Analytical ultracentrifugation - diffusion

In addition to the forces to which a particle is exposed in the centrifugal field (centrifugal force, buoyancy, friction), it is also subject to undirected and directed (mutual) diffusion. Undirected diffusion due to BROWNian motion is the subordinated process compared to diffusion caused by the concentration gradient at the sedimentation boundary. The latter leads to a broadening of the sedimentation boundary, mimicking a broader s distribution.

Fig. 1 shows diffusion in the course of time. The left figure shows the differential distribution of a monomodal species in the cell radius domain. The scans registered at different time points were superimposed for better comparability; in reality, the distribution's center moves to the cell bottom, i. e. to the right. The right figure shows the data converted into the s-domain.

The essential information of Fig. 1 lies in the scans' order: In the cell radius domain, the distribution is initially narrow and widens as time progresses. In the s domain, the distribution is initially broad and becomes narrower with time. This shows that sedimentation outweighs diffusion, as will be described in more detail in the following section.

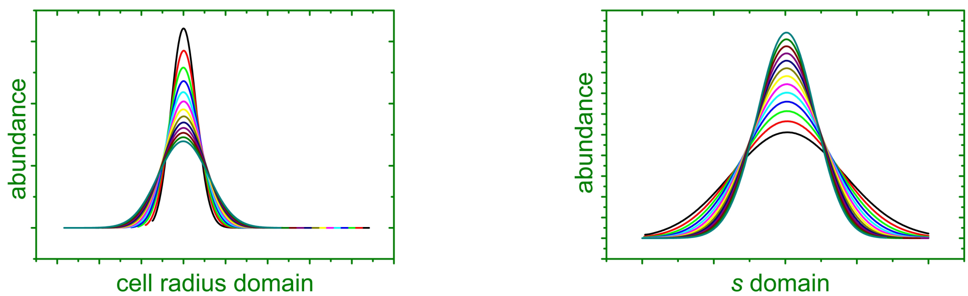

Figure 1: Diffusion broadening in the course of a sedimentation velocity experiments. Raw data (cell radius domain, left panel) and transformed s distributions (s domain, right panel).

For certain experiments, it may be necessary to take the effect of diffusion into account. In principle, two courses of action exist:

- Diffusion can be *experimentally* suppressed, typically by high rotational speeds and short experimental duration.
- Diffusion can be eliminated during evaluation, requiring assumptions to be made.

However, the effect of diffusion broadening can also be utilized to determine diffusion coefficients. For this purpose, *classical* and *more modern* variants exist, which are discussed comprehensively in the article on *diffusion broadening*. This article is limited to the theoretical description of the phenomenon.

However, it should already be noted at this point that alternative methods for the measurement of diffusion coefficients are more accurate and easier to apply (dynamic light scattering, field flow fractionation). Under certain circumstances, however, analytical ultracentrifugation can also be useful in this respect.

Impact of diffusion onto sedimentation coefficient distributions

Mutual diffusion of *one* particle species along a spatial coordinate x is described by FICKs 2nd law, according to which the temporal change of its concentration c_i depends on its diffusion coefficient D and the concentration gradient:

$$\left(\frac{\partial c_i}{\partial t}\right)_x = D\left(\frac{\partial^2 c_i}{\partial x^2}\right) \tag{1}$$

The index i refers to the solvent or solute in question. The equation assumes that the diffusion coefficient is independent of the location; an approximation that is often not applicable. From the solution of this differential equation, the mean pathlength $\bar{x}$ which a particle with the diffusion coefficient D has traveled after t seconds is accessible:

$$\bar{x} = \sqrt{\langle x^2 \rangle} = \sqrt{\int_0^\infty \frac{c_2}{c_{02}}\, x^2 \,\mathrm{d}x} = \sqrt{2\,D\,t} \tag{2}$$

where c_2 is the concentration of the solute at time t and c_{02} at time $t = 0$ at location x. In a first approximation, the distance a particle has covered out of the sedimentation boundary due to diffusion depends on the square root of time. In contrast, *sedimentation* velocity is *directly* proportional to time. Thus, for large t, sedimentation overcomes diffusion. After infinite running time in an infinitely long measuring cell, the width of a sedimentation coefficient distribution would represent polydispersity alone.

In reality, however, the FICKs 2nd law must be generalized such that the diffusion coefficient is not assumed to be independent of concentration. This results in the expression:

$$\left(\frac{\partial c_i}{\partial t}\right)_x = \frac{\partial}{\partial x}\left(D\frac{\partial c_i}{\partial x}\right) \tag{3}$$

The fundamental equation of ultracentrifugation, LAMMs differential equation, takes the local dependence of the transport processes into account and links diffusion and sedimentation. The radial position r is used as the spatial coordinate:

$$\frac{\partial c}{\partial t} = \frac{1}{r}\frac{\partial}{\partial r}\left[r \cdot D\,\frac{\partial c}{\partial r} - s\,\omega^2\,r^2\,c\right] \tag{4}$$

In the LAMM equation, the different dependencies of sedimentation and diffusion on time are not apparent; moreover, there are no constant solutions. Numerous attempts have been made to find manageable solutions for the LAMM equation. A common approximation was suggested by FUJITA:

$$c_2\,(r,\,t) = \frac{c_{02}\,e^{-\tau}}{2} \cdot \left[1 - \Phi\left(\frac{\tau - z}{2\sqrt{\varepsilon\tau}}\right)\right] \tag{5}$$

with the abbreviations

$$\tau = s\omega^2 t; \qquad z = 2\ln\frac{r}{r_m}; \qquad \varepsilon = \frac{2D}{s\omega^2 r_m^2} \tag{6}$$

It gives the particles' concentration c_2 at a given time t and a given cell radius r as a function of the sedimentation coefficient s, the diffusion coefficient D, the meniscus position r_m, the angular velocity ω and the initial concentration c_{02}. $\Phi(x)$ is the Gaussian error function, which well resembles the sedimentation boundary's shape.

It turns out useful to introduce a relative concentration w with values between 0 and 1, indicating the mass-weighted fraction of the plateau concentration at the respective point of the sedimentation boundary. Such, w, usually normalized to 1, corresponds to the function value of the $G(s)$ function, which is introduced in the article on sedimentation velocity.

If s_w^* is defined as the apparent sedimentation coefficient at the relative concentration w, diffusion broadening can be described by the inverse error function $\Phi^{-1}(x)$:

$$s_w^* = s - \frac{2\sqrt{D}}{r_m \omega^2} \cdot \Phi^{-1}(1 - 2w) \cdot \frac{1}{\sqrt{t}} \qquad (7)$$

Equation (7) shows:

- that the sedimentation boundary will not change at $w = 0.5$,
- that the impact of diffusion grows towards the upper and lower plateau,
- that the impact of diffusion grows with increasing angular velocity,
- that diffusion broadening is reciprocal to the square root of runtime,
- that diffusion broadening is proportional to the square root of the diffusion coefficient.

As said before, eq. (7) can serve two purposes:

- It can be used to eliminate diffusion broadening from s distributions.
- It can be used to calculate diffusion coefficients from diffusion broadening.

The second aspect is discussed in the article on *diffusion broadening.* Table 1 shows diffusion broadening calculated for the sedimentation of a polystyrene latex with a diameter of 50 nm (i. e., with a relatively *small* diffusion coefficient!) in water ($s = 83$ S) at different rotational speeds.

Drehzahl [rpm]	t [s]	Δs [S]	Δs [%]
1000	$1{,}69 * 10^6$	43,9	53
3000	$1{,}88 * 10^5$	14,6	18
10000	$1{,}69 * 10^4$	4,4	5
30000	1880	1,5	2
50000	677	0,9	1

Table 1: Diffusion broadening at $w = 0.2$ and 0.8 depending on angular velocity, after displacement of the sedimentation boundary by one cm. t is the time required for this distance. The table shows diffusion broadening to multiply at low angular velocities.

The example shows that s distributions can be considerably distorted by diffusion broadening. The *mean* sedimentation coefficient is correctly determined, but not the *distribution*. However, the entire distributions are of interest, taken to be too broad if not corrected for the contribution of diffusion.

Experimentally, diffusion broadening can be can be minimized by keeping runtimes short and rotational speeds high. However, this is not always possible, depending on the system. In this case, the data must be evaluated using an estimated diffusion coefficient according to eq. (7). A rough estimate can be sufficient for this purpose.