

Comparison of Size Distribution Data from Dynamic Light Scattering and Size-Exclusion Chromatography*

L. MRKVIČKOVÁ,^{1,†} B. PORSCHE,¹ and L.-O. SUNDELÖF²

¹Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, 162 06 Prague, Czech Republic;

²Physical Pharmaceutical Chemistry, Uppsala University, BMC, Box 574, S-751 23 Uppsala, Sweden

SYNOPSIS

The intensity-defined distribution functions of hydrodynamic radii of equivalent spheres, R_h , obtained from dynamic light-scattering experiments using the CONTIN procedure via the Stokes formula were compared with distributions of gyration radii, R_g , determined by size-exclusion chromatography. The number-, weight-, and intensity- (z)-defined R_g distributions accessible from size-exclusion chromatography experiments were calculated using the Flory–Fox relation. Reliable ratios of average radii, R_g/R_h , for linear polystyrenes having narrow, broad, or bimodal molecular weight distributions were obtained in toluene. Care should be taken to utilize properly averaged experimental quantities. For instance, the CONTIN DLS data evaluation procedure yields the z -average of the inverse of the hydrodynamic radius, $\langle 1/R_h \rangle_z^{-1}$. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Dynamic light scattering (DLS) has become a technique¹ often used to determine size distributions of polymers and dispersions. A translational diffusion coefficient, D , is measured by DLS and a hydrodynamic radius of the coil, R_h , (i.e., the radius of an equivalent sphere having the same D) is inversely related to D through the Stokes–Einstein formula. The scattered light intensity from a very dilute solution of macromolecular particles of R_h is proportional to the number of these particles per volume unit and to the square of their molecular weight, M , if the Mie effect is neglected. In polydisperse systems, each particle size contributes by its z -fraction to the total measured intensity of scattered light. A z -average diffusion coefficient² is obtained in this case; the intensity-defined distribution of D and the corresponding distribution³ of R_h is obtained from a multiexponential autocorrelation function by the inverse Laplace transformation.⁴

Hence, the inverse z -average R_H (cf. Ref. 5) is usually obtained from DLS data. In cases when an unambiguous relation between D and M exists as a secondary calibration, the distribution of M for a polymer sample is also accessible from a DLS experiment.^{6,7}

High-performance size-exclusion chromatography (SEC) is nowadays a routine technique for determination of the molecular weight distribution of macromolecules. The hydrodynamic coil volume governs the SEC separation⁸ proportionally to the product $M[\eta]$, where $[\eta]$ is the intrinsic viscosity. $M[\eta]$ is known as a universal calibration parameter.⁸ The weight distribution of the radii of gyration, R_g (static coil dimension, determined, e.g., from the angular dissymmetry in an integral light-scattering experiment), can be calculated if the existing universal calibration of an SEC system is transformed to R_g calibration via the Flory–Fox relation.⁹ The use of low-angle light-scattering detection (LALLS) on line together with refractometric (RI) detection simplifies an “intensity weighting” of SEC data and the comparison of proper averages of R_g and R_h . A dimensionless quantity $\rho = R_G/R_H$ [cf. eq. (8)] has been suggested¹⁰ as a measure of polymer conformation in terms of branching density and/or intrinsic flexibility of a polymer chain.

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[†] To whom correspondence should be addressed.

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Although DLS is a nonseparation technique, it is often claimed as being competitive^{6,7} or even superior¹¹ to SEC for determination of molecular weight and size distributions. On the other hand, DLS has also been claimed to be not very promising² in polydispersity analysis. The "ill-conditioning" of the inverse Laplace transformation,¹² low resolution of sums of exponentials,¹³ and a great sensitivity to base-line errors¹⁴ seem to be the weakest points of the DLS technique. In view of such widely differing statements, this work has a twofold aim: (1) to provide a comparison of particle-size distributions as obtained by DLS experiments with corresponding distributions calculated from SEC experiments with on-line LALLS and RI detection, and (2) to provide a comparison of resolution by both techniques in the case of narrow, broad, and bimodal polystyrene samples.

EXPERIMENTAL

Materials

Well-characterized polystyrene standards (PS1 and PS2 with $\langle M \rangle_w = 4.98 \times 10^5$, $\langle M \rangle_w / \langle M \rangle_n \sim 1.1$ and $\langle M \rangle_w = 1.8 \times 10^6$, $\langle M \rangle_w / \langle M \rangle_n \sim 1.3$, respectively, from Pressure Chemical Co.) and commercial polydisperse polystyrene (PS 3 with $\langle M \rangle_w = 3 \times 10^5$, $\langle M \rangle_w / \langle M \rangle_n \sim 5.5$ from BASF Ludwigshafen, Germany) were used.

DLS Experiment

An Inova 70-series 4W argon-ion laser (Coherent Laser Division, Palo Alto, CA) tuned to 514.5 nm was focused into a precision scattering cell (Hellma GmbH, Mullheim Baden, Germany). The optical components were mounted on a massive steel bench (Newport Research Corp., Fountain Valley, CA). The scattered photon flow was monitored at the angle 90° using a commercially available Brookhaven photomultiplier unit and a 128 channel BI-8000 digital correlator (Brookhaven Instruments Corp., Holtsville, AL). The Brookhaven particle distribution software package¹⁵ contains five of the most common size-analysis procedures; the CONTIN (including Mie correction) procedure was used here. For the DLS measurements, the concentration of solutions of PS in toluene was selected in the range $(5-10) \times 10^{-4} \text{ g cm}^{-3}$.

SEC Experiment

The chromatographic equipment consisted of a pump (HPP 5001), an injection valve LC-30 with a 100 μL loop, a RIDK-102 differential refractometer (Laboratory Instruments, Prague, Czech Republic), and a LALLS detector (KMX-6, Chromatix, Sunnyvale, CA), both detectors being connected through an A/D convertor 2308 (Black*Star Ltd., St. Ives, Huntingdon, England) to an IBM compatible computer with a printout facility. The software (Copyright M. Netopilík, Inst. of Macromolecular Chemistry, Prague, Czech Republic) allows on-line data accumulation as well as calculation of M or R_g distributions and averages of M or R_g . A commercial stainless-steel column HP (7.5/600 mm) packed with PLGel 10 μm MIX (Polymer Laboratories, Inc., Shropshire, England) was used with toluene as the eluent; sample concentrations were selected in the range $(1-3) \times 10^{-3} \text{ g cm}^{-3}$. In the universal calibration,⁸ the Mark-Houwink-Sakurada equation for PS in toluene¹⁶ [eq. (1)] was used:

$$[\eta] = 7.5 \times 10^{-3} M^{0.75} \quad (1)$$

Under given experimental conditions of SEC separation, the axial dispersion was negligible; therefore, the chromatographic curves were not corrected.

RESULTS AND DISCUSSION

Weight-defined molecular weight distributions were transformed to the corresponding distributions of radii of gyration using the relation

$$R_g = \left(\frac{1}{6}\right)^{1/2} ([\eta]M/\phi_0)^{1/3} \quad (2)$$

where $\phi = 2.86 \times 10^{23}$ is the Flory universal constant. Number- and z -defined R_g distributions were then obtained from the corresponding concentration and molecular weight profiles, the number fraction N_i and the z -fraction z_i being defined as $N_i = (c_i/M_i) / \sum c_i/M_i$ and $z_i = (c_i M_i) / \sum c_i M_i$. The resulting number-, weight-, and z -defined R_g distributions of the narrow polystyrene standard PS1 as plotted in Figure 1 illustrate a moderate effect caused by different weighting when the z -defined R_g distribution is compared with the z -defined R_h distribution as calculated by the CONTIN procedure. The ratio of $\langle R_g^2 \rangle_z^{1/2} / R_H = 1.3$ [cf. eqs. (8) and (9)] was shown experimentally to be approximately constant for several polymers (including PS) in different solvents.¹⁷ The observed shift of the R_h distribution

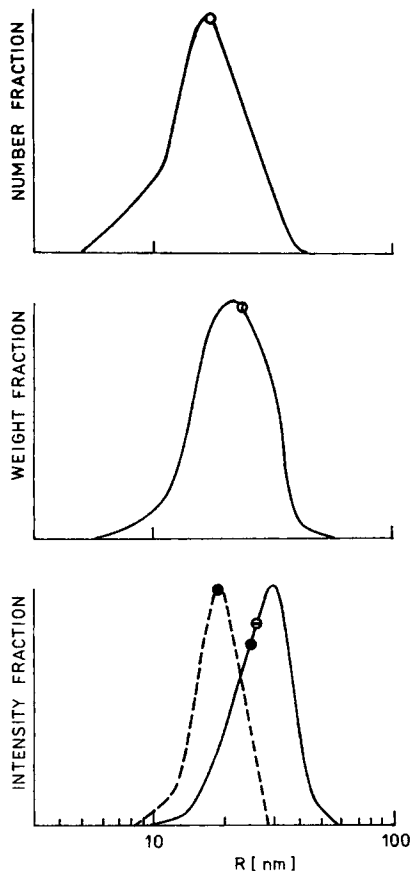


Figure 1 Number-, weight-, and z -defined size distributions of narrow PS1: (—) R_g ; (---) R_h ; (○) $\langle R \rangle_n$; (⊙) $\langle R \rangle_w$; (●) $\langle 1/R \rangle_z^{-1}$; (⊖) $\langle R \rangle_z$.

against the R_g distribution matches well this value with the exception of lowest sizes, where DLS becomes insensitive¹⁸; nevertheless, the agreement between DLS and SEC must be considered quite satisfactory.

The same comparison of distributions in the case of a broad polystyrene sample PS3 (see Fig. 2) illustrates a loss of DLS sensitivity at low sizes due to intensity weighing. Almost no molecules having a diameter smaller than $\langle R_g \rangle_n$ are "seen," although their weight fraction is by no means negligible. Also, the shift between R_h and R_g intensity distributions increased (from 1.36 to 1.63); a known overcompensation for dust⁴ combined with the effect of experimental noise on the inverse Laplace transformation is the most probable explanation. This result confirms the observed shortcoming⁷ of the DLS technique that it normally gives narrower distributions of broadly distributed samples than the real distributions are.

R_g and R_h distributions (defined as above) and obtained for mixtures of the polystyrene samples

PS1 and PS2 are shown for the weight compositions 4 : 1 and 18 : 1 in Figures 3 and 4, respectively. A similar behavior is observed for the broad sample (Fig. 2) concerning the shift and narrowing of the DLS R_h distribution. The resolution of the two peaks at 4 : 1 composition (adjusted to give approximately equal scattering intensities of both components) could be obtained only when the size range of the CONTIN calculation procedure was set closely to the curve evaluated, i.e., some a priori knowledge^{4,6} had to be used. The two peaks at composition 18 : 1 could not be resolved at all by DLS. The ratio of molecular weights of samples PS2 and PS1 is 3.6 (their R_h ratio is 1.85), PS2 being broader than PS1. Hence, the resolution limit of the Brookhaven CONTIN version is obtained as $M_2/M_1 > 3.6$, when both components give roughly the same scattering power, in agreement with the value $M_2/M_1 > 3.5$ found recently⁶ in a similar case. On the other hand, an SEC experiment always resolves both components (Figs. 3 and 4) and no a priori information— with the exception of a general knowledge of the

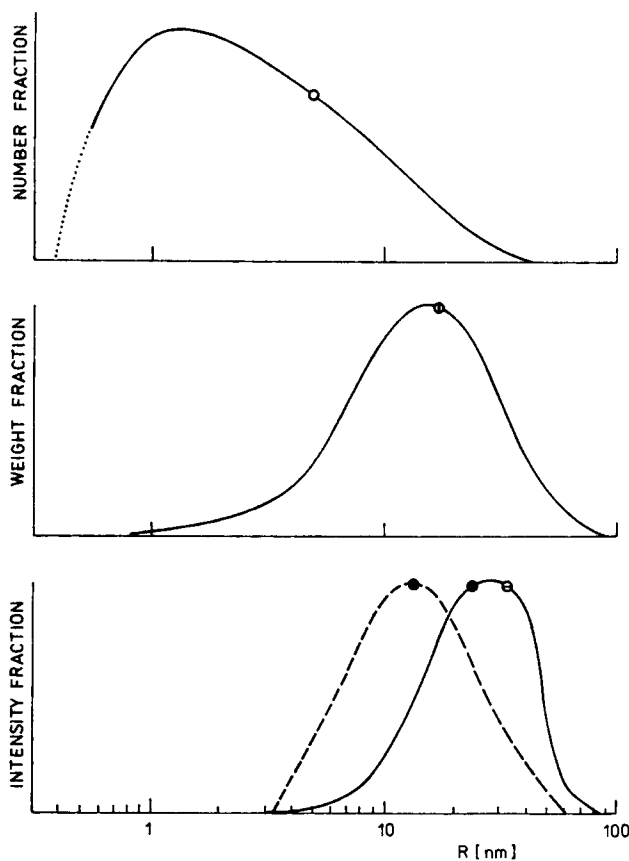


Figure 2 Number-, weight-, and z -defined size distributions of very broad PS3; curves and points are denoted as in Figure 1.

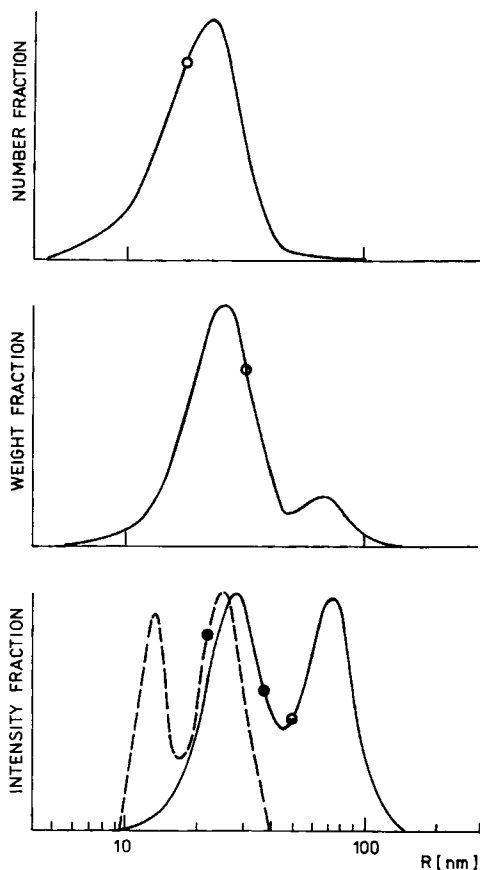


Figure 3 Number-, weight-, and z -defined size distributions of a mixture of PS1 and PS2 with 4 : 1 relative concentrations; curves and points are denoted as in Figure 1.

sample behavior in an SEC—is needed. Moreover, the SEC resolution can easily be increased to get base-line separation of the two peaks, if one more SEC column is added to the system.

The DLS-average hydrodynamic diameter is defined^{5,19} as the inverse z -average:

$$\left(\sum w_i M_i / R_{h,i} \right) / \sum w_i M_i = \langle 1/R_h \rangle_z = 1/R_H \quad (3)$$

and, except for a “monodisperse” polymer, $\langle R_h \rangle_z = \langle 1/R_h \rangle_z^{-1}$. The relative width of the z -distribution of the diffusion coefficients (z -average normalized variance), w_h , in terms of R_h ,

$$w_h = (\langle (1/R_h)^2 \rangle_z - \langle 1/R_h \rangle_z^2) / \langle 1/R_h \rangle_z^2 \quad (4)$$

$$\langle (1/R_h)^2 \rangle_z = \left(\sum w_i M_i / R_{h,i}^2 \right) / \sum w_i M_i \quad (4a)$$

is also obtained from DLS data through the use of cumulants and inverse Laplace transform techniques.⁴

Having access to the R_g distributions, the conventional statistical averages could be calculated:

$$\langle R_g \rangle_n = \sum N_i R_{g,i} / \sum N_i \quad (5)$$

$$\langle R_g \rangle_w = \sum w_i R_{g,i} / \sum w_i \quad (6)$$

$$\langle R_g \rangle_z = \sum w_i M_i R_{g,i} / \sum w_i M_i \quad (7)$$

By replacing R_h by R_g in eqs. (3) and (4), the analogous R_g and w_g values were also evaluated. Finally, the well-known structure-sensitive parameter¹⁰

$$\rho = \langle R_g^2 \rangle_z^{1/2} / R_H \quad (8)$$

which combines static LS and DLS data, was calculated, $\langle R_g^2 \rangle_z$ being defined²⁰ as

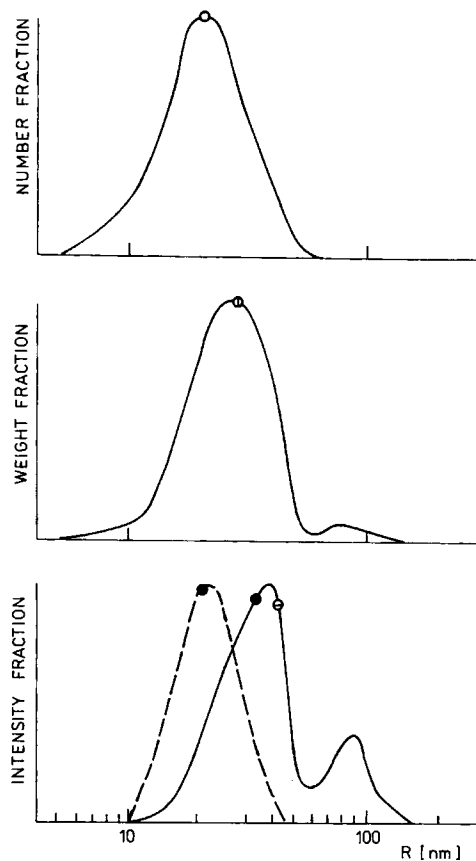


Figure 4 Number-, weight-, and z -defined size distributions of a mixture of PS1 and PS2 with 18 : 1 relative concentrations; curves and points are denoted as in Figure 1.

$$\langle R_g^2 \rangle_z = \sum w_i M_i R_{g,i}^2 / \sum w_i M_i = R_G^2 \quad (9)$$

All averages, polydispersity criteria, and values of the parameter ρ are summarized in Table I. From the examination of Table I, it follows that $\langle \langle R_g \rangle_n \rangle < \langle R_g \rangle_w < \langle 1/R_g \rangle_z^{-1} < \langle R_g \rangle_z < \langle R_g^2 \rangle_z^{1/2}$. The ratio $\langle R_g \rangle_w / \langle R_g \rangle_n$ is equal to $(\langle M \rangle_w / \langle M \rangle_n)^{0.5+\vartheta}$, where $\vartheta = (2a - 1)/6$ and a is the exponent in eq. (1). $\langle M \rangle_w / \langle M \rangle_n$ is the conventional polydispersity index. The widths w_h of the size distributions as given by eq. (4) are consistently lower than the corresponding w_g values as calculated from SEC data. The difference is higher the wider is the distribution. In the case when the CONTIN procedure failed to resolve two peaks (sample 5), a drop in w_h to the value obtained for the narrowest distribution (sample 1) was found. Obviously, the same explanation of the SEC and DLS distributions applies, again illustrating a weakness of DLS polydispersity determination when compared to the SEC technique.

The calculated conventional ρ parameter¹⁰ [eq. (8), the second to last line in Table I] has been shown to depend not only on the polymer structure, but also on its polydispersity.¹⁰ In the case of broad distributions, the difference may be quite pronounced as follows from a comparison of values of ρ and ρ' calculated from proper averages, i.e.,

$$\rho' = \sum \langle 1/R_g \rangle_z^{-1} / R_H \quad (10)$$

to eliminate any polydispersity effect. In the case of a narrow sample (column 1 in Table I), the difference is small and ρ' approaches the experimental value ca. 1.3 (cf. Ref. 17). For the very broad sample (col-

umn 3) and for a bimodal mixture (column 4), the ρ value increases to 2.46 and 2.62, respectively. When the effect of polydispersity is eliminated, ρ' values do not show any essential deviation from the theoretical values¹⁷ of 1.5 (theta-solvent) or 1.86 (good solvent). The question whether such a theoretical treatment may need to be refined is still open for discussion.¹⁷ On the other hand, there is no theoretical support for ρ being equal to 1.3. Moreover, it must be remembered that the above-mentioned feature of the DLS experiment, a shift of R_H to lower values, should lead to higher values of ρ . Anyway, our experiments clearly illustrate the general importance of proper averaging before making conclusions concerning the structure of broad and/or bimodal polymer systems, based on static and dynamic light scattering.

CONCLUSION

The SEC technique is superior to DLS insofar as a high molecular-size distribution resolution is the main aim. The SEC method also gives more direct results. Current state-of-the-art SEC columns have pore sizes of packings up to 400 nm. In the case of particles larger than this size, DLS should be selected. Under the circumstances when proper SEC (no interaction) conditions cannot be found, for instance, in systems containing water-soluble amphiphilic substances, DLS will be the technique of choice. When DLS is applied, care must be taken to use appropriate averages of measured quantities, especially in the case of broad-size distributions.

Table I Average Dimensions, Polydispersity Indices, and ρ Parameter Polystyrene Samples Determined in Toluene at 25°C

Parameter	Sample				
	1 [PS1]	2 [PS2]	3 [PS3]	4 [PS1 : PS2 = 4 : 1]	5 [PS1 : PS2 = 18 : 1]
$\langle R_g \rangle_n$	18	30	6	19.5	20
$\langle R_g \rangle_w$	23.5	53.4	17.3	31.6	29.1
$\langle R_g \rangle_w / \langle R_g \rangle_n$	1.3	1.8	2.9	1.6	1.5
$\langle 1/R_g \rangle_z^{-1}$	25.7	61	23.6	37.9	33.8
$\langle 1/R_h \rangle_z^{-1}$	18.9	35	14.5	21.5	25
$\langle R_g^2 \rangle_z$	26.7	69.4	33.7	50.5	42.8
$\langle R_g^2 \rangle_z^{1/2}$	27.6	72.6	35.65	56.4	49.6
w_g	0.14	0.216	0.539	0.32	0.23
w_h	0.074	0.113	0.189	0.123	0.074
ρ	1.46	2.07	2.46	2.62	2.42
ρ'	1.36	1.74	1.63	1.76	1.61

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