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Determination of Molecular Weight Distributions from Frequency-Analyzing (Dynamic) Light Scattering†

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ABSTRACT: A general method for the determination of molecular weight distributions from frequency-analyzing (=dynamic) light scattering is described. The measured autocorrelation functions are used to construct a curve which is not influenced by interaction between different particles and intramolecular interferences. This is done by a special extrapolation method. The method is applied to polystyrene samples with molecular weights $\bar{M}_w$ from 2.5×10^5 to 5×10^6 and different polydispersities, using toluene as solvent. The investigated system shows pronounced concentration and intramolecular interferences (scattering function and intramolecular chain motions) which are successfully eliminated. The molecular weights and polydispersities obtained by the new procedure are in good agreement with those from independent measurements.

For frequency-analyzing light scattering (FAN LS)[23] of polymers in solution a number of methods have been proposed[1-14] which permit characterization of the molecular weight distribution (MWD) of the scattering particles. Most of these methods are quite special and somewhat restricted in application. Often concentration effects are neglected, though this is not always permissible. We describe here a method which eliminates all dependencies on scattering parameter and concentration.

We introduce the autocorrelation function (ACF) in the usual form as a function of the scattering parameter $k = (4\pi n/\lambda_0) \sin(\vartheta/2)$ and the correlation time t, where λ_0, n, and ϑ have their usual meanings. The influence of concentration c is taken into account by regarding the ACF as a function of $h(c)$, which must strictly increase with c, be differentiable, and vanish for $c \to 0$; then the inverse function h^{-1} with $h^{-1}(h(c)) = c$ exists. The concentration effects, and with them the correlation of different particles, are eliminated by extrapolating the measured ACF to $h(c) \to 0$.

To provide a generally applicable procedure, it is necessary to construct from the measured autocorrelation curves a function such that the effects of intramolecular motions and of the scattering function are eliminated, while at the same time full information on the MWD is retained.

For the theoretical derivations we thus restrict ourselves to small scattering parameters k for which only translational diffusion influences the scattered light. In this case[15-18] the expression for the autocorrelation function C_{ex}^{hm} of the homodyne technique is independent of the shape of the scattering particles and is given by

$$\lim_{h(c)\to 0} C_{ex}^{hm}[k, t, h(c)] = A\left\{\bar{M}_w^{-1} \int_0^\infty Mf(M)P(k, M)e^{-k^2 D_0(M)t}\, dM\right\}^2 + B \quad (1)$$

where $f(M)$ is the weight-fraction MWD of the scattering molecules with molecular weight M, $P(k, M)$ is the scattering function, and $D_0(M)$ reflects the M dependence of the diffusion coefficient at vanishing concentration. A and B are linear parameters still to be specified. Application of eq 1 requires that values of k be sufficiently small; however, in general, it is not known just how small they have to be.

Theory

In order to solve the problem of using sufficiently small scattering parameters k, we replace the generalized coordinates $\{k, t\}$ of the optical system in the ACF by

$$\{g(k), t'\} := \{g(k), k^2 t\} \quad (2)$$

For $g(k)$ the same conditions as for $h(c)$ have to be fulfilled. With the generalized or optical time t' eq 1 then becomes

$$\lim_{h(c)\to 0} C_{ex}^{hm}[g(k), t', h(c)] = A\left\{\bar{M}_w^{-1} \int_0^\infty Mf(M)P\{g^{-1}[g(k)], M\}e^{-D_0(M)t'}\, dM\right\}^2 + B \quad (3)$$

For finite scattering parameters, eq 1 and 3 contain the same information about the scattering system and are thus equivalent. But when the identity

† Dedicated to Professor G. V. Schulz on the occasion of his 75th birthday.

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$$\lim_{k\to 0} P\{k, M\} = \lim_{g(k)\to 0} P\{g^{-1}[g(k)], M\} = 1 \quad (4)$$

is used, eq 1 gives for all real times in the limit of vanishing k

$$\lim_{\substack{h(c)\to 0 \\ k\to 0}} \{C_{ex}^{hm}[k, t, h(c)] - B/A\} = 1 \quad (5)$$

Thus, when the calculation is based on eq 1, the elimination of the influence of the shape of the particles leads to elimination of all information concerning the dynamics of the scattering system and the MWD.

In contrast, the limiting value of eq 3 for $g(k) \to 0$ leads to

$$\lim_{\substack{h(c)\to 0 \\ g(k)\to 0}} C_{ex}^{hm}[g(k), t', h(c)] = A\left\{\bar{M}_w^{-1}\int_0^\infty Mf(M)e^{-D_0(M)t'}\,dM\right\}^2 + B \quad (6)$$

This mathematically constructed function decreases monotonically with t' and for all values of M is described by the translational diffusion, while the scattering function is eliminated.

It is observed that eq 6 may also be applied to coiled molecules in solvents which are not at a Θ temperature. Only the function $D_0(M)$ has to be known in order to determine $f(M)$. In the following, eq 6 will be called the "basic equation".

Experiments

Six thermally polymerized styrene samples[19] and the NBS standard polystyrene NBS 706 were tested. The solvent was toluene at 20 °C. The polymers have high molecular weights and/or broad molecular weight distributions. Therefore the selected conditions were quite severe and complicated for the test because the exact scattering function $P(k, M)$ is not known and the second virial coefficients exhibit pronounced concentration effects. The wavelength λ_0 = 514.5 nm was used. The scattered light was registered by a Malvern correlator at 96 points.

In order to evaluate an ACF from the measured curves which could be described by the basic equation (6), the following conditions were fulfilled:

(a) For all concentrations c_j (j = 1, ..., 5) and all angles ϑ_i (i = 1, ...) the real-time difference Δt for two neighboring points of the measured ACF was chosen so as to result in a constant $\Delta t'$ of the optical-time difference

$$\Delta t' = k^2\Delta t = \text{const} \quad (7)$$

This means that in the experiment only one real correlation time difference may be associated with a given angle. For each sample, $\Delta t'$ was selected to cover the essential part of the extrapolated ACF.

In order to minimize the influence of statistical fluctuations the ACFs were smoothed by spline polynomials by applying a method described in ref 22. Only these values, resulting in continuous curves, were used for further evaluations.

(b) The parameter A and, for many correlator types, also the parameter B depend on experimental conditions (intensity, illuminated detector area, etc.). Thus A and B for each measurement are different and unknown.

Therefore all the functions C_{ex}^{hm} as obtained from spline polynomials were subject to linear transformations so that the resulting ACFs C_s^{hm} obey the following condition:

$$C_s^{hm}[g(k), t_1', h(c)] = 2 \quad \text{for } t' = t_1' = 6\Delta t'$$

$$C_s^{hm}[g(k), t_2', h(c)] = 1 \quad \text{for } t' = t_2' \to \infty \quad (8)$$

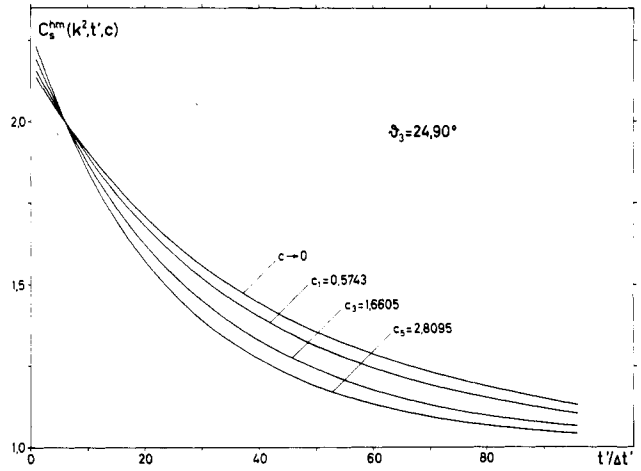

Figure 1. Autocorrelation functions C_s^{hm} of PST ThA60 at ϑ_3 = 24.90° at three finite concentrations and zero concentration as a function of $t'/\Delta t'$.

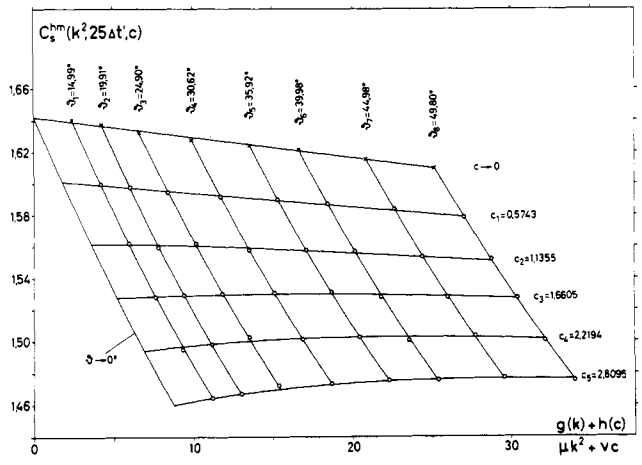

Figure 2. Extrapolation diagram of C_s^{hm} of PST ThA60 for t' = 25$\Delta t'$ as a function of $g(k) + h(c) = \mu k^2 + \nu c$.

For the evaluations reported here the ACFs are regarded as functions of $h(c) = \nu c$ and $g(k) = \mu k^2$, where ν and μ are constants.

When conditions a and b were satisfied, the functions C_s^{hm} could be extrapolated: at first for each angle ϑ_i to $h(c) = \nu c \to 0$ and then at zero concentration to $g(k) = \mu k^2 \to 0$. Both extrapolations were done numerically.

Figure 1 shows C_s^{hm} functions evaluated from the measuring curves of the sample ThA60 with $\bar{M}_w = 1.62 \times 10^6$ and a broad MWD ($\bar{M}_w/\bar{M}_n \simeq 2$) for the angle ϑ_3 = 24.90° at several concentrations. Concentrations in all figures are in 10^{-3} g/cm^{-3}. In this case the optical-time difference used according to eq 7 was $\Delta t' = 1.413 \times 10^5$ s cm^{-2}. The uppermost curve (for $t' = 6\Delta t'$) results from extrapolation $h(c) = \nu c \to 0$.

This extrapolation is done by a special numerical procedure which is illustrated by the diagrams given in Figures 2 and 3 for the optical times $t' = 25\Delta t'$ and $t' = 77\Delta t'$, respectively. The figures clearly show that the numerical extrapolation $h(c) \to 0$ of the ACF values at the given t' values (circles in the diagrams) to zero concentration can be and was done here by a polynomial of second order for a fixed angle ϑ_i. The values thus obtained (crosses in the figures) were extrapolated to $g(k) = \mu k^2 \to 0$ by linear regression. In Figures 2 and 3 the extrapolation to $g(k) \to 0$ for finite and constant concentrations is also given; since it was not used in the calculations, this extrapolation was done only graphically.

Table I
Molecular Weights $\bar{M}_w$ and Polydispersities $U = \bar{M}_w/\bar{M}_n - 1$ Determined by Frequency-Analyzing (=Dynamic) Light Scattering (FAN LS) as Compared with Results from Frequency-Averaging (=Classical) Light Scattering (FAV LS), GPC,[19] and Other Methods

	FAN LS		FAV LS	GPC		other		
sample	$\bar{M}_w/10^6$	U	$\bar{M}_w/10^6$	$\bar{M}_w/10^6$	U	$\bar{M}_w/10^6$	U	technique
NBS 706	0.284	1.23	0.278	0.259	1.28	0.258		FAV LS[a]
						0.288	1.10	SE[a]
ThA60	1.62	1.01	1.59	1.69	0.98	1.64	0.91	FAV LS[b]
ThA20	4.52	0.98	4.43	4.79	1.33			
ThA60 6F10	0.881	0.33	0.919	0.833	0.29			
ThA60 5F11	1.37	0.25	1.39	1.44	0.22			
ThA60 5F7	2.00	0.27	1.94	2.14	0.27			

[a] NBS data. [b] References 20 and 21.

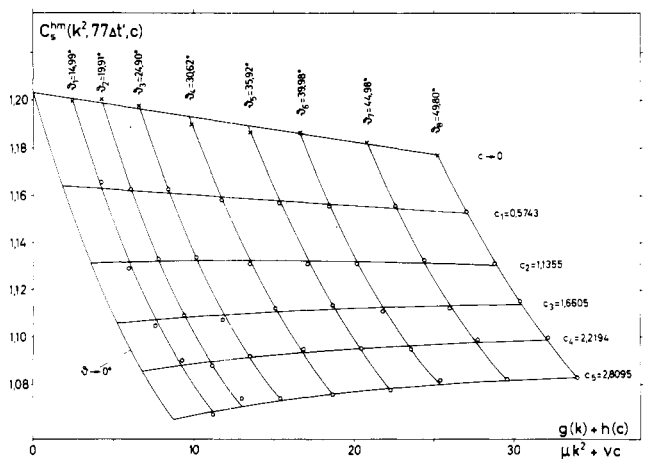

Figure 3. Extrapolation diagram of C_s^{hm} of PST ThA60 for $t' = 77\Delta t'$ as a function of $g(k) + h(c) = \mu k^2 + \nu c$.

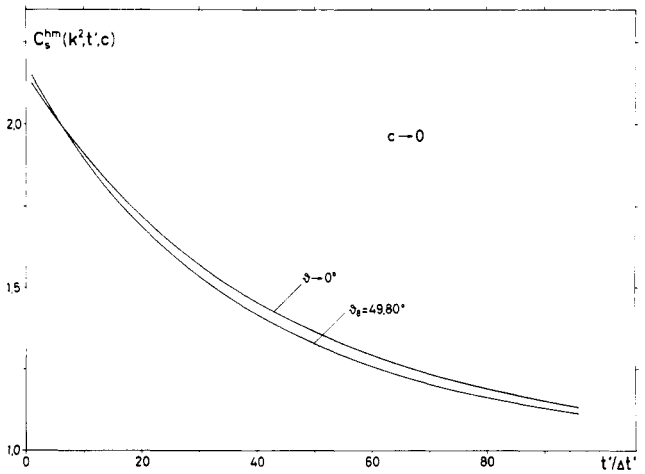

Figure 4. Extrapolated curves of C_s^{hm} of PST ThA60 at $h(c) = \nu c \rightarrow 0$ for $\vartheta_8 = 49.80°$ and for $g(k) \rightarrow 0$ as a function of $t'/\Delta t'$.

The numerically obtained ACF for $g(k) \rightarrow 0$ (or equivalently $\vartheta \rightarrow 0°$) which results from the extrapolations at many t' is shown in Figure 4 together with the curve for $c \rightarrow 0$ and $\vartheta_8 \simeq 50°$; all C_s^{hm} for $0° < \vartheta < 50°$ lie between the curves drawn.

The same procedure was used for further polystyrene samples with $0.25 \times 10^6 < \bar{M}_w < 5 \times 10^6$ and various polydispersities $\bar{M}_w/\bar{M}_n - 1$ for which other values of $\Delta t'$ had to be chosen. For the samples with higher molecular weights the influence of intramolecular motions on the measured ACF is more pronounced than in the given example. Thus the diagrams are more complicated than in Figures 2 and 3. Our extrapolation technique nevertheless eliminated this effect.

All $h(c) \rightarrow 0$ and $g(k) \rightarrow 0$ extrapolated ACFs can be represented by the basic equation (6). The necessary numerical approximation of the curves was based on a Schulz-Flory MWD substituted for $f(M)$ in eq 6. This distribution type is expected for the broad samples according to the (radical) polymerization process of their production. For the narrow samples the same distribution type could always be used; in this case the type only very weakly influences the ACF.

For the molecular weight dependence of the diffusion coefficient for polystyrene in toluene at 20 °C

$$D_0(M) = (3.32 \times 10^{-4})M^{-0.574} \text{ (cm}^2 \text{ s}^{-1}) \quad (9)$$

has been taken.

Table I shows the results of the evaluation together with comparative measurements by gel chromatography (GPC) and by frequency-averaging light scattering (FAV LS), i.e., classical LS. Both $\bar{M}_w$ and the polydispersity parameter $U = \bar{M}_w/\bar{M}_n - 1$ are given. Evidently the method results in acceptable $\bar{M}_w$ and U also for high molecular weights; with the exception of sample ThA20 the agreement is good.

Conclusion

As can be seen from the diagrams in Figures 2 and 3 the angular dependencies of the ACFs for finite concentrations are different from those after extrapolation to $h(c) \rightarrow 0$. The differences become more pronounced with increasing optical time and increasing concentration. This behavior could indicate problems for the direct evaluation of the MWD, as reported very recently,[9-12] where additionally a Θ solvent was required. In some cases[10-12] the exponent of eq 1 was replaced by $D_c(M) = D_0(M)\{1 + k_c(M)c\}$. This requires knowledge of the additional parameter $k_c(M)$, perhaps with the exception of very low concentrations, which, of course, could exhibit broader error limits.

We believe that we have presented an improved method for the characterization of MWDs by FAN LS. Details of this method as well as further details of the extrapolation technique will be given elsewhere.

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(23) Since both types of light scattering techniques are applied in our laboratory, we prefer to name them according to the measuring process: frequency-averaging LS and frequency-analyzing LS for classical LS and dynamic LS, respectively. The latter takes dynamic effects into account.
We think that most of the other names for frequency-analyzing LS should be avoided. For instance, the terms inelastic, quasi-elastic, and elastic cannot unequivocally be connected with the two LS types. There are good reasons to call both quasi-elastic or perhaps both quasi-inelastic. Moreover, the term laser LS is not reasonable since, if MWDs are to be characterized, a laser should be used for FAV LS as well as FAN LS. Furthermore, FAN LS is not a form of spectroscopy.

Helix End Effects in Block Copolypeptides, Proteins, and Protein–Detergent Complexes

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ABSTRACT: Average configuration-dependent properties (mean-square radius of gyration, mean-square end-to-end distance, helical content, helix probability profile, and average number of amino acid residues in a helical segment) have been evaluated for unperturbed partially helical polypeptides by using two weighting schemes. The weighting schemes differ with regard to whether end effects in a helical segment are assigned in like manner to amino acid residues at each end or are instead associated with only one amino acid residue. Molecules considered are block copolypeptides and specific sequence copolypeptides with amino acid sequences corresponding to those found in 44 proteins. Without exception, helix formation in the proteins is found to exhibit greater cooperativity if amino acid residues at each end of a helical segment contribute to the end effects. The helix probability profile for proteins is also found to be much smoother with this weighting scheme. Block copolypeptides show more dramatic changes in helicity, helix probability profile, and average number of amino acid residues in a helical segment. Unperturbed dimensions for both proteins and block copolypeptides are nearly independent of the weighting scheme adopted. Modification of the statistical weights used for arginyl, histidyl, and lysyl residues can produce helicities and unperturbed dimensions in reasonable agreement with helicities and dimensions deduced from circular dichroism spectra and viscosity measurements of complexes formed by reduced bovine serum albumin and sodium dodecyl sulfate.

Matrix methods are widely used to compute statistical mechanical averages of configuration-dependent properties for unperturbed macromolecules.[1] A statistical weight matrix, $\mathbf{U}_i$, is formulated for each bond (or virtual bond) in the main chain. The configuration partition function, Z, for an unperturbed linear chain molecule is extracted from the sequential product of these statistical weight matrices. Appropriate modification of $\mathbf{U}_i$ permits computation of the average occupancy of a rotational state accessible to bond i. Dimensional properties, such as the mean-square end-to-end distance, $\langle r^2 \rangle$, and mean-square radius of gyration, $\langle s^2 \rangle$, are obtained by using supermatrices formulated from the statistical weight matrices and generator matrices appropriate for the molecule in a specified configuration.[1] These procedures can be rigorously extended to encompass treatment of branched molecules.[2,3]

Application of matrix methods to partially helical homopolypeptides is most easily achieved by using a 2×2 statistical weight matrix[4,5]

$$\mathbf{U}_i = \begin{bmatrix} 1 & \sigma s \\ 1 & s \end{bmatrix}_i \qquad (1)$$

Columns index the state of amino acid residue i, rows index the state of amino acid residue $i - 1$, and the order of indexing is coil (c), helix (h). The statistical weight for amino acid residue i is unity if it is not in a helical state. Its statistical weight is σs if it initiates a sequence of helical amino acid residues and s if it propagates an existing helix. The configuration partition function for a homopolypeptide containing n amino acid residues is

$$Z = \text{row } (1, 0)\ \mathbf{U}_1\ \mathbf{U}_2 \ldots \mathbf{U}_n \text{ col } (1, 1) \qquad (2)$$

The serial product of statistical weight matrices can be replaced by $\mathbf{U}^n$ for a homopolypeptide. Statistical weight matrices in eq 1 can be combined with suitably constituted generator matrices in order to evaluate the mean-square unperturbed dimensions of partially helical homopolypeptides, both in the absence[5] and in the presence[6] of interchain cross-links. Asymmetry of the spatial distribution for partially helical homopolypeptides has been characterized[7] by using a priori and conditional probabilities extracted from the 2×2 statistical weight matrix in eq 1.

The subscript i for $\mathbf{U}$ must be retained if the treatment concerns a copolypeptide. If eq 1 is used for $\mathbf{U}_i$, a helical segment starting at amino acid residue i and extending to amino acid residue j will have a statistical weight given by $\sigma_i s_i s_{i+1} \ldots s_{j-1} s_j$. The burden of helix initiation is placed solely on amino acid residue i, while none of that burden

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