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FIELD-FLOW FRACTIONATION

EXTENDING THE MOLECULAR WEIGHT RANGE OF LIQUID CHROMATOGRAPHY TO ONE TRILLION (10^{12})

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SUMMARY

The principal driving force for the explosive renaissance in liquid chromatography in the last decade can be found in the failure of gas chromatography to accommodate compounds of medium and high molecular weight. However, even liquid chromatography, including its subclass of exclusion chromatography, weakens at very high molecular weights and eventually becomes inapplicable. The primary difficulties stem from unbalanced phase distribution and interfacial adsorption. These problems are largely avoided in field-flow fractionation (FFF).

FFF is a methodology resembling chromatography in its dynamical aspects. However, it is a one-phase system, and is therefore, technically, not chromatographic. In FFF, external fields or gradients in one phase replace the partition and adsorption forces that, in chromatography, distribute solute between differential flow regions.

Working in one phase, one can virtually eliminate the distortion in phase distribution and the interfacial adsorption that eventually occur with increasing molecular weight in all forms of chromatography. FFF analysis can be extended freely into the polymer and even the particulate range. So far, a billionfold mass range has been explored, roughly from 10^3 to 10^{12} daltons.

In this paper, the principles, applications, and characteristics of FFF are described; some limitations of exclusion chromatography are noted; and the basic factors involved in extending the tractable molecular weight range are discussed.

INTRODUCTION

Field-flow fractionation (FFF) is a concept now 10 years old[1]. A crude separation of two polystyrene fractions was reported in 1967[2]. The separation of polystyrene fractions has been steadily refined since then[3–5]; at least nine components are presently separated in a single run[5]. Also in the intervening years FFF has been shown applicable to proteins[6,7] viruses[7,8] and polystyrene latex beads[7,9–11], and, in preliminary effects, to cell nuclei and other particles and polymers. Its special advantages are now becoming apparent in handling "massive" and complex materials over a wide range of molecular weight (MW), polarity and stability.

Field-flow fractionation is an appropriate topic for a conference on liquid chromatography (LC) because the fundamental mechanism of chromatography —in which solute is partitioned reversibly between regions of different velocities[12,13]— applies also to FFF. In addition, the experimental train and the elution pattern are generally similar, and there are also some overlapping applications.

FFF does not, however, technically belong to the chromatographic class of separations because partitioning occurs in one phase, not two, as demanded by accepted definitions. However, the method can, with some liberties taken, be described as "liquid chromatography in one phase"[14], or as "one-phase chromatography" as it first appeared in an abstract in 1969[15]. The term "field-flow chromatography" is also descriptive.

FFF works by using an external field or gradient to selectively partition solute into different flow regions of a flow channel. The channel is characteristically empty of packing, smooth in cross section, and theoretically tractable in its performance. The preferred configuration is illustrated in Fig. 1.

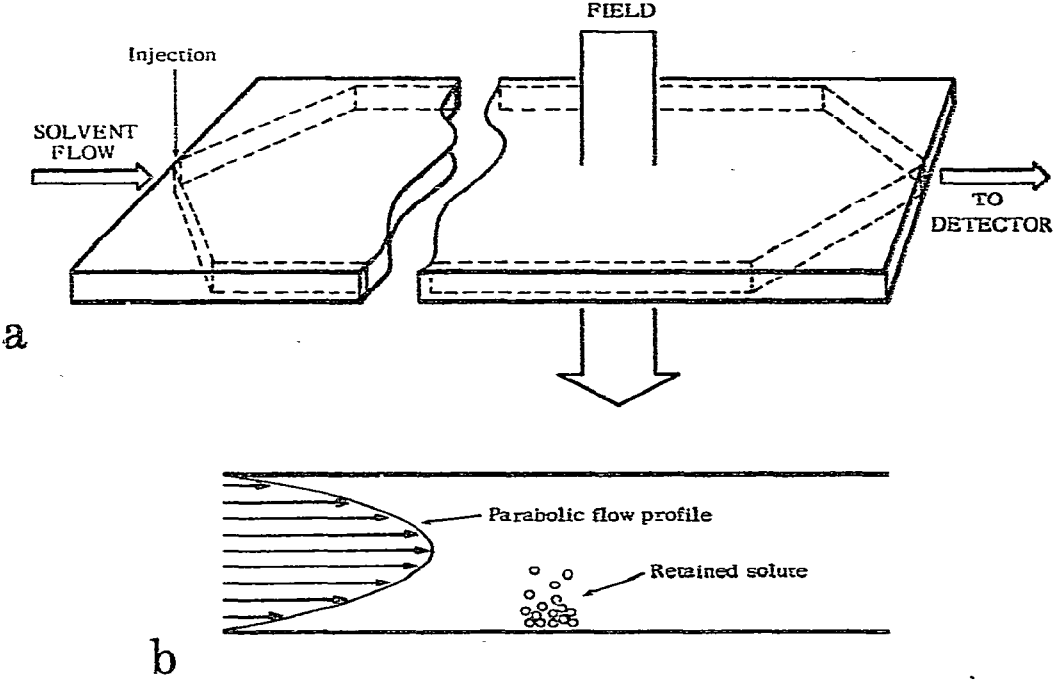

Fig. 1. (a) Configuration of parallel-plate channel for field-flow fractionation. (b) Side view.

In practice, the channel is arranged in an experimental sequence like that of liquid chromatography: a liquid pump controls flow; injection of a narrow sample is made at the head of the channel; a detector and a recorder monitor the eluting fluid. Only the nature of the channel —and the mechanism of fractionation— are significantly different.

In essence, an FFF channel resembles a chromatographic column containing

only a mobile phase. It is, of course, difficult for many chromatographers to imagine any selective influence on solutes without a stationary phase. However, in FFF, an external field takes over the role ordinarily played by the stationary phase. By acting in a direction lateral to flow, it forces solute into relatively quiescent flow regions near one of the channel walls. These semi-stagnant regions of FFF are analogous to the stationary phase in chromatography, and the fields that draw them there are analogous to the attractive forces generated by the stationary phase.

In theory, there should be roughly as many variations of FFF as one now finds in chromatography. The most basic variations stem from the various kinds of "fields" used to force retention in FFF. This is analogous to the types of stationary phases in chromatography. With chromatography, there are liquids, solid surfaces, ion exchangers, porous exclusion media, liquid crystals, etc. So far in FFF, we have worked with thermal gradients[4,5], electrical fields[6], cross-flow fields[7,11], sedimentation fields[8–10] and concentration gradients. These subtechniques are named, respectively, thermal-, electrical-, flow-, sedimentation-, and concentration-FFF. Other fields are possible also.

In addition to the basic variations, many techniques can be imagined for programming, recycling, varying the channel configuration and dimensions, combining different fields, and even combining the technique with chromatography so that the two retention influences act simultaneously.

It is clear, therefore, that FFF, like chromatography, is versatile enough in its many variations to use with both aqueous and non-polar solvents, with charged and uncharged species, with random chain molecules as well as particles, and with components spanning an enormous MW range.

At first sight then, the potential versatility of FFF is comparable to that of chromatography. However, such a conclusion is only moderately useful because it does not define the major strengths and weaknesses of the two methodologies. These arise out of the fundamental nature of the technique independent of the number of forces (phases) or variations applicable.

In subsequent material, we will consider some of the fundamental factors operating in both chromatography and FFF that bear on fractionation. We will emphasize applicability over a large MW range, and to species of extremely high MW. First, however, we sketch the essential elements of the theory of FFF.

THEORY OF FFF

The lateral field or gradient of FFF compresses solutes into layers against the channel wall. The steady state distribution of these layers is usually exponential[16,17]

$$(c/c_0) = \exp(-x/l) \tag{1}$$

where c_0 is the concentration of solute at the channel wall, c is the concentration at altitude x, and l is a characteristic parameter —roughly the mean thickness— of the distribution. Simple theory shows it to be given by

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

where D is the solute-solvent diffusion coefficient and U is the mean velocity of solute induced by the field. More useful than l is dimensionless parameter λ

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

where w is the channel width.

The retention ratio, R, is related to λ by the straightforward equation[15]

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

At high retention (small R), this equation reduces to the simple expression

$$R = 6\lambda \tag{5}$$

This equation is often applicable in practice.

Eqns. 4 and 5 relate the experimental retention ratio R (void volume/peak retention volume) to λ, a parameter dependent on the simple physical and physicochemical quantities of eqn. 3.

Retention can, of course, be expressed directly in terms of retention volume, V_r, and void volume, V_0. Since $V_r = V_0/R$, we have, using eqns. 4 and 5

$$V_r = V_0/6\lambda\,[\coth(1/2) - 2\lambda] \approx V_0/6\lambda \tag{6}$$

The theory of plate height has also been developed, but its details are far less simple. Plate height, H, should theoretically be dominated by the non-equilibrium term[16],

$$H = \chi\, w^2 \langle v \rangle / D \tag{7}$$

where $\langle v \rangle$ is mean channel flow velocity and χ is a coefficient that depends on λ in a complicated way.

The dimensional form of eqn. 7 resembles that of chromatographic non-equilibrium terms[13]. This is not surprising considering their similar origin.

MOLECULAR WEIGHT OF PARTICLES

FFF is applicable to an exceptionally wide mass range of molecules and particles. Most particles (with some exceptions, including viruses) are characterized by particle diameter, d_p, more often than by MW. However, in order to provide a continuity of scale, we use "molecular weight" to describe all species, including those in both globular and linear-chain configurations.

The molecular weight, M, of a spherical particle of diameter d_p is

$$M = (1/6)\, N \pi \varrho\, d_p^3 \tag{8}$$

where N is Avogadro's number and ϱ is mean particle density. This equation reduces to

$$M = 0.31534\, \varrho\, d_p^3 \tag{9}$$

providing d_p is expressed in Ångstrom units and ϱ in g/cm^3.

MOLECULAR WEIGHT RANGE AND RESOLUTION

It is important to emphasize that a rigid limit on the capability of chromatographic-like columns (including those of FFF) is imposed by two parameters: (a) number of theoretical plates, N, and (b) retention volume range, V_n/V_1, equal to the elution volume of the final peak to that of the first peak. Years ago it was shown that the largest number of resolvable peaks (the peak capacity, n) compatible with these two parameters is[18]

$$n = 1 + \frac{N^{\frac{1}{2}}}{4\,R_s} \ln \frac{V_n}{V_1} \tag{10}$$

Here, the original equation has been modified to indicate the resolution, R_s, between adjacent peaks.

Given, then, a limited n —with, for example, a polymer sample— one may imagine having the choice of two extreme cases: (1) one can separate extremely close-lying peaks such as homologs differing only by δM in MW; or (2) one can separate peaks across an extremely broad range, ΔM, in MW. In case 1 the high resolvability would be associated with a restricted range which could span at most *ca.* $n\delta M$ daltons. In case 2, the large range would carry a limitation of $\delta M \approx \Delta M/n$ average discrimination between adjacent peaks. Of course, in reality, some intermediate case may prevail, or some combination representing different capabilities in different regions. However, given N and V_n/V_1, these basic compromises exist between range and resolution. These compromises, as they limit exclusion chromatography and FFF, have been elaborated theoretically[3].

In order to throw light on the range *versus* resolution question in particular systems, it is necessary to examine the elution spectrum: the values and increments in elution volume with increasing MW (or with changes in other properties). The elution spectrum shows fundamental differences between the main classes of chromatography and FFF, and these in turn help determine the respective capabilities and limitations of the different methods.

Before proceeding, we distinguish two basic classes of chromatography. First are the most common forms that include both adsorption and partition chromatography. We call this class *retention chromatography*, because there are positive forces acting at the interfaces to prevent the unrestrained movement of solute into the mobile phase. Thermodynamically, the retention originates largely in enthalpy differences of solute in the two phases.

The second fundamental class is *exclusion chromatography*, which is well named because solute tends to be expelled, or excluded, from narrow pores constituting the stationary phase. The forces of expulsion arise in the loss of entropy that large molecules suffer when they are in a constricted region[19,20].

We can now formulate the elution spectrum of retention chromatography by utilizing Martin's classical assumption that chemical potential effects are the additive sum of group contributions[21]. This gives an exponential dependence of retention volume on M

$$(V_r/V_0) = 1 + a e^{bM} \tag{11}$$

The exponential elution spectrum of retention chromatography compares to an almost linear spectrum in FFF. An unpublished analysis of the latter shows that we can write

$$(V_r/V_0) = \omega M^q \tag{12}$$

where q ranges from 1/3 to 1.

Exclusion chromatography is at the other extreme, yielding a spectrum generally described as logarithmic

$$V = A + B \log M \tag{13}$$

The implications of the distinct classes of retention spectra, as summarized in the last three equations, are as follows. The exponential spectrum of retention chromatography provides excellent resolution, but soon the successive peaks become so well separated from their precursors that they occupy the practical limit of retention volume, and no more peaks can be accommodated. Thus, range is limited. The near-linear spectrum of FFF provides a compromise situation between range and resolution. The logarithmic spectrum of exclusion chromatography is inherently good for range, but the limited retention volume, $(V_n/V_1) \approx 2$–2.5, makes this property difficult to utilize. Ranges are typically only about two orders of magnitude in MW. At the same time, resolution is impaired by the logarithmic dependence. One can extend the range by mixing pore sizes, but only by diluting resolution further. The nature and limitations on such compromises has been discussed[3].

With programming, the retention spectrum of both retention chromatography and FFF can be altered to incorporate a much greater range without a significant loss in resolution. Inasmuch as the range of FFF is already broad without programming, it can, in theory, be extended to very broad limits. The example of Fig. 2, using thermal FFF on polystyrene polymers, suggests this capability, but by no means explores its full potential[5].

The thermal FFF used for Fig. 5 is in many ways typical of FFF methodology: A channel of approximately 0.4 m length and 2 cm width is formed by clamping plates together over a 0.025 cm spacer. However, in thermal FFF, the plates are metallic

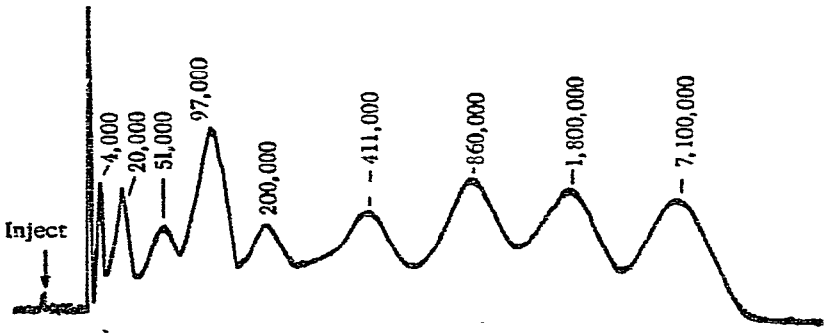

Fig. 2. The programming of thermal FFF has made possible the separation of these polystyrene peaks in a single run[5]. The MW range covered in this run is 1775.

bars, one heated and one cooled. The "field" shown in Fig. 1 is thus nothing more than a temperature gradient capable of inducing thermal diffusion. Programming is achieved by steadily lowering the temperature of the heated plate.

SOME DRAWBACKS OF EXCLUSION CHROMATOGRAPHY

Exclusion chromatography (EC) —both gel filtration and gel permeation— has had an unprecedented success in macromolecular analysis[22], and its remarkable value will undoubtedly continue for this class of materials. However, exclusion chromatography as a system has some serious drawbacks that are infrequently discussed; these require a better understanding in order to gain perspective and to continue extending the MW range. Accordingly, some of the drawbacks are listed below. A few are discussed in some detail and new results are presented.

Limited peak capacity

As discussed earlier, the peak capacity of EC, at a given plate number, N, is limited; it is about 3–5 times smaller than for retention chromatography and for FFF[18]. Column efficiency, as measured by N, must be increased roughly 9–25 times to offset this disadvantage. This factor makes more difficult the extension of EC across a broad molecular weight range, except when a serious loss in resolution can be tolerated[3].

Lack of retention control and programming

In a given column, very little control can be exerted over retention by the normal control mechanisms existing in retention chromatography (mainly solvent type and temperature). For the same reason, programming is exceptionally difficult although minor success has been achieved[23].

Difficulties in the calibration of retention

The undefined pore space of exclusion media has so far frustrated a theoretical description of retention. Thus no *a priori* theory, applied to a real porous material, has succeeded in correlating retention volume with molecular characteristics. Moreover, it is not even clearly understood which molecular dimension parameter is most suitable. Hydrodynamic volume has been widely used[24], but it can be shown that this parameter is *not* a universal calibration factor, because in model pore systems where calculations are possible, species of different shapes but equal hydrodynamic volumes are unequally retained[19,25].

Shear degradation

The shear degradation of polymers during EC analysis becomes important at an MW of *ca.* 10^7. This effectively imposes a ceiling on the MW limit of EC for these materials.

The gravity of the shear problem is greatest in high-efficiency systems, because these employ small particles and large pressure gradients to maintain high flow velocities. At a given mean flow velocity, the shear gradient is inversely proportional to particle size. Therefore the avoidance of shear degradation and the use of high-efficiency systems appear to be incompatible goals.

Large surface area

Exposed surfaces become increasingly important as molecular size expands. Adsorption becomes more severe and less inclined to reversibility at high MW's because the free energy of the adsorption process grows in rough proportion to size. Surface catalytic effects can also occur with some fragile macromolecules. A current review listed seven recent references in which retention is perturbed by adsorption effects[26].

Minimum surface effects can be sought by a combination of reducing the activity of surfaces and by reducing their area. However, the latter is subject to very little control in EC because surface area is a fundamental parameter for controlling retention.

Exclusion chromatography requires supports of relatively high surface area. This is so because the exclusion process is caused by pore walls crowding in so closely that the macromolecule's freedom of motion (and corresponding entropy) is severely curtailed. Significant exclusion, therefore, implied substantial pore-wall area.

Theoretical justification exists for this view. The distribution coefficient, K, in a random-plane pore network model can be shown to be related rigorously to the surface area, s, per unit free volume of the support by[19]

$$K = \exp(-s\bar{L}/2) \tag{14}$$

where $\bar{L}$ is the *mean external length* (or effective diameter) of a molecule of any arbitrary shape. (The equation suggests that parameter $\bar{L}$ might come closer to being a universal calibration parameter for macromolecules than hydrodynamic volume.) This equation shows that, for balanced retention ($K = 0.6$) s must be of the order of $1/\bar{L}$. If the pore space consisted of the volume between *parallel* random planes, with $s = 1/\bar{L}$, the average spacing between planes would be $2\bar{L}$. If we envision the solute as a sphere of diameter $\bar{L}$, the average distance from the surface of the sphere to the nearest wall would be of the order of $\bar{L}/2$.

In FFF, by contrast, a macromolecule averages about one "mean layer thickness," l, in distance from the nearest wall.

The relative influence of any surface effect —absorption, catalysis, etc.— is proportional to the crowding of the species against a surface, or inversely proportional to the average distance of the molecule or particle from the nearest surface. Thus, the relative surface effects in EC and FFF are approximately

$$\frac{\text{EC (surface effect)}}{\text{FFF (surface effect)}} = \frac{l}{\bar{L}} \tag{15}$$

assuming a like surface material. For globular materials, assumed to be perfect spheres of diameter $\bar{L}$ and unit density, the ratio $l/\bar{L}$ varies with MW as shown in Fig. 3. For linear polymers, the ratio would be somewhat smaller. It is possible to formulate this ratio for common supports by substituting mean pore diameter for $\bar{L}$. A value of 10 μm is typical for l, although this can be varied, if necessary, to make surface effects even less obtrusive in FFF.

Fig. 3 shows that the ratio of surface effects given in eqn. 15 is indeed quite large. It is the order of 1000, for example, at MW = 10^6. This could be a significant drawback in further extensions of the MW range of EC.

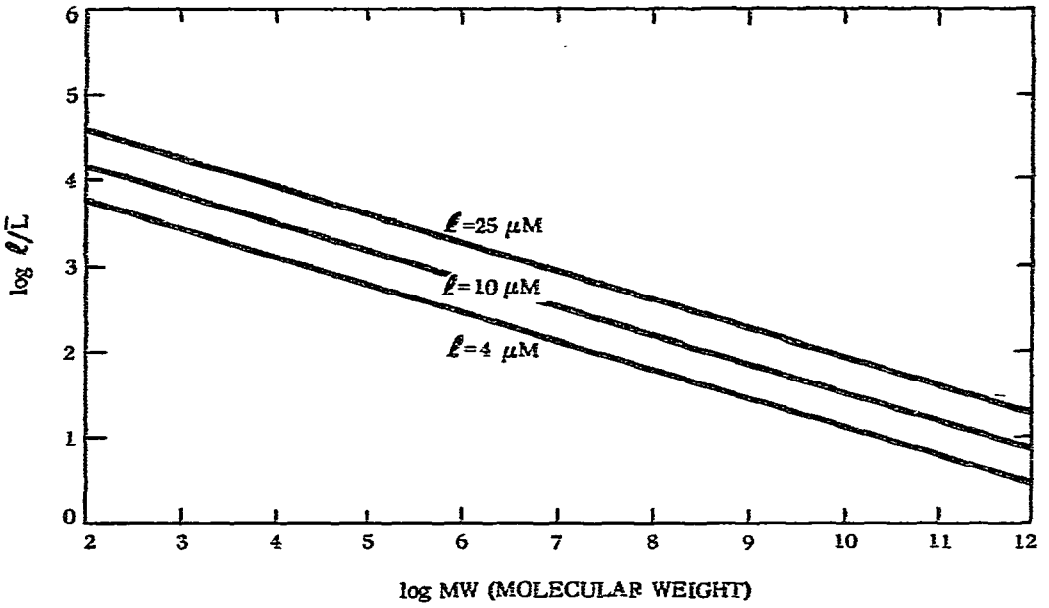

Fig. 3. Plots showing l/L as a function of molecular weight, M, for globular species of unit density. The ratio, l/L, is the approximate ratio of surface effects in EC and FFF, as indicated by eqn. 15.

BASIC CHARACTERISTICS OF FFF

Although the elution fractograms in FFF are superficially similar to those in chromatography, the unique one-phase characteristic and the unlike forces responsible for fractionation create fundamental differences in the way that solutes migrate and separate. An understanding of these differences is important in delineating the advantages and limitations of the two methodologies, and the possibility for extending the tractable MW to very high values.

First, we explore the unique features that are introduced mainly by the existence of a one-phase system.

Minimum surface effects

The fact that relative surface areas are much smaller in FFF than in chromatography has been detailed. The reason, of course, is that surfaces in FFF exist only to the extent necessary to define the boundaries of the channel. Furthermore, the surface material is generally subject to control and substitution. The principle requirement is that the surface material be capable of transmitting the field. Usually, a wide range of materials satisfy this requirement.

In chromatography, surfaces are more extensive and at the same time less subject to control. In retention chromatography, the nature of the surface is fixed by the mobile and stationary phases used. There is generally no independent control for obtaining the desired surface properties. In exclusion chromatography, more control exists but the nature of the surface is still largely determined by the limited range of materials that can be used in forming pores of precisely determined size and size distribution, and in forming particles of the desired size, uniformity and rigidity.

Theoretical tractability and calibration

The unobstructed, well defined geometry of a FFF channel makes possible the rather exact description of retention in a FFF system[17]. By contrast, as noted earlier, the complexity of the pore geometry in exclusion chromatography has so far made it impossible to predict retention exactly in terms of basic parameters.

The utility of theoretical predictability is multiple. First of all, one can more simply predict optimum operating conditions. Perhaps more importantly, complex systems can be characterized to a first approximation without any calibration curve. Material in each region of the elution spectrum is directly characterized by some value of a physicochemical parameter such as diffusivity, or by simple groups of physicochemical parameters. These parameters can, in turn, be related to MW, charge distribution, and other important properties of the solute.

It is very probable that calibration and the use of internal standards will sometimes be useful in FFF, but these will assume the role of sharpening the definition rather than in defining the basic nature of the elution spectrum.

Near linear retention spectrum

The approximate linearity between retention volume and MW yields a compromise; an elution spectrum that provides a reasonably broad range of MW and reasonable resolution for a given plate number, the range can be extended by means we note below.

The near-linear retention spectrum can be attributed to the linear increase in flow velocity with distance above the channel wall in the wall's vicinity[16], and to the inverse dependence of l on MW.

Minimum shear

The openness and smoothness of the FFF channel; the distribution of flow over a relatively large cross section; and the lack of a stationary phase that will immobilize segments of macromolecules against flow; should make the FFF system capable of avoiding shear degradation up to very high values of molecular weights.

We now turn to those distinctive features of FFF that stem from the use of lateral fields or gradients to achieve retention.

External control of retention

By simply varying the field strength, retention can be controlled to any desired level. This control is instantly applicable and can even provide mid-course corrections should that prove desirable. With this mode of control, peaks that are grouped too near to the void peak can be spread out and their resolution improved, as illustrated in Fig. 4. In addition, peaks with excessive retention time can be eluted more rapidly through a reduction of field strength.

Another useful feature of this control is that it is capable of eliminating retention altogether. This makes unnecessary such strategies as back flushing to rid the column of highly retained components. In FFF, one can simply reduce the field to zero and wait for the passage of one void volume in order to sweep the residue out of the column.

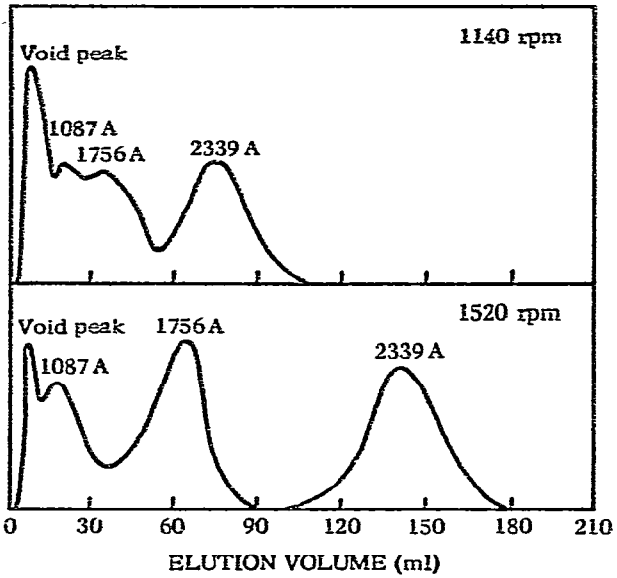

Fig. 4. Increase in retention and improvement of resolution of polystyrene latex particles in sedimentation FFF by increasing the rotor speed from 1140 to 1520 rpm[9].

Versatile programming capability

The strength of the external field in FFF can be varied continuously in accord with any desired program[5,10]. Most advantageously, the program can be designed to increase the MW range of FFF. Thus, if the field strength is continuously reduced during a run, lower MW peaks will elute first, then successively higher MW components will follow as the field is reduced to a level compatible with their reasonable migration and separation. If ultra-high MW components are present, the field can be gradually reduced to whatever levels are necessary for their elution. This open endedness is generally unachievable in chromatography because it is rarely possible to eliminate the phase distribution forces totally. Even when the forces can be eliminated, as in some cases of solvent programming, the point of zero retention cannot be approached in a smooth, continuous and precisely controlled way[27].

The capability of programmed thermal FFF in separating polystyrene polymers over a 1775-fold MW range —from 4,000 to 7,100,000— was shown in Fig. 2. This result was achieved by gradually reducing temperature increment between the plates of a thermal FFF column.

Gentleness of forces

The forces of retention in FFF are continuous and generally quite uniform from one wall to another of the channel. There are no abrupt discontinuities in the forces that might prove destructive to fragile species.

The retention forces of chromatography are diametrically opposite: they are discontinuous by nature because they originate at the interface betwen two phases. These discontinuities can be associated with adsorption and with alterations in the structure of complex species.

Different basis of selectivity

The differential migration of species in FFF is based on a different group of factors than in chromatography. Highly selective solvent–solute interactions are, of course, gone. In its place one has differential levels of coupling between the external field and the components of the mixture, and also differential values of the diffusion coefficient, which is the second selectivity factor reflected in eqn. 2.

Ordinarily we do not expect the coupling of external fields with solutes to be highly selective. In rare instances one would have, perhaps, small clusters of electrical charge that would interact selectively with an electrical field, or unusual chemical groups that might act in some selective way with, possibly, a thermal gradient or concentration gradient.

Ordinarily, high selectivity in complex systems is unnecessary. For example, in polymer analysis one is more interested in a reasonable spectrum across a broad range of MW's than in the effect of any special chemical group. In fact, if there are single groups which may have some unusual effect on the polymer, then molecules containing these groups can be isolated in prior treatment through processes such as extraction.

The degree to which selectivity is achievable or desirable in FFF has not been thoroughly defined. Undoubtedly, chemical properties will influence retention with some types of fields. The importance of this effect must await further work for clarification.

The above compilation defines some of the most important characteristics of FFF, and significant points of contrast between chromatography and FFF systems. The most important implication of this, insofar as the present work is concerned, is the way that FFF characteristics lend themselves to the study of ultra-high MW materials. The minimal surface and shear effects, the inherent range, the gentle forces controllable to zero, and the theoretical tractability combine to provide a tool of utmost promise in going to MW of one trillion and above.

IMPLEMENTATION OF FFF

The implementation of FFF has been difficult because of certain rigorous requirements in the uniformity of the channel, and other normal problems associated with developing a new technology. Nonetheless, the power of the method has expanded considerably in the first decade of its existence —especially in just the last year— and application has been shown to an increasing variety of high MW materials. The evaluation of the applicability of the technique in our laboratories is illustrated in Fig. 5.

Among other things, Fig. 5 shows that several particle systems of MW $> 10^8$ have been successfully retained in various FFF systems. Most commonly we have used polystyrene latex beads (shown in the figure as PS beads) because of their availability in well defined sizes of narrow distribution. Both sedimentation FFF and flow FFF have been used quite successfully with these particles[9–11], and some preliminary results have been noted with electrical FFF as well. We note also that some viruses are in this range. Particles with MW slightly greater than 10^{12} have also been retained in preliminary studies. These are cell nuclei and gel beads (see legend to Fig. 5) of diameters >1 μm. For such systems, the column has been tipped on end to

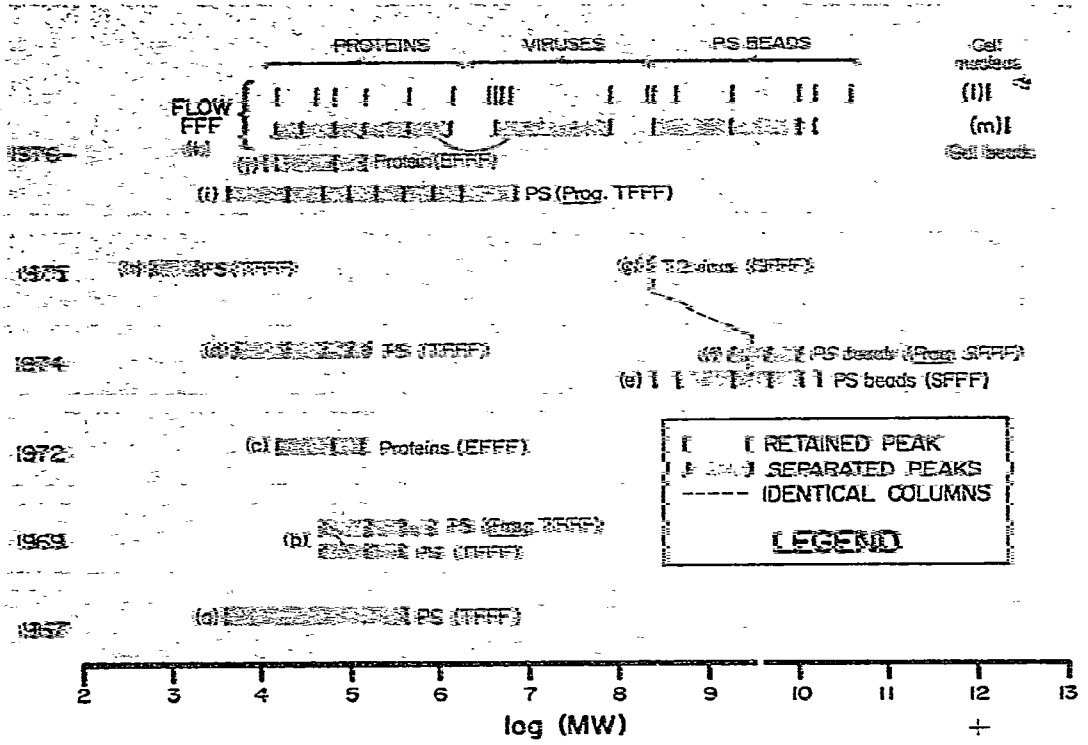

Fig. 5. Components of widely varying MW retained by FFF since inception of the University of Utah FFF program. A number of components of unknown MW have been left out and a few have been estimated. Those of polystyrene beads and gel beads have been calculated using eqn. 9. Each short, verticle bar represents a retained peak distinguishable from the void peak. Each shaded horizontal bar connecting peaks represents a fractionation in which each peak was resolved sufficiently from its neighbors to be distinguishable. PS = linear polystyrene polymer; PS beads = polystyrene latex beads; Prog = programmed FFF; TFFF = thermal FFF; SFFF = sedimentation FFF; EFFF = electrical FFF. (a) Ref. 2; 3525 MW peak barely retained, barely separated from 411,000 MW peak. (b) Ref. 28; first programmed FFF. (c) Ref. 6; flexible membrane channel. (d) Retention data published in ref. 29, but fractogram not published until 1975 in ref. 3. (e) Ref. 9. (f) Ref. 10; two programming methods used—solvent and field strength. (g) Ref. 8; MW of virus determined by SFFF. (h) Ref. 4; an intentional effort to incorporate lower MW's, here down to 600. (i) Ref. 5; nine solutes spanning a 1775-fold MW range separated in a single programmed run. (j) Ref. 30; rigid membrane channel. (k) Parts of this work appear in Refs. 7 and 11; other parts are yet unpublished. (l) Unpublished; retention of nuclei of baby rat brain cells noted. Retention not yet well characterized. (m) Unpublished. Beads are Affi-Gel® 702, nominally 1–3 μm in diameter, from Bio-Rad Labs. (Richmond, Calif., U.S.A.). The size range extends well to either side of the value shown.

avoid the superposition of gravitational effects, although the two fields might, in some case, be used advantageously in combination (sedimentation forces are somewhat more selective than flow forces). The large size of these particles seems to present no important barrier to further extensions in MW.

The bacteriophage T2 retained by sedimentation FFF, while not unique among the components of Fig. 5, shows how the close theoretical relationship between retention and physicochemical parameters can be used to advantage. Retention in sedimentation FFF depends solely on effective mass. Thus from measured retention, we have

arrived at MW = $230 \cdot 10^6$ for the bacteriophage T2, and have thereby helped characterize this system[8].

Fig. 5 also illustrates the dramatic improvement in the resolution, range, and speed of FFF, as well as in the upper MW limit. The first crude separations of 1967 and 1969 (shown as (a) and (b)) were improved many times in resolution by 1976 (i); the time required for a programmed run was reduced from 50 h to less than 4, while the peak capacity increased from 4 resolvable components to 9. We are still nowhere near the theoretical limit of performance in FFF, and we expect comparable gains in the future.

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REFERENCES

1 J. C. Giddings, *Separ. Sci.*, 1 (1966) 123.
2 G. H. Thompson, M. N. Myers and J. C. Giddings, *Separ. Sci.*, 2 (1967) 797.
3 J. C. Giddings, Y. H. Yoon and M. N. Myers, *Anal. Chem.*, 47 (1975) 126.
4 J. C. Giddings, L. K. Smith and M. N. Myers, *Anal. Chem.*, 47 (1975) 2389.
5 J. C. Giddings, L. K. Smith and M. N. Myers, *Anal. Chem.*, in press.
6 K. D. Caldwell, L. F. Kesner, M. N. Myers and J. C. Giddings, *Science*, 176 (1972) 296.
7 J. C. Giddings, F. J. Yang and M. N. Myers, *Science*, in press.
8 J. C. Giddings, F. J. F. Yang and M. N. Myers, *Separ. Sci.*, 10 (1975) 133.
9 J. C. Giddings, F. J. F. Yang and M. N. Myers, *Anal. Chem.*, 46 (1974) 1917.
10 F. J. F. Yang, M. N. Myers and J. C. Giddings, *Anal. Chem.*, 46 (1974) 1924.
11 J. C. Giddings, F. J. Yang and M. N. Myers, *Anal. Chem.*, 48 (1976) 1126.
12 J. C. Giddings, *J. Chem. Educ.*, 44 (1967) 704.
13 J. C. Giddings, *Dynamics of Chromatography, Part I: Principles and Theory*, Marcel Dekker, New York, 1965.
14 J. C. Giddings, *163rd National Meeting, American Chemical Society, Boston, April 9–14, 1972*, Anal 016. American Chemical Society, Washington, 1972, Anal 016.
15 J. C. Giddings, *157th National Meeting, American Chemical Society, Minneapolis, April 13–18, 1969*, American Chemical Society, Washington, 1969, Anal 003.
16 J. C. Giddings, *J. Chem. Educ.*, 50 (1973) 667.
17 E. Grushka, K. D. Caldwell, M. N. Myers and J. C. Giddings, in E. S. Perry, C. J. Van Oss and E. Grushka (Editors), *Separation and Purification Methods*, Vol. 2, Marcel Dekker, New York, 1974, p. 127.
18 J. C. Giddings, *Anal. Chem.*, 39 (1967) 1927.
19 J. C. Giddings, E. Kucera, C. P. Russell and M. N. Myers, *J. Phys. Chem.*, 72 (1968) 4397.
20 E. F. Casassa and Y. Tagami, *Macromolecules*, 2 (1969) 14.
21 A. J. P. Martin, *Biochem. Soc. Symp.*, 3 (1949) 4.
22 K. H. Altgelt and L. Segal (Editors), *Gel Permeation Chromatography*, Marcel Dekker, New York, 1971.
23 J. C. Giddings and K. Dahlgren, *Separ. Sci.*, 5 (1970) 717.
24 H. Benoit, Z. Grubisic, P. Rempp, D. Decker and J. G. Zilliox, *J. Chim. Phys.*, 63 (1966) 1507.
25 E. F. Casassa, *J. Polym. Sci., Part B*, 5 (1967) 773.
26 V. F. Gaylor, H. L. James and H. H. Weetall, *Anal. Chem.*, 48 (1976) 44R.
27 J. C. Giddings, *J. Gas Chromatogr.*, 5 (1967) 143.
28 G. H. Thompson, M. N. Myers and J. C. Giddings, *Anal. Chem.*, 41 (1969) 1219.
29 M. N. Myers, K. D. Caldwell and J. C. Giddings, *Separ. Sci.*, 9 (1974) 47.
30 J. C. Giddings, G. C. Lin and M. N. Myers, *Separ. Sci.*, 11 (1976) in press.