An Experimentally Fast and Straightforward Method for the Direct Determination of Axial Dispersion σ_{Ad} as Occurring in Size Exclusion Chromatography

Irene Schnöll-Bitai

Institut für Physikalische Chemie, Währinger Str. 42, A-1090 Wien, Austria
E-mail: irene.schnoell-bitai@univie.ac.at

Summary: The method for the direct determination of σ_{ad} (the extent of axial dispersion) under normal working conditions makes use of the peak variance of Poisson distributions and narrow peaks, which can be determined from the points of inflection. For such distributions the experimentally measured peak variance is the sum of the pristine or true peak variance and that stemming from the broadening processes during SEC measurements. The advantage of this method is that commercially available narrow polymer standard can be used which is demonstrated for four series of polymer standards. Knowledge of σ_{ad} finally enables the application of different correction procedures.

Keywords: axial dispersion; Poisson distributions; size exclusion chromatography

Introduction

Whenever the chain length distribution (CLD) of monodisperse or narrow polymers is measured by size exclusion chromatography (SEC) the influence of axial dispersion will become obvious as a broadening effect (c.f. Figure 1). This leads to an adulteration of the determined average degrees of polymerization and polydispersities (used as a figure of merit in quasi living polymerization) as well as to slightly incorrect values for the location of the points of inflection[1-3] which are of especial interest in the PLP-SEC method[4] (pulsed laser polymerization plus analysis of the resulting molecular weight distribution). The discrepancies become obvious as soon as the k_p values (rate constant of propagation in free radical polymerization) of different working groups are compared.[5,6] In order to improve the accuracy it is necessary to have reliable information about the operative extent of axial dispersion and to develop simple correction procedures.[7]

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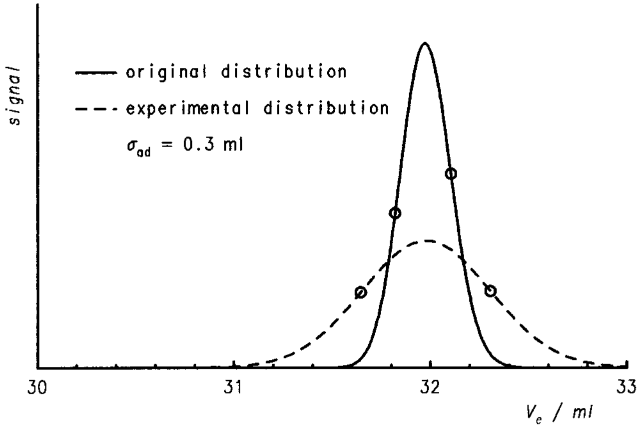

Figure 1. A Poisson distribution with i_{max}=800 was converted to a chromatographic distribution; axial dispersion was taken into account by application of the Tung equation.[15] The points of inflection are marked by circles.

Most of the already existing techniques[8-14] make use of the Tung-equation[15] together with a Gauss distribution, some of them even take into account the skewness[16,17] of peaks (essential for monodisperse samples). Besides elaborate mathematical inversion methods which do not yield satisfactory results under all conditions[18], in some cases even a change of the experimental set up is necessary in order to determine the parameters characteristic for axial dispersion. As an alternative the new method[19] works acceptably well with commercially available polymer standards which can in many cases be regarded as being close to Poisson distributions and are used for the generation of the calibration curve.

Preliminaries

The direct determination of σ_{ad} is based on the fact that the relative peak width, δ, defined as the ratio of the chain lengths i_{high} and i_{low} belonging to the points of inflection is an invariant quantity with respect to the number, n, molar mass, w, and hyper distribution, h[20] - and therfore independent of the detector type - from an experimental point of view for narrow distributions:

$$\delta_{rel,\,theory} = \frac{i_{high}}{i_{low}} = \delta_n \cong \delta_w \cong \delta_h > 1 \qquad (1)$$

As can be seen in Figure 1, axial dispersion will always increase the relative peak width of Poisson (and other narrow) distributions. Furthermore, the experimental peak variance $\sigma_{\mathrm{SEC}}^2 = \sigma_{\mathrm{peak}}^2 + \sigma_{\mathrm{ad}}^2$ is a linear combination of the (pristine or ideal) peak variance, σ^2_{peak}, and the contribution of axial dispersion, σ^2_{ad}. The experimental peak width, σ_{SEC}, correlates to the relative peak width by:[21]

$$2\sigma_{\mathrm{SEC}} = V_{\mathrm{e,low}} - V_{\mathrm{e,high}} = \frac{1}{k}\left\{\lg M_{\mathrm{high}} - \lg M_{\mathrm{low}}\right\} = \frac{1}{k}\lg\frac{i_{\mathrm{high}}}{i_{\mathrm{low}}} = \frac{1}{k}\lg\delta_{\mathrm{h}} \qquad (2)$$

when a linear calibration curve ($\lg(M) = a - kV_e$, a and k are constants, M is the molar mass, V_e elution volume) is used. Hence, knowledge of the experimental and ideal variances suffices for the direct determination of axial dispersion:[2,19,20]

$$\sigma_{\mathrm{ad}} = \sqrt{\sigma_{\mathrm{SEC}}^2 - \sigma_{\mathrm{peak}}^2} = \sqrt{\sigma_{\mathrm{SEC}}^2 - \left\{\lg\left(\delta_{\mathrm{rel,\,theory}}\right)/2k\right\}^2} \qquad (3)$$

The advantage of the relative peak width is that a generalization for different types of distributions is thus feasible and the peak width is also quantifiable.

Table 1: Theoretically derived expression for $\delta_{\mathrm{rel,\,theory}}$

type of distribution	$\delta_{\mathrm{rel,\,theory}}$	Comment
Monodisperse	1	per definition
Poisson	$\frac{i_{\max} + \sqrt{i_{\max}}}{i_{\max} - \sqrt{i_{\max}}}$	$i_{\max}$ = chain length of the peak maximum
Gauss	$\frac{i_{\max} + \sigma}{i_{\max} - \sigma}$	σ = standard deviation
distributions obtained by QUIPS RS-ME	$\frac{j(t_{\mathrm{In}} + t_{\mathrm{D}})}{(j-1)(t_{\mathrm{In}} + t_{\mathrm{D}}) + t_{\mathrm{D}}}$ j=1,2,...	t_{In}, t_{D} = duration of the initiation, dark period j = number of the peak

More complex (monomodale or multimodale) CLDs are obtained with pseudostationary (RS-ME: rotating sector polymerisation in microemulsion) or quenched instationary radical polymerization (QUIPS) in which the peaks are the weighted sum of adjacent Poisson distributions. For narrow peaks (with $\delta_{\mathrm{peak}} \leq 1.2$) the validity of the additive peak variances was proven by simulations.

Results and Discussion

Analysis of standards (ps = polystyrene, pmma = polymethyl methacrylate, p-t-bma = poly-tert-butyl methacrylate, pb = polybutadiene) yielded decreasing peak widths with increasing peak molecular weight (c.f. details in reference 19) which is in agreement with theory. In some cases (i.e. molar masses < 5 kD) was the peak width even lower than expected, but the first and the last standard used for the construction of the linear calibration curve might be considered less reliable for the extraction of σ_{ad}. An increase of the calculated $\sigma_{ad}k$ values (Equation (4)) with $\lg i_{max}$ can be seen for all systems (c.f. Table 2) but p-t-bma. This was also observed by other groups[14,22,23] but its origin is still a matter of debate. Replicate measurements gave almost identical results and can be used to exclude results adulterated by baseline problems. Finally, the concentration of the polymers has a non negligible influence on the extent of axial dispersion especially when too high a concentration is used; lower concentrations gave identical results but a worse signal to noise ratio. It should be kept in mind, that the quality of the standards is essential and that alternative ways to synthesize Poisson or narrow distributions will help to elucidate the band broadening effects.

Table 2: $\sigma_{ad}k$ values determined from the relative peak widths via Equation 4; the indices 1 and 2 signify the first and second peak, respectively

Pmma		Ps		p-t-bma		pb		ps / rs-me	
$\lg i_{max}$	$k\sigma_{ad}$	$\lg i_{max}$	$k\sigma_{ad}$	$\lg i_{max}$	$k\sigma_{ad}$	$\lg i_{max}$	$k\sigma_{ad}$	$\lg i_{max}$	$k\sigma_{ad}$
1.187	0.0488	1.621	0.0378	2.552	0.0553	2.293	0.0417	2.125$^{1)}$	0.0576
1.140	0.0269	1.623	0.0297	2.848	0.0504	2.967	0.0427	2.153$^{1)}$	0.0518
1.132	0.0307	1.900	0.0439	3.406	0.0488	3.671	0.0551	2.279$^{1)}$	0.0544
1.960	0.0554	2.199	0.0415	3.896	0.0508			2.281$^{1)}$	0.0525
1.973	0.0534	2.465	0.0436					2.409$^{1)}$	0.0569
1.993	0.0544	2.465	0.0435					2.426$^{1)}$	0.0522
2.002	0.0549	2.965	0.0595					2.437$^{2)}$	0.0441
2.916	0.0512	2.966	0.0596					2.444$^{1)}$	0.0573
2.924	0.0513	3.134	0.0497					2.458$^{2)}$	0.0456
2.999	0.0550	3.503	0.0561					2.555$^{1)}$	0.0534
3.008	0.0554	3.762	0.0537					2.563$^{2)}$	0.0500
3.011	0.0550	3.763	0.0515					2.565$^{2)}$	0.0518
3.016	0.0554	4.017	0.0583					2.579$^{1)}$	0.0528
3.510	0.0675	4.196	0.0612					2.693$^{2)}$	0.0439
3.911	0.0679	4.214	0.0656					2.704$^{2)}$	0.0489
3.915	0.0699							2.708$^{1)}$	0.0483
4.168	0.0588							2.729$^{2)}$	0.0503
4.173	0.0588							2.831$^{2)}$	0.0498
4.193	0.0585							2.858$^{2)}$	0.0502
4.204	0.0584							2.974	0.0559

Analysis of multimodale distributions of polystyrene prepared by rotating sector polymerization in microemulsion (light to dark ratio 1:5) yielded $\sigma_{ad}k$ values which are in agreement with those derived from the standards.[24] The values deduced from the first peak were slightly too high, whereas those from the second were marginally smaller. A comparable result was found when theoretical distribution curves calculated for pulsed laser polymerization were analysed.[25] In all, this means that polymers prepared by completely different polymerization mechanism (anionic and free radical polymerization) are equally well suited for the direct determination of σ_{ad} when the relative peak width of the latter peaks is smaller than 1.2 according to theoretical considerations. Polymerization in microemulsion yield extremely well resolved adjacent[26] peaks as bimolecular termination processes will take place mainly during the initiation period as the confinement of the radicals in the micelle reduces the possibility for bimolecular termination during the dark period drastically.

These results encourage reflections on a simple correction procedure that should yield the genuine relative peak width and/or location of the points of inflection for narrow peaks. Whenever σ_{ad} is known or experimentally accessible the true standard deviation , $\sigma_{\text{peak, corr.}}$, can be calculated:

$$\sigma_{\text{peak, corr.}} = \sqrt{\sigma_{\text{SEC}}^2 - \sigma_{\text{ad}}^2} \tag{4a}$$

Knowledge of the true standard deviation of a Gauss distribution allows the calculation of the true distribution.[21] The location of the points of inflection of Gauss and Poisson distributions depends on the true standard deviation as for sufficiently high values of i_{max} (> 50) the slight asymmetry of the peak of the latter distribution cannot be detected experimentally.

$$i_{\text{pi}} = i_{\text{max}} \pm \sigma \tag{5}$$

If the corrected standard deviation, $\sigma_{\text{peak, corr}}$, is inserted in Equation (5) it is possible to obtain the corrected values for the points of inflection. If the peak is unsymmetrical the observable shift between the genuine values and the experimental values is given by:

$$\text{shift} = \sigma_{\text{SEC}} - \sqrt{\sigma_{\text{SEC}}^2 - \sigma_{\text{ad}}^2} \tag{6}$$

$$i_{\text{pi, corr.}} = i_{\text{pi}} \pm \text{shift} \tag{7}$$

A simple summation or subtraction will give the genuine points of inflection and comparison with theoretical curves is possible. Furthermore, as Schweer at al.[3] already suggested, a point-wise comparison of distributions measured with SEC and MALDI-TOF is at least within reach. The new approach is not exclusively restricted to Poisson distributions but can be carried out for all narrow distributions with $\delta \leq 1.2$; no assumptions need to be made concerning the nature of the distribution curves.

The results presented thus far show that two to three different regions can be distinguished in the behaviour of σ_{ad}. For intermediate molar masses the change in σ_{ad} values is very small and they might be regarded as almost constant. The use of an averaged constant σ_{ad} value seems to be justified. For low molar masses σ_{ad} values are sometimes far smaller whereas, at higher molar masses a maximum in the σ_{ad} values can be observed in some cases. As it is not yet clear whether this is caused by the standards deviating from the Poisson distribution the individual value for the respective molecular weight should be used at the moment. Preparation of polymers with approximately the same peak molar masses by microemulsion polymerization will help to verify this behaviour.[27] Furthermore, comparison with results obtained with other methods (e.g. SEC-measurements using light scattering detectors) is aimed at.[27]

Conclusion

The method for the direct determination of axial dispersion is highly efficient as was demonstrated for different sets of narrow polymer standards and more complex distributions (obtained by pseudostationary polymerization). For practical application the generation of the calibration curve and the determination of axial dispersion are achieved by measurement of narrow standards.

Knowledge of σ_{ad} is a prerequisite for the application of different correction procedures. Simple corrections enable the calculation of corrected points of inflection which are of especial interest for the extraction of kinetic information from experimentally measured distribution curves.

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