Molecular Weight Distributions from the Analytical Ultracentrifuge

Neil Errington, NCMH

Alternative sedimentation velocity method

An alternative method for obtaining distributions uses g(s*) or G(s) distributions and knowledge of the Mark-Houwink relationship parameters for the system under study:

$$s = k' M^b$$

Data analysis methods available

There are several methods available for determining molecular weight distributions from analytical ultracentrifuge (AUC) data:

- Mainly from Sedimentation equilibrium e.g. (Lechner[1,2])
- Also from Sedimentation Velocity (e.g. Schuck[3])

1. Lechner, M.D. in"Analytical Ultracentrifugation in Biochemistry and Polymer Science" (1992), Chapter 16.
2. Lechner M.D. & Machtle, W. Makromol. Chem (1991) **192** 1183-1192
3. Schuck, P., (2000) Biophysical Journal, **78** 1606-1609.

Equilibrium method

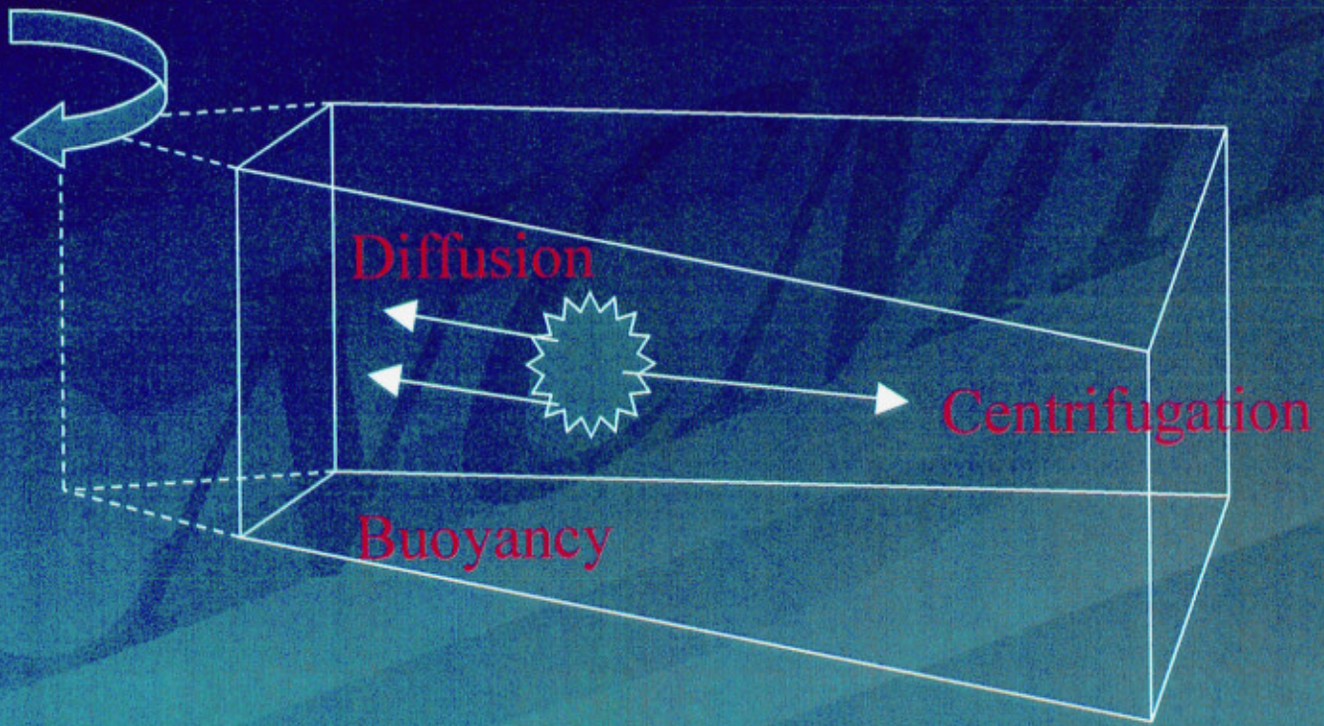
- Does give distributions but relies on careful extrapolations to zero concentration (messy) and multiple concentrations/ rotor speeds.
- This takes a long time (several days) and a lot of careful analysis.
- A version of this analysis has been coded up into a Windows program (polyfit, Les Holladay) but the numbers returned need checking properly with known standards. Also relies on Model distributions and extrapolations.

SedimentationVelocity Methods

Finite element fitting to Lamm equation models and then regularisation using CONTIN-type analysis to give **size** distributions. Knowledge of the frictional ratio is required to obtain a molecular weight distributions.

This analysis is available in the program Sedfit by Peter Schuck and downloadable freely from http://www.analyticalultracentrifugation.com

About Sedimentation Velocity



Centrifugation + Buoyancy + Diffusion =0
therefore particle acquires a velocity through the solution just enough to make the overall force zero

Expressed mathematically

Centrifugation + Buoyancy + Diffusion = 0

$\omega^2 rm \quad - \omega^2 rm_o - \quad fv \quad = 0$

as $m_o = mv\rho$ so we can write:

$\omega^2 rm(1-v\rho) - fv = 0.$

Multiply by Avogadro's number to get a molar basis:

$M(1-v\rho)/Nf = v/\omega^2 r = s$

$$M(1-v\rho)/Nf = v/\omega^2 r = s$$

Therefore sedimentation coefficient (s) depends upon M and f
or MOLECULAR WEIGHT and SHAPE

How it works

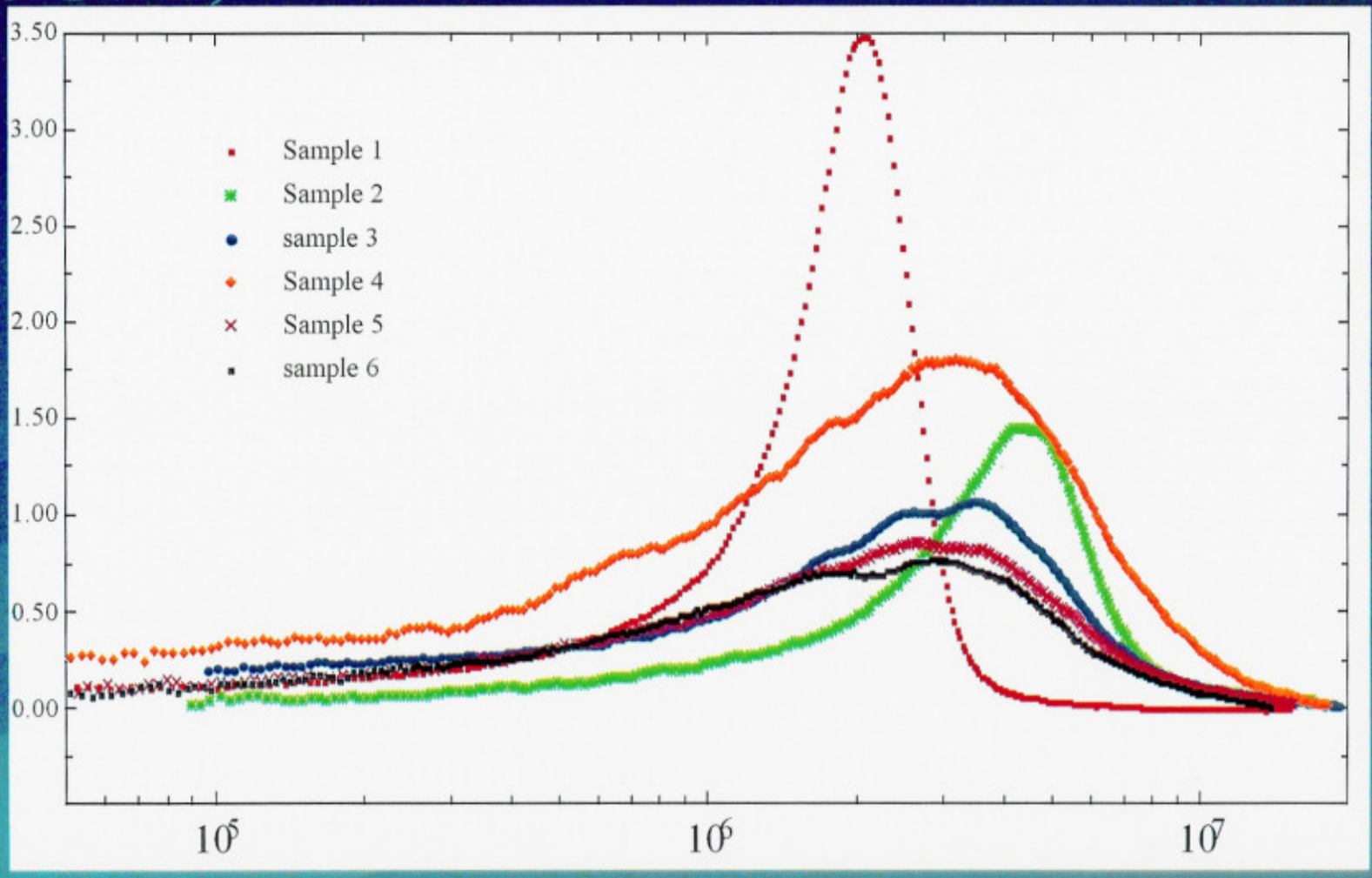
From a sedimentation velocity experiment you can obtain a g(s*) distribution by time-derivative methods, or a G(s) distribution This MUST be converted to $s_{20,w}$!!
The g(s*) distribution is an APPARENT distribution of sedimentation coefficient, uncorrected for diffusion.
The G(s) distribution is diffusion corrected.

When the previous equation is rearranged we get

$$\log_{10} M = \log_{10}(s/k') /b$$

Therefore we can convert the s* axis into an M axis and get a molecular weight distribution.

Example: (Nitro-cellulose in THF by g(s*))

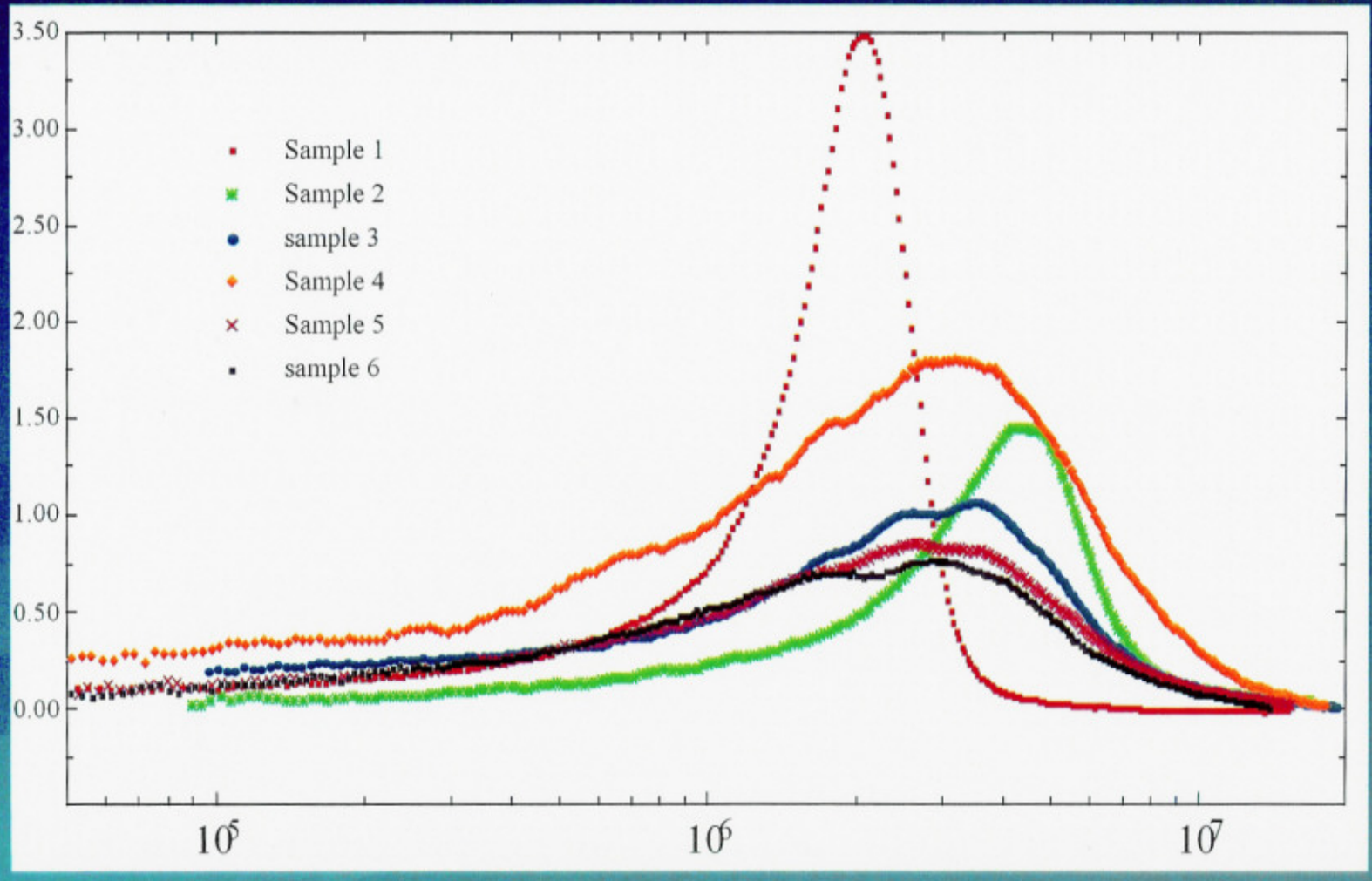


Comments

In the previous example it would seem that sample 2 is the source material and samples 1,3-6 are degraded samples. Sample 4 does not fit into the trend and seems to have a different distribution.

Sample 1 is also slightly different in that it has a narrower distribution than the others but a smaller maximum.

Example: (Nitro-cellulose in THF)



Limitations and Considerations:

- ABSOLUTE NEED to have **relevant** (and good) MHKS parameters otherwise conversion is meaningless. A possible work-around would be to use sedimentation equilibrium to compute Mw and then adjust the calculation from MHKS parameters accordingly.
- Using $g(s_{20,w})$ rather than G(s) will cause problems with small molecules as diffusion is not corrected for, and this also contributes to the apparent distribution of sedimentation coefficients.
- With very large and/or asymmetric molecules this diffusion correction will be less quite small.
- To obtain G(s) the data need to be extrapolated to infinite time (1/sqrt time = 0), this removes the effect of diffusion. (van-Holde & Weischet, 1978).

Advantages

- No stationary Phase (as in GPC etc) therefore no column interactions or exclusions.
- If a comparison only is needed rather than absolute values, this method is very useful indeed as actual MHKS values do not matter.
- Much quicker than sedimentation equilibrium (5-6 hours only).
- Relies on only one "model" - the MHKS parameters.
- No problems with deconvoluting multiple exponentials.
- If diffusion can be removed or ignored (G(s)) get correct.molecular weight distribution.
- Easy to compute averages using standard equations.

Disadvantages

- Need to have good extrapolation to infinite time, especially for smaller, symmetrical molecules in order to remove diffusion broadening of the distribution.

- With very asymmetric molecules, or highly solvated molecules, self-sharpening of the boundary can occur at high speeds (e.g. Sample 1 of the Nitro-cellulose). This will result in artificially narrow distributions and lower Mw values. This is a problem for all velocity methods. If this occurs, the sample should be run again at a lower rotor speed.

- It is necessary to have the correct MHKS parameters for your solute in this solvent, otherwise conversion of s to M is invalid. Unless only a qualitative answer is required.

Conclusions

- The analytical ultracentrifuge can provide quick estimates of molecular weight and/or size distributions.
- The limitations are not insurmountable, and are not important if qualitative data is all that is required.