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ABSOLUTE PEAK BROADENING CALIBRATION IN SIZE-EXCLUSION CHROMATOGRAPHY USING A POLYMER-BOUND CHROMOPHORE

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SUMMARY

A recently developed theory permits the determination of peak broadening in size-exclusion chromatography by combining the simultaneous signals obtained from two different detectors, one being sensitive to the mass concentration and the other to the molecular weight of the solute eluted. Based on this methodology, a new experimental technique employing polymer-bound chromophores and a standard UV detector was developed to provide information normally obtained with an absolute detector. The advantages of the method are its simplicity and the utilization of a single cell for measurements, which relieves much of the detection geometry problems inherent to other techniques. Fractions of polystyrene labelled with azobenzene groups and synthesized by radical polymerization were used. The experimental broadening parameters are critically evaluated with respect to the possible sources of error and compared with results obtained from quasi-monodisperse polymer fractions.

INTRODUCTION

Since its introduction in 1964[1], size-exclusion chromatography (SEC) has gained wide acceptance as a standard tool for the determination of the molecular weight (MW) distribution in polymers.

The major limitation of SEC, in common with all other chromatographic techniques, is that it is a secondary analytical tool. Precise evaluation of experimental data in terms of the molecular weight distribution depends on the availability of accurate polymer standards for the transformation of the elution volume into a scale of molecular masses. In this respect, the hydrodynamic volume approach of Benoît *et al.*[2,3] and the recent advent of reliable MW-sensitive detectors, like the continuous viscometer[4] and on-line low-angle laser light scattering photometer (LALLSP)[5], reduced much of the effort and time spent on mass calibration.

One persistent problem is the lack of a simple procedure to calibrate axial dispersion in SEC. Heretofore, several methods have been proposed, but they are generally time-consuming[6,7], indirect[8] or require sophisticated instrumentation and computation facilities[9] not readily available to the average polymer laboratory.

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In this paper, we will describe a novel experimental technique to determine instrumental broadening, based on the application of polymer-bound chromophore. The approach is practical and the technique may be used on any standard liquid chromatograph equipped with a variable wavelength UV detector, or even better with a photodiode-array detector where signals at different wavelengths can be processed simultaneously.

The idea of using polymer molecules labelled with chromophore groups for the purpose of molecular counting is not new in itself and has been employed for over 20 years in the colorimetric end-group titration of polystyrene[10]. Recently, the usefulness of this class of compounds for absolute molecular weight calibration in SEC has been noted[11]. In any event, it does not seem that the potential of the chromophore method for axial dispersion calibration has been duly considered. Part of this neglect stems from the fact that polymer samples having the required properties for this type of application are difficult to obtain:

the number of chromophore groups per chain must be constant, otherwise its dependency on the polymer molecular weight within the sample must be precisely known;

the absorption spectrum of the labelled group should preferably be distinct from that of the polymer to ensure minimum peak overlap;

an high absorption coefficient of the chromophore is needed for a good signal-to-noise ratio of the detector signal;

absence of adsorption on the stationary phase;

good thermal and photochemical stability of the chromophore;

a low polydispersity of the sample is an asset although not mandatory, providing the first condition for stoichiometry is properly fulfilled.

The versatility of anionic polymerization makes it the method of choice for labelled polymer synthesis[12]. However, finding appropriate conditions for anionic polymerization requires time and expertise. In a first step, to evaluate the feasibility of the technique, we rely on the simpler radical polymerization system initiated with a chromophore-labelled peroxide.

POLYMER SYNTHESIS

The monomer used was styrene. A peroxide containing azobenzene as the chromophore (Fig. 1) was selected for the radical initiation of polymerization. It was synthesized according to the protocol described by Kämmerer *et al.*[13]. The symmetric isomer, bis(4-phenylazo)benzoyl peroxide, although more easy to synthesize, could not be used as an homogeneous polymerization initiator due to its limited solubility in styrene and in methyl methacrylate.

The UV absorption spectrum of bound azobenzene has a maximum at 318 nm; its minimum is nearly coincident with the absorption maximum at 262 nm of the

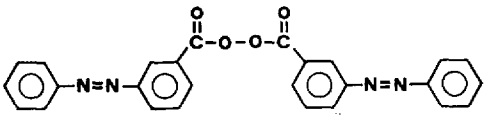

Fig. 1. Chemical structure of bis(3-phenylazo)benzoyl peroxide.

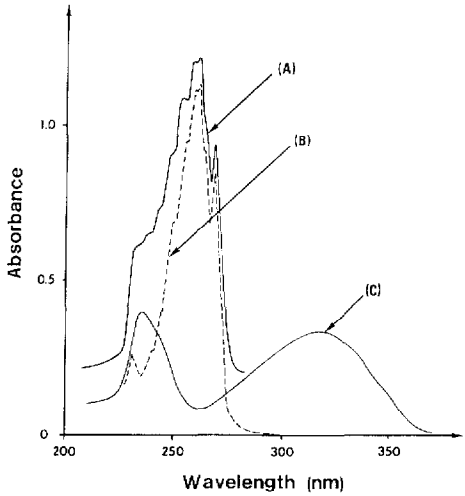

Fig. 2. UV absorption spectrum of chromophore-bound PS, deconvoluted into the azobenzene and styrene absorption bands (polymer fraction synthesized at $4 \cdot 10^{-3}$ *M* peroxide, concentration = 0.500 mg/ml dichloromethane). (A) Spectrum of chromophore-bound PS; (B) spectrum of thermally initiated PS; (C) spectrum of the chromophore obtained by the difference between (A) and (B).

styrene units in polystyrene (PS) (Fig. 2). This insures a good selectivity in the detector signals obtained at these two wavelengths. Use of the azobenzene-labelled peroxide has the added advantage that its decomposition kinetics has already been investigated in styrene and in methyl methacrylate[10].

In the course of polymerization, azobenzene moieties become attached to the chain backbone principally during the initiation stage but also by a chain-transfer mechanism, as revealed by detailed characterization of the synthesized polymers.

Degassed solutions of styrene, containing $2.5 \cdot 10^{-4}$–$10 \cdot 10^{-3}$ *M* peroxide, were polymerized in bulk at 70°C, in vacuum-sealed Pyrex tubes. The polymerization was stopped at conversion yields below 5% to avoid side reactions. The polymer was recovered by precipitation and repeatedly extracted with methanol to remove unreacted initiator and monomer.

Characterization of labelled polymer

The molecular weight distributions of the synthesized polymers were determined by SEC on a Waters 150C equipped with a set of Ultrastyragel columns. The variable wavelength UV detector (Perkin-Elmer LC-75) was interfaced to a Waters Data Module and a personal computer (Hewlett-Packard 9816S) for data acquisition and treatment.

The presence of a few additional azobenzene groups in the labelled polymer is unlikely to perturb significantly the hydrodynamic radius of the molecular coil as compared to homo-PS. For this reason, we used for mass calibration the same curve as established with PS standards without any further adjustment. As explained in the next section, a small correction was applied to the styrene absorbance at 262 nm to allow for band overlap with the absorbance of the chromophore group.

The decadic molar extinction coefficient of PS-bound azobenzene, measured in chloroform, is equal to $1.67 \cdot 10^7$ cm^2 mol^{-1} at the absorbance maximum of 319 nm[10]. Referring to this value, we determined the equivalent extinction coefficient in dichloromethane which is the eluent solvent for SEC: $\varepsilon_2(318\text{ nm}) = 1.64 \cdot 10^7$ cm^2 mol^{-1}.

The average number of chromophore groups per chain, $\bar{n}$, was calculated according to the Lambert–Beer law as given by the following relationship

$$\text{Absorbance (318 nm)} = \varepsilon_2(318\text{ nm}) \cdot CL\bar{n}/M_n \quad (1)$$

where C = mass concentation of the polymer in g cm^{-3}, L = optical length of the cell in cm and M_n = number average molecular weight of the sample in g mol^{-1}.

The results, reported in Table I, showed that $\bar{n}$ decreased with the number average molecular weight of the polymer. The sample prepared at $2.5 \cdot 10^{-4}$ M peroxide is an exception: at this low concentration, thermal initiation competed with peroxide decomposition and the average number of azobenzenes incorporated into the polymer during this step decreased accordingly. To avoid uncontrolled uncertainty in the distribution of chromophore groups which may result from this complex kinetics, the polymer synthesized at the lowest peroxide concentration was omitted from the present studies.

Using the UV absorption spectrum of thermally initiated PS (without additive) as the reference, the absorption curve of labelled polymers was deconvoluted by difference into separate bands belonging to the styrene units and to the azobenzene group (Fig. 2). With proper normalization, all the individual bands are directly superimposable regardless of the sample, which seems to indicate that the chromophore absorption spectrum is independent of the polymer molecular weight. At 262 nm, the absorbance germane to the styrene units is given by the following relationship which was used to correct for band overlap

$$\text{Absorbance(PS)} = A(262\text{ nm}) - 0.23\,A(318\text{ nm}) \quad (2)$$

where A = absorbance at the designated wavelength.

To determine the variation of n as a function of chain length, each polymer synthesized was fractionated into eight to ten fractions by preparative SEC, with the polydispersity ranging from 1.07 to 1.10. Each fraction was then analyzed for M_n by

TABLE I

CHARACTERIZATION OF THE LABELLED POLYMERS

Polymerization temperature: 70°C.

Concn. of peroxide (M)	M_w	M_n	M_w/M_n	$\bar{n}$
0	1470 000	661 000	2.2	0
$2.5 \cdot 10^{-4}$	464 000	153 000	3.0	1.07
$1 \cdot 10^{-3}$	236 000	101 000	2.3	1.60
$4 \cdot 10^{-3}$	85 700	31 500	2.7	1.29
$10 \cdot 10^{-3}$	40 200	12 200	3.3	0.89

SEC and for the absorbances at 262 and 318 nm. The average number of chromophores for each fraction, n_f, was calculated from the ratio of absorbances originating respectively from the azobenzene groups and from the styrene units

$$n_f = (a_1/\varepsilon_2)\, M_n A(318\text{ nm})/[A(262\text{ nm}) - 0.23A(318\text{ nm})] \quad (3)$$

where a_1 is the mass extinction coefficient at 262 nm of PS in dichloromethane, equal to 2270 $cm^2\ g^{-1}$.

The results of the characterization (Fig. 3) revealed one of the essential features of the polymerization mechanism: the number of chromophore groups per chain increases both with the chain length and the initial concentration of peroxide. The decrease of $\bar{n}$ with the initiator concentration, as indicated in Table I, merely reflects the averaging effect over the molecular weight range present in each sample. This trend is consistent with a chain-transfer mechanism where a growing macroradical is added to the chromophore group, without modifying the UV absorption property of the azobenzene double bond[10].

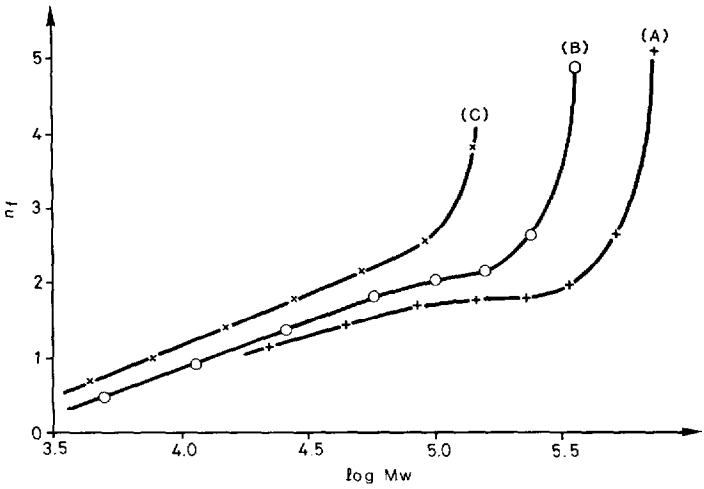

Fig. 3. Variation of chromophore stoichiometry with polymer molecular weight (n = number of azobenzene groups per polymer chain). Polymer synthesized at: (A) $1 \cdot 10^{-3}$; (B) $4 \cdot 10^{-3}$; (C) $10 \cdot 10^{-3}$ M peroxide.

THEORETICAL BASIS FOR AXIAL DISPERSION CALIBRATION

The equations used are in essence based on the general relationships developed by Hamielec[9] for absolute detector systems. According to the methodology described, the axial dispersion parameters, and with certain approximations even the axial dispersion function, can be obtained by combining the signals of the same sample arising simultaneously from two different detectors. One of these detectors should be sensitive to the mass concentration and the other to the molecular weight of the eluted polymer. We will succinctly rewrite below some equations of interest for the case of a molecular detector, keeping whenever possible the same notation as in ref. 9.

Let $F_1(V)$ be the response of the UV detector at the elution volume, V, when tuned to the maximum absorption wavelength of the polymer (262 nm for PS), and $F_2(V)$ the detector signal at the absorption maximum of the chromophore (318 nm in dichloromethane).

For the present discussion, we will assume that $F_1(V)$ has already been corrected for any band overlap, using eqn. 2.

The corrected absorbance $F_1(V)$ is proportional to the mass concentration of polymer and is given by

$$F_1(V) = a_1 L \int_0^\infty C(V,Y)\, \mathrm{d}Y \tag{4}$$

where $C(V,Y)$ is the mass concentration of polymer within the elution range V and $V + \mathrm{d}V$, due to species with mean retention volumes between Y and $Y + \mathrm{d}Y$, and L is the optical length of the detector cell.

According to Tung[14], $F_1(V)$ can be written as a Fredholme integral of the first kind

$$F_1(V) = a_1 L \int_0^\infty C(Y)G(V,Y)\, \mathrm{d}Y \tag{5}$$

where $G(V,Y)$ is the normalized instrumental dispersion function to be determined.

At the chromophore wavelength, the detector signal, $F_2(V)$, gives the concentration of azobenzene groups which is directly related to the molar concentration of the polymer

$$A(318\ \mathrm{nm}) = F_2(V) = \varepsilon_2 L \int_0^\infty n(Y)C(V,Y)/M(Y)\, \mathrm{d}Y \tag{6}$$

where $M(Y)$ is the calibration curve in the absence of peak broadening, and $n(Y)$ is the number of chromophore groups per chain for species eluted between Y and $Y + \mathrm{d}Y$ as given in Fig. 3.

By definition, the instantaneous number average molecular weight of the detector cell content at the elution volume, V, is given by:

$$M_n(V) = \frac{\int_0^\infty C(V,Y)\, \mathrm{d}Y}{\int_0^\infty C(V,Y)/M(Y)\, \mathrm{d}Y} \tag{7}$$

This quantity is proportional to the ratio of the detector signals obtained at the two wavelengths selected, 262 and 318 nm, regardless of the shape of the spreading function, $G(V,Y)$:

$$F_1(V)/F_2(V) = (a_1/\varepsilon_2)M_n(V)/n(V) \tag{8}$$

In order to relate $M_n(V)$ and $M(V)$ with an analytical expression, a common practice is to approximate $G(V,Y)$ with a variable gaussian function of standard deviation, $\sigma(V)$, changing with the elution volume:

$$G(V,Y) = G(V - Y) = \frac{1}{\sigma(V)\sqrt{2\pi}} \cdot \exp\left[-\frac{(V-Y)^2}{2\sigma(V)^2}\right] \tag{9}$$

With this simplification, a simple relationship between $M_n(V)$ and $M(V)$ can be obtained:

$$\frac{M_n(V)}{M(V)} = \frac{F_1(V)}{F_1[V + D_2(V)\,\sigma(V)^2]} \cdot \exp\{-1/2[D_2(V)\,\sigma(V)]^2\} \tag{10}$$

Written differently, eqn. 10 provides a means to determine the spreading parameter, $\sigma(V)$, from a combination of the experimental traces $F_1(V)$ and $F_2(V)$:

$$F_1[V + D_2(V)\,\sigma(V)^2] = [a_1/n(V)\,\varepsilon_2]F_2(V)M(V) \cdot \exp\{-1/2[D_2(V)\,\sigma(V)]^2\} \tag{11}$$

In this equation, the constants D_1 and D_2 of the locally linearized calibration curve in the absence of peak broadening must be measured beforehand:

$$\ln M(V) = D_1(V) - D_2(V) \cdot V \tag{12}$$

For narrow MW distributions, the position at the peak apex is insensitive to the instrumental axial dispersion[9]. A good approximation to $M(V)$ is actually provided by the calibration graph established with sharp PS standards.

RESULTS AND DISCUSSION

Eqn. 11 is valid regardless of the shape of the functions $F_1(V)$ and $F_2(V)$, and hence of the sample MW distribution. For practical purposes, we will distinguish however the situation where the polymer is of large polydispersity from the case of a nearly monodisperse fraction.

Broad MW distribution

First, $n(V)$ must be known precisely from separate measurements and the appropriate values substituted into eqn. 11. The ensuing steps are then exactly similar to the approach employed with an on-line mass-sensitive detector[9]: for any given elution volume, a single variable search permits the determination of the best value of $\sigma(V)$ which fits the left-hand term of eqn. 12 to its right-hand counterpart.

The results of this fit are reported in Fig. 4 for one of the labelled PS fractions polymerized at $4 \cdot 10^{-3}$ *M* peroxide.

The detector signals are collected at discrete sampling intervals to give a total of *ca.* 100 data points across each single chromatogram. Values situated between two data points are extrapolated by a third-order polynomial before the variable search is commenced, using in the present case the Fibonacci technique of linear optimization[15]. This procedure however does not work near the peak maximum where any deviation of the signal will significantly influence the value of σ and should be replaced by a more elaborate optimization technique. Since $\sigma(V)$ is a monotonous function of V, the few missing points at the peak apex are more readily obtained by extrapolation from the fitted values on the ascending and descending slopes of the SEC trace (Fig. 4).

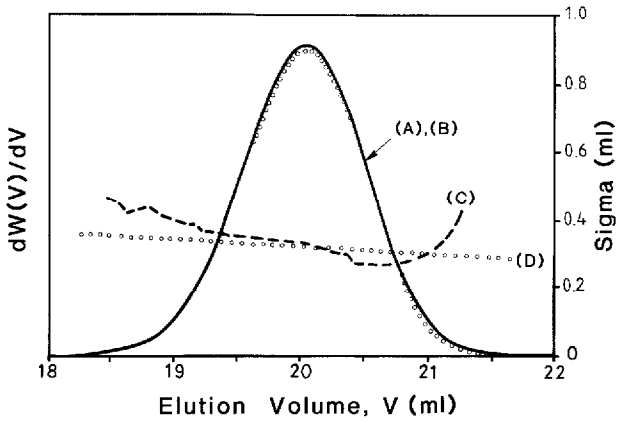

Fig. 4. Axial dispersion constant as a function of elution volume fitted with a broad MW distribution ($4 \cdot 10^{-3}$ *M* peroxide, μStyragel columns). (A) Detector signal at 262 nm, corrected for band overlap according to eqn. 2. (B) Curve calculated from the right-hand side of eqn. 11, mostly indistinguishable from (A). (C) Results of a variable search using eqn. 11 (right ordinates). (D) Results from Fig. 6 obtained with narrow fractions of labelled polymer (right ordinates).

Theoretically, eqn. 11 should allow determination of $\sigma(V)$ over a wide range of elution volumes with a single polymer sample. In practice, the precision of the fit is largely dependent on baseline fluctuations, on the accuracy of the MW calibration graph and in the determination of $n(V)$. These sources of error are cumulative, making the reliability of the results questionable. For example, the fitted $\sigma(V)$ curve in Fig. 4 shows systematic deviation from a similar curve determined with narrow MW fractions (Fig. 5), although they have identical values when averaged over the elution range of the sample.

Narrow MW distribution

Samples with $M_w/M_n \approx 1.01$–1.02 are obtained from the crude polymer by multiple refractionation of the middle portion on an analytical column (Table I).

The narrow elution range which encompasses each fraction allows several levels of simplification in the determination of the axial dispersion constant. The choice of

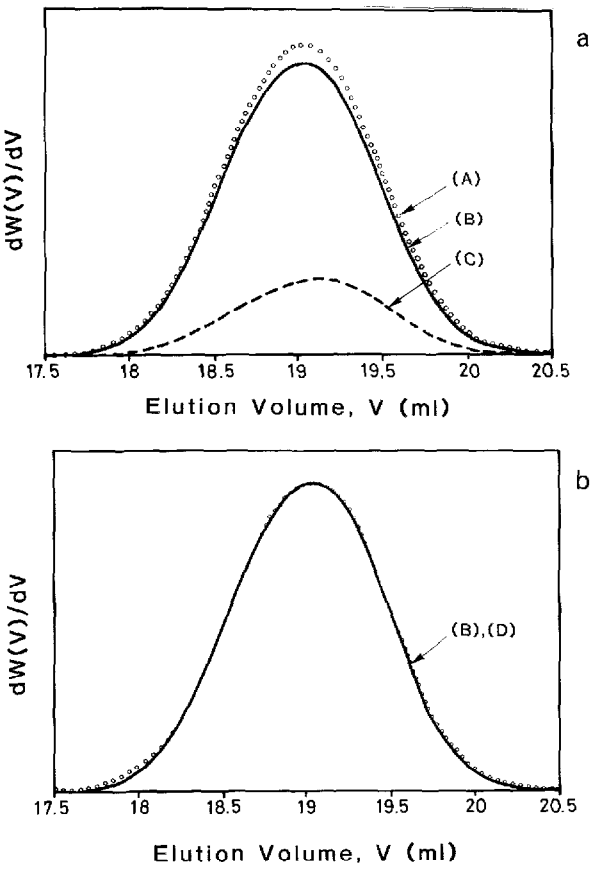

Fig. 5. Determination of σ by curve fitting with a narrow polymer fraction ($4 \cdot 10^{-3}$ M peroxide, μStyragel columns). (a) Detector signals at 262 nm (A), at 262 nm corrected for band overlap (B) and at 318 nm (C). (b) (B) Corrected detector signal at 262 nm; (D) curve calculated from the right-hand side of eqn. 11.

the method is mainly dictated by the degree of precision and the kind of information which may be needed.

(a) Both $n(V)$ and $\sigma(V)$ change slowly with the elution volume. The low polydispersity of the labelled fractions permits the replacement in eqn. 11 of $n(V)$ and $\sigma(V)$ with average values, n_f and σ.

Over a limited range of the elution volume, $D_2(V)$ can be considered constant. Let $D_2\sigma^2 = \Delta V$, eqn. 11 simplifies to:

$$F_1(V + \Delta V) = F_2(V)\{M(V) \cdot \exp[-1/2(D_2\sigma)^2]\ (a_1/n_f\varepsilon_2)\} \tag{13}$$

Since the right-hand-side expression between the round brackets is constant for a given elution volume, proper normalization of the functions $F_1(V)$ and $F_2(V)$ permits account to be taken of this factor without the need to know the exact value of each individual term.

A simple translation by ΔV permits a superimposition of the two normalized curves, and hence the determination of σ.

(b) At the same level of approximation, *i.e.*, constant n_f and D_2, and a constant gaussian for the axial dispersion function, the spreading constant can be simply calculated from the properties of the whole polymer average molecular weights[16].

At the polymer wavelength (262 nm):

$$M_n(\text{uc1}) = \frac{\int_0^\infty F_1(V)\mathrm{d}V}{\int_0^\infty F(V)/M(V)\mathrm{d}V} = M_n(\text{c}) \cdot \exp[-1/2(D_2\sigma)^2] \tag{14}$$

$$M_w(\text{uc1}) = \frac{\int_0^\infty F_1(V)M(V)\mathrm{d}V}{\int_0^\infty F_2(V)\mathrm{d}V} = M_w(\text{c}) \cdot \exp[+1/2(D_2\sigma)^2] \tag{15}$$

At the chromophore wavelength (318 nm):

$$M_n(\text{uc2}) = \frac{\int_0^\infty F_1(V)M(V)\mathrm{d}V}{\int_0^\infty F_2(V)\mathrm{d}V} = M_n(\text{c}) \cdot \exp[+1/2(D_2\sigma)^2] \tag{16}$$

$$M_w(\text{uc2}) = \frac{\int_0^\infty F_1(V)M(V)\mathrm{d}V}{\int_0^\infty F_2(V)M(V)\mathrm{d}V} = M_w(\text{c}) \cdot \exp[+3/2(D_2\sigma)^2] \tag{17}$$

The indices "uc" and "c" in the above equations mean respectively "uncorrected" and "corrected" for axial dispersion.

D_1 and D_2 are coefficients obtained by linear regression of the calibration graph over the useful elution range of the polymer fraction.

Values obtained by combining pairs of eqns. 14–16 and 15–17 to eliminate $M_n(c)$ and $M_w(c)$ give a good concordance with the values obtained from whole-curve fitting using the procedure described under (d) below. This simple technique may be employed for a rapid determination of the axial dispersion constant without the use of

a computer. The extra information contained in the entire envelope of the detector signals can be used in principle for the determination of the axial dispersion function (see technique "c").

(c) If the spreading function is independent of the elution volume, it is possible to solve simultaneously the set of equations given by the detector signals at the two wavelengths and determine $G(V,Y)$[18]. This method should be particularly useful in the high MW range where $G(V,Y)$ may deviate significantly from the pure gaussian[9].

(d) First, $\sigma(V)$ is considered constant over the elution range of interest. In this case, the same curve-fitting method as described for a polymer of broad MW distribution can be used but the errors involved are much smaller due to the limited distribution of molecular masses (Fig. 5).

When several narrow samples with different MWs are available, $\sigma(V)$ can be refitted by iteration if necessary.

We found the present procedure (d) to be the most reliable among the above-mentioned techniques for the determination of $\sigma(V)$.

The values of σ obtained by applying technique (d) for the three narrowest fractions of labelled polymers are plotted in Fig. 6. Two different sets of μStyragel and Ultrastyragel columns (10^5 Å + 10^4 Å) are used. As expected, μStyragel columns with larger particles have dispersion constants nearly twice the values obtained with Ultrastyragel columns, particularly in the high MW range.

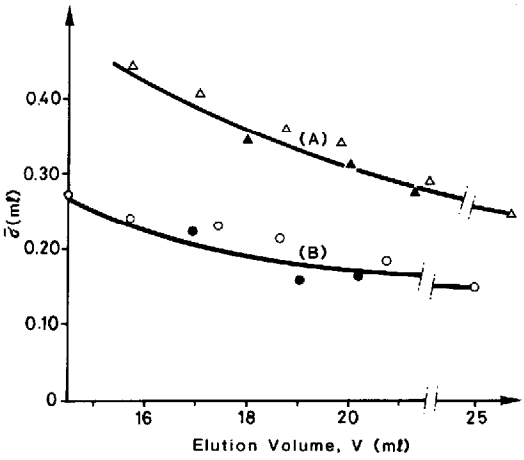

Fig. 6. Axial dispersion constant as a function of elution volume using sharp polymer fractions. (A) μStyragel columns (10^4 Å + 10^5 Å): ▲, polymer-bound chromophore method; △, quasi-monodisperse fraction method. (B) Ultrastyragel columns (10^4 Å + 10^5 Å): ●, polymer-bound chromophore method; ○, quasi-monodisperse fraction method.

As an independent check on the consistency of the technique, we measured $\sigma(V)$ for nearly monodisperse PS obtained by repeated fractionation on Ultrastyragel columns ($M_w/M_n \approx 1.01$ estimated with the recycling technique[19]). The influence on the peak width due to sample polydispersity is negligible at this level. The peak width at half maximum ($= 2.35\sigma$) can then be used for the evaluation of the axial dispersion constant.

Visual inspection of the data points in Fig. 6 shows a good concordance between the two methods, any deviation being within the expected experimental errors involved.

Precision of the technique

In order to evaluate the reliability of the technique, we examine the influence of possible sources of errors on the accuracy of the results.

Since the signals $F_1(V)$ and $F_2(V)$ are obtained from two separate experiments, fluctuations of the pump flow-rate may cause additional uncertainty in the determination of σ. A flow-rate calibration using a low MW solute (toluene) showed that the effect is relatively small and accounted at most for 6% error on the measured values, providing the columns and the pump are both in perfect working order.

Variation of the band overlap factor in eqn. 2 from 0.1 to 0.4 resulted in the expected change in the ratio a_1/ε_2, without any significant influence on the value of σ.

The use of constant average instead of locally linearized values for D_1 and D_2 (eqn. 12) tends to ab overestimation of σ, but the effect is small and amounts to less than 10% for a sample of broad MW distribution with $M_w/M_n = 1.3$ even in a portion of the calibration graph where departure from linearity is appreciable.

From the SEC traces of nearly monodisperse PS samples, it was verified that the spreading function is symmetric with a gaussian shape within the elution range attainable with currently available fractions of labelled polymer (*cf.*, also Fig. 5). However, starting from molecular masses of $\geqslant 10^6$ daltons, some peak skewness was observed at the same flow-rate (1.0 ml/min). For a precise determination of the MW distribution, the complete dispersion function should be used in this high MW range.

Error in the determination of chromophore stoichiometry. Taking the extreme case where any dependency of n on the chain length was neglected, we determined the spreading constant for samples of similar M_n but with different polydispersities. The

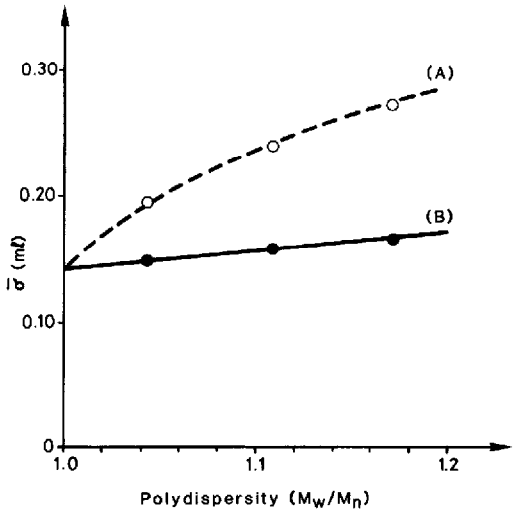

Fig. 7. Effect of chromophore stoichiometry on σ ($10 \cdot 10^{-3}$ M peroxide, Ultrastyragel columns). (A) $n(V)$ considered constant; (B) $n(V)$ from Fig. 3.

fraction with the largest M_w/M_n shows also the largest deviation from the correctly determined spreading constant (Fig. 7). When the chromophore stoichiometry was properly taken into account, the variation of σ with polydispersity was much weaker, as expected from the shape of the curve in Fig. 6. This experiment reveals in a vivid manner the importance of using the correct stoichiometry for a precise determination of σ.

CONCLUSIONS

The results from the present study showed that the utilization of a chromophore-bound polymer is a direct, reliable and versatile technique for the calibration of instrumental dispersion in SEC. Unlike the LALLS photodetector, the equipment is simple and if high accuracy is not required the spreading constant can even be obtained from average molecular weights without the need for any computer data treatment.

The use of a single cell to collect complementary information on mass and molar solute concentration is a definite advantage as compared to other techniques: sampling geometry is known to give rise to non-trivial problems in LALLSP where two different detection cells are placed in series[20].

As mentioned before, the whole dispersion function, $G(V-Y)$, is theoretically accessible with the present technique[18] and will be the subject of further investigation.

It is currently assumed that the spreading constant depends on the molecular mass but not on the nature of the polymer[9]: another application of the present technique would be to use different polymer matrices bearing the chromophore to test for the validity of this hypothesis.

The aim underlying this work was to provide SEC practicians with polymer standards which can be used both for mass and axial dispersion calibration. At the present stage of development, the method still presents several limitations which need further improvement.

The change of the chromophore number with molecular weight, which requires careful stoichiometry analysis, can be avoided by using anionic polymerization. Except for the difficulties of chemical synthesis, the best solution would be to employ an anionic initiator bearing the chromophore group. In this case, chain termination due to impurities or side reactions cannot interfere with the chemical stoichiometry which would be exactly one chromophore group per polymer chain. The synthesis of such a compound presents probably the most challenging aspect of the problem.

Modern micro-packed columns necessitate injection volumes below 50 μl of dilute polymer solution (typically <0.2 mg/ml for MW above 10^6). Working with such infinitesimal quantities requires a very high absorption coefficient of the chromophore group to obtain a measurable detector signal.

Even with azobenzene, which has one of the highest extinction coefficients amongst synthetic dyes, the workable range of molecular weight is still limited to $<300\,000$. Straightforward calculations show that in order to analyze polymers in the 10^6 MW range, chromophore groups with extinction coefficients exceeding $2 \cdot 10^8$ cm^2 mol^{-1} are necessary. For laboratories having access to a fluorescence detector, another alternative to improve the signal-to-noise ratio would be to use polymer-bound fluorescent groups instead of absorbing chromophores.

The choice of PS as the support polymer bearing the chromophore group

facilitates MW calibration due to the wide availability of good commercial standards. The presence of PS requires however that the UV absorption spectrum of the chromophore possesses a window in the vicinity of 250–270 nm to minimize band overlap. This actually excludes compounds containing benzene rings in their structures. One exception seems to be provided by coronene which has a very high absorption coefficient at 305 nm but is virtually transparent at 262 nm. Compounds belonging to this category are worth consideration in future investigations.

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