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J. C. Giddings[a]; M. N. Myers[a]; K. D. Caldwell[a]
[a] DEPARTMENT OF CHEMISTRY, UNIVERSITY OF UTAH SALT LAKE CITY, UTAH

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# Field-Flow Fractionation: Methodological and Historical Perspectives

J. C. GIDDINGS, M. N. MYERS, and K. D. CALDWELL

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF UTAH
SALT LAKE CITY, UTAH 84112



## ABSTRACT

Field-flow fractionation (FFF) is briefly introduced with respect to its working nature, mechanism of retention, types of applications, and relationships to chromatography. Nine fundamental characteristics of FFF are then outlined. The nine characteristics are used (Table I) to distinguish FFF from other separation methods which employ an external field perpendicular to the flow axis.

Three brief accounts of the early history of FFF are given. These accounts relate the individual experiences of the three authors of this articles.

## INTRODUCTION

Field-flow fractionation (FFF) developed rather slowly in the first decade after its introduction in the mid-1960's (1). In recent years, techniques have improved and areas of application have expanded dramatically (2). The rapid improvements have attracted widened attention; about a dozen groups have now launched FFF projects of their own. Major results from some of these projects are contained in this special issue on FFF. These projects are likely

to have an autocatalytic effect, spurring new projects, new methodology, and a quickened pace of new applications. The potential applicability of FFF to macromolecules and particles appears to be very broad, reinforcing the notion that FFF work will expand considerably in the near future.

The present article is designed to provide perspective on this expanding technique. There are three specific objectives. First, because the methodology of FFF is not yet widely understood, this article will serve to introduce basic FFF concepts to the unfamiliar reader. Second, the characteristics which distinguish FFF from related techniques will be explored in order to more clearly establish the place of FFF in the field of separations. Third, in view of the present impetus to uncover the roots of diverse scientific disciplines, we will strive to present a concise history of early developments in FFF, which originated in our laboratory.



We undertake the historical project fully aware that FFF is not yet a widely used technique. However, waiting for scientific maturity removes the process of writing even further from the events to be described; this sacrifices freshness and accuracy. We prefer taking the risk now that FFF will become a major scientific tool, so that the history will have been worth the writing.

## THE NATURE OF FFF

FFF takes advantage of the fact that fluid flowing laminarly in a narrow conduit assumes different velocities at different points of the conduit's cross section. For example, the velocity approaches zero near conduit walls due to the viscous drag of the walls, and it reaches a maximum in the center of the conduit (see Figure 1). If we could place different kinds of molecules in different flow regions, separation would follow as a consequence of their differential migration along different flow paths.

In FFF, external fields and gradients are used to push molecules and particles into selected flow paths within a fluid

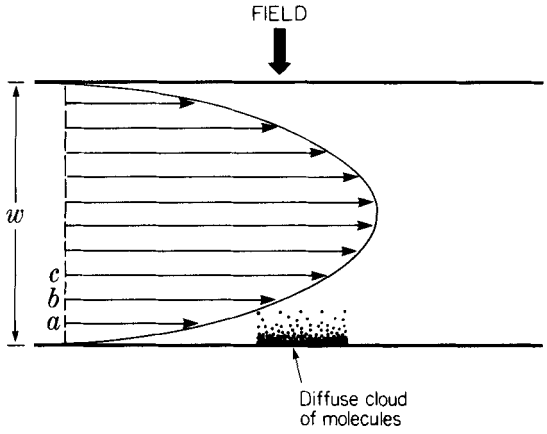


FIGURE 1. Conduit in which a flowing fluid assumes different velocities (the magnitude indicated by the length of arrows) at different points of its cross section. In FFF, a perpendicular field forces specific molecules or particles into a diffuse cloud near the lower wall, thus forcing it into the vicinity of a given streamline, say a. Clouds of other molecules, interacting differently with the field, may have a center of gravity around streamlines b or c, etc., and thus be swept along at a velocity proportional to the length of arrows b or c, and so on. The ensuing differential migration of components leads to the separation of components characteristic of FFF.



flowing in a narrow conduit or channel. The field direction is perpendicular to the flow axis as shown in Figure 1. This is required to force species laterally into the selected cross sectional regions in which the desired flow is taking place. Usually, species are forced close to one wall, as illustrated by the figure. The slow flow near the wall is then responsible for the retention of the species.

## Retention

In FFF, the degree of retention is controlled by the strength of the field and can be easily varied by changes in field strength. Strong fields force a given component into a thin layer of mean thickness $\ell$ against a channel wall. The degree to which the component's velocity is retarded relative to the mean carrier velocity is designed by retention ratio R, related to $\ell$ by (2,3)

$$R = \frac{6\ell}{w} \left[\coth\left(\frac{w}{2\ell}\right) - \frac{2\ell}{w}\right] \quad (1)$$

When the ratio $\lambda = \ell/w$ is small ($< 0.1$), as it must be for effective FFF separation, the above equation approaches the simple limiting form

$$R = 6\lambda \quad (2)$$

Separation, reflected in unequal R values, occurs by virtue of differences in $\ell$ and thus in $\lambda$ between different components. Additional theoretical detail can be found elsewhere (4-7).



## Types of Separations

We have used centrifugal, gravitational, and electrical fields, thermal gradients, and cross-flow to force species into desired flow paths (2). We have attempted to use lateral concentration gradients (8). We have proposed magnetic fields (1) and have made preliminary investigations of other forces for this purpose. The fields so far exploited have provided the multicomponent separation of ionic and nonionic components in aqueous and nonaqueous media. The components have had their origins in biological, environmental, energy, and industrial materials. Existing systems in our laboratory can separate effectively over a $10^{15}$-fold mass range--from $10^3$ molecular weight polymers to $10^2$ μm particles. Separations have been achieved in times as short as a few minutes (9).

## FFF and Chromatography

Experimentally, FFF operates much like chromatography. A small sample is injected at the head of the separation channel and separation takes place as the components are carried along at different velocities by the flow. The components therefore emerge at different times, on which occasion they can be detected and/or collected for further use or study.

FFF, however, does not fall in the category of chromatographic methods because retention is not induced by a distribution between two or more phases, as required by nearly all definitions of chromatography. The Commission on Analytical Nomenclature, Analytical Chemistry Division, International Union of Pure and Applied Chemistry, recommends that chromatography be defined as, "A method, used primarily for separation of the components of a sample, in which the components are distributed between two phases, one of which is stationary while the other moves" (10). With FFF, by contrast, retention is induced by a field forcing species into different flow regions within a given phase. However, the close dynamical similarity of chromatography and FFF has led us to use the term "one-phase chromatography" for FFF to emphasize the relationship (11). Lightfoot, et.al., use the term "polarization chromatography" for their version of FFF (12).



## Characteristics of FFF.

Because FFF utilizes an external field or gradient whose direction is perpendicular to flow, the method falls in the F(+)c or F(+)cd category of separations (13,14). It is thus in the same family with thermogravitational (Clusius-Dickel) columns, electrodecantation methods (including Kirkwood's electrophoresis-convection system), and related methodologies. Such approaches were used by the ancient Greeks who combined gravity and horizontal flow to sort out valuable mineral constituents (15). Few of the other approaches, however, offer much promise for multicomponent separations, particularly if we require any reasonable level of speed, convenience, and resolution. We wish, therefore, to outline some of the principal characteristics responsible for the efficacy of FFF. These characteristics are listed below.

1. Unidirectional flow. FFF utilizes a fluid stream passing essentially one way through the separation channel. This is in contrast to thermogravitational (16) and electrodecantation (17) methods for which flow is countercurrent. Countercurrent flow is well suited for binary separations, but adapts only with difficul-

ty (such as by the staging of individual units) to multicomponent systems.

We note that FFF flow, while essentially unidirectional, can have a countercurrent component without changing its basic nature and capabilities. Such is the case with thermogravitational FFF (18).

Unidirectional flow allows accurate pump control, which control can be independent of the dimensions of the channel. This feature greatly aids optimization. Most countercurrent flow in this family is, by contrast, driven by convection and cannot be controlled independently of other important system parameters. Unidirectional flow also leads conveniently to the continuous-flow elution of separated samples, aiding detection and sample collection.



2. Sample elution. In FFF, the fractionated components are carried out of the separation system by the continuous stream of fluid. This greatly simplifies the detection and collection of components, as just noted.

Elution follows rather naturally from unidirectional flow, but the latter does not guarantee the former. Some unidirectional flow system (for example, hydraulic classifiers) leave part of their contents deposited within the flow chamber.

3. Elongated channel. The length L of FFF flow channels (measured along the flow axis) is much greater than the thickness w over which the field is applied: $L >> w$. We have employed L/w ratios of almost $10^4$; values over $10^3$ are common. While many related methods benefit from large L/w ratios, such ratios are not always employed (as in electrodecantation), and they are often limited (especially with respect to w) by the weak driving force of convective flow.

Channel elongation allows one to multiply a weak, field-induced enrichment effect acting over thickness w into complete separation. The multiplication effect is like that occurring in chromatography (14).

4. Use of carrier. FFF generally utilizes a carrier fluid to entrain the components of interest and to establish a differential flow pattern. The components separate within the chosen carrier, although modifications exist for exchanging carrier liquids and for concentrating components within the carrier (19). While electrophoresis-convection and the present electrodecantation method (17) also employ a carrier (an aqueous solution), their close relative, thermogravitational columns, usually operate with binary mixtures (16).

Carrier properties can be chosen to control component migration and optimize separation. In view of the unidirectional flow of FFF, carrier properties (such as density and pH) can be changed continuously to generate programmed carrier systems to enhance separation characteristics (20).

5. Lateral steady-state. The major part of FFF separation occurs with components distributed approximately in a steady-state configuration over channel thickness w. Thus, once separation is well under way, any flux of a component toward a wall due to the action of the field is offset by a nearly equal outward flux due to diffusion. The steady-state condition need not apply in each cross section but applies to the overall distribution of the components along the field axis.

In order to achieve steady-state, allowance must be made for relaxation time $\tau$, the time required for a component introduced into the FFF channel to closely approach its steady-state condition. Usually, part of the component must migrate across most of thickness w to achieve steady-state, leading to $\tau = w/U$, where U is the field-induced velocity. Steady-state operation requires that retention time $t_r$ be considerably larger (an order of magnitude as a rough minimum) than $\tau$, $t_r > \tau$ or that flow be stopped (the stop-flow method) for a time $\geq\tau$ at the beginning of the run (21).

The steady-state condition assures the uniform migration of components down the channel at a rate calculable from simplified steady-state considerations.

6. Rapid lateral equilibration. Steady-state profiles, once formed in a given cross section, are disturbed by the differential flow of the FFF system. (This does not alter the steady-state condition discussed above which does not require applicability in each cross section.) Effective FFF requires that the profiles repair themselves rapidly via the ongoing lateral transport processes. Specifically, the equilibration time for repair $\tau_e$ should be much less (at least two orders of magnitude) than the retention time, $\tau_e << t_r$.

In a properly functioning FFF channel, the equilibration time introduced here is much shorter than the relaxation time described above. The difference is due to the small thickness $\ell$ of the steady-state layer (over which transport for "equilibration" occurs) compared to the channel thickness w (from which the steady-state layer is formed by "relaxation"). Typically, $\lambda = \ell/w$ lies in the range 0.1 - 0.01. However, for some non-FFF techniques, particularly those employing thermal diffusion with gases or low molecular weight liquids, $\ell$ and w are of comparable magnitude and the two "relaxation times" $\tau$ and $\tau_e$ merge into one and become indistinguishable.

Rapid equilibration makes possible sharp component pulses, high resolution, and fast separation. In this respect, FFF is like chromatography where rapid equilibration between phases leads to high theoretical plate counts, resolution, and speed (22). Rapid equilibration is encouraged by high field strength, thin steady-state layers, and low carrier viscosity.

7. Axial nonsteady-state. Component concentrations continually vary along the flow axis as pulses of material pass through the channel. This is consistent with time-based multicomponent separations typical of elution techniques such as chromatography. The pulses (or "fronts" in the case of frontal analysis) are kept sharp by the rapid equilibration condition above. Only in continuous FFF systems (23) is an axial steady-state realized.

In many F(+)cd techniques such as electrophoresis-convection or thermogravitation columns, an axial steady-state is approached

under ideal circumstances near the end of the run. Excessive times are usually required to achieve such axial steady-state conditions.

8. Axial Separation. Although the underlying enrichment step occurs in the field direction, the separation is finally realized by virtue of differential migration along the flow axis. This axial separation is then converted into a time based separation by virtue of the steady flow. While some of the original enrichment (and a good deal of sample concentration) could be realized by splitting the outlet stream into different lateral components, the degree of lateral separation is trivial compared to that of axial separation.

The magnification of weak lateral enrichment into powerful axial separation occurs by virtue of characteristic 4 (lateral steady-state) amplified by characteristic 5 (rapid lateral equilibration). Methods which realize separation along the field coordinate by stream division or other lateral sampling methods have no magnification by flow, and must depend on field effects alone. In this case flow plays no integral role in the actual separation, although it facilitates continuous operation through the transport and collection of samples (14). Such is the case with continuous flow electrophoresis (17), which category includes the STAFLO method of Mel (24), and with the thermal diffusion flow cell (25,26). The latter is not even generally used for separation because the thermal diffusion effect is so intrinsically weak (14). However, FFF, using flow magnification, produces effective multicomponent polymer separations based on thermal diffusion (27).

9. Integral flow. We note that not all techniques discussed here fall in the F(+)c or F(+)cd categories. To be so categorized requires that the flow play an integral role in the separation, as noted in the discussion of magnification above. To test for integral flow, we can often simply inquire if equal resolution is obtainable without the flow, without regard to the final position or arrangement of the separated materials or the continuous-noncon-

tinuous nature of the separation. A more complete discussion of integral flow is found elsewhere (14).

Integral flow is related to characteristics 5, 6, and 8 and to some extent to characteristics 3 and 7, but it is not clear if its presence can be uniquely related to these other characteristics. For example, axial separation may be necessary to realize integral flow, but it is not sufficient. The axial separation observed in hydraulic classifiers (28) presents no greater resolution than gravity sedimentation alone, although it has the practical advantage of continuous operation.

Comparison of FFF and Related Methods.

Table I has been constructed to provide a comparison of FFF and related techniques revolving around the nine FFF characteristics noted above. All techniques listed employ a field or gra dient perpendicular to a flow axis. The list is not exhaustive, mainly because of the plethora of related methods. There are also occasional variations in the stated techniques which reverse the sense of one or more characteristics. For example, thermal diffusion flow cells are not designed with any particular emphasis on achieving lateral steady-state conditions, but they can be easily operated in such a mode. The table shows only if the given feature is characteristic of and important to the techniques as they are commonly employed.

Table I shows that a substantial gulf exists between FFF and the other methods. Nearly two-thirds (40/63) of the entries in the table below FFF are "nos". Thus, only about one-third of the important features of FFF are, on the average, also characteristic of the related techniques. The table suggests that electrophoresis-convection (29) is the technique closest to FFF, but even here four of the nine characteristics fail to correspond.

Table 2 shows the "similarity" of the various methods to FFF based on the scale of nine characteristics. The scale is, of course, somewhat arbitrary because each characteristic is weighted equally, but it clearly indicates the magnitude of the gap between

TABLE 1.

Nine Essential Features of FFF. These Characteristics are: 1-Unidirectional Flow, 2-Sample Elution, 3-Elongated Channel, 4-Use of Carrier, 5-Lateral Steady State, 6-Rapid Lateral Equilibrium, 7-Axial Nonsteady State, 8-Axial Separation, 9-Integral Flow. The Table shows which of these Features are Characteristic of each of the Other Listed Separation Techniques which Employ a Field Perpendicular to a Flow Axis.

| Method | Characteristics | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1. | 2. | 3. | 4. | 5. | 6. | 7. | 8. | 9. |
| FFF | yes | yes | yes | yes | yes | yes | yes | yes | yes |
| Thermogravitational column (16) | no | no | yes | no | yes | no | no | yes | yes |
| Thermal Diffusion flow cell (25,26) | yes | yes | yes | no | no | no | no | no | no |
| Electrodecantation (17) | no | no | no | yes | no | no | no | yes | no |
| Electrophoresis-convection (29) | no | no | yes | yes | yes | no | no | yes | yes |
| Continuous flow Electrophoresis (17) | yes | yes | no | yes | no | no | no | no | no |
| Hydrocyclone (29) | yes | no | no | yes | no | no | no | yes | no |
| Hydraulic classifier (28) | yes | no | no | yes | no | no | no | yes | no |

TABLE 2.

Approximate Similarity of Various Related Methods to FFF Based on the Scale of Nine Characteristics from Table 1.

| | |
|---|---|
| 9- | FFF |
| 8- | |
| 7- | |
| 6- | |
| 5- | electrophoresis-convection |
| 4- | thermogravitational column |
| 3- | continuous flow electrophoresis, th. diff. flow cell, hydrocyclone, hydraulic classifier |
| 2- | electrodecantation |
| 1- | |
| 0- | |



FFF and the other methods and thus the uniqueness of FFF among such methods.

While most of the "yes" and "no" entries underlying Tables I and II are clearly appropriate, a few are "borderline" and require justification. Perhaps most clouded in this respect is the hydrocyclone (30) which, even to get on the list, requires that the perpendicular field (centrifugal) be considered as valid for this category even though arising in the fluid motion rather than being applied from the outside. Then the flow folds in and returns along the axis in a countercurrent type motion, but we do not consider the flow countercurrent because there is no substantial exchange of material between the two streams. Finally, the small particles are eluted with the emerging stream but, since the large particles deposit in the system, it is not considered to be an elution system.

Some categorical decisions such as the above may be reversed by subsequent workers, but it is doubtful if such changes will

substantially alter the magnitude or the significance of the methodological gulf between FFF and related methods.

## EARLY HISTORY OF FFF

In order to provide some historical breadth, this section includes separate accounts from each of the three authors of this article. The three accounts appear as successive subsections by J. Calvin Giddings (JCG), Marcus N. Myers (MNM), and Karin D. Caldwell (KDC), respectively.

### Origins of FFF (JCG).

The FFF concept first seriously occupied me in about 1960. The concept grew out of my interest in the fundamentals of chromatography and the mechanism for achieving retention and separation. I very frequently considered and evaluated alternate ways of approaching chromatography in those days.

The idea occurred to me that centrifugal forces could be used to impel molecules into a stationary region of a flow system, replacing the affinity of the stationary phase. This alternate mechanism of retention would lead to elution sequences depending specifically on component molecular weights. I called the method "centrifugal chromatography". The idea appeared sufficiently promising that I developed a theoretical treatment of retention and zone broadening. I still have rather extensive notes from those calculations in my possession.

I did not pursue FFF at that time because my interest was, unfortunately, narrowly focused on low molecular weight species. Centrifugal forces were simply not powerful enough to do much with such components. However, the idea occupied me periodically in the next year or two, as evidenced by other notes I compiled listing proposed research projects. Then the idea slipped from consideration at the conscious level.

In the next few years I developed an interest in separating macromolecules. Chromatography does not work as well with macro-

molecules as it does with small molecules. The macromolecular focus set the stage for the most important area of FFF applications, and provided the motivation for most FFF development work. However, there was an interesting step: FFF was first launched to solve a problem of the other extreme of the molecular weight spectrum.

Among my many chromatographic projects in the early 1960's (31) was a strong and persistent interest in developing a rapid method for separating isotopes. Isotopes, of course, demonstrate very little selectivity in partitioning between two phases. Consequently, chromatography is not a very effective approach. A much greater selectivity can be achieved based on diffusion rates; indeed, this underlies the sluggish gaseous diffusion process developed for the large scale enrichment of uranium-235. But I wanted to do it faster and bypass the plumbing, something on the scale of simplicity and speed exhibited by chromatography. To do so would require diffusion distances at sub-millimeter levels to rapidly multiply the effects of any single diffusion step envisioned.

I attempted many times in the early 1960's to think of a way of multiplying or cascading a simple diffusion step to obtain chromatographic-like separations. The problem proved to be very difficult.

In the latter part of 1965, I was vacationing in Wyoming and elected to spend the night in a motel in Evanston, a cowboy town. It would be dramatic to say that saloon noises and shootings kept me awake that night, but in fact it was a banging radiator that disturbed my night's rest. My thoughts came back to the diffusion problem. I imagined using some kind of force field to restrain a mixture within a narrow layer while differential diffusion acted to allow some species to escape further from the layer than others. I was trying to think of an appropriate field and imagine how best to couple this with differential flow when suddenly it occurred to me that thermal diffusion might solve all the problems at once.

It is well known that a strong temperature gradient will cause components in a mixture to migrate one way or another along the gradient axis. Clearly, if thermal diffusion causes the buildup of a layer of some species against the wall, that species will undergo diffusion in such a direction as to expand and dilute that layer. One gets a steady-state layer, the thickness of which will be different for different species, depending on diffusion coefficients and the strength of thermal diffusion effects.

The unique thing about using thermal diffusion (as compared to other forces) is that the applied temperature gradient creates a viscosity gradient. Thus any flow passing through the system perpendicular to the temperature gradient would move parts of the layer that had been formed more rapidly than other parts. It would, furthermore, move some layers faster than others, depending on their thickness. Consequently, separation would be achieved.

I was so fascinated with the idea that I spent most of the remainder of the night thinking about ways to improve and implement it. I called the method "thermal diffusion chromatography", having totally forgotten about my earlier work on "centrifugal chromatography".

It became clear immediately that field-induced layering could be coupled with the natural flow inequalities always present in any conduit, whether or not a temperature gradient existed. This meant that the method could be used with any kind of externally applied field or gradient, allowing one to choose the kind most suitable for the mixture at hand. An obvious field type was centrifugal; I had come full circle in five years. However, the concept now had versatility provided by all conceivable fields.

The thermal diffusion mode (thermal FFF) seemed the most easy to implement, so I focused most early attention on this method. I believed that thermal FFF could be applied to both isotopes and macromolecules.

I should add that I immediately realized that the method was not, in general, strictly based on differences in diffusion coefficients, in accordance with my original goal. At that point

I didn't care; the method was novel, intriguing, and showed considerable practical promise. As it turns out, however, the separation of polymers by thermal field-flow fractionation is based largely on differential diffusivities and the retention behavior of another FFF system, flow FFF, is based entirely on diffusion coefficients.

That fall we initiated an experimental program in thermal FFF, to be described by Dr. Marcus N. Myers in the next subsection. Dr. Myers, then a postdoctoral with considerable industrial experience, has played a key role in the experimental design of nearly all our FFF systems since the beginning. The next subsection, written by Dr. Karin Caldwell, describes some FFF projects with which she was involved in the late 1960's and early 1970's. Dr. Caldwell has helped initiate work on many FFF systems and has coordinated our biochemical work.



First Attempts and Successes with FFF (MNM).

One morning late in 1965, Professor Giddings called Margo Hovingh, me, and one other person to his office in the old Chemistry Building at the University of Utah. He had just returned from a recreational trip, and was excited about a new separation method he had formulated. He then presented the basis for field-flow fractionation. A few days later he presented a seminar to his research group, repeating this information and pointing out that any field could be used which would cause movement of molecules, such as electrical, gravitational, or thermal fields. He thought the thermal field would be particularly easy to apply.

The same afternoon that the seminar was given, Lillian MacLaren attempted to make a thermal field-flow fractionation column by wrapping 28 gauge ( ~ 1 mm O.D., 0.75 mm I.D.) ultrathin teflon tubing around a piece of 1 inch copper pipe through which hot water or oil could be passed to heat one side of the tubing. A water jacket was placed on the outside of the coil for cooling. She made several unsuccessful attempts to separate gases. At the same time, we began to formulate the design and

collect materials for improved columns. By the end of December, materials had been acquired and construction started. Two systems of the same type were built, each consisting of two 2-inch thick discs of aluminum clamped together over a flat coil of teflon tubing. The top plate was heated by Calrod heaters such as are used for electrical stove units. The bottom plate was cooled by passing tap water through channels milled in the bottom of the plate. One unit was about 14 inches in diameter, accommodating a coiled tube nearly 54 meters long. The maximum temperature difference across the teflon tubing was 220° when working with helium as a carrier. Both rectangular (1.3 x 2.0 mm I.D.) and round teflon spaghetti tubing (0.56 mm I.D.) were used for the column. I ran many experiments during the spring and summer of 1966 with no evidence of retention. Convection and inadequate thermal diffusion prevented successful results.

Gary Thompson began using a thermal field in a liquid system in June 1966. He attempted to separate proteins and dyes. He was hampered by the lack of a good detector and reliable pulse-free pumps. The Waters R-4 RI monitor became available and solved (to some extent) the detector problem, while ingenuity provided a stable flow gravity feed pumping system. He built several types of systems with glass and teflon capillary tubes, heated and cooled in several ways. He then worked with an aluminum disc system as described above using the spaghetti tubing in lengths from 6.6 to possibly 55 meters. He first observed retention in this column of 860,000 molecular weight polystyrene August 29, 1966. He devoted considerable effort over the next months to the separation of low molecular weight polystyrene (10,000 and 5525, etc.) with little success. However, when he again used molecular weights greater than 100,000, reproducible retention was being observed. The fractionation of high and low molecular weight polymers was thus clearly demonstrated (32), but resolution was poor.

In November 1967, he started to construct a 10-foot long straight column consisting of two polished 1 inch square stainless steel tubes separated by a teflon spacer, and clamped together by

"C" clamps 3 inches apart. When completed in January 1968, it was a wonder to behold (see Figure 2)! Hot oil was pumped through the top tube and tap water through the bottom tube. Greatly improved separation was obtained (33).

Subsequently, gold plated and gold sheathed copper bars were used in studies attempting to obtain retention in aqueous systems. This work will be described by Dr. Karin Caldwell in the next subsection.

In the summer of 1971, an 18 inch long column was constructed using two polished copper bars clamped together over a mylar spacer. The top bar was heated by cartridge heaters and the bottom cooled by passing tap water through drilled holes (34). All thermal FFF columns built since then have been basically the same design.



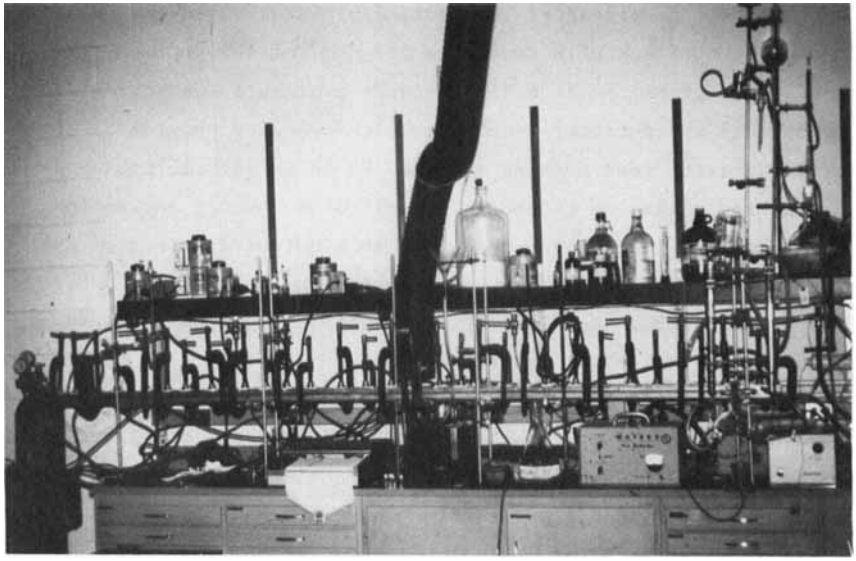

FIGURE 2. First thermal FFF unit to yield reproducible retention of low molecular weight polymeric samples. The ten-foot long column was held together by C-clamps.

We started to develop a sedimentation FFF system in the spring of 1967. The biggest problem was to obtain a rotating seal with low internal volume and no leakage that would provide nonmixing entering and exiting streams. We purchased and modified a B20 centrifuge and seal from International Equipment Co. in November 1967. Eli Grushka attempted to obtain retention of Bacteriophage T2 and polystyrene latex beads for the next year using 1 mm I.D. round and 0.75 mm I.D. square steel tubes (the latter 24 m long) coiled inside a centrifuge basket. The material eluted in a smaller volume than a void peak! The IEC seal also leaked badly until redesigned. Because of these problems, sedimentation FFF was set aside for a time.

Frank Yang, then a graduate student, revived the project in the late summer of 1972. By then, we had decided that the early elution was due to secondary flow. The channel was redesigned to give a thin wide rectangular cross section. The seal was also redesigned. Immediately, he obtained retention agreeing remarkably well with theory (35), and by January 1, 1973, he had explored density and field programming along with other studies indicating the potential value of this technique (20,36).

Since then, simple and inexpensive centrifuges with reliable seals and channels up to 1 meter in length have been produced in our laboratory for a large variety of experiments up to 500 gravities.

Investigation of electrical FFF began in the spring of 1969. This work will be described by Dr. Caldwell in the next subsection.

Flow (cross flow) as a field was mentioned in our group discussions by Professor Giddings sometime around 1970. Little was done to utilize this field until the spring of 1972 when Karin Caldwell tried using a Visking membrane system. This work is described in the next subsection.

Wayne Yared continued the flow FFF project in the fall of 1973 using an Amicon PM10 membrane on the bottom wall of the channel. Some of the early results with albumin appeared promising,

but membrane and contamination problems arose. Hemoglobin was retained much longer than theory predicted. After Dr. Yared left in 1974, I continued some work aimed at flow stabilization and the removal of bacteria.

Frank Yang returned in June 1975 for postdoctoral study, and took over the flow FFF work. By Autumn 1975, he had obtained data agreeing quite well with theory for a number of proteins. The equipment used was essentially the same as in previous studies, with a cast cellulose acetate membrane on the upper wall of the channel and a UM 20 or cast cellulose membrane on the bottom of the channel. The method of casting and curing the cellulose acetate was modified to produce a stronger more adherent membrane. No retention studies were done with random coil polymers, which had proven so difficult in the earlier work.

Work has continued using the flow fields. We have found that Millipore PTGC membrane gives less adsorption than UM20 and PM10 membranes. Flow equilibration problems have been essentially eliminated by using a pump to remove carrier from the column (an "unpump") at a specific rate, rather than relying on valve control.

As FFF techniques were extended to larger particles, theoretical considerations by Prof. Giddings led to steric FFF. The basic theory was published in a paper submitted May 20, 1977 (37). The specific method was proposed in writing on September 14, 1977. At this time we built an apparatus to use the earth's gravity consisting of two 0.5 inch thick glass plates and a Mylar spacer clamped together with Plexiglas bars to provide visibility in the channel. I used some 5-40 μm glass beads and obtained immediate fractionation (38).

In February 1978, while discussing steric FFF, Prof. Giddings and I realized that we should be able to combine steric FFF and sedimentation to produce a continuous separation method for particles. Accordingly, a channel was constructed with ports for removing particles at various distances along one side of the channel. The channel was tilted 20° from the vertical and a sus-

pension of particles pumped in continuously. Good separation of a mixture of glass beads according to size was obtained (23).

Aspects of Thermal, Electrical, and Flow FFF (KDC).

In 1967, Prof. Giddings invited Prof. Jerker Porath of the Department of Biochemistry at the University of Uppsala, Sweden, to send a postdoctoral to Utah. I graduated from Uppsala in November of 1968 and immediately left for Salt Lake City for "a year of training" in separation theory, and to apply the new thermal FFF technique to the separation of proteins.

It was realized that the early separations of polystyrene could be improved by polishing the walls of the flow channel to a smooth mirror finish, such that the depth of existing pits and scratches would be less than the thickness of the field induced layer of solute near the wall. I spent a couple of months polishing copper bars, which subsequently were plated with gold in order to provide an inert column surface suitable for work in aqueous buffers. The early thermal FFF apparatus (Figure 2) had used compressed thin-walled teflon tubing as a spacer between the hot and cold wall. This construction lacked rigidity and geometrical definition. The "new" 1969 modification used a spacer in the form of a teflon sheet (0.01 inch thick) from which the channel void had been cut out. In spite of substantial temperature differences between the hot and cold plates (about 50°C), there was no evidence of retention of either proteins (serum albumin MW 68,000; gamma-globulin MW 150,000), nucleic acids (t-RNA MW 23,000), or dextran (MW 2,000,000) in an aqueous medium. The same temperature drop in the same apparatus gave observable retention of a polystyrene sample whose molecular weight was as low as 5000 using toluene as a carrier. A study of the dextran sample in carriers of different ratios of DMSO and water showed that the lower the water content, the more significant was the retention (39).

During the Spring of 1969, much time was devoted to designing a workable unit for electrical FFF. Early suggestions to let metal plates serve as both channel walls and electrodes were put

aside, since a uniform field could not be established and an upper limit to useful voltages at the electrolysis of water would be imposed by gas bubble formation. I suggested that we move the electrodes outside the flow channel and make the walls out of semipermeable membranes. For this purpose we investigated regenerated cellulose dialysis tubing of Visking type (4" flat width). This membrane showed low resistance to ionic transport, was durable, and could be stretched to tautness, which made it seem desirable to form a channel whose geometry had to be well defined. The membrane sheets were assembled around a teflon spacer and clamped together with lucite frames. Foil electrodes were mounted on lucite sheets. The whole unit was immersed in buffer. By early August 1969 the system was ready for use; the retention of albumin was soon demonstrated. Although acceptable retention was recorded for a number of sample proteins under different conditions of field strength and carrier pH (40), the resolution was not in any way comparable to theoretical predictions.

One important problem with the early electrical FFF channel construction was a gradual sagging of the membrane walls. The channel geometry was also easily altered by pressure fluctuations, which in turn drastically affected both retention and zone spreading (41). In 1975, this design was replaced by one with membranes cast directly onto a porous rigid polymeric frit material for support. The new modification was geometrically stable but its electrical resistance was considerably higher and undesirable heat effects had to be dealt with (42).

Experimental work on flow FFF (another system with a semipermeable-wall-channel) was initiated in early 1972. In flow FFF, one simply forces a lateral stream of carrier across the channel walls, which concentrates macromolecules in the wall region. The first attempts at forcing liquid through the regenerated cellulose membranes were negative, as the large pressures needed for even modest fluxes caused serious deformation of the membrane walls. On August 4, 1972 a new apparatus had been built with porous polymer frits in place of the unbacked membranes. These frits were

coated with a cellulose acetate membrane which was cast in our laboratory from an acetone-formamide-water solution. The membrane was allowed to air dry for 30 minutes, and was subsequently immersed in water. This skin membrane gave fluxes even at modest pressures which were of correct magnitude to retain proteins and other macromolecules. Blue Dextran (MW $2x10^6$) was injected and was estimated to elute after 16 ml at the imposed drift velocity ($8.25 \times 10^{-5}$ cm/sec). No peak had appeared at 16.7 ml, after which the field was turned off and a blue zone released.

In October, retention was recorded also for hemoglobin, but the value for retention ratio (R = 0.81) was larger than that predicted (R = 0.67) by theory. On November 21, retentions were compared for Blue Dextran and two suspensions of polystyrene latex beads whose diameters were 0.091 μm and 0.312 μm, respectively. The cross flow ($8.3 \times 10^{-5}$ cm/sec) was held constant, and so was the axial flow (12 ml/h). The recorded retentions for these samples were R = 0.37, 0.51, and 0.46, respectively, and it was thus clear that differential migration was achieved in this flow FFF channel.

The problem of "infinite" retention of dextran at high cross flows still persisted, and in December a more elaborate study was made of retention of Blue Dextran as a function of axial velocity at constant cross flow. The retention volume at 24 ml/hr was 1 column volume V°, whereas at 15.5 ml/h it had increased to 5 V° and at 14.4 ml/h no zone had appeared after 12 V°. At this point the longitudinal flow was increased to 24 ml/h and the zone appeared. Such results were poorly understood, and the project was temporarily interrupted in the hope that better membrane casting techniques might develop such that a truly uniform porosity could be assured. We decided not to publish our work at that time due to the stated problems, despite the fact that the basis of separation had been clearly demonstrated.

Even today using commercial membranes, the nagging problem of infinite retention persists at high cross flows for long chain polymer samples. Thus the early unit, with all its imperfections, was in qualitative agreement with today's flow FFF equipment.

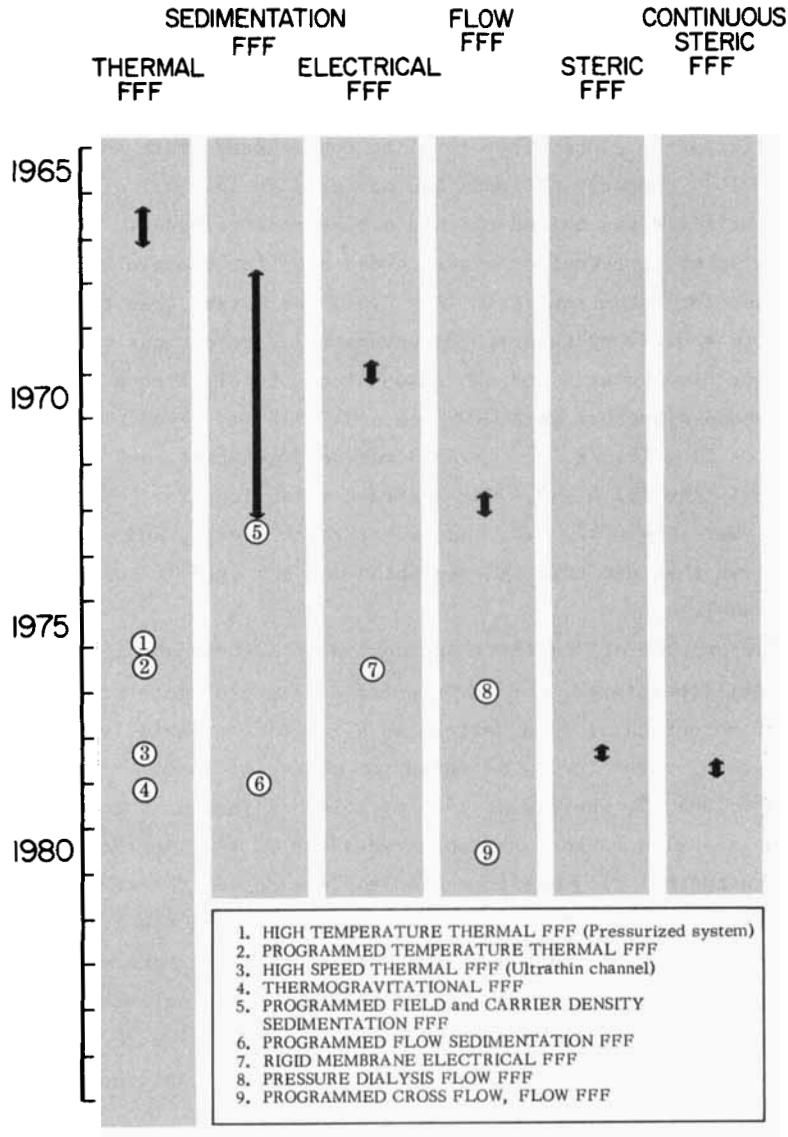


FIGURE 3. Schematic representation of the period of experimental development for various FFF projects in the University of Utah laboratory (see text).

Synopsis.

We summarize the experimental development of FFF systems at Utah by means of Figure 3. The figure shows when experimental work was initiated for each of the major FFF techniques (the top of the arrows) and when fractionation was first demonstrated (the bottom of the arrows). Sedimentation FFF clearly took the longest time to implement--over five years. Steric FFF, by contrast, was almost instantly successful.

The development of some derivative techniques in different categories is identified on the time scale of Figure 2 by numbered triangles. None of the derivative techniques listed required extensive development time.



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