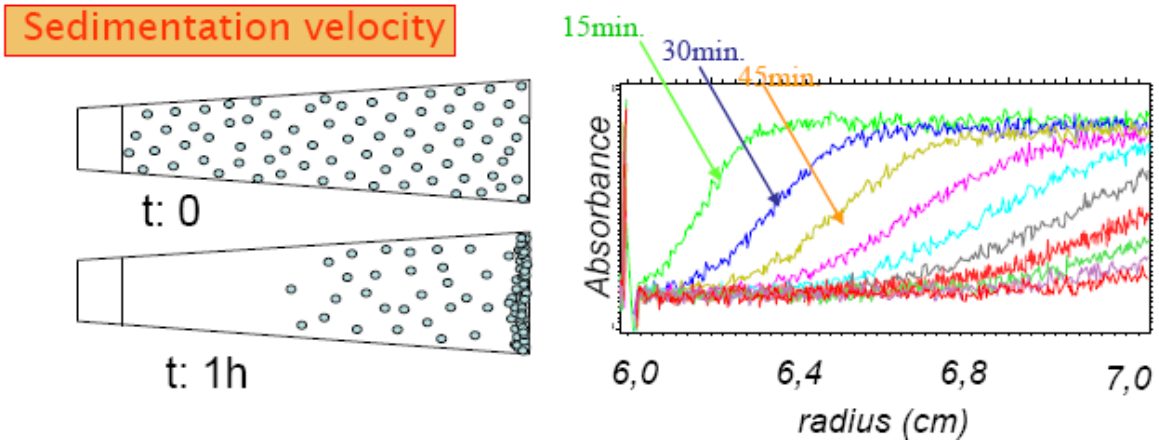
PRINCIPLE :

Sedimentation velocity experiments observe the movement of macromolecules in solution when submitted to centrifugal force. Absorbance and interference fringe shifts are measured as a function of the distance to the rotation axis at different times. They describe the evolution of the particles concentrations in solution with time.



The sedimentation is related, for each solute, to at least two parameters:

- The sedimentation coefficient, *s*, which translates the velocity of the particle, *v*, in response to the centrifugal field, $\omega^2 r$. ($s=v/\omega^2 r$)
- The diffusion coefficient, *D*, which translates the capacity of the particle to diffuse in response to concentration gradient.
- For particles in interaction, it is necessary to introduce parameters translating the fact that some of the particles disappear while others appear (kinetic constant of association /dissociation, equilibrium constant …: the case will not be considered here).

The Lamm's equation describes the evolution of the concentration *c* of one type of ideal particles (without interaction) as a function of time, *t*, and distance to the rotation axis, *r*:

Lamm's Equation: $(\partial c/\partial t). = - 1/r \,.\, \partial/\partial r \,[\, r\,(c\, s\omega^2\, r - D.\, \partial\, c\, /\partial\, r)]$

ω : angular velocity in rad/s.

The sedimentation coefficient, *s*, depends of the particle molecular weight, *M*, partial specific volume, $\bar{v}$, (inverse of particle density), and size, stated by the hydrodynamics radius, or Stokes radius, *Rs* (also written R_H). *s* depends also of the solvent density and viscosity, $\rho°$ and $\eta°$:

Svedberg's Equation: $s = M.(1- \bar{v}\rho°) / (N_A.6\pi\, \eta°\, Rs)$

Moreover, ***D* and *Rs* are linked, via the frictional coefficient *f*:**

Stokes relation: $f=6\,\pi\,\eta°\,Rs$
Einstein-Stokes relation: $D=RT / N_A.f$

R: constant of gas *T*: temperature (°K)

Frictional ratio f/f_{min}: We can compare the frictional coefficient, *f* (and so the Stokes radius) to the minimum frictional coefficient, f_{min}, corresponding to the volume of the particle as a compact sphere of radius R_{min}.

$f = f/f_{min}\, f°$ $Rs = f/f_{min}\, R_{min}$

R_{min} is easily obtained from *M* and $\bar{v}$ (representing the volume by gram of particle). f/f_{min} is larger than 1, because of particle hydration, surface roughness and shape anisotropy or non-compacity. For globular compact particle, f/f_{min} is between 1.2 and 1.3: we consider a default value of 1.25. For slightly asymmetric particle f/f_{min} reaches 1.5. For tobacco mosaic virus (elongated rod): f/f_{min} =2.6. For unfolded coil-like proteins, it depends on *M* and reaches f/f_{min} = 3 for *M*=220 kDa.

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) and viscosity (viscosity-meter AMVn)
2- Select the angular velocity for AUC experiments: SEDFIT
3- Start the experiment : XLI
4- Analyze the data : SEDFIT

PRINCIPLE OF DATA ANALYSIS

We use the data analysis program SEDFIT, developed by P. Schuck, NIH, (http://www.analyticalultracentrifugation.com). The program uses numeric solutions of the Lamm's Equation. It simulates sedimentation profiles for particles characterised by *s* and *D*, considering the experimental parameters (angular velocity, times, geometry of the cell).

1 Our analysis will consist:

1: Characterization of a distribution of sedimentation coefficients

We consider a large number of particles (typically 200), for which we link the sedimentation coefficient and diffusion coefficient, (*i.e. M* and *Rs*) via input values of f/f_{min} (typical value 1.25) and $\bar{\nu}$. We search the best combination of the signal of these particles that models the experimental data. It is named the distribution of sedimentation coefficients *c*(*s*), also named particle distribution.

Comment: This method is extremely powerful and relatively robust. This is because the transport process is dominated by the sedimentation of the particles. Their diffusion only affects boundary spreading. But, because the analysis considers the diffusion process, in an approximate but enough precise way- high resolution particle distributions are obtained. Because a large number of sedimentation profiles are globally analyzed, systematic noises are easily evaluated, which increases also the potential of the analysis.

Warning: the mathematic treatment may conduct at artefact "oscillations". It is possible that a large and regular distribution of particles appears as a succession of "peaks", or the contribution of a particle appears as two peaks. There is the possibility of over- interpretation, particularly in the case of poly disperse systems.

- This is the reason why a procedure of data regularization (typically using maximum of entropy method), which imposes the search of regular distributions, is generally used.
- The superimposition of the *c*(*s*) from different *c*(*s*) analysis (input parameters, selection of experimental profiles), or from data obtained for the same sample with different optical system, or for different samples at different concentrations (giving data with different signal/noise ratio) helps to decipher discrete species and a continuum of species.
- The effect of the choice of the input $\bar{\nu}$ has to be evaluated when $\bar{\nu}$ is unknown or when particles with different $\bar{\nu}$-values are present in the same sample.

2: The interpretation of distributions of sedimentation coefficients with mass.

This requires hypothesis for f/f_{min} (shape) and $\bar{\nu}$ (particle density). f/f_{min} = 1.25 corresponding to globular compact particles is generally used for estimating *M* .A particle with anisotropic or extended shape will have under-evaluated *M*-value. $\bar{\nu}$ is known or well-approximated ($\bar{\nu}$=0.74 ml/g) for proteins.

3: The characterization of one compound in s and D (M or M_b and Rs)

The non-interacting species model analysis is possible only for homogeneous non interacting samples. The superimposition of the *c*(s) obtained for samples at different concentrations helps to decide if this model can be considered .The 2 or 3-non interacting species analysis or the hybrid analysis (one specie + c(s)) can be interesting only in some specific cases.

4: Signal and concentration

The peaks from *c*(*s*) are characterized by their "signal", absorbance, *A*, or fringe shift, *J*, which are related to concentration, *c*, in mg/ml, optical path lenght, λ (cm), extinction coefficient $E_{0.1\%}$ (Absorbance for 1 mg/ml and *l*=1cm) and increment of index of refraction, $\partial n/\partial c$ (ml/g), of the particle, and laser wavelength (λ=6.75 10^{-5}cm):

$$A = E_{0.1\%}\, lc \qquad ; \qquad J = c\,(\partial n/\partial c)\,/\,\lambda$$