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POLYMER ANALYSIS AND CHARACTERIZATION BY FIELD-FLOW FRACTIONATION (ONE-PHASE CHROMATOGRAPHY)

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SUMMARY

Field-flow fractionation (FFF) is introduced as a tool for polymer analysis and characterization and a theoretical summary is given of its operation. The existing scope of published experimental results is noted with several illustrative fractograms. New experimental results are presented which deal with extending that scope in the direction of (a) high-speed analysis, (b) non-polar polymers other than polystyrene, and (c) water-soluble polymers. Finally, we have analyzed the basic factors that may ultimately determine the limits of application of various FFF subtechniques to different classes of polymers.

INTRODUCTION

Field-flow fractionation (FFF) is a separation method having special advantages in dealing with mixtures of macromolecules and particles¹⁻⁵. Not only is it adept at analytical-scale separations, but also peak elution volumes can be related in rather exacting ways to important characteristics such as diffusion coefficients, molecular weights and thermal diffusion factors. Consequently, the FFF technology has become a tool capable of characterization as well as fractionation⁶⁻⁹. At its best it combines these two functions in such a way that the constituents (even trace components) of a complex mixture are separated upon emergence from an FFF column and are individually characterized (and possibly identified) by the point of emergence.

In its relatively short history, FFF has been applied to biological particles, non-biological particles, biological polymers and non-biological polymers. It is the latter class that we shall address here, looking both at specific experimental examples and then trying more broadly to define the scope and ultimate potential of FFF in dealing with this important class of materials.

THEORY

The concepts and theory of FFF have been described in a number of papers, of which only a few are cited here¹⁰⁻¹². Below we summarize some of the major theoretical conclusions of direct relevance in polymer separations and characterization.

In FFF a field or gradient is applied in a direction perpendicular to the axis

of a narrow flow channel. At the same time a solvent is forced steadily through the channel forming a cross-sectional flow profile of a more or less parabolic shape. When a polymer sample is injected into the channel, it is forced toward one wall by the applied field. The buildup of concentration at the wall is opposed by diffusion. A steady-state condition is soon reached in which the field-induced motion and diffusion are exactly balanced. In this condition the polymer forms an exponential distribution of approximate mean thickness l .

Parameter l is different for polymers of different sizes because of unequal interactions with the field and unequal diffusion coefficients. Usually l is greatest for the smallest polymer components. If we imagine a two component mixture of polymers A and B—where the molecular size of polymer B is less than that of polymer A—then polymer B will form a thicker, more diffuse layer against the channel wall where accumulation occurs. This is illustrated in Fig. 1.

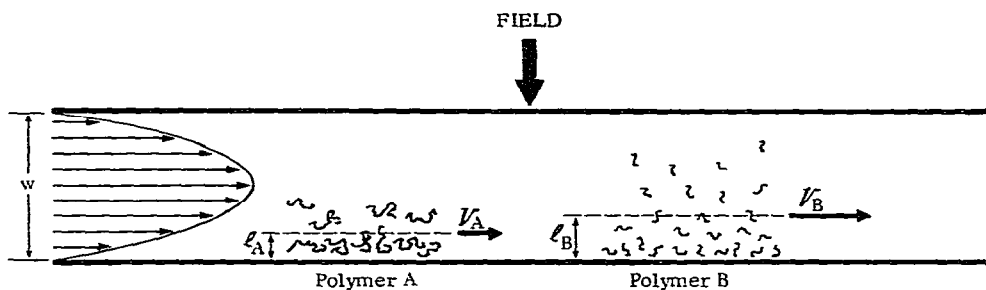


Fig. 1. Illustration of the principles of polymer separation by FFF.

Because the layer for polymer B has a greater thickness than that for polymer A ($l_B > l_A$), polymer B occupies, on the average, regions of higher mean velocity within the parabolic flow profile. Therefore polymer B is carried more rapidly down the channel than polymer A, as suggested by Fig. 1. Consequently, there is a separation of the two polymers.

By the direct extension of this argument, it is clear that a continuous distribution of polymers according to size will migrate with a continuous spectrum of velocities and will emerge at the end of the column with a continuous time distribution. When processed through a detector and its associated electronics, this time distribution becomes an elution diagram or fractogram.

For a polymer of a given molecular size the mean thickness of the layer determines both retention and plate height (peak broadening) characteristics. It is most convenient to express this mean thickness in the following dimensionless form

$$\lambda = l/w \quad (1)$$

where w is the channel thickness, usually only a fraction of a millimeter. The steady-state equations for solute transport lead to the following equivalent expression⁸ for λ

$$\lambda = \frac{D}{Uw} = \frac{\mathcal{R}T}{Fw} \quad (2)$$

where D is the polymer-solvent diffusion coefficient, U is the mean velocity of the polymer induced by the field, \mathcal{R} is the gas constant, T is the temperature, and F is the force or effective force imposed on a mole of the polymer by the applied field.

Retention

In most FFF systems an excellent approximation for retention ratio R is found in the equation^{2,12}

$$R = 6\lambda [\coth (1/2\lambda) - 2\lambda] \quad (3)$$

We thus note that relative retention depends only on dimensionless layer thickness λ . The retention volume V_r depends both on λ and void volume V^0 . It is given by V^0/R , which, with eqn. 3 yields

$$V_r = V^0/6\lambda [\coth (1/2\lambda) - 2\lambda] \quad (4)$$

in which void volume V^0 is simply the internal volume of the channel. Since the channel is without obstructions, V^0 can be calculated from channel dimensions.

In practical FFF operation λ is small. It is usually less than 0.1 and sometimes less than 0.01. Under these conditions the retention volume is described by the following limiting equation¹³:

$$\frac{V_r}{V^0} = \frac{1}{6\lambda} + \frac{1}{3} \quad (5)$$

When the first part of eqn. 2 is substituted for λ , we have

$$\frac{V_r}{V^0} = \frac{Uw}{6D} + \frac{1}{3} \quad (6)$$

A reasonable approximation to the last two equations can be obtained by dropping the fraction 1/3. (This is equivalent to subtracting one third of a void volume from the predicted retention volume V_r , a fair approximation when $V_r \gg V^0$.) We obtain the highly simplified equation

$$\frac{V_r}{V^0} = \frac{1}{6\lambda} = \frac{Uw}{6D} \quad (7)$$

which serves as an idealized model for discussing polymer behavior in FFF.

It is well known that random-coil polymers are governed by a diffusion coefficient-molecular weight equation of the following approximate form¹⁴:

$$D = \text{constant} \times M^{-a} \quad (8)$$

where ideally $a = 0.5$ but in practice a is often in the range 0.5-0.6. Furthermore, we will show subsequently that for most FFF systems applicable to polymers quantity

U is approximately independent of molecular weight M . Under these circumstances the retention volume increases roughly in proportion to M^a .

$$V_r/V^0 \approx M^a \approx M^{0.5} \quad (9)$$

The latter expression provides our idealized model for the retention spectrum of random coil polymers in FFF systems. It has been shown that this kind of spectrum provides an intrinsic resolution considerably superior to that of gel permeation chromatography⁴.

The existence of departures from eqn. 9 will be noted later in this section.

Plate height and separation speed

The plate height in an ideal FFF system in which we ignore longitudinal diffusion and end effects is determined by non-equilibrium processes analogous to those in chromatography^{10,12}. It is also a function of λ , but in the form

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

in which parameter χ is a complicated function of λ (ref. 10). In the limiting case of small λ values (equivalent to high retention) χ becomes

$$\chi(\lambda) = 24\lambda^3 \quad (11)$$

$$\lambda \rightarrow 0$$

In this same high retention limit the maximum number of theoretical plates that can be generated in a unit of time is given by³

$$\dot{N}_{\max.} = D/4\lambda^2 w^2 \quad (12)$$

It is clear from the last three equations that minimum peak dispersion and maximum analysis speed are to be expected in those channels having minimum width w and in which the solute is compressed into a layer of minimum dimensionless thickness λ . Rapid diffusion, measured by diffusion coefficient D , also enhances these objectives.

The above theory and conclusions are quite general, applying for the most part to any type of lateral field. The specific differences encountered in going from one field to another are detailed next.

The subtechniques of FFF

The field or gradient responsible for the formation of solute layers and thus responsible for retention in FFF can be of any form in which there is an interaction with the polymer producing a lateral motion of the polymer molecules. Of the many kinds of interactive fields or gradients possible, four principal field types have proven to be most practical. These have led to the following subtechniques of FFF: (1) sedimentation FFF, (2) flow FFF, (3) thermal FFF, and (4) electrical FFF. The subtechniques depend, respectively, upon (1) sedimentation in a gravitational or centrifugal field, (2) lateral transport by cross flow of solvent, (3) the induction of thermal dif-

fusion by a temperature gradient, and (4) mobility in an applied electrical field. Details of these four subtechniques can be found in the cited literature, and in references contained therein.

In each subtechnique a λ value is established that depends upon the strength of the field or gradient, the diffusion coefficient D , and the magnitude of the coupling between the field and the molecular species. These conclusions are in accord with eqn. 2. The following four equations can be obtained from eqn. 2 under specific circumstances, and describe the λ values of the four respective techniques. Sedimentation FFF^{6,8}:

$$\lambda = \frac{D}{sGw} = \frac{\mathcal{R} T}{GM(1 - \bar{v}_s \rho) w} \quad (13)$$

flow FFF¹⁵:

$$\lambda = DV^0/\dot{V}_c w^2 \quad (14)$$

thermal FFF^{8,16}:

$$\lambda = \frac{D}{D_T w (dT/dx)} = \frac{T}{\alpha w (dT/dx)} \quad (15)$$

electrical FFF¹⁷:

$$\lambda = D/\mu Ew \quad (16)$$

where:

- s = sedimentation coefficient;
- G = acceleration;
- \bar{v}_s = partial molar solute volume;
- ρ = solvent density;
- \dot{V}_c = volumetric cross flow-rate;
- D_T = coefficient of thermal diffusion;
- x = distance from lower channel wall;
- α = thermal diffusion factor;
- μ = mobility;
- E = electrical field strength.

Most of these equations are quite exact. However, eqn. 15 for thermal FFF is a simplified version of more elaborate equations that account for the distortion of the parabolic flow profile by viscosity variations and for the variability of solvent thermal conductivity^{7,15}.

Each of the four preceding equations—at least in the form first presented where two forms are used—is proportional to diffusion coefficient D . This would suggest the validity of the simple retention volume behavior of eqn. 9, which had its origin in the first part of eqn. 2. However eqn. 9 can only be correct if the principal dependence on molecular weight M is through diffusion coefficient D . In the sedimentation case, eqn. 13, this is notably untrue in that sedimentation coefficient s also

has a strong dependence upon molecular weight. In this case a second equality is shown which provides the true molecular weight dependence of λ —an inverse proportionality to M . As a consequence of this dependence the elution volume V_r —as seen by means of eqn. 7—is directly proportional to M . However, in the case of eqn. 14, applicable to flow FFF, λ is rigorously proportional to D and the conclusions of eqn. 9 will therefore apply in the high retention limit.

In thermal and electrical FFF, eqns. 15 and 16, the principal molecular weight dependence is expected to originate in D . However, perturbations will be encountered. In thermal FFF, for example, the coefficient of thermal diffusion D_T has long been recognized as being approximately independent of molecular weight¹⁸. However, there is no rigorous basis for this. Our evidence, in fact, suggests a small decrease in D_T with M according to a power of approximately 0.03–0.05⁷.

In the case of electrical FFF, it has long been recognized that mobility μ is approximately constant for species of a given chemical type but of different size. However, some departure from this constancy is expected for applicable polymers.

The foregoing λ equations can be substituted back into the general retention, plate height and separation speed equations presented earlier in this section in order to predict the behavior of each of the FFF subtechniques. In accord with past experimental results, it is now recognized that the retention equations work with considerable accuracy. Unfortunately the plate height commonly exceeds its theoretical value.

We note that each of the four subtechniques of FFF yields a λ value and thus a retention volume that depends on molecular weight through such parameters as D , s , D_r and μ . Thus each subtechnique is capable of producing a fractionation according to molecular mass. Moreover, given an observed retention volume for a given component, fraction or cut, the retention equations (such as eqn. 4) can be used to fix an experimental λ value for that fraction. With λ established, eqns. 13–16 can then be used—depending on the subtechnique employed—to determine for that fraction values of molecular weight M , diffusion coefficient D , thermal diffusion factor α or the diffusion/mobility ratio D/μ . Thus by this means FFF becomes a powerful tool for the characterization of components in complex materials.

OVERVIEW OF PUBLISHED EXPERIMENTAL RESULTS

The first feeble polymer fractionation by FFF was reported in 1967¹⁹. Two polystyrene fractions were partially separated from one another by thermal FFF in a capillary tube 55.5 m in length. Two years later the rather complete separation of four polystyrene fractions was reported in an improved 3-m channel²⁰. A crude programming system was introduced to aid the latter separation. Shortly thereafter it was shown that retention measurements in thermal FFF could be used to determine the value of the thermal diffusion factor α for polymers²¹.

The more recent efforts in polymer analysis by FFF have employed the subtechnique of thermal FFF also. (We shall note one exception in a subsequent section.) However, the column length has shrunk to approximately 0.4 m and the resolution and speed have been greatly enhanced. Present day channels consist of a ribbon-like space between solid copper bars. The channel thickness (or width) w is typically 0.25 mm. These devices may be programmed²². Also temperature drops of up to 158°

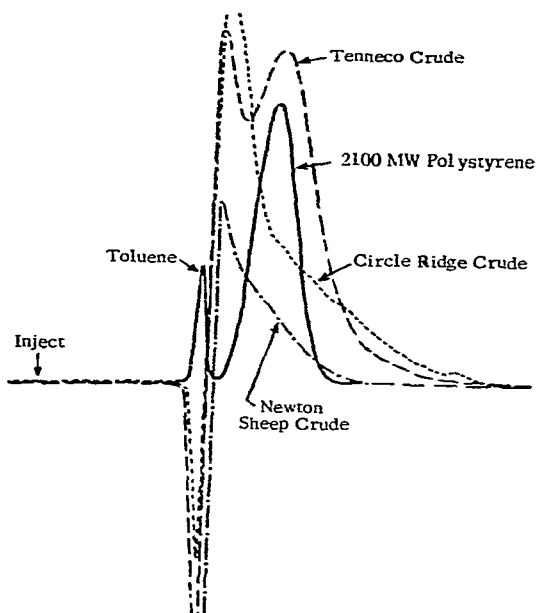


Fig. 2. Fractionation pattern of several crude oil samples achieved with a temperature drop of 158° in thermal FFF. A 2100 molecular weight polystyrene peak is shown for comparison²³.

can be applied in order to extend retention to the low-molecular-weight species²³. As an example of the latter, Fig. 2 shows the retention pattern generated for several crude petroleum samples in comparison with 2100 molecular weight polystyrene. The significance of the pattern cannot presently be fully deciphered because the dependence of the thermal diffusion phenomenon on molecular structure and constitution has not yet been established.

Fig. 3 illustrates a five-component separation obtained with one of the new columns operated under non-varying temperature conditions. However, the resolving

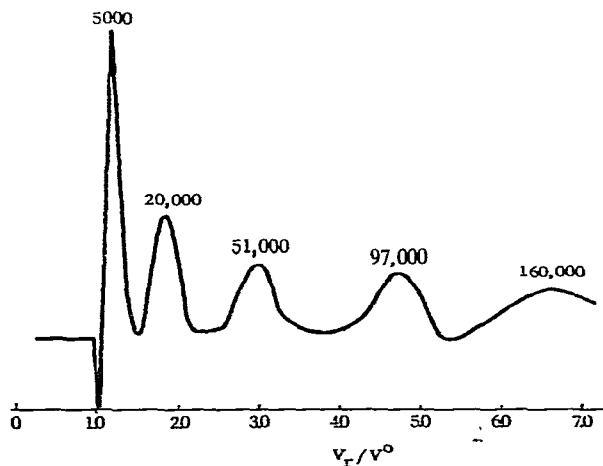


Fig. 3. Five-component separation of polystyrene polymers by thermal FFF⁴.

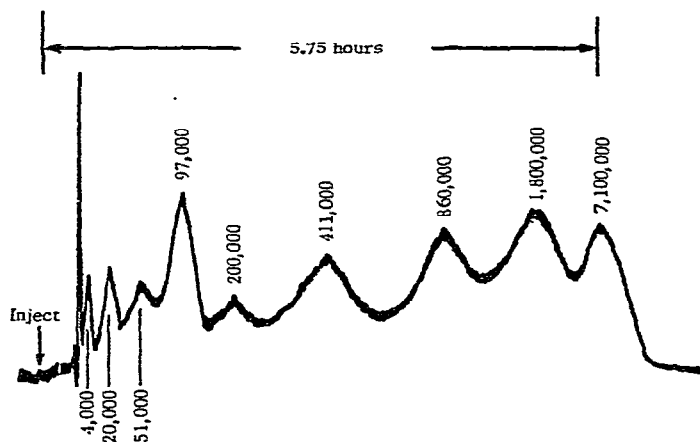


Fig. 4. Programmed separation of polystyrene fractions by a linearly programmed temperature drop in thermal FFF²².

power and range of thermal FFF is best illustrated by various programmed runs²². Fig. 4 is an example of such a run using a linear program (the temperature drop decreases linearly with time). Even better resolution has been obtained with a parabolic program. The separation, as shown in the figure, requires about 6 h. This separation can be hastened by simply increasing the flow-rate and the program-rate, although

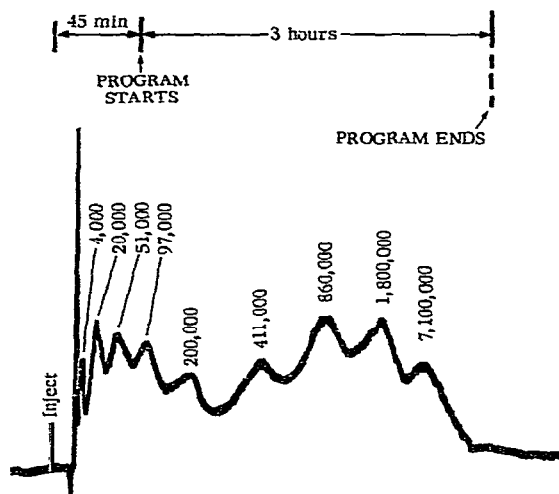


Fig. 5. Programmed separation of polymer fractions using a parabolic program. The program is initiated 45 min after injection²².

some resolution is lost. Fig. 5 shows an example of a separation achieved with a 3-h parabolic program following a 45-min time lag after injection.

The fractionation studies reported above have been accompanied by fundamental studies of column and retention behavior of polymers in thermal FFF. Improved equations have been developed for retention¹⁶ and more accurate means have been developed for extracting thermal diffusion factors from thermal FFF data⁷. A rather extensive study of peak broadening factors has also been completed²⁴. In addition, a fundamental study comparing thermal FFF with exclusion chromatography shows clearly the higher intrinsic resolving power and peak capacity of the FFF methodology⁴.

NEW DIRECTIONS

The short tradition of FFF in polymer analysis is centered around fractionation within a single polymer class—linear polystyrenes. While steady improvements in resolution and molecular weight range have been made for this polymer, as noted in the last section, the results are still too limited to establish this method among the stable of techniques used for polymer analysis. In order to become generally applicable to polymer materials, other polymer classes must be brought within the purview of FFF. In addition, analysis time—now typically measured in hours—must be shortened. We report in this section some preliminary progress toward these goals. In the subsequent section we speculate on the ultimate scope of different FFF subtechniques in polymer analysis.

Separation speed

Eqn. 12 suggests that the maximum rate of theoretical plate production, $\dot{N}_{max.}$, increases with $1/w^2$. This provides a strong motivation for reducing channel width w . Most previous attempts to use channels with w below the typical value of 0.254 mm have not yielded noticeable improvements, perhaps because surface flatness is correspondingly more critical at small w .

Recently we have succeeded in constructing an efficient thermal FFF channel with $w = 0.127$ mm. The construction procedures were conventional¹⁶, except for the use of a spacer of only 0.127 mm thickness. The column was 42.7 cm in length and 2 cm in breadth. Its void volume was 1.04 ml. Samples of narrow-distribution polystyrenes (Waters Assoc., Milford, Mass., U.S.A.) were injected by syringe. The eluted samples were detected by a differential refractometer (Waters R401 or LDS 1103). A temperature drop of 60° was established between copper bars, the colder of which was 24°. Continuous flow was used and relaxation effects were thus uncompensated.

Fig. 6 illustrates the partial resolution of six polystyrene samples in less than 20 min. The fraction with a molecular weight of 2000 was eluted with the void peak and that with a molecular weight of 5000 had sufficient retention to appear as a shoulder only. A higher temperature drop, of course, would increase the retention of the early peaks.

Fig. 7 shows the effect of further increases in speed. As in chromatography, resolution (information) is lost, but time is reduced. In this case three components are partially resolved in less than 4 min at a flow-rate of 76.3 ml/h.

The results of Figs. 6 and 7 demonstrate only a small fraction of the theoretical

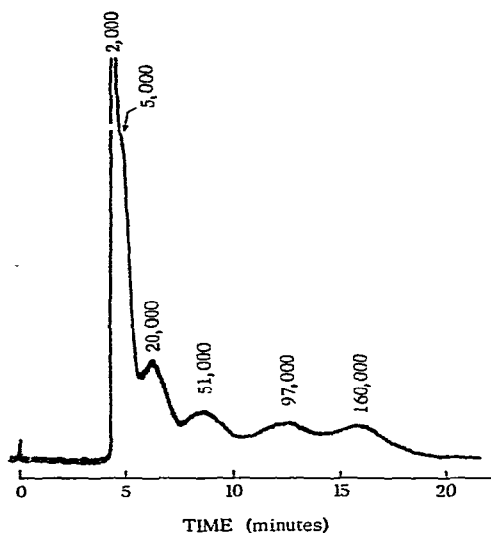


Fig. 6. Partial resolution of six polystyrene fractions (including a shoulder with a molecular weight of 5000) by thermal FFF in a 1.04-ml channel of width $w = 0.127$ mm. Flow-rate = 14.0 ml/h.

effectiveness of FFF, but they represent a clear advance over previous experimental results. We regard them as providing only an opening into the study of high-speed polymer analysis by FFF.

Polymers subject to thermal FFF

Thermal FFF has been the workhorse among FFF subtechniques insofar as polymer separations are concerned. From the beginning we have wondered how widely among polymer classes thermal FFF would apply. The question is still unanswered because no systematic study of polymer thermal diffusion has been carried out.

It would certainly be unusual if the only solute-solvent system subject to

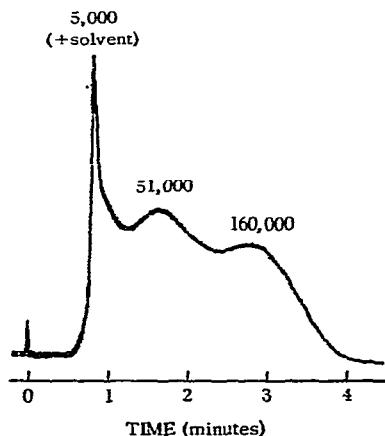


Fig. 7. Partial resolution of three polystyrene components in the narrow ($w = 0.127$ mm) channel. Flow-rate = 76.3 ml/h.

thermal FFF was polystyrene-ethylbenzene. Indeed our previously published work shows that a broad variety of solvents work successfully with polystyrene. Beside ethylbenzene, we have used toluene, chloroform, ethyl acetate, cyclohexane, dimethylformamide, 2-butanone and dioxane^{7,16}. Other solutes, too, are subject to retention, as most simply demonstrated by the retention pattern of crude oils shown in Fig. 2. This retention is especially notable because of the low molecular weight of the crudes, in that retention increases with molecular weight roughly in accord with eqn. 9. Some systems, however, have not yet produced an observable retention, including proteins and carbohydrates in aqueous solution¹⁵.

In the recent past we have gathered some additional data, little of which is conclusive, but which nonetheless merits brief note because of the paucity of information in this important area.

Polymers other than polystyrene have been examined in a limited way. Several difficulties hampered the investigation. Several of the polymers had only limited solubility in the solvent, ethylbenzene, resulting in precipitation on the cold wall of the thermal FFF apparatus when its temperature was *ca.* 16°. This limitation was, of course, especially important in the case of polyethylene. This problem was overcome by raising the temperature of the cold wall to about 100°, but this procedure led to a decrease in the temperature drop available. A second problem was the unavailability of samples of low polydispersity and high molecular weight. Most samples had polydispersities, \bar{M}_w/\bar{M}_n , ranging from 2 to 20 and $M < 50,000$.

In spite of these difficulties, retention was observed or indicated for a variety of polymers including vinyl, acrylates, methacrylates, and polyethylene. In some instances, *R* values of the order of 0.5 were observed with a temperature drop of 50–70°. However, data interpretation was hindered by peak broadening, tailing, and shoulders, much of which could be a product of the severe polydispersity of the samples.

These preliminary results are encouraging but should be followed by a more thorough study in order to clarify the potential role of thermal FFF in analyzing these and other polymers. The polydispersity problem requires a solution, either by means of finding less polydisperse samples, or by taking cuts from the eluent and cycling these again through the FFF column.

Water-soluble polymers

As noted previously, our efforts to retain macromolecules in aqueous solutions by thermal FFF have failed. One of the strengths of the FFF methods, however, is that its subtechniques form a versatile set of such a nature that when one approach fails, another can be expected to take its place. Thus we have turned to flow FFF to fill the apparent gap in the capability of thermal FFF. Flow FFF by itself, as we have noted previously, is one of the most versatile of the FFF subtechniques. It is an obvious candidate for the challenging field of water-soluble polymers.

We have prepared sulfonated polystyrenes by a standard method²⁵. These have been retained in several flow FFF columns. Fig. 8 shows the elution pattern generated by a mixture of three polymers injected into a 1.65-ml column. The peaks are identified with respect to the molecular weight of the polystyrenes prior to sulfonation. A clear fractionation is indicated.

Retention in flow FFF is governed by diffusion coefficients alone. Thus one

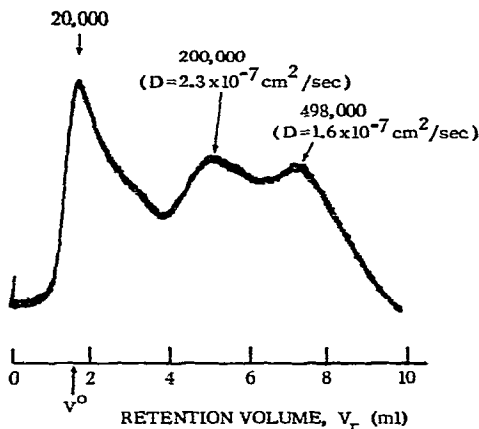


Fig. 8. Fractionation of sulfonated polystyrenes by flow FFF in an aqueous solution containing 0.02 *M* triethanolamine adjusted to pH 8.5 by nitric acid. The numbers indicate the molecular weights of the parent polystyrenes. Diffusion coefficients, *D*, are shown for the two retained peaks. The void volume, 1.65 ml is shown as V^0 . The channel flow-rate was 2.7 ml/h and the cross flow-rate was 16.6 ml/h.

can determine a diffusion coefficient (or Stokes radius) for each retained fraction, or alternatively, calculate a diffusion coefficient (or Stokes radius) distribution. The former is shown in the figure. This rigorous theoretical association of retention and diffusion makes flow FFF a promising tool for characterizing mixtures of polymers. Unfortunately the diffusive behavior of our water-soluble polymers appears to be concentration sensitive, responding to extremes of dilution, so the values given cannot be taken as infinite dilution values.

Again this study is preliminary. It illustrates the growing scope of FFF in polymer analysis but it is in no sense a complete study of this promising new area.

ULTIMATE SCOPE OF FFF IN POLYMER ANALYSIS

Notwithstanding the fact that a large majority of the results obtained by FFF in polymer analysis has been limited to thermal FFF with polystyrene polymers, the ultimate scope of FFF in polymer studies is likely to be very broad. We can only guess at the potential scope of applications at this point although theory provides some important guidelines.

Our discussion of the last section confirmed the applicability of FFF to polymers beyond the conventional limits. Furthermore, in the theory section we showed that the four conventional subtechniques of FFF are all capable of size related fractionations for any polymers for which the methods are applicable. We also showed that important physicochemical parameters could be deduced from the observed retention pattern. The overall prospects, then, are favorable.

We now wish to examine more thoroughly the conditions necessary for the the application of a certain technique of FFF to different classes of polymers. It will be assumed that the resolving power and speed of all FFF subtechniques will continue to improve with the accumulation of experience.

The basic requirements for the applicability of FFF are rather straightforward.

They consist of the following major elements: (1) the field must be of a type of sufficient strength to interact with the solute sufficiently to force it into a narrow layer against one wall (this simply means that the field-polymer interaction must exceed by a factor of 10 or more the mean thermal energy $\mathcal{R}T$); (2) a polymer must be soluble or dispersible in a solvent that is compatible with the FFF system; (3) the surfaces of the channel must be compatible with the polymer in such a way that adsorption, trapping, or polymer loss is slight.

With regard to requirement 1, we note that in some cases the interaction is so weak that a particular methodology is not applicable to a certain polymer. Thus uncharged particles or polymers would not interact sufficiently with an electric field to cause retention. Very often the interaction of a field with a polymer is sufficient to meet the requirement for high-molecular-weight species but drops below satisfactory levels for the low-molecular-weight components.

Requirement 2 above is related most often to the polymer polarity, which sets limits on solvent polarity. Solvents of a certain polarity may dissolve or swell various seals, membranes, and other components of the system. More fundamentally, they may destroy the polymer-field interaction by affecting electrical charges and phenomena such as thermal diffusion.

Requirement 3 is a less fundamental criterion than the first two. Success in this area hinges largely on the improved engineering of channel surfaces. However, we will note one important limitation established by this requirement in our discussion of flow FFF below.

Using the theory and results presented above as guidelines, we will now attempt to formulate some elements of the potential and limitations of various FFF subtechniques in polymer analysis and characterization.

Sedimentation FFF

The subtechnique of sedimentation FFF is basically compatible with both polar and non-polar solvents, so that requirement 2 above is rather easily satisfied. The channel walls are polished metal surfaces which could be coated with special layers if necessary to reduce adsorption. Therefore requirement 3 is expected to impose very few limitations on the application of sedimentation FFF to polymers.

The major limitation in the application of this subtechnique is requirement 1, as shown by eqn. 13. Unless acceleration G is large, parameter λ will not be small enough to represent a reasonable level of retention and resolution. The implications of this limitation have been discussed elsewhere⁶. For present purposes we shall assume that λ should not exceed 0.1 and that w should not be greater than 1 mm. We shall assume conditions near room temperature and let $(1 - \bar{v}_s \rho) = 0.25$. With these rough limitations, eqn. 13 can be applied to show that the molecular weight must exceed the approximate value

$$M \approx 10^{10}/g \quad (17)$$

in order to provide a strong enough interaction with the sedimentation field. The quantity g is the acceleration expressed in units of gravity. Present sedimentation FFF systems, limited to approximately $10^3 g$, will therefore function only for M values of approximately 10^7 or larger. However, it is obvious that high-speed centrifugal tech-

niques in which g values exceed 10^5 would be applicable to polymers with molecular weights of less than 10^5 . This would be an exciting area of application in view of the fact that the theory (eqn. 13) shows that retention in sedimentation FFF is determined by molecular weight M alone. Furthermore, the retention volume becomes linear in M for well retained solutes. Therefore one would not only obtain the mass spectrum from the elution diagram, but it would be a mass spectrum on a linear mass-time scale. This approach must be regarded as promising for polymers of high molecular weight.

Flow FFF

In flow FFF a cross flow of solvent is established between walls made of semipermeable membranes. In theory the method can be applied to any polymer-solvent system for which a semipermeable membrane can be found. Requirement 1—the requirement for an adequate field strength—is largely a matter of increasing the pressure differential across the channel to a level that will force solvent flow at the required speed across the channel. The requirements, then, are mainly mechanical in nature and not very restrictive. Without much equipment sophistication we have applied the method to proteins in the vicinity of $M \approx 10^4$. The upper molecular weight limit is also very broad—no intrinsic operational problems are expected for M up to *ca.* 10^{12} (ref. 5). Even the problem of shear degradation is expected to be less severe than in competing methods⁵.

In an earlier section we noted the applicability of flow FFF to water-soluble polymers. The application of this subtechnique to non-polar polymers is an equally bright prospect. In both cases, as shown by eqn. 14, the retention is controlled by the polymer diffusion coefficient D so that one gets a spectrum of diffusion values. The diffusion coefficient, of course, is related directly to the Stokes radius or diameter of a species. Therefore one obtains directly a spectrum of size as gauged by the Stokes radius. Once again, the elution volume or time is linear in this parameter so that one can substitute—after a small initial period—a size scale for the time scale of the elution diagram.

Thermal FFF

While most of our polymer experience has accumulated in thermal FFF systems, little is known about the fundamental limits imposed by requirement 1 as noted earlier. Our work has shown clearly that some polymer-solvent systems are more prone to a thermal diffusive interaction than are others. Macromolecules soluble in aqueous systems, for example, appear from our limited data to have interactions too weak for practical applicability. However, it is very possible that some water-soluble polymers will eventually prove to be an exception to this rule.

Eqn. 15 can be used to establish the criterion for successful polymer fractionation in terms of the thermal diffusion factor α —a basic physicochemical parameter. Quantity w (dT/dx) is approximately the channel temperature drop ΔT , so that eqn. 15 becomes

$$\lambda \approx T/\alpha\Delta T \quad (18)$$

If the maximum practical λ is *ca.* 0.1 and the maximum achievable ($\Delta T/T$) *ca.* 0.5, then the minimum α value for marginal FFF retention is *ca.* 20. Unfortunately, no

broad compilation of α values exists for polymers, so that the feasibility of applying thermal FFF to new polymer types is still largely an empirical matter.

In the last section we noted some of our experience with various solute-solvent systems. Much more work, however, will be required to get a clear and systematic picture of the limits of the method. It is now obvious that the thermal FFF system works very efficiently for some polymers. The ease of construction of the thermal FFF apparatus dictates that this method will remain an important tool among the FFF subtechniques applied to polymers.

Electrical FFF

Electrical FFF requires the presence of charged species in order to achieve retention. It is mainly of interest, therefore, in the area of water soluble polymers. It is anticipated that the method would work well for most such polymers although the electrical FFF method has so far been more difficult to apply than the other subtechniques of FFF.

The above analysis shows that one or more subtechniques of FFF should be applicable to virtually any non-biological polymer of importance. As resolution, accuracy, speed, and data handling improve, it is anticipated that FFF will become one of the prime tools in polymer analysis and characterization.

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