# AUC equilibrium experiment

## Aim of the experiment

Sedimentation equilibrium experiments are used for determining molecular weights. The measuring principle is to establish a stationary state within the measurement cell, so that no transport processes take place. Although the molar mass can also be calculated from sedimentation velocity data, using the Svedberg equation (ref. to introduction article), an equilibrium experiment can provide higher precision and reliability. For sedimentation velocity, two transport quantities, namely the sedimentation constant and the diffusion coefficient, are required for evaluation. Errors for these two parameters add up.

In a sedimentation equilibrium experiment, this problem is avoided - no transport processes take place, and all hydrodynamic parameters are eliminated. The formalism describing the steady state is outlined in the following.

## Conducting an equilibrium experiment

The system is subjected to a *moderate* gravitational field. The field causes particles to sediment; this increases the concentration of particles at the bottom of the cell. In consequence, diffusion from the bottom along the concentration gradient, opposing the gravitational field, steadily increases. After sufficient run time, a stationary state is established, forming an exponential concentration profile within the measuring cell (Fig. 1). This profile contains information on the molar mass and can be evaluated using the experimental parameters,

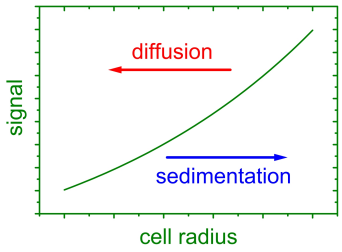


Figure 1: Concentration profile for sedimentation equilibrium

In the following, two evaluation procedures are described. Both refer to systems containing only one species. For multimodal or polydisperse mixtures, the evaluation procedures have to be extended accordingly. For more

complex systems, e. g. self-aggregating molecules, other methods have to be applied.

## Mathematical description

Sedimentation equilibrium is described by a mass balance. A volume element is balanced, located between the distances $r$ and $r+dr$ from the rotation axis (Fig. 2). Sedimentation causes particles to enter at cell radius $r$ and to exit

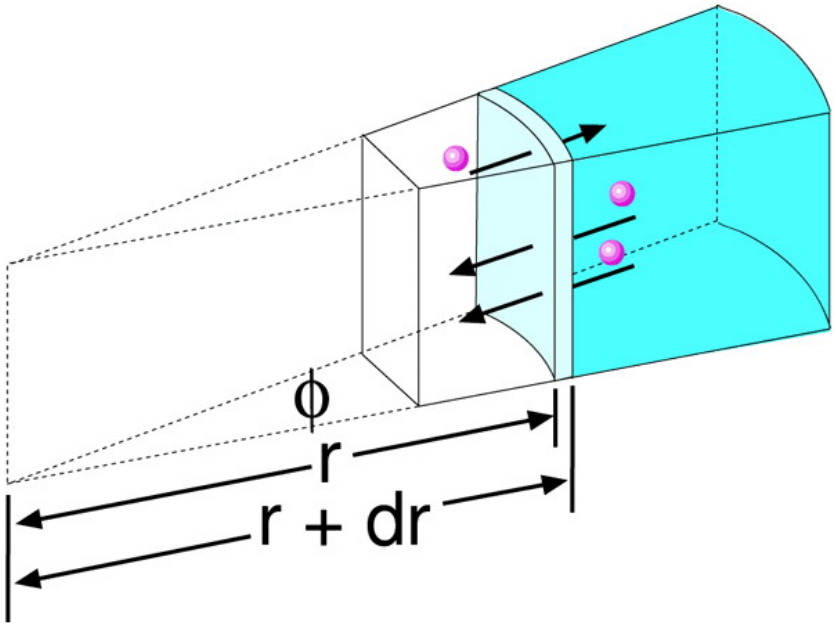


Figure 2: Bidirectional mass flows through a volume element

at cell radius $r+dr$. Due to diffusion, particles enter at cell radius $r+dr$ and exit at cell radius $r$. This consideration is the basis for the derivation of the Lamm differential equation, a fundamental equation of ultracentrifugation. For the derivation of the sedimentation equilibrium, however, it is sufficient to reduce the volume element to a surface $A$:

$$A = \phi\, r\, a, \tag{1}$$

Where $\phi$ is the opening angle of the sector-shaped cell and $a$ its height. A mass flow into the surface $A$ occurs due to sedimentation:

$$\frac{\mathrm{d}m_s}{\mathrm{d}t} = c \cdot\ s\omega^2 r \cdot A \tag{2}$$

$c$ is the concentration of the solute.

Diffusion in the opposite direction is described by FICK's second law; thus, the mass flow in the opposite direction is given by

$$\frac{\mathrm{d}m_D}{\mathrm{d}t} = -D \cdot \frac{\partial c}{\partial r} \cdot A. \tag{3}$$

In equilibrium, the flows given by eq. (2) and eq. (3)are identical, resulting in the following relationship:

$$\frac{\mathrm{d}m_D}{\mathrm{d}t} = -\frac{\mathrm{d}m_s}{\mathrm{d}t} \qquad \Rightarrow \qquad \frac{s}{D} = \frac{\frac{1}{c}\,\partial c}{r\,\partial r \cdot \omega} = \frac{M \cdot (1 - \bar{v}\varrho)}{RT}, \tag{4}$$

where $\bar{v}$ is the partial specific volume of the solute, $\varrho$ the solvent density, and $\omega$ is the angular velocity of the rotor. $M$ is the molar mass and $D$ is the diffusion coefficient of the solute. With

$$r\,\partial r = \frac{1}{2}\,d(r^2) \tag{5}$$

and

$$\frac{1}{c}\,\partial c = d\,\ln c \tag{6}$$

the result is obtained after rearranging:

$$M = \frac{2\,RT}{(1 - \bar{v}\,\varrho)\,\omega^2} \cdot \frac{d\,(\ln c)}{d\,(r^2)} \tag{7}$$

At equilibrium, therefore, an exponential concentration profile is formed in the measuring cell. Plotting the logarithm of concentration vs. the square of the radial coordinate $r$ yields a straight line, allowing to calculate the particles' molar mass from the slope.

For polydisperse systems, the molar mass is obtained as the weight average $M_w$:

$$M_w = \frac{\sum n_i\,M_i^2}{\sum n_i\,M_i} = \frac{\sum c_i\,M_i}{\sum c_i} \tag{8}$$

Where $n_i$ is the abundance, $c_i$ is the concentration, and $M_i$ is the molar mass of particle $i$.

Eq. (7) can also be derived thermodynamically. The free energy of a particle in the measurement cell is a function of pressure, concentration, and distance from the rotation axis. At force equilibrium $\mathrm{d}F = 0$, the total differential is:

$$\mathrm{d}F = \frac{\partial F}{\partial r}\,\mathrm{d}r + \frac{\partial F}{\partial p}\,\mathrm{d}p + \frac{\partial F}{\partial c}\,\mathrm{d}c \tag{9}$$

The three differentials can be expressed as:

$$\frac{\partial F}{\partial r} = -M\,\omega^2 r; \qquad \frac{\partial F}{\partial p} = M\,\bar{v} \qquad \frac{\partial F}{\partial c} = \frac{RT}{c} \tag{10}$$

Furthermore

$$\mathrm{d}p = \varrho\,\omega^2 r\,\mathrm{d}r, \tag{11}$$

so that the following expression is obtained:

$$\mathrm{d}F = -M\,\omega^2 r\,\mathrm{d}r + M\,\bar{v}\,\varrho\,\omega^2 r\,\mathrm{d}r + \frac{RT}{c}\,\mathrm{d}c = 0 \tag{12}$$

Rearranging eq. (12) gives the previously derived eq. (7).

Eq. (7) is valid for a solution of only *one* type of particles; also, the solution must be thermodynamically ideal, i. e. the activity coefficient of the particles in solution must be 1. For multimodal mixtures and nonideal behavior, other, more complex functions have to be used, requiring an underlying model (sums of exponential functions). A model-independent evaluation method for the determination of molecular masses from sedimentation equilibrium was proposed by Scholte; however, this evaluation is complicated and requires complex calculations.

A simple alternative is provided by the model-independent $M^*$ function suggested by Creeth and Harding:

$$M^*(r) = \frac{c(r) - c_m}{k c_m\,(r^2 - r_m^2) + 2k\int_{r_m}^{r} r\,(c(r) - c_m)\,dr} \tag{13}$$

with the cell radius $r$, the cell radius and concentration of solute, respectively, at the meniscus $r_m$ and $c_m$, and the constant $k = (1 - \bar{v}\,\varrho)\,\omega^2/2\,RT$, which contains the partial specific volume of the solute $\bar{v}$, the density of the solvent $\varrho$, and the angular velocity of the rotor $\omega$. The most important property of this function is its value at the cell bottom - the apparent molecular mass of the solute:

$$M^*(r = r_b) = M_{w,app} \tag{14}$$

Another important difference between eq. (13) and eq. (7) is that differentiation, being very sensitive to noise, has been replaced by the much more stable integration. This is advantageous for practical work.

Both the use of model-dependent fit functions and the $M^*$ function result in an apparent molar mass $M_{w,app}$, which must be extrapolated to infinite dilution by measuring a concentration series. There are also methods that provide a global fit to the exponential profiles of an entire concentration series.

If the concentration at the meniscus ($c_m$ in eq. (13)) is different from zero, it needs to be determined by a mathematical procedure before using the $M^*$ function. In the case of model-dependent fits, this is expressed in terms of an additional degree of freedom for the baseline. Experimentally, rotational speed can be chosen such that the solution at the meniscus does not contain any particles ("*meniscus depletion*"), circumventing this problem ($c_m = 0$). However, this can lead to complete sedimentation of a part of the material, resulting in the risk of determining molar mass averages that are too low. Thus, especially in the case of high molar masses and polydisperse systems, rotational speed for this experiment must be chosen carefully.