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Evaluation of diffusion coefficients by means of an approximate steady-state condition in sedimentation velocity distributions

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ABSTRACT

This investigation examined the feasibility of manipulating the rotor speed in sedimentation velocity experiments to spontaneously generate an approximate steady-state condition where the extent of diffusional spreading is matched exactly by the boundary sharpening arising from negative s–c dependence. Simulated sedimentation velocity distributions based on the sedimentation characteristics for a purified mucin preparation were used to illustrate a simple procedure for determining the diffusion coefficient from such steady-state distributions in situations where the concentration dependence of the sedimentation coefficient, $s = s^0/(1 + Kc)$, was quantified in terms of the limiting sedimentation coefficient as $c \rightarrow 0$ (s^0) and the concentration coefficient (K). Those simulations established that spontaneous generation of the approximate steady state could well be a feature of sedimentation velocity distributions for many unstructured polymer systems because the requirement that $Kc_0\omega^2 s^0/D$ be between 46 and 183 cm^{-2} is not unduly restrictive. Although spontaneous generation of the approximate steady state is also a theoretical prediction for structured macromolecular solutes exhibiting linear concentration dependence of the sedimentation coefficient, $s = s^0(1 - kc)$, the required value of k is far too large for any practical advantage to be taken of this approach with globular proteins.

In sedimentation velocity experiments on a non-interacting macromolecular solute, the spreading of the migrating boundary by diffusion is countered to some extent by negative concentration dependence of the sedimentation coefficient (s). Therefore, allowance needs to be made for this boundary sharpening in order to obtain a meaningful estimate of the diffusion coefficient (D). Advantage was taken long ago [1–4] of an approximate analytical solution [5,6] of the basic sedimentation equation [7] under constraints of linear s–c dependence but concentration-independent diffusion to evaluate D for globular proteins.

An alternative analytical approach entails manipulation of the sedimentation conditions to achieve an approximate steady-state condition in which the extent of diffusional spreading is matched exactly by the boundary sharpening arising from negative s–c dependence [8,9]. Unfortunately, the technical complexities associated with the suggested procedure for achieving that steady-state condition in the sedimentation of globular proteins have led to virtual disregard of the approach for evaluating diffusion coefficients. However, subsequent numerical solution of the Lamm equation under the same constraints [10] has revealed that the steady-state condition should arise in conventional sedimentation velocity experiments on systems exhibiting a sufficiently large s–c dependence. This time-invariant shape for sedimentation velocity distributions has also been observed as the limit of boundary spreading analysis based on the general solution of the Lamm equation by the SEDFIT program [11].

In this investigation, we employed the more recent SEDFIT computer program [12,13] for numerical solution of the Lamm equation to provide additional confirmation of that earlier observation [10] as well as to show that the steady-state condition would have been attained in normal sedimentation velocity distributions for the mucin preparation examined by Creeth [9]. We also shed further light on the range of solute sedimentation characteristics (s^0, D, and Kc_0, the product of initial solute concentration and the

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concentration coefficient) compatible with spontaneous steady-state generation.

Theory

In terms of solute flow for a single non-interacting solute in solvent, the Lamm equation may be written as

$$J(r) = \omega^2 rsc - D(\mathrm{d}c/\mathrm{d}r), \quad (1)$$

where $J(r)$ is the flow of solute at radial distance r from the center of rotation of a rotor spun with angular velocity ω. Being a function of concentration, s refers to the sedimentation coefficient pertaining to the solute concentration (c) at radial distance r. The flow of solute mediated by the centrifugal field is opposed by diffusional flow, which is depicted in Eq. (1) as the product of the diffusion coefficient D (assumed constant) and the solute concentration gradient ($\mathrm{d}c/\mathrm{d}r$) at radial distance r. In that regard, the absence of a concentration gradient in the plateau region means that the flow of solute there is given by

$$[J(r)]_\mathrm{p} = \omega^2 r s_\mathrm{p} c, \quad (2)$$

where s_p is the invariant sedimentation coefficient describing solute migration throughout the plateau region because of the constant concentration c_p. Thus, the establishment of a steady state in the boundary region is conditional on identity of the flow defined by Eq. (1) with that, $[J(r)]_\mathrm{p}$, in the plateau region, a situation described by the relationship

$$\omega^2 rc(s - s_\mathrm{p}) - D(\mathrm{d}c/\mathrm{d}r) = 0, \quad (3)$$

or, on rearrangement,

$$\frac{1}{rc}\frac{\mathrm{d}c}{\mathrm{d}r} = \frac{\omega^2(s - s_\mathrm{p})}{D}. \quad (4)$$

For relatively unstructured macromolecular solutes, the concentration dependence of the sedimentation coefficient is described adequately by the relationship

$$s = s^0 \big/ (1 + Kc_\mathrm{p}), \quad (5)$$

where s^0 is the limiting sedimentation coefficient as $c_\mathrm{p} \to 0$ and where the uppercase notation is used for the concentration coefficient in order to distinguish it from the corresponding parameter for globular proteins, which exhibit a linear concentration dependence of sedimentation coefficient, $s = s^0(1 - kc_\mathrm{p})$. From Eq. (5), the difference between the magnitudes of the sedimentation coefficient for concentrations c and the plateau concentration c_p may be written

$$s - s_\mathrm{p} = s^0 K \frac{(c - c_\mathrm{p})}{(1 + Kc)(1 + Kc_\mathrm{p})} \quad (6)$$

which, on combination with Eq. (4) and integration, leads to the expression [9,10]

$$Y = \ln(c/c_\mathrm{o}) - (1 + Kc_\mathrm{p})\ln[(c_\mathrm{p} - c)/c_\mathrm{o}] = \frac{\omega^2 s^0}{2D}\frac{Kc_\mathrm{p}}{1 + Kc_\mathrm{p}}\left(r^2 - r_\mathrm{b}^2\right) \quad (7)$$

where r_b denotes the boundary position. For these systems, the location of r_b is not straightforward because of boundary asymmetry and, hence, the need to determine the boundary position as the square root of the second moment of the boundary [14,15]. This reference radial position has also been described [10] as the radius at which $\ln c = (1 + Kc_\mathrm{p})\ln(c_\mathrm{p} - c)$. Fortunately, the determination of r_b is not crucial to the analysis in that an estimate of D is already available [10] from the slope of the dependence of $[(1 + Kc_\mathrm{p})\ln(c_\mathrm{p} - c) - \ln c]$ on r^2 provided that values for s^0 and K have been predetermined. For comparisons of boundary spreading as a function of time (or distance migrated), therefore, it suffices to regard r_b as the radial distance at which $Y = 0$.

Practical considerations

The application of this approach for the determination of D from the extent of boundary spreading in a sedimentation velocity experiment is, of course, dependent on the conformity of solute distributions with the steady-state criterion. In that regard, the previous test for compliance [9] was based on availability of the distribution in Schlieren format (the radial dependence of $\mathrm{d}c/\mathrm{d}r$)—the usual optical record of sedimentation velocity distributions at that time. We now require a criterion for the radial dependence of concentration (or absorbance), the form in which sedimentation velocity distributions are currently recorded—a requirement that is verified in the current simulative study by return of the input value of the diffusion coefficient.

Feasibility of spontaneous generation of steady-state distributions for a mucin

Currently, the only evidence for the possibility of steady-state distributions being generated spontaneously in sedimentation velocity experiments resides in Fig. 15 of the computer simulation study by Dishon and coworkers [10], which demonstrates conformity of distributions with Eq. (7) for two situations: one in which $\omega^2 s^0/D = 23.5$ cm^{-2} and $Kc_\mathrm{o} = 5$ (Fig. 15A of Ref. [10]) and the other in which $\omega^2 s^0/D = 117$ cm^{-2} and $Kc_\mathrm{o} = 1$ (Fig. 15B of Ref. [10]). Although the former possible situation is unlikely to be encountered experimentally because of its requirement for an extremely large s–c dependence, the sedimentation characteristics ($s^0 = 11.1$ S, $K = 0.12$ L/g, $D = 1.5 \times 10^{-7}$ cm^2 s^{-1}) of the B-specific blood group substance [15] studied by Creeth [9] conform fairly closely with the second possibility in that $\omega^2 s^0/D = 130$ cm^{-2} and $Kc_\mathrm{o} = 0.9$ for a 7.5-g/L solution of the mucin subjected to centrifugation at 40,000 rpm.

To provide sedimentation velocity distributions for testing the above theoretical predictions, concentration profiles were generated by the SEDFIT program [12,13], a moving hat adaptation [12,16] of the Claverie finite element approach [17] to solving the differential equations numerically. Simulated distributions were recorded at 5-min intervals for a solution of solute subjected to centrifugation at selected rotor speeds; a random error of 0.02 fringes was superimposed on the simulated distributions to replicate the experimental situation [18].

Distributions at 5-min intervals were generated for a 7.5-g/L solution of mucin subjected to centrifugation at 20,000–50,000 rpm. Representative concentration patterns are shown in Fig. 1A, which presents distributions generated after 25–200 min of simulated centrifugation at 40,000 rpm in experiments with $c_\mathrm{o} = 7.5$ g/L for $6.2 \le r \le 7.2$ cm as the initial boundary condition. A striking feature of these patterns is the similarity of shape for all distributions as the boundary migrated the entire cell length—a characteristic that contrasts with the time-dependent increase in diffusional spreading that is usually encountered in sedimentation velocity studies on smaller solutes at higher rotor speeds. Analyses of these distributions in accordance with Eq. (7) exhibited the predicted linear dependencies of Y on $(r^2 - r_\mathrm{b}^2)$