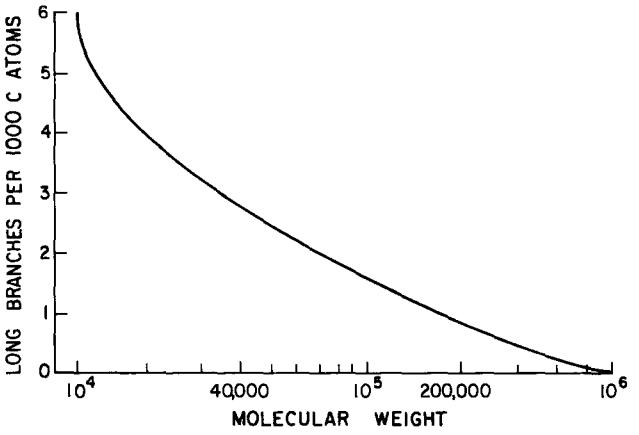

Figure 3

Long chain branching frequency versus molecular weight for ethylene-octadecene-1 copolymer.

expected. Long branches are formed in free radical polymerizations by chain transfer to polymer (19). Since larger dead polymers offer bigger targets it is assumed that encounters with growing macroradicals and long chain branching will be greater at higher molecular weights. This reasoning is plausible for isothermal polymerizations. Low density polyethylene polymerization reactions span a wide range of temperatures, however, and the molecular weight of the polymers produced decreases with increasing temperature. Chain transfer reactions are also enhanced at higher temperatures. The activation energy for chain transfer to polymer is greater than for the polymerization reaction (23). Thus chain transfer to polymer and long branch formation are most frequent under conditions where lower molecular weight polyethylenes are being formed.

The measurements reported here suggest that macroradicals undergo chain transfer reactions primarily with dead polymer that was formed in the same microregion of the flow-through reactor. This is not very surprising, since most polyethylene reactors are not designed to provide back-mixing.

Our conclusions differ from those of previous workers, who found either that long chain branching in low density polyethylene increased with molecular weight or was independent of molecular weight (18,20). The present data are believed to be more realistic, because they are derived with newer and more sensitive analytical methods.

There has also been some question as to whether the presence of short branches should be discounted in the estimation of long branch frequency (24,25). The present data show that short chain branching can be ignored, if a short branch is defined as one with six or less carbons.

Acknowledgment

The authors thank the Natural Sciences and Engineering Research Council of Canada for financial support. The ethylene/1-olefin copolymers were kindly supplied by C. T. Elston and Dupont Canada Ltd.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1823-1830 (1984)

APPLICATION OF GEL PERMEATION CHROMATOGRAPHY FOR INVESTIGATION OF 9-(2,3-EPOXYPROPYL)-CARBAZOLE OLIGOMERS

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Kaunas Antanas Sniečkus Polytechnic Institute

ABSTRACT

Gel permeation chromatography on dextran gels "Sephadex LH-20" and "Sephadex LH-60" in the solution of dimethyl formamide was used for the estimation of molecular weight and molecular weight distribution of 9-(2,3-epoxypropyl)carbazole oligomers and for the investigation of the process of their obtaining as well. The simple numerical method of the instrumental dispersion correction as well as the principle of "the differential chromatograms" was used to interpret the gel permeation chromatography data. The method used permits to separate 9-(2,3-epoxypropyl)carbazole oligomers within the range of molecular weights 150-15000.

INTRODUCTION

9-(2,3-epoxypropyl)carbazole (EPC) oligomers obtained by anionic and cationic polymerization are known as perspective organic semiconductors for electrophotography (1,2).

As the value of molecular weight (MW) and molecular weight distribution (MWD) of oligomers of this type considerably affects their properties the choise of a good determination method of these parameters is an important factor in the field of

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these investigations. Gel permeation chromatography (GPC) is fit the best for the determination of the MW and MWD of poly/9-(2,3-epoxypropyl)carbazole/ (PEPC) especially as the UV-absorbtion intensity of the solution of PEPC and a comparatively high MW of the elementary unit give possibility to use a simple equipment - a single short column furnished with the hydrostatic feeding of eluant, a continuous UV-absorbtion measuring cell made of ordinary optical glass and the UV-spectrophotometer as a detector.

EXPERIMENTAL

The GPC of EPC oligomers was carried out in the solution of dimethylformamide (DMFA) using dextran gels "Sephadex LH-20" and "Sephadex LH-60". The UV-spectro - photometer "Specord UV VIS" operating as a detector at the constant wavelength 294 nm was used. Samples (0.5 ml) were injected as a solution in eluant (0.05-0.4%). The elution rate was 20 ml/h. The elution volume measurement was carried out by means of graduated glass tube with the accuracy of 0.1 ml and marked on the elution curve at 2-3 ml intervals by means of the special button on the recorder. The detector's response - optical density of the PEPC solution in DMFA at the wavelenght 294 nm does not depend on the degree of polymerization in the investigated range of MW and can be transformed directly into the concentration.

RESULTS AND DISCUSSION

The numerical method of the correction of instrument spreading for the GPC data treatment was used (3,4). The essence of the method is as follows: a chromatogram described in general by the equation of instrument spreading 1 (5) is divided into a sufficient number of little intervals. This equation

$$\int_0^\infty F(v) = \int_0^\infty A(v,y)\ W(y)\ dy \qquad (1)$$

is expressed in the matrix form with a sufficient degree of accuracy:

$$F = A \cdot W \qquad (2),$$ where

F - the matrix of the experimental chromatogram function values,

A - the matrix of the instrument spreading function values,

W - the matrix of the corrected chromatogram function values.

When the function of instrument spreading represents the Gaussian distribution, every member of the matrix A is calculated by means of the equation:

$$a_{ik} = \delta (h_k/\pi)^{1/2} \exp/-h_k(i-k)^2\delta^2/ \qquad (3),$$ where

δ - the interval of the division, ml or counts,

h_k - the coefficient of instrument spreading at the elution volume v_k, ml^{-2} or $counts^{-2}$,

i and k - numerical variables.

The corrected chromatogram in the matrix form was calculated by means of the equation:

$$W = A^{-1} F \qquad (4).$$

For this purpose by the use of a standard computer program the inverse matrix A^{-1} is calculated and multiplied by the matrix F or by multiplying the matrixes $A \cdot W$ according to the equation 1 and obtaining the system of equations:

$$\begin{aligned} a_{11}w_1 + a_{12}w_2 + \dots + a_{1k}w_k &= f_1 \\ a_{21}w_1 + a_{22}w_2 + \dots + a_{2k}w_k &= f_2 \\ \vdots \quad\quad \vdots \quad\quad\quad\quad \vdots \quad &\vdots \\ a_{i1}w_1 + a_{i2}w_2 + \dots + a_{ik}w_k &= f_i \end{aligned} \qquad (5).$$

After designing $f_1 = a_{1,k+1}$; $f_2 = a_{2,k+1}$; $\dots$ $f_i = a_{i,k+1}$ the system 5 is solved by means of a standard computer program. The solution of this system represents the desired matrix W - the corrected chromatogram.

The division interval of a chromatogram was so chosen that the determinant value of the matrix A was no less than $4 \cdot 10^{-9}$ as the inversion of such "singular" matrixes as well as the solution of the systems of equations based on them is impossible. Thus the interval value for the caculations was 1 and 2 ml for "Sephadex LH-20" and 3 ml for "Sephadex LH-60". In order to decrease the division interval to 1 ml the first point was transferred by 1 ml and the calculations were carried out twice or three times respectively. The results were arranged to corresponding points at 1 ml intervals. The values h_K were determined by method mentioned (4) and were assumed equal throughout the interval of elution volume as their changes were negligible.

In order to demonstrate the possibilities of the method used the experimental and corrected (h_K=0.352) elution curves of the typical product of anionic polymerization of EPC under the action of KOH having the number-average MW (M_n) 1030 and weight-average MW (M_w) 1100 are shown in Figure 1. It is evident the method permits to separate the lower PEPC oligomer homologues to the polymerization degree of n=4. For this experiment the "Sephadex LH-20" gel column was used. The MW range of oligomers separated by this column was 150~2000.

For the investigation of EPC oligomers having a higher MW, for example, the polymerization products of EPC under the action of the alluminium isopropoxide - zinc chloride catalytic system the "Sephadex LH-60" gel was used. The corresponding elution curves of the PEPC sample (M_w=4270, M_n=3020) are shown in Figure 2 (h_K= 0.091). It is obvious that this product differs in the considerably higher polydispersity than PEPC obtained by using KOH. The process itself is more complicated by

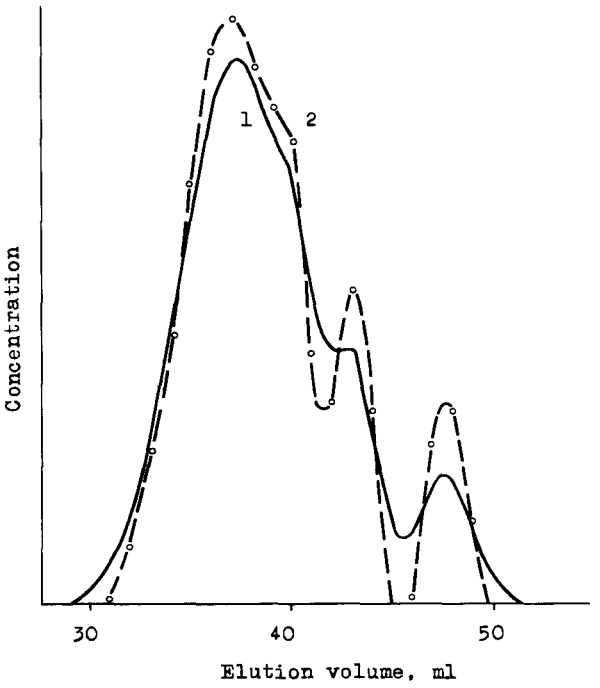

Figure 1

Experimental (1) and corrected (2) elution curves of the EPC anionic polymerization product.

instrument spreading as the low h_K value indicates. It should be noted that low h_K value results the considerable oscilation in the corrected chromatogram what is characteristic for most other methods of the instrument spreading correction (6). The PEPC MW range that is separated by this column was 200-15000. The data (see Figures 1 and 2) show that the application of both GPC variants allows to obtain the information about MW and MWD of PEPC in the range of MW 150-15000.

GPC was used not only for the analysis of the EPC polymerization products, but for the investigation of

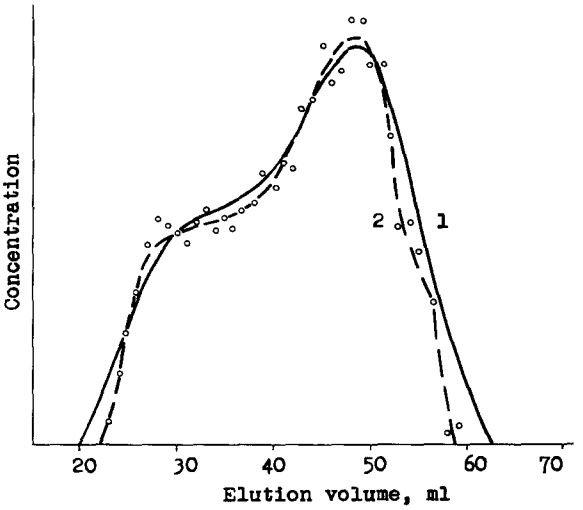

Figure 2

Experimental (1) and corrected (2) elution curves of the EPC polymerization product under the action of catalytic system aluminium isopropoxide - zinc chloride.

this process mechanism by observation on the changes of the MWD at the different monomer conversion as well. For this purpose the "differential chromatogram" principle (7) was used giving possibility to estimate the MWD of the product formed in the definite interval of time as well as the substances from which this product was formed. The investigation of this type was demonstrated by an example of the EPC polymerization in the dioxane solution under the action of sodium phenoxide in the presence of dibenzo-18-crown-6 (8). The differential MWD curves of the product formed at the different stages of monomer conversion are shown in Figure 3 a, b ("Sephadex LH-20" GPC, h_k=0.488). Each Figure has two MWD curves corresponding to different

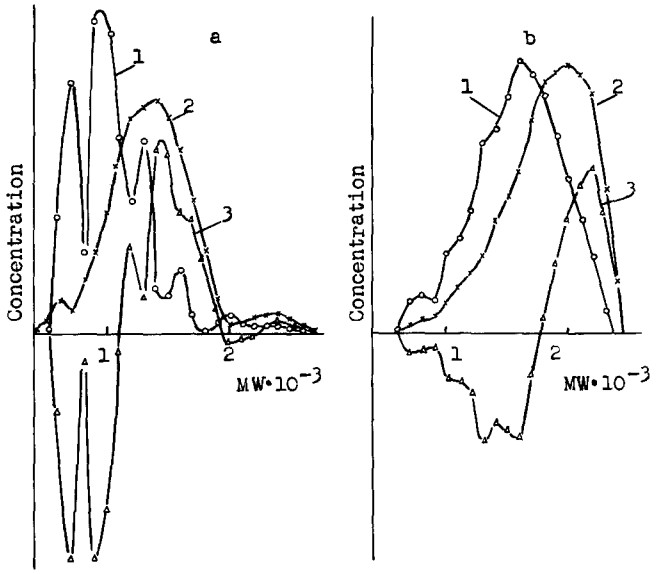

Figure 3

MWD curves of EPC polymerization products at different degrees of monomer conversion: a - 3.2% (1), 8.8% (2), MWD difference curve (3); b - 15.8% (1), 32.0% (2), MWD difference curve (3).

degrees of a monomer conversion as well as a "MWD difference curve" obtained on their basis. The monomer conversion vas 3.2 and 8.8% (Figure 3 a), and 15.8 and 32.0% (Figure 3 b). A part of the MWD difference curve below the base line corresponds to fractions of the EPC oligomer having reacted in the given time interval whereas the upper part of this curve corresponds to the reaction products. Thus it is evident that in the initial polymerization period (the change of the EPC conversion from 3.2 to 8.8%) the lower PEPC homologues (MW 700 and 900) add a monomer to form products having MW 1200, 1400, 1500 and higher. At more considerable polymerization degree (see Figure 3 b) the decrease of

the content of low-molecular fractions with MW 600-1800 and the formation of much higher MW products is obvious too. Thus the investigation proved the stepping chain growth mechanism in the process.

Hence, the application of GPC by using the instrument spreading correction for the data treatment in the investigation of the synthesis and properties of EPC oligomers allows to estimate the MW and MWD of the products obtained as well as to get the definite information about the mechanism of the process.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1831-1840 (1984)

GEL CHROMATOGRAPHY WITH SILICA GELS
I. COLUMN SYSTEMS FOR CONVENTIONAL POLYMER SEPARATIONS EXTENDED TOWARDS LOWER MOLAR MASSES [+]

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ABSTRACT

Three procedures for preparation of silica gels with small pore diameters suitable for gel chromatography were tested. The materials with optimum properties were not obtained, however, it was shown that the column set with the separation range extended towards lower molar masses can be built using appropriately chosen silica gels.

INTRODUCTION

Since recently, SiO_2 based column filling materials have been rather frequently used in both conventional and high performance gel chromatography (size exclusion chromatography).

Silica based gels posses several advantages:

1. Their structure and, consequently, their both external and pore geometry is essentially independent of pressure, temperature and eluent.

[+] Presented at 7th Symposium on Column Liquid Chromatography, Baden Baden, May 1983

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2. They are compatible with mobile phases of different polarities.
3. They allow to prepare column packings with
   - open pore structure exhibiting high separation efficiency due to fairly high velocity of the mass transfer
   - large pore volumes allowing to increase separation selectivity
   - desired pore sizes from about six nanometers up to few hundreds of nanometers
   - matched pore size distribution to obtain possibly highest separation selectivity
   - strictly spherical particle shape with diameters ranging from few micrometers up to hundreds of /um.
4. It is possible to modify their surface by simple chemical reactions.

High mechanical and thermal stability, universality as to the eluent and versatility in physico-chemical properties make SiO_2 aero-gels a welcome completion to the organic polymers based column fillings for gel chromatography (GPC).

On the other hand, the silica gels and porous glasses exhibit some drawbacks:

1. Free silanol groups cause the surface activity of the packing and, consequently, unwanted interactions with both electroneutral and charged samples.
2. Both SiO_2 matrix and its bonds with organic groups of the surface modifier are unstable in eluents with pH 7 - 8 and, generally, in aqueous mobile phases.
3. So far silica gels are not available with sufficiently large pore volume and narrow pore

size distribution with the large pores above 400 nm and with the small pores between about 2 and 6 nm in diameter. The column fillings with very large pore sizes are needed for fractionations of extremely large synthetic and biological macromolecules and for separations of particles of dispersions. On the other hand, gels with small pore dimensions would be used:

i. For both analytical and preparative selective GPC fractionation of oligomers or at least for their quantitative separation from high polymers

ii. For construction of GPC column sets with calibration dependences: log molar mass versus elution volume linear down to few hundreds g mol^{-1} molar mass values

iii. For separation of peaks of polymers from various ghost peaks caused by gases, water and other low molecular impurities and polymer additives often present in the injected solutions.

The linearity of the calibration curve simplifies substantially the GPC data processing while the quantitative separation of oligomeric and low molecular substances from the analysed polymer is a necessary condition for obtaining reliable data on mean molar masses and molar mass distribution: It is known that the disturbances of GPC traces in the region of high elution volumes decrease the precision of the values of number average molar mass of the macromolecular substance measured by gel chromatography.

In present paper, we describe our results concerning experimental work devoted to the solving the latter problem. The obtained results are presented in the form of calibration curves.

EXPERIMENTAL

The GPC calibration dependences were measured by means of a simple device assembled in this Laboratory. The eluent was transported by means of a single piston reciprocating membrane pump, Model VMC 300 (Workshops of Czechoslovak Academy of Sciences, Prague, Czechoslovakia) that was provided with a pulse damper according to (1). The three-way six-port injection valve was equipped with the loops 0.5 - 3 mL depending on the column set. The column dimensions were 1,220 or 610 or 500 mm in length and 8 or 4 mm in diameter. The detector was a differential refractometer, Model 2025/50 (Knauer K. G., Bad Homburg, FRG). The elution volumes were measured either by means of an automatic siphon system that was provided with a device diminishing the loss of eluent by evaporation (2) or with a drop counter Model DC 1002 (Laboratory Instruments Works, Prague, Czechoslovakia). Both siphon system and drop counter were calibrated by weighing.

Tetrahydrofuran (THF) was used as eluent after purification described in (3). Narrow polystyrenes with molar masses in the range from 6×10^2 up to 10^7 g mol^{-1} were products of Pressure Chemicals, Pittsburgh, PA, USA,or Toyo Soda MfG. Co., Ltd., Tokyo, Japan.

The column packings were products of Electro Nucleonics, Fairfield, NJ, USA (various types of porous glass CPG - 10); Merck, Darmstadt, FRG (set of Fractosils); Waters Inc., Milford, MA, USA (set of Porasils) and Glassworks Kavalier, Votice, Czechoslovakia (Silpearl). Silpearl sorbent was originally intended for TLC. Its surface area was about 600 m^2 g^{-1}, pore volume approx. 0.5 mL g^{-1}. In our experiments the particle fraction 30 - 60 μm was selected. All columns were dry packed by the classical tap-and-fill procedure.

RESULTS AND DISCUSSION

Figs. 1 - 3 show the GPC calibration curves for various commercial SiO_2 based column packings. Evidently, the selectivity of the separation is rather poor in the molar mass range below about 4×10^3 g mol^{-1} for all gels studied.

In an effort to change pore sizes of silica gels to make them more suitable for separation in lower molar mass region, three different procedures were used:

a. Diminishing the pore sizes by depositing various materials into wide-pore packings. The easiest way seemed to be the polymerization of different monomers on the pore walls of silica gels. Various epoxy resins were formed within the pores. The amount of resin varied from 10 to 60 mass % calculated on the starting silica gel. The results were not promising: While the smallest pores had been already completely blocked by the resin the large pores have still remained too large. If the amount of deposited resin was further increased, both the pore diameters and pore volumes decreased simultaneously so that the resulting material with desired pore size had too small pore volume.

b. Controlling the polymerization of silicic acid so that presumably small pores were formed in resulting silica gels. Various conditions for fine pore formation were tested: Concentration of starting sol of silicic acid, time and temperature of polymerization as well as postpolymerization treatment. The results obtained were again not satisfactory (cf. Fig. 4). The silica gels so far prepared had either too large mean pore diameters or too small pore volumes.

c. Increasing the pore diameters of the silica gel with very small pores. In our experiments, we have chosen

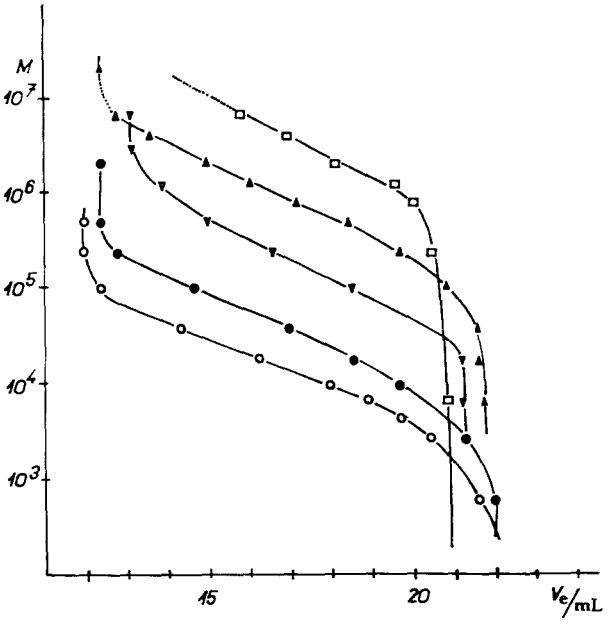

FIGURE 1. GPC calibration curves for Fractosils. □ - Fractosil 500 nm; ▲ - Fractosil 250 nm; ▽ - Fractosil 100 nm; ● - Fractosil 50 nm; ○ -Fractosil 15 nm. The numbers represent the mean pore diameters of the gels given by the producer.

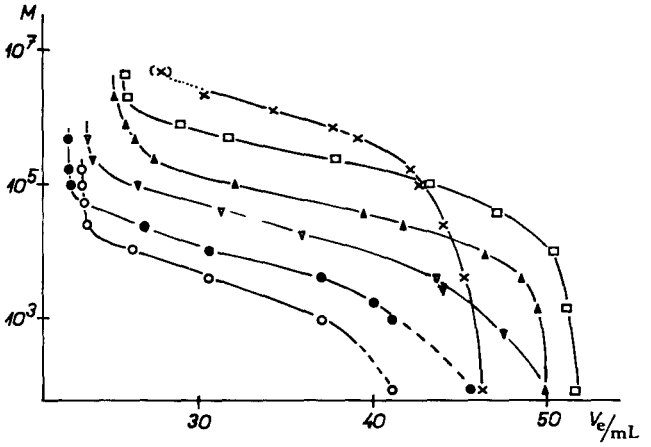

FIGURE 2. GPC calibration curves for Porasils. ○ - Porasil A; ● - Porasil B; ▽ - Porasil C; △ - Porasil D; □ - Porasil E; × - Porasil F. Column dimensions 610 x 8 mm.

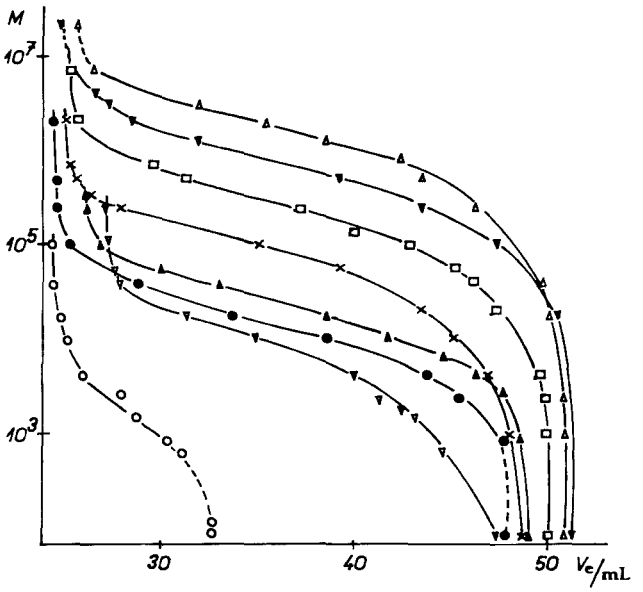

FIGURE 3. GPC calibration curves for CPG - 10 Porous Glasses. △ - 204.5 nm; ▼ - 142.2 nm; □ - 72 nm; × - 36.8 nm; ▲ - 15.6 nm; ● - 11.8 nm; ▽ - 7.5 nm; ○ - 4 nm. Column dimensions 1220 x 8 mm.

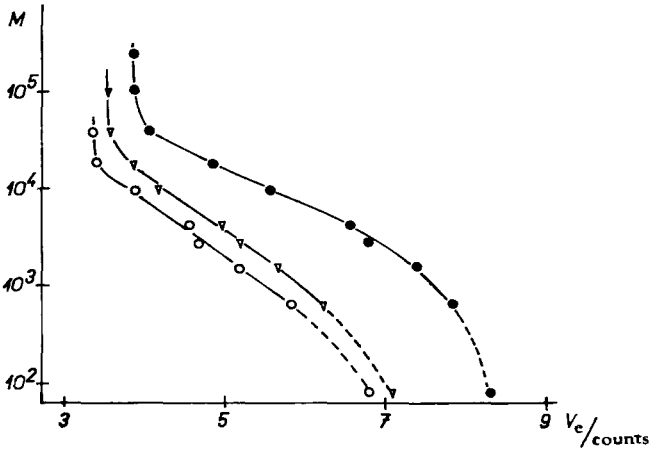

FIGURE 4. GPC calibration curves for experimental irregular silica gels. ● - SG-3N; ▽ - SG-15N; ○ - SG-10N. Column dimensions 500 x 6 mm. 1 count represents 0.7 mL.

silica gel Silpearl despite of its rather small pore volume. We used various leaching techniques applying solutions of alkaline (NaOH, KOH) or acidic (HF) leaching agents at different concentrations, temperatures and reaction times. Here again, we have found that probably due to the interface tension between leaching agent and silica gel, as well as due to the restricted diffusion rate of the leaching agent into small pores, the dissolution of gel matrix started preferably in larger pores. In other words, leaching resulted again in materials with too large pore diameters. The calibration curves for some materials obtained by leaching of Silpearl silica gel are shown Fig. 5.

Finally, we have decided to use the columns packed with original and leached Silpearl in combination with the above mentioned commercial wide-pore silica gels in order to prepare column sets with the separation ranges extended towards lower molar masses.

The examples of the calibration dependences for some column sets are shown in Fig. 6. The experimental points are compared with the courses of the calibration dependences obtained by simple addition of the elution volumes for particular single columns. The agreement is surprisingly good.

From the presented results it can be concluded that special types of narrow pore silica gels can be used for extending the separation range of the conventional wide pore SiO_2 based GPC packings towards lower molar masses. However, due to generally smaller pore volume of these materials, several columns packed with narrow pore silica gels must be added to the column systems in order to obtain linear calibration curves down to 10^3 g mol^{-1} and lower molar masses or, at least,

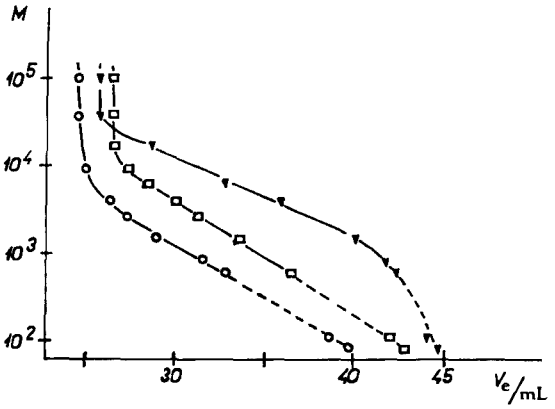

FIGURE 5. GPC calibration curves on Silpearl. ○ - starting sample; □ - sample leached by HF (0.5 hour in 3 % aqueous HF solution at 25 °C) ∇ - sample leached by NaOH (1 hour in boiling 2 % NaOH solution). Columns dimensions 1220 x 8 mm.

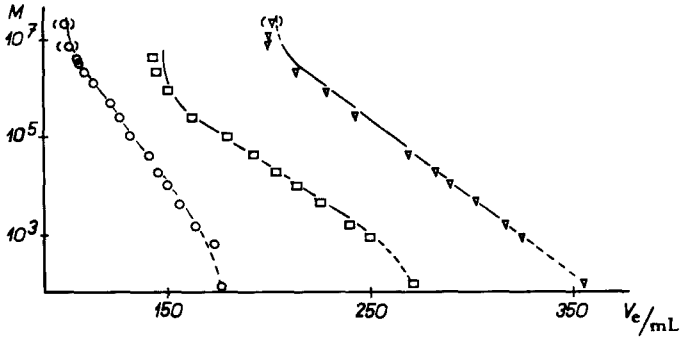

FIGURE 6. Combined calibration curves. ○ - Fractosils: 1 x 500 nm, 2 x 250 nm, 1 x 100 nm, 2 x 15 nm, plus 1 x Silpearl modified by NaOH, 1 x Silpearl modified by HF, columns 8 x 610 mm; □ - Porasils: C, D, E, 1 x original Silpearl, 1 x Silpearl modified by NaOH, 1 x Silpearl modified by HF, columns 8 x 1220 mm; ∇ - 1.5 x Fractosil 500 nm, 1 x CPG Porous Glass 142.2 nm, 1 x CPG Porous Glass 36.8 nm, 1 x CPG Porous Glass 15.6 nm, 1 x original Silpearl, 1 x Silpearl modified by NaOH, 1 x Silpearl modified by HF, columns 8 x 1220 mm; solid line - calculated curve, points - experimental.

column systems that resolve the peaks of polymers from the peaks of oligomeric or low molecular accompanying substances. Consequently, both the dead volume of the system and the time of analysis increase and the overall separation efficiency is partially sacrificed.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1841-1850 (1984)

HYDRODYNAMIC VOLUME FLUCTUATION OF POLYSTYRENE BY COLUMN TEMPERATURE AND ITS EFFECT TO RETENTION VOLUME IN SIZE EXCLUSION CHROMATOGRAPHY

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ABSTRACT

The fluctuation in hydrodynamic volume of polystyrene in several solvents was evaluated by changing solvent temperature and the relation between the magnitude of the fluctuations and the retention volume change was experimentally examined. Limiting viscosity numbers of polystyrene in tetrahydrofuran(THF), chloroform, toluene, cyclohexane, and a benzene-methanol mixed solvent (77.8/22.2 vol/vol) were measured by using a Ubbelohde-type viscometer. Temperature dependence of limiting viscosity number of the polystyrene solution was observed in some range of temperature, where a change of the retention volume of polystyrene would be assumed to be observed with the change of column temperature because of the change of its hydrodynamic volume in solution. The examination of the temperature dependence of retention volume in SEC for polystyrene standards confirmed this effect. The recommended column temperature is in the range where the temperature dependence of the limiting viscosity number of polystyrene solutions is negligible; e.g., at 25° - 50°C for THF, 35° - 65°C for toluene, and 20° - 30°C and 45° - 55°C for chloroform. Column temperature in the range of 30° - 45°C in chloroform is also recommended because of counterbalance of several effects to retention volume fluctuation.

INTRODUCTION

The measurement of molecular weight averages and their distributions for polymers by size exclusion chromatography (SEC, or GPC)

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requires the construction of a calibration curve obtained by plotting retention volumes vs. molecular weight of the corresponding polymers. A reliable molecular weight - peak retention volume relationship is the most important factor for obtaining the accurate and precise molecular weight averages. The retention volume changes with changing column temperature [1,2]. In the previous paper [3], we reported the effect of column temperature on the retention volumes of solute polystyrenes and it was stressed that a 10°C change in column temperature caused a decrease of about 1% in retention volume, which corresponds to an error of more than 10% in molecular weight at the same retention volume. Two main factors which cause the retention volume fluctuation were assumed to be (1) an expansion or a contraction of the mobile phase in the column due to the temperature difference between column and solvent and (2) the adsorption effects of a solute to the gel phase.

In SEC, solutes are separated according to their hydrodynamic volumes in solution. Cantow et al. [1] and Little and Pauplis [2] have explained the effect of column temperature to retention volume by the variation of polymer coil size. However, at a molecular weight of 100,000 and an increase in temperature of 115°C from 35 °C, the linear expansion coefficient of polystyrene in 1,2,4-trichlorobenzene was 1.05 [1], so that the estimated change in retention volume caused by the increase in hydrodynamic volume was about 0.07% when an increase in temperature was 10°C [3]. In the previous paper [3], it has been assumed that the estimated change in hydrodynamic volume caused by the change in column temperature was negligibly small.

However, it is obvious that temperature of a polymer solution affects its limiting viscosity number [4,5]. which increases or decreases with temperature and exhibits a maximum in an limiting viscosity number vs. temperature curve when it is measured over sufficiently wide range of temperature [5]. Because the value of hydrodynamic volume of a polymer in solution is proportional to the product of its limiting viscosity number $[\eta]$ and molecular weight M, the change in limiting viscosity number with temperature will

also affect the retention volume of the polymer. In other words, the evaluation of the change of the limiting viscosity number with temperature can estimate the change of the retention volume caused by the variation of the hydrodynamic volume with column temperature.

In this paper, the temperature dependence of limiting viscosity number of polystyrene dissolved in several solvents commonly used in SEC is demonstrated and the contribution to the retention volume of hydrodynamic volume fluctuation with temperature is estimated.

EXPERIMENTAL

Determination of limiting viscosity number.

Limiting viscosity number of polymer solutions in the range of concentration 0.2 - 1.0 g/dl was measured by using Ubbelohde-type viscometers (one has the range of dynamic viscosity 1.7 - 5 cst and the viscometer coefficient 0.00463 cst/s and the other 3 - 10 cst and 0.00980 cst/s). A sample polymer used for the measurement of limiting viscosity number was a commercial polystyrene ESBRITE ($\bar{M}_w = 2.87 \times 10^5$ and $\bar{M}_n = 1.08 \times 10^5$). Viscosity numbers of the polymer solutions were calculated by dividing the specific viscosities (which are relative viscosities minus 1) by the corresponding concentrations. The limiting viscosity number of a polymer solution was then obtained by plotting viscosity numbers vs. concentrations and by extrapolating the line to the intersection at zero concentration. Solvents used for the measurements of limiting viscosity number of polystyrene were tetrahydrofuran (THF), toluene, chloroform, cyclohexane and a benzene-methanol mixed solvent (77.8/22.2 vol/vol).

Determination of retention volume.

A Jasco (Japan Spectroscopic Co. Ltd., Hachioji, Tokyo 192, Japan) TRIROTAR high performance liquid chromatograph was used with a Shodex Model SE-11 differential refractometer (Showa Denko Co.,

Minato-ku, Tokyo 105, Japan). Column systems were DuPont Bimodal SEC columns (PSM-60S and PSM-1000S) packed with deactivated silica gel and two Shodex A80M SEC columns packed with polystyrene gel. Columns were thermostated at specified temperatures to an accuracy of 0.1°C in a water bath. Mobile phases were toluene, chloroform for DuPont columns and cyclohexane for Shodex columns.

Monodisperse polystyrene (PS) standards (Pressure Chemical Co., Pa., USA) were used as test samples. A portion of 0.1 ml of 0.05% polystyrene solutions was injected into columns. The flow rate of the pump dial was adjusted to 0.5 ml/min for PSM columns and 1.0 ml/min for Shodex columns and the actual flow rate was checked at the outlet of the RI detector by measuring the time required to fill a 10-ml measuring flask with solvent eluted from the system.

Measurement of peak retention volumes was performed five times and average values were obtained. These retention volumes were first taken in units of time and then calculated by multiplying the retention time by the flow rate measured at the outlet of the RI detector. The retention volume thus obtained was then corrected by subtracting or by adding the amount due to the difference between column temperature and the mobile phase temperature in the reservoir. The partition coefficient (K_{SEC}) of each polystyrene standard was calculated as follows:

$$K_{SEC} = (V_c - V_o) / V_i$$

where V_c is the corrected retention volume of a polystyrene standard, V_o the void volume or interstitial volume of gel in the columns (V_o for PSM 5.33 ml and for A80M 20 ml), and V_i inner volume of gel in the columns (V_i for PSM 4.37 ml and for A80M 21 ml).

RESULTS and DISCUSSION

The dependence of limiting viscosity number on temperature for PS (unfractionated PS) in five solvents is shown in Figures 1 and 2. In these figures, we can see that a range of temperature where

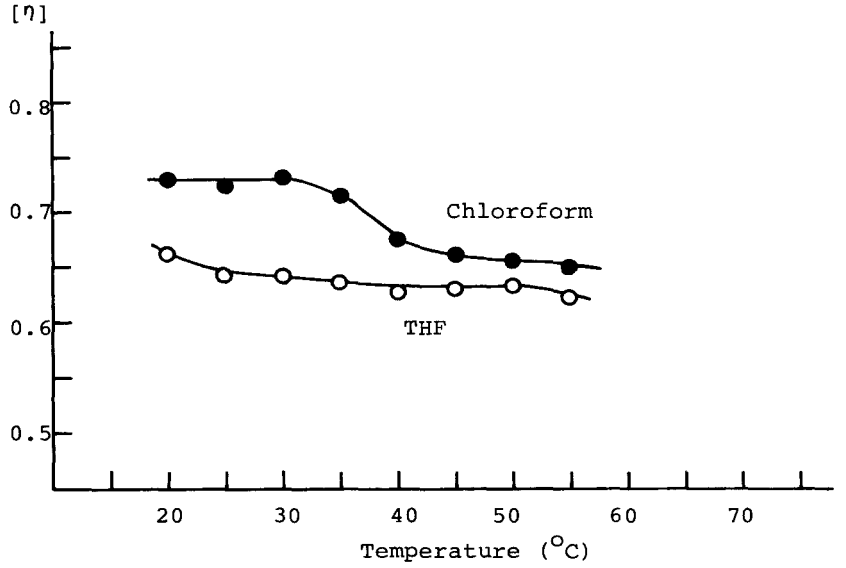

FIGURE 1. Plot of limiting viscosity number versus temperature for unfractionated polystyrene in chloroform and THF.

a variation of limiting viscosity number is below 0.01 is at 25° - 50°C for THF, 35° - 65°C for toluene, 20° - 30°C and 45° - 55°C for chloroform, and 25° - 45°C for benzene/methanol (77.8/22.2 vol/vol). In these temperature ranges, the contribution of hydrodynamic volume difference due to temperature fluctuation to retention volume would be negligible. The limiting viscosity number of PS in cyclohexane increased with increasing temperature and that in benzene/methanol increased similarly over 45°C. The mechanism of temperature dependence of limiting viscosity number is out of our scope.

In the previous paper [3], it was observed that a 10°C change in column temperature caused about a 1% shift of retention volume and resulted in errors of more than 10% in molecular weight at the same retention volume. In this experiment, a 10°C increase from 25°C for toluene or a 15°C increase from 30°C for chloroform was found to cause an about 10% increase or decrease in hydrodynamic volume of PS in these solvents, respectively, resulting in the

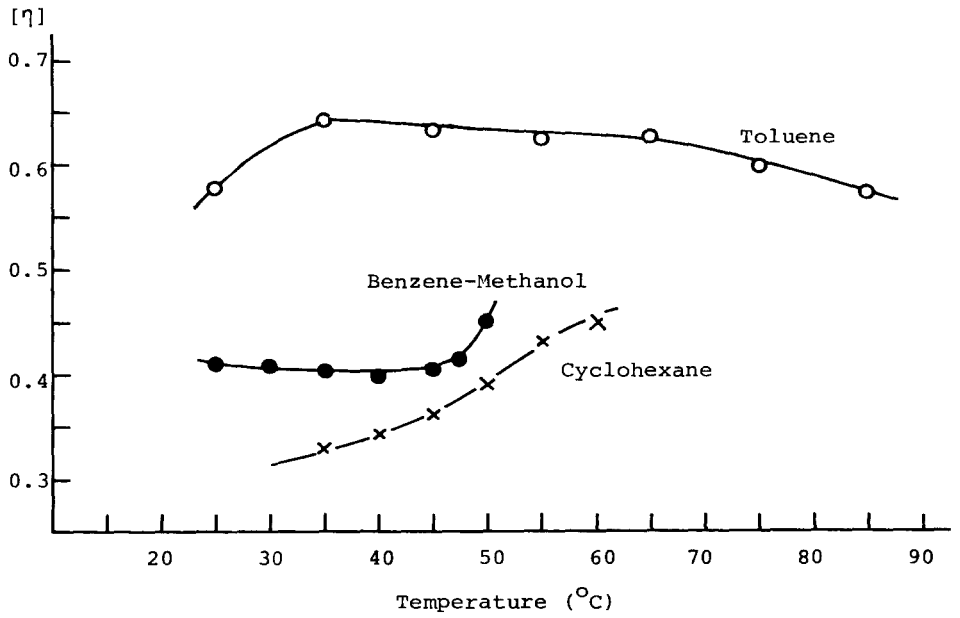

FIGURE 2. Plot of limiting viscosity number versus temperature for unfractionated polystyrene in toluene, cyclohexane and a benzene - methanol mixed solvent.

change of 0.5% in retention volume which corresponds to a 5% change in molecular weight.

A shift of retention volume with increasing column temperature will be resulted in next three main factors:

(i) An expansion of the mobile phase in the column due to the difference of temperatures between the column and a solvent reservoir (and a pumping system).

(ii) The adsorption effects of a solute to the gel phase.

(iii) A change of hydrodynamic volume of a solute.

In the previous paper [3], the third factor was excluded. In the experimental range of 25° - 45°C in the mobile phase THF, it is observed to be correct (see Figure 1). However, in the range where the limiting viscosity number fluctuates, the effect of a change of

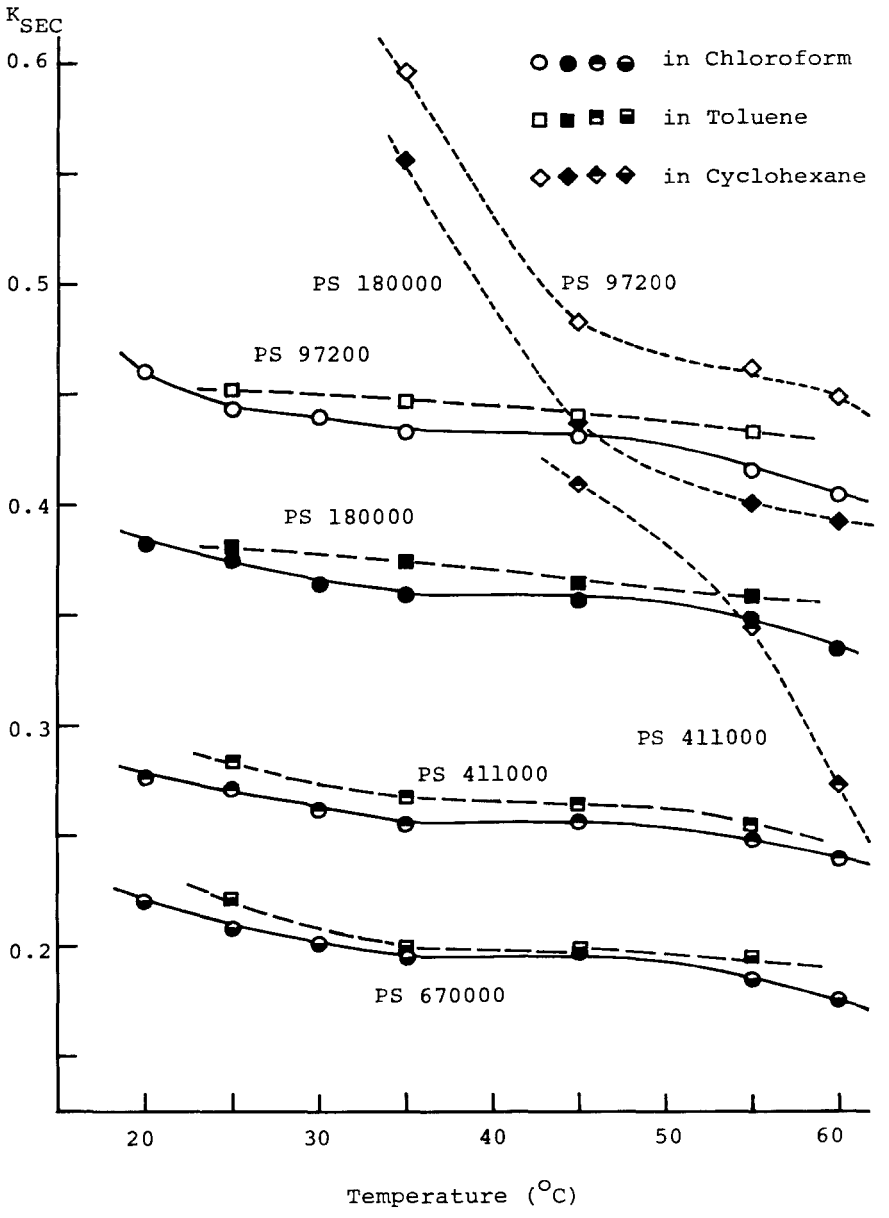

FIGURE 3. The relationship between column temperature and partition coefficients for polystyrene standards in chloroform, toluene and cyclohexane.

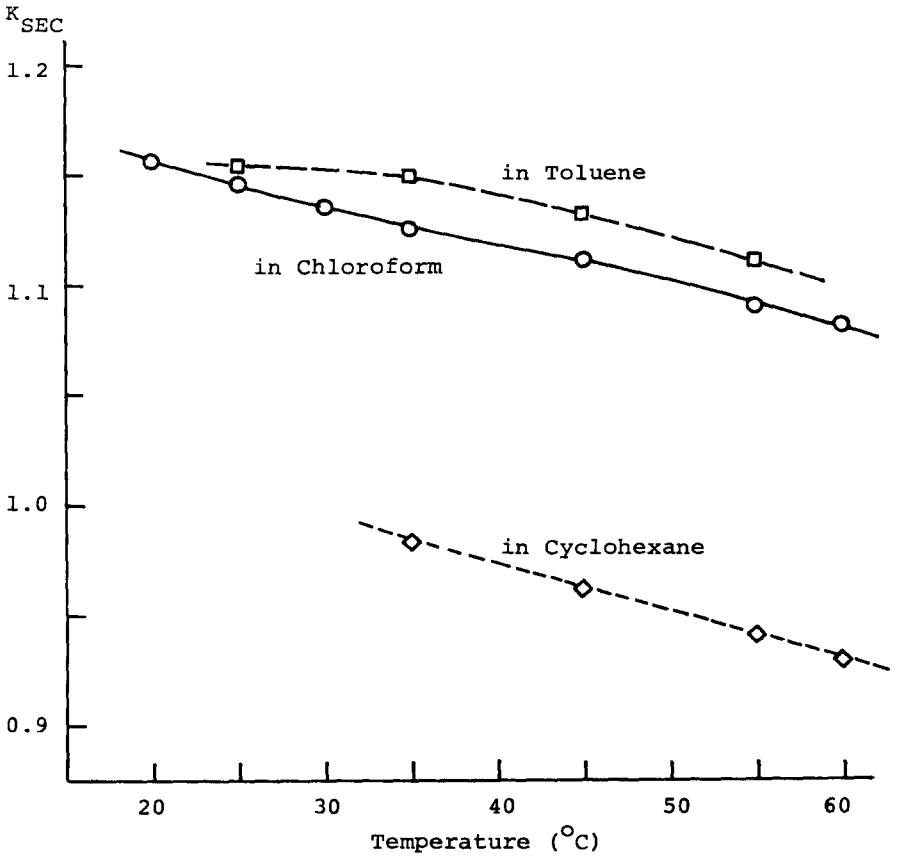

FIGURE 4. The relationship between column temperature and partition coefficients for n-hexane in chloroform, toluene and cyclohexane.

hydrodynamic volume can not be ignored. The increase in column temperature causes the expansion of the mobile phase in the column and the reduction of the adsorption effect of a solute to the gel phase, resulting in the decrease of the retention volume. On the other hand, in case of the temperature dependence of the limiting viscosity number, as shown in Figures 1 and 2, there are three types; constant over some range of temperature difference, the increase or the decrease with increasing temperature. When the lim-

iting viscosity number increases with temperature, then the retention volume decreases, as is the case in (i) and (ii). If the limiting viscosity number decreases with increasing temperature, the retention volume will increase and result in the counterbalance to the effects of expansion of the mobile phase and of the adsorption to the gel phase. This will be the case for the range 30° - $45^{\circ}C$ in chloroform.

Figures 3 and 4 show the relationships between column temperature and the partition coefficients K_{SEC} for four PS standards and n-hexane in three different mobile phases. In chloroform, a plateau in the curve is observed at 35° - $45^{\circ}C$ as is expected from the above discussion.

In toluene, the deviation of the values of K_{SEC} between $25^{\circ}C$ and $35^{\circ}C$ is much than those between $35^{\circ}C$ and $45^{\circ}C$ and between $45^{\circ}C$ and $55^{\circ}C$, which corresponds to the increase in the limiting viscosity number at $25^{\circ}C$ with increasing temperature.

In cyclohexane, the values of K_{SEC} decrease significantly with increasing in temperature. This phenomena may be attributed to the increase of the limiting viscosity number with temperature in addition to the decrease of the adsorption effect with increasing in temperature.

The values of K_{SEC} for n-hexane decrease uniformly with increasing in temperature, suggesting the participation of the hydrodynamic volume effect in the temperature dependence of the values K_{SEC} of polystyrene solutes.

In conclusion, the recommended column temperature is in the range where the temperature dependence of the limiting viscosity number of polystyrene solutions is negligible; e.g., at 25° - $50^{\circ}C$ for THF, 35° - $65^{\circ}C$ for toluene, and 20° - $30^{\circ}C$ and 45° - $55^{\circ}C$ for chloroform. Column temperature in the range of 30° - $45^{\circ}C$ in chloroform, though the temperature dependence of limiting viscosity number is significant, the counterbalance of the three effects makes the temperature dependence of the retention volume minimum. Though hydrodynamic volume or size of polymer in solution should essentially be measured by light scattering technique, our discus-

sion would still be effective in the point of the factors that affect the reliability to a molecular weight - retention volume relationship.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1851-1865 (1984)

CONCENTRATION DEPENDENCE IN GEL PERMEATION CHROMATOGRAPHY

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ABSTRACT

Four narrow distribution polystyrene samples with molecular weights from 3.5×10^4 to 2.7×10^6 and six linear or branched polyvinyl acetate samples were used in the study. GPC experiments were performed in two solvents (THF and MEK), three column sets (different permeation limits) and five different concentrations ranging from 0.05% to 3%.

The elution curves were normalized while average retention volumes and peak width were calculated. The data of the same sample with different concentrations can thus be compared on the same graph. The following conclusions were drawn.

(1) At very low concentration, elution curves were independent of the concentration. On increasing the concentration, peak positions were first moved to longer retention volumes and then the whole curves broaden appreciably.

(2) Concentration dependence increases with the increase in molecular weight and goodness of the solvent power.

(3) The plots of the retention volume vs concentration deviate from linearity. Extrapolation at higher concentrations is not reliable.

(4) The peak widths of the elution curves expressed by the variance σ increase with the increase of concentration.

(5) The initial slopes of the peak-concentration plot of the branched PVAc samples are proportional to the hydrodynamic volumes expressed as $[\eta]M$ of the samples.

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INTRODUCTION

Gel permeation chromatography is the most widely used method for determining the molecular weight and molecular weight distribution of high polymers (1). The reliability of the results depends both on the correct manipulation of the experimental technique and on the appropriate ways of data treatments. Many experimental conditions such as concentration, rate of flow, injection volume and temperature will have significant effects on the chromatograms. It is necessary to make proper choice and control of these factors.

Because of their universality, differential refractive index detectors are commonly used to monitor the concentration of the polymer in the eluate. The sensibility of RI detector, however, depends strongly on Δn, the difference in refractive index of the polymer and the solvents. In certain cases, only a few solvents with unfavorable Δn are available. Chromatographers have to use higher concentrations in order to obtain larger signal. Therefore studies on the concentration dependence in GPC are essential both for theoretical and practical reasons.

The existence of concentration dependence in GPC was already reported in literature (2). Many experimental results showed that retention volume tends to increase with increasing concentration. The effect is more pronounced the higher the molecular weight of the polymer and on the solvent goodness. The origin of the occurrence of the concentration dependence in GPC was explained in different ways. Moore (3) explained it from a hydrodynamic point of view. Since there is a large difference in viscosity of the solution and solvent, the plug flow of the eluate will be perturbed and distortion of chromatogram shape and excessive tailing result. Janča (4,5) showed by theoretical calculation and experimental verification that 80-90% of

the concentration effect can be attributed to hydrodynamic factors. Recently the concentration effect has been explained thermodynamically as a consequence of the reduction of the effective hydrodynamic volume of the solvated polymer coil with increasing concentration (6,7). Both theories can explain qualitatively the concentration effect in GPC.

In this work, GPC experiments were performed on 4 narrow distribution polystyrene samples in three column sets with different permeation limits, five concentrations (0.05% to 3%) and two solvents (THF and MEK) respectively. In addition, six polyvinyl acetate fractions with different degree of branching were carefully selected so that three of them have nearly same intrinsic viscosities and the other three have nearly same $\bar{M}_w$. They were chromatographed in four different concentrations using THF as the solvent. All the chromatograms thus obtained were normalized and comparisons were made on the same sample at different concentrations. The effects of molecular weight, degree of branching, solvent, column sets and concentration on the peak retention volume, average retention volumes and peak widths were examined.

EXPERIMENTAL

Samples:

Four narrow distribution polystyrene samples with molecular weights of 2.7×10^6, 6.7×10^5, 2.0×10^5 and 3.5×10^4 were obtained from Waters Associates Inc. Six PVAc fractions were prepared and fractionated in this laboratory. The characterization data of these six PVAc samples are listed in Table 1.

Solvents:

THF, Analytical pure; MEK, Chemical pure.

GPC equipment:

TABLE 1

Characterization Data For Six PVAc Fractions (8)

Sample	$\bar{M}_w \times 10^{-6}$	$[\eta]$	Conversion,%	G*	n_w**
PVAc-1	2.10	282	65.8	0.673	8.46
PVAc-2	2.04	316	51.1	0.768	4.69
PVAc-3	2.07	361	21.8	0.871	1.71
PVAc-4	0.92	230	7.06	1	0
PVAc-5	1.14	230	43.8	0.84	2.22
PVAc-6	1.78	229	65.8	0.815	4.95

* $G = [\eta]_b / [\eta]_l$

** n_w characterizing the number of branches in one molecule.

Waters LC/GPC Model 244 chromatograph was used. Two of the three column sets consisting of two 1-meter column connected in series each were packed with deactivated porous silica gel prepared in this laboratory. The permeation limits of these two sets are 2.7×10^6 and 9.5×10^5 in PS molecular weight respectively. The third column is a commercial one consisting of one 50 cm Shodex-Pak A-80M with a permeation limit of 5×10^6. In all cases, the flow rate was kept at 1 ml/min.

Data treatment:

All the chromatograms were normalized in order to make comparison on the same graph. The ordinates of the curves were transformed into $h_i / \sum h_i$, where h_i is the height of the species having retention volume of V_i. Figure 1 to Figure 6 are part of the typical curves obtained. Average retention volumes , $\bar{V}$, were calculated according to $\bar{V} = \sum (h_i V_i / \sum h_i)$. The widths of the curves were characterized by σ which were calculated

according to $\sigma = \sum [(h_i / \sum h_i) (V_i - \bar{V})^2]$. V_p vs c plots and $\bar{V}$ vs c plots were illustrated in Figure 7 and σ vs c plot was shown in Figure 8.

RESULTS AND DISCUSSIONS

Concentration Dependence of GPC Chromatograms:

Chromatograms of four PS samples in five different concentrations (approximately 0.05%, 0.1%, 0.3%, 1% and 3%) with THF or MEK as the solvent were illustrated in Figure 1 to Figure 5. With the exception of the PS-3.5 x 10^4 sample which has the lowest molecular weight, all the curves exhibit three different stages of variation of the shape on increasing the concentration of the sample. When the concentrations were sufficiently low, no concentration effect was seen and the curves coincide. This situation can be easily explained by both the hydrodynamic or the thermodynamic reasoning, since low concentration induces low viscosity difference and larger inter-molecular distances in the solution. On increasing the concentration to a certain level, the curves began to show distortion and the peaks moved to larger retention volumes. The spans of the chromatograms remained unchanged. This phenomenon can easily be realized through the theoretical consideration of reduction of coil dimension at finite concentration. The higher molecular weight portion of the sample will exhibit concentration effect at that concentration level while the lower molecular weight portion did not. As a result, the span of the chromatogram remained unchanged but the shape of the curves distorted. Elsdon (8) recently studied polydispersed samples and reached the same conclusion. On further increasing the concentration of the samples , the curves were broadened significantly along with severe tailing. This is obviously caused by very large difference in viscosity as well as

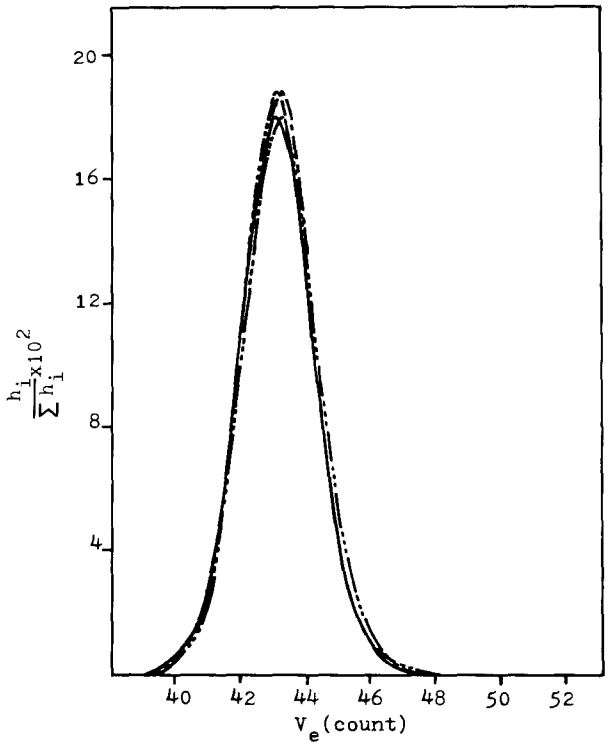

Fig. 1 Chromatograms of Five Different Concentrations of Sample PS-3.5 x 10^4 in MEK

by the further reduction of coil dimension and overloading of the column. Figure 6 is the result of high performance GPC. Serious concentration effects were observed along with appreciable tailing.

Effects of Molecular Weight, Solvent and Column Type on the Concentration Dependence:

Molecular weight and goodness of the solvent are found to be closely related with the concentration dependence. From the normalized chromatograms obtained for four PS samples, certain classification can be made to

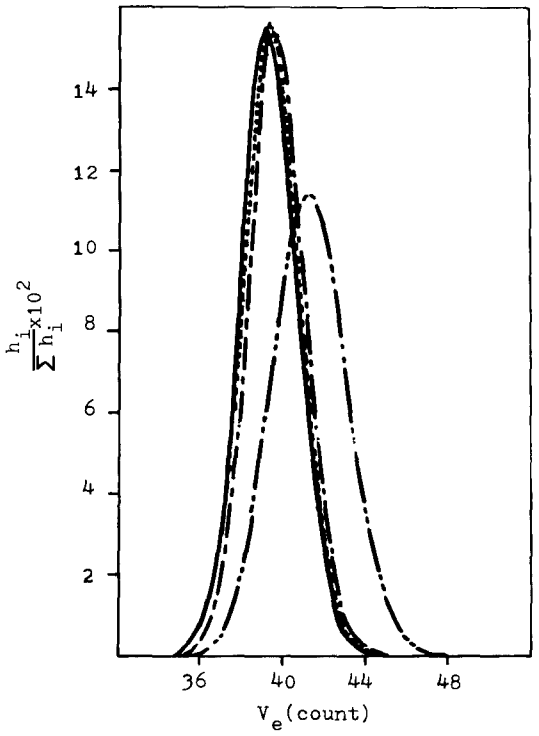

Fig. 2 Chromatograms of Five Different Concentrations of Sample PS-2.0 x 10^5 in MEK

show different behavior in concentration dependence as illustrated in Table 2. It was shown that the concentration dependence increases with molecular weight and goodness of the solvent, in agreement with those reported in literature (2). The third and fourth vertical columns in Table 2 listed the concentration regions which showed respectively ' no concentration effect ' and ' roughly beginning of the concentration effect '. The values of the fourth column should be quite close to the overlapping concentration c* proposed by de Gennes in his scaling treatment of the polymer solution (9). Graessley (10)

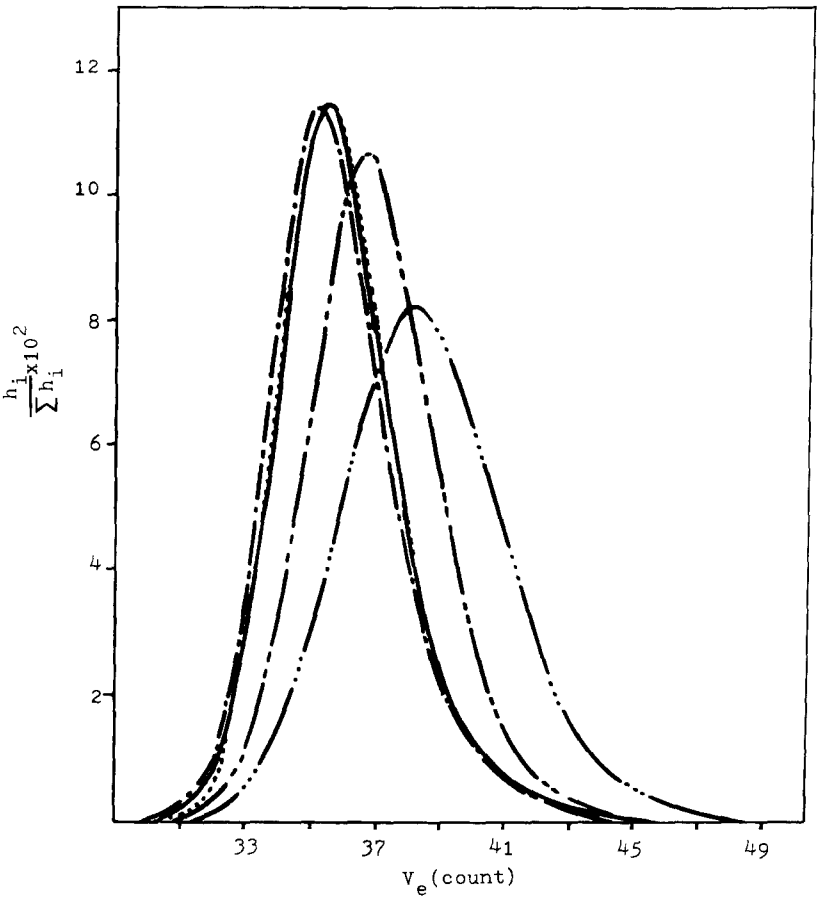

Fig. 3 Chromatograms of Five Different Concentrations of Sample PS-6.7 x 10^5 in MEK

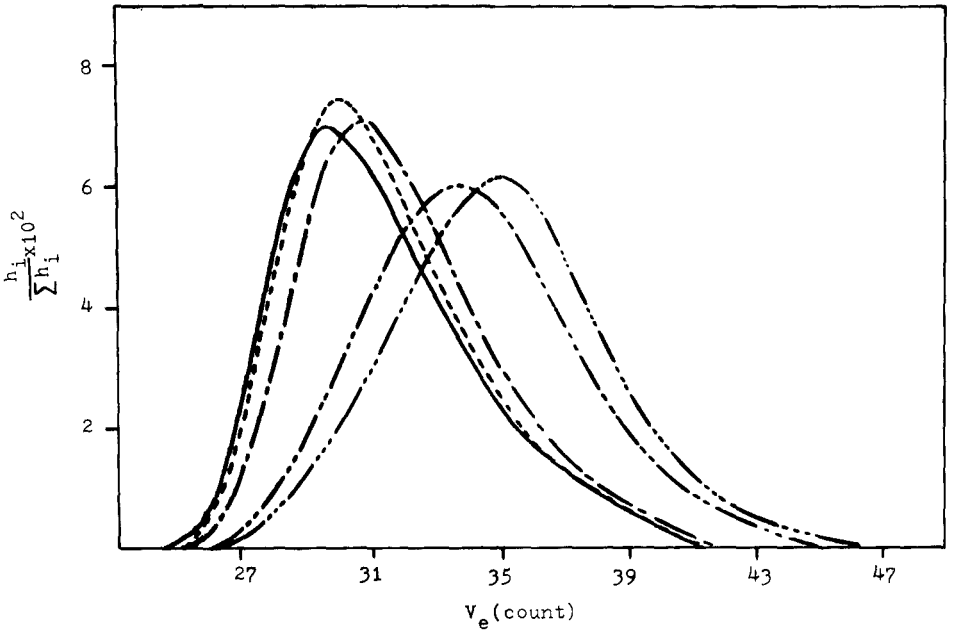

Fig. 4 Chromatograms of Five Different Concentrations of Sample PS-2.7 x 10^6 in MEK

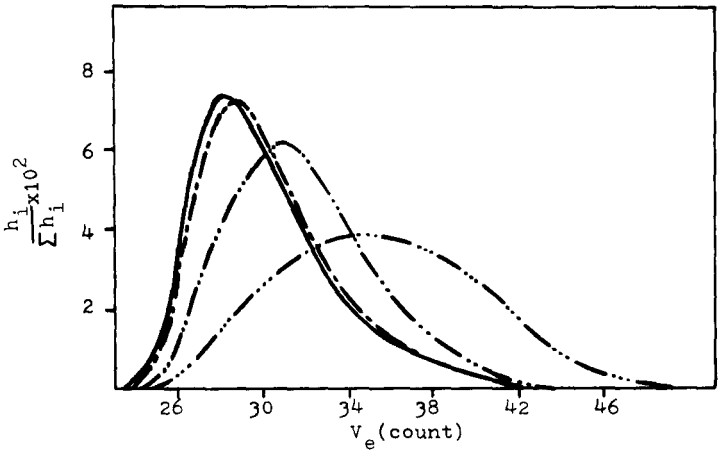

Fig. 5 Chromatograms of Five Different Concentrations of Sample PS-2.7 x 10^6 in THF

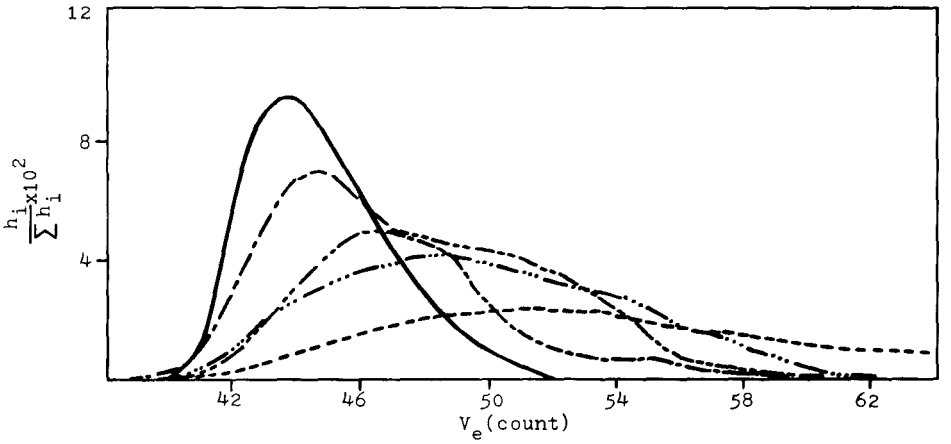

Fig. 6 Chromatograms of Five Different Concentrations From 0.01% to 3% of Sample PS-2.7 x 10^6 in THF in Shodex-Pak A-80M Column

TABLE 2

Concentration Dependence of Four PS Samples

Sample MW	Solvent	No Conc. Effect,%	Distortion Span same,%	Broaden Tailing,%
2.7x10^6	THF,MEK	0.05;0.1	0.3	1; 3
6.7x10^5	THF,MEK	0.05, 0.1, 0.3	1	3
2.0x10^5	THF	0.05, 0.1, 0.3	1	3
2.0x10^5	MEK	0.05,0.1,0.3, 1	-	3
3.5x10^4	THF	0.05,0.1,0.3, 1	-	3
3.5x10^4	MEK	0.05,0.1,0.3,1, 3	-	-

TABLE 3

c* Values of 4 PS Samples in THF and MEK

Samples	$c^* \times 10^2$	
	THF	MEK
PS-2.7 x 10^6	0.13	0.33
PS-6.7 x 10^5	0.39	0.79
PS-2.0 x 10^5	0.98	1.70
PS-3.5 x 10^4	3.74	5.10

proposed a simle equation for calculating this overlapping concentration c* as $c^* = 0.77 / [\eta]$. It will be interesting to see whether our experimental results correspond with those calculated theoretically. If the following Mark-Houwink equations are employed,

$$[\eta]_{THF} = 0.682 \times 10^{-2} M^{0.766}$$

$$[\eta]_{MEK} = 1.95 \times 10^{-2} M^{0.635}$$

the values of c^* calculated for the four PS samples are listed in Table 3. Comparison of the values of the 4th column in Table 2 with those in Table 3 show that the agreement is good in the case of THF and fair in the case of MEK.

Figure 7 gives the plot of V_p and $\overline{V}$ against concentration for different molecular weight and different solvents. Only sample PS-3.5 x 10^4 gives linear plot. Plots of other samples with higher molecular weight deviate from linearity in higher concentrations. Suggestions in literature of eliminating concentration dependence by extrapolation to infinite dilution are not justified by our data. The influence of concentration effect on the curve width was shown in Figure 8. In all cases, the curve width expressed with σ increases with the increase of concentration.

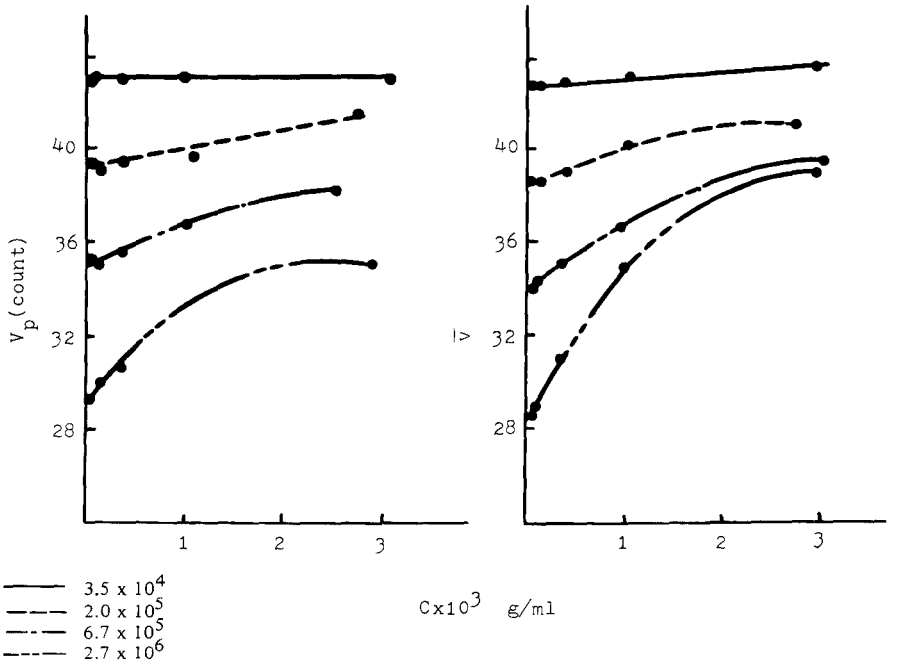

Fig. 7 V_p vs C Plots of Four PS Samples in THF (Right) and in MEK (Left)

Concentration Dependence of Branched Samples:

Concentration effects on the GPC studies of branched polymers have not been reported in literature. Since difference in degree of branching induces difference in segmental densities of the samples in solution which will have significant effect on the concentration dependence of the GPC behavior. V_p vs c plots for the three branched PVAc samples with nearly same intrinsic viscosities and the other three with nearly same $\bar{M}_w$ were illustrated in Figure 9 and Figure 10. It is obvious that when the concentrations were below 0.6%, V_p vs c plots were linear with different slopes for different samples. Since these six samples represent different degree of branching, the

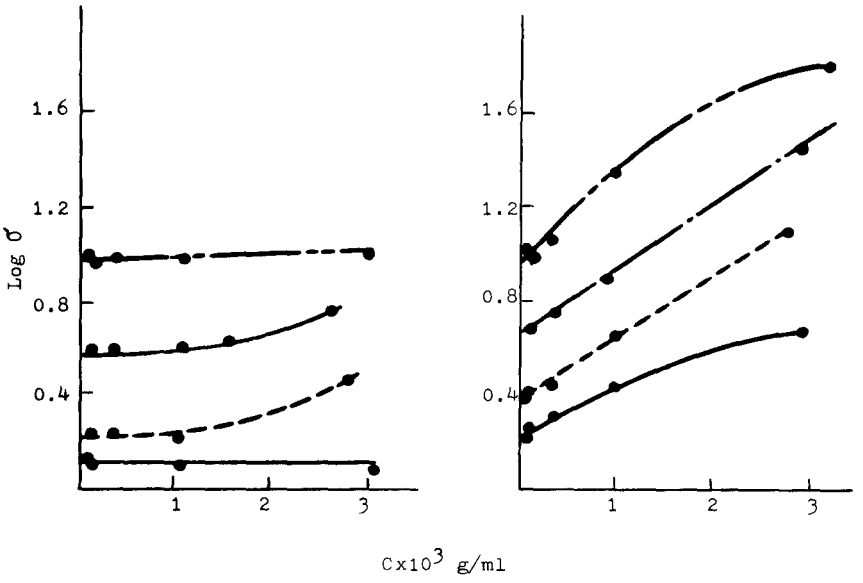

Fig. 8 Log σ vs C Plots of Four PS Samples in THF (Right) and in MEK (Left)

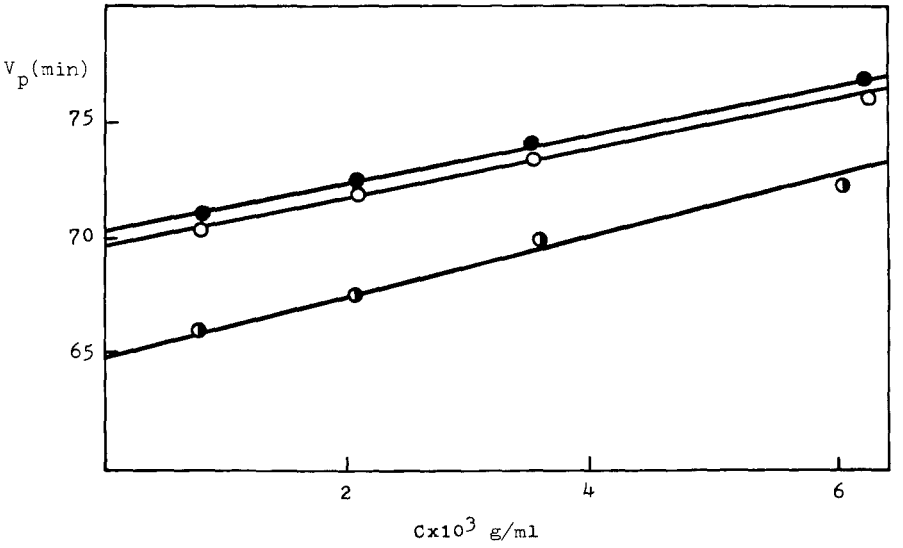

Fig. 9 V_p vs C Plots of Three PVAc Samples With Nearly Same $[\eta]$

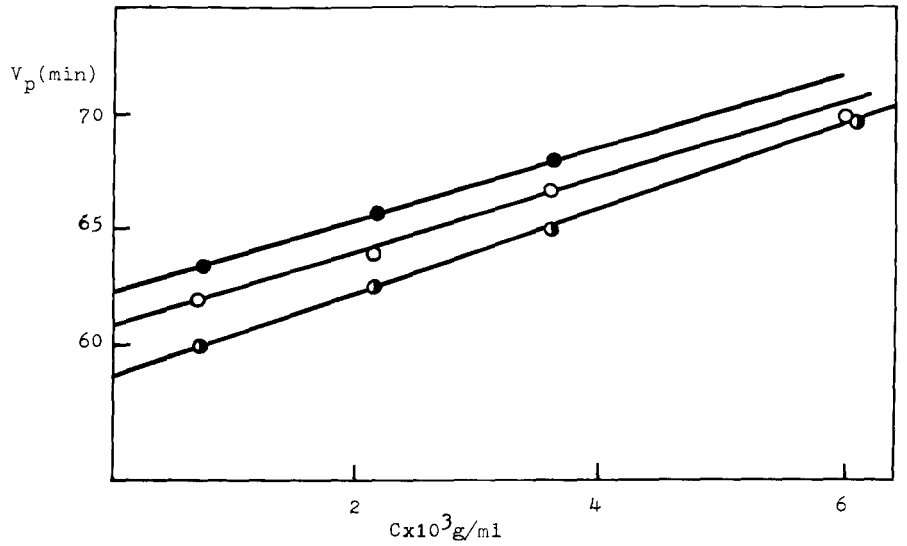

Fig. 10 V_p vs C Plots of Three PVAc Samples With Nearly Same $\bar{M}_W$

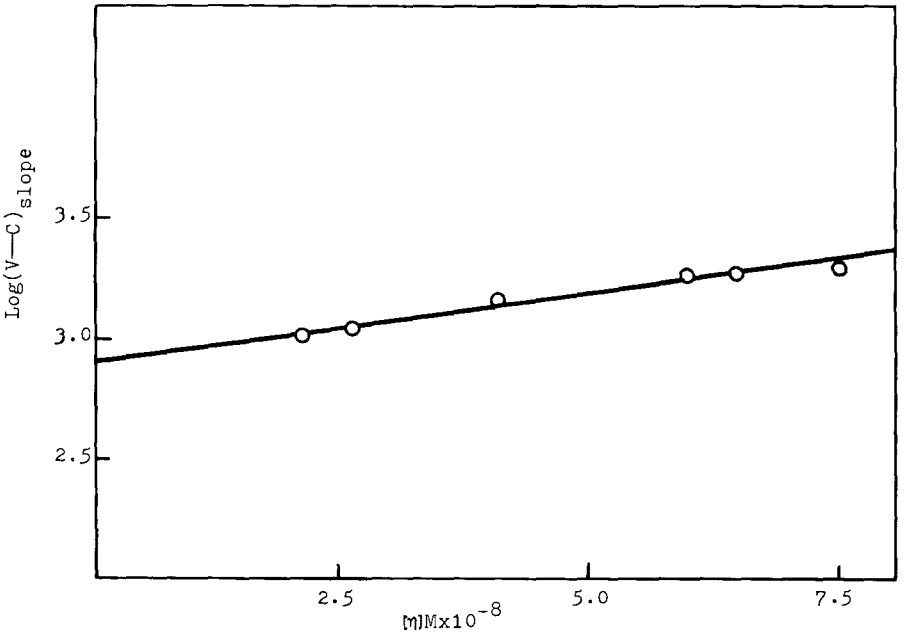

Fig. 11 Log (V-C)$_{slope}$ vs $[\eta]\bar{M}_W$ Plots for Six PVAc Samples With Different Degree of Branching

concentration effect should be different. If we take the slope of the V_p vs c plot as an indication of magnitude of concentration dependence, we found that these slopes depend on the hydrodynamic volumes , $[\eta]M$, of the samples. Figure 11 illustrates the slope vs $[\eta]M$ plots which gives a good straight line. From our data, it can be concluded that the concentration dependence of GPC behavior for branched samples of different degree of branching can still be realized through the variation of the hydrodynamic volume.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1867-1885 (1984)

CONCENTRATION DEPENDENCE OF ELUTION VOLUMES IN SIZE EXCLUSION CHROMATOGRAPHY OF POLYMER MOLECULES. 1. EFFECT OF VISCOSITY AND OF COIL CONTRACTION IN GOOD SOLVENT

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ABSTRACT

The contribution from viscosity phenomena and coil size contraction to the shift of polymer elution volumes with increasing concentration has been evaluated in size exclusion chromatography through a practical experimental procedure. It is shown that the viscosity effect is operative to different extents, depending on the different column systems. For most of the investigated polymer samples, however, macromolecular coil contraction seems to be the main contributing effect to the total concentration dependence of polymer elution volumes.

INTRODUCTION

It is well known that, in size exclusion chromatography (SEC) of polymer molecules, when the concentration of the injected sample is increased the peak maximum is shifted toward higher elution volumes. The change of elution volumes is particularly evident for narrow distribution polymers such as the standards normally used for calibration and, in the same chromatographic system, it increases with increasing both the sample molar mass and the thermodynamic quality of the solvent.

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This concentration dependence of elution volumes in SEC has been generally attributed either to an effect of contraction of the polymer coils with increasing concentration in the injected solution [1], or to a sum of contributing processes [2], namely, the mentioned coil contraction, the effect of the viscosity of the solutions in the interstitial volume of the columns, and the so-called secondary exclusion due to the reduction of the accessible pore volume of the packing when polymer molecules are already present in the pores. The reduction of the effective hydrodynamic volume of solvated polymer coils with increasing concentration can be accounted for by using the model developed by Rudin [1]. Results from this model are in reasonable agreement with Yamakawa's theory relating concentration and hydrodynamic volumes of solvated polymers and were shown to describe SEC behaviour of several practical systems [3]. The fact that the concentration dependence of elution volumes is lower in thermodynamically poor solvents [4-6] gives support to the hypothesis on which Rudin's theory is based. Quantitative relationships between concentration effects in SEC and thermodynamic quality of the solvents have been recently discussed [7].

The relative importance of the different contributing effects to the total concentration dependence of elution volumes was investigated by Janca [8-10]. The viscosity of the injected polymer solutions was experimentally shown to drastically affect elution volumes and chromatogram widths of polymer standards which elute in the column interstitial volume only [8]. Relationships for the quantitative description of this phenomenon and of the ratio of individual contributions to the overall concentration dependence were derived [9,10] and the application to experimental results led to an estimation of the viscosity effect as approximately 80% of the total concentration effect. The reported comparisons of expe-

rimental elution volumes with the predictions of Rudin's theory are therefore, according to Janca [11], incorrect because it is not considered that during the chromatographic process dilution along the column occurs; when this effect is not taken into account the polymer concentrations are obviously overestimated.

Attempt was also made of evaluating the contribution due to secondary exclusion [12]: experimental results showed that, at least under stationary conditions, this process is probably operative to a very small extent. As a consequence it might be assumed that in SEC the increase of peak elution volumes with sample concentration is completely due, in real chromatographic systems as well, to the sum of the contributions from the macromolecular coil contraction and from the solution viscosity in the interstitial column volume.

In this paper we present results of an investigation intended to evaluate the relative amounts of the two contributing effects over the total concentration dependence of elution volumes under some practical experimental conditions. The study has been carried out in a good solvent, with narrow distribution polymer standards eluting either in the interstitial volume only, or in the permeation range of chromatographic columns with different pore sizes.

METHODOLOGY

The following procedure has been used to evaluate the different contributions to the concentration effect in our systems. The overall concentration dependence of elution volumes is obtained from direct injections of several standards at different concentrations. In general, for values of concentrations not too high the measured elution volumes vary linearly with the injected concentration. Injection in the same column system of a totally excluded polymer gives the shift of elution volumes due to the pure vi-

scosity effect taking place in the interstitial volume. It has been shown [8] that for such a polymer a linear relationship exists between the specific viscosity of the injected polymer solution and the elution volume, at least up to viscosity values where the solution behaviour is still newtonian. Owing to the fact that such a viscosity effect occurs in the interstitial volume, outside the gel pores, it might be assumed that a very same effect occurs for permeating polymers as well. In other words, for polymer solutions with the same specific viscosity, the same viscosity contribution should result, independently of the fact that the polymer molecules can diffuse into the gel pores. Therefore, such a viscosity contribution can be evaluated, for each injected sample, from the elution volume shift of the excluded polymer at the same specific viscosity. The specific viscosities of the polymer samples are calculated by the Huggins equation [13] at the different concentrations, and the amount of the viscosity effect can be subtracted from the total increase of the elution volumes.

EXPERIMENTAL

The chromatographic columns employed (25 cm length, 0.46 cm I.D.) were slurry packed with microparticulate spherical silica gels (average particle diameter 10 μm) supplied by E. Merck (Darmstadt, Germany). Mean pore sizes of the gels were 10 nm, 50 nm and 100 nm.

Two different column combinations were used in order to cover different molar mass ranges; their characteristics are reported in the next section.

Narrow distribution polystyrene (PS) standards (ArRo Laboratories, Joliet, Illinois, and Waters Associates, Milford, Massachu-

setts) were injected as tetrahydrofuran (THF) solutions at different concentrations; THF was used as eluent as well; injection volumes were 10 μl. An UV spectrophotometer (Zeiss PM2) at 260 nm wavelength was used as a detector. The chromatographic flow rate was approximately 0.5 cm^3/min.

All the measurements were done in triplicate and the results were averaged.

RESULTS AND DISCUSSION

The calibration curve of the first column system, two columns in series with 100 nm and 50 nm respectively as nominal pore size of the silicas, is shown in Fig. 1. The narrow distribution PS standards reported in Table 1 were injected at different concentrations (up to $2 \cdot 10^{-2}$ g/cm^3 for some of the samples) and the resulting peak elution volumes were measured.

As it appears from Fig. 1, the polymers with molar masses 17500, 111000, 200000, 390000 and 670000 are all eluting in the fractionation range of the columns, whereas the 10^7 standard is completely excluded from the pores. For the latter sample it has been checked by using Rudin's model [3] that even at the highest injected concentration, $2 \cdot 10^{-3}$ g/cm^3, his hydrodynamic volume is still large enough to make the molecules excluded.

With increasing polymer concentrations, not only peak elution volumes V_e and widths increased, but distorted chromatograms were obtained, especially for the high molar mass samples. Some examples are shown in Fig. 2 for two permeated polymers and for the excluded one. When the asymmetry and distortion of the chromatograms were severe, the average elution volumes of the polymer samples were obtained by calculating the first statistical moment of the peaks.

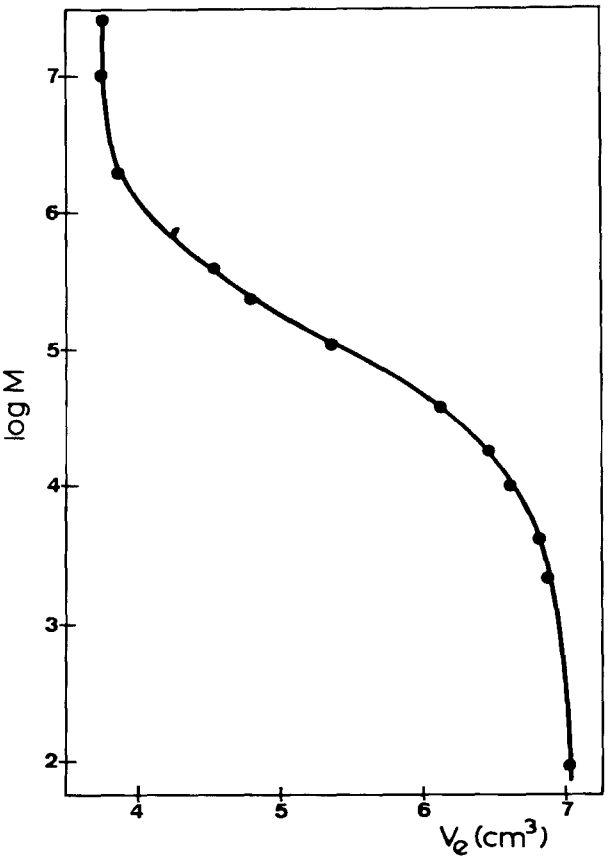

FIGURE 1. Calibration curve for the column system 100 nm + 50 nm.

The experimentally observed elution volumes at increasing concentrations, c, are reported in Fig. 3; the concentration dependence of V_e increases with the polymer molar mass as expected, and is linear either in the low concentration or in the whole range, depending on the different molar masses.

For each polymer concentration of Fig. 3 the specific viscosities were calculated by means of the Huggins equation

TABLE 1

Huggins Constants, k_H, and Contribution of Hydrodynamic Volume Contraction to the Concentration Effect for the PS Standards in the Column System 100 nm + 50 nm.

Polymer Molar Mass	k_H	$[d(\Delta V_s)/dc]/[d(\Delta V_t)/dc]$
17500	0.40	0.82
111000	0.33	
200000	0.31	0.77
390000	0.28	
670000	0.25	0.71
10^7	0.24	

$$\eta_{sp} = [\eta]\, c + k_H [\eta]^2 c^2 \qquad (1)$$

where the intrinsic viscosities $[\eta]$ were obtained from the equation [14]

$$[\eta] = 1.11 \cdot 10^{-2} M^{0.723}. \qquad (2)$$

The values of the Huggins constant, k_H, for PS in the investigated molar mass range, in THF solution, were interpolated from the data by Spychaj et al.[15] and are reported in Table 1.

From Fig. 3 the elution volumes $V_{e,o}$ extrapolated at c=0 can be obtained. The total increment of elution volumes, $\Delta V_t = (V_{e,c} - V_{e,o})$, where $V_{e,c}$ is the elution volume of the polymer at concentration c, can be plotted against the polymer specific viscosities, as it is shown in Fig. 4. The increase of elution volumes for the

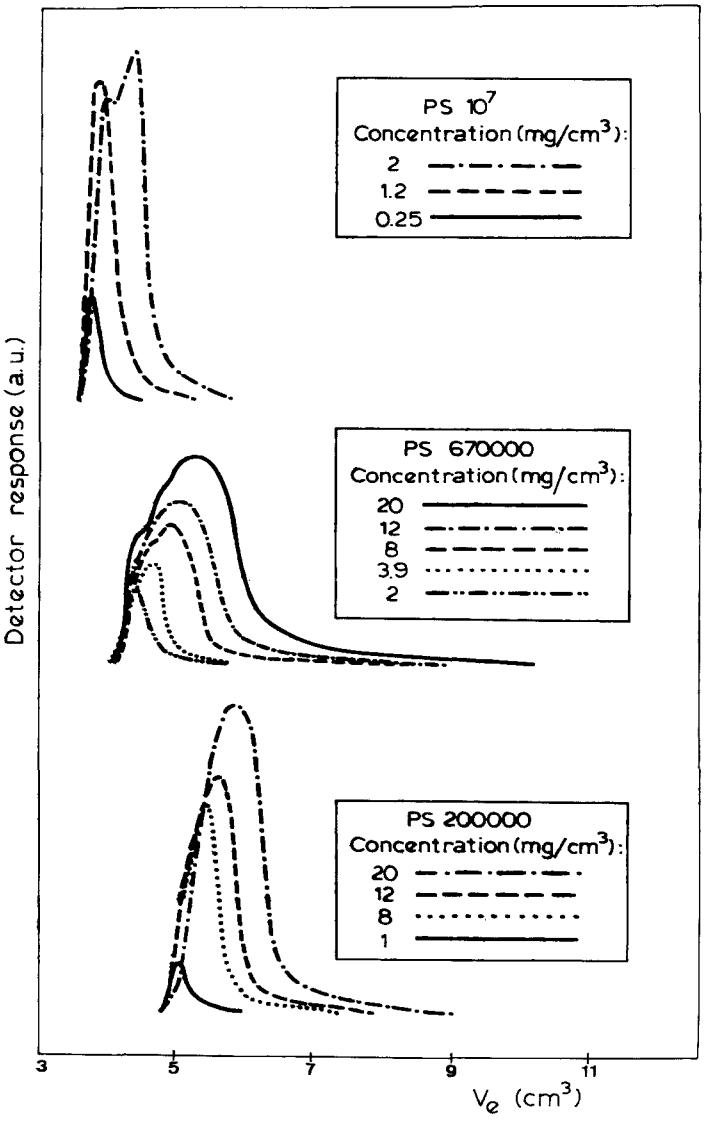

FIGURE 2. Chromatograms of permeated and excluded PS samples at different injected concentrations.

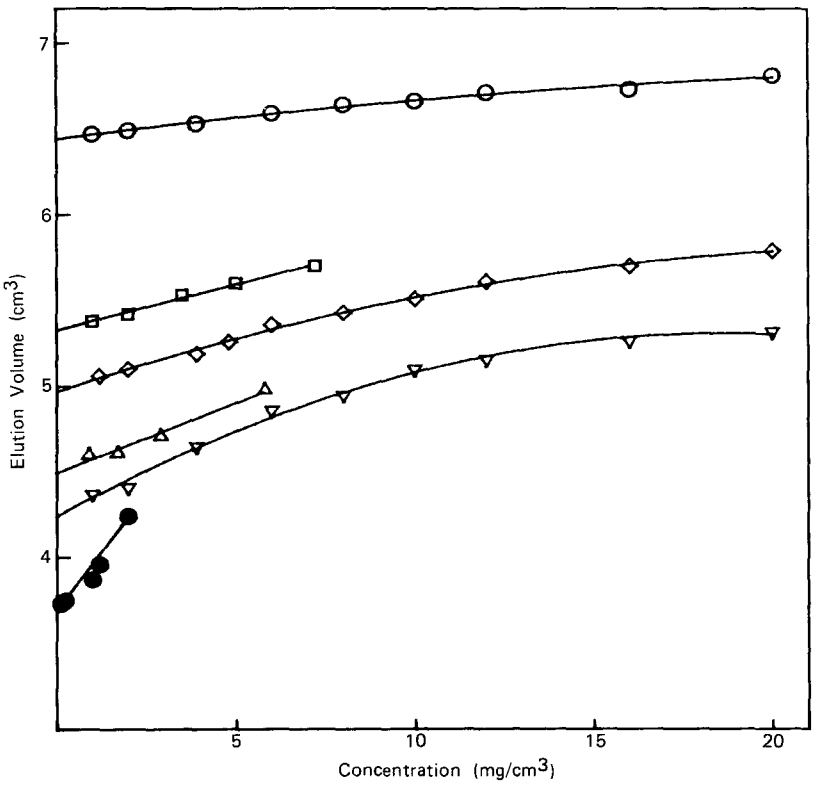

FIGURE 3. Elution volumes of PS standards at different concentrations; ○:17500; □ :111000; ◇:200000; △ :390000; ▽:670000; ●:10^7.

excluded polymer, PS 10^7, is completely due to the viscosity effect in the interstitial volume. For all of the other samples, at each η_{sp} value, the ΔV_t values are higher than those of the excluded polymer, suggesting that in addition to viscosity, the effect of coil contraction is operative. The increase of ΔV_t is higher for the lower molar mass samples due to the fact that, for a same η_{sp} value, these polymer samples have a higher concentration.

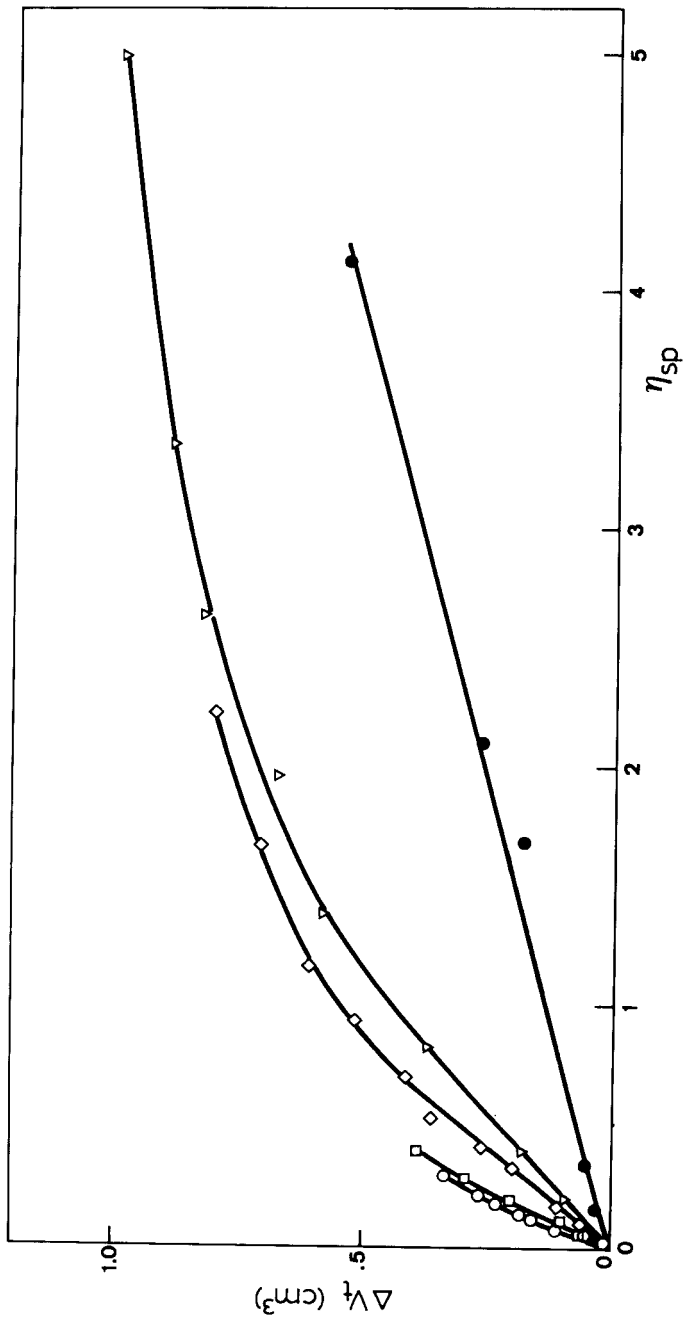

FIGURE 4. Changes of elution volumes, ΔV_t, with specific viscosities. Columns 100 nm + 50 nm. Symbols as in Fig. 3.

The contribution to the total concentration effect on elution volumes from viscosity phenomena is given, under the experimental conditions adopted and the assumptions already made, for each of the permeated polymer samples by the ΔV_t of the 10^7 PS sample at the same η_{sp} value;this contribution can be subtracted from the total change of elution volumes ΔV_t at the appropriate concentration and the resulting increment of elution volumes, ΔV_s, is due to the macromolecular coil contraction only.

In Fig. 5 the change of ΔV_t with injected polymer concentration is shown for three different molar mass samples, eluting in three different parts of the column permeation range, i.e. at the beginning (PS 670000), in the middle (PS 200000) and at the end (PS 17500) of the practically linear part of the calibration curve (see Fig. 1). After subtraction of the viscosity contributions, the resulting increment of elution volumes, ΔV_s, at different concentrations are plotted in Fig. 6 for the three PS standards. Both in Figs. 5 and 6 an initial linear dependence of the incremental elution volumes ΔV_t and ΔV_s on concentration is evident. For each polymer sample, the ratio of the slopes $d(\Delta V_s)/dc / d(\Delta V_t)/dc$ will give an estimate of the fractional amount of the contribution due to coil size contraction over the total concentration dependence of elution volumes. This ratios are reported in the last column of Table 1 for the samples considered. One can see that the contribution of coil size contraction is dominant, in our experimental system, in determining the increasing of elution volumes. The viscosity effect seems to be responsible for 20-30% only of the total elution volume change.

Similar experiments were also performed with a different column set (2 columns packed with 10 nm pore size silica gel) covering the molar mass range 10^3-10^5, as it is shown by the calibration curve

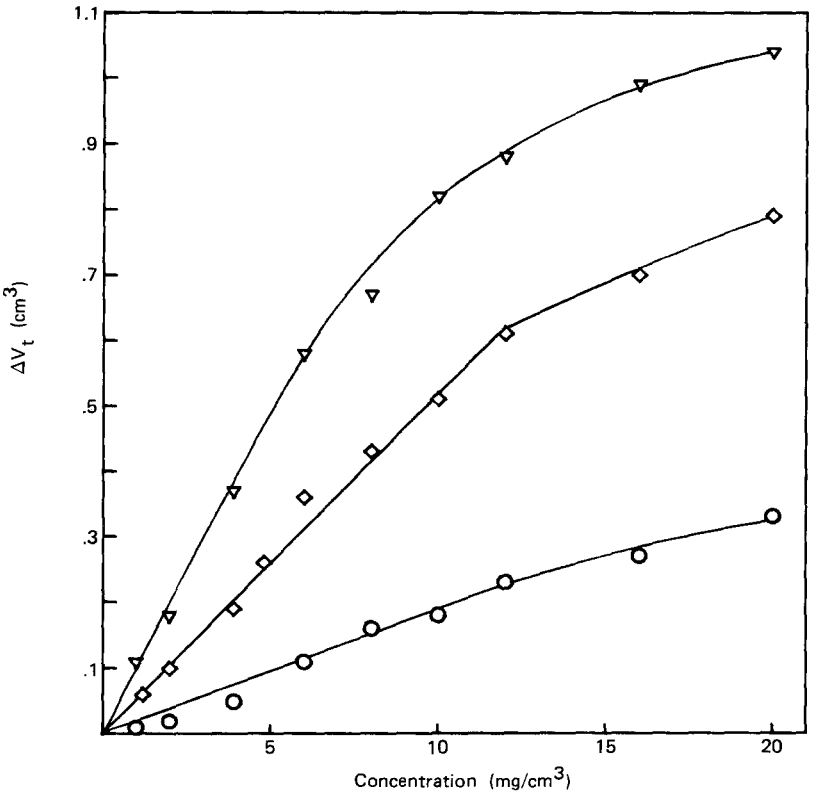

FIGURE 5. Changes of elution volumes, ΔV_t, with injected polymer concentration. Symbols as in Fig. 3.

in Fig. 7. The PS standards listed in Table 2 were injected at increasing concentrations. The results are shown in Fig. 8.

All the chromatograms were regular in shape, with only slight distortion for the peaks of the excluded polymer, PS 470000, shown in Fig. 9. For these latter samples the elution volumes were obtained from the first moment of the peaks.

It can be seen from Fig. 8 that the changes of elution volumes with concentration are small for the low molar mass permeating sam-

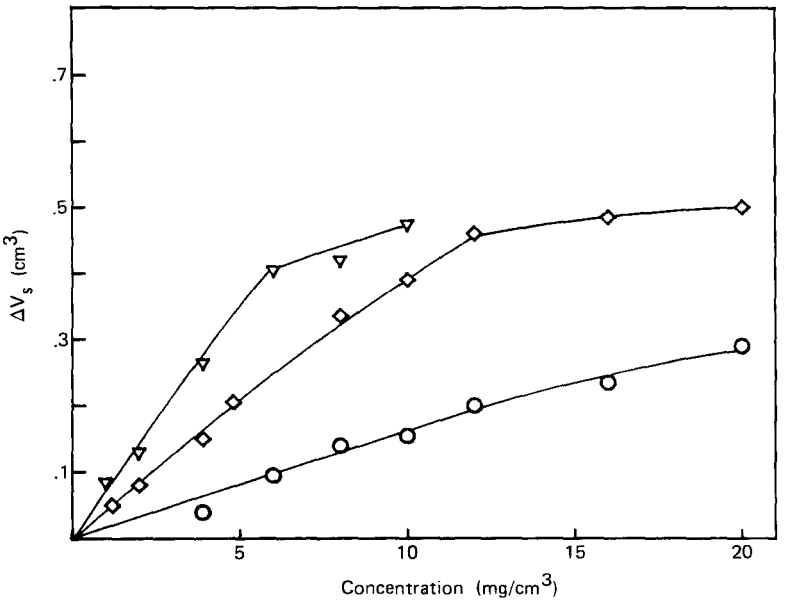

FIGURE 6. Increment of elution volumes for macromolecular coil contraction. Symbols as in Fig. 3.

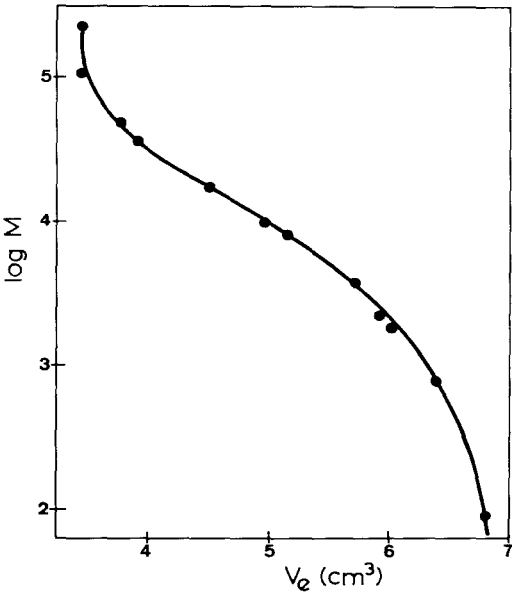

FIGURE 7. Calibration curve for the column system 2 x 10 nm.

TABLE 2

Huggins Constants, k_H, and Contribution of Hydrodynamic Volume Contraction to the Concentration Effect for the PS Standards in the Column System 2 x 10 nm.

Polymer Molar Mass	k_H	$[d(\Delta V_s)/dc]/[d(\Delta V_t)/dc]$
1800	0.50	0.65
8500	0.50	0.63
17500	0.40	0.47
50000	0.35	0.36
470000	0.27	

ples. The changes for PS 17500 are also slightly smaller than those observed for the same polymer in the first column system investigated. The specific viscosities of the injected samples were calculated at the different concentrations by using the Huggins constant values reported in Table 2. The $[\eta]$ values were obtained from Eq. (2) for all the standards, with the exception of PS 1800 and PS 8500, for which the viscometric equation [16]

$$[\eta] = 1.0 \cdot 10^{-1} \; M^{0.50} \qquad (3)$$

recommended for low molar mass samples in good solvents, was employed. The k_H value for these two latter polymers were taken from reference data relative to low molar mass polystyrene samples in different solvents [16].

The total increments of elution volumes are plotted in Fig. 10 against the η_{sp} values: one can see that the viscosity effect, as

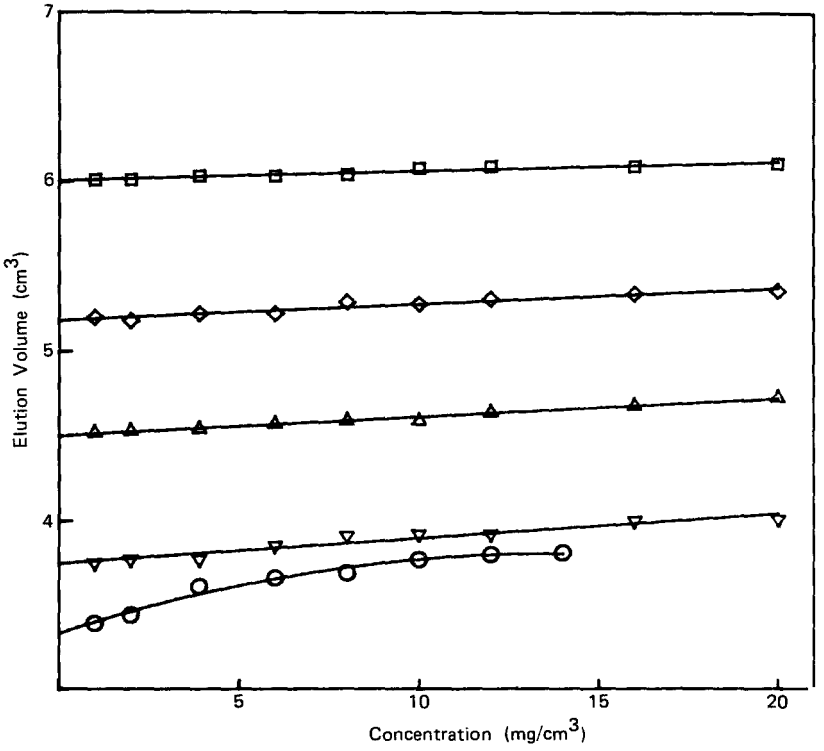

FIGURE 8. Elution volumes of PS standards in the column system 2 x 10 nm. □ :1800; ◇ :8500; △ :17500; ▽ :50000; ○ :470000.

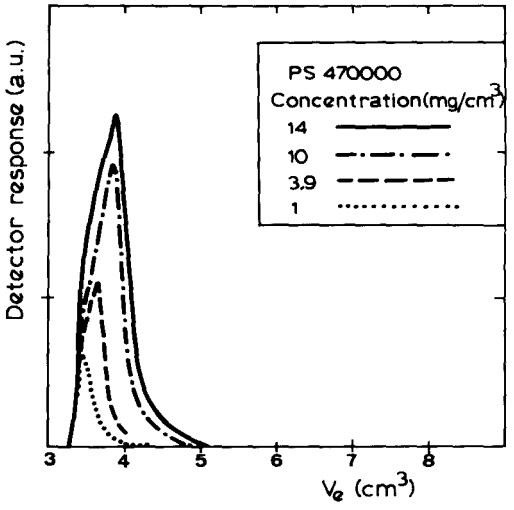

FIGURE 9. Chromatograms of excluded PS 470000 standard at different concentrations.

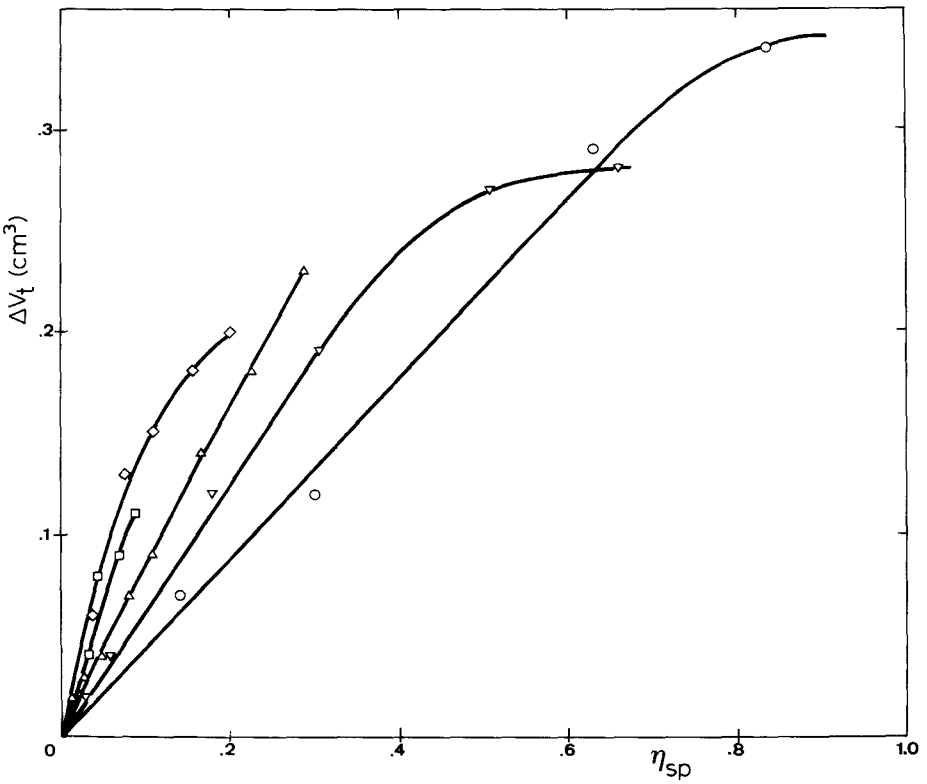

FIGURE 10. Changes of elution volumes, ΔV_t, with specific viscosities. Columns 2 x 10 nm. Symbols as in Fig. 8.

represented by ΔV_t changes of the excluded PS 470000 sample, is in this column system higher than in the previous case.

After obtaining at the different concentrations the viscosity contributions to ΔV_t with the procedure described above, the values of ΔV_s could be calculated, and these are plotted in Fig. 11 vs injected polymer concentrations for three of the standards. The relative amount of the concentration dependence of elution volumes due to the hydrodynamic volume reduction was again estimated

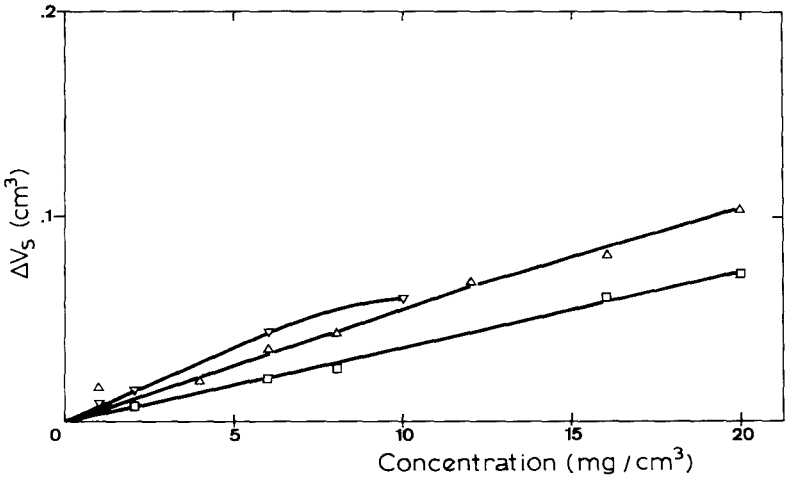

FIGURE 11. Increment of elution volumes for macromolecular coil contraction. Columns 2 x 10 nm. Symbols as in Fig. 8.

from the ratio $d(\Delta V_s)/dc/d(\Delta V_t)/dc$, for the different standards, and the results are shown in the last column of Table 2.

The effect of macromolecular coil contraction turns out to be reduced in respect of the results of Table1; particularly, for the samples PS 17500 and PS 50000 this contribution is acting either at the same level than the viscosity effect, or with a lower relative importance. In practice, in this column system, the increased viscosity contribution overcomes the one from the higher pore permeation of the macromolecules. The results relative to the lower molar mass samples, PS 1800 and PS 8500, cannot be given too much confidence, because it is questionable wether, for such short polymer chains, the same concentration dependence of molecular sizes and of solution viscosities used for random coil molecules can be still employed. On the other hand, elution volume changes occurring for these samples are quite low, and large differences in the calculated contributions can result from small errors in measurements.

CONCLUSIONS

A practical experimental procedure for evaluating the ratio of the viscosity effect to the total change in elution volumes in SEC of polymers at different concentrations has been applied to real chromatographic systems. It is assumed that all the concentration effect comes from two main contributions: the frictional forces acting in the interstitial volume during sample elution in the columns (viscosity effect) and the higher pore volume permeated because of the macromolecular size contraction with increasing concentrations.

The calculated relative amount of these contributions show that the viscosity effect can be operative to different extents, depending on the column system; under the experimental conditions investigated,however, the macromolecular coil contraction seems to account for 50-80% of the total elution volume changes for most of the investigated samples.

In the analysis of data herewith shown use has been made of the injected nominal concentrations of the polymer solutions. It is known that during the chromatographic elution a dilution process occurs, and this should be taken into account when looking for quantitative relationships [11]. The employed methodology, however, of plotting elution volume changes against nominal concentrations and against the correspondent specific viscosities is not incorrect if it is assumed that the dilution of the sample equally affects the concentration and viscosity dependence of elution volumes. To check that this assumption is reasonable the experimental elution volumes were also correlated to the average effective concentration of the samples estimated by using the procedure suggested by Janca [11] and the different contributions to the changes of elution volumes were evaluated. The results so obtained were only slightly different

from those reported here, showing in general an even bigger effect of the hydrodynamic volume contraction.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1887-1901 (1984)

ON THE CONCENTRATION EFFECTS IN STERIC EXCLUSION CHROMATOGRAPHY UNDER STATIONARY EQUILIBRIUM CONDITIONS

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ABSTRACT

The effect of concentration on the distribution of macromolecules between solution inside the pores and outside the porous medium after mixing of polymer solution with the medium was investigated. Experimental measurements were carried out for polystyrene standards in a thermodynamically good solvent - tetrahydrofuran, and in a mixed theta solvent tetrahydrofuran-methanol. The results of measurements, particularly in the theta solvent, suggested a considerable effect of secondary non-exclusion interactions. A comparison between the distribution coefficients calculated theoretically using various models and those determined experimentally revealed a considerable discrepancy. It is obvious that the reported theoretical models of concentration dependence of the distribution coefficients under stationary conditions do not adequately reflect the real situation. The individual likely causes of this discrepancy have been critically discussed.

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 0148-3919/84/0709-1887$3.50/0

INTRODUCTION

In our preceding paper (1) we reported an experimental investigation of the concentration dependence of the equilibrium distribution coefficient K_{SEC}. The measurements were carried out under stationary conditions, by mixing a known quantity of the porous medium with a known volume of polymer solution of the given concentration. After the equilibrium was established the polymer concentration in the supernatant was determined. If the dissolved polymer was excluded at least from one part of the pores accessible to pure solvent, the polymer concentration in the supernatant increased. Using the values of polymer concentration in solution before mixing with the porous medium and after mixing the known volume of the solution and the known pore volume in the porous medium, the equilibrium distribution coefficient can be calculated. Its dependence on the polymer concentration in solution is interesting, because values thus determined are free from the effect of dynamic factors, such as, e.g., viscosity phenomena operative to a considerable degree in concentration effects which have been studied by us and quantitatively described in a series of our preceding papers (for review cf. (2)). The results of our preceding paper (1) showed that the experimentally determined increase in the distribution coefficient with increasing concentration is larger than corresponds to the theoretical calculations based on the assumed role played merely by a change in the effective size of dissolved macromolecules with varying concentration. This has proved that the contribution of secondary exclusion, the role of which had been considered earlier, may be only very small, if any. If secondary exclusion was also operative in the concentration effects, this would lead, on the

contrary, to a decrease in the distribution coefficient with increasing polymer concentration in solution, or a smaller increase in the distribution coefficient with increasing concentration than corresponds to a change in the effective size of macromolecules.

Anderson and Brannon (3) reported a theoretical model of concentration dependence of the distribution coefficient of rigid spherical macromolecules in the porous structure. Their model is interesting, since it predicts concentration effects also in the thermodynamically poor theta solvent.

This study supplements our preceding measurements (1) and extends experimental measurements of the equilibrium distribution coefficients K_{SEC} under stationary conditions, also using the theta solvent. By comparing all experimental results thus obtained with the theoretically calculated distribution coefficients, various theoretical models were critically analyzed which, in principle, may elucidate the observed concentration dependence of the distribution coefficients.

THEORY

No detailed analysis of the individual theoretical models of concentration effects is offered in this part. Only basic theoretical relations are given which are used in the interpretation of the experimental results and which make possible a basic understanding of the problem of concentration effects. For details, we refer to our earlier papers.

For the concentration ratio of the polymer solution before mixing with the porous medium, c_1, and after the mixing, c_2, relation

$$c_1/c_2 = 1 + (V_p/V_1)(K_{SEC}-1) \quad (1)$$

is valid, in which V_p is the total pore volume in the porous medium added, V_1 is the volume of polymer solution before mixing, and K_{SEC} is the distribution coefficient (1). It is assumed that for infinite dilution the equality between the distribution coefficients under stationary conditions holds also in a dynamic chromatographic experiment. The distribution coefficient under dynamic chromatographic conditions is defined by

$$V_R = V_i + K_{SEC}\, V_p \quad (2)$$

where V_R is the retention volume of the given polymer and V_i is the interstitial or dead volume of the solvent between grains of the column packing. For the totally excluded polymer, $K_{SEC} = 0$. If there is no other interaction between the separated macromolecules and porous medium apart from steric exclusion, for permeating macromolecuIs $0 \leq K_{SEC} \leq 1$. The dependence of the distribution coefficient on the effective dimensions of permeating macromolecules may be described by an empirical calibration function

$$K_{SEC} = f\ (v.\varepsilon) \quad (3)$$

where v is the volume of unswollen macromolecule and ε is the dimensionless swelling factor. The central part of the calibration curve may be approximated by a linear function

$$K_{SEC} = P + Q\ \ln\ (v.\varepsilon) \quad (4)$$

According to Rudin and Wagner (4), the swelling factor

ε is a function of the concentration c

$$1/\varepsilon = 1/\varepsilon_o + (c/c_x)(\varepsilon_o - 1)/\varepsilon_o \quad (5)$$

where ε_o is the swelling factor for infinite dilution (c=0) and c_x is the critical concentration at which the size of the macromolecule is the same as under the theta conditions (ε=1). These quantities can be calculated from

$$\varepsilon_o = [\eta]/[\eta]_\theta \quad (6)$$

$$c_x = M/(N_o\ v) \quad (7)$$

$$v = 4\pi[\eta]_\theta M/(3.\Phi) \quad (8)$$

where $[\eta]$ is the intrinsic viscosity; in the theta solvent, $[\eta]_\theta$ is given by

$$[\eta]_\theta = K_\theta \cdot M^{0.5} \quad (9)$$

N_o is the Avogadro number, M is molecular weight and $\Phi = 3.1 \times 10^{24}$ is the universal Flory constant.

Anderson and Brannon (3) have suggested a model which describes the distribution of rigid spherical macromolecules between solution in pores of various shape, on the one hand, and bulk solution, on the other, assuming steric (hard sphere - hard wall) and long-range (screened electrostatic) interactions. These authors described, in terms of virial series expansion, the concentration dependence of the distribution coefficient for the single particle distribution function in a restricting medium, where the effect of the porous medium is represented by position-dependent potential energy acting upon each macromolecule. They explained the concentration effect

on the distribution function by coupling between the macromolecule-macromolecule and macromolecule-pore interactions. The basic idea underlying the effect of concentration on the local distribution coefficient consists in a reduced spherical symmetry inside the pore which causes a decrease in the macromolecule--macromolecule interaction inside the pore. As a consequence, the same interactions outside the pores enhance the distribution of macromolecules towards solution in the pore, compared with the exclusion effect of the single macromolecule-pore wall interaction. The result of complicated operations involving mathematical statistics was approximated (3) by means of an empirical virial expansion

$$K_{SEC} = K_o \ (1 + \alpha_1 \ c_\infty + \alpha_2 \ c_\infty^2 + \ ...) \qquad (10)$$

where K_o is the distribution coefficient at zero concentration and c_∞ is the polymer concentration in the supernatant. Hence, in the stationary experiment, $c_2 = c_\infty$. The first virial coefficient α_1 was then given by

$$\frac{\alpha_1}{v} = 8 - 7.92 \ K_o - 8.48 \ K_o^2 + 8.40 \ K_o^3 \qquad (11)$$

The preceding theoretical conclusions offer two limiting hypothetical alternatives for the explanation of the concentration effect under stationary conditions. First, it may be assumed that a change in the distribution coefficient is due only to a change in the effective dimensions of the macromolecule with changing concentration. In this case, however, the distribution coefficient should not be affected by concentration in the theta solvent. In the second case of the Anderson-Brannon model (3), the distribu-

tion coefficient ought to be a function of concentration also for rigid macromolecules; hence, it should vary also in the theta solvent, in spite of the fact that there is no change in the effective size of macromolecules with varying concentration. Our experimental work was planned so as to make possible a critical comparison of the alternatives just outlined with real experimental data.

EXPERIMENTAL

Polystyrene standards (PS) were used; their molecular parameters are given in Table 1. Tetrahydrofuran (THF) representing a thermodynamically good solvent and a mixture of THF and 28.7 % (v/v) methanol which at 25 °C is a theta solvent (5) were used as solvents. A detailed description of experimental procedures has been given in our preceding paper (1), from which also one part of experimental results has been taken. In this study, a constant temperature of 25 °C (accuracy ± 0.1 °C) was maintained in all experiments by means of a thermostat. The porous medium (silicagel Merckogel Si 500 Å deactivated by a reaction with trimethyl chlorosilane and hexamethyl dichlorosilane, particle size 40-63 μm) was mixed with a solution of PS after evacuation of the mixing vessel containing the porous medium. This eliminated the possibility of air microbubbles being caught in the pores. The ratio of concentrations of PS solutions before and after mixing with the porous medium was determined chromatographically as reported earlier (1).

Porosity (the total pore volume of the given amount of porous medium) was determined by the method of

TABLE 1

Molecular parameters of polystyrene standards

Standard	Manufacturer	$M_w x10^{-3}$	$M_n x10^{-3}$
PS 694 000	Waters Assoc.,Inc.	694	not given $M_w/M_n < 1.05$
PS 303 000	Chrompack,Holland	303	288
PS 111 000	Waters	111	111

Note: Molecular weights are given by the manufacturer

mercury porosimetry. The total pore volume of Merckogel Si 500 Å was V_p = 0.641 ml/g.

The composition of the theta solvent before and after mixing with Merckogel Si 500 Å was determined by gas chromatography. 0.2275 g of silicagel was mixed with 0.4677 g of the theta solvent. The methanol content in the supernatant dropped from the original 28.7 % v/v to 27.2 % v/v. This fact indicates that under the given conditions, methanol is probably preferentially sorbed on the surface of the porous medium, and consequently the composition of the solvent varies near the surface. For this reason, a one-component theta solvent seems more suitable. On the other hand, however, the unpublished results of our preceding paper (1) demonstrated a considerable adsorption of PS on the surface of the porous medium, if cyclohexane was used as the theta solvent. A similar adsorption was observed with deactivated Merckogel Si 500 Å. Virtually, the adsorption was so strong that the polymer concentration in the supernatant decreased distinctly, so that, at low concentrations,

the polymer could not be detected in the supernatant at all. It is of interest that, although in the stationary arrangement the polymer was almost quantitatively adsorbed from the solution in cyclohexane in the dynamic chromatographic experiment with cyclohexane as solvent the experimental result did not suggest adsorption.

RESULTS AND DISCUSSION

In addition to direct experimental data obtained by the stationary measurement, the Mark-Houwink equation for PS in THF at 25 °C was also needed for the calculations. We used an equation from our earlier work (6)

$$[\eta] = 1.17 \times 10^{-2} M^{0.717} \qquad (12)$$

The constant of Eq. (9) was calculated as $K_\theta = 8.12 \times 10^{-2}$ (1). The slope and abscissa of the calibration function (Eq. (4)) were calculated for Merckogel Si 500 Å by using the least squares method from chromatographic data obtained by the calibration of the PS series by employing a procedure described earlier (1). The values thus obtained were $P = -4.5178$ and $Q = -0.1201$.

The experimental c_1/c_2 and V_p/V_1 values are reviewed in the first part of Table 2. The second part of Table 2 contains the distribution coefficients K_{SEC} calculated using Eq. (1) from direct experimental data (A) in THF (a,b) and in the theta solvent (c). Also, the Table 2 contains the K_{SEC} values calculated theoretically assuming that a change in concentration will affect the change in K_{SEC} only

TABLE 2

Change in the distribution coefficients K_{SEC} due to a change in concentration in the stationary experiment - direct experimental values and theoretical calculation according to various models

c_1 (% w/v)	c_1/c_2	V_p/V_1	K_{SEC} A	K_{SEC} B	K_{SEC} C
PS 694 000[a]					
4.21	0.9109	0.286	0.689	0.273	0.612
3.11	0.8802	0.281	0.574	0.246	0.581
1.41	0.8390	0.267	0.396	0.179	0.473
1.13	0.8377	0.241	0.327	0.162	0.436
0.52	0.7359	0.324	0.185	0.122	0.327
0.125	0.7023	0.325	0.084	0.077	0.153
0				0.057	0.057
PS 303 000[b]					
1.87	0.9611	0.159	0.755	0,085	0.100
0.88	0.9052	0.133	0.288	0.053	0.070
0.43	0.8395	0.207	0.225	0.034	0.048
0.21	0.8286	0.187	0.083	0.024	0.032
0				0.013	0.013
PS 303 000[c]					
1.85	0.8917	0.204	0.469	0.013	0.099
0.92	0.8596	0.204	0.312	0.013	0.078
0.45	0.8516	0.191	0.223	0.013	0.050
0.22	0.8087	0.198	0.032	0.013	0.033
0				0.013	0.013
PS 110 000[a]					
2.00	0.9642	0.216	0.834	0.509	1.392
1.00	0.9330	0.240	0.721	0.492	1.016
0.50	0.8885	0.259	0.570	0.482	0.779
0.13	0.8765	0.278	0.556	0.473	0.553
0.03	0.8834	0.225	0.483	0.470	0.490
0			0.469	0.469	

Note:

A - calculated from experimental data using Eq.(1)

B - calculated theoretically: only the effect of a change in the effective dimensions of macromolecules; Eq.(4).

C - calculated theoretically: only the effect of macromolecule-macromolecule interactions; Eqs. (10) and (11).

a - experimental values from ref. (1) in tetrahydrofuran

b - stationary experiment in tetrahydrofuran

c - stationary experiment in theta solvent

as a consequence of the change in effective dimensions of macromolecules in solution. Using the dynamic chromatographic experiment, the empirical calibration function, i.e. the dependence of K_{SEC} on the effective dimensions of PS of various molecular weights, and the P and Q values were determined. The effective dimensions of PS macromolecules for various concentrations were calculated from Eqs. (5-9). Finally, the distribution coefficients were calculated from Eqs. (10) and (11). From the results summarized in Table 2, several conclusions may be drawn:

1. K_{SEC} calculated from experimental data for PS 303 000 varies in an important way also in the theta solvent. As K_{SEC} of the same PS in THF changes much more with changing concentration than values for PS 694 000, is seems that also interactions other than mere steric exclusion take place on the deactivated Merckogel Si 500 Å. Measurements with PS 694 000 and PS 111 000 obtained earlier (1) were performed with Porasil DX, but other interactions also cannot be excluded even in this last case.

2. The K_{SEC} values calculated only from a change in the effective dimensions of macromolecules in solution are distinctly lower in all cases than corresponds to experimental ones. This finding has been reported earlier (1). It is quite obvious, therefore, that a mere change in effective dimensions with changing concentration cannot explain the overall observed change in K_{SEC}, even under conditions of a stationary experiment. Already from these facts it can be seen that, as long as one does not succeed in excluding or quantitatively describing some interactions other than mere steric exclusion, the stationary experiment has only a limited

importance in the investigation of concentration effects in SEC, and also in corroborating any model of the separation mechanism in SEC (7).

3. The K_{SEC} values calculated using the Anderson and Brannon model suggest an apparent accord with the experimental values for PS 694 000. For PS 303 000 they are substantially lower than the experimental ones. For PS 111 000, on the other hand, they are much higher, reaching even extreme and physically unlikely values $K_{SEC} > 1$. Only the first virial coefficient was considered in the calculation of K_{SEC}. To elucidate the causes of this artefact, the K_{SEC} vs. K_o dependences calculated from Eqs. (10) and (11) were plotted in Fig. 1 for various values of the volume fraction φ. Fig. 1 shows that for $\varphi = 0.5$ and $\varphi = 1.0$ the calculated dependences pass through a distinct maximum which exceeds in a wide range of K_o the value $K_{SEC} > 1$. Since for flexible chain macromolecules which strongly swell in a thermodynamically good solvent φ reaches considerably high values already at relatively low weight concentrations (depending on molecular weight), it is obvious that for swelling macromolecules this model is nonrealistic. The physically unreal K_{SEC} values may result from the calculation also for a relatively low-molecular weight polymer (cf. PS 111 000), if K_o is high.

4. A shortcoming of the model based on a change in effective dimensions consists in that the $c_2 = c_p$ (concentration in the supernatant equals that in the pore). The Anderson and Brannon model does not introduce this assumption.

5. Thus, the results of measurements of the distribution coefficients under stationary conditions are not very encouraging with respect to the possible

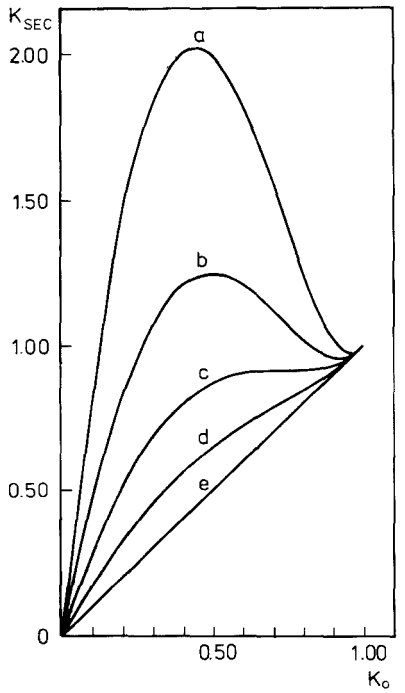

Fig. 1.
The K_{SEC} vs K_o functions for various values of volume fractions φ

a: $\varphi = 1.0$, b: $\varphi = 0.5$, c: $\varphi = 0.25$, d: $\varphi = 0.1$, e: $\varphi = 0$.

comparison with the dynamic chromatographic experiment. A question remains whether it is possible to rule out or evaluate the contribution of secondary (nonexclusion) interactions. It is quite probable that also kinetic aspects (i.e. the sorption-desorption rate) in the dynamic chromatographic process may become operative. The Anderson-Brannon model was worked out for the behaviour of rigid particles in a porous medium, which is a limiting factor of its suitability

in the case of a coil of the flexible chain macromolecule. On the other hand, however, the calculations of Casassa (8) and Giddings (9) demonstrate that differences between the behaviour in the SEC of statistical coils and rigid spherical macromolecules are not too drastic. Although our attempts to quantify exactly the role played by the mechanisms and models described above in the elucidation of concentration effects in the SEC of macromolecules have failed, our previous conclusions regarding the effect of dynamic viscosity effects remain valid (10).

It is difficult to draw conclusions, as further investigation of the problem of distribution coefficients under stationary conditions. Undoubtedly, further development of the Anderson-Brannon model for the case of macromolecules other than rigid ones would be useful. From the experimental point of view, it is desirable to investigate also other types of macromolecuIs, and rigid particles in the first place. Both theoretical research and experiments in this direction will continue.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1903-1905 (1984)

NOTE ON COMPLEXITY OF CONCENTRATION EFFECTS IN SEC

Josef Janča

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The paper by Chiantore and Guaita (1) published in this special issue deals with concentration dependence of the elution volumes in Steric Exclusion Liquid Chromatography (SEC) of polymers. The observed dependence is surprisingly high. Their main conclusion is that macromolecular coil contraction seems to account for 50-80 % of the total elution volume changes. This result is in quantitative variance with our findings cited in their paper, that viscosity phenomena account for about 80 % of the total concentration effects in the central part of the calibration curve.

The total increments of the elution volumes $\Delta V_t = (V_{e,c} - V_{e,o})$, where $V_{e,c}$ and $V_{e,o}$ are the elution volume at the concentration c and the elution volume extrapolated to c = 0, respectively, were taken from Fig. 3 of the ref. (1) for some polystyrene standards and for the concentrations of 10 and 20 mg/cm^3. The values of ΔV_t were multiplied by the corresponding $\left[d(\Delta V_s)/dc\right] / \left[d(\Delta V_t)/dc\right]$ values (a factor of the

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fractional amount of the contribution due to the coil size contraction over the total change of the elution volume), taken from Table 1, ref. (1). The resulting values of ΔV_s were added to the corresponding $V_{e,o}$ thus obtaining the corrected elution volumes $V_{e,c}^{corr}$ at given concentrations minus the contribution of the viscosity phenomena. By using these corrected elution volumes, the apparent molecular weights M_{app} were taken from the calibration graph on Fig. 1, ref. (1). Further the change of the swelling factor ε (see ref. (2)) with the change of concentration from zero to the given concentration and the ratio $\varepsilon/\varepsilon_o$ (ε_o is the swelling factor at $c = 0$ and ε is the swelling factor at given concentration) were calculated. The ratio $\varepsilon/\varepsilon_o$ is proportional to the contraction of the macromolecular coil when changing the concentration from zero to the given value. If the ratio $\varepsilon/\varepsilon_o$ is equal to the ratio of the true molecular weight of the polystyrene standard in question to the apparent molecular weight M_{app} found from the calibration graph by a procedure described for $V_{e,c}^{corr}$, the whole change of the elution volume corrected for the viscosity phenomena would correspond to the contraction of the macromolecular coils in solution. All the results are summarized in Table 1.

As can be seen from Table 1, the ratios M/M_{app} are systematically higher than the $\varepsilon/\varepsilon_o$ values by about 60 % or more. It means that the contribution of the macromolecular coil contraction calculated by using Rudin's theory (3) is lower than considered (1). On the other hand it means at the same time that also other phenomena (e.g. adsorption) are probably operative at the given experimental conditions and should be considered to explain the whole change of the elution volumes as a consequence of the concentration effects.

TABLE 1

Polystyrene molecular weight M	$V_{e,c}^{corr}$ c_1	$V_{e,c}^{corr}$ c_2	M_{app} c_1	M_{app} c_2	M/M_{app} c_1	M/M_{app} c_2	$\varepsilon/\varepsilon_o$ x) c_1	$\varepsilon/\varepsilon_o$ x) c_2
17500	6.63	6.75	10000	6400	1.75	2.73	1.05	1.09
200000	5.40	5.60	112900	85000	1.77	2.35	1.31	1.61
670000	4.85	5.02	243000	183300	2.76	3.66	1.92	2.84

x) Taken and calculated from ref. (2);
c_1 = 10 mg/cm^3, c_2 = 20 mg/cm^3

The support for the relative importance of the viscosity phenomena was given by applying Rudin's theory (3) to experimental data (4). However, the results by Chiantore and Guaita (1,5) as well as numerous previously published papers by other authors indicate the enormous complexity of the processes underlying the concentration effects in SEC of polymers and the need for further investigation.

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JOURNAL OF LIQUID CHROMATOGRAPHY, 7(9), 1907-1910 (1984)

LC CALENDAR

1984

AUGUST 21 - 24: 24th Int'l Conf on Analytical Chem. in Development, Sri Lanka. Contact: Secretary, Organizing Committee, Centre for Anal. Chem R & D, Dept. of Chem., University of Colombo, P. O. Box 1490, Colombo 3, Sri Lanka.

AUGUST 26-31: National ACS Meeting, Philadelphia, PA. Contact: Meetings, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

SEPTEMBER 10-14: Advances in Liquid Chromatography, including the 4th Annual American-Eastern European Symposium on LC and the Int'l Symposium on TLC with Special Emphasis on Overpressured Layer Chromatography, sponsored by the Hungarian Academy of Sciences' Chromatography Committee & Biological Research Center and the Hungarian Chemical Society, in Szeged, Hungary. Contact: Dr. H. Kalasz, Dept. of Pharmacology, Semmelweis University of Medicine, P.O.Box 370, H-1445 Budapest, Hungary, or Dr. E. Tyihak, Research Inst. for Plant Protection, P.O.Box 102, H-1525 Budapest, Hungary.

SEPTEMBER 16 - 21: Federation of Analytical Chemistry & Spectroscopy Societies (FACSS), Marriott Hotel, Philadelphia, PA. Contact: D. B. Chase, DuPont Co., Experimental Station 328, Wilmington, DE, 19898, USA.

OCTOBER 1-5: 15th Int'l. Sympos. on Chromatography, Nurenberg, West Germany. Contact: K. Begitt, Ges. Deutscher Chemiker, Postfach 90 04 40, D-6000 Frankfurt Main, West Germany.

OCTOBER 8 - 10: ASTM Committee E-19 on Chromatography, St. Louis Sheraton Hotel, St. Louis, MO. Contact: F. M. Rabel, Whatman, Inc., 9 Bridewell Place, Clifton, NJ, 07014, USA.

OCTOBER 24 - 26: Third Workshop/Symposium on LC/MS and MS/MS, Montreux, Switzerland. Contact: R. W. Frei, Dept. of Anal. Chem., Free University, De Boelelaan 1083, NL-1081 HV Amsterdam, The Netherlands.

OCTOBER 28 - NOVEMBER 1: 2nd International Congress on Computers in Science, Washington, DC. Contact: S. R. Heller, EPA, PM-218, Washington, DC, 20460, USA.

NOVEMBER 13 - 16: 23rd Eastern Analytical Symposium, New York Penta Hotel, New York City. Contact: S. D. Klein, Merck & Co., P. O. Box 2000/R801-106, rahway, NJ, 07065, USA.

DECEMBER 10-12: "TLC/HPTLC-84: Expanding Horizons in TLC," Sheraton-University City, Philadelphia, PA. Contact: J. C. Touchstone, University of Pennsylvania, Dept. OB-GYN, 3400 Spruce eStreet, Philadelphia, PA.

DECEMBER 10-12: Fourth International Symposium on HPLC of Proteins, Peptides and Polynucleotides, Baltimore, MD. Contact: Shirley E. Schlessinger, Symposium Manager, 400 East Randolph, Chicago, IL, USA.

DECEMBER 16-21: International Chemical Congress of Pacific Basin Societies, Honolulu, Hawaii, sponsored by the Chemical Inst. of Canada, Chemical Soc. of Japan, and the American Chem. Soc. Contact: PAC CHEM '84, International Activities Office, American Chem. Soc., 1155 Sixteenth St., NW, Washington, DC, 20036, USA.

1985

FEBRUARY 11-14: Polymer 85, Int'l Symposium on Characterization and Analysis of Polymers, Monash University, Melbourne, Australia, sponsored by the Polymer Div., Royal Australian Chemical Inst. Contact: Polymer 85, RACI, 191 Royal Parade, Parkville Victoria 3052, Australia.

FEBRUARY 25 - MARCH 1: Pittsburgh Conference on Analytical Chemistry & Applied Spectroscopy, New Orleans, LA. Contact: Paul E. Bauer, 1985 Pittsburgh Conference, 437 Donald Rd., Dept. FP, Pittsburgh, PA, USA.

APRIL 15 - 17: Second International Symposium on Instrumental TLC, Wurzburg, West Germany. Contact: H. M. Stahr, 1636 College Veterinary Medicine, Iowa State University, Ames, IA, 50011, USA., or Prof. S. Ebel, Institute for Pharmacies, A. M. Hubland, D-8700 Wuerzburg, West Germany.

APRIL 28 - MAY 3: 189th National ACS Meeting, Miami Beach. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

JUNE 24 - 28: 59th Colloid & Surface Science Symposium, Clarkson College of Technology, Potsdam, NY. Contact: J. P. Kratohvil,

Institute of Colloid & Surface Science, Clarkson College of Technology, Potsdam, NY, 13676, USA.

JULY 1-5: Ninth International Symposium on Column Liquid Chromatography, sponsored by the Chromatography Discussion Group and by the Royal Society of Chemistry's Chromatography & Electrophoresis Group, Edinburgh, Scotland. Contact: Prof. J. H. Knox, 9th ISCLC Secretariat, 26 Albany Street, Edinburgh, EH1 3QH, Great Britain.

JULY 4: 4th International Flavor Conference, Greece. Contact: C. J. Mussinan, IFF R&D, 1515 Highway 36, Union Beach, NJ, 07735, USA.

SEPTEMBER 1-6: 6th International Symposium on Bioaffinity Chromatography & Related Techniques, Prague, Czechoslovakia. Contact: Dr. J. Turkova, Institute of Organic Chemistry & Biochemistry CSAV, Flemingovo n. 2, CS-166 10 Prague 6, Czechoslovakia.

SEPTEMBER 8-13: 190th National ACS Meeting, Chicago. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

SEPTEMBER 29 - OCTOBER 4: Federation of Analytical Chemistry & Spectroscopy Societies (FACSS), Marriott Hotel, Philadelphia, PA. Contact: T. Rains, NBS, Center for Analytical Chemistry, Chemistry B-222, Washington, DC, 20234, USA.

1986

APRIL 6-11: 191st National Am. Chem. Soc. Mtng., Atlantic City, NJ. Contact: A. T. Winstead, ACS, 1155 16th Streeet, NW, Washington, DC, 20036, USA.

SEPTEMBER 7-12: 192nd National Am. Chem. Soc. Mtng., Anaheim, Calif. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA

SEPTEMBER 21-26: XVIth International Symposium on Chromatography, Paris, France. Contact: G.A.M.S., 88,Boulevard Malesherbes, F-75008 Paris, France.

1987

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AUGUST 30 - SEPTEMBER 4: 194th National Am. Chem. Soc. Mtng., New Orleans, LA. Contact: A. T. Winstead, ACS, 1155 16th Street, NW, Washington, DC, 20036, USA.

1988

JUNE 5 - 11: 3rd Chemical Congress of the North Americanmn Continent, Toronto, Ont., Canada. Contact: A. T. Winstead, ACS, 1155 Sixteenth St, NW, Washington, DC, 20036, USA.

SEPTEMBER 25 - 30: 196th ACS National Meeting, Los Angeles, CA. Contact: A. T. Winstead, ACS, 1155 Sixteenth Street, NW, Washington, DC, 20036, USA.

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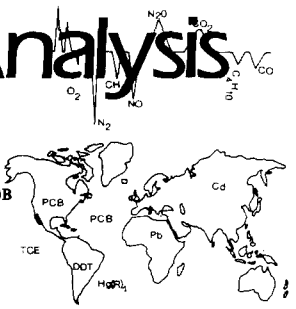

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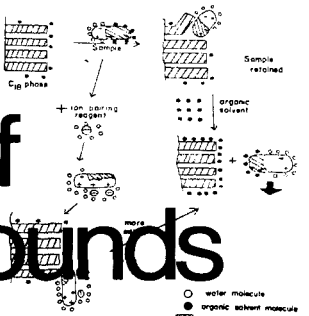

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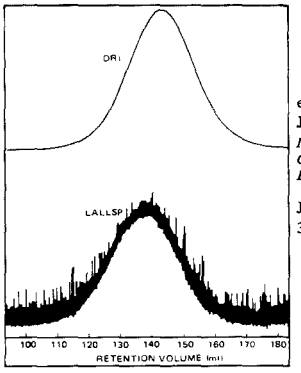

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P. O. Box 1440-SMS
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A SEMI-AUTOMATIC TECHNIQUE FOR THE SEPARATION AND DETERMINATION OF BARIUM AND STRONTIUM IN SURFACE WATERS BY ION EXCHANGE CHROMATOGRAPHY AND ATOMIC EMISSION SPECTROMETRY

F. D. Pierce and H. R. Brown
Utah Biomedical Test Laboratory
520 Wakra Way
Salt Lake City, Utah 84108

3. Abstract: Three lines below the addresses, the title ABSTRACT should be typed (capitalized and centered on the page). This should be followed by a single-spaced, concise, abstract comprising less than 10% of the length of the text of the article. Allow three lines of space below the abstract before beginning the article itself.

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