Analysis of polymer heterogeneity by sedimentation transport: 1. Method of moments, a new approach

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A novel procedure for the treatment of sedimentation velocity data by the method of moments is proposed for determination of polydispersity indices, such as M_z/M_w, etc. This method is based on linear approximations and enables the reliable exclusion of diffusion and concentration effects. It is particularly effective when applied to polymer solutions with strong intermolecular interactions and noticeable diffusion mobility of the macromolecules.

(Keywords: polymolecularity; sedimentation boundary; diffusion and concentration effects)

INTRODUCTION

Velocity ultracentrifugation is a valuable analytical tool for the measurement of molecular weight heterogeneity of a polymer sample by an absolute method[1]. The effectiveness of the method depends strongly on the sensitivity of the limiting sedimentation constant, s_0, to change in molecular weight, M, and also to a considerable extent on the reliable exclusion of diffusion and concentration effects, which disturb the sedimentation boundary profile. A possible way to solve the latter problem is presented in this work; it involves the method of moments and is based on linear approximations.

In a classic sedimentation velocity experiment, the macroscopic flow of the polymer molecules in the centrifugal field is known to lead to formation of a boundary between the solution and solvent, termed the sedimentation boundary. This boundary migrates in a radial direction at a rate equal to that for the isolated macromolecules, and spreads with time. Ahead of the boundary, there is a plateau region with approximately constant solute concentration equal to the initial one, c_0. Boundary curves that are not restricted by the meniscus and leave the plateau regions ahead of their maxima, according to Fujita[2], are referred to as freely sedimenting boundary curves. The short phenomenological description of boundary spreading presented here is valid for such curves.

EXPERIMENTAL

The method of moments

The boundary curve (profile) means the distribution of displacements (x-spectrum) of the macromolecules in the sedimenting boundary range i.e. $(1/c_0)\ (\partial c/\partial x)$, where x is the distance measured from the axis of rotation. The ith moment, m_i, of a freely sedimenting boundary curve about the centre of rotation is defined by:

$$m_i = \int_0^\infty x^i (\partial c/\partial x)\, \mathrm{d}x \tag{1}$$

The integration range here $(0, \infty)$ is limited in practice by the cell dimensions, namely from the meniscus level, x_{men}, to plateau regions, x_{p}. The dispersion of the x-spectrum (the second moment about the mean of the boundary gradient curve) is defined by:

$$\sigma_x^2 = \overline{(x-\bar{x})^2} = m_2/m_0 - (m_1/m_0)^2 \tag{2}$$

Boundary spreading with time is caused by both the polymer heterogeneity and the Brownian spreading of the concentration boundary. Let us define σ_{xs}^2 and σ_{xD}^2 as the contributions of these phenomena to x-spectrum dispersion, and accept a postulate about the reciprocal independence of the sedimentation and diffusion flows of the macromolecules valid at vanishing concentration:

$$\sigma_{\mathrm{x}}^2 - \sigma_0^2 = \sigma_{\mathrm{xD}}^2 + \sigma_{\mathrm{xs}}^2 \tag{3}$$

In this equation, σ_{x}^2 and σ_0^2 denote the dispersions of the curve that defines the sedimentation boundary at times $t=t$ and $t=0$, $\sigma_{\mathrm{xD}}^2 = 2Dt\ (1 + s\omega^2 t + \ldots)$, where D and s are the weight-average values of diffusion and sedimentation constants, respectively, t is the time of centrifugation at the angular rotor rotation velocity $\omega = 2\pi n/60$, and n is expressed in rev min^{-1}. The σ_0^2 value is especially significant when a sedimentation velocity experiment is carried out with a synthetic boundary cell[3].

The heterogeneity of a polymer sample can be quantified by the standard deviation, σ_{s}, of the distribution of s (s-spectrum) which is related to σ_{xs}^2 by[4,5]:

$$\sigma_{\mathrm{xs}}^2 = x_{\mathrm{m}}^2 [\sigma_{\mathrm{s}}\omega^2 t + (\sigma_{\mathrm{s}}\omega^2 t)^3/3! + (\sigma_{\mathrm{s}}\omega^2 t)^5/5! + \ldots]^2 \tag{4}$$

with $x_{\mathrm{m}} = m_1$. Thus, to a good approximation $\sigma_{\mathrm{s}}\omega t^2 < s\omega^2 t \ll 1$, and the observed σ_{x}^2 *versus* t relation is described

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quite accurately by:

$$\sigma_x^2-\sigma_0^2=2Dt+x_m^2\sigma_s^2\omega^4t^2 \tag{5}$$

Equation (5) was derived in the manner below by using the definition of s given by:

$$x=x_0\exp(\omega^2st) \tag{6}$$

where x_0 is equal to x at the initial time $t=0$ and coincides with x_{men} in experiments with a conventional cell. The difference in displacements, Δx, of two molecules at time $t=t$, after the expansion of the exponents in a power series, was recalculated to the difference in sedimentation constants; as a result, equation (5) was obtained on the assumption that $s\omega^2t\ll 1$.

When sedimentation velocity does not depend on the solute concentration, in accordance with equation (5), the σ_x^2 is the quadratic function of time, and σ_s^2 is obtained by:

$$\sigma_s^2=(\sigma_x^2-\sigma_0^2-2Dt)/x_m^2\omega^4t^2 \tag{7}$$

At fixed time $t=t$, the σ_x^2 may be calculated by equation (2), and D measured separately in a diffusion experiment or with the aid of the same analytical ultracentrifuge under low rotation velocity (low n).

Calculation of σ_s^2 is, however, more complicated when sedimentation velocity depends on the solute concentration. The value of s generally decreases with increasing c. For this reason, the macromolecules situated at the lower c sediment faster than the same ones at a higher c. As a result, the sedimentation boundary becomes sharper (the so-called self-sharpening effect for a homogeneous solute[2] and the Johnston–Ogston effect[6] for a polymolecular solute). These effects can be taken into account by inserting the functions f and φ in equation (3):

$$\sigma_x^2-\sigma_0^2=\sigma_{xD}^2/f+\sigma_{xs}^2/\varphi \tag{8}$$

The influence of both effects weakens with decreasing c and may be completely excluded by the extrapolation for infinite dilution, $c=0$, with the selected suitable approximation function, preferably the linear one. An analysis of the available experimental sedimentation data has shown that f and φ in equation (8) may be well approximated by[7]:

$$f=(1+K_1\omega^2\Delta s^{1.5}t^{0.8})^2 \tag{9}$$

$$\varphi=(1+K_2\omega^2\Delta s^2t^{0.3})^2 \tag{10}$$

where K_1 and K_2 are arbitrary constants independent of c and t, $\Delta s=s_0-s$, where s is the sedimentation constant measured at concentration c and s_0 is the value extrapolated to $c=0$. Hence, the dependence of σ_x on Δs was shown[7] to be an approximately linear function convenient for the σ_x extrapolation to $\Delta s\to 0$ (i.e. $c=0$) with the subsequent exclusion of diffusion effects carried out by means of equation (7). However, in a region of very low c this approximation is not always linear, and therefore becomes insecure. Among the more linear dependences is that of Gralén, described briefly below.

Gralén–Eriksson method

Gralén[8] defined the width of the sedimentation boundary, B, as the ratio of the area under the curve to its maximum height:

$$B=\int_{x_0}^{\infty}(\partial c/\partial x)\,\mathrm{d}x/(\partial c/\partial x)_{\max} \tag{11}$$

As the quantity of a solute heterogeneity Gralén used the slope of B as a function of x_m, and when it depended on x_m and c he proposed that the slope at the origin be extrapolated to vanishing concentration:

$$\partial B/\partial x=\lim_{c\to 0}\lim_{x_m\to x_0}(\partial B/\partial x_m) \tag{12}$$

Such exclusion of concentration effects was supplemented by Eriksson[9] who took into account diffusion spreading of the sedimentation boundary by subtracting the diffusion dispersion:

$$\mathrm{d}B/\mathrm{d}x=\lim_{c\to 0}\lim_{x_m\to x_0}\mathrm{d}[(B^2-4\pi Dt)^{1/2}]/\mathrm{d}x_m \tag{13}$$

This $\mathrm{d}B/\mathrm{d}x$ value was taken as a quantity related to solute heterogeneity in the sedimentation behaviour and, hence, as a parameter of its molecular-weight distribution, MWD[10].

One can see from equation (13) that the applicability of this method is limited by the requirement:

$$B^2\geqslant 4\pi Dt \tag{14}$$

In a polymer solution with strong $s(c)$ dependence, this condition is often not satisfied[7], particularly for the semiflexible macromolecules with intensive intermolecular hydrodynamic interactions[11]. However, it is more important to note that relation (3) is valid, in general, only when $s\neq s(c)$ or at $c=0$. This means that the subtrahend in equation (13) is not $4\pi Dt$ but $4\pi Dt/f$ (see equation (8)), i.e. an unknown a priori value, accounting for the self-sharpening of the sedimentation boundary. Hence, this procedure for the exclusion of diffusion effects, suggested by Eriksson[9], disregards the shelf-sharpening of the sedimentation boundary and is not therefore applicable to the polymer–solvent systems with concentration-dependent sedimentation velocity, even if the required condition (equation (14)) holds. (One can see that a formal application of Eriksson's procedure to a polymer solution with $s=s(c)$ leads to erroneous, lowered values of the heterogeneity indices.) Probably for this reason the method has not found wide application.

Nevertheless, it is an experimentally established fact that B very often depends on x_m as a linear function, in a wide range of c. It would be tempting, therefore, to use this regularity in the convenient method for determining solute heterogeneity. In the present approach, we exclude the diffusion spreading not before, but after the exclusion of concentration effects, and by adding the more complete description of the sedimentation boundary width as a function of x_m at vanishing concentration.

RESULTS AND DISCUSSION

New approach to determination of heterogeneity

The method mentioned above was developed for the unimodal sedimentation curves with a Gaussian form. To avoid this assumption, we use (instead of boundary width) the standard deviation σ_x of the x-spectrum and study its change with varying time. The slope of the σ_x dependence on x_m at vanishing concentration is defined by:

$$(\partial\sigma_x/\partial x_m)_0=\lim_{c\to 0}(\partial\sigma_x/\partial x_m) \tag{15}$$

Let σ_{x0} represent the σ_x value at $c=0$. Since $\sigma_{x0}\to 0$ at

$x_m \to x_0$, we can write:

$$\sigma_{x0} = (\partial\sigma_x/\partial x_m)_0 (x_m - x_0) \qquad (16)$$

Inserting this σ_{x0} into equation (3), which is valid at $c=0$, we have:

$$\sigma_s^2 = [(\partial\sigma_x/\partial x_m)_0^2 (x_m - x_0)^2 - 2Dt]/x_m^2 \omega^4 t^2 \qquad (17)$$

Replace t by x_m here. On the assumption that $s\omega^2 t \ll 1$, from equation (6) we have:

$$t = (1/\omega^2 s)(x_m - x_0)/x_0 \qquad (18)$$

Substitution of this t relation in equation (17) yields:

$$\sigma_s^2 = [(\partial\sigma_x/\partial x_m)_0^2 (x_m - x_0)^2 - (1/\omega^2 s_0) 2D \ln(x_m/x_0)] / [x_m^2 s_0^{-2} \ln^2(x_m/x_0)] \qquad (19)$$

Here D is clearly the infinite value of the weight-average diffusion coefficient as far as equation (19) is deduced for $c \to 0$ conditions.

Note that equation (3), and hence equation (19), should be valid at any time, i.e. at any x_m satisfying the requirement $\ln(x_m/x_0) \ll 1$ adopted above. Substitution of $x_0 = 6.0$ cm and $x_m = 6.3$ cm in equation (19) leads, for instance, to:

$$\begin{aligned}\sigma_s^2/s_0^2 &= 0.95(\partial\sigma_x/\partial x_m)_0^2 - D/(\omega^2 s_0)\\ &= 0.95(\partial\sigma_x/\partial x_m)_0^2 - [RT/\omega^2(1-\bar{v}\rho_0)]M^{-1} \qquad (20)\end{aligned}$$

where R is the gas constant and $(1-\bar{v}\rho_0)$ the buoyancy factor. The numeric coefficient in equation (20) at $(\partial\sigma_x/\partial x_m)_0$ must be constant but exhibits slight dependence on the x_m value, as a consequence of the approaches made above. The subtrahend in equation (20), in turn, is the contribution of the diffusion spreading of the sedimentation boundary with varying time. One can see that an absolute value of this contribution is inversely proportional to M, and that a relative part of it is inversely proportional to M and to the heterogeneity index. In other words, the part of diffusion spreading becomes more significant for both the low molecular weight and the moderately heterogeneous polymer samples.

Thus, the method presented above for the treatment of sedimentation velocity data consists of the following steps.

1. A series of experiments with a freely sedimenting boundary is performed at several different dilutions (with decreasing values of c down to the minimum possible value) and under identical experimental conditions (ω, temperature, cell).
2. Molecular weight, M, and diffusion constant, D, are determined independently.
3. Standard deivation σ_x is calculated at different time moments using the sedimenting boundary profile, and the σ_x value is plotted *versus* x_m.
4. Slopes of these dependences at different c values are extrapolated to vanishing concentration in coordinate axes $\partial\sigma_x/\partial x_m$ *versus* c (or Δs). In this way, the $(\partial\sigma_x/\partial x_m)_0$ value is obtained.
5. Finally, the diffusion spreading is excluded by means of equations (19) and (20) whence σ_s/s_0 follows.

Reduced dispersion of the s-spectrum, σ_s^2/s_0^2, is known to be an absolute quantity of the polymer sample heterogeneity. Further transition to MWD indices, i.e. to indices of polymolecularity, may be carried out by means of known expressions that relate to each other the second moments of the s-spectrum and the M-spectrum, for instance by[12]:

$$\sigma_M^2/M_w^2 = (M_z/M_w) - 1 = (1-b)^{-2}\sigma_s^2/s_0^2 \qquad (21)$$

where b is the exponent in the equation $s_0 = KM^{1-b}$, M_z and M_w are the z- and weight-average values of M.

CONCLUSIONS

The proposed treatment of the sedimentation data may be regarded as the transformed Gralén–Eriksson procedure. However, in contrast, the proposed method:

1. takes into account more rigorously all concentration effects and does not require special knowledge of $D(c)$ dependence;
2. is insensitive to the type of cell used (either a conventional or a synthetic boundary cell) because not σ_x, but its change with varying x_m is used in the calculations;
3. is based on linear approximations that provide a higher reliability of the results;
4. requires no assumption as to the form of the distribution.

Conclusion 2 is especially important, as with the aid of the synthetic boundary cell it is more often possible to satisfy the conditions of freely sedimenting boundary formation and hence to increase the applicability of the method, eliminating the problem connected with negative s values. The method of moments seems to be useful in the preliminary treatment of sedimentation data, which helps to choose the proper method to obtain MWD according to the Gralén–Lagermalm or any other method[2].

The peculiarities of the proposed (partly empirical) method are believed to be of great significance when it is applied to moderately heterogeneous polymer samples with considerable diffusion and concentration effects. Such a contingency has been met, for instance, in the analysis of sedimentation velocity diagrams obtained for poly(benzimidazole terephthalamide) and carboxymethyl cellulose. An account of these studies will be submitted at a later date.

Finally, it should be noted that, when the sedimentation velocity of the macromolecules does not depend on c (or at $c \to 0$), the sedimentation boundary broadens with time in accurate proportion to the path passed by the macromolecules ($\sigma_x \sim x_m$, which follows from equation (20) and was mentioned by Eriksson[9]). However, the similar linear character of the σ_x dependence on x_m for the polymer–solvent systems with strong $s = s(c)$ dependence at definite solute concentrations (an experimental result which does not follow from equation (8)) still awaits theoretical explanation.

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