# AUC - Measuring diffusion constants

## Introduction

The effect of diffusion broadening can be used to determine diffusion constants with the analytical ultracentrifuge. There are different approaches that may be divided into two categories:

- *Established methods* use an experiment developed specifically for this purpose, requiring special measurement cells. In practise, these techniques encounter certain limitations. They usually fail for polydisperse systems, i. e. they fail because of the the superposition of polydispersity and diffusion, which *both* lead to a broadening of the interface.
- *New methods* use a normal sedimentation velocity experiment and do not require any special equipment. However, they have not yet been extensively tested in practice.

The following section first describes the classical *synthetic-boundary experiment*. Its evaluation procedures are, to some extent, used in the new method as well.

## Conducting a *synthetic-boundary*-experiment

At low centrifugal speed, the solvent is overlaid with solution in a special cell. With time, the interface will broaden. This broadening is optically monitored and converted into the Fick diffusion coefficient. The methods applied are described below.

In this experiment, sedimentation is undesirable; thus, no information about the sedimentation rate or the molar mass is obtained. However, the diffusion coefficient can be used to evaluate an *independently conducted* sedimentation velocity experiment in respect to the molar mass of the particle.

## Diffusion along a moving interface

In a *synthetic-boundary* experiment, it is attempted to restrict the particles' motion to diffusion. Though the rotor is spinning, the centrifugal force is

kept small, causing the particles to sediment as slowly as possible. The AUC is actually only used with regard to its optical systems, not for creating a centrifugal field.

In principle, however, boundary broadening can also be observed with *sedimenting* particles as well. Thus, a conventional sedimentation velocity experiment can be evaluated with respect to diffusion broadening by means of additional evaluation procedures. This approach has several advantages:

- No special equipment (cells) is required.
- Heavy particles, that would (undesirably) sediment in a *synthetic-boundary* experiment, can be investigated.
- In one single experiment, an $s$ distribution is obtained as well.
- From the combination of the results concerning *sedimentation* and *diffusion* from a single experiment, further results are accessible. In this manner, all experimental experimental parameters are identical, ensuring comparability to a maximal extent.

As to the calculation of diffusion constants from AUC velocity runs, there are several options. All methods evaluate the diffusion broadening of the sedimentation front. Their common assumption is that the particles in the sedimentation front are Gaussian distributed. Thus, the sedimentation front can be described by the Gaussian error function.

For evaluation, the following possibilities are available:

1. *classical:* Evaluation of the raw data (Gauss)
2. *classical:* Evaluate the differential $s$-distribution $g(s)$ (Gaussian).
3. *classical:* Evaluation of the integral $s$-distribution $G(s)$ according to Van Holde and Weischet
4. *new approach:* Isolation of diffusion broadening from polydispersity, then: Evaluation as a *synthetic-boundary* experiment.
   (a) ... according to Chervenka.
   (b) ... by the steepness of the sedimentation boundary.

In the first three methods, the influence of polydispersity on the broadening of the sedimentation front is not separated from diffusion, therefore diffusion coefficients are usually found too large. The new approach separates diffusion from polydispersity and is realized in the fourth method.

**Evaluating the raw data (Gaussian)**

Diffusion broadening can be derived directly from the temporal broadening of the sedimentation front. A plot of the squared standard deviation of the GAUSS distribution vs. time yields the diffusion coefficient in means of the slope. This evaluation corresponds to the one described in the following section, except that it is performed in the radial domain, rather than in the $s$ domain.

**Evaluate differential $s$-distribution (Gaussian)**

A monomodal sedimentation coefficient distribution is well described by a GAUSS function. The standard deviation $\sigma$ as a function of runtime $t$ is determined. The raw data (left panel in Fig. 1) show the apparent decrease in diffusion broadening. For evaluation, the standard deviation is converted from the $s$ domain $\sigma_s$ back into the $r$ domain ($\rightarrow \sigma_r$),

$$\sigma_r(s,\, \sigma_s) = r_m \left[ \exp\left( (s + \sigma_s) \cdot \int \omega^2 \, \mathrm{d}t \right) - \exp\left( s \cdot \int \omega^2 \, \mathrm{d}t \right) \right] \qquad (1)$$

yielding the actual progression of diffusion with time when plotting the squared standard deviation. FICK's Second Law

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

states that the change in concentration $c_i$ over time depends on the diffusion coefficient $D$ and the concentration gradient. From the solution of this differential equation, it is possible to calculate the mean value of the distance $\bar{x}$ which a particle with a diffusion coefficient $D$ has traveled after $t$ seconds:

$$\bar{x} = \sqrt{\langle x^2 \rangle} = \sqrt{\int_0^\infty \frac{c_2}{c_{02}} \, x^2 \, \mathrm{d}x} = \sqrt{2\, D\, t} \qquad (3)$$

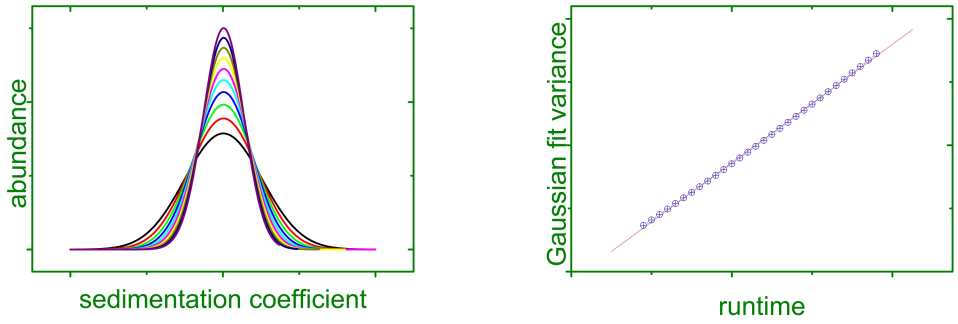


Figure 1: Diffusion broadening in the transformed $s$ distribution (left). With increasing runtime, the distribution is narrowed because sedimentation outweighs diffusion. Right: Variances of the GAUSS fits to the $s$ distributions, transformed into $r$-space, as a function of runtime $t$.

Identifying the variance of the GAUSSIAN distribution to be the squared averaged expected value for the distance of the particles from the center of the sedimentation front from Eq. (3), the diffusion coefficient is obtained as half the slope of a plot of $\sigma^2$ vs. time (Fig. 1).

$$\sigma^2 = \overline{x^2} = 2\,D\,t \tag{4}$$

Even with absolute monodispersity (Fig. 1 shows simulated data), it is actually a parabola, causing linear regression to yield a diffusion coefficient that is too high, in this case by 17%. However, the derivative of the plot is linear, yielding the diffusion coefficient as an intercept with $D = 1.003 \cdot 10^{-7}$ cm$^2$/s ($1.000 \cdot 10^{-7}$ cm$^2$/s was given for data simulation).

### Evaluation according to VAN HOLDE/WEISCHET

The previous evaluation methods fail for polydisperse systems, because polydispersity will be attributed to diffusion. The evaluation method according to VAN HOLDE and WEISCHET yields an average diffusion coefficient for a monomodal distribution, which may, by theory, be *polydisperse*. For evaluation, the $G(s)$ distribution, normalized to values between 0 and 1, is divided into horizontal slices, which are then subject to further calculation. The $y$ value of the slice is denoted by $w$; this is actually a concentration, since $G(s)$ is proportional to concentration. For every slice $w$, the corresponding $s$ values of all recorded measurements are determined and plotted vs. the

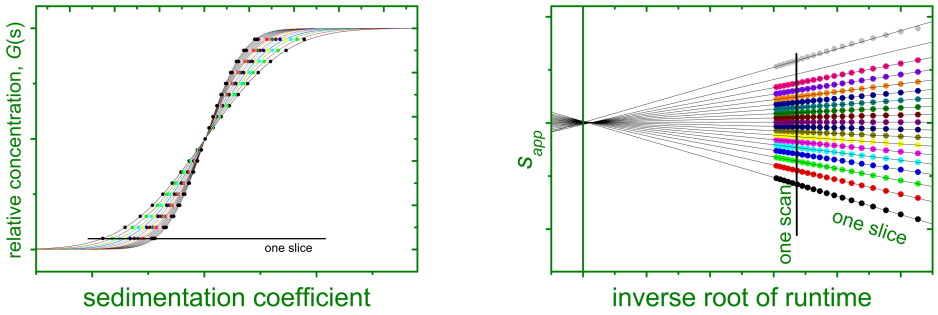


Figure 2: Analysis of $s$ distributions according to VAN HOLDE/WEISCHET

reciprocal root of runtime. All $w$ are processed in this manner. Fig. 2 shows the $G(s)$ and the resulting VAN HOLDE/WEISCHET plot for simulated data.

At infinite runtime, diffusion vanishes against sedimentation, and the apparent sedimentation coefficients $s^*$ converge into one point, as the system is monodisperse. Diffusion broadening depends on the relative concentration $w$. Regression of all $s^*$ vs. the reciprocal root of runtime yields the diffusion coefficient, according to the following equation:

$$s_w^* = s - \frac{2\sqrt{D}}{r_m\omega^2} \cdot \Phi^{-1}(1-2w) \cdot \frac{1}{\sqrt{t}} \tag{5}$$

where $\Phi^{-1}$ is the inverse GAUSS error function, describing the shape of the sedimentation boundary. The modified argument ensures that it is normalized to arguments between 0 and 1. $r_m$ is the meniscus, $\omega$ is the angular velocity of the rotor, and $w$ the relative concentration of the respective slice. Eq. (5) is based on a description of the sedimentation boundary given by FUJITA.

Plotting the slopes for all $w$ versus concentration, we can fit the inverse error function and obtain the factor $2\sqrt{D}/r_m\omega^2$ as a stretching parameter, from which the diffusion coefficient can be calculated:

$$\mathit{Slope} = \frac{2\sqrt{D}}{r_m\omega^2} \cdot \Phi^{-1}(1-2w) \tag{6}$$

Fig. 3 shows the slopes of all 19 slices fitted to the inverse error function (left axis). On the right axis, the inverse error function itself is shown.

As an additional fit parameter, a shift of the curve in y-direction is required.

This occurs if the typical fan structure of the VAN HOLDE-WEISCHET plot is not symmetric with respect to the corrected $s$-value.

Although this fit is obvious, it is not discussed in literature. However, for monodisperse data, it provides quite accurate results. For the simulated data shown, an error of 4% is obtained.

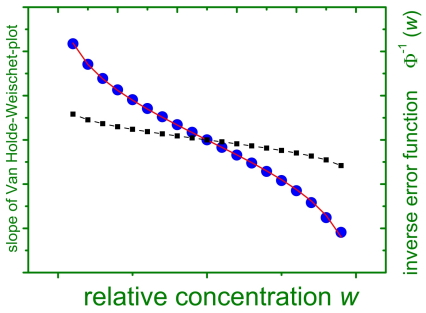


Figure 3: Fit of the GAUSSian error function to the slopes of a VAN HOLDE-WEISCHET plot.

In the classical evaluation, a diffusion coefficient is calculated from the slope of each slice according to Eq. (6). Theoretically, this should yield a $D$ distribution over all $w$. In practice, however, the mean values are disturbed by the singularity at $w = 0$, so that a reasonable result can only be obtained by omitting the values in the center and averaging over the remaining ones. This is shown in Fig. 4. This example produces an error of only 2%.

However, the average is strongly dependent on the number of selected datapoints. In the novel procedure described above, *all* datapoints are included in evaluation.

Moreover, the classical approach will fail for the previously discussed case of $s$ distributions that are not symmetrically distributed around the expectation value.

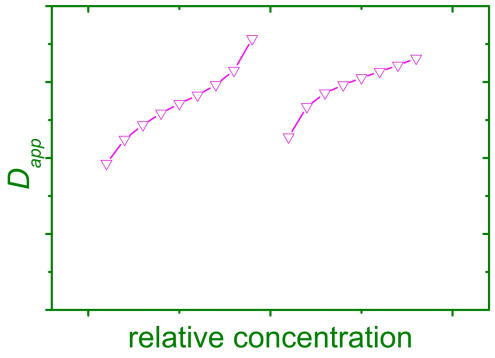


Figure 4: Result of a classical VAN HOLDE-WEISCHET evaluation

### Evaluation of the diffusion-corrected *s*-distribution

A further result of the VAN HOLDE/WEISCHET evaluation is the diffusion-corrected $s$ distribution, extrapolated to infinite runtime. When this distribution is reconverted into the $r$ domain for each slice and subtracted from the raw data, the information of the measurement is reduced to pure diffusion. The motivation lies in application to polydisperse systems. In principle, the obtained data correspond to a *synthetic-boundary* experiment. However, their acquisition requires neither special cells nor an appropriately designed experiment - the data are obtained from a normal velocity run. For their evaluation, the usual procedures for *synthetic-boundary* experiments apply.

These evaluation methods take advantage of the fact that the diffusing particles are Gaussian distributed. This means that the curves shown in the figure 5 obey the GAUSS error function (or sums thereof).

Conventional methods limit the evaluation to one or two points, for constructing the entire GAUSS curve. Fig. 5 shows the distributions of simulated data widening with increasing runtime.

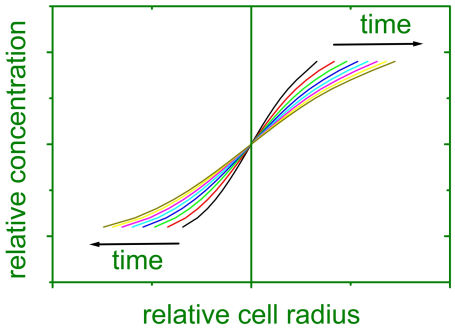


Figure 5: Diffusion broadening excluding polydispersity from a VAN-HOLDE/WEISCHET plot.

Two x values of a GAUSS curve enclose an area represented by the difference of the corresponding error function values. This area can be used to uniquely determine the variance of the GAUSS function. The calculation is simplified if the two values are symmetrically distributed around 0. With the GAUSS-function

$$\phi(r) = \frac{1}{\sigma\sqrt{2\pi}} \cdot e^{-\frac{1}{2}(r/\sigma)^2} \tag{7}$$

and the error function

$$\Phi(r) = \frac{2}{\sqrt{\pi}} \cdot \int_0^r e^{-y^2} \mathrm{d}y, \tag{8}$$

which takes on values between -1 and 1, and the derivative of which is a GAUSS curve with an area of 2 and a variance of $\sqrt{2}$, the area $A$ between the

x values -$r$ and $r$ will be

$$A = \frac{\Phi(r)+1}{2} - \frac{\Phi(-r)+1}{2} = \Phi(r), \tag{9}$$

and $r$ and $\sigma$ will be proportional for the standard function:

$$\sigma = x \cdot r = \sqrt{2} \tag{10}$$

Thus,

$$\sigma^2 = \frac{2}{\left[\Phi^{-1}(A)\right]^2} \cdot r^2 = 2\,D\,t \tag{11}$$

where $A$ is given as the difference of the chosen y values. Typically, the radii at $G(s) = 0.25$ and 0.75 are selected, where the slope is the 0.227fold of the diffusion coefficient. This procedure was introduced by Chervenka.

Another option, first described by Ralston, is to determine the variance of the Gaussian distribution by means of its maximum. For $r = 0$, the Gauss function in eq. (7) takes on the value $1/\sigma\sqrt{2\pi}$, which is identical with the slope of $G(s)$ in its inflection point:

$$\left(\frac{\mathrm{d}c}{\mathrm{d}r}\right)_{r=0} = \frac{1}{\sigma\sqrt{2\pi}} \qquad \Rightarrow \sigma = \frac{1}{\sqrt{2\pi}} \cdot \left(\frac{\mathrm{d}r}{\mathrm{d}c}\right)_{r=0} \tag{12}$$

Thus, plotting the reciprocal slopes will yield a slope being the $4\pi$fold of the diffusion coefficient.

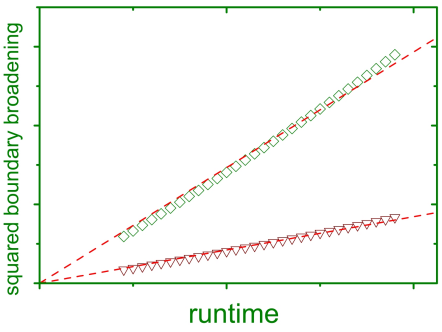


Figure 6: *Synthetic-boundary* analogous evaluation applying methods by Ralston and Chervenka.

Alike the preceding Gauss fits, these plots are actually not linear, but parabolic. The linear fit yields a diffusion constant that is approximately 15% too high. As before, an extrapolation of the derivative to zero gives the correct value. Applied to real data, however, this method is often impractical. The strength of the method lies in separating polydispersity from diffusion. In specific, the standard deviation of the diffusion corrected distribution provides valuable information. If the extrapolation to $1/\sqrt{t} = 0$ does not yield a reasonable distribution,

interactions may be present and can be investigated in this way. In contrast, a method described in the literature, extrapolating over $1/t$ is not feasible for determining of a diffusion-corrected distribution.

In any case, the the *pseudo-synthetic-boundary* procedure requires data of high quality. Its strength lies in the separation of diffusion and polydispersity.