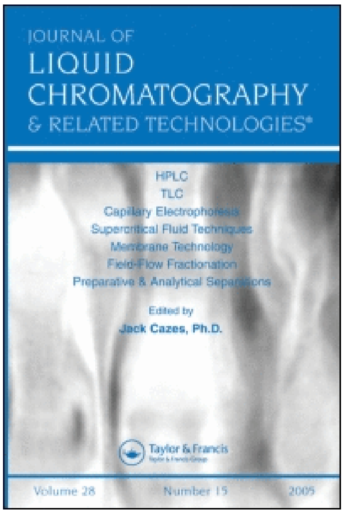

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OPTIMIZATION OF FIELD-FLOW FRACTIONATION WITH RESPECT TO RELAXATION AND USE OF STOP-FLOW TECHNIQUE AT CONSTANT FIELD OPERATION

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ABSTRACT

The relaxation phenomena developing after the injection of the sample into the channel during Field-Flow Fractionation were analyzed theoretically. Relaxation times, necessary to stop the flow immediately after the injection, were calculated on the basis of various models describing the relaxation processes. A stop-flow time long enough so that the deviation from the equilibrium would not influence the retention within the limits of experimental errors and so that the maximum efficiency would be reached was required. Sedimentation Field-Flow Fractionation was used to verify the theoretical analysis. The diffusion coefficient of latex used for the study is small and thus the long relaxation times allow to reach sufficient precision of the measurement. The experiments performed at different flow rates and using different ways of sampling proved a good agreement between the experimental relaxation times and the values calculated theoretically. The stop-flow technique was proved both theoretically and experimentally to be advantageous from the viewpoint of shortening the time of fractionation

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The disagreement with the previous experimental data obtained by other authors was explained. There was a good agreement between our experimental data and the theoretical predictions. Field-Flow Fractionation using the stop-flow technique at the constant field operation was found to be very suitable for exact physico-chemical measurements.

INTRODUCTION

Field-Flow Fractionation (FFF) has progressed rapidly in the recent years both in theory and in practical applications. This progress is naturally accompanied by the appearance of the new problems that have to be solved. One of them is related to the relaxation processes.

Immediately after the injection of the sample into the channel, the solute is expected to be distributed homogeneously across the channel thickness. Only due to the action of the field does the concentration gradient across the channel thickness start its formation until the steady state is reached.

The distribution of the solute concentration across the channel thickness (in the direction of x-axis), c(x), in steady state can be described by (1)

$$c(x) = c_o \exp\ (-x/l) \qquad (1)$$

where l is the distance of the centre of gravity of the solute distribution from the accumulation wall of the channel, c_o is the solute concentration at accumulation wall (x = 0). The average velocity of the zone movement in the longitudinal direction z along the channel is given by

$$V = \langle c(x)\rangle\langle v(x)\rangle/\langle c(x)\rangle \qquad (2)$$

where v(x) is the actual velocity of the streamline at the coordinate x, brackets $< >$ designate the average values. Retention ratio R is then defined as a ratio of the average velocity of the solute zone movement along the channel to the average velocity of the fluid by

$$R = V/\langle v(x)\rangle = \langle c(x) \cdot v(x)\rangle / \langle c(x)\rangle\langle v(x)\rangle \quad (3)$$

For the isothermal, isoviscous flow of a Newtonian liquid between the two parallel infinite planes that is not affected by any outer field, it applies that

$$v(x) = \Delta Px(w - x)/2L\mu \quad (4)$$

where ΔP is the pressure drop along the channel of the length L, the thickness w is the distance between the walls of the channel, and μ is the viscosity of the liquid. For the average velocity it holds

$$\langle v(x)\rangle = \Delta Pw^2/12L\mu \quad (5)$$

The solution of the Equation (3) then gives (2)

$$R = 6\lambda\left[\coth(2\lambda)^{-1} - 2\lambda\right] \quad (6)$$

where $\lambda = l/w$. This equation is a basic theoretical relationship that describes quantitatively the retention in FFF. On the other hand, zone spreading in FFF is characterized quantitatively by the height equivalent to a theoretical plate (2)

$$H = \sigma^2/L = 2D/R\langle v(x)\rangle + \chi w^2\langle v(x)\rangle/D + \sum H_i \quad (7)$$

where σ is the standard deviation of the concentration zone at the end of the channel, D is the diffusion coefficient of the solute, and χ is a dimensionless parameter. The first term on the RHS in Equation (7) describes the longitudinal diffusion, the second one the nonequilibrium effects and the third one the sum of different contributions following from relaxation processes, finite width of the injection, etc. In the limit when λ tends to zero, it holds simply (2)

$$\lim_{\lambda \to 0} \chi = 24\lambda^3 \qquad (8)$$

The time period till the approach of the steady state is called the relaxation time. Relaxation processes contribute to zone spreading according to the relationship (1,2)

$$H_r = (17\ n/140L)\ (\lambda w^2 \langle v(x) \rangle / D)^2 \qquad (9)$$

where H_r is the contribution to the height equivalent to a theoretical plate due to relaxation processes, n is an effective number of relaxation processes along the channel, usually equal to one. While increasing the total zone spreading undesirably, this contribution can be eliminated by stopping the flow through the channel after the injection for the time period that makes it possible to approach the steady state or quasiequilibrium.

Relaxation processes occurring after the injection of the solute obviously affect also the retention. The relaxation time of the solute, t_r, was defined by Giddings et al. (1) as the time necessary for overcoming the distance between the centre of the channel and the centre of gravity of the quasiequilibrium zone. Then the relationship between the apparent retention ratio, R', and the theoretical equilibrium R, i.e., not including the relaxation processes, is given by (2)

$$1/R' = 1/R - n\langle v(x) \rangle t_r (1-R)/RL \qquad (10)$$

and the relaxation time is given by (2)

$$t_r = \frac{w^2\lambda}{D}\left[\frac{1}{2} - \lambda + (\exp(1/\lambda) - 1)^{-1}\right] \qquad (11)$$

The choice of the above relaxation distance is rather arbitrary. In practice, it depends also on the arrangement of the given FFF experiment. Actually, in another paper by Giddings and co-workers (3), this distance is defined as the whole thickness of the channel w, and consequently t_r is expressed as

$$t_r = \frac{w^2\lambda}{D} \qquad (12)$$

by neglecting the second and the third terms in rectangular brackets of the right hand side of Equation (11). In fact, each of the above simple equations is only an approximation to the real situations after the injection of the sample into the channel. If the flow is not stopped after the injection of the sample for a period of time necessary for relaxation, a zone of solute with two concentration maxima along the z axis is formed. The resulting fractogram also has two maxima. Their ratio is given by the ratio between the transversal flux and the longitudinal flow of the solute inside the channel (2). This theoretically foreseen phenomenon (2) was experimentally proved in the paper by Yang, Myers and Giddings (4) who studied Flow Field-Flow Fractionation. These authors reached a very good qualitative agreement between the theory and the experiment. However, the quantitative evaluation of the results indicated important differences.

Kirkland and co-workers (5) investigated the time necessary to obtain the retentions not influenced by relaxation when using the stop-flow technique. Their experimental results indicated much longer times than expected on the basis of the above simple equations. Jayaraj and Subramanian (6) published a detailed theoretical study of relaxation phenomena in FFF. Using a numerical method of finite differences, they were able to model the process occurring in the FFF channel in various phases of the zone movement. Their results indicated a complex dependence of the concentration profiles across the channel on the axial coordinate during the relaxation. The use of the stop-flow procedure was not considered in their paper. Smith (7) has found theoretically the pretreatment stop-flow to be very effective procedure that can improve the separation. This conclusion was based on the differences found between the fractograms calculated for the equilibrium relaxed initial distribution of the solute and those calculated for the uniform initial concentration of the solute across the channel.

Quite obviously, an additional study, both theoretical and experimental, is needed to evaluate the relaxation phenomena and the related stop-flow method in order to optimize FFF experimental conditions.

THEORY

The time dependence of the relaxation processes

If the solute after the injection is distributed homogeneously across the channel, the time necessary to reach the theoretical equilibrium, characterized by exponential distribution (Equation 1), will be infinitely long. Thus in practice we are interested to know how the deviation from the equilibrium will influence the retention and the zone spreading, or, how far from the equilibrium we can work to keep the retentions and the zone spreading uninfluenced within the limits of experimental errors. Mason and Weaver (8) have already studied the distribution of the solute in the dependence on the relaxation time and thus on the deviation from the equilibrium. They derived an equation for the distribution of the sedimenting solute $c(\rho,t)$.

$$\frac{c(\rho,t)}{c_o} = \frac{e^{\rho/\lambda}}{\lambda(e^{1/\lambda}-1)} + e^{\rho/2\lambda} \sum_{m=1}^{\infty} T_m(t)\Big[\sin(m\pi\rho) + 2\pi m\lambda \cos(m\pi\rho)\Big] \qquad (13)$$

where

$$T_m(t) = \frac{16\lambda^2\pi e^{(-(\lambda m^2\pi^2 + \frac{1}{4}\lambda)\ t/\beta_m)}\big[1-(-1)^m\ e^{-1/2\lambda}\big]}{(1 + 4\pi^2 m^2\alpha^2)^2} \qquad (14)$$

The meaning of the symbols in Equations (13) and (14) is the same as above, $\rho = x/w$ a $\beta_m = \lambda w^2/D$. Developing the theory further, Weaver (9) obtained the relations for time t_ε necessary to reach the state distant from the equilibrium by the factor ε

$$t_\varepsilon = w^2 F(\lambda)/D \qquad (15)$$

The function $F(\lambda)$ is given by the relation

$$F(\lambda) = -\frac{1}{\pi^2 U(\lambda)} \ln\Big[\frac{\pi^2 U^2(\lambda)\varepsilon}{4(1+\cosh(1/2\lambda))}\Big] \qquad (16)$$

and it holds for $U(\lambda)$

$$U(\lambda) = 1 + (1/4\pi^2\lambda^2) \qquad (17)$$

The factor ε is defined (10) by the relation

$$\varepsilon = (\Delta c_{eq} - \Delta c_t)/\Delta c_{eq} \qquad (18)$$

or by the relation

$$\varepsilon = 2\pi\lambda^2 \sum_{m=1}^{\infty} m\, T_m(t) \left[1 - (-1)^m e^{1/2\lambda} \right] \qquad (19)$$

It follows from Equation (18) that the factor ε expresses quantitatively a fractional relative deviation of the concentration difference Δc_t in time t, per distance w, i.e., in coordinates x=0 and x=w; $\Delta c_t = c_{x=0} - c_{x=w}$, and of the concentration drop Δc_{eq} in equilibrium. Thus the factor ε acquires the values from 0 to 1. Let us note the similarity of Equation (15) and Equations (11) and (12), all of them describing various retention times.

Yau and Kirkland (11) have discussed the causes of deviations of experimental retention data from the equilibrium theory for both the high-speed separations with the constant field force, as well as for the use of time-delayed exponential force field decay in FFF. They have introduced a partial differential equation describing the transport phenomena in the channel, however, they have not given a detailed derivation of their kinetic theory (11).

The relaxation time

First of all, let us notice the difference between the relaxation times calculated from Equation (12) in comparison with the relaxation times for different deviations from the equilibrium, ε, calculated from Equations (15)-(17). Fig. 1 shows the results of this comparison as the dependence of the ratio $F(\lambda)/\lambda$ in the range of retentions most often used in practice (λ=0.01-0.2) and for different values of ε. It is evident, from Fig. 1, that in the proximity of the equilibrium, it means for $\varepsilon \leq 0.05$, the ratio $F(\lambda)/\lambda$ (or the ratio of the retention times

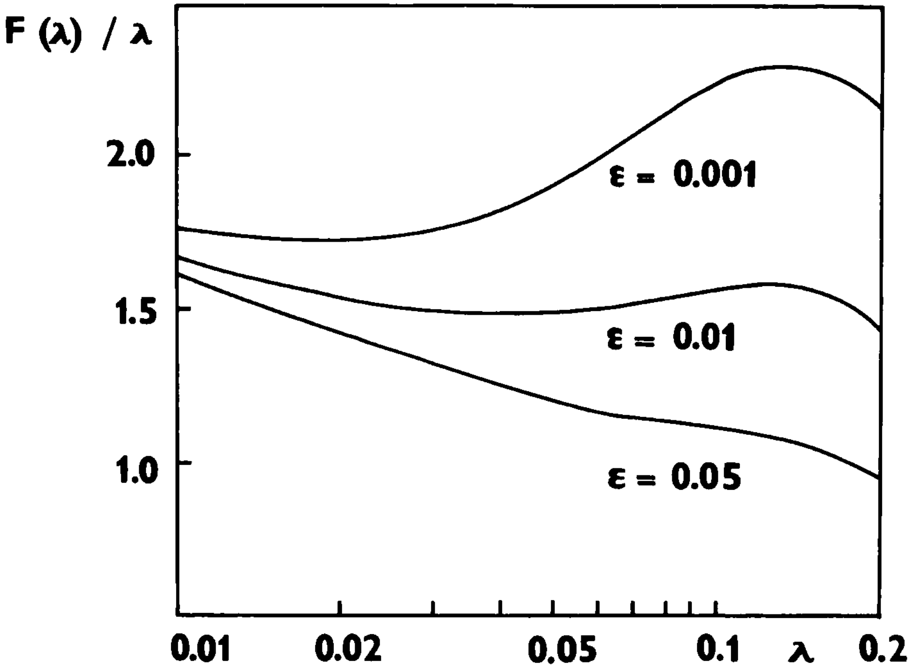

FIGURE 1. Dependence of $F(\lambda)/\lambda$ ratio on the value of λ for various deviations from the equilibrium ε (see Equations (16) and (17)).

calculated according to the given relations) is always more than 1. For example, for the value $\varepsilon=0.01$ (i.e., for a relative deviation of 1 % from the equilibrium) the average value is $F(\lambda)/\lambda \doteq 1.5$, within the range of $0.01 \leq \lambda \leq 0.2$. If Equation (11) instead of Equation (12) (with the constant value of 1 instead of 1/2 in square brackets) is used to express the relaxation time, then the calculated ratio $F(\lambda)/\lambda$ is even higher especially for higher values of λ. It is due to the relaxation time calculated for the particle relaxing the distance from the farther wall of the channel to the centre of gravity, instead of the distance from the centre of the channel to the centre of gravity of the solute equilibrium distribution across the channel.

Relaxation without the stop-flow

Equation (10) can be rearranged

$$t_o = t_r(1-R)/\delta \qquad (20)$$

where $t_o = L/\langle v(x)\rangle$ is the mean elution time of the unretained solute and $\delta = 1 - (R/R')$ is the relative change of the retention ratio caused by the relaxation processes. If the change in the retention has to be within the limits of experimental errors in the FFF separation without the stop-flow, the relative change should not exceed the error in the determination of R. Regarding both the contemporary technical possibilities of instrumentation and the experimental experience the precision of the determination of R can be considered approximately ± 1 % relative.

Then it follows from Equation (20) that it will approximately hold $t_o = (10 \text{ up to } 100) \cdot t_r$ within the practically employed range of retentions (ca. $0.02 \leq R \leq 0.9$). It means that the total time of the separation will be many-fold longer than t_r.

Relaxation with the stop-flow

It is evident that if the injected solute is relaxed at the stopped flow, the total time of the FFF separation can be essentially reduced. In a real experiment, the solute will be relaxed at the stopped flow until the required deviation from the equilibrium is obtained and then it will be eluted at an essentially higher velocity. It is possible to calculate the difference between the theoretical equilibrium retention ratio R and the retention ratio

R_ε if the solute is relaxed at the stopped flow for a time period necessary to obtain the deviation from the equilibrium characterized by the factor ε. The equilibrium value R is given by Equation (6) and the value R_ε by the relation

$$R_\varepsilon = \frac{\int_0^1 c(\rho,t).v(\rho)\ d\rho}{\int_0^1 c(\rho,t)\ d\rho \int_0^1 v(\rho)\ d\rho} \qquad (21)$$

where $c(\rho,t)$ follows from Equations (13) to (17) and $v(\rho)$ will be obtained by transformation of Equation (4) to a dimensionless coordinate ρ. The analytical integration of Equation (21) has not been found. We have thus calculated the values of the ratio R_ε/R for different values of λ and ε by numerical integration. Sinclair ZX Spectrum (Cambridge, U.K.) personal computer with 16 K bytes RAM was used for calculations. The integration difference $d\rho$ and the number of terms m of the series in Equation (13) have been chosen so that the result of the calculation would be within the required limits of precision. The values $d\rho = 10^{-4}$ and $m = 10$ fulfilled this requirement for the whole investigated range of λ and ε. The precision of the calculation was verified by intentional changes of $d\rho$ and m, and by calculating R_n/R where the values of R_n were calculated by numerical integration of Equation (3). In the last case the average precision of the numerically calculated values was better than 0.1 % relative for λ within the range from 0.02 to 0.2. The nomogram in Fig. 2 illustrates the results of the calculations for the chosen values of λ and ε. R_ε/R ratio does not depend on the values of ε only (i.e., on the deviation from the equilibrium), but for the given ε it also depends on the value of λ.

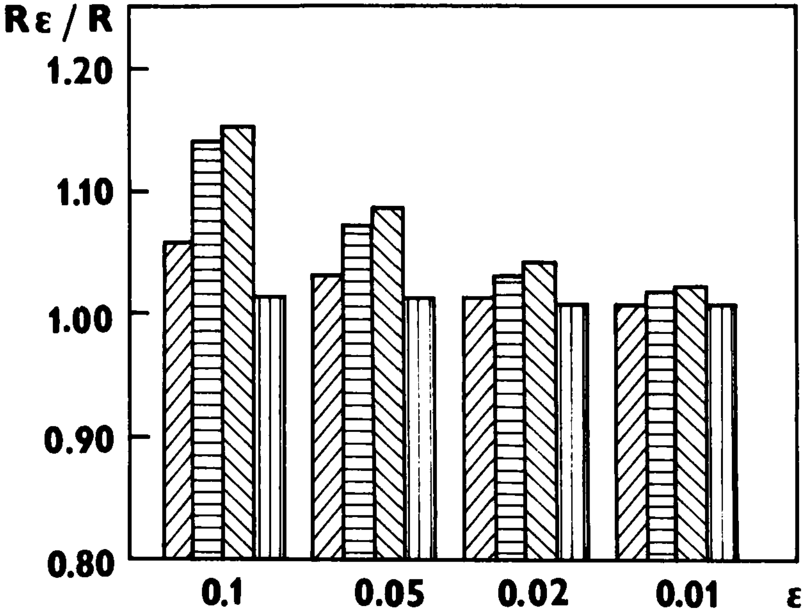

FIGURE 2. Ratio of R_ε/R values (see Equations (6) and (21)) calculated numerically for various values of ε and λ.
▨ $\lambda = 0.2$; ▤ $\lambda = 0.1$; ▧ $\lambda = 0.05$; ▥ $\lambda = 0.02$.

R_ε/R ratio surpasses the values of the acceptable experimental error in the whole interesting range of λ from 0.2 to 0.02 if $\varepsilon = 0.1$. On the other hand, if $\varepsilon = 0.01$, the ratio R_ε/R is less then 1.02 for all the calculated and illustrated values of λ. However, in all the mentioned cases, we neglect the fact that the relaxation processes continue also after the stop-flow period so that the resulting R_ε/R ratio will be still lower at the end of the separation. From the viewpoint of time economy it is interesting to know the relation between the ε value and the time or the $F(\lambda)$ value, respectively, as it follows from Equations (15)-(17).

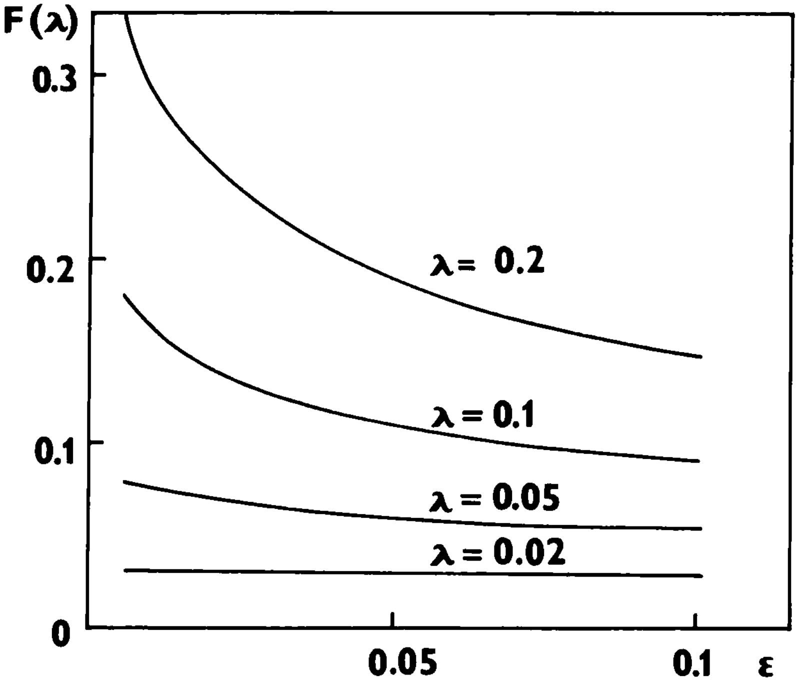

FIGURE 3. Dependence of the value of F(λ) (Equations (16) and (17) on the deviation from the equilibrium ε for various values of λ.

This relation is demonstrated in Fig. 3 for different values of λ. It follows from the results demonstrated in Figs. 2 and 3 that relaxation processes will not affect the resulting value of the retention within the range of experimental errors provided that the state deviated by 1 % from the equilibrium ($\varepsilon = 0.01$) is reached during the stop-flow period. Moreover, the difference in times necessary to reach the state with $\varepsilon = 0.05$ or $\varepsilon = 0.01$ (especially for low values of λ) is not very important.

It follows from the theoretical reasoning mentioned above that the stop-flow technique is advantageous as its use allows to reduce the whole separation time up to several-times without changing the retention ratio above the limits of experimental errors. When reducing the time of separations by FFF the changes in efficiency (i.e., changes in H) must also be taken into account. These changes occur because of differences between the continuous-flow technique and the stop-flow technique with a subsequent higher flow rate. By applying the stop-flow technique up to the state when $\varepsilon = 0.01$, it is justified to expect that the value of $H_r = 0$ (see Equation (9)). It means that the total value of H will decrease (Equation (7)). If the flow rate after the stop-flow period is higher than with the use of the continuous-flow technique the zone spreading increases due to the nonequilibrium processes. It is convenient to use the ratio between H_r and the other term of the right hand side of Equation (7), expressing the nonequilibrium processes, for quantitative evaluation of the changes in the efficiency. The other terms of Equation (7) can be neglected. Then it holds

$$\frac{H_r}{H_n} \doteq 0.005 \frac{w^2 \langle v(x) \rangle}{LD\lambda} \tag{22}$$

The extent of the contribution of the relaxation phenomena to the total value of H is evident from Equation (22). Its contribution will be especially important for highly retained solutes (small values of λ) with low diffusion coefficient D. If we want the resulting value of H to remain unchanged or even to be lower when applying the stop-flow technique, we must consider these circumstances.

EXPERIMENTAL

We have chosen Sedimentation Field-Flow Fractionation (SFFF) to verify experimentally the theoretical reasoning. This technique is suitable for this purpose as the fractionation of the particles is based on the first physical principle and that is why the retention characteristics have a straightforward relation to the properties of the particles under separation. Regarding the size of the particles under fractionation, and thus the values of their diffusion coefficients, the relaxation times are long enough and they can be determined with a sufficient precision. Under appropriate experimental conditions the steric effects are still negligible. After all, it is advantageous that the disturbing effects following, e.g., from the deformation of the shape of the flow velocity profile under non-isothermal non-isoviscous conditions do not play any important role so that the theoretical relations for both the retention and the efficiency are quite simple.

The apparatus for SFFF consisted of a linear displacement pump LD 2 (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia), a six-port injection valve and a centrifuge, both of them designed and manufactured in the Institute of Analytical Chemistry, Czechoslovak Academy of Sciences, Brno, Czechoslovakia, an UVM-4 UV detector with a variable wavelength (Development Workshops of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia) and a TZ 4200 line recorder (Laboratory Instruments, Prague, Czechoslovakia). The rotor for SFFF was home-made of our own design, the dimensions of the channel in the rotor were 18.5 x 0.2 x 496 mm. A more detailed description of the rotor will be published later (12). All experiments were performed under constant rotation of 2100 rpm that corresponded to the acceleration of 389 g with the given channel. The injection valve, the rotation seal (13) as well as the detector cell including all the connecting capillaries contributed to the total zone broadening less than 1 % relative (12). The solution (0.1 %) of Tween 60 surfactant (polyoxyethylene-sorbitane-monostearate) (Fluka AG, Buchs SG, Switzerland) in distilled water was used as the solvent. This solution was degassed before use. The flow rate of the solvent and the retention volumes were measured by means of a DC 1002 drop counter (Laboratory Instruments, Prague, Czechoslovakia) and verified by an occasional control by weighing the solvent flown out at a certain time inter al. All the measurements were performed at a temperature of 21 °C.

A sample of monodisperse latex of polyglycidyl methacrylate having nominal particle diameter of 130 nm, provided by Dr K. Bouchal (Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, Prague, Czechoslovakia), was used. The diffusion coefficient of this latex was calculated from Stokes-Einstein equation

$$D = \frac{kT}{6 \pi \eta r} \qquad (23)$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity of the liquid and r is the radius of the particles. We have used the value of $D = 3.3 \times 10^{-8}\ \mathrm{cm}^2\ \mathrm{sec}^{-1}$ calculated for the given experimental conditions.

RESULTS

The first series of experiments was intended to verify the time necessary for stopping the flow, calculated theoretically from Equation (15) for $\varepsilon = 0.01$ (i.e., for conditions under which the retention does not change within the limits of experimental errors). A flow rate of 200 μl/min was chosen for this experiment. This flow rate was sufficiently high to show clearly the changes in the values of H. After the injection (during rotation) when all the sample was safely inside the channel, the flow was stopped for a certain time and then re-established and kept till the end of the elution. Fig. 4 illustrates the results of this experiment. In spite of certain variance of the experimental values, it can be seen that the minimum value of H is reached for an approximate stop-flow time $t_{stop} = 12$ min. The stop-time calculated theoretically for the given experimental conditions and for $\varepsilon = 0.01$, $t_{stop} = 12.5$ min, was in a very good agreement with the experiment. Considering that the initial concentration of the solute in the direction across the channel thickness is not homogeneous (first of all because of the convective transport within the parabolic velocity profile) we can explain an important decrease in the value of H for shorter time $t_{stop} = 5$ min. In fact, the average relaxation distance of the solute will be somewhere between the values of w and w/2 if the experiment is executed in this way. Moreover, the relaxation processes is the course of the further elution must also be taken into account. It would be rather difficult to reach experimentally homogeneous concentration across the channel immediately after

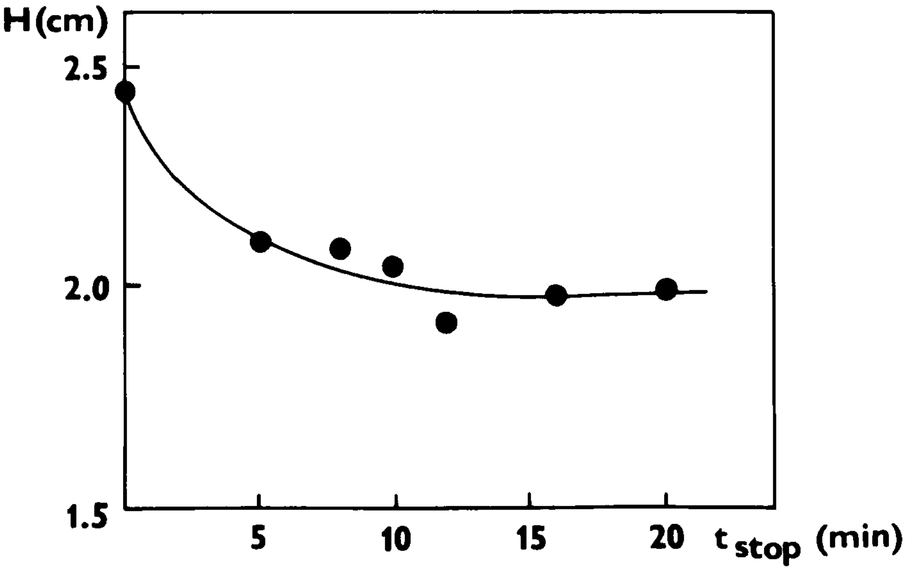

FIGURE 4. Dependence of the height equivalent to a theoretical plate H on the stop-flow time t_{stop} at a flow rate of 200 µl/min.

the injection. We should have to inject the sample without the rotation (with a zero field intensity). Then the flow should have to be stopped for the time necessary to form a more homogeneous distribution of the concentration owing to the diffusion. However, the practical value of this experiment would be low.

The dependence of retention ratio R on the stop-flow time t_{stop}, illustrated in Fig. 5, confirms the achievement of the maximum value of R for t_{stop} = 12 min. This dependence is (in agreement with the theory (7)) less profound than the dependence of the width of the elution curve or of the value of H on t_{stop}.

In the next series of experiments we have investigated the dependence of H on $\langle v(x) \rangle$ under

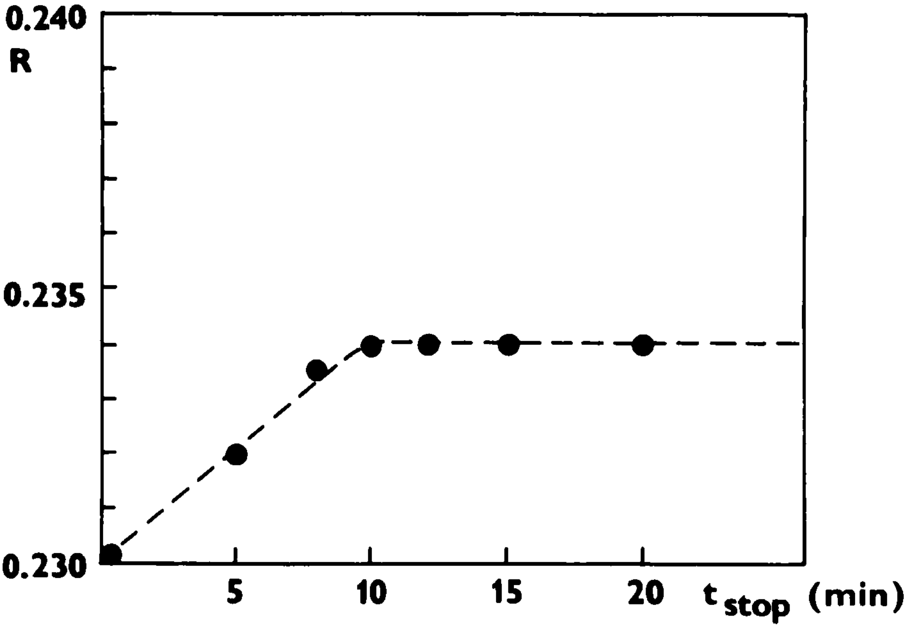

FIGURE 5. Dependence of retention ratio R on the stop-flow time t_{stop} at a flow rate of 200 µl/min.

various initial conditions of the experiment during the injection of the sample into the channel and after it. First of all the samples were injected at different flow rates and eluted without stopping the flow under constant rotation. Further, the samples were injected under rotation and when all the volume of the sample was safely inside the channel the flow rate was stopped for 10 minutes and then re-established and kept till the end of the elution. Finaly, the samples were injected into the channel in a similar way but at a lower flow rate (always 50 µl/min). The flow rate was then stopped for 10 minutes and re-established to the given nominal value. Fig. 6 shows the results of these experiments together with the dependence of H vs. $\langle v(x) \rangle$ calculated theoretically from Equations (7)-(9). Several interesting findings follow from the comparison of the experimental results with the theoretical dependences of H vs. $\langle v(x) \rangle$. In contradiction to foregoing studies of the zone spreading by using Thermal Field-Flow Fractionation by Giddings et al. (2,14), the contribution of the relaxation phenomena, calculated from the theoretical relations, is important within the whole practical range of the flow rates. This is caused by lower diffusion coefficients of particulate solutes in SFFF. However, the values of H measured experimentally without stopping the flow do not follow the theoretical course of H vs. $\langle v(x) \rangle$. At the highest flow rate of 200 µl/min, the experimental value of H is essentially lower than it would correspond to the theory; at a flow rate of 100 µl/min it is in a good agreement with the theory, and at a flow rate of 50 µl/min the experimental value of H is higher than the theoretical one. The deviation of the theory from the experiment can partly be explained by the contribution of the polydispersity of the sample. This contribution appears as an increase in an apparent value of H, especially at lower velocities $\langle v(x) \rangle$ at which the total value of H is lower. Further, it is necessary to take into account that the concentration of the solute immediately after the injection cannot be considered as distributed homogeneously in the direction of the field. The concentration distribution of the solute after the injection is much more complicated due to the convective flow, as mentioned above. The lower experimental value of H at a high flow rate of 200 µl/min as compared to the theory corresponds qualitatively to this idea. The exact quantitative evaluation is difficult due to the complexity of the flow at the beginning of the channel. When the flow is stopped for the relaxation time, the experimental values under all the studied flow rates are lower than those in the previous case. The stop-flow then causes a favourable increase in the efficiency. For the last case, when the flow rate was always 50 μl/min during the time period of the solute injection and the sample was eluted at the nominal value of the flow rate only when the stop-flow time was over, the experimental values of H are the lowest ones at all the flow rates studied. The H values are in a very good agreement with the theoretical dependence of H vs. $\langle v(x) \rangle$ whithin the range of higher flow rates. As mentioned above, the experimental values of H measured by using the stop-flow technique are lower than those measured in the experiment without the stop-flow period. The differences are so important that it is much more advantageous, from the viewpoint of the time economy, to inject the sample at a low flow rate (50 μl/min, e.g.), to stop the flow for the calculated relaxation time and then to continue the elution at higher flow rate (200 μl/min, e.g.). In our experiments a better efficiency was reached together with reduction in the elution time of the whole sample of the latex used (from ca. 100 min to ca. 60 min) it means by 40 %, in comparison with the experiment at a flow rate of 100 μl/min but without stopping the flow.

Applying the stop-flow technique also at the lowest studied flow-rate of 50 μl/min in the course of the whole elution, the value of H decreased and, consequently, the efficiency was improved (see

Fig. 6). This fact further demonstrates the advantage of the application of the stop-flow technique. The advantages of this technique were also demonstrated by Giddings et al. (3) who injected the sample at a low flow rate and a higher intensity of the field. By means of this procedure they have reached an advantageous solute concentration at the beginning of the channel.

With a decrease in the flow rate, a mere disappearance of the leading peak of the unrelaxed solute can be seen (see Fig. 7), but it still does *not mean that the minimum* value of H and thus the maximum efficiency was reached.

CONCLUSION

In this study we intended to clear up from both theoretical and experimental points of view which model of the relaxation processes describes best the real situation after the injection of the solute into the channel. The theoretical analysis showed that providing the solute is homogeneously distributed across the channel immediately after the injection and the flow is stopped during the relaxation time then the use of Mason and Weawer's model (8) is correct. However, it is difficult to approach such conditions experimentally and it is not useful for practical applications of SFFF. Moreover, the theoretical calculations based on this model showed that it is sufficient to reach the deviation from the equilibrium $\varepsilon = 0.01$ so that the retention would not be influenced within the limits of experimental errors. Then the relaxation time, necessary to reach this state with the flow stopped, warrants the

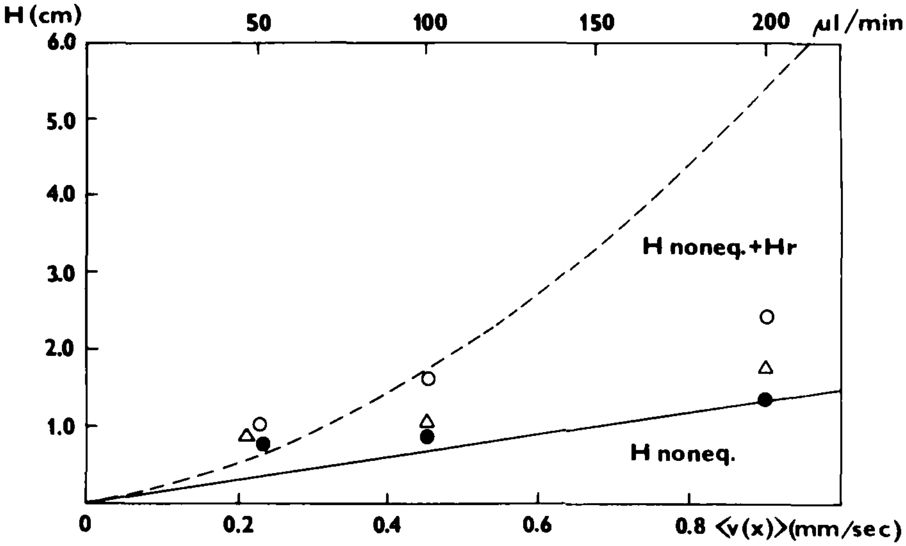

FIGURE 6. Dependence of the height equivalent to a theoretical plate H on the mean linear velocity of the solvent $\langle v(x) \rangle$.

——————— theoretical dependence calculated for nonequilibrium processes only

- - - - - - - - theoretical dependence calculated for the sum of nonequilibrium and relaxation processes

○ experimental values for $t_{stop} = 0$

△ experimental values for $t_{stop} = 10$ min

● experimental values for $t_{stop} = 10$ min and for injection at a reduced flow rate of 50 μl/min

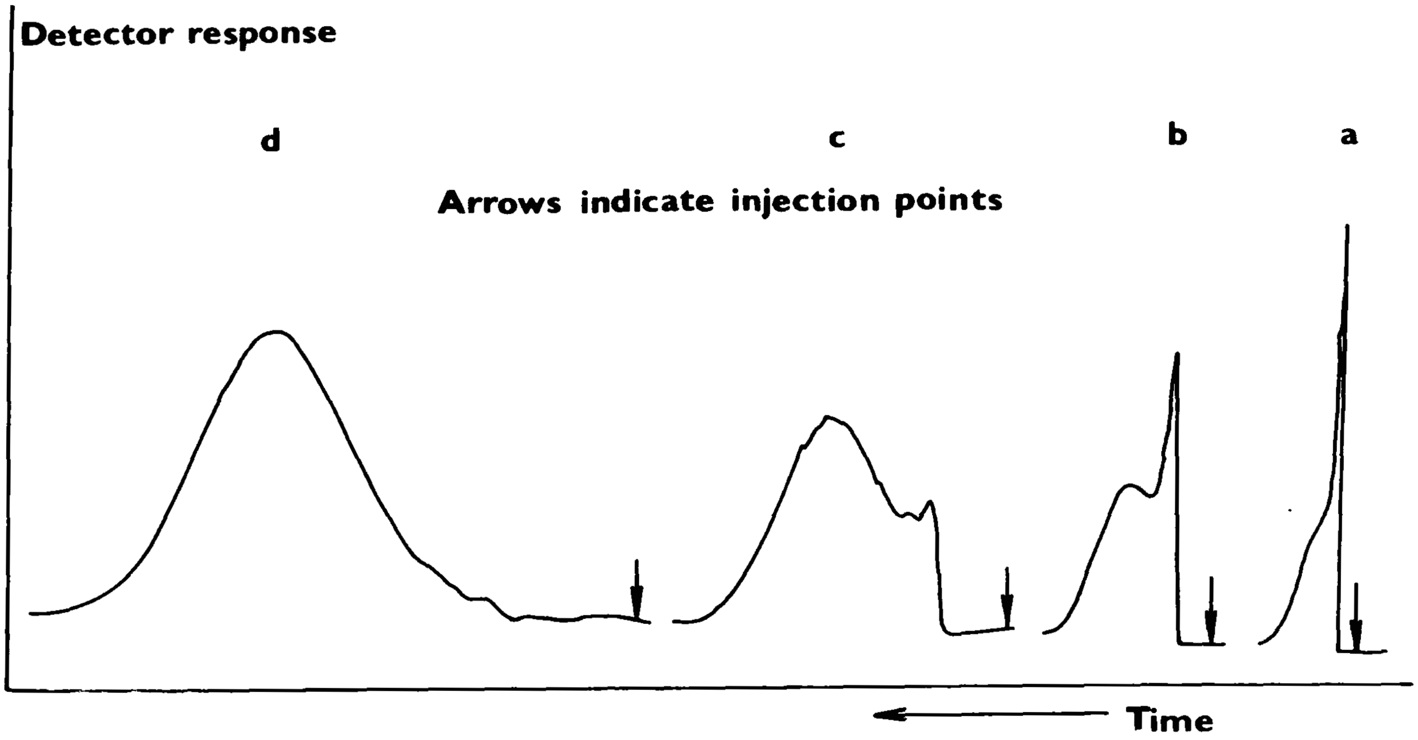

FIGURE 7. Fractograms of the studied latex obtained at 1400 rpm, $t_{stop} = 0$, flow rates: a) 500 µl/min, b) 200 µl/min, c) 100 µl/min, d) 50 µl/min.

independence of the retention on the relaxation processes providing the following elution is performed without changes in the intensity of the field.

The stop-flow technique together with injection at a reduced flow rate proved itself to be very advantageous since it reduces the whole fractionation time and, in general, higher efficiency is reached. When programming the intensity of the field the problem of relaxation of the solute with lower diffusion coefficients appears more complicated as Yau and Kirkland have indicated (11). Consequently, physico-chemical measurements by means of SFFF should be performed under clearly and well defined conditions of the constant intensity of the field. The investigation in this direction still continues.

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