Analytical ultracentrifugation basics

Essentially, two fundamental properties feature analytical ultracentrifugation (AUC):

1. AUC is a dispersive measurement method. Therefore, it is particularly suitable for the characterization of *mixtures*. In principle, all quantities are obtained not as averages, but as *distributions*. These can be molar mass, size, or density distributions.

2. AUC is an absolute method. It does not require calibration.

Analytical ultracentrifuges can be equipped with various optical systems, making a large scope of chemical systems accessible. Absorbance optics are feasible for particles and molecules exhibiting UV/VIS absorbance. In contrast, Rayleigh interference optics is sensitive to differences in refractive index. By synchronously operating both systems or measuring absorbance at different wavelengths, different particle species can be registered simultaneously and, to some extent, specifically.[1]

Particle target properties such as mass, density or size are obtained as *absolute quantities*. For multimodal mixtures, relative concentrations of different species are obtained correctly if

- the particle species have similar extinction coefficients (absorbance),
- the particle species are chemically similar and thus have similar refractive index increments with respect to the solvent (interference), and
- the particle species do not exhibit very large size differences (Mie theory, absorbance).

If these conditions are not met, it becomes more complex to calculate the relative concentrations for various particle species. However, the *properties of the particle species* are obtained correctly.

[1] Other common detectors are based on fluorescence, turbidity or the Schlieren effect - the latter detector is not being built anymore. Further detectors are in development - based on the Raman effect or light scattering. With multiwavelength detectors, existent as prototypes, complete spectra of absorbance or fluorescence emission can be registered rather than single wavelengths.

Properties amenable to AUC

AUC registers the *mass* or *density* of particles by means of their sedimentation in a gravity field. The primary target property is the *sedimentation coefficient*, which specifies how fast a particle sediments in a normalized gravitational field by one unit of length. The sedimentation coefficient or distribution is often already sufficient to answer certain questions about the system in question. It is obtained directly from the measured data without further assumptions or interpretation.

Furthermore, the following quantities are accessible from AUC experiments:

Size Particle size distributions can be directly calculated from sedimentation coefficient distributions if the particles' density is known. If not, particle size distributions are accessible via the density variation method.

Density Particle density distributions are measured by density variation or with density gradients.

Mass Molar masses are obtained as weight averages; highest precision is achieved with sedimentation equilibrium experiments. In principle, molar masses can also be obtained from sedimentation velocity experiments.

Interaction Concentration series yield interaction parameters, such as k_s (describes interparticle interactions) or the Second Osmotic Virial Coefficient A_2.

Geometry Hydrodynamic calculations allow for deeper characterization, e. g. the axis ratios of non-globular particles.

Diffusion Diffusion coefficients can be determined by analyzing the broadening of the sedimentation boundary.

In contrast to other methods, AUC encounters virtually no limitations in respect to applicable solvents.

Summary of AUC experiments

In principle, there are two categories of AUC experiments:

Equilibria Sedimentation and diffusion are brought into equilibrium. Transport processes and, thus, hydrodynamic parameters are eliminated (sedimentation equilibrium, density gradient).

Dynamic experiments Transport processes induced by sedimentation and diffusion are monitored and evaluated. The first is in the focus of sedimentation velocity experiments, the latter of *synthetic-boundary* experiments. A combination of both is the *pseudo-synthetic-boundary* experiment which monitors diffusion during a process of sedimentation.

The various methods are discussed in more detail in other theoretical abstracts on this website. Here, we give a brief description for basic AUC experiments:

Sedimentation velocity experiment: Sedimentation is monitored, mostly under high centrifugal fields.
Yields: sedimentation coefficient distribution, particle size distribution, molecular mass distribution.

Density variation: Variant of the previous, additionally yielding a **density distribution**.

Sedimentation equilibrium: Establishing an equilibrium of sedimentation and diffusion, mostly with moderate centrifugal fields.
Yields: molecular mass (weight average).

Density variation: Variant of the previous, does *not* require particle density for evaluation.
Yields: molecular mass (weight average), particle density (weight average).

Density gradient: Establishing a gradient of an additional solvent or solute under mostly high centrifugal fields. Analyte particles will accumulate at the position where their own density is matched.
Yields: density distribution.

***Synthetic-boundary* experiment:** Monitor interface broadening as a consequence of diffusion while suppressing sedimentation (extremely low centrifugal fields).
Yields: diffusion coefficient.

***Pseudo-synthetic-boundary* experiment:** Monitor boundary broadening (as a consequence of diffusion) while monitoring boundary movement (as a consequence of sedimentation), usually at high centrifugal fields. This experiment combines the sedimentation velocity technique with the *synthetic-boundary* experiment.
Yields: sedimentation coefficient distribution, particle size distribution, diffusion coefficient.

The table below summarizes the four classical experimental techniques.

experiment	parameters required	parameters obtained
sedimentation velocity	–	s
	$\bar{v}$, D	M
	$\bar{v}$, M	D bzw. R_h
- with density variation	–	$\bar{v}$ as well as M / D / R_h
- *pseudo-synthetic-boundary*	–	D / R_h
sedimentation equilibrium	$\bar{v}$	M
- with density variation	–	$\bar{v}$, M
density gradient	–	$\bar{v}$
synthetic boundary	–	D

Table 1: Compilation of AUC basic experiments