AUC - Solvent density variation

Introduction

This section describes how sedimentation coefficient distributions can be converted into particle size distributions without prior knowledge of particle density. This description is limited to globular particles; but the concept can be generalized to particles of other geometries. In principle, an s distribution can be converted into a particle size distribution as follows:

$$d^2 = \frac{18\,\eta_1\,s}{\varrho_P - \varrho_1}, \tag{1}$$

where d represents the particle diameter, s its sedimentation constant and ϱ_P its density. ϱ_1 and η_1 represent the density and viscosity of the solvent. Eq. (1) is obtained from a combination of the SVEDBERG equation with the STOKES-EINSTEIN relationship.

For the conversion of a sedimentation coefficient distribution into a particle size distribution, the density of the sedimenting particle is required according to eq. (1). If this is not known, a second experiment in a solvent of different density can be performed, where the same particles will sediment at a different velocity, according to the different density (and viscosity) of the second solvent. Thus, a density distribution can be calculated, and, in consequence, a size distribution based on the individual density of all particle species. The method of solvent density variation, preferably in a solvent and its deuterated analogue (or other isotope variants), have been described decades ago both for sedimentation velocity as well as for sedimentation equilibrium experiments. The following description is limited to the sedimentation velocity experiment. This needs less experimental time and has the advantage of yielding density and size *distributions* rather that weight averages, though sedimentation equilibrium does not require any transport variables and is therefore - in principle - more exact.

Mathematical description

The sedimentation of a sample in two solvents of different density is shown in Fig. 1. In the solvent of higher density, a particle usually has *smaller s*

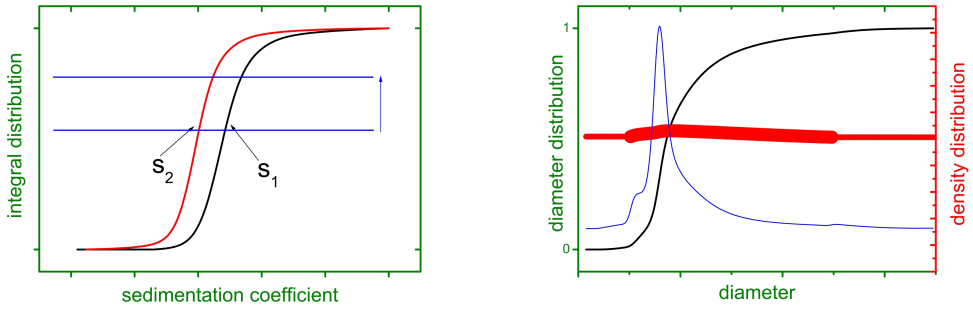

Figure 1: Left panel: Sedimentation coefficient distribution of a sample in two solvents of different density. Right panel: obtained density (right ordinate) and diameter distributions (left ordinate).

values. The method is based on the comparison of the corresponding s values, as it is shown in the figure: For each s_a (the index a always represents the solvent of lower density) there is a corresponding s_b with the same $G(s)$. The entire distribution is calculated for the integral values from 0 to 1 as follows:

Each pair of values (s_a,s_b) must yield the same particle density and diameter when they are inserted in eq. (1). Thus, there is a distribution of sets with two equations and two unknowns for all datapoints:

$$d_i^2 = \frac{18\,\eta_{1,a}\,s_{i,a}}{\varrho_{i,P} - \varrho_{1,a}} = \frac{18\,\eta_{1,b}\,s_{i,b}}{\varrho_{i,P} - \varrho_{1,b}} \tag{2}$$

and

$$\frac{\eta_{1,a}\,s_{i,a}}{\varrho_{i,P} - \varrho_{1,a}} = \frac{\eta_{1,b}\,s_{i,b}}{\varrho_{i,P} - \varrho_{1,b}}. \tag{3}$$

After transforming, equations for density

$$\varrho_{i,P} = \frac{\eta_{1,a}\,s_{i,a}\,\varrho_{1,b} - \eta_{1,b}\,s_{i,b}\,\varrho_{1,a}}{s_{i,a}\,\eta_{1,a} - s_{i,b}\,\eta_{1,b}} \tag{4}$$

and diameter

$$d_i = \sqrt{\frac{18\,\left(\eta_{1,a}\,s_{i,a} - \eta_{1,b}\,s_{i,b}\,\right)}{\varrho_{1,b} - \varrho_{1,a}}} \tag{5}$$

are obtained for each individual species observed at corresponding integral values. Subsequently, the integral and the density distribution are plotted over the calculated diameters. In Fig. 1, the result of the evaluation is shown in the right panel.

Density variation on swollen systems

A swollen particle, e. g. a polymer coil in a good solvent, contains co-sedimenting solvent, not contributing to the effective mass of the total particle, since its mass is equal to its buoyant mass. Thus, density variation yields the *bulk* density of the polymer. However, its volume contributes to the diameter and thus the friction of the coil. In the following, a simple approach for the treatment of this additional degree of freedom is given.

Since eq. (1) is valid only for compact spheres, the approach (2) must be extended accordingly. If the content of co-sedimenting solvent is taken into account in the form of the parameter Φ, representing the solvent volume fraction, then the density of the total object, assuming volume additivity, is given by

$$\varrho_{sed} = \Phi \cdot \varrho_1 + (1 - \Phi) \cdot \varrho_{02}, \tag{6}$$

where ϱ_1 is the density of the solvent and ϱ_{02} is the density of the polymer. ϱ_{sed} is the average density of the sedimenting polymer coil. Setting eq. (6) as the particle density into the approach for the density variation (2), then one obtains for all pairs $(s_a; s_b)$

$$\frac{\eta_{1,a}\, s_a}{[\Phi\, \varrho_{1,a} + (1 - \Phi)\ \varrho_{02}] - \varrho_{1,a}} = \frac{\eta_{1,b}\, s_b}{[\Phi\, \varrho_{1,b} + (1 - \Phi)\ \varrho_{02}] - \varrho_{1,b}} \tag{7}$$

Simplification yields the unchanged eq. (3)

$$\frac{\eta_{1,a}\, s_a}{\varrho_P - \varrho_{1,a}} = \frac{\eta_{1,b}\, s_b}{\varrho_P - \varrho_{1,b}} \quad , \tag{8}$$

which shows that for polymer coils and other swollen particles the density obtained from the density variation is the bulk density of the polymer *without* solvent contribution. The situation is different for the diameter, where the approach

$$d_h = \sqrt{\frac{18\, \eta_{1,a}\, s_a}{\varrho_{2,sed} - \varrho_{1,a}}} = \sqrt{\frac{18\, \eta_{1,b}\, s_b}{\varrho_{2,sed} - \varrho_{1,b}}} \tag{9}$$

in combination with eq. (6) provides a result for d that is governed by Φ:

$$d_h = \sqrt{\frac{18\ (\eta_{1,a}\, s_a - \eta_{1,b}\, s_b)}{(1 - \Phi) \cdot (\varrho_{1,a} - \varrho_{1,b})}} \tag{10}$$

To solve the equation, independent information is required either for the volume fraction Φ or for the diameter. The particle diameter can be derived independently, either from AUC or from other methods. Φ is then adjusted to yield the correct diameter in eq. (10). This is most simply done by determining ϱ_{sed} according to the rearranged eq. (1), now using ϱ_{sed} rather than ϱ_P:

$$\varrho_{sed} = \frac{18\,\eta_1\,s}{d^2} + \varrho_1 \tag{11}$$

and calculating Φ by

$$\Phi = \frac{\varrho_{sed} - \varrho_{02}}{\varrho_1 - \varrho_{02}} \tag{12}$$

In summary, the following can be concluded about the application of density variation on swollen particles:

1. The density variation method provides the *bulk density* of the polymer.
2. The *diameter* of the swollen particle needs be determined independently, but the parameter Φ obtained may prove useful for describing the particle architecture.

Density variation on biological systems

Biomolecules are often already well characterized in terms of their molar mass. However, the density and hydrodynamically related molecular parameters such as the swelling degree are of great interest and difficult to access with common methods. This information is often indispensable for understanding the function of such molecules.

A method for determining the degree of swelling based on the characterization in a poor solvent and in a good solvent has been described. This method is preparatively laborious, since the molecules have to be transferred from the poor solvent to the good solvent by dialysis. The method of density variation, in contrast, has the advantage that a stock solution only has to be *mixed* with deuterated solvent.

Since hydration water does not contribute to buoyancy, density variation yields the bulk density of the biomolecule. This is also accessible via macroscopic density measurements, requiring, however, large substance quantities.

For this reason, increment methods have been developed to calculate density from the amino acid sequence. AUC density variation provides an experimental access, requiring but small sample amounts.

Further evaluations, once density has been calculated, yield comprehensive information on the biomolecule, including the amount of hydration water. We have described this in a publication using apoferritin as an example.

Density variation on systems with excess parameters

Many systems exhibit excess densities or volumes that complicate the evaluation of density variation experiments and require additional experimental boundary conditions. Most easily accessible for this purpose is the determination of the partial specific volume of the solute using the Kratky balance.

For the case of micelles of amphiphilic block copolymers, a method is described in my doctoral thesis to include their excess properties in a comprehensive characterization, utilizing dynamic light scattering and macroscopic density measurements. Table 1 shows the required data and the accessible quantities.

Input		
w_{sol}	mass fraction of the soluble block	synthesis data
R_h	hydrodynamic radius	DLS
ϱ_{dv}	mean polymer bulk density[1]	UC-DV
ϱ_2; $\bar{v}$	partial specific density[2]	Kratky balance
ϱ_1; η_1	density and viscosity of the solvent	literature/measurement
$\varrho_{02,sol}$	bulk density of the soluble polymer block[3]	literature/measurement
$\varrho_{02,nsol}$	bulk density of the unsoluble polymer block[4]	literature/measurment
Output		
R_c	core diameter	
M_w	molar mass (only polymer)	
m_{LM}	Mass of associated solvent per micelle	
Φ	weighting parameter for co-sedimenting solvent	
Φ_c	polymer ratio in the core	
$\Phi_{LM,Cor}$	solvent ratio in the corona	
ϱ_{Cor}	mean density of the corona (including solvent)	
ϱ_c	mean density of the core	
control/comparison		
M_w	micelle molar mass (only polymer)	SLS
M_w	micelle molar mass (with solvent contribution)	AUC-EQ
ϱ_2; $\bar{v}$	partial specific density	AUC density gradient

Table 1: Micelle parameters accessible from AUC measurements in combination with other methods. Footnotes: (1) *mean* density = weighted by block lengths; $\varrho_{dv} = \varrho_{02}$ for systems without excess volume; (2) required for systems with excess volume; (3) used to calculate R_c; (4) used to calculate Φ_c.

Particularly useful is the determination of the micelle core diameter R_c; the experimental alternative would be by time-consuming and expensive neutron scattering. From the extended density variation method, the bulk density and the mass of the polymer part of the micelle are obtained. From this and prior knowledge of the block length ratio and the bulk density of the soluble block, the core diameter can be calculated.

For *hairy latices*, the "naked" diameter can be calculated accordingly. The steric stabilizers are regarded as "hairs", and the latex itself as the "core". As a prerequisite, the mass fraction of stabilizer must be known from preparation.

Table 2 shows how an increasing complexity of the sedimenting particle is countervailed by supplementing additional ultracentrifugation methods. The good complementarity of the methods towards each other is based on the fact that a "simple" sedimentation run, a density variation evaluation and a macroscopic density measurement each establish different density balances:

In a macroscopic measurement, the total mass is regarded, and the excess volume is *not compensated*. In sedimentation velocity, it is the effective mass, where the excess property is *partially compensated*. In density variation, only the *effective* portion of the *difference between light and heavy solvent* is taken into account, the other part is eliminated.

particle	method	information
compact sphere	AUC-DV	density, mass, size
	+ DLS	+ Φ, Thickness of solvent shell
coil	ditto	Φ, mass, density
hairy latex	+ w_{sol}	+ R_c
micelle	+ $\bar{v}$-determination	+ solvent contribution

Table 2: Required methods with increasing complexity of the colloidal particle.

Influence of concentration and diffusion

Good data quality is indispensable for a density variation evaluation. However, two effects can considerably falsify the evaluation of qualitatively good distributions:

- Concentration dependence of the s distribution
- Diffusion broadening

Measurements on latices and other hard spheres usually do not cause any problems, so that no corresponding corrections have been made in the previous literature. This is because

- k_s[1] is very small for hard spheres and
- diffusion broadening can be suppressed by high rotational speeds.

For other systems, the influence of concentration and diffusion can be considerable. In principle, it is reasonable to conduct the measurements in the two solvents under identical concentration and diffusion conditions. Such, the error is included in both s distributions to a similar extent, which does not eliminate the error in the result, but keeps it small. With large k_s and with strong diffusion broadening, however, the errors for both measurements can be considerable even under identical conditions.

[1] k_s indicates the influence of the concentration on the sedimentation rate.

Contamination of the results due to the concentration dependence of sedimentation is the significantly stronger effect. k_s should therefore be known, which requires measuring a concentration series. The diffusion correction, if necessary, is carried out on the basis of an independently determined particle diameter. The s distribution is thereby corrected according to the VAN-HOLDE/WEISCHET method. This method is described in the article on diffusion.

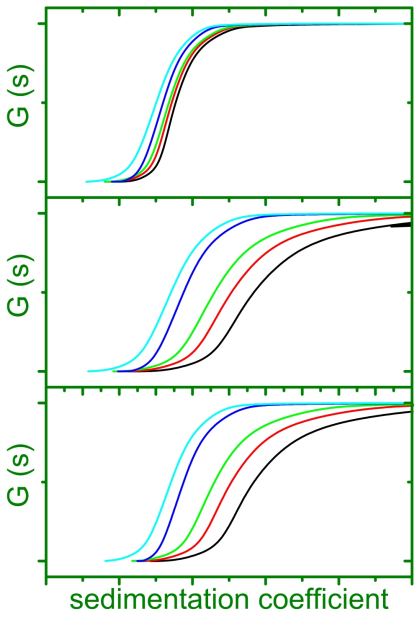

Figure 2: Effects of the corrections on s distributions. Raw data (top), concentration correction (middle), plus diffusion correction (bottom).

For certain systems, the corrections can have significant effects. In Fig. 2, the effects of the correction for a micellar system in five different mixtures of protonated and deuterated solvent are shown. The concentration correction shifts the s distributions to higher s values and expands them. The diffusion correction narrows the distribution.

Fig. 3 shows the impact of concentration correction on evaluated results. The left panel shows an uncorrected evaluation. The density is found far too high and dispersive, the diameter is found too small. In the right panel, it can be seen that the density distribution is actually nearly constant and the particle size distribution is narrow. In this evaluation, diffusion correction has not even been included!

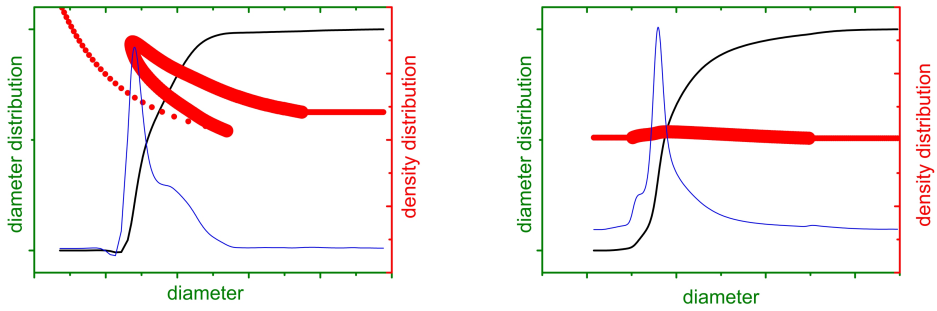

Figure 3: Effect of concentration correction on the density variation evaluation. Left: without, right: with correction on the concentration dependence of s.

It can be mathematically shown that the impact of concentration on the calculated *density* is eliminated if the concentration of both solutions is identical - although the s distributions are shifted. This does not apply for the derived *diameter* which is affected by concentration in either case.