

THE PRINCIPLE :

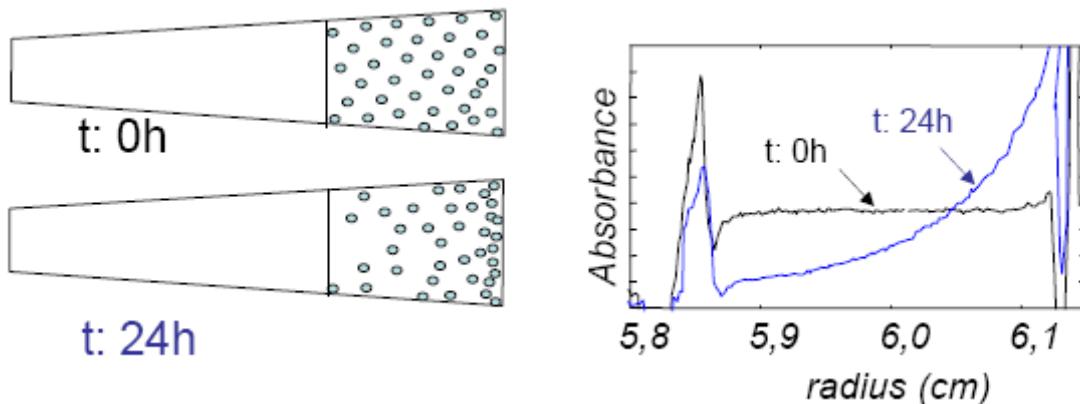
Equilibrium sedimentation (ES) allows describing the concentrations of macromolecules in solution when submitted to centrifugal force, in conditions of equilibrium, typically using absorbance optics.

ES gives information on the **molecular weight** of the macromolecules and on the **association-dissociation equilibrium constant** of the complexes.

The solutions of macromolecules are centrifuged at different angular velocities and during some days. At equilibrium -after typically 24 hours for each velocity- a smooth gradient of concentration is obtained. The experiment is performed at quite low angular velocity and with small volumes of sample (< 200 µl). For an optical path of 12 mm :

- If the sample is really stable : 180µl Ech + 10µl FC43 / 200µl solvent
- If the sample is stable : 110µl Ech + 10µl FC43 / 130µl solvent
- If the sample perhaps not so stable : 80µl Ech + 10µl FC43 / 100µl solvent
- If the sample is poorly stable : 40µl Ech + 10µl FC43 / 60µl solvent

A central piece with 2 or 6 channel is chosen depending on the volumes of the samples.

**THE EXPERIMENT :**

- 1- Determine the parameters of the sample and solvent : vbar, molecular weight, s, density et viscosity : **SEDNTERP**, S-RHouMnew.xls and/or measurement of the solvent density (density-meter DMA5000)
- 2- Select the angular velocities for AUC experiments: **SEDFIT**
- 3- Start the experiment : **XLI**
- 4- Analyze the data : **SEDFIT**, **WIN MATCH** and **SEDPHAT**

PRINCIPE DE L'ANALYSE :

In the condition of equilibrium, the Lamm equation (see the file : sedimentation velocity) is simplified:

$$c(r) = \sum c_{0i} \cdot \exp [\omega^2 M_{bi} / 2RT] \cdot (r^2 - r_0^2) + \delta$$

with :

- $c(r)$ the concentration at radial distance r
- c_{0i} the concentration of the species i at radial distance r_0 (usually the first point for the fit)
- ω the angular velocity (s^{-1}) - M_{bi} the buoyant molar mass of the species i : $M_{bi} = M_i \cdot (1 - \bar{v}_i \rho^0)$

- R : gaz constant - T : température ($^{\circ}\text{K}$) - δ : noise (solvent signal considered as constant)

For an equilibrium of association, the concentrations c_{0i} of the species are linked by the association constant, and the **molecular masses** by the stoichiometries.

Typically, different profiles of sedimentation equilibrium obtained at different concentrations and different velocities are globally fitted.