A New Procedure for Simultaneous Calibration of Separation and Axial Dispersion in SEC

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Summary

A new computational procedure for the simultaneous calibration of separation and axial dispersion by using SEC coupled with a LALLS detector is presented. Its performance is tested by SEC/LALLS experiments with commercially available samples of broad and narrow polystyrene standards. The peakbroadening parameters were calculated by a novel method which utilizes the different effects of axial dispersion on polymers with narrow and broad molecular weight distribution. The dispersion function was assumed to be gaussian. The variation of the dispersion parameter with molecular weight is in good agreement with reports in the literature. Experimental chromatograms have been corrected for the axial dispersion effect.

Introduction

The accurate interpretation of data from size exclusion chromatography (SEC) in terms of molecular weight averages and molecular weight distributions requires calibration of the separation and axial dispersion corrections: this has been reviewed by Dawkins [1], Hamielec [2] and Belenkii [3]. There have been many approaches to the evaluation of the dispersion parameter and these have made use of different methods such as reverse flow [4] and recycling methods [5], runs of chromatographic monodisperse polymers [6] or of samples with known molar mass distribution [7], and the use of a molecular mass detector [8–11]. Plate-height data from various polymer standards has also been used for estimation of the dispersion parameter [12]. Obviously most of these methods require sophisticated instrumentation and complex data evaluation procedures. In SEC coupled with a low-angle laser light scattering (LALLS) photometer as detector of molecular mass, some special methods permit a simultaneous calibration of separation and axial dispersion especially when no calibration standards are available [8, 10, 11].

In this paper, we present the application of a recently developed computation procedure for the simultaneous calibration of separation and axial dispersion by using SEC coupled with a LALLS photometer on various polystyrene samples as model substances.

Development

The effect of axial dispersion on the signals of the concentration and the lightscattering detector are described by two differential equations. Tung's equation relates the normalized concentration signal e(v) (the eluogram) to the true mass distribution of retention volume $v(v_0)$ [13]

$$e(v) = \int w(v_0) \cdot D(v, v_0)\, dv_0 \tag{1}$$

(v_0 = retention volume of a certain compound of the solute, $D(v, v_0)$ = dispersion function). All functions are normalized. The response of a LALLS detector E(v) is given by [14]

$$E(v) = \int w(v_0) \cdot D(v, v_0) \cdot M(v_0)\, dv_0 \tag{2}$$

E(v) is normalized, so that $E(v)/e(v) = M_w(v)$ and $\int E(v) = \overline{M}_w$. Since the measurement of the shape of the dispersion function requires truly monodisperse polymers which are generally not available with synthetic polymers, $D(v, v_0)$ is frequently assumed to be a gaussian function with the variance σ^2. For polymers with narrow mass distribution the spreading may be assumed uniform and eq. (1) degenerates to a convolution integral. With the previous assumptions and a linear calibration curve $M(v_0) = A - B \cdot v$ the relationship

$$M_w(v) = \frac{e(v - B\sigma^2)}{e(v)} \cdot \exp(B^2\sigma^2/2) \cdot M(v). \tag{3}$$

has been derived [15]. $M_w(v)$ is the weight average molecular weight within the detector cell content at the retention volume v. Eq. (3) has been rearranged for E(v) as well as e(v) (details of development see ref. [11])

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$$E(v) = e(v - B\sigma^2) \cdot \exp(B^2\sigma^2/2) \cdot \exp(A - Bv) \qquad (4a)$$

$$e(v) = E(v + B\sigma^2) \cdot \frac{\exp(B^2\sigma^2)}{\exp(A - Bv)} \qquad (4b)$$

Both equations are equivalent. The goal of our approach is to solve either eq. (4a) or (4b) for the unknown coefficients of M(v) and σ by nonlinear regression methods.

Evaluation Procedure and Axial Dispersion Correction

The program EVAL-DISP requires the input of the measured e(v) and E(v)-functions. To reject errors from low signal domains only data which have values higher than a threshold – generally in the range of 5–20% of the height at peak maximum – are accepted. The spreading parameter σ is evaluated by fitting the light scattering function calculated from the measured eluogram according to eq. (4a) to the experimental determined scattering function E(v). The stop criterium used for the fit was $ABS(\sigma_{old} - \sigma_{new}) < 0.1$ microliter. Generally the condition was satisfied after 50–100 iterations.

With broad and narrow samples the values of A, B and σ can be calculated in an iterative procedure without knowing M(v) a priori. Starting values for the coefficients A and B were obtained from $M_w(v)$ of the broad sample PS7, since the $M_w(v)$ of a broad distribution sample is generally very close to the true M(v) as has been proved by model calculations [16]. E(v) of the narrow sample was calculated from the measured e(v) on the basis of eq. (4a) using A and B from $M_w(v)$ of PS7 and σ was varied [17] until the best fit between the calculated and the measured E(v) was obtained (Fig. 1). All the narrow samples were fitted one by one, assuming the dispersion parameter to be constant within the range of the respective eluogram. Taking an average of the obtained σ-values, E(v) of the broad sample PS7 was treated in a similar way, now optimizing A and B [18]. This procedure results in an increase of the slope of $M_w(v)$ of PS7 (Fig. 2). Step 1 was repeated with the new coefficients of the calibration line of PS7. After 3–4 repetitions of these steps $M_w(v)$ of PS7 converged to M(v) and A, B and σ became constant within the precision of measurement.

A widely used axial dispersion correction method (Ishige et al., method 2, [19]) was applied to the experimental chromatograms using the calculated dispersion parameters. The required input for this procedure includes the eluogram of the respective sample, the coefficients A and B of M(v) and σ. The stop criterium

$$| w(v)_{i+1} - w(v)_i | \, dv < 0.01$$

was used as given in ref [19].

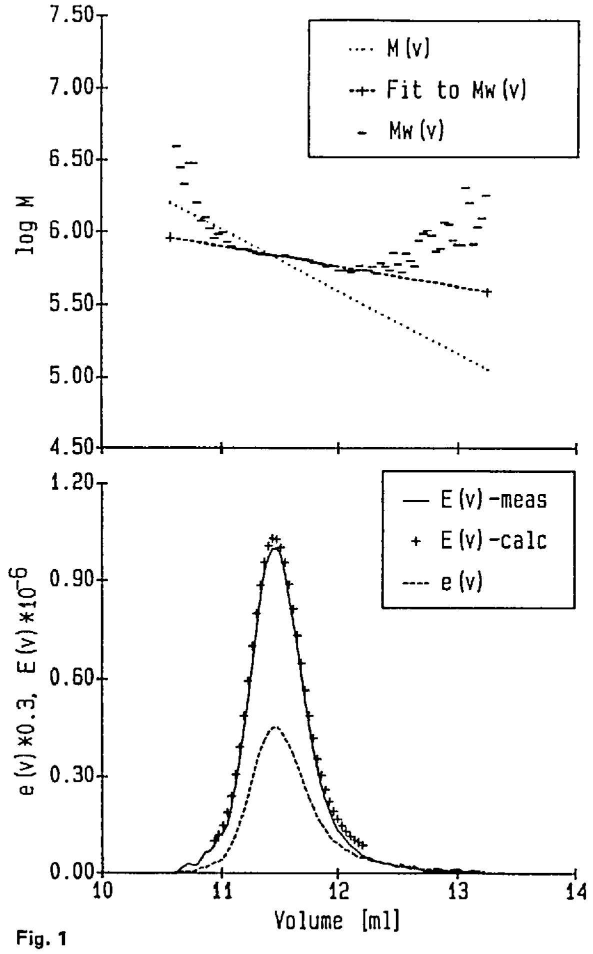

Fig. 1

Narrow sample PS4, single parameter search for σ assuming $M_w(v)$ of PS7 to be a close approximation of the true M(v) (cf. text).

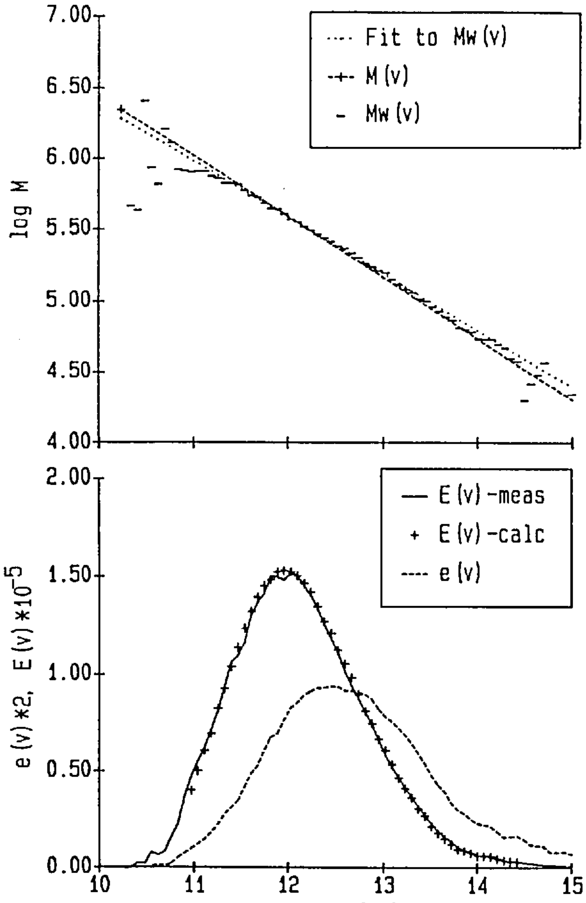

Fig. 2

Broad sample PS7, evaluation of new coefficients of M(v) using σ calculated from narrow samples.

Experimental

Materials

Samples of polystyrene standards (PS1, PS3, and PS6) were obtained from Polymer Laboratories Ltd. (Church Stretton, Shropshire, UK), PS2, PS4, PS5 from Knauer (Berlin, FRG) and PS7 from National Bureau of Standards (Washington, D.C., USA). The nominal molecular weight averages are given in Table I. Tetrahydrofurane (THF) "pro analysi", Merck (Darmstadt, FRG), was used as supplied.

Apparatus

The measurements were carried out at an ambient temperature on a high-performance SEC system consisting of the following components: a HPLC-pump Modell 510 (Waters Associates, Milford, MA, USA), an injection valve with 25μl loop (Knauer, Berlin FRG), a PL mixed gel column (Polymer Laboratories Ltd., Church Stretton, Shropshire, UK) of 60cm length and 10μm particle diameter. Two detectors were used in series. The concentration signal was monitored by a UV-photometer (model Uvicord SII, LKB, Bromma, Sweden) and a low angle laser light scattering photometer (LALLS), (model KMX-6, LDC/Milton Roy, Hasselroth, FRG), was connected in series. The flow rate (1.07ml/min) was checked by measuring the weight of the eluent. GPC data were collected by a personal computer (IBM PC-XT), equipped with a 4 channel high speed data acquisition system of 12 bit resolution. Computer programs for data acquisition and interactive processing of the SEC data including graphical selection of baseline, integration limits and calculation of the normalized signals e(v) and E(v) were developed and are to be published. The computer program for the simultaneous calibration of separation and axial dispersion including peakbroadening correction was developed on an ATARI 1040 ST (Atari Corp., Deutschland, GmbH) and implemented on a VAX 11/750 microcomputer by one of us [20].

Results and Discussion

The molecular weight averages, peak elution volumes and dispersion parameters calculated from the experimental chromatograms are listed in Table II.

Fig. 1 and 2 show the experimentally determined $M_w(v)$-functions and the fit of the calculated E(v) to the experimental one for a narrow and a broad sample respectively. With narrow standards, axial dispersion results in a serious decrease of the slope of the measured $M_w(v)$-function. Therefore this type of sample is recommended for the estimation of σ, as has been previously reported [11]. Due to the small elution volumes in modern SEC systems, some problems arise with the volume lag between the two detectors, which must be determined very carefully [21, 22]. After considerable investigation into the estimation of the volume lag we recommend a crosscorrelation of concentration and lightscattering signals from a run without columns. The volume lag was determined to be 0.07 ±0.005ml. To reject errors from oscillations in

Table I. Specifications of the polystyrene samples (SEC calibration standards).

Code	$\overline{M}_p$	$\overline{M}_w/\overline{M}_n$
PS1	68,000	1.04
PS2	110,000	1.05
PS3	170,000	1.05
PS4	390,000	1.06
PS5	670,000	–
PS6	1,100,000	1.06
PS7 (SRM 706)	$\overline{M}_w$ = 256,000	2.10

Table II. Weight average molecular weights, peak elution volumes and dispersion parameters calculated from the experimental chromatograms.

Code	$\overline{M}_w$ [g/mol]	V [ml]	σ [ml]
PS1	66,500	14.08	0.19
PS2	112,500	13.41	0.21
PS3	171,000	13.06	0.21
PS4	397,000	12.11	0.19
PS5	687,000	11.64	0.23
PS6	1,076,000	11.21	0.25

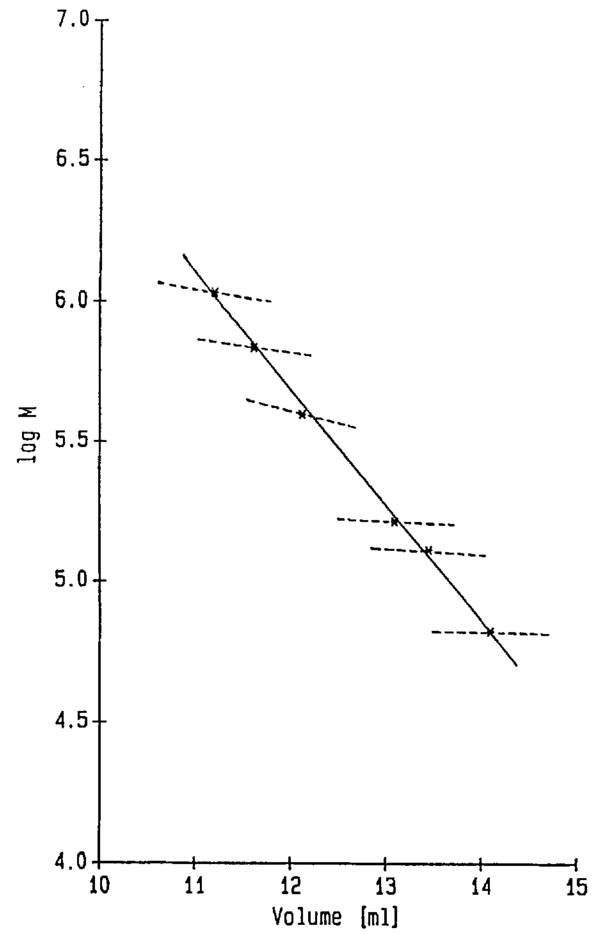

Fig. 3

Calibration function of the SEC system, * points from narrow standards calibration. – – – narrow samples $M_w(v)$ fitted by a straight line, —— M(v)-calculated by the iterative procedure (cf. text).

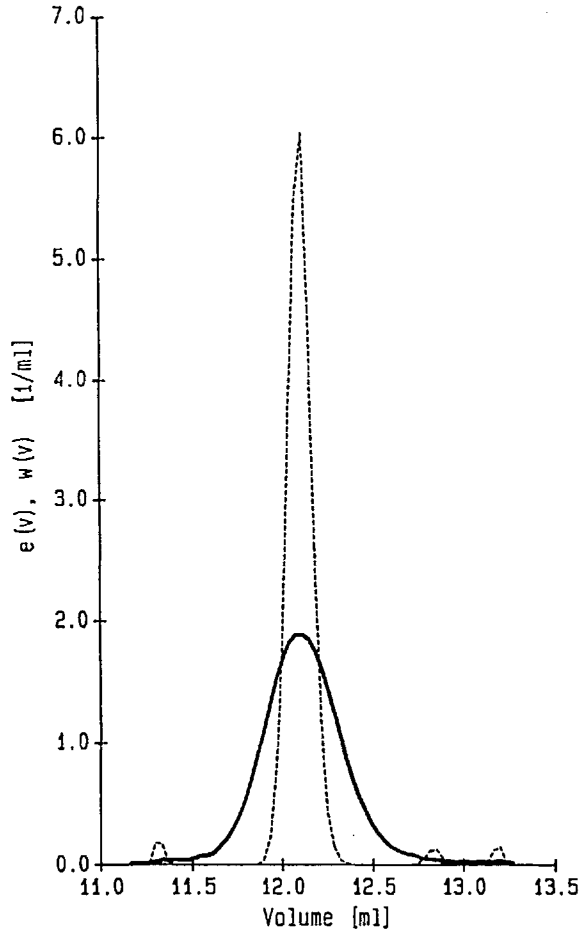

Fig. 4

Narrow sample PS4, —— measured eluogram, – – – mass distribution w(v) obtained after peakbroadening correction.

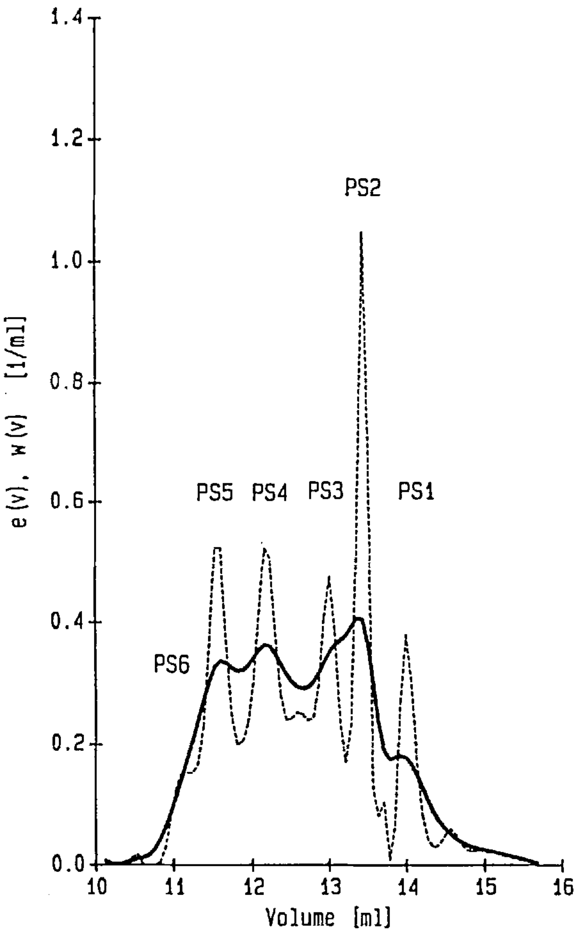

Fig. 5

Broad multimodal mixture consisting of Hostyren N7000 (64.7%), PS1 (4.6%), PS2 (9.2%), PS3 (5.3%), PS4 (3.4%), PS5 (9.1%), and PS6 (3.7%). —— measured eluogram, – – – mass distribution w(v) obtained after peakbroadening correction.

the measured $M_w(v)$-function, the calculations were performed in that range only, where the E(v) and e(v)-functions had values higher than 10% of the maximum. Fig. 3 shows the linear ranges of the measured $M_w(v)$-functions and the M(v)-function calculated by the iterative procedure. The calculated M(v)-function is in very good agreement with the calibration line obtained from narrow standards calibration. The dispersion parameter increases with increasing molecular mass (cf Table II). Similar behaviour has already been reported [8, 12, 23, 24]. A maximum of σ was not found since our samples lie within the inner part of the separation of the column. The use of eq. (4b) instead of eq. (4a) results in almost the same σ-values. The differences are within ± 1.5% of the values in Table II.

Peakbroadening correction was done according to the iterative method proposed by Ishige et al. Since this iterative reshaping method is very sensitive to noise, the eluograms have been smoothed using cubic spline functions. Fig. 4 shows e(v) of PS4 and w(v) obtained after 3 iterations. Another example of the correction procedure on a broad multimodal sample consisting of a PS injection moulding grade mixed with six narrow PS standards of the composition given in the legend to Fig. 5 is shown in that figure. The individual peaks of the six added standards are clearly developed from the eluogram, but, in addition, some ghost peaks appear due to experimental noise. The polydispersities calculated from SEC/LALLS, from calibration without correction and after dispersion correction are given in Table III. SEC/LALLS generally results in significantly lower polydispersity ratios than conventional SEC [25].

Table III. Polydispersities $(\overline{M}_w/\overline{M}_n)$ calculated from SEC/LALLS directly (A), calibration without correction (B) and after dispersion correction (C).

Code	A	B	C
PS1	1.02	1.06	1.04
PS2	1.03	1.04	1.02
PS3	1.02	1.04	1.03
PS4	1.03	1.05	1.03
PS5	1.05	1.15	1.12
PS6	1.02	1.07	1.04

The new procedure here presented allows the simultaneous calibration of separation and axial dispersion in SEC coupled with LALLS within a short time and includes generally useful devices such as smoothing and peakbroadening correction procedures.

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