

## THE PRINCIPLE :

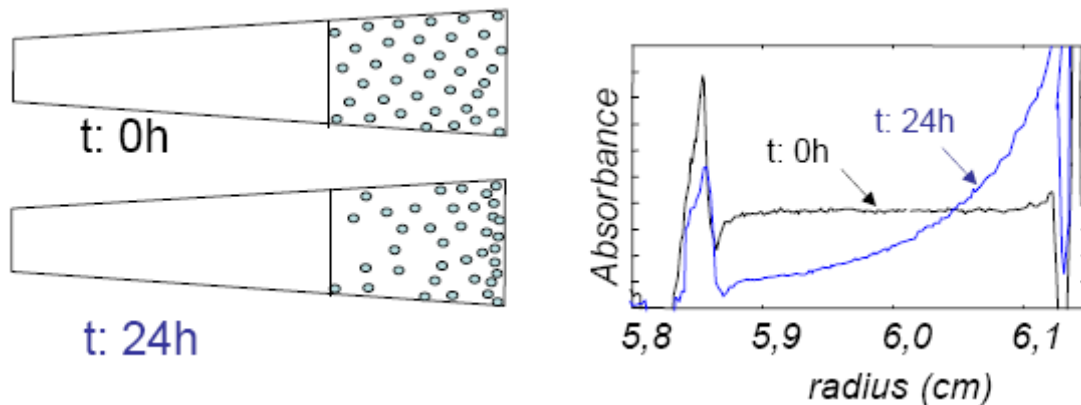
Equilibrium sedimentation (ES) allows describing the concentrations of macromolecules in solution when submitted to centrifugal force, in conditions of equilibrium, typically using absorbance optics.

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 :  $\bar{v}$ , 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)
- $\omega$  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 ( $^{\circ}K$ )      -  $\delta$ : 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 stœchiometries.

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