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## HIGH-SPEED POLYMER SEPARATIONS BY THERMAL FIELD-FLOW FRACTIONATION

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### SUMMARY

Factors controlling speed in field-flow fractionation (FFF) are noted, and a basic analogy is shown with high-speed chromatography, which is based on similar dynamical requirements. The theory of speed optimization in FFF is developed along lines parallel to the theory of high-speed chromatography. However, allowance is made for the case in which continuous polymer distributions must be analyzed in place of discrete molecular species. We show that analysis time is proportional to the square of the channel thickness, among other things, thus demonstrating that the reduction of thickness is an important avenue for gaining speed. Accordingly, we describe two FFF column systems with channel thicknesses of 0.127 mm and 0.051 mm, two and five times thinner than conventional channels. A comparison of the performance of the two systems with polystyrene polymer fractions is shown under various flow conditions. A comparison is also made between some of the new results and published results for a conventional system. Plate height plots are also shown and discussed. These studies show—more or less in accord with theory—a dramatic improvement in speed with decreasing channel thickness. Using the thinner-channel system, 0.051 mm, we have obtained the separation of three polymer fractions in about 4 min. A partial separation was obtained in 1 min. Speeds considerably greater than this are suggested by theory under conditions of high retention and high flow.

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### INTRODUCTION

Field-flow fractionation (FFF) is a separation method applicable to various classes of macromolecules and colloidal particles<sup>1-3</sup>. It is an elution technique, comparable in its external experimental features to column liquid chromatography. However, the mechanism of separation is different. Each solute component is pushed by an external field toward a wall of the separation channel where it forms a layer whose thickness is peculiar to each species. The differential velocity profile present in laminar flow forces the zones to migrate downstream at different velocities depending on how far the layer extends into the faster streamlines toward the center of the channel.

Several types of external fields can be used to induce retention in FFF. Each type leads to a distinct subtechnique of FFF. The major subtechniques are thermal, sedimentation, electrical and flow FFF<sup>4-7</sup>. Each subtechnique has areas of special

strength that relate to its capabilities with regard to molecular-weight range, selectivity, type of solvent, and so on.

Theoretical analysis shows that FFF has a considerable potential for polymer analysis because, for a given number of theoretical plates, it is capable of producing higher resolution and generating more separated peaks than is exclusion chromatography (EC)<sup>8</sup>. Indeed, its high peak capacity has been demonstrated<sup>9</sup>. Yet in its experimental realization, plates have been generated slowly and effective separations have required hours. Such separations are slow by comparison to the latest generation of EC columns, for which analysis times are measured in minutes<sup>10</sup>. Hence, despite FFF's intrinsic advantages which include low surface interactions, theoretical tractability, precise solute characterization, low pressure drops, external retention control and programming, it has not been competitive in a practical sense with EC because of the low speed of FFF. This problem has been magnified by the unfamiliarity of the scientific community with the advantageous characteristics of FFF methods and the unavailability of appropriate equipment. In this paper we hope to point the way to the removal of the first of the above stated barriers to the wider acceptance of FFF.

It is important to recognize that FFF is not an intrinsically slow method. It has been relatively slow in practice because the experimental techniques and apparatus needed for slow separation are much less demanding than those required for fast separations. In that FFF is still in the early stages of its evolution, the slow separations were realized first in the course of characterizing and proving the FFF methodology.

In FFF, solute components are forced into a diffuse layer against a channel wall, as mentioned. The mean thickness,  $l$ , of this layer varies depending upon overall channel thickness and the magnitude of the effective potential drop of the field across the channel thickness. It differs from component to component, a fact which is responsible for differential migration. That is,  $l$  controls retention and separation. However, layer thickness  $l$  has another significant role. Solute diffuses across distance  $l$  (or more exactly, a distance scaled to  $l$ ) in establishing equilibrium between the slow moving portion of the zone near the wall and the faster moving parts extending out from the wall<sup>11</sup>. The speed of this process controls the speed of analysis just as in chromatography. Analysis time is established largely by the speed of diffusion (mass transfer) between and across stationary and mobile phases<sup>12</sup>. In the chromatographic case this ordinarily involves diffusion over approximately one support particle diameter,  $d_p$ . The trend of modern liquid chromatography toward smaller support particles (and attendant high pressures) is explained by the need to establish rapid diffusion across or through those particles for fast analysis. An equal incentive exists for achieving small  $l$  values in FFF. This qualitative conclusion is supported by theory. Thus it has been shown that the maximum rate of generation of theoretical plates,  $\dot{N}_{\max.}$ , in an FFF channel is given approximately by the equation<sup>13</sup>

$$\dot{N}_{\max.} = D/l^2 \quad (1)$$

where  $D$  is the diffusion coefficient. Thus the intrinsic speed of separation varies strongly with changes in  $l$ , making a reduction in  $l$  the primary requirement for high-speed separation. We will elaborate on this conclusion in the theory section.

In principle,  $l$  can be made to approach zero through unlimited increases in

field strength. This limiting condition corresponds to a separation time approaching zero. There are, of course, practical limits (such as surface imperfections and finite particle size) that prevent  $l$  from approaching too closely to zero, but the magnitude of the limitations are not yet understood. A discussion of some of these limits appears elsewhere<sup>13</sup>.

In this paper, following the development of the theory, we will report experimental results in which  $l$  has been reduced to approximately  $3.7 \mu\text{m}$  by reducing channel thickness. This has made it possible to separate three polymer fractions on a time scale of minutes.

## THEORY

### *Resolution requirements*

The closeness in both the dynamical behavior and in the experimental organization between FFF and chromatography would suggest that the theory of time optimization in FFF could be patterned after that already developed in the chromatographic literature. We regard this as a viable and desirable approach. We note, however, that time optimization in chromatography has generally focused on separating two discrete components in the least possible time<sup>12</sup>. With most polymers, the distributions we encounter are for all practical purposes continuous since one cannot at this time resolve individual high-molecular-weight species. One seeks in this case to obtain information on the chain length or molecular-weight distribution curve. The information is always subject to distortion, a major part of which relates to "band spreading", that is, the spreading of species of one molecular weight into the spatial territory of other species in the column. Because of band spreading and species overlap, one cannot hope to obtain a direct measure of the abundance of individual species. One can hope only to obtain—through deconvolution techniques—a molecular-weight distribution curve that has fidelity to the true distribution over a broad range of molecular weights, but which lacks fine structure due to band spreading and the attendant overlap. The degree to which detail can be sacrificed depends upon requirements imposed from the outside by the polymer chemist or process engineer.

In general, one loses significant information on the relative distribution of two species (the relative heights of the distribution curve at two points) whenever their overlap is substantial. The degree to which information is lost depends on the intrinsic smoothness of the distribution curve, the experimental noise and other factors, but the loss is basically coupled to species overlap. Therefore, the degree to which fine structure can be sacrificed in the distribution curve can be stated in terms of some minimum requirement in the resolution of various species within the polymer series. Hence the problem can ultimately be reduced to one of setting resolution requirements for discrete species. At this point the problem becomes largely equivalent to the classical chromatographic problem of minimum analysis time: one has certain requirements imposed from the outside for the resolution of discrete species, and this resolution is to be achieved in the least possible time.

For the above reasons, in this paper we will approach the minimization of analysis time as a problem of achieving, in the least time, a fixed minimum resolution between discrete species. The treatment and the resulting conclusions are expected to be applicable to continuous polymer distributions for the reasons detailed above.

However, our experimental studies are made much simpler by using polymer samples with a limited molecular-weight range that behave much like the discrete species described in our time optimization theory.

*General approach to time optimization: chromatography and FFF*

A conceptually simple procedure for establishing minimum time conditions in chromatography has been developed<sup>12</sup>, and here we will extend it to FFF. First of all, minimal resolution requirements are established. Following this, the number of theoretical plates,  $N$ , needed to achieve that resolution are calculated. In the next step, the time necessary to evolve these plates is written simply as

$$t_R = Nt_p \quad (2)$$

where  $t_p$  is the time necessary to evolve one plate. (This is the reciprocal of  $\dot{N}$ , the number of plates generated per second.) Finally, the elaboration of eqn. 2, which follows below, leads to the desired expression for  $t_R$  in terms of separation requirements and column characteristics.

We now express  $t_p$  as the length or height of the plate,  $H$ , divided by the mean velocity at which it is traversed by solute,  $R\langle v \rangle$

$$t_p = H/R\langle v \rangle \quad (3)$$

With eqn. 3 substituted into eqn. 2, we get the required expression for analysis (or retention) time

$$t_R = \frac{NH}{R\langle v \rangle} \quad (4)$$

Theoretical equations or empirical correlations must then be used for  $H$  and for retention ratio  $R$  in order to express  $t_R$  in terms of basic column parameters and operating conditions. Finally,  $t_R$  can be optimized with respect to those parameters.

In polymer analysis the basic resolution requirement can be stated in terms of the difference in molecular weight,  $\Delta M$ , that can be allowed between two species requiring unit resolution. As  $\Delta M$  decreases, the intrinsic resolving power required of the system increases. Thus intrinsic resolving power is proportional to  $1/\Delta M$ . This can be formalized by defining the dimensionless ratio,  $M/\Delta M$ , as the fractionating power of the system<sup>8</sup>. The fractionating power is given by the expression

$$\frac{M}{\Delta M} = \frac{N^{\pm}}{4} \left| \frac{d \log V_R/V^0}{d \log M} \right| \quad (5)$$

where  $V_R$  is retention volume,  $V^0$  is void volume and  $M$  is molecular weight or mean molecular weight.

If one is working with two discrete polymer fractions having an increment  $\delta M$  in molecular weight, their resolution will be

$$R_s = \delta M/\Delta M = (\delta M/M)(M/\Delta M) \quad (6)$$

in that  $\Delta M$  is defined as the increment for which we have unit resolution. Solving for  $M/\Delta M$  and substituting this in eqn. 5, we get

$$\frac{R_s M}{\delta M} = \frac{N^{\frac{1}{2}}}{4} \left| \frac{d \log V_R/V^0}{d \log M} \right| \quad (7)$$

From this equation we find that the number of plates required for achieving a resolution between these two fractions of  $R_s$  is

$$N = \frac{16R_s^2 M^2}{(\delta M)^2} \frac{1}{[d \log(V_R/V^0)/d \log M]^2} \quad (8)$$

When this is substituted into the basic expression for analysis time, eqn. 4, we obtain

$$t_R = \frac{16H}{R\langle v \rangle} \frac{R_s^2 M^2}{(\delta M)^2} \frac{1}{[d \log(V_R/V^0)/d \log M]^2} \quad (9)$$

In this equation, the practical requirements for separation can be stated in terms of the resolution  $R_s$  required for fractions spaced  $\delta M$  apart. The term  $|d \log(V_R/V^0)/d \log M|$  must be evaluated for the specific fractionating column being used. Examples of this have been shown both for FFF and exclusion chromatography<sup>8</sup>. The plate height  $H$  and retention ratio  $R$  must also correspond to the column in use.

Up to this point the equations and the discussion are applicable to both FFF and exclusion chromatography. The difference between them appears when we evaluate  $H$  and  $R$ , along with the ubiquitous logarithmic term.

#### *Time optimization in FFF*

Plate height contributions in FFF have been studied previously<sup>14-16</sup>. They stem from longitudinal molecular diffusion, non-equilibrium considerations and such non-ideal effects as external dead volumes, relaxation processes, solute heterogeneity, concentration effects and channel irregularities. In a system working with a minimum of extraneous disturbances, the nonideal effects may be neglected. Also, due to the very low diffusion coefficients of polymers, the longitudinal molecular diffusion contribution to plate height is rarely observed. Therefore, the most important contribution to  $H$  is due to non-equilibrium. In that case, the plate height equation has a very simple form<sup>14</sup>

$$H = \chi w^2 \langle v \rangle / D \quad (10)$$

where  $D$  is the average solute-solvent diffusion coefficient,  $w$  is the channel thickness and  $\chi$  is a complicated function of the dimensionless parameter  $\lambda$ <sup>17</sup>. Quantity  $\lambda$  is the ratio of the mean layer thickness  $l$  of the exponential solute zone divided by the channel thickness  $w$

$$\lambda = l/w \quad (11)$$

This parameter is related to a good approximation to the retention ratio  $R$  by

$$R = 6\lambda\mathcal{L}(1/2\lambda) \quad (12)$$

where  $\mathcal{L}$  is the Langevin function

$$\mathcal{L}(y) = \coth y - 1/y \quad (13)$$

We note with respect to the logarithmic term of eqn. 9 that

$$\frac{d \log(V_R/V^0)}{d \log M} = \frac{d \log R}{d \log M} \quad (14)$$

This is a direct consequence of the equation defining the retention ratio:  $R = V^0/V_R$ . Furthermore, eqn. 12 shows that  $R$  is a function of  $\lambda$  only, so that

$$\frac{d \log R}{d \log M} = \frac{d \log R}{d \log \lambda} \frac{d \log \lambda}{d \log M} \quad (15)$$

With the two equations above used to replace the logarithmic term, and eqn. 10 replacing  $H$ , eqn. 9 becomes

$$t_R = \frac{16w^2}{D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \frac{\chi}{R \left( \frac{d \log R}{d \log \lambda} \right)^2} \frac{1}{\left( \frac{d \log \lambda}{d \log M} \right)^2} \quad (16)$$

The last term of this equation can be obtained from the relationship for thermal FFF<sup>18</sup>

$$\lambda = \varphi/\Delta TM^{0.5} \quad (17)$$

in which  $\varphi = 1420 \text{ }^\circ\text{C}(\text{g/mole})^{1/2}$  for polystyrene in ethylbenzene. This equation yields

$$\gamma = d \log \lambda/d \log M = 0.5 \quad (18)$$

However, there is empirical evidence that 0.6 is a better value for  $\gamma$  in this particular system<sup>19</sup>. We will use the symbol  $\gamma$  for this dimensionless number for now to maintain generality. Eqn. 16 then assumes the form

$$t_R = \frac{16w^2}{\gamma^2 D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \frac{\chi}{R(d \log R/d \log \lambda)^2} \quad (19)$$

In order to complete our analysis, we must evaluate the last term, each component of which is a function of  $\lambda$ . Retention ratio  $R$  is given by eqn. 12, and from this one can also obtain  $d \log R/d \log \lambda$ . Coefficient  $\chi$  can be found in the literature<sup>17</sup>.

*Limiting equations for high retention*

The above  $\lambda$ -dependent expressions are for the most part rather complicated. For this reason we examine first the very simple but important limit applicable when  $\lambda$  approaches zero and retention thus approaches infinity. In practice, the limiting equations are good approximations for medium as well as high retention. This will be shown shortly.

It can be shown that  $R$  in eqn. 12 approaches the limit<sup>15</sup>

$$R = 6\lambda \quad (20)$$

under high retention (low  $R$ ) conditions. Consequently the term  $d \log R/d \log \lambda$  approaches unity. Finally, the complicated expression for  $\chi$  approaches the simple limit<sup>14,17</sup>

$$\chi = 24\lambda^3 \quad (21)$$

With the above limiting expressions, the last term of eqn. 19 becomes  $4\lambda^2$  and eqn. 19 itself assumes the form

$$t_R = \frac{64\lambda^2 w^2}{\gamma^2 D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} = \frac{64l^2}{\gamma^2 D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \quad (22)$$

The latter expression involves  $l$  directly, and shows its importance in accord with our earlier discussion. The expression has been obtained by substituting  $l$  for  $\lambda w$ , which follows from eqn. 11.

The coefficient,  $64/\gamma^2$ , assumes the value of 256 or 178, depending on whether  $\gamma$  is 0.5 or 0.6. As noted, empirical evidence favors the latter for polystyrene in ethylbenzene solvent. Thus, to an approximation,

$$t_R = \frac{178\lambda^2 w^2}{D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \quad (23)$$

Similar limiting equations that express  $t_R$  in terms of  $R$  rather than  $\lambda$  can be deduced from the following discussion (see eqns. 26 and 28).

*Departure from high-retention limit*

When retention is not high and the above limiting equations are therefore not valid, we can account for the departure from eqn. 22 by incorporation of the departure term,  $\xi$ .

$$t_R = \frac{64\lambda^2 w^2}{\gamma^2 D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \xi \quad (24)$$

This term can be obtained by comparing eqns. 22 and 19

$$\xi(\lambda) = \chi/4\lambda^2 R(d \log R/d \log \lambda)^2 \quad (25)$$

Although  $\xi$  is a function of  $\lambda$ , it can also be evaluated in terms of  $R$  because of the relationship of  $R$  and  $\lambda$  shown in eqn. 12. It is actually more practical to express  $\xi$  in terms of  $R$  because the latter is an observable parameter. In Fig. 1 this dependence is shown. We note that the departure of  $\xi$  from unity amounts to nearly 25% at  $R = 0.5$  ( $V_R = 2V^0$ ), and decreases rapidly as  $R$  approaches zero. It can be shown that  $\xi$  approaches  $1 - R/3$  as  $R$  approaches zero.

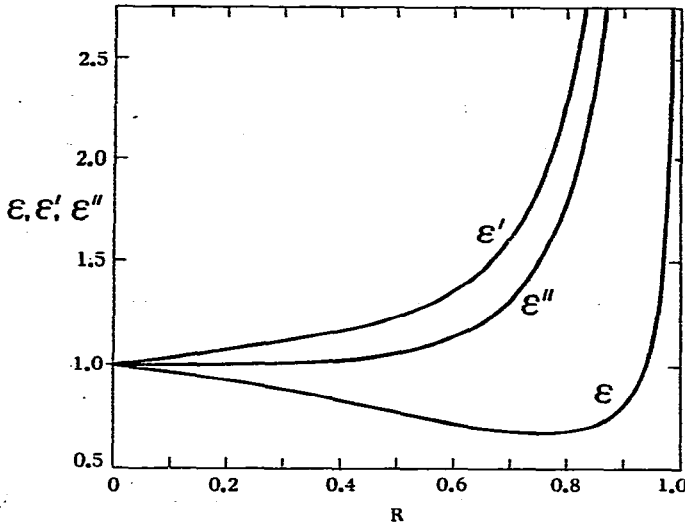


Fig. 1. Departure terms representing the relative increase in analysis time as  $R$  approaches unity. The terms are defined by eqns. 24, 26 and 28.

As an alternative, eqn. 24 in its entirety can be expressed in terms of  $R$  instead of  $\lambda$ . We do this by employing eqn. 20 for  $\lambda$ , which yields  $\lambda = R/6$ . With this substituted into eqn. 24, we get

$$t_R = \frac{16R^2w^2}{9\gamma^2D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \xi' \quad (26)$$

where  $\xi'$  is a function slightly different from  $\xi$  for finite values of  $\lambda$  and  $R$  because the substitution of  $R/6$  for  $\lambda$  is an approximation except at  $\lambda = 0$ . We have for  $\xi'$

$$\xi' = 9\chi/R^3 (d \log R/d \log \lambda)^2 \quad (27)$$

This parameter is also shown in Fig. 1. It approaches  $1 + R/3$  as  $R$  approaches zero. If we wish to include this factor in the  $t_R$  expression rather than in  $\xi'$ , we can write

$$t_R = \frac{16R^2(1 + R/3)w^2}{9\gamma^2D} \left\{ \frac{R_s^2 M^2}{(\delta M)^2} \right\} \xi'' \quad (28)$$

where

$$\xi'' = 9\chi/R^3 (1 + R/3) (d \log R/d \log \lambda)^2 \quad (29)$$



The flatness of  $\xi''$  in Fig. 1 shows that eqn. 28 gives a more accurate (although slightly more cumbersome) picture of the dependence of  $t_R$  on  $R$ . Of course, in the high retention limit,  $\xi'$  or  $\xi''$  can be dropped from eqns. 26 and 28, respectively, and we have the appropriate limiting expressions corresponding to eqns. 22 and 23, except in the present case  $t_R$  is given in terms of  $R$ .

### Theoretical conclusions

Our discussion of time optimization will now proceed in reference to eqns. 24, 26, and 28, all similar in form. We note that these equations consist of three groups of terms. We will proceed from the last to the first.

The terms  $\xi$ ,  $\xi'$  and  $\xi''$ , shown in Fig. 1, reflect small (and not too significant) variations in time for medium and high retention levels, up to  $R \approx 0.7$ . However, they show that a major deterioration in fast analysis occurs for inadequate retention,  $R > 0.8$ . The deterioration in resolution under conditions of poor retention is a phenomenon that occurs in both chromatography and FFF<sup>18</sup>. With decreased resolution, more plates—and thus more time—are required to achieve the desired separation.

The middle term,  $[R_s^2 M^2 / (\delta M)^2]$ , expresses the level of resolution  $R_s$  required, and the relative "distance"  $\delta M / M$  between the polymers to be separated. All quantities here are squared, demonstrating the highly sensitive dependence of  $t_R$  on the requirements of the separation. The proportionality of  $t_R$  to  $R_s^2$  shows that there is a trade-off between speed and resolution, as in chromatographic systems.

The final term,  $64\lambda^2 w^2 / \gamma^2 D$ ,  $16R^2 w^2 / 9\gamma^2 D$  or  $16R^2(1 + R/3) w^2 / 9\gamma^2 D$  for eqns. 24, 26, or 28, respectively, reflects a group of individual contributions to  $t_R$ . Some can be changed to advantage and others have little flexibility. In the latter category is  $\gamma$ , defined by eqn. 18. There are probably small solvent and temperature effects on  $\gamma$ , but these are unlikely to exceed a few percent. For all practical purposes (within the limited framework of present knowledge), this term is essentially constant, and offers little potential for the optimization of speed.

Diffusion coefficient  $D$ , on the other hand, can be increased significantly both by increasing the mean temperature and by utilizing a less viscous solvent. A several-fold gain in speed is expected. This strategy is much like that proposed recently for exclusion chromatography, and, like the latter, could be carried beyond the normal boiling point and perhaps into the dense gaseous region using high pressures<sup>20</sup>.

Finally, the product term,  $\lambda w$  or  $Rw$ , must be considered as the most important focus for time optimization. The term  $\lambda w$ , of course, equals the effective thickness of the solute layer,  $l$ . The importance of this term was emphasized earlier. The dependence of  $t_R$  on the square of  $\lambda$  and  $w$ , and thus its proportionality to  $l^2$ , confirms the significance of this parameter. (Because  $R$  is proportional to  $\lambda$  at high retention, the same conclusions apply to  $Rw$  as to  $\lambda w$ , and they are both proportional to  $l$ .)

The two components of  $l$  ( $\lambda$  and  $w$ ) must be reduced in quite opposite ways in thermal FFF. Quantity  $\lambda$  is best reduced by increasing the temperature increment,  $\Delta T$ . By means of a pressurized system,  $\Delta T$  values of about 160 °C have been obtained<sup>18</sup>. There is also a solvent effect, although this is usually small<sup>21</sup>.

Channel thickness  $w$ , by contrast, can only be reduced in the construction process: thinner channels must be made. This is the basis of the present experimental work. We have reduced  $w$  up to five-fold relative to conventional channels, which

corresponds to  $w$  values down to 0.051 mm. Because of the square dependence, this should represent a 25-fold gain in speed. We will report the experimental results for the system shortly.

Another aspect of the  $\lambda w$  or  $Rw$  product is its proportionality to retention ratio  $R$ , and the consequent proportionality of  $t_R$  to  $R^2$ . Thus, speed is predicted to increase sharply with increasing retention across the entire retention range. (The  $\xi$  terms, discussed earlier, complement this dependence, but show significant changes in speed only at  $R$  values near unity.) This result is unexpected and is contradictory to chromatography where increasing retention corresponds to increasing delays in elution and thus increasing analysis time. The offsetting factor in FFF is that plate height drops so rapidly with increased retention that one can get by with much shorter columns or much higher velocities for the highly retained peaks. The net effect is a significant increase in speed.

At this point, we show some calculated numerical values for  $t_R$ . The values of Table I are based on a channel with  $w = 0.127$  mm (0.005 in.). These results will vary strongly if  $w$  is changed, as just noted. The table illustrates a rapid increase in analysis time as the separation becomes more difficult, as reflected in going from  $M/\delta M$  values of unity to 10. An  $M/\delta M$  of 3 would apply, by way of example, to polymer fractions of 83,000 and 117,000 molecular weight.

TABLE I

VALUES OF ANALYSIS TIME DERIVED FROM EQN. 24

Parameters:  $w = 0.127$  mm,  $D = 5 \times 10^{-7}$  cm<sup>2</sup>/sec,  $R_s = 1$ ,  $\gamma = 0.5$ .

$R$	$M/\delta M$			
	1	3	5	10
0.1	24 sec	3 min 30 sec	9 min 50 sec	39 min 30 sec
0.3	3 min 50 sec	34 min 30 sec	1 h 36 min	6 h 23 min
0.5	11 min 50 sec	1 h 46 min	4 h 54 min	19 h 35 min

## EXPERIMENTAL

The two systems used in this work were constructed and assembled in the same way as those used in previously reported thermal FFF studies<sup>21</sup>. For each column two copper bars with highly polished faces were clamped together over a sheet of Mylar in which the channel space had been cut. This space was rectangular with tapered ends. The breadth was 2.0 cm, and the distance between the inlet and outlet holes (located at the apices of the tapered ends) was 44.6 cm. The Mylar spacers were of two thicknesses, creating channel widths  $w$  of 0.127 mm and 0.051 mm. We designated these as the thin channel and the ultra-thin channel, respectively. The void volume of the thin channel was 1.04 ml and that of the ultra-thin channel was 0.44 ml. Special attention was given to applying the same stress on each of the bolts clamping the two bars together, in order to reduce the non-uniformity of thickness across the channel. Without this precaution, two or three peaks were sometimes obtained for a single unretained compound.

A pneumatic pump consisting of a 600-ml holding coil contained the solvent. This was kept at constant pressure by a nitrogen stream from a tank. The volume of the coil allowed the system to operate for periods of several hours to several days without refilling. Since the pressure drop between the channel inlet and outlet was very small (500 mbar under the mere drastic conditions used in this study, and only 1 mbar in more usual operation), a restrictor was used to generate pressure drops and to control flow. The restrictor consisted of a small column packed with fine glass beads inserted between the coil and injection septum. Different restrictor columns with different permeabilities or lengths were used to extend the flow range.

A Waters Assoc. differential refractometer model R-401 was used as a detector under most circumstances; an LDC model 1103 monitored column effluent at the higher flow-rates.

All the solute materials but one were ultra-narrow polystyrene fractions ( $M_w/M_n \approx 1.009$ ) supplied by Waters Assoc. (Milford, Mass., U.S.A.). The exception was the 51,000 molecular weight ( $M_w/M_n \approx 1.06$ ) sample used in the plate height studies, which was from Mann Labs. (New York, N.Y., U.S.A.). Ethylbenzene supplied by Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.) was used as the carrier solvent. The polymer samples were first mixed together in a small amount of toluene. These dilutions contained on the order of 3–40 mg polystyrene per ml of toluene. Samples ranging in size from 1–10  $\mu$ l were injected into the flowing solvent stream through a silicone septum located at the inlet of the column. A Microliter No. 701 syringe was used to inject the samples. No stop-flow techniques were used.

The ethylbenzene caused the silicone septum to swell, and after several injections some pieces of this weakened structure were cut loose by the syringe needle. These were swept downstream, partially plugging the channel, and decreasing the flow-rate. It was necessary to remove the damaged septum, backflush the column and replace the septum. Using a PTFE-coated silicone septum was no improvement.

In most of the experiments, the temperature drop  $\Delta T$  between the hot and cold walls was 60 °C, with cold temperature of 21 °C and 33 °C for the thin and ultra-thin channels, respectively. However, a maximum  $\Delta T$  of 56° C, with a cold wall temperature of 35 °C, was used for the plate height studies involving the ultra-thin channel. This, of course, led to slightly reduced retention levels.

## RESULTS AND DISCUSSION

Fig. 2 shows the separation of a six-component mixture of polystyrenes having molecular weights ranging from 2000 to 160,000, using the thin-channel (0.127 mm) system. A satisfactory baseline resolution was obtained for all the components except for 2000 and 5000 molecular weight samples. Also, the 2000 molecular weight component was not separated from the unretained toluene (sample solvent) peak. The loss in resolution among the toluene, 2000 and 5000 peaks compared to the 20,000 and 51,000 pair is largely illustrative of the loss of fractionating power at low retention (high  $R$ ), reflected in part in the rise in the  $\xi$  terms of Fig. 1.

The fractogram of Fig. 2 is nearly identical to the one shown in Fig. 1B of ref. 9, for which the total retention time was 7 h. In that the channel thickness  $w$  in the present case was half of that for the study noted, retention times are expected to be reduced four-fold. Indeed, the seven hour analysis was effectively achieved

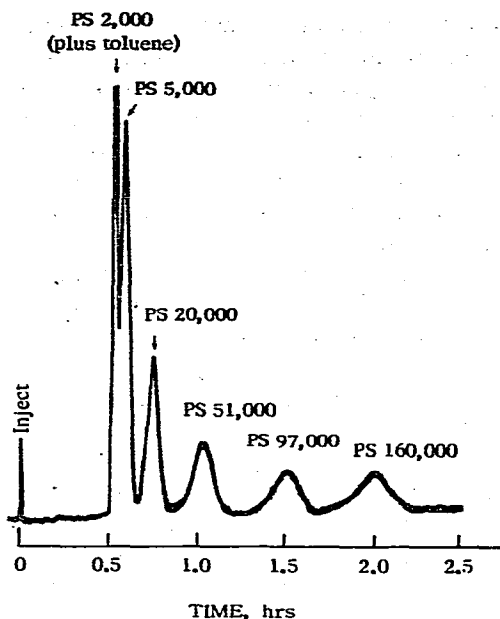


Fig. 2. Fractogram of a six-component mixture of polystyrenes separated in the thin (0.127 mm) channel.  $\Delta T$  was 60 °C,  $\dot{V}$  (the volumetric channel flow-rate) was 1.96 ml/h, and  $\langle v \rangle$  was 0.022 cm/sec.

in slightly over 2 h in the present thin channel system, a time reduction more or less in accord with expectation.

In view of the trade-off noted earlier between speed and resolution, we expect to be able to gain speed if we allow some sacrifice in resolution. This trade-off is most simply achieved by increasing the flow velocity,  $\langle v \rangle$ . This directly reduces time, but it reduces the number of plates in the column and thus reduces resolution. This phenomenon can be seen by comparing Figs. 3 and 2. The velocity employed in obtaining Fig. 3 was over four times greater than that used for Fig. 2. Analysis time, therefore, has been reduced from *ca.* 2 h to *ca.* 0.5 h, but the degradation of resolution is immediately apparent. Each experimentalist, of course, must decide how far such a trade-off can be profitably carried.

In quantitative terms, the resolution between fractions of 51,000 and 97,000 molecular weight is approximately 1.85 in Fig. 2 and 1.0 in Fig. 3. (These numbers correspond to a fractionating power of 1.6 and 2.9, respectively, as can be seen from eqn. 6.) The average time of elution of the two components is approximately 76.2 min and 17.4 min for Figs. 2 and 3, respectively. However, the ratio of the retention times between these two figures ( $76.2/17.4 = 4.4$ ) is not completely explained by the square of the ratio of the resolution [ $(1.85/1.0)^2 = 3.4$ ], as is predicted from eqn. 24. This discrepancy most likely originates in terms other than non-equilibrium that contribute to peak broadening. In such cases eqn. 10 cannot be substituted for  $H$  in eqn. 9: Under these conditions  $H$  generally acquires a different velocity dependence than the simple proportionality of eqn. 10, and the ratio  $H/\langle v \rangle$  in eqn. 9 itself retains a velocity dependence because  $\langle v \rangle$  terms no longer cancel out. Analysis time  $t_R$  will

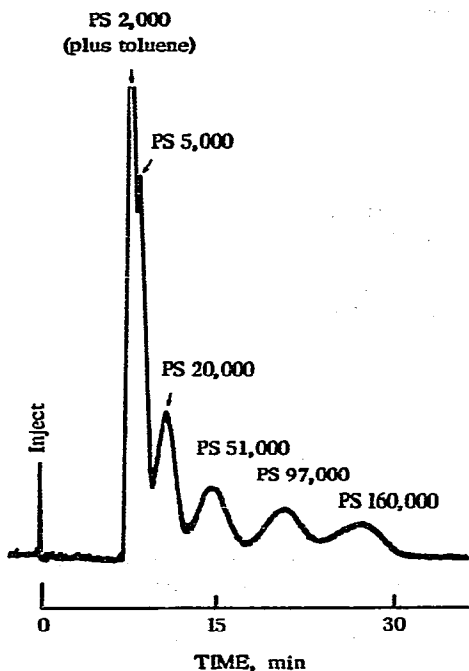


Fig. 3. Separation of a six-component mixture of polystyrenes in the thin channel.  $\Delta T$  was 60 °C,  $\dot{V}$  was 8.07 ml/h, and  $\langle v \rangle$  was 0.092 cm/sec.

then depend on velocity. The form of this dependence will hinge on the nature of  $H$  versus  $\langle v \rangle$  curves.

While plate height studies have been made for a number of thermal FFF systems<sup>16</sup>, none has involved channels as thin as those utilized in this study. Therefore, we have obtained several  $H$  versus  $\langle v \rangle$  curves for the present channels to clarify the basic time and resolution questions raised.

Plate height data obtained both with the thin and ultra-thin channels using 51,000 and 160,000 molecular weight fractions are shown in Fig. 4. While the data exhibit considerable scatter, the trends are indicated by the straight lines of the figure. These lines were fit to the data by least squares. Clearly, there is a tendency in all systems to intercept the positive  $H$  axis. In the thin channel (0.127 mm) case the intercepts are low enough and the slopes high enough that the assumption (as in eqn. 10) of a proportionality between  $H$  and  $\langle v \rangle$  is not an unreasonable description of the data. For the ultra-thin channel the assumption of proportionality is unreasonable.

It is not our objective to go deeply into the plate height question here, but a few major considerations merit discussion because, in the end, plate height trends rule the efficacy of separation.

First of all, a positive intercept on the  $H$  axis is expected for any sample with finite polydispersity. This intercept can be calculated in terms of the molecular weight ratio,  $\mu = M_w/M_n$ , if the latter is known<sup>16</sup>. If we assume  $\mu = 1.009$  for the 160,000 molecular weight Waters Assoc. sample and 1.06 for the 51,000 Mann Labs. sample,

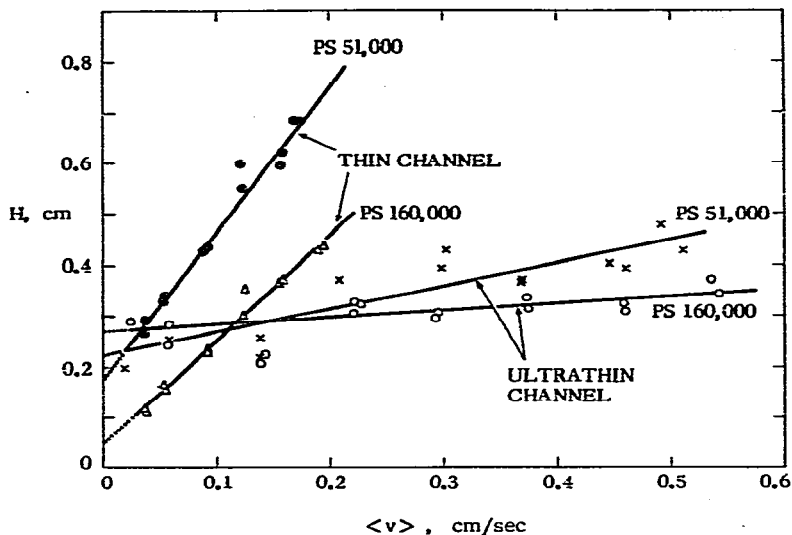


Fig. 4. Plate height-velocity data and corresponding least square plots for the 51,000 molecular weight Mann Labs. polystyrene fractions and the 160,000 molecular weight Waters Assoc. polystyrene fractions in both the thin and ultra-thin FFF channel. The thin channel had a  $\Delta T$  of 60 °C, with retention values of  $R = 0.50$  for the 51,000 molecular weight material and  $R = 0.27$  for the 160,000 molecular weight material. The ultra-thin channel had a  $\Delta T$  of 56 °C, with  $R = 0.6$  for the 51,000 molecular weight material and  $R = 0.37$  for the 160,000 molecular weight material.

we find that the calculated intercepts due to polydispersity are greater than the observed intercepts (by factors ranging from 1.1 to 1.9) for all cases except the 160,000 sample in the ultra-thin channel. In the latter case the observed intercept is four times too high. In view of errors introduced by the probable unreliability of the  $\mu$  values and the scatter of the data, we see that the intercepts can be largely accounted for by polydispersity, although other factors may enter to some degree.

If polydispersity is the chief factor, there is no need to modify our plate height expression by adding a constant to eqn. 10 because the polydispersity contribution is a term originating in fractionation. It is a broadening that occurs because the larger components of a sample are pulled away from the smaller components. A large polydispersity contribution is therefore an asset as far as fractionation is concerned, demonstrating a degree of separation, and should not be considered as a liability in the form of another plate height contribution. It is an apparent plate height contribution only in the sense that it appears in the description of polydisperse fractions like those used here, but it detracts nothing from the fidelity with which molecular weight distribution curves can be generated.

The slopes as well as the intercepts of the lines shown in Fig. 4 are in the general range described by theory, but again considerable scatter is apparent. The ratio of empirical slopes to those predicted on the basis of eqn. 10 are 1.1 and 0.5 for the ultra-thin channel and 1.3 and 2.3 for the thin channel, the two numbers in each case representing 51,000 and 160,000 molecular weight samples, respectively. Again, there is no substantial case for abandoning eqn. 10, which enters and influences the subsequent analysis time expressions. We note, however, that if more

detailed studies show the need to modify eqn. 10, the changes can be carried easily through the remaining equations of the theory section.

The annoying scatter in Fig. 4, which clouds a more finely tuned interpretation, may be largely associated with injection problems, and these might also act to increase plate height. Relaxation effects contribute also to increased plate height, but these are probably not very important, even for the fastest analyses, since the calculated relaxation times<sup>15</sup> of the 51,000 and 160,000 molecular weight fractions are only about 10 sec for the thin channel and 2 sec for the ultra-thin channel.

In the case of the ultra-thin system, dead volume may have made a substantial contribution to  $H$ , and it may also have influenced the apparent  $R$  value. Thus in going from the thin to the ultra-thin system, the apparent  $R$  of the 160,000 molecular weight sample increased from 0.27 to 0.37. The temperature increment, at the same time, dropped from 60 °C to 56 °C, but this is not enough to explain the change in  $R$ . An increasing dead-volume effect might be responsible for part of this increase in  $R$ . (The apparent  $R$  value increases with increasing dead volume, and will actually approach unity when the dead volume greatly exceeds the channel volume.) Unfortunately, our efforts to measure the effective dead volume were not successful. Of course, every effort was made to keep it minimal.

While the plate heights of Fig. 4 cannot be considered to hold an exact correspondence to theory, for the reasons noted above, the figure does verify the strong advantage of reducing channel thickness  $w$ , particularly at high flow velocities. In this sense the figure fully verifies the trend predicted by eqn. 10 and the subsequent equations based on it. The advantages expected for increasingly thin channels are therefore confirmed by these data. The realization of these advantages is demonstrated more fully in Figs. 5, 6 and 7.

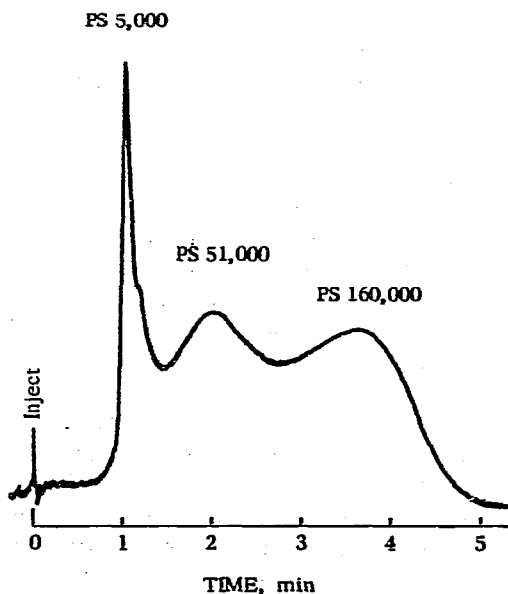


Fig. 5. Fractogram of a three-component mixture of polystyrenes separated in the thin channel.  $\Delta T$  was 60 °C,  $\bar{V}$  was 62.2 and  $\langle v \rangle$  was 0.71 cm/sec.

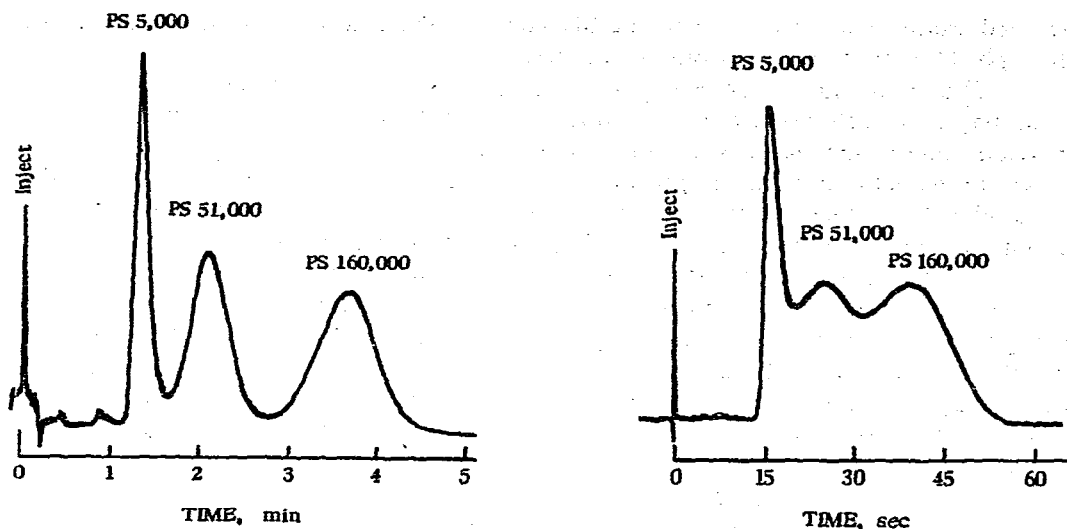


Fig. 6. Separation of a three-component mixture of polystyrenes in the ultra-thin (0.051 mm) channel.  $\Delta T$  was 60 °C,  $\dot{V}$  was 21.1 ml/h and  $\langle v \rangle$  was 0.56 cm/sec.

Fig. 7. Fractogram showing the separation of a three-component mixture of polystyrenes in the ultra-thin channel.  $\Delta T$  was 60 °C.  $\dot{V}$  was 115 ml/h and  $\langle v \rangle$  was 3.05 cm/sec.

Figs. 5 and 6 show the elution of three polystyrene fractions in comparable periods of time (4–5 min) from the thin and ultra-thin channel system, respectively. The mean flow velocities  $\langle v \rangle$  are relatively high, 0.71 and 0.56 cm/sec, respectively. While the partial resolution shown in Fig. 5 may be adequate for some purposes, it is far inferior to the separations and therefore the potential informational content contained in Fig. 6. While these figures do not directly show a speed advantage for the ultra-thin channel, the speed advantage clearly exists because of the trade-off already demonstrated between resolution and speed. Thus the superior resolution for the ultra-thin channel can be exchanged for increased speed in this channel. This is demonstrated in Fig. 7. The flow velocity—and thus the speed—of the ultra-thin system has been increased over five-fold (to 3.05 cm/sec) relative to that used in Fig. 6. The resolution has degraded to a level comparable to that shown for the thin-channel system in Fig. 5, but the speed is nearly six times greater. Elution is complete in less than 1 min. Thus, to a very good level of approximation, the prediction of eqns. 24, 26 and 28 that analysis time for a given resolution increases with  $w^2$  (a factor of 6.25) is verified.

The ultra-thin-channel system demonstrates that FFF can be made competitive with fast exclusion chromatography using present technology. However, the remarkable thing is that we are still far removed from the theoretical potential of FFF. For instance, if we could reduce the average  $R$  value of the 51,000 and 160,000 molecular weight fractions from approximately 1/3 down to 1/10, analysis time, according to eqn. 28, would be reduced over ten-fold relative to the best results presented here. Lower  $R$  values would show rapidly increasing improvements in speed. We note that  $R$  values as low as *ca.* 0.02 have been realized in sedimentation FFF<sup>3</sup>. We conclude,



therefore, that significant gains in speed beyond those observed here are a likely prospect for the future of FFF.

#### ACKNOWLEDGEMENT

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