FIELD–FLOW FRACTIONATION OF POLYMERS: ONE–PHASE CHROMATOGRAPHY

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Abstract – Field-flow fractionation (FFF) is introduced as a one-phase
chromatographic system utilizing an external field to differentially
retain high molecular weight polymeric and particulate species. The
principles and theory of FFF are described. FFF and exclusion chroma-
tography are then compared on the basis of their underlying separative
mechanisms, and the way that these mechanisms influence and limit experi-
mental capabilities. This comparison is continued in a more quantitative
way by examining fundamental column selectivity requirement for polymer
fractionation. Several examples of polymer fractionation by FFF are then
shown. Finally, some of the extreme limits of FFF performance are discussed,
including resolution, separation speed and high and low molecular weight
limits.

INTRODUCTION

One of the outstanding challenges in the broad discipline of chemical separations is the
general achievement of high-resolution polymer fractionation. The magnitude of the
technical hurdle is not unlike that confronted in separating isotopes over three decades
ago. One of the common problems is that in each case the fractional difference in
transport or equilibrium properties between close-lying species is extremely small and
separations based on these differences are accordingly hard to achieve. Polymer fractiona-
tion has its own unique problems, however, in that most chromatographic systems are at
best only marginally, if at all, applicable to polymers (1). Exclusion methods of
chromatography (gel filtration and gel permeation chromatography) have been developed,
but these have inherent limitations and drawbacks that will be pointed out later. Non-
chromatographic methods, including solubility fractionation and ultracentrifugation,
have failed to provide high resolution. It is apparent, therefore, that effective
approaches to polymer fractionation are extremely limited in scope. This fact makes
the appearance of any truly new approach to polymer separations an uncommon event deserving
close scrutiny to see if new capabilities have thereby been introduced.

Field-flow fractionation (FFF) is a broad methodology capable of separating many complex,
high molecular weight species, including nonpolar, synthetic polymers. It can be applied
to biological particles such as viruses, nonbiological particles such as latex beads and
chromatographic support particles, biological macromolecules such as proteins, and non-
biological macromolecules such as polystyrene and polyacrylic acid. Its scope appears to
be very broad throughout this high mass range. However, this report will be limited to
the potential applicability of FFF to nonbiological polymers alone.

The concept of FFF was first proposed by the author in 1966 (2), and experimental work has
been underway since that time. Progress at first was slow due to the difficulties of
initiating a new type of experimental system. Recent years have witnessed increasing
versatility, resolution and speed in the separation of both polymers and particles by FFF.
Separations have now been carried out over the effective molecular weight range 10^3–10^{15},
a trillion-fold mass range. In terms of particle size, this corresponds to diameters
ranging from about 0.001 µm to over 10 µm. In another direction, separation times for
polymers have been reduced to approximately one minute (3).

FFF resembles chromatography in both the experimental and dynamical aspects of its opera-
tion, and it can therefore be thought of as a chromatographic method. However, there is
no stationary phase: separation occurs in an open channel containing a single moving
fluid. For this reason FFF has been described as one-phase chromatography (4,5).

Because FFF functions like a chromatographic system, the theoretical analysis of intrinsic
resolving power and separation speed is subject to the same principles and definitions
used in exclusion chromatography (EC) and other chromatographic methods. However, in

many respects, the theory is more exact. This will be seen when theoretical aspects
are developed in a later section.

It is the object of this paper to describe the principles, preliminary results and
advantages of FFF operation. We shall also discuss the basic question dealing with
factors that limit polymer resolution in chromatographic systems. In addition, FFF and
EC will be compared in selectivity. Finally, some of the theoretical limits of FFF
will be explored, including the limits to resolution, speed, and the upper and lower
molecular weight limits.

PRINCIPLES OF FFF

The principles of FFF are illustrated in Fig. 1. A lateral field acting across the faces
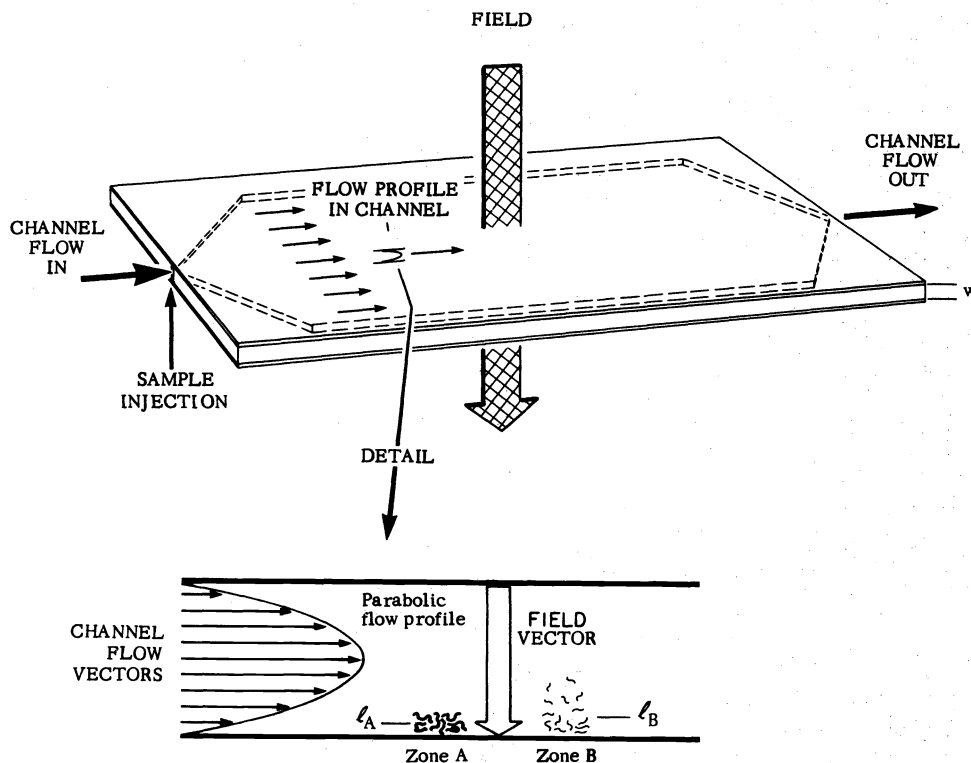

Fig. 1. Schematic illustration of FFF channel (top) and of flow profile
and solute layer distribution in channel (bottom).

of a narrow flow channel forces the solute into a thin, steady state cloud, hugging the
channel wall. The thickness of the cloud is different for each distinct polymer or
particulate species, depending on the physical basis of the coupling between the field
and the species, and on the solute-solvent diffusion coefficient. In Fig. 1, for
instance, the cloud for species A is compressed more by the field than that for species
B. Therefore, very little of A extends into the faster flow streamlines shown near the
center of the channel, and the zone of A consequently moves downstream slowly (that is,
A is highly retained). The cloud for species B extends into these fast streamlines,
and the mean velocity of B molecules is therefore relatively fast (not highly retained).
Other species may form clouds of different mean thickness and therefore exhibit various
intermediate levels of retention. Because of differential retention, zones are eluted
and collected at different times, thus providing separation (6,7).

Types of fields used with FFF.
Any field (or effective field) that interacts significantly with polymers can be used in
FFF. Each different field type gives rise to a new methodological subtechnique having
its own unique advantages and experimental problems. Among the possible fields are:
1) electrical, 2) sedimentation, 3) thermal, 4) cross-flow, 5) concentration, 6) dielectric,
and 7) magnetic. These give rise to FFF subclasses named, respectively, electrical FFF,
sedimentation FFF, thermal FFF, flow FFF, and so on. We have achieved effective separa-
tions with the first four of these subtechniques and have gained the best separations and
the most experience with 3), thermal fields that act through thermal diffusion. These

thermal FFF systems have quite effectively separated polymers in organic solvents, but they have so far been ineffective in most aqueous solutions. For water soluble polymers we have used cross-flow as our effective field. Higher molecular weight polymers may eventually be separated effectively by sedimentation FFF and charged polymers may be subject to electrical FFF analysis. Some of the limitations of these various subtechniques in polymer analysis have been described elsewhere (8).

Theory of retention

The solute cloud held in place by the field of an FFF system follows an exponential distribution much like the atmosphere of the earth as it is held in a distribution that is approximately exponential by the force of gravity (7). In the case of FFF, solute distribution can be represented by Eq. 1.

$$c/c_o = \exp(-x/\ell) \tag{1}$$

where c/c_o is the concentration relative to its value at the lower wall, x is the distance from that wall, and ℓ is a characteristic length parameter termed the mean layer thickness. It can be shown that the latter parameter is given by

$$\ell = D/U \tag{2}$$

where D is the diffusion coefficient and U is the mean lateral velocity introduced by the presence of the field.

The treatment of retention is simplified if we introduce the dimensionless layer thickness $\lambda = \ell/w$, where w is the channel thickness. This definition combined with Eq. 2 leads to the equation

$$\lambda = D/Uw \tag{3}$$

It is clear on intuitive grounds that retention depends on parameter λ. In our earlier discussion of the basis of retention, we noted that retention depends upon the relative penetration of molecules or particles into the fast streamlines of the flow channel. This relative penetration clearly depends upon ℓ which measures how far out from the wall the molecules or particles extend and channel thickness w which governs the dimensions of the parabolic flow profile in the channel. A more exacting mathematical treatment shows that the relationship between retention and λ is

$$R = 6\lambda[\coth(1/2\lambda) - 2\lambda] \tag{4}$$

where R, the retention ratio, is the void volume of the column V^o divided by the retention volume V_r. Consequently, the retention volume can be expressed as

$$V_r = V^o/6\lambda[\coth(1/2\lambda) - 2\lambda] \tag{5}$$

In the limiting case of highly compressed solute layers and high retention (λ small) Eq. 5 assumes the limiting form (5,9)

$$V_r = V^o[(1/6\lambda) + 1/3] \tilde{=} V^o/6\lambda \tag{6}$$

In this limit, which is approximately valid for almost the entire practical range of operating conditions in FFF, retention volume assumes a simple reciprocal dependence on λ. On top of this simplifying relationship we note that λ itself is specified in terms of physicochemical parameters and column dimensions as shown in Eq. 3. By comparison, the theory of EC is less direct and entails parameters that depend on the detailed pore size distribution of the porous support. The latter is so complicated that a satisfactory theory that will work for real column supports has not yet been formulated, and nearly all retention is described empirically.

Theory of peak dispersion

The theoretical plate height, which describes peak dispersion in chromatography and chromatographic-like columns, can be expressed by the equation (10)

$$H = \frac{2D}{R\langle v \rangle} = \chi \frac{w^2 \langle v \rangle}{D} + \Sigma H_i \qquad (7)$$

This expression, which is analogous to the H equations of chromatography, accounts for longitudinal diffusion, nonequilibrium and extraneous contributions, respectively. Under ideal circumstances, only the nonequilibrium term contributes to H, and we have the simplified expression

$$H = \chi \frac{w^2 \langle v \rangle}{D} \qquad (8)$$

Parameter χ is a complicated function of λ and thus of R, and at high retention levels (R<<1) can be approximated by the limiting form R3/9 (11). Thus H assumes the ideal, limiting form

$$H = (R^3/9)w^2\langle v\rangle/D \qquad (9)$$

This equation predicts a strong decrease in H with decreasing channel thickness w and with increasing retention (decreasing R).

Theory of separation speed

Satisfactory separation requires that a certain number of theoretical plates, N, be developed by the column. (N is equal to column length, L, divided by H.) Maximum speed therefore requires that these N plates be developed in the least possible time, t, i.e., that $\dot{N} = N/t$ be maximized. The maximum rate of generation of plates can be shown to equal (12)

$$\dot{N}_{max} = D/4\ell^2 \qquad (10)$$

which shows the importance of working with small ℓ values (maximally compressed zones), which is virtually equivalent to operating at high retention levels.

COMPARISON OF FFF AND EC

While FFF and EC are both chromatographic-like methods capable of fractionating polymers, they differ in many fundamental respects. These differences are expected ultimately to reflect themselves in fractionating performance. In this section we wish to discuss some of the ties between separative mechanisms and experimental capabilities. We note at the outset that the mechanism underlying both techniques is rather unique among chromatographic methods.

Exclusion chromatography methods are singular in that partitioning between the mobile and stationary phases (the pore space) is governed by entropy effects related to the reduced configurational freedom of large molecules in narrow pores. Entropy favors exclusion from the pores, and it is in the (size-dependent) degree of exclusion that selectivity if found. Because of entropy-based exclusion, the retention volume range is limited to the volume of the pores. Furthermore, retention tends to be temperature and solvent independent, so that it is very difficult to influence retention in any given EC column, or to utilize programming methods (13). Finally, the entropy mechanism requires intimate contact between solute molecules and pore walls, which tends to maximize any adverse surface effects. All of these features of EC are disadvantages to effective versatile operation.

Field-flow fractionation systems are equally unique. They are one phase systems in which retention is governed by the interaction of solute with an external field or gradient. Selectivity occurs as a result of the differential layering of solute molecules in the quasi-stagnant liquid adjacent to one wall.

The fundamental properties of FFF lead, generally, to operational advantages. However, we note at the outset that the formation of thin solute layers reduces sample capacity and puts rather severe demands on detector sensitivity.

The open, one-phase channel of an FFF system is subject to rather exacting theoretical treatment, as noted earlier. This makes it possible to correlate observed retention parameters with fundamental physicochemical constants. In some cases, unknown constants may be obtained by the exacting measurement of retention, even for trace constituents in a complex mixture. This facet of FFF has been explored, utilizing different FFF subtechniques, with respect to diffusion coefficients (14), Stokes diameters (9), molecular weight (15,16) and thermal diffusion factors (17).

The open channel of an FFF column also has the advantage of minimizing solute-surface interactions. While some surface perturbations are unavoidable, they are relatively slight, and can be reduced by the proper choice of wall material and field strength. A quantitative comparison of potential solute-surface interactions in EC and FFF is presented elsewhere (4).

The use of an external field to control retention is perhaps the greatest advantage of FFF. External field strength--and along with it retention--can be varied over wide limits, either from one run to the next, or as a function of time within a single run. Generally, then, field strength conditions can be adjusted to the requirements of nearly any complex mixture within a single column. A reduction to zero (which has no analog in most conventional chromatography) allows for complete column flushing. Different time-based variations lead to versatile programming systems. Altogether, the versatility and speed with which retention can be controlled has no parallel among the other chromatographic methods.

Other advantages of FFF, particularly when compared to EC methods, include the reduction of shear stresses due to the parallel flow pattern within the channel, and the relative insensitivity of experimental molecular weights to slight errors in flow velocity.

Other aspects of the comparison of EC and FFF have been presented in another paper (4).

Because EC and FFF are based on intrinsically different mechanisms, their elution and selectivity characteristics are expected to be quite different from each other. This is apparent, first of all, in elution order, which is opposite for the two methods: in EC large molecules elute before small molecules, and in FFF the small species appear first.

The differences, however, go much more deeply than simple elution order. The ability to generate selectivity and to deal with a wide molecular weight range is also quite different between techniques. The major advantages in this regard appear to lie with FFF. In order to establish this on a sound basis, it is necessary to examine in some detail the general column requirements for polymer fractionation. This is the subject of the next section.

COLUMN SELECTIVITY REQUIREMENTS FOR POLYMER FRACTIONATION

Whether using FFF or chromatography, the polymer analyst will generally seek a column that can resolve two fractions that are ΔM apart in molecular weight so that the information from the fractogram will accurately reflect changes in polymer distribution over $\sim \Delta M$ in range. Quantity ΔM must be small to provide detailed information, but it can be large if the demands of the analysis are minimal. Generally a ΔM will be required that is some fraction of the average molecular weight, M, in that working range, so that a specific value of $\Delta M/M$ can be considered as a fixed requirement (or at least a desired goal) to a first approximation.

The essence of elution methods like EC and FFF is that a molecular weight spectrum develops with elution volume, V_r. Each volume cut corresponds theoretically to a specific molecular weight M, so that there is some functional relationship between V_r and M

$$V_r = V_r(M) \tag{11}$$

which is to be determined by theory, calibration, or some combination or extrapolation of the two. Although Eq. 11 lies at the heart of the analysis, its exact form may elude definition for many practical polymer studies. We assume here that some exact or approximate form does exist.

Because of the column fractionation defined by Eq. 11, a small change, δM, in molecular weight elicits a shift, δV_r, in elution volume of the magnitude

$$\delta V_r = (dV_r/dM) \, \delta M \tag{12}$$

where the derivative, dV_r/dM, is defined by Eq. 11. For larger changes, ΔM, Eq. 12 is still valid as a first approximation.

$$\Delta V_r \cong (dV_r/dM) \Delta M \tag{13}$$

Thus a required resolution of magnitude ΔM translates into a required volume increment, ΔV_r. The problem is that not all columns will actually resolve components that appear within ΔV_r of each other because of zone spreading. Criteria must be established to judge this capability.

For two components appearing ΔV_r apart, the resolution is

$$R_s = \Delta V_r / 4 \bar{\sigma}_V \qquad (14)$$

where $\bar{\sigma}_V$ is the average value of the standard deviations of the individual components, measured in volume units. In order to achieve a resolution of unity, the peaks must be sufficiently narrow ($\bar{\sigma}_V$ adequately small) that $R_s = 1$ in Eq. 14. This requires, as we see from Eq. 14, that $\bar{\sigma}_V$ not exceed the value

$$\bar{\sigma}_V = \Delta V_r / 4 \qquad (15)$$

In chromatographic studies, the magnitude of $\bar{\sigma}_V$ is usually expressed in terms of the average number of theoretical plates, $\bar{N}$, in the column. Since $\bar{N} = (V_r/\bar{\sigma}_V)^2$, we have

$$\bar{\sigma}_V = V_r / \bar{N}^{\frac{1}{2}} \qquad (16)$$

The combination of Eqs. 15 and 16 yields an equation for the number of plates required for unit resolution

$$\bar{N} = 16(V_r/\Delta V_r)^2 \qquad (17)$$

The substitution of Eq. 13 for $\bar{\Delta V}_r$, followed by rearrangements, yields

$$\bar{N} = 16 / \left(\frac{d \ln V_r}{d \ln M}\right)^2 \left(\frac{\Delta M}{M}\right)^2 \qquad (18)$$

In words, the minimum number of plates required for a specific resolution depends on two things: the _fractionating power_, $M/\Delta M$, needed to reach the goals of polymer characterization and the _column selectivity_, $|d \ln V_r/d \ln M|$.

Importance of column selectivity

We wish to emphasize the importance of the latter term, which we give the special symbol, S

$$S = |d \ln V_r/d \ln M| \qquad (19)$$

Absolute value bars have been used because it makes no difference to resolution whether V_r increases or decreases with M. Quantity S expresses the fractional change in elution volume with a given fractional change in M. The essence of "selectivity" as an intuitive concept is that it represents a large change in elution volume or time with a small change in a solute property such as molecular weight M. This concept is clearly represented by the S expression of Eq. 19. Its importance is indicated by substituting S for $d \ln V_r/d \ln M$ in Eq. 18.

$$\bar{N} = 16/S^2 (\Delta M/M)^2 \qquad (20)$$

This equation shows, in comparing two columns, that a doubling of S reduces the plate requirements by a factor of four. Thus--given equal plate heights at equal solute velocities--a column with a doubled S will be only one-quarter as long as the original column, and require only one-fourth the time for elution, without a loss of resolving power. Thus it is clear that S is a parameter of major importance in describing column performance, and in comparing different columns that may be used for polymer fractionation.

Column selectivity for FFF

A detailed examination of column selectivity, S, for FFF and EC systems shows that higher values of S can be obtained for FFF, and that they persist over a wider molecular weight range than they do for EC. This matter will now be explored.

Eq. 5 can be written in the form

$$V_r = V^o / 6 \lambda f \qquad (21)$$

where

$$f = [\coth(1/2\lambda) - 2\lambda] \tag{22}$$

Taking the logarithm, then the derivative, of Eq. 21 leads to the selectivity expression

$$S = \left|\frac{d \log V_r}{d \log M}\right| = \left|\frac{d \log}{d \log M}\right| \left(1 + \frac{d \log f}{d \log \lambda}\right) \tag{23}$$

In the high retention limit, $d \log f/d \log \lambda$ is negligible, so that

$$S_{max} = |d \log \lambda/d \log M| \tag{24}$$

The value of $d \log \lambda/d \log M$ varies from one FFF subtechnique to another, and also depends on the configuration of the solute. For random coil polymers the values of Table 1 apply. Table 1 shows that sedimentation FFF is superior to the other methods, but unfortunately it

TABLE 1. Maximum values of column selectivity, S_{max}, for various
          FFF subtechniques.

Subtechnique	S_{max}
Thermal FFF	0.5-0.6
Flow FFF	0.5-0.55
Sedimentation FFF	1

is difficult to achieve significant retention in this system unless $M > 10^5$ (8).

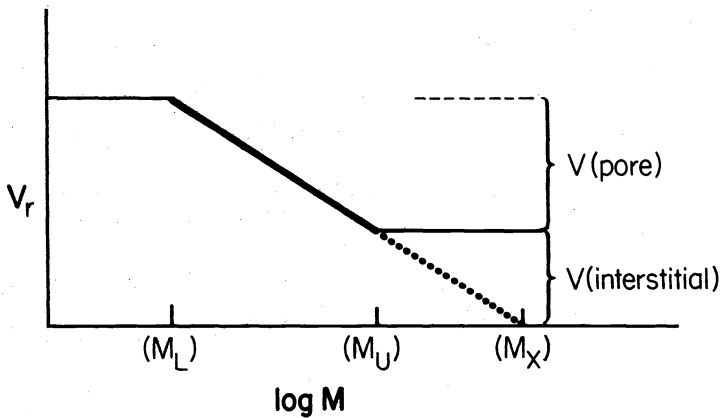Fig. 2. Elution volume function (calibration curve) for exclusion chromatography. The heavy line is described by the equation, V_r = A-B log M.

Column selectivity for EC

In the case of EC, we must start with the common empirical logarithmic calibration curve in order to specity the form of Eq. 11.

$$V_r = A - B \log M \tag{25}$$

This function is plotted in Fig. 2. The figure serves to define upper and lower molecular weight limits, M_U and M_L, and the extrapolated value M_X. The molecular weight range, r, can be defined as

$$r = M_U/M_L \tag{26}$$

It has been shown that S as defined by Eq. 19 is equivalent to (18)

$$S = 1/\ln(M_X/M) \tag{27}$$

Thus, selectivity reaches a maximum at the highest possible values of M, which is near the upper molecular weight limit, M_U. Below, we show how S values can be simply derived.

If we consider the two extreme components at M_U and M_L, we note according to Fig. 2 and Eq. 25 that the elution volume separating them is the total pore volume, V(pore). Eq. 25 yields

$$V(\text{pore}) = B \log(M_U/M_L) = B \log r \tag{28}$$

from which we get B

$$B = V(\text{pore})/2.3 \ln r \tag{29}$$

In the same way that V(pore) can be related to $\log M_U/M_L$ in Eq. 18, V(interstitial) can be related to M_X/M_U

$$V(\text{interstitial}) = B \log(M_X/M_U) = 2.3 B \ln (M_X/M_U) \tag{30}$$

We note again that the maximum of S occurs for $M = M_U$

$$S_{\text{max}} = 1/\ln(M_X/M_U) \tag{31}$$

Substituting $\ln M_X/M_U$ obtained from Eq. 30 into 31, and using Eq. 29 for B, we obtain

$$S_{\text{max}} = \frac{V(\text{pore})}{V(\text{interstitial})} \frac{1}{\ln r} \tag{32}$$

By similar reasoning, the value of S_{min}, reached at the lower molecular weight limit, M_L, is given by

$$S_{\text{min}} = \frac{V(\text{pore})}{V(\text{interstitial}) + V(\text{pore})} \frac{1}{\ln r} \tag{33}$$

These equations show the potential value of maximizing internal pore space and of reducing interstitial space, perhaps by compressing the chromatographic bed.

Good EC packings have V(pore) $\sim$ V(interstitial) and r $\sim$ 10^2. For such a column, $S_{\text{max}} = 0.22$ and $S_{\text{min}} = 0.11$. Values of S_{max} and S_{min} for these and a few other parameters are tabulated in Table 2. This table illustrates the detrimental effect on selectivity of

TABLE 2.   Minimum and maximum values of column selectivity, S, for EC columns having various parameters

Range r (M_U/M_L)	$\dfrac{V(\text{pore})}{V(\text{interstitial})}$	S_{min} to S_{max}
10^2	1	0.11–0.22
10^2	0.5	0.07–0.11
10^3	1	0.07–0.14
10^3	0.5	0.03–0.05
10^4	1	0.05–0.11

attempting to increase range, r, in any given column system (including a system of coupled columns), and of giving up pore space within a particle in order to achieve a more rigid packing.

The comparison of Tables 1 and 2 shows that all forms of FFF have a considerable advantage over EC in column selectivity. This is all the more striking when it is realized that theoretical plate requirements vary with the inverse square of S, as shown by Eq. 20. Thus an EC column with S = 0.1 requires 25 times more plates to reach a specific fractionating power than an FFF column with S = 0.5.

The above comparison illustrates only one of the advantages of FFF over EC that stem from intrinsic column characteristics. Another potential advantage relates to range, r. We note from Table 2 and Eq. 32 that any effort to increase range in EC leads to a reduction in selectivity. In contrast, the molecular weight range of FFF, at least in theory, is virtually infinite, with no reduction in selectivity with increased range. This advantage stems from the fact that FFF relies on an active retention mechanism, rather than on an exclusion mechanism. The positive retention of FFF means that fractionation can occur over the entire range from one void volume, V^o, up to 5-50 V^o, and possibly more. Therefore, virtually any requirement for increased range can be met by utilizing another increment of the normal elution volume range. This range might best be augmented by programming methods in some cases (19,20).

The matter of fractionating power and its interaction with molecular weight range and peak capacity in both EC and FFF has been explored more fully elsewhere (18).

POLYMER FRACTIONATION BY FFF

Polymer fractionation by thermal FFF was first noted in a paper published in 1967 (21). Since that time, work has been done to improve column efficiency, to increase speed (3), to introduce programming methods and thus extend the molecular weight range (19), to develop high field strength methods for dealing with low molecular weight species (22), and to utilize flow FFF for water soluble polymers (23). In this section we will show selected results from these studies to illustrate the present state of advancement of FFF in polymer separations.
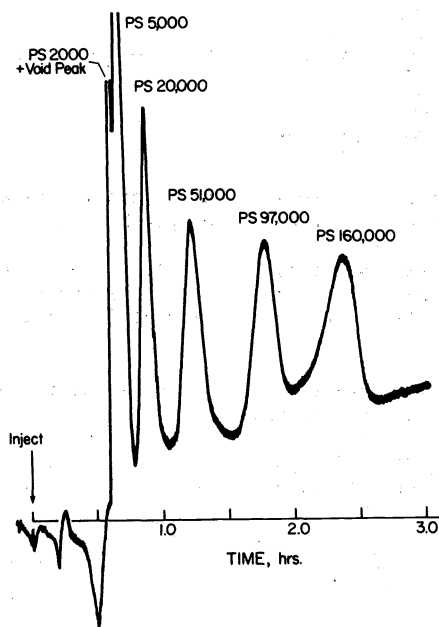

Fig. 3. Separation of polystyrene fractions of the molecular weight indicated by a new thermal FFF system.

Figure 3 illustrates the separation of six narrow polystyrene fractions using a new, high efficiency column system. These results, unpublished until now, were recently collected by Dr. Michel Martin in our laboratory.

In a previous study, we showed that the molecular weight range that could be covered in a single run could be extended by the use of programming (19). By this method, the temperature drop in a thermal FFF system is started at some high value (say 80°C) appropriate to low molecular weight species, and gradually reduced to zero so that it passes successively through conditions appropriate to various higher molecular weight polymers. Figure 4 illustrates the application of this concept. Using a parabolic

temperature program (curved line), we were able to identify the nine peaks of a nine-component polymer mixture, plus the void peak. The molecular weight range for this single run extends from 4000 to 7,100,000, a ratio of nearly 2000.
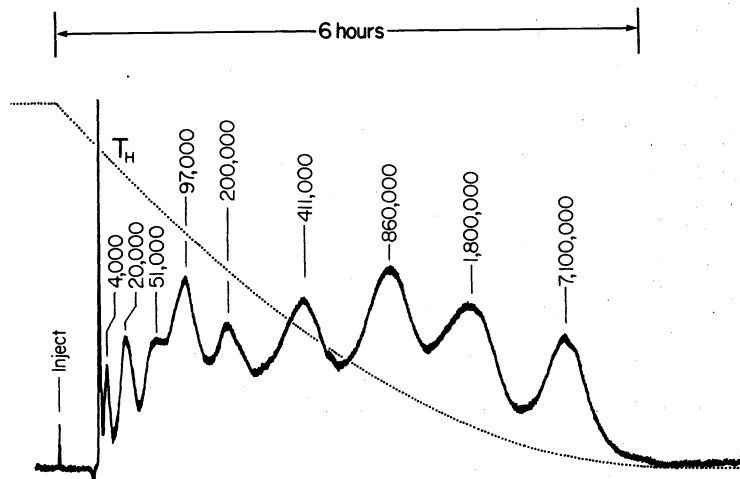

Fig. 4. Separation of polystyrene fractions in ethyl benzene solvent by programmed thermal FFF. The curved line indicates the progression of the hot wall temperature during the course of the run. The temperature drop started at 70°C and ended at zero.

Recently we have developed an ultrathin column system (w = 51 μm) to increase the speed of polymer separations (3). The results of this approach are illustrated in Fig. 5. Both a 5-minute and a 1-minute separation are shown. The former shows superior resolution but reduced speed due to the lower flow velocity employed.
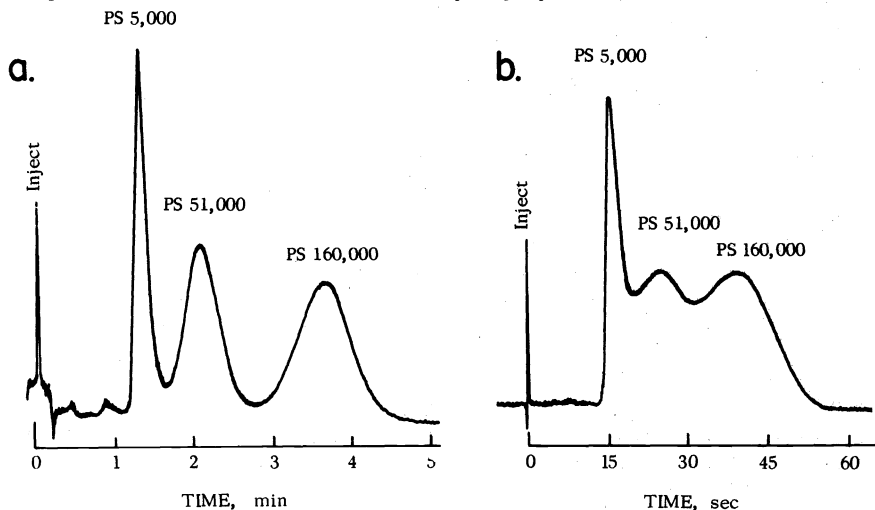

Fig. 5. Separation of polystyrene fractions in a 0.446 m long ultrathin thermal FFF column system with a temperature drop of 60°C. Mean flow velocities, <v>, are a) 5.6 mm/sec and b) 30.5 mm/sec.

Finally, in Fig. 6 we show the extension of FFF to water soluble polymers (23). In this case, flow FFF was employed because thermal FFF has not been effective in aqueous solutions. This switch to an alternate subtechnique illustrates one of the strengths of FFF that may prove important in future polymer studies: when one subtechnique does not function properly under the conditions necessary for separation, several other subtechniques are available to fill the void. Consequently, it is likely that all soluble polymers will eventually be subject to one form or another of FFF analysis.
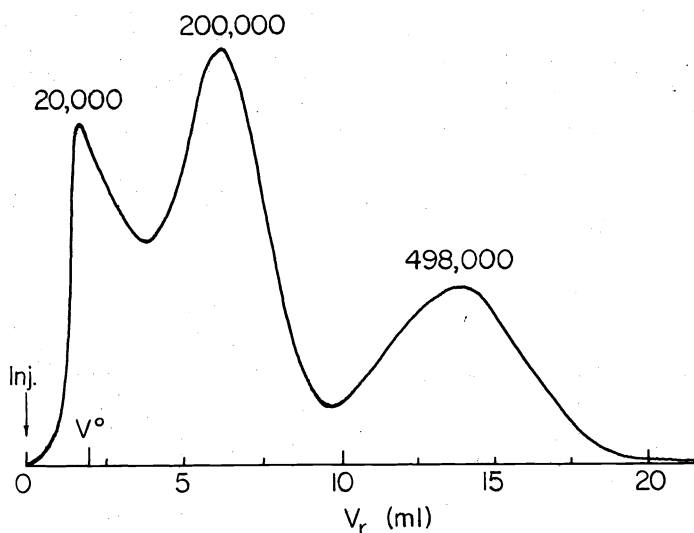
Fig. 6. Separation of three sulfonated polystyrenes by flow FFF in an
aqueous solution. Molecular weights refer to values prior to sulfonation.

LIMITS OF FFF PERFORMANCE

The versatility of the FFF methodology has been discussed on several occasions in this
paper. In some respects, the simple theory of FFF suggests rather remarkable characteristics
that cannot possibly be realized in practical systems. This section will deal with some of
the limiting factors that now impose themselves or will eventually impose themselves on
FFF performance.

Resolution

Eq. 9 predicts that plate height decreases without limit as retention increases (R decreases).
A plate height approaching zero is equivalent to a resolution approaching infinity.
Clearly, such a limit is not possible in real systems. We wish to investigate here some
of the factors that eventually interject themselves between real and ideal systems.

Increasing retention in FFF is associated with an increasing compression of solute layers.
The mean layer thickness, ℓ, will assume lower and lower values in this process. However,
it is impossible to approach the limit $\ell = 0$ for a number of reasons. First of all,
imperfections at the column wall would negate the formation of perfect layers of zero
thickness. Second, such highly compreseed layers would require such high sample dilutions
to avoid solute-solute interactions that the resulting solute profile would be undetectable.
Third, molecular size imposes a limit on ℓ roughly equal to particle radius (24). Fourth,
infinite field strength would be required to approach this limit. Fifth, finite elution
speed would require infinite solvent velocity, which would require infinite pressure drop
within the column. Finally, even if the nonequilibrium contribution of Eq. 9 could be
reduced to zero, the ubiquitous contributions due to injection, detector dead volume
spreading in external tubing, and so on, would maintain H at a finite level.

Despite the obvious failure of Eq. 9 at the extreme limit of retention, this expression
provides valuable guidance on the direction to be taken to improve both column efficiency
(plate number) and resolution. It is increasingly apparent, from this equation and our
laboratory experience, that the outstanding potential of FFF can best be realized under
conditions of high retention. Experimentally, these conditions have been more difficult to
realize with polymers than with particles, due undoubtedly to strong nonidealities in
compressed layers of polymer solutions. While we have been able to work with particles
at retentions up to 50 void volumes, we have found it difficult to work with polymers
beyond about five void volumes. Fortunately, programming methods, as illustrated in Fig. 4,
are able to partially offset this problem.

In a fundamental sense, small ℓ values are sought in FFF because ℓ is the approximate
distance over which molecules must diffuse to approach equilibrium between different
velocity lamina. Therefore, ℓ is a parameter analogous to particle diameter in liquid
chromatography.

Separation speed

Eq. 10 shows that the rate of generation of theoretical plates increases inversely with the
square of ℓ. Thus, with separation speed as well as with resolution, the degree to which ℓ

can be reduced determines the practical limits of performance.

Recently, we have made significant progress in the speed of polymer separations by working with reduced channel thickness, w, which, as noted above, leads to reduced ℓ. Separation times have been reduced to $\sim$ 1 min, whereas previously separation required $\sim$ 1 hr. An example of a high speed separation was shown in Fig. 5.

Factors limiting the practical reduction of ℓ were outlined in our discussion of resolution, and need not be repeated here.

Upper molecular weight limit

There is increasing interest in the fractionation and characterization of polymers having molecular weights of 5-10 x 10^6 and higher. Polyolefins and various water soluble polymers are notable examples. There is no intrinsic reason why such polymers cannot be fractionated by FFF, although practical problems may be encountered in the form of nonidealities and viscosity increases in the thin layers of solute developed. Therefore, it will be especially important to work with small samples (high dilutions) and sensitive detectors in seeking to extend the molecular weight limit upward.

One of the brightest prospects for high molecular weight polymers is the potential application of sedimentation FFF, whose field strength is not adequate to retain lower molecular weight species. As shown by Table 1, the column selectivity, S, of sedimentation FFF is unity, a value higher than that for any other chromatographic method. Coupled with this, retention in sedimentation FFF is related directly to molecular weight. The latter, therefore, need not be extrapolated from measurements that are essentially related to molecular dimensions, as are the retention volumes in EC systems.

Some limits that would be encountered with increasing molecular weight, aside from the nonideality and viscosity effects already noted, would be shear degradation, and finally the effects of increasing molecular size, which introduces a lower limit to ℓ (24). We have noted previously that the parallel streamlines of FFF constitute a favorable system for avoiding shear. With regard to molecular size, it is doubtful if any serious limit would be encountered at less than 10^7 - 10^8 molecular weight. Any analysis beyond this extreme of molecular weight may need to utilize the recently developed technique of steric FFF, which is applicable to particles from 1-100 μm in effective diameter (25).

Lower molecular weight limit

In many cases small polymer molecules fail to interact sufficiently with external fields to form the thin solute layers necessary for FFF. The obvious solution to this problem is to increase field strength to such a level that the necessary interactions occur. In thermal FFF, however, the field strength is essentially the temperature increment, ΔT, between hot and cold walls, and is limited eventually by the freezing point and boiling point of the solvent.

In a recent study, we were able to extend the liquid range by imposing a pressure of eight atmospheres on the system, thus raising the boiling point nearly $100^\circ C(22)$. With this we were able to work at $\Delta T = 158^\circ C$, approximately twice the value of the nonpressurized system. Polystyrene of molecular weight 600 was measurably retained in this system, whereas the previous limit had been over 2000.

Other approaches to reducing the molecular weight limit include the use of columns with surface grooves, which act as "traps" to enhance the retention of solute (26), and the use of vertical columns where retention can be augmented by convective flow.

In flow FFF systems, where the field strength is essentially the cross-flow velocity, low molecular weight polymers would require the use of small-pore membranes with adequately low molecular weight cutoffs, and the construction of columns able to withstand the high pressures accompanying the increased flow rate and the decreased permeability.

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