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ORIGINAL CONTRIBUTION

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The preparative dynamic density gradient method

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Abstract In this work the development of a new ultracentrifugation method is described. The preparative density gradient method (PDDG) allows the fractionation of colloidal systems according to the density of their components. The fractions—collected separately—can be subjected to further investigations with any appropriate physical or analytical method. The particle density of a colloidal system is a very valuable information that can be used in many ways. Here for the first time a method is presented that could make the access to that information much easier for laboratories throughout the world. The preparative dynamic density gradient is performed in a preparative ultracentrifugation tube by layering heavy water under a sample that is dispersed or dissolved in water. The water and heavy water molecules undergo a fast diffusion process finally leading to a homogeneous solvent mixture. While reaching this equilibrium the water–heavy water concentration varies over the cell radius. Thus a density gradient is built up. Fractions of the sample will move to the radial position where their densities are matched. They can then be collected separately. In this work basic experiments and results that lead to the development of the PDDG will be presented. Calculations based on basic ultracentrifugation theory confirm the described experimental findings. An example will demonstrate the problem solving capability of this new method.

Keywords Preparative ultracentrifugation · Analytical ultracentrifugation · Density gradient · Dynamic density gradient · Colloidal systems · Polymeric dispersion · Fractionation · Diffusion theory

Introduction and motivation

The analysis of the particle densities of especially colloidal systems is, still a topic that is, to the authors' understanding not addressed sufficiently. The scientific power of such results is not recognized widely. This may be due to the fact that right now very few laboratories exist that are able to gain the information. Especially in the case of core shell particles or multiple particles the particle density obtained by analytical ultracentrifuge (AUC) has proved to be very valuable [1–4]

Basically, the AUC is an apparatus that is used to fractionate samples according to two principles. Depending on the experiment performed on the samples they can either be fractionated according to size or density. The latter is achieved in density gradient experiments. There are two kinds of density gradients: (a) Static density gradients that do not change with time after they have reached equilibrium. (b) Dynamic density gradients that do change with time and are therefore non-equilibrium gradients.

The dynamic density gradient was originally developed by Lange [5]. It is performed in a synthetic boundary cell by overlaying H_2O onto a sample that is dispersed or dissolved in D_2O. The H_2O and D_2O molecules undergo a fast diffusion process finally leading to a homogeneous solvent mixture. While reaching this equilibrium the H_2O–D_2O concentration varies over the cell radius. Thus a density gradient over the cell radius is built up. Fractions of the sample will move to the radial position where their densities are matched. In the classical experiment the radial position is then monitored by Schlieren optics [6] to yield the densities directly.

As already stated, the fractionation of a sample according to density requires the generation of a density gradient inside a centrifugal field. The centrifugal field is created by centrifugation of the sample inside an ultracentrifuge (UC). Concerning this experiment, basically two transport mechanisms have to be taken into account: diffusion and sedimentation. In the dynamic density gradient directed diffusion according to Fick's second law creates the density gradient, whereas sedimentation drives the fractions of the sample to their radial position in the density gradient.

It has to be emphasized that the centrifugal force is not sufficient to fractionate H_2O and D_2O. For these molecules diffusion is the sole transport process of importance in this kind of experiment.

In this work we describe the development of a preparative dynamic density gradient performed in an OPTIMA XL. The most important advantages of this new method are: Creation of density gradient without any other medium but water. Separate analysis of fractions by AUC or any other technique. Create amounts even sufficient to apply application tests. Thereby complex colloidal systems become more important and can be analyzed with respect to synergistic or anti-synergistic effects.

We first will present basic experiments and results that lead to the development of the preparative density gradient method (PDDG). Calculations based on basic UC theory confirm the described experimental findings. In the second part of the presentation one impressive example is given, which elucidates how the PDDG can help in problem solving and the research on new products based on polymeric colloids.

Theoretical considerations

The basic law for diffusion processes was given by Fick and is called Fick's first law [7]. The combination of Fick's first law with the equation of continuity results in an equation which rules the concentration dependence of diffusion processes and which is called Ficks second law of diffusion [7]:

$$\partial C/\partial t = (\partial/\partial R)[D\partial C/\partial R)] \tag{1}$$

or in the case that D is independent of R (Fig. 1):

$$(\partial C/\partial t)_R = D(\partial^2 C/\partial R^2)_t. \tag{2}$$

Integration of eq. 2 yields

$$C(R,t)/C_0 = (1/2)\{1 - erf[(R - R_\mathrm{u})/(4Dt)^{1/2}]\} \tag{3}$$

With the starting condition $C(R, t) = C_0$ at $t = 0$ and $erf(\mathrm{x}) = (2/\pi^{1/2}) \int_0^\mathrm{x} \exp(-s^2)\mathrm{d}s$. D is the mutual diffusion coefficient, i.e., $D_\mathrm{AB} = D_\mathrm{BA}$. Eq. 3 is the final equation for calculating the concentration of the samples A and B at any time t and for any distance R in a diffusion cell under the assumption that the diffusion coefficient D and the initial concentration C_0 of the system are known. If the initial state of the diffusion experiment consists of the pure substances the following relationships hold: $C_{0,\mathrm{A}} = \rho_\mathrm{A}$ and $C_{0,\mathrm{B}} = \rho_\mathrm{B}$ where ρ_A and ρ_B are the densities of the pure samples A and B, respectively.

For sector shape cells the radial dilution (or thickening) effect has to be taken into account:

$$C(R,t)_\mathrm{corr} = C(R,t)\ (R_\mathrm{u}/R)^2 \tag{4}$$

In case the volume additivity of the mixture is valid with respect to the samples A and B the concentration of A in B and vice versa is proportional to the density $\rho(R,t)$ of the mixture.

$$\rho(R,t) = \rho(R_\mathrm{m}) + [\rho(R_\mathrm{b}) - \rho(R_\mathrm{m})]C_\mathrm{B}(R,t)/C_{0,\mathrm{B}} \tag{5}$$

A similar equation is obtained with respect to sample A. $C_\mathrm{B}\ (R,t)$ is the concentration of sample B at distance R and time t and $C_{0,\mathrm{B}}$ the initial concentration at $t = 0$ in the region between $R = R_\mathrm{u}$ and $R = R_\mathrm{b}$. $\rho(R_\mathrm{b})$, and $\rho(R_\mathrm{m})$ are the densities of the pure substances A and B.

The diffusion coefficient D may be calculated with the well-known Stokes–Einstein relation:

$$D = kT/(3\pi\eta_1 d) \tag{6}$$

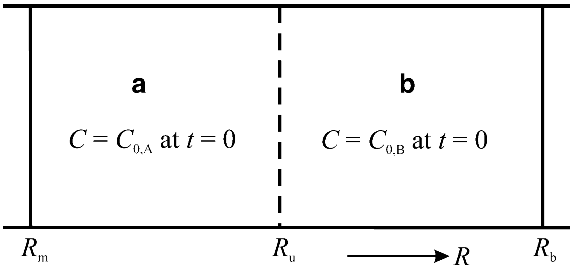


Fig. 1 Diffusion of two components A and B

where η_1 is the viscosity of the solvent and d the diameter of the particle. On the other hand D may be measured. In a couple of cases D is tabulated for various systems [8]. For the system H_2O/D_2O the situation is very comfortable because both the mutual diffusion coefficient of H_2O/D_2O and the densities of H_2O and D_2O are known [8, 9]: Mutual diffusion coefficient H_2O/D_2O at $T = 25$ °C: $D = 2.2 \cdot 10^{-5}$ cm^2/s [8]

Density of light water, H_2O at $T = 25$ °C: $\rho = 0.997$ g/cm^3 [9]

Density of heavy water, D_2O at $T = 25$ °C: $\rho = 1.105$ g/cm^3 [9]

If the diameter of the H_2O molecule could be assumed as 0.2 nm [10] and the viscosity of light water as 0.00893 g/(cm s) [8] the Stokes–Einstein relation, eq. 6 exhibits for the diffusion coefficient $D = 2.4 \cdot 10^{-5}$ cm^2/s; this value is in the same magnitude as the measured value of D from [8].

Testing of theoretical predictions

The diffusion model described in the previous section may be tested with the Analytical Ultracentrifuge OPTIMA-XLS with Schlieren optics which has been developed in the BASF factory [6]. According to Lange [5] a synthetic boundary cell is partly filled with pure H_2O and then underlayed with D_2O. The diffusion process can then be controlled and measured with Schlieren optics. The density profile is obtained according to [5]:

$$\rho(R) = \rho(R_\mathrm{m}) + [\rho(R_\mathrm{b}) - \rho(R_\mathrm{m})] \int_{R_\mathrm{m}}^{R} (\mathrm{d}n/\mathrm{d}R)\mathrm{d}R \Big/ \int_{R_\mathrm{m}}^{R_\mathrm{b}} (\mathrm{d}n/\mathrm{d}R)\mathrm{d}R \qquad (7)$$

where dn/dR is the physical quantity measured with Schlieren optics. To test the theoretical con-siderations mentioned above, a pure dynamic density gradient was performed in the AUC. Figure 2 shows a Schlieren photo taken after 9 min experimental time.

In Fig. 3 the calculated density profile and the experimentally obtained density profile are compared: Agreement is achieved within the experimental error.

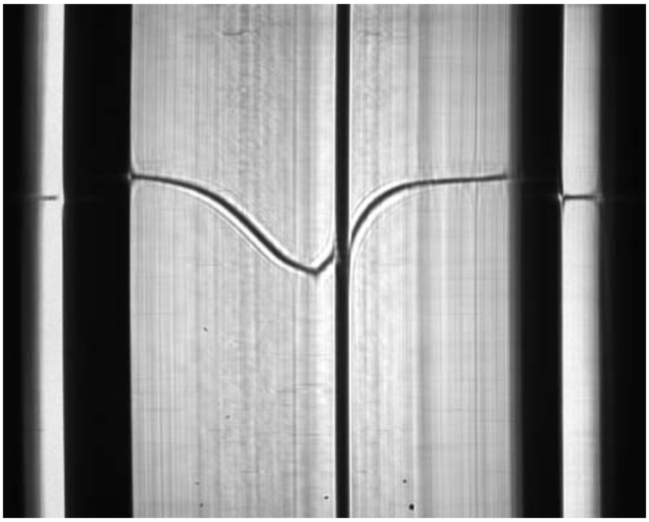


Fig. 2 Schlieren photo of H_2O/D_2O in the Analytical Ultracentrifuge OPTIMA-XLS, cell length: 1.2 cm, run time: 9 min, rotor speed: 40,000 min^{-1}, temperature: 25 °C, wavelength 546 nm

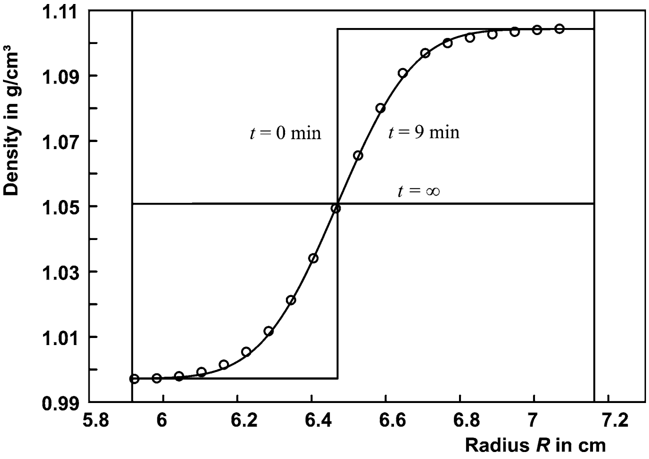


Fig. 3 Density profile of H_2O/D_2O in the Analytical Ultracentrifuge OPTIMA-XLS, Schlieren optics, cell length: 1.2 cm, run time: 9 min, rotor speed: 40,000 min^{-1}, temperature: 25 °C, wavelength 546 nm. *Lines* denote calculated curve according to eqs. 3, 4, and 5 and *open circles* denote experimental values

Transfer of theory to preparative scales

The dimensions of the preparative centrifugation tube are given in Fig. 4.

With the help of eqs. 3 and 5, the parameters of H_2O and D_2O (given in the previous section) and the dimensions of the centrifugation tube (Beckman, Palo Alto, USA) the density profile may be calculated. Figure 5 demonstrates the results at different times and Fig. 6 compares the calculated values with the experimental ones. The determination of the experimental values in the preparative centrifugation tube was done by stopping the centrifuge at different times, taking the tube out of the centrifuge and measuring the distance of the test sample from the center of rotation. Due to fairly large experimental errors the agreement is sufficient.

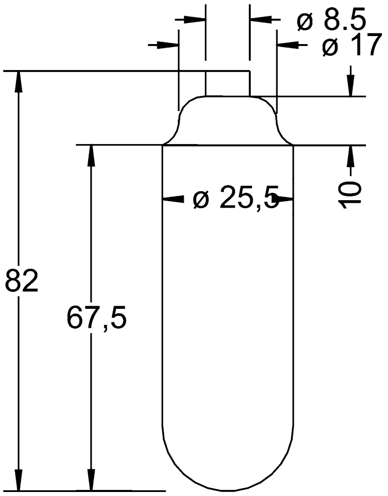


Fig. 4 Dimensions of the preparative centrifugation tube in mm

Experimental procedure

Creation of density gradient

Unlike the analytical dynamic density gradient in the case of the preparative dynamic density gradient the creation of the density gradient cannot be performed applying a synthetic boundary cell. Thus a special procedure had to be developed for PDDG.

The big advantage of such a synthetic boundary cell is that it allows overlaying of water onto the dispersed sample during centrifugation. The centrifugal field inside the spinning rotor stabilizes the meniscus, t. i. the air–water interface at the top of the dispersion column. This reaches the creation of a well-defined boundary between H_2O and D_2O. Therefore, special care had to be spent in the development of PDDG to ensure an undisturbed boundary.

Different possible procedures for dispersing the sample have been tested: the sample dispersed in the upper H_2O-, in the lower D_2O-phase and both phases. It can be summarized that in the case of the preparative dynamic density gradient experiment the best results are obtained when the sample is dispersed in the upper H_2O-phase. Very careful layering of D_2O under the dispersed sample in the described way creates the density gradient.

Preliminary Tests

As a first proof of the concept, blue ink and a polymeric dispersion were subjected to a PDDG experiment. Then ink was dissolved in the lower heavy water fraction, whilst the polystyrene particles were dispersed in the upper H_2O phase (Fig. 7).

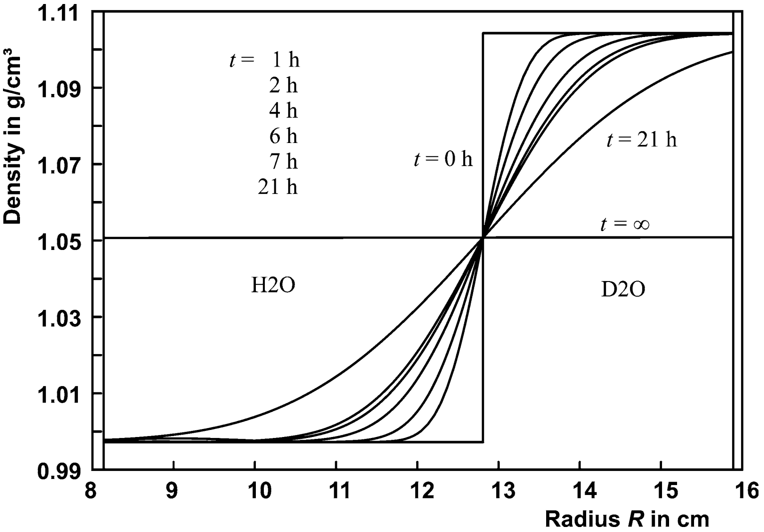
After 24 h of centrifugation the ink is distributed homogeneously through the cell, whereas the polystyrene particles are accumulated in a separate discoid band. This experiment proves that the cumulated sample did not disturb the diffusion of the ink assuming that the same will be true for the diffusion of the H_2O/D_2O solvent.

Secondly a sample of a 30 nm PS dispersion was analyzed in a PDDG experiment to demonstrate that the known density of a technical product can be reproduced by PDDG.

In Fig. 8 the pictures of the preparative tube after different experimental times are shown. The centrifugation was performed at 26,000 RPM, pursuant to a relative centrifugal field (RCF) of 90,000*g*.

In the first picture—taken before the start of the centrifugation—the effectual layering of D_2O becomes apparent. The picture after 3 h already shows the accumulation of the sample in one turbid band located approximately at the middle of the centrifugation tube.

Fig. 5 Calculated density profile of H_2O/D_2O in the preparative centrifugation tube at different diffusion times according to eqs. 3 and 5



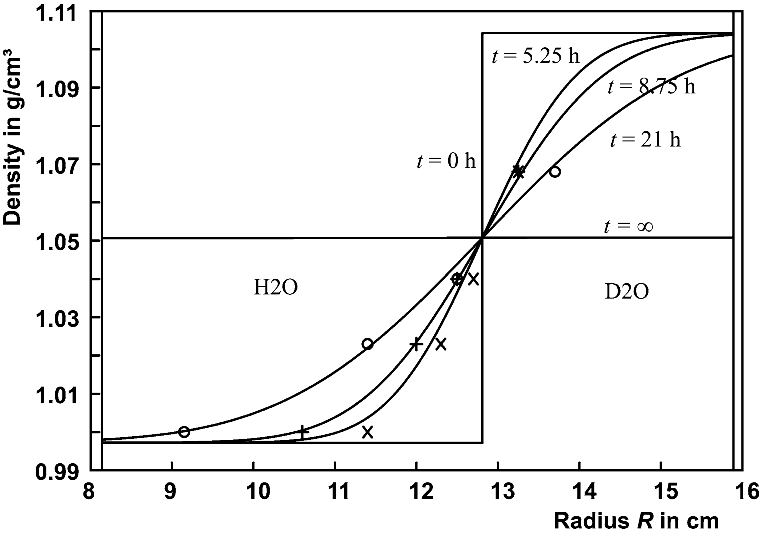


Fig. 6 Density profiles of H_2O/D_2O in the preparative centrifugation tube, run time: 7 h and 21 h, rotor speed: 26,000 min^{-1}, temperature: 25 °C. *Lines* indicate calculated curves according to eqs. 3 and 5 and *open circles* and *plus* indicate experimental values, ρ = 1.000, 1.023, 1.040, 1.068, 1.088 g/cm^3

The gradient of H_2O ($\rho = 0.997$ g/cm^3) and D_2O ($\rho = 1.105$ g/cm^3) is formed and the polymer accumulates at the position corresponding to its density. This means that even by eye it can be judged that the known density of the sample $\rho \approx 1.050$ g/cm^3 is found in this experiment as well. The turbid band splits up to form two separate fractions after 24 h. Remarkably the yellowish colour of the sample is located in just one of the fractions, whereas the other fraction is colourless. This suggests the existence of impurity in this lot of the technical product.

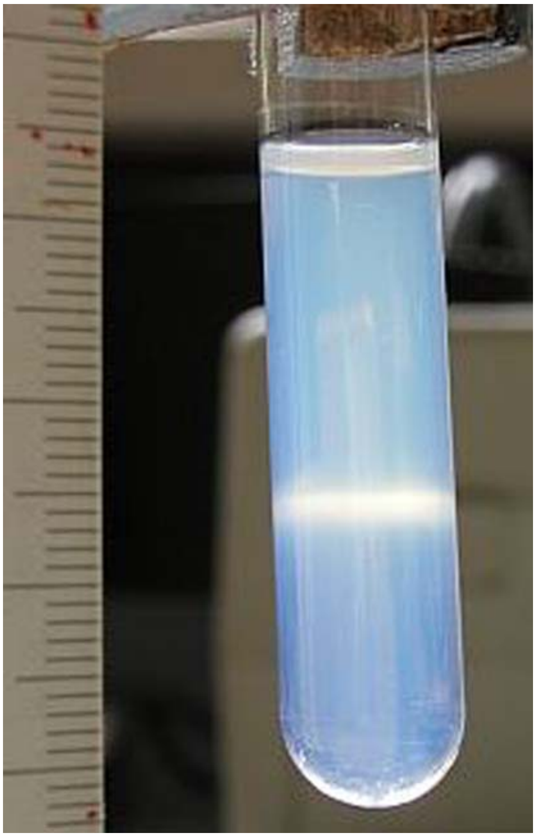


Fig. 7 Accumulation of polystyrene particles while ink is distributed homogeneously

Withdrawal of fractions

The most important feature of PDDG is that sufficient amounts of the density fractions can be isolated. The

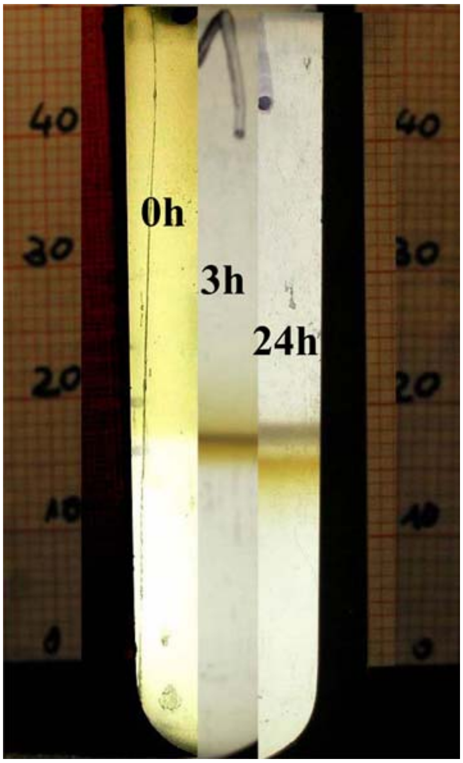


Fig. 8 Fine seed sample at different experimental times

Fig. 9 Cutting facility

Fig. 10 Pumping facility

sole fractions can then be subjected to further treatment. Many different approaches to the optimum method of isolating the fractions have been tested. Only the two most successful methods are addressed here: a) Withdrawal applying a home-made cutting facility. b) Withdrawal applying a home-made pumping facility.

The cutting facility is applicable when large amounts of the sample are sedimenting or flotating. A blade is driven through the centrifugation tube to separate the desired upper fraction from the lower rest. Successive cuts can be made to withdraw several fractions in distinguished heights (Fig. 9).

Due to leakiness and disturbances while cutting, this apparatus cannot be used for separating flat discoid accumulations. The pumping facility is made of a syringe containing oil, which is connected to the top of the centrifuge tube via a thin hose. When the oil is pressed into the tube it floats on the tube content and presses the lower sample through a thin steel tube from the bottom of the sample tube into a small glass bottle.

The spherically formed bottom of the centrifugation tube concentrates the discoid fractions to the entry of the steel tube. This is applicable for all low viscous fractions from bottom to top of the centrifugation tube.

The amount of withdrawn fractions and the yielded concentrations varies from some drops to several milliliters and from some grams per liter to pasteous consistence with polymer contents up to nearly 100%, depending on the property of the original sample.

In practice, one centrifugation tube with an average dispersion sample produces around 1–5 ml per fraction and concentrations from 0.5 to 10 g/l. One centrifugation run includes six preparative tubes, multiplicating the above-mentioned volumes (Fig. 10).

Example: PDDG on a mix of 11 chemically differing polymeric lattices

In order to demonstrate the capability of PDDG experiments one example may be given here with a mix of 11 chemically differing polymeric lattices. Eleven EHA/MA copolymer lattices have been used to demonstrate the fractionating ability of PDDG experiments. They are also used to calibrate the system and to compare results from PDDG to the theoretical predictions that arise from the theory described above.

The lattices exhibit differing chemical composition and therefore different particle densities (see Table 1). The characterization of these lattices has already been described in detail elsewhere [1]. The theoretical densities given in Table 1 were verified by measurements using a Paar DMA5000 Kratky-densitometer. The accordance is given within $\Delta\rho = \pm 0.003$ g/cm^3.

Table 1 Chemical composition of 11 lattices used to calibrate PDDG

No.	Chemical composition (mass %)		Theoretical density (g/cm^3)
	EHA	MA	
1	100	0	0.980
2	90	10	1.000 *
3	80	20	1.021 *
4	70	30	1.043 *
5	60	40	1.066 *
6	50	50	1.089 *
7	40	60	1.114
8	30	70	1.140
9	20	80	1.167
10	10	90	1.196
11	0	100	1.225

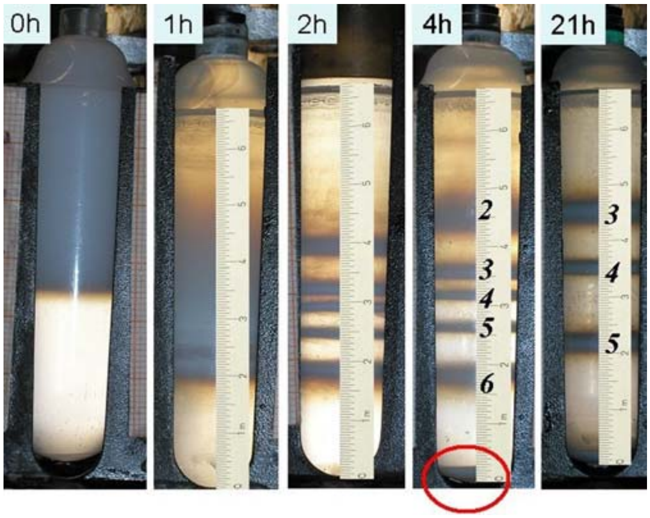


Fig. 11 PDDG experiment at different centrifugation times (at 90,000 g)

According to the densities of the particles five different fractions (*) are in the density range between 1.0 and 1.1 g/cm^3. Thus, we expect to find five fractions in the PDDG experiment. The other particles should either float or sediment out of the density gradient.

Figure 11 shows pictures of the PDDG experiment performed at the mixture of the 11 lattices. The pictures have been taken at different experimental times. The picture at 0 h experimental time again affirms the successful layering of D_2O under the sample. After 1 h nearly no effect is visible. The floating of the fractions exhibiting a density below 1.0 g/cm^3 is completed. After 2 h fractionation can be seen for the first time. The five expected fractions are already distinguishable. The fractionation becomes even better after 4 h. In this picture the sediment consisting of the five dispersions exhibiting a density $>$ 1.1 g/cm^3 is visible.

After 21 h the density gradient has flattened so that the two fractions consisting of samples no. 2 and 6 are not caught in the gradient but have already floated or sedimented. This example demonstrates the high fractionation power of the new technique impressively.

Summary and outlook

We present here the development of a new ultracentrifugation method called PDDG. It proves its capability to be a valuable tool in research and development on colloidal systems, especially polymeric dispersions.

Even small density differences are sufficient to separate the fractions for further analysis. The withdrawal of the fractions will be mechanized to earn more accuracy and to simplify the preparation. Other solvents will be tested to expand the density range to values smaller than 0.997 and more than 1.105 g/cm^3.

There is no principle limit to the available amount of density fractions. Several runs of the 6-hole rotor can be made and the withdrawn fractions can be added.

In some cases larger amounts of polymer can be separated with a sedimentation and floatation process. Thereby the density of the solvent must be adjusted to a value exactly between the two polymer densities. Added D_2O is used to trim the liquid phase to the desired density. An Excel sheet exists in the AUC laboratory to calculate the necessary amount of D_2O to be added.

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