AUC - sedimentation velocity experiment

Aim of the experiment

A sedimentation velocity experiment aims at the determination of the sedimentation constant s. For a given molar mass, density and shape, it also depends on pressure, temperature and solvent. Therefore, the experiment is either performed at standardized conditions, or the measured sedimentation coefficient is corrected to these conditions.

As AUC is a fractionating method, s is obtained as a distribution of all sedimenting material.

Conducting a sedimentation velocity experiment

A *strong* centrifugal field is applied, causing dissolved or dispersed particles to sediment rapidly. In selectable time intervals, the concentration distribution along the cell radius r (this is not a fixed length, but a coordinate that indicates the distance from the axis of rotation) is registered by optical systems. Fig. 1 shows scans of a sedimenting species taken at fixed time intervals.

The first scan shows the sedimentation boundary to be near the meniscus, whose cell radius is given as r_m. As the experiment progresses, the boundary is displaced towards the cell bottom, where the sedimented material is pelleted in a thin layer. The radius at which the cell bottom is located is denoted by r_b.

Mathematical description

The boundary's inflection point r_{bnd} is a good approximation for its second moment, which would be the precise approach. The temporal change of r_{bnd}, i. e. the migration velocity u of the boundary, is related to the sedimentation coefficient s:

$$u = \frac{\mathrm{d}\, r_{bnd}}{\mathrm{d}\, t} \; ; \qquad s = \frac{u}{\omega^2\, r} \tag{1}$$

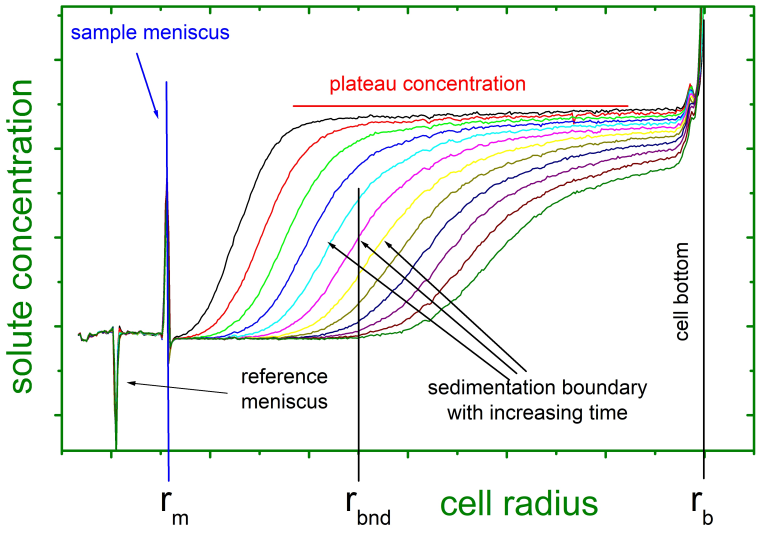

Figure 1: Movement of a sedimentation boundary with time during a sedimentation velocity experiment. Absorbance was measured in a double sector cell (sample vs. pure solvent).

Integrating eq. (1) yields:

$$\ln \frac{r_{bnd}}{r_m} = s \int \omega^2 \, \mathrm{d}\, t \tag{2}$$

$\int \omega^2 \, \mathrm{d}\, t$ is known as the *runtime integral* and is a measure for the centrifugal acceleration to which the particle has been exposed until the time t. A logarithmic plot of the ratio r_{bnd}/r_m vs. the runtime integral results in a straight line with the slope s.

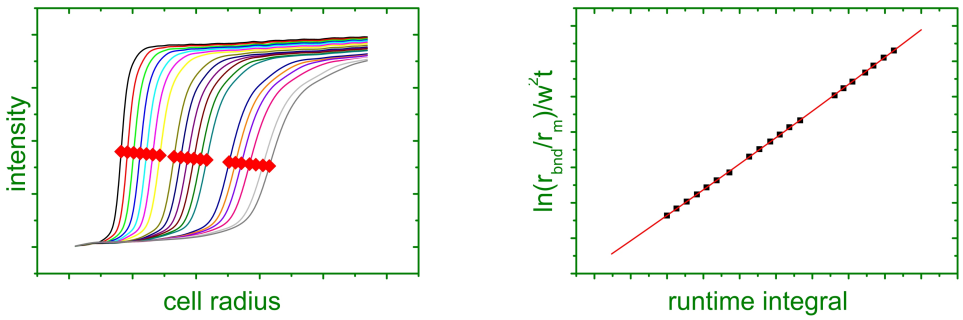

Figure 2: Evaluation of the sedimentation boundary's displacement with time according to ea. (2).

In Fig. 2, the steepest point, i. e. the middle, was taken from the concentra-

tion profiles, representing the center of the distribution for each scan. The corresponding cell radii were calculated according to eq. (2) and plotted vs. the runtime integral. The slope yields s.

Concentration dependency of s

In many systems, the sedimentation coefficient turns out to be dependent on concentration. Typically, it is extrapolated to infinite dilution by means of a reciprocal plot:

$$\frac{1}{s} = \frac{1}{s_0} + \frac{1}{s_0} k_s \, c \tag{3}$$

Here, s_0 is the sedimentation coefficient corrected to infinite dilution and c is the solute concentration. k_s is a measure for the concentration dependency of s. Fig. 3 shows an extrapolation based on a concentration series of six dilutions.

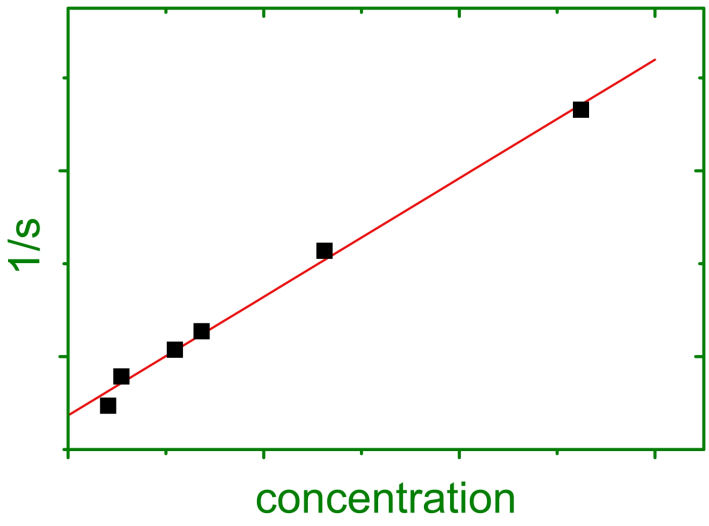

Figure 3: Reciprocal extrapolation of s to infinite dilution.

Sedimentation coefficient distribution

Rather than converting r_{bnd} as a single value from the scan, the entire x-axis in Fig. 1 can be converted into the s domain over all cell radii r - a sedimentation coefficient *distribution* is obtained. In this manner, the capability of the

ultracentrifuge to measure dispersively is better utilized than by evaluating a single value.

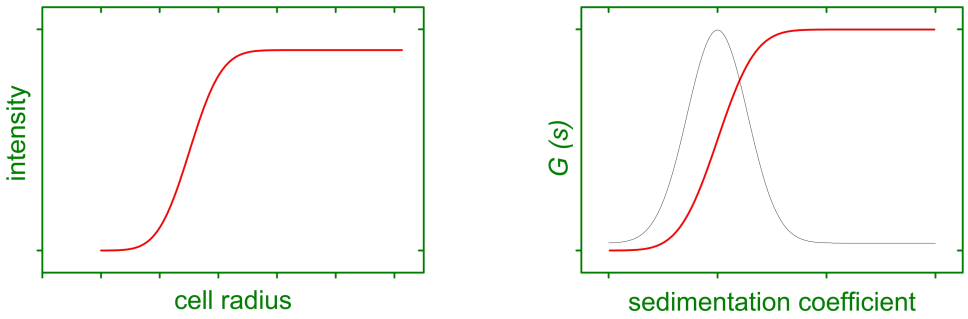

Figure 4: Evaluation of the entire concentration profile to a sedimentation coefficient *distribution* (shown additionally as its derivative).

Fig. 4 illustrates how a sedimentation coefficient distribution is obtained in this manner. When evaluating multiple concentration profiles taken at different times (e. g., the scans from Fig. 2 left), the converted distributions should overlay, extending more and more towards small sedimentation coefficients with time. If this is not the case, other processes may be present (diffusion, aggregation, chemical conversion), which can be revealed in this way.

Radial dilution

In order to avoid convection, the measuring cells are sector-shaped. Therefore, as sedimentation progresses, *radial dilution* occurs, as can be seen in Fig. 1 where the plateau concentration decreases with time. With knowledge of r_{bnd} for the respective scan, it is calculated according to

$$c = c_0 \cdot \left(\frac{r_{bnd}}{r_m} \right)^2 \tag{4}$$

where c is proportional to the signal amplitude under given circumstances. c_0 represents the solute's initial concentration. In fact, it is common to use the function $g(s)$ rather than a intensity or concentration, which contains a correction for radial dilution, additionally normalizing concentration:

$$g(s_i) = \frac{1}{c_{0i}} \cdot \frac{dc_i}{dr} \cdot \left(\frac{r_{bnd,\,i}}{r_m} \right)^2 \cdot r\omega^2\, t \tag{5}$$

Another approach is to evaluate the time derivative changes of concentration, yielding a $g(s)$ with lower experimental noise.

The integrated expression of $g(s)$ is denoted as $G(s)$.

Further evaluations

Often, the sedimentation coefficient is already an appropriate quantity for characterizing the sedimenting particle. It can, however, be subject to further evaluation. The introduction article describes how the *particle size* can be calculated from the sedimentation constant with knowledge of particle density and solvent density and viscosity. In analogy to single sedimentation coefficients or sedimentation coefficient distributions, particle sizes can be calculated as *distributions* (psd = particle size distribution).

For this conversion, it must be taken into account (especially for organic solvents) that due to the solvent's compressibility of the solvent, its density is not constant over the cell radius, but increases towards the bottom. Therefore, a local pressure correction is often already included in the calculation of $g(s)$ (eq. 5).

Another property to be calculated from the sedimentation constant is the *molar mass* of the particle. The relationship is described in the introduction article. However, this requires another transport property: the diffusion coefficient. Since the errors resulting from the determination of two transport parameters add up, molar masses were traditionally determined from equilibrium experiments, in which no transport processes occur. In principle, however, molar masses can be determined from sedimentation velocity experiments in high accuracy. Modern computer programs fit the measured data to approximate solutions of the Lamm differential equation, describing both sedimentation and diffusion processes. Thus, the complete set of variables is addressed, and molecular mass distributions can - under certain conditions - be calculated quite precisely from sedimentation velocity data.