

Introduction to Analytical Ultracentrifugation

Analytical ultracentrifugation was developed in the 1920s. Originally, it was developed to measure the particle size distributions of gold sols. Subsequently, it was used mainly in biochemistry for the determination of molar masses and sedimentation coefficients. After commonly being substituted in the 1970s by other, more modern methods (light scattering, gel permeation chromatography, gel electrophoresis), there has been a renaissance for AUC in the recent decades, due to newly emerging fields in colloid research and the investigation of complex systems in biochemistry. New applications have been found in addition to the traditional fields of application. Among others, the following properties prove AUC to be valuable in colloid and biochemical research:

1. AUC is an *absolute method*.
2. AUC is a *fractionating method*, particularly feasible for investigating mixtures.
3. AUC targets on *geometric* (size, shape, structure) und *thermodynamic* properties (G , H , S , interaction constants).
4. A maximum rotational speed of 60,000 rpm, equivalent to a 260,000fold of Earth's gravity, makes AUC feasible for a large range of particles sizes (1 to 1000 nm) and masses (1000 to millions of Da).
5. A large scope of solvents can be used.
6. Detection is versatile, due to various optical systems that may be, in part, operated synchronously.
7. Complex mixtures are fractionated with a high level of statistical security, as *all* particles are registered.
8. Resolution of particles is possible down to an Ångström range.

For industrial applications, semi-automated instruments are in use for product control. In the biochemical context, AUC is widely used in research and development, for approval procedures by authorities and for batch verification.

Forces in the sedimentation cell

A particle in a sedimentation field is exposed to three forces: the centrifugal force F_z is opposed by buoyancy F_b and friction F_f . In addition, it is subject to diffusion, which is discussed elsewhere.

As these forces lie on one spatial coordinate, they need not be treated as vectors.

The centrifugal force F_z is proportional to the gravitational field:

$$F_z = \omega^2 r \cdot m, \quad (1)$$

where m is the particle's mass, ω is the rotor's angular velocity, and r the radial distance from the center of rotation. Thus, centrifugal force will increase with increasing r during the course of sedimentation. The opposing force of buoyancy according to ARCHIMEDES

$$F_b = -\omega^2 r \cdot m_{01}, \quad (2)$$

is proportional to the mass of displaced solvent m_{01} . The frictional force F_f is also directed into the opposite direction, it is proportional to the particle's velocity u :

$$F_f = -f \cdot u, \quad (3)$$

where f is the frictional coefficient. Equilibrium of the three forces is quickly attained (< 1 ms)

$$F_z + F_b + F_f = 0, \quad (4)$$

establishing a stationary state with a constant sedimentation rate u at the given position r .

The mass of the displaced solvent is the product of its density ρ and the particle's volume V_P . The latter can be expressed in terms of its mass m and

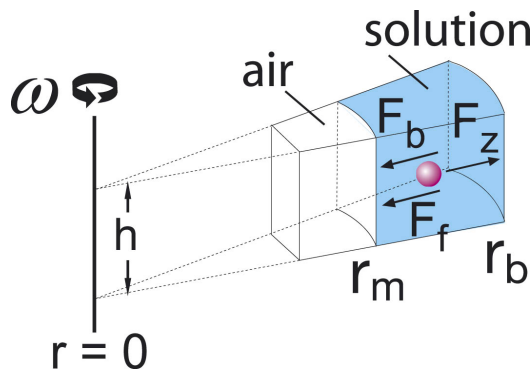


Figure 1: Forces in the sedimentation cell

it partial specific volume \bar{v} :¹

$$m_{01} = V_P \cdot \rho_1 = m \bar{v} \rho \quad (5)$$

Eq. (1) through (4) lead to:

$$\frac{m(1 - \bar{v} \rho)}{f} = \frac{u}{\omega^2 r} \equiv s \quad (6)$$

Thus, the ratio of sedimentation velocity to centrifugal force is constant and referred to as the sedimentation coefficient s . Its dimension is time; the unit is usually *Svedberg* [S], where 10^{-13} s = 1 S. The frictional coefficient f can be expressed by the diffusion coefficient d according to EINSTEIN:

$$f = \frac{kT}{D}, \quad (7)$$

transforming eq. (6) into the SVEDBERG equation:

$$M = \frac{sRT}{D(1 - \bar{v} \rho)}, \quad (8)$$

which allows to calculate the particle's molecular mass from the transport properties s and D .

For a rigid sphere, the frictional coefficient f_0 may be replaced according to STOKES

$$f_0 = 6 \pi \eta R_h, \quad (9)$$

yielding R_h , the hydrodynamic radius for this sphere. η is the solvent's viscosity. This transforms eq. (7) into the STOKES-EINSTEIN relationship

$$D = \frac{kT}{6 \pi \eta R_h}, \quad (10)$$

and from eq. (6)

$$s = \frac{m(1 - \bar{v} \rho)}{6 \pi \eta R_h}, \quad (11)$$

allowing to calculate the particle's radius from readily available parameters. This applies to rigid spheres where $f = f_0$. Furthermore, the sphere has a fixed relationship between mass, size, and density. Replacing density by

¹In AUC literature, the symbol \bar{v} has been established - the thermodynamically correct expression would be \tilde{V}_2 .

$\rho_P = 1/\bar{v}$ and replacing its mass m by the product of ρ_P and its volume $(\frac{4}{3} \pi R_h^3)$, eq. 11 is simplified:

$$d^2 = \frac{18 \eta s}{\rho_P - \rho} \quad (12)$$

For particles that are no compact spheres, the simplifications due to $f = f_0$ do not apply, and using the SVEDBERG equation (8) to calculate molar masses from sedimentation coefficients requires either a diffusion coefficient or other terms of expressing this property. Commonly, the frictional properties of an object other than compact and spherical are expressed in terms of the *frictional coefficient* f/f_0 , giving a dimensionless factor for how much slower the particle sediments, as would the compact sphere of equal mass and density.

The frictional coefficient is defined as

$$\frac{f}{f_0} = \frac{M(1 - \bar{v}\rho)}{N_A \cdot 6\pi\eta s} \cdot \sqrt[3]{\frac{4\pi N_A}{3\bar{v}M}} \quad (13)$$

In modern data evaluation, it is common to use f/f_0 as a fit parameter. Global fits of measurement data to approximate solutions of LAMMS differential equation then yield not only a sedimentation coefficient distribution, but also a fit result for f/f_0 and, thus, a molecular mass and/or size distribution.