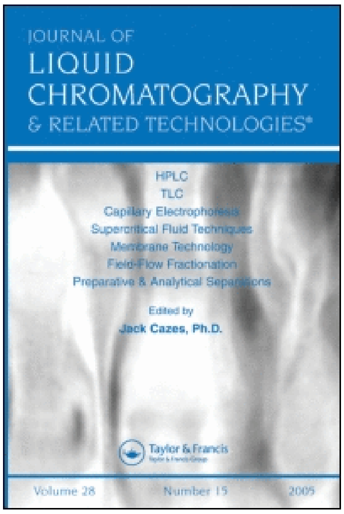

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SEPARATION AND DISPERSION IN SEDIMENTATION FIELD-FLOW FRACTIONATION

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ABSTRACT

Contributions of separation and dispersion to the total zone width of a polydisperse latex sample under various flow rates and retentions were studied. Both the direct flow and the reverse flow techniques were employed and the experimental results were compared with theoretical relationships. The results showed a good agreement between the theory and the experiment under different conditions. A good agreement between the mean particle diameters calculated from our experimental data and those given by the producer was found. The width of the particle size distribution characterized by the standard deviation was found about two fold higher than that given by the producer.

INTRODUCTION

Dispersion processes cause undesirable broadening in the solute zone in every separation. The original mixture of low molecular weight monodisperse substances is separated into discrete zones and dispersion operates against the separation processes. As a result, the zones of the separated species occur or, in an unfavourable case, the individual zones are not completely resolved. The latter case usually occurs when fractionating the macromolecules or particles. These substances are not monodisperse either from the molecular weight distribution or particle size distribution viewpoint, but the polydispersity in the distribution is typical for them. The individual components of such polydisperse mixture of macromolecules of particles cannot be separated into discrete zones. Thus the result of separations including Field-Flow Fractionation (FFF) is a wide zone. Its shape and width are the result of both separation and dispersion processes. This zone passes through a detector situated at the end of the separation system. The record of the detector (fractogram) contains the quantitative information on both of these processes and, implicitly, the information on molecular weight distribution or particle size distribution of the fractionated sample. However, the accurate and precise evaluation of molecular weight distribution or particle size distribution is possible only if the contribution of the dispersion causing the broadening of the resulting fractogram is taken into account.

This study was aimed to evaluate in detail the contributions of separation and dispersion by applying various experimental procedures as well as to compare the results of these experiments with the theory. The Sedimentation Field-Flow Fractionation (SFFF) was chosen as a model system. However, the results hold in general for all the other techniques of FFF.

THEORY

Theoretical relationships describing the retention ratio as a factor of separation and the height equivalent to a theoretical plate as a factor of dispersion were used for the quantitative evaluation of the separation and dispersion in SFFF. The retention ratio R is given (1) by a relationship

$$R = 6\lambda\,(\coth\,(2\lambda)^{-1} - 2\lambda) \qquad (1)$$

where the reduced layer thickness λ is the ratio of the mean thickness of the solute zone to the thickness of the channel for FFF. From the experimental point of view, R expresses the ratio between the retention volume of the unretained solute V_o and the retention volume of the retained solute V_R, thus

$$R = V_o/V_R \qquad (2)$$

V_o equals the volume of the fractionation channel. It holds (2) for λ in SFFF

$$\lambda = 6\ kT/(\pi d_p^3\ g\ w\Delta\rho) \qquad (3)$$

where k is the Boltzmann constant, T is the absolute temperature, d_p is the diameter of the spherical particles, g is the centrifugal acceleration, w is the channel thickness and $\Delta\rho$ is the difference between the density of the particles and of the carrier liquid. The dispersion is quantitatively characterized by the height equivalent to a theoretical plate H (1)

$$H = 2D/(R\langle v\rangle) + \chi w^2\langle v\rangle/D + \sum H_i = \sigma^2/L \qquad (4)$$

where D is the diffusion coefficient of the solute, <v> is the mean linear velocity of the carrier liquid, χ is the dimensionless parameter, σ is the standard deviation of the eluted zone, and L is the length of the fractionation channel. The first term in Equation (4) describes the longitudinal diffusion, the second one nonequilibrium processes, and the third one describes the sum of various contributions such as the extrachannel zone broadening and the contribution of relaxation processes. The dependence of the dimensionless parameter χ on λ was derived by Giddings et al. (3)

$$\chi = \frac{24\,\lambda^3 \cdot B}{1 + e^{-1/\lambda} - 2\,\lambda\,(1 - e^{-1/\lambda})} \qquad (5)$$

where

$$B = (28\,\lambda^2 + 1)(1 - e^{-1/\lambda}) - 10\lambda\,(e^{-1/\lambda} + 1) - \frac{1}{3\,\lambda^2} - \frac{2}{\lambda} + 4 - \frac{1/\lambda}{(1 - e^{-1/\lambda})}\left[4\lambda\left(1 + \frac{1/\lambda}{(1 - e^{-1/\lambda})}\right) - \frac{1}{3\lambda} - 6\right] \qquad (6)$$

The diffusion coefficient D for rigid spherical particles can be calculated from Stokes-Einstein equation

$$D = kT/(3\pi\eta\, d_p) \qquad (7)$$

where η is the viscosity.

The above relationships were derived for an idealized channel formed between two infinite parallel planes. The real channel has, of course, the finite width and thus a given ratio of the thickness to the width - so called "aspect ratio". This study will show how reliably the real experimental system is described by the idealized theoretical model.

EXPERIMENTAL

The rotor for SFFF was described in detail in our recent paper (4), as well as the other equipment. The fractionation channel inside the rotor had the thickness w = 0.02 cm, the length L = 48.4 cm, and the volume V_o = 1.8 ml.

Using the direct flow technique, the sample is injected into the channel and eluted unidirectionally. Then the width of the fractogram is the result of both separation and dispersion of a polydisperse sample. When using the reverse flow technique, the direction of the flow inside the channel is reversed at a given moment (before the front of the sample zone begins to leave the channel) and the sample is eluted from the channel in the reverse direction. It means, that the fastest particles (those which travelled the longest distance inside the channel during the first part of the elution) are again the fastest, and, since they travel the same distance back, they leave the channel together with the particles moving more slowly. The separation processes are eliminated in this way and the fractogram is the result of the dispersion processes only. This technique is used for similar purposes in steric exclusion liquid chromatography (5), few results were obtained also with SFFF (6).

Applying the reverse flow technique, another six-port valve was inserted into the hydraulic circuit behind the six-port injection valve. This second valve permitted to reverse the flow inside the channel in the course of the experiment. A diagram of the hydraulic circuit with the six-port valve for reversing the flow can be seen in Figure 1. In the course of reverse flow experiments, the flow rate was reversed when the

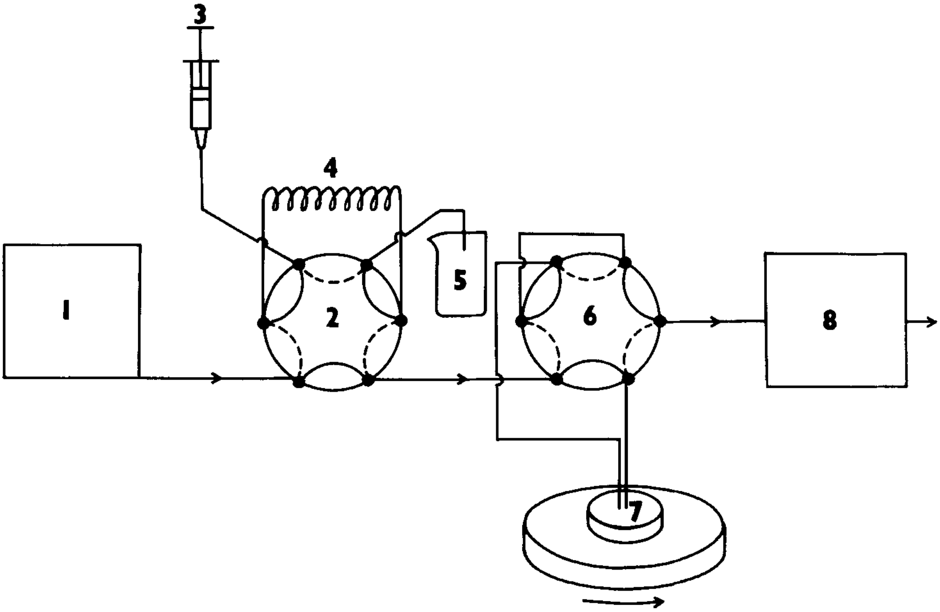

FIGURE 1. Schematic representation of the separation system for reverse flow experiments.
1-pump, 2-injection valve, 3-syringe, 4-loop, 5-waste, 6-valve for reversing the flow, 7-rotor, 8-detector.

elution volume reached one half of the value of the mean retention volume of standard latex sample measured by direct flow experiment under identical conditions. The resulting mean retention volume in reverse flow experiments was nearly the same as in direct flow experiments.

0.1 % solution of Tween 60 surfactant (Fluka AG, Buchs SG, Switzerland) in distilled water was utilized as the carrier liquid. The solution was degassed before use by heating up to 90 °C and by the action of ultrasound. Polystyrene standard latex (Duke Scientific, Palo Alto, California) was used for the study. The producer's data for this standard are: mean particle diameter $\bar{d}_p$ = 109 nm, standard deviation of the distribution width - 2.5 %, and density - 1.05 g/cm^3. The volume of 30 μl of this standard latex suspension of about 0.05 % concentration was injected. Prior to the injection, the sample was shortly (60 sec) agitated with ultrasound in order to destroy the occasionally formed aggregates. The measurements were effectuated at flow rates of 100, 200, 400 and 1000 μl/min and under various rotation velocities of 3900, 4400, 5500 and 6000 rpm that corresponded to the accelerations of 1343, 1710, 2671 and 3179 gravities. The temperature during measurements was kept at 21 ± 0.5 °C. In our previous papers we studied the extrachannel contributions to the zone spreading (4) and the influence of the relaxation processes on both the zone spreading and retention (7). We have found out that employing our device for SFFF, the extrachannel elements of the separation system, i.e., injector, rotation passage and detector, contribute by less than 1 % to the total zone width, i.e., within the limit of experimental errors (4). Consequently we neglected this extrachannel contribution in further calculations. The contribution of the relaxation processes to the zone spreading can be minimized to a level comparable with experimental errors by a suitable mode of injection followed by stopping the flow. The sample is injected under a very slow flow rate of 50 μl/min. When all the sample volume is safely at the beginning of the channel, the flow rate is stopped at a constant rotation for a time permitting to reach the state distant by about 1 % from the equilibrium. The stop--flow period always took 10 minutes. Then the flow rate is restarted at some of the above nominal values. Under these conditions and using the mentioned device for SFFF it holds for the last term in Equation (4) $\Sigma H_i = 0$.

A detailed description of the whole experimental arrangement for SFFF (except the rotor) can be found in our previous paper (7).

RESULTS AND DISCUSSION

The first series of the measurements was aimed to find out experimentally the dependence of the height equivalent to a theoretical plate for variously retained solute on the linear velocity of the carrier liquid. This dependence was compared with the theory. The dimensionless parameter λ can theoretically be calculated either from Equation (3) if the correct and precise values of d_p and $\Delta \rho$ are given by the producer of the standard latices, or it can be calculated from SFFF experimental data.

We preferred to use the values of λ , calculated from Equation (1) by means of iteration method, utilizing our experimental data for the mean values of $\bar{R}$. The mean values of $\bar{R}$ were calculated from the relationship

$$\bar{R} = \Sigma h_i / \Sigma (h_i / R_i) \qquad (8)$$

where h_i are the heights of the fractogram in the elution volume V_i corresponding to the retention ratio R_i. The mean retention volume $\bar{V}_R$ is defined by

$$\bar{V}_R = \Sigma (V_i \ h_i) / \Sigma h_i \qquad (9)$$

The experimental values of $\bar{R}$ were somewhat dependent on the flow rate (see Figure 2). This dependence can be caused by lift-forces observed by Giddings et al. (8). The values of $\bar{R}$ extrapoled by

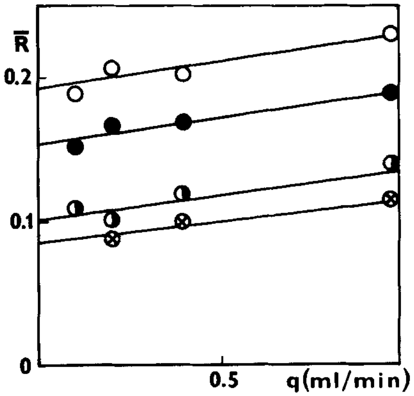

FIGURE 2. Dependence of the mean retention ratio $\overline{R}$ on the flow rate q for various rotations.
o 3900 rpm, ● 4400 rpm, ◑ 5500 rpm, ⊗ 6000 rpm.

linear regression to the zero flow rate were utilized for further calculations. Figure 3 illustrates the theoretical linear dependences of H on <v> and the experimental values of H_{df} (from direct flow measurements) at given flow rates. The experimental values of H_{df} were calculated from equation

$$H_{df} = L\Sigma((V_i - \overline{V}_R)^2 h_i)/(\overline{V}_R^2 \Sigma h_i) \tag{10}$$

As can be seen from Figure 3, the experimental dependences of H_{df} vs. <v> are paralel to the theoretical ones. However, the experimental values of H_{df} are higher. This is caused (at least partially) by the separation of the polydisperse latex sample. We calculated the differences between the experimental values of H_{df} and the theoretical values of H.

$$\Delta H = H_{df} - H \tag{11}$$

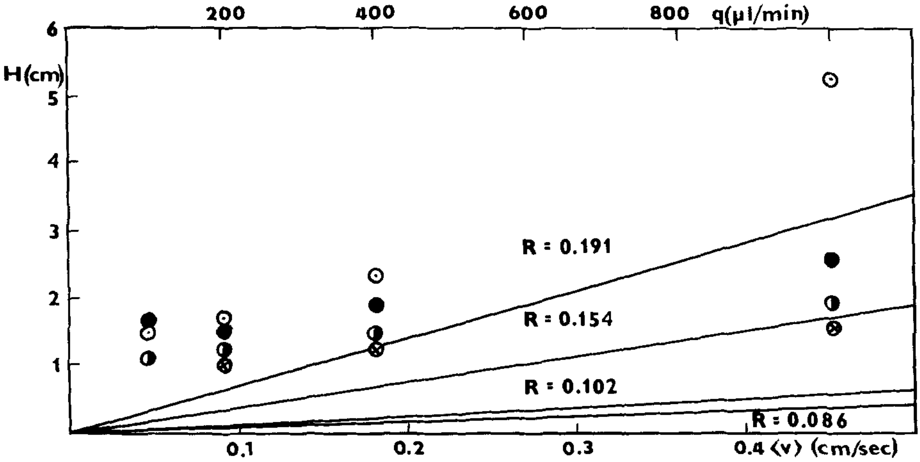

FIGURE 3. Dependence of the height equivalent to a theoretical plate H on the carrier fluid velocity ⟨v⟩ (or on the flow rate) for variously retained solute. Solid lines illustrate the theoretical dependence. Experimental points correspond to various retention ratios. Designation as in Figure 2.

These differences are summarized in Table 1. The values of ΔH in Table 1 are almost identical for various retentions within the limits of experimental errors which proves high reproducibility and reliability of SFFF measurements.

From the principal physical viewpoint it is not correct, to consider ΔH as a polydispersity contribution to the zone spreading because of real separation processes. On the other hand the height equivalent to a theoretical plate always expresses the dispersion processes. However, from a formal viewpoint, ΔH can be interpreted as measure of polydispersity of the sample studied.

In the second series of the measurements the reverse flow technique was applied. The values

TABLE 1

Height Equivalents to a Theoretical Plate, Mean Particle Diameters and Standard Deviations of Particle Size Distribution Calculated from Direct and Reverse Flow Experiment Data

Rotation (rpm)	Flow rate (μl/min)	ΔH (cm)	$\Delta H'$ (cm)	$\bar{d}_p$ (nm)	$\sigma_{d,rel}$ (% rel)	$\Delta\sigma_{d,rel}$ (% rel)
3900	100	1.2		119	6.6	5.8
	200	1.1		116	7.2	5.4
	400	1.1		116	8.2	5.4
	1000	1.9		113	14.2	7.5
4400	100	1.5		119	6.7	6.3
	200	1.3	0.8	116	6.6	5.8
	400	1.2	0.8	115	7.3	5.6
	1000	0.9	0.8	110	8.7	4.9
5500	100	1.1		115	5.4	5.2
	200	1.2	1.1	118	5.7	5.4
	400	1.2	1.1	112	6.0	5.5
	1000	1.4	1.1	105	7.3	6.0
6000	200	1.1		115	5.2	5.1
	400	1.2		112	5.8	5.5
	1000	1.3		107	6.5	5.7

of H_{rf} obtained, correspond only to the dispersion processes inside the channel, i.e., to the second term in Equation (4). These measurements were effectuated only at 4400 and 5500 rpm and at flow rates of 200, 400 and 1000 μl/min. The latex sample was injected several times and the flow was reversed in various moments to verify whether the behavior of the solute under the reverse flow experiments corresponds to the theory. It means that the zones of the solute were at various distances from the injection point in the moment when the flow was reversed. The dependence of the fractogram width, expressed as the square of the standard deviation σ^2, on the mean retention volume should be linear if the channel is homogeneous and each of its incremental length contributes uniformly to the final spreading. In an ideal case, the extrachannel contributions to dispersion are negligible and this dependence should intersect the origin of coordinates. Figure 4 demonstrates the experimental results. Both of the linear dependences obtained by the measurement at 4400 and 5500 rpm intersect the axis of σ^2 very close to each other and close to zero.

The values of $\Delta H'$ represent the differences between the direct flow experimental data H_{df} and H_{rf} values calculated from the results of the reverse flow experiment

$$\Delta H' = H_{df} - H_{rf} \tag{12}$$

The corresponding values of H_{rf} were calculated using an identical relationship as Equation (10). Thus obtained raw H_{rf} values were recalculated by interpolation inside the interval of the experimental retention volumes shown in Figure 4, in order to

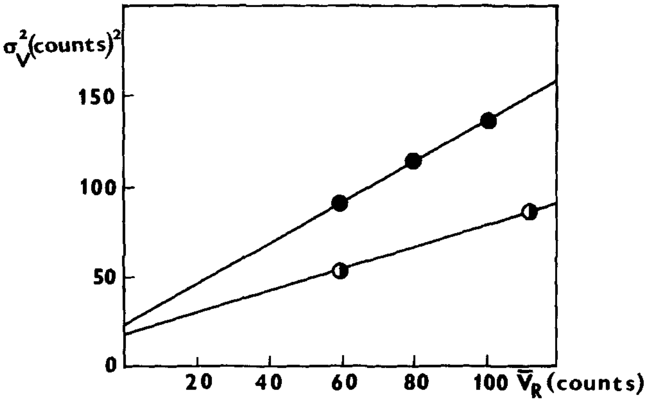

FIGURE 4. Dependence of the fractogram variance σ_V^2 on the mean retention volume in reverse flow experiment for various rotational speeds (designation as in Figure 2) and under flow rate of 200 μl/min (1 count = 0.129 ml).

obtain the corrected values of H_{rf}, corresponding exactly to the retention volumes in direct flow experiment. With regard to the slope of the linear dependences on Figure 4, these corrections were almost negligible.

The calculated values of H' are also given in Table 1. Although the values of $\Delta H'$ are lower than the difference between the experiment and the theory, a significant quantitative agreement can be found.

To characterize the particle size distribution the value of the weight average particle diameter $\bar{d}_p$, was calculated from the relationship

$$\bar{d}_p = \sum d_i \, h_i / \sum h_i \tag{13}$$

where d_i is the value of the diameter of a particle eluting in i-th elution volume. The width of the particle size distribution was characterized by percent standard deviation $\sigma_{d,rel}$ calculated from the relationship

$$\sigma_{d,rel} = (\Sigma(d_i - \bar{d}_p)^2 h_i/\Sigma h_i)^{1/2} \cdot 100/\bar{d}_p \qquad (14)$$

In this way we obtained the values of $\bar{d}_p$ and $\sigma_{d,rel}$ directly from the original fractograms. Further we calculated the values of $\Delta\sigma_{d,rel}$. The calculations were based on the values of ΔH, the contributions of the dispersion process being subtracted. The values of the standard deviations of the fractograms in the retention volume units were calculated first

$$\Delta\sigma_V = \bar{V}_R\ (\Delta H/L)^{1/2} \qquad (15)$$

and then converted to the relative percent standard deviations of the particle size distribution $\Delta\sigma_{d,rel}$. To convert $\Delta\sigma_V$ into $\sigma_{d,rel}$ Equation (2) was used first to calculate ΔR corresponding to ΔV_R from $\Delta\sigma_V$, and then Equations (1) and (3) were used to calculate $\sigma_{d,rel}$ by iterations. All the calculated values of $\bar{d}_p$, $\sigma_{d,rel}$ and $\Delta\sigma_{d,rel}$ are given in Table 1. From the results, a conclusion can be drawn, that the calculated values of $\bar{d}_p$ are in good agreement with the data of the producer. Coincidentally, the influence of lift forces can be observed (the values of $\bar{d}_p$ decrease with increasing flow rate). The values of $\sigma_{d,rel}$ uncorrected for dispersion increase with increasing flow rate under the given rotational speed and they decrease with increasing retention under the given flow rate, both in agreement with the theory. The values of $\Delta\sigma_{d,rel}$ remain constant within the limits of experimental errors under all rotations and flow rates. Only the value of $\Delta\sigma_{d,rel}$ at 3900 rpm and 1000 μl/min differs greatly. In this case a not negligible part of the sample elutes unretained at the void volume of the channel V_o. The numerical calculation using Equation (14) could not be applied, but the standard graphical evaluation of $\sigma_{d,rel}$ from the intersection of tangentials in inflexion points of the fractogram with the base line was possible.

It is necessary to remark that we have neglected the correction for the detector response. The values of h_i should be corrected as the response of the detector is a function of both the mass concentration and the size of the particles. However, the neglecting of this type of the correction was justifiable with respect to the narrow particle size distribution of the latex used for the present study.

CONCLUSIONS

1. The course of the experimental dependence of the height equivalent to a theoretical plate on the linear velocity of the carrier fluid flow is in good agreement with the theoretical relationships if the separation of the standard polydisperse latex sample is considered and if the extrachannel contribution and the contribution of the relaxation processes to the total dispersion are minimized. This agreement was found for various retentions of the given solute.

2. The zone width formally characterized by ΔH, corresponding to the separation processes only, is the same within the limits of experimental errors if the theoretical relationships or the experimental data of the reverse flow experiments were used for the calculation.

3. The standard deviation of the particle size distribution calculated after subtracting the

contribution of the dispersion processes is the same, within the limits of experimental errors, for various retentions and flow rates. However, it is twice as big compared with the data given by the producer.

4. All the mentioned results of both the direct and reverse flow experiments proved a good agreement with the theoretical relationships and thus, in fact, confirmed the validity of the theory of both the retention and the dispersion in SFFF.

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