AUC - density gradient

Aim of the experiment

Often the density of a sedimenting particle is unknown or may even be the main object of investigation. The *density gradient* experiment is designed to determine *exclusively* particle densities. For this purpose, an additional component with high density is added to the system in question. When a high centrifugal field is applied, a concentration and thus a density gradient is established along the radial coordinate. Dispersed particles accumulate in the cell at the position where the density of the solvent mixture corresponds to their own density. In addition to a density distribution, the approximate molar masses of the separated components are also accessible. The accuracy of the separation of individual components reaches the fourth decimal place of the density.

Conducting a density gradient experiment

The additional component needs to be chosen with care. Typical gradient materials are sugars and salts for aqueous systems, mixable organic compounds of high density for organic solutions. Then, the appropriate concentration ratio is selected on the basis of theoretical calculations. The following criteria must be taken into account:

- The expected density at the meniscus must be smaller, the density at the cell bottom larger than the (assumed) density of the particles in question.
- The initial density of the mixture at the beginning of the experiment should be higher than the density of the particles. Otherwise the particles could sediment and pellet irreversibly at the cell bottom before the gradient is established.
- The higher density component added to the solvent must have a *substantially* higher density than the solvent to generate a significant gradient.

- The expected density of the particle should be as close as possible to the center of the gradient.

The last point is aimed at the fact that the steepness of the gradient depends not only on solvent composition but also on rotor speed. An optimal choice of the solvent composition allows to *zoom* into the center of the gradient by reducing the rotor speed. However, the particles must neither completely sediment nor float.

The cell radius at which the particles accumulate is recorded and the solution density at this location is measured via the refractive index or calculated from the run parameters using certain assumptions. The calculation by means of the classical HERMANS-ENDE approach is still subject to current research. For detection, the otherwise rarely used SCHLIEREN optics is best suited. Absorbance optics is limited to systems with absorbing particles (and non absorbant gradients); interference optics often cannot resolve the steep refractive index gradients.

The system should not be chemically changed by the addition of the gradient forming component. This applies especially for *micellar* and other self assembling systems.

Mathematical description

In the following a simplified derivation of the density gradient is given. The density of the solvent mixture is sought as a function of the cell radius r. Experimental parameters are the composition of the mixture, expressed by the volume fractions ϕ_k, and the angular velocity ω of the rotor.

The gradient is governed by the equilibrium of sedimentation and diffusion, as formulated in LAMM's differential equation. In the following, however, a thermodynamic derivation is used. According to this, the equilibrium condition of ultracentrifugation according to GOLDBERG can be written as:

$$M_k\,\omega^2 r \left(1 - \tilde{V}_{2,k}\,\varrho\right) = \frac{\mathrm{d}\mu_k}{\mathrm{d}r} \tag{1}$$

where μ_k is the chemical potential of component k and M_k its molar mass, ω is the rotor's angular velocity, r the respective observation radius, $\tilde{V}_{2,k}$ is the molar specific volume of component k, and ϱ is the density of the solution at this cell radius. The density of the mixture is

$$\varrho = \sum_{k=0}^{s} \frac{\phi_k}{\tilde{V}_{2,k}} \tag{2}$$

ϕ_k is the volume fraction of component k. The use of the volume fraction instead of the mass fraction assumes volume additivity. In the following, a binary mixture of solvents is assumed, usually a mixture of the solvent suitable for the substance to be investigated (index 1) with a significantly denser component (index 2), which must not influence the behavior of the solution or dispersion. If eq. (2) is written as

$$\varrho = \phi_1\,\varrho_1 + \phi_2\,\varrho_2, \tag{3}$$

then eq. (1) can be written as

$$M_2\,\omega^2 r\,\phi_1\,\frac{\varrho_2 - \varrho_1}{\varrho_2} = \frac{\mathrm{d}\mu_2}{\mathrm{d}r} \tag{4}$$

The following applies to the chemical potential:

$$(\mathrm{d}\mu_k)_{T,\,p} = (\mathrm{d}\Delta\mu_k)_{T,\,p} \tag{5}$$

The right term can be expressed by the relation

$$(\Delta\mu_k)_{T,\,p} = \left(\frac{\partial \Delta G_m}{\partial n_k}\right)_{T,\,p}, \tag{6}$$

because the relation between the mixing energy G_m and the amount of substance n_k of component k can be described according to GIBBS via the equation:

$$\Delta G_m = RT\,(n_1 \ln\phi_1 + n_2 \ln\phi_2) \tag{7}$$

Here, ideal miscibility of the components is assumed (same molar volumes, no excess volume, no mixing heat). Taking into account

$$\phi_1 = f(n_2) = \frac{n_1 V_1}{n_1 V_1 + n_2 V_2} \qquad \text{and} \qquad \phi_2 = f(n_2) = \frac{n_2 V_2}{n_1 V_1 + n_2 V_2} \tag{8}$$

(with the molar volumes V_k of the components) we obtain the derivative

$$\frac{\partial \Delta G_m}{\partial n_2} = (d\mu_2)_{T,\,p} = RT\left[\ln\phi_2 + \left(1 - \frac{V_2}{V_1}\right)(1 - \phi_2)\right] \tag{9}$$

The derivative of eq. (9) with respect to ϕ_2 gives

$$\frac{d\mu_2}{d\phi_2} = RT\left[\frac{1}{\phi_2} + \frac{V_2}{V_1} - 1\right], \tag{10}$$

and with $\phi_0 + \phi_1 = 1$ under (preliminary) neglect of density effects, the chain rule is applied:

$$\frac{d\mu_2}{dr} = \frac{d\mu_2}{d\phi_2} \cdot \frac{d\phi_2}{dr} \tag{11}$$

establishing a relationship between the composition and the experimental parameters from eqs. (4) and (11):

$$M_2\,\omega^2 r\,\phi_1\,\frac{\varrho_2 - \varrho_1}{\varrho_2} = RT\left[\frac{1}{\phi_2} + \frac{V_2}{V_1} - 1\right] \cdot \frac{d\phi_2}{dr} \tag{12}$$

With the additional simplification of comparable molar volumes of the two components, the integration results in

$$\frac{\phi_2}{\phi_1} = \alpha\, e^{\beta\, r^2}; \qquad\qquad \beta = \frac{\omega^2\, M_2}{2\, RT} \cdot \frac{\varrho_2 - \varrho_1}{\varrho_2} \tag{13}$$

Equation ((13) is known as the HERMANS-ENDE-equation. Another notation is:

$$\phi_2(r) = \frac{\alpha\, e^{\beta\, r^2}}{1 + \alpha\, e^{\beta\, r^2}} \tag{14}$$

The composition of the mixture is obtained as a function of the cell radius. The integration constant α is calculated from the mass balance as a boundary condition for a component at the beginning and end of the experiment:

$$\int_{r_m}^{r_b} \phi_2(r)\, dr = \phi_2^{initial} \cdot \frac{r_b^2 - r_m^2}{2} \tag{15}$$

Here r_m and r_b are the cell radii of the meniscus and the cell bottom, respectively, and one obtains α as

$$\alpha = \frac{\exp\left[\beta\,\left(r_b^2 - r_m^2\right)\,\phi_2^{initial}\right] - 1}{\exp\left[\beta\, r_b^2\right] - \exp\left[\beta\, r_b^2 \phi_2^{initial} + \beta\, r_m^2\, \phi_1^{initial}\right]} \tag{16}$$

Now one of the volume fractions and thus the density is obtained from Eq. (14):

$$\varrho(r) = \left[1 - \phi_2(r)\right] \cdot \varrho_1 + \phi_2(r) \cdot \varrho_2 \tag{17}$$

Pressure correction

A pressure correction is applied to the final calculated density gradient. With the compressibility of the mixture κ_{ges}, the corrected density ϱ_{korr} as a function of the local pressure $p(r)$ is obtained according to

$$\varrho_{korr}(r) = \varrho(r) \cdot [1 + \kappa_{ges}(r) \cdot p(r)] \tag{18}$$

The local pressure is calculated from the uncorrected local density:

$$p(r) = \frac{1}{2}\varrho(r)\,\omega^2 \left[r^2 - r_m^2\right] \tag{19}$$

and the total compressibility is composed additively from the compressibilities of the components using the local volume fractions:

$$\kappa_{ges}(r) = [1 - \phi_2(r)] \cdot \kappa_1 + \phi_2(r) \cdot \kappa_2 \tag{20}$$

Evaluation

The density gradient is calculated from the experimental parameters for all radial positions. The detected intensity profile is plotted vs. the equivalent densities. Fig. 1 illustrates this by superimposing the (calculated) density gradient (right axis) and the registered concentration (left axis).

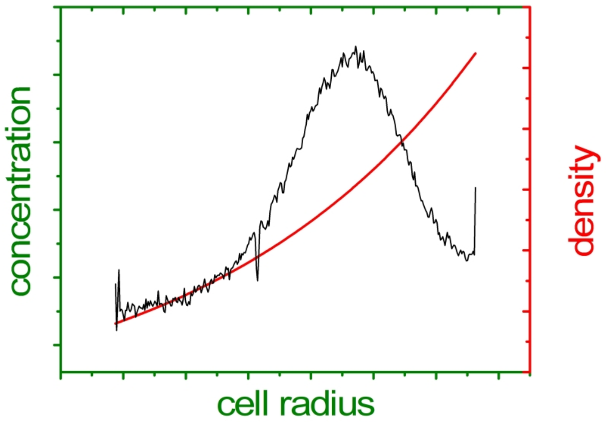

Figure 1: Density gradient in equilibrium (right axis) and accumulation of the material at the cell radius where its density is matched.