Journal of Chromatography, 504 (1990) 247–258
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 282

Two-dimensional field-flow fractionation

J. CALVIN GIDDINGS
Field-Flow Fractionation Research Center, Department of Chemistry, University of Utah, Salt Lake City, UT 84112 (U.S.A.)
(Received December 7th, 1989)

SUMMARY

Multidimensional field-flow fractionation (FFF) is described in two major forms: one in which different separative stages are coupled together and one in which two independent displacements, at least one of them FFF, are carried out in a generally planar channel structure. The latter, the subject of this paper, is relatively promising for implementation in FFF systems because in most cases the geometry of the FFF channel is already planar; the channel structure needs mainly to be broadened along the second dimension and modified with different inlets and outlets for this two-dimensional use.

The large number of potential two-dimensional FFF systems is discussed. These systems are described at greater length in four categories: (1) FFF displacement used in both dimensions, (2) FFF along one axis and chromatography along another, (3) FFF along one axis and a field-induced displacement along the other, and (4) FFF separation combined with bulk or flow displacement at right angles.

Finally, theoretical equations are obtained for the deflection of the trajectories away from the main flow axis z. The sensitivity of deflection to component properties is described in terms of the *deflection selectivity*. Several examples are discussed in which the deflection selectivity is remarkably high.

INTRODUCTION

Multidimensional separation methods are those techniques that utilize, according to well-defined criteria, two or more relatively independent separative stages to achieve the resolution of sample components[1,2]. Well-designed multidimensional systems have a number of advantages over normal one-dimensional or linear separation systems. These advantages include enhanced resolving power, increased flexibility, and in some cases an improved compatibility in the matching of the separation system to the sample[2].

The primary limitation of multidimensional separation systems is their complexity. Perhaps the simplest means for realizing multidimensional capabilities is to couple or link different separative stages to one another. In coupled column chromatography,

0021-9673/90/$03.50 © 1990 Elsevier Science Publishers B.V.

for example, partially resolved fractions taken from the eluent of the first column are shunted one at a time into a subsequent column, usually with a different stationary phase. Such systems have great flexibility and they are relatively easy to operate[3,4]. However, a conceptually simpler multidimensional [in this case, two-dimensional (2D)] approach involves carrying out two different displacement (usually separation) steps along the two axes of a surface, most often a planar bed[5]. This powerful approach has been used extensively in thin-layer chromatography and in electrophoresis[6–8]. While coupled column operation can be usefully applied to field-flow fractionation (FFF)[9], it is the "planar" 2D system, in which a FFF mechanism is used for one or both of two right angle displacement steps, that will be considered in this paper.

For a number of reasons (see below), the dominant geometry for FFF channels has been that of a thin rectangular space enclosed between close-lying parallel plates. In most cases, separation has been realized only along the principal or flow axis of the system. The thin dimension, across which the field is applied, is used for the enrichment process that underlies separation[10,11]. This leaves a final dimension, the "breadth" coordinate, available for further separative manipulations. Thus without a change in basic geometry, this latter coordinate, perhaps physically expanded somewhat, can in theory be used as a second dimension along which separation can be realized. The feasibility of this approach has been demonstrated by the development of continuous steric FFF, a 2D method in which gravitational sedimentation is applied along the breadth dimension in order to convert steric FFF into a continuous separation system[12].

In this paper we develop the conceptual foundations for 2D-FFF in a broader context, outlining a large number of possible experimental configurations. We examine the use of the second (breadth) dimension both for continuous separations and for discrete methods yielding true 2D fractograms. For the convenience of the ensuing discussion, the coordinates of all such systems are defined in Fig. 1.

The reasons for the dominance of thin parallel plate channels in FFF are instructive; some of these considerations carry over to 2D-FFF. First we note that the field in FFF should generally impinge on the channel surface at right angles to the

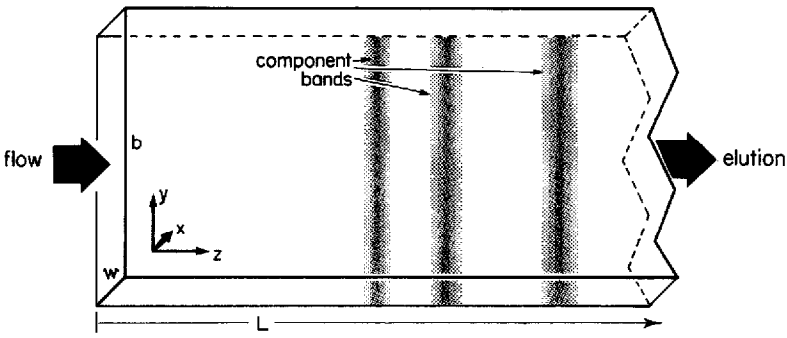

Fig. 1. Illustration of coordinate system of FFF channel: z = flow direction (forming principal separation axis), x = field direction, y = lateral direction (available as a second dimension for separation). Component bands extending from $y = 0$ to $y = b$ and separating along axis z demonstrate normal one-dimensional FFF.

surface plane in order to avoid spurious transport parallel to the plane. Because a majority of fields and gradients are simplest to apply with all field lines more or less parallel in the apparatus, flat channel surfaces are preferable. Furthermore, if one wants to avoid possible convective effects due to temperature gradients in the channel (thermal FFF) or density gradients induced by the sample, then the surface should be flat and normal to the earth's gravity. Finally, the parallel plate configuration is relatively easy to construct with carefully controlled dimensions.

The substantial breadth b (several centimeters) ordinarily found in FFF channels is employed to diminish edge effects, increase sample capacity, and increase channel volumes so that extraneous volumes are negligible by comparison.

If a radial field can be generated, then an annular thin channel configuration is also desirable. (We define an annular channel as one in which the flow direction is parallel to the axis of symmetry of the field. Normal sedimentation FFF, in which the ribbon-like channel is wrapped around the inside of a centrifuge basket, employs a flow direction at right angles to the axis of rotation with all the attributes of a parallel plate channel of finite breadth b.) True annular channels have the advantage of doing away with edge effects entirely. However, in any configuration chosen, an annular channel is subject to possible disturbances by gravity-induced convection. (In at least one case, thermogravitational FFF, convective flow can be used to advantage[13].) Annular channels as well as parallel plate channels are expected to be convenient for 2D separations. An exception is the subtechnique of shear FFF in which the breadth dimension (measured around the circumference) must be employed to generate the shear-induced forces[14]. Consequently, there will be no further reference to shear FFF in this paper.

CATEGORIES OF 2D-FFF

Following the general pattern of enormous variability in 2D separation methods[5], the more specific implementation of 2D-FFF methods can, in principle, assume many forms. We can identify a number of major categories and subcategories; these are summarized in Table I (see explanation of Table I below). These categories vary, as shown, with respect to possible elution axes and regarding the possibility of simultaneous operation, in which both displacements take place at the same time. Operation in the simultaneous mode is a potentially streamlined approach to discrete analytical separations; it is essential for continuous separations[5].

Table II, applicable only to one-dimensional operation, is suggestive of the range of possibilities. The elements of the table's matrix represent different potential kinds of one-dimensional FFF subtechniques, based on the combination of six fundamentally different modes of operation of FFF and various displacement forces originating in some of the different fields and gradients conceivable for FFF. Each subtechnique (element) can, in theory (many are untested), be operated under a wide range of conditions and field strengths. For 2D operation, we can combine each FFF subtechnique with most other FFF subtechniques or with other displacement effects (of a form indicated by Table I) to generate hundreds of possible 2D-FFF approaches. These are clearly too numerous to detail here, but by examining different categories we can better understand the potential characteristics, advantages, and limitations of FFF in various 2D forms.

TABLE I

MAJOR CATEGORIES AND SUB-CATEGORIES OF 2D-FFF SEPARATION

See text.

2D category	*2D sub-categories*	*Possible elution axes*	*Simultaneous/ continuous operation*
(1) FFF × FFF	$F_jFF \times F_kFF$ $F_jFF(1) \times F_jFF(2)$ $A_1FFF \times A_2FFF$	FFF	No[a]
(2) FFF × chromatography	FFF × open channel FFF × packed channel	FFF chromatography	No[a]
(3) FFF × field displacement	$F_jFF \times F_j$ $F_jFF \times F_k$	FFF	Yes
(4) FFF × bulk/flow displacement	FFF × bulk FFF × flow	FFF flow	Yes

[a] Subject to occasional exception.

Category 1: FFF × FFF

The two FFF displacements in this category must generally be carried out sequentially. The reason for this, as explained more fully in the original 2D publication[5], is that in most cases of simultaneous operation, all components take the same vector direction, which is the axis of flow. (Exceptions can be developed by using different mechanisms of flow in the two orthogonal directions.) In the absence of differences in the deflection angle, discrete separations are no better (and much more complicated) than their one-dimensional form; continuous separations are impractical[5].

The sequential operation of the FFF × FFF mode allows a great deal of

TABLE II

GREAT VARIETY OF FFF METHODS IS SUGGESTED BY THIS MATRIX, MOST ELEMENTS OF WHICH ARE POTENTIAL FFF SUBTECHNIQUES

The table is not comprehensive as some potential displacement phenomena, *e.g.*, photophoretic, have been omitted.

Operating mode	*Displacement force*						
	Sedimentation (Sd)	*Thermal (Th)*	*Electrical (El)*	*Dielectrical (Dl)*	*Flow (Fl)*	*Shear (Sh)*	*Magnetic (Mg)*
Normal (Nl)	SdNlFFF	ThNlFFF	ElNlFFF	DlNlFFF	FlNlFFF	ShNlFFF	MgNlFFF
Steric (St)	SdStFFF	ThStFFF	ElStFFF	DlStFFF	FlStFFF	ShStFFF	MgStFFF
Hyperlayer (Hy)	SdHyFFF	ThHyFFF	ElHyFFF	DlHyFFF	FlHyFFF	ShHyFFF	MgHyFFF
Cyclical-field (Cy)	SdCyFFF	ThCyFFF	ElCyFFF	DlCyFFF	FlCyFFF	ShCyFFF	MgCyFFF
Secondary equilibrium (Sy)	SdSyFFF	ThSyFFF	ElSyFFF	DlSyFFF	FlSyFFF	ShSyFFF	MgSyFFF
Chromatographic hybrid (Ch)	SdChFFF	ThChFFF	ElChFFF	DlChFFF	FlChFFF	ShChFFF	MgChFFF

flexibility. Different carrier fluids can be applied along the two axes with operation possible at different temperatures, pressures, etc. Even more, the edge strip along which the first FFF displacement occurs can have its own thickness and surface characteristics; the wall along this strip can be designed to transmit a different external field than that applied over the bulk of the channel for the second displacement.

As shown in Table I, there are some distinct sub-categories of FFF × FFF operation. The use of different fields for the two displacements, say fields j and k, is symbolized by $F_jFF \times F_kFF$. The same field applied under two different sets of conditions, (1) and (2), which may involve different field strength, flow velocity, carrier density, pH, etc., is indicated by $F_jFF(1) \times F_jFF(2)$. Separations combining two fundamentally different modes of operation, A_1 and A_2 (see above), are designated by $A_1FFF \times A_2FFF$. In some cases more than one of these characteristics may differ between the two component runs.

Category 2: FFF × chromatography

The combination of FFF and chromatographic mechanisms is subject to many of the same considerations (such as sequential operation) that apply to category 1. With thin enough channels having walls coated with a retentive phase (except along an edge strip reserved for FFF), effective chromatography could be carried out in the second dimension. However, channels of extraordinary thinness are needed for effective chromatographic operation in open channels, particularly for macromolecules[15]. Such extreme thinness is not needed in FFF because the diffusion path is reduced by the field[11]. It is possible that a channel in which the thickness is stepped would help resolve the unlike requirements of FFF and chromatography.

Alternatively, the part of the flat channel used for chromatography could be carefully packed, the remainder left open for FFF. Either mechanism could be used first in sequence but with FFF used first along a channel formed at one edge, flow control might be simpler.

Category 3: FFF × field displacement

By applying an external field at right angles to the FFF displacement (and also at right angles to the primary FFF field), simultaneous operation is possible, yielding either discrete or continuous separation. The previously cited example of continuous steric FFF (better specified as Sd/StFFF × gravitational sedimentation) illustrates simultaneous, continuous separation in this category. In this example, both displacements are selective (separative), but this is no particular advantage for continuous separation[5]. For example, it should be possible to combine electrophoresis, which provides little size selectivity for colloidal particles of fixed surface composition, with sedimentation FFF (in essentially any mode), or with some other size-selective FFF methods to realize continuous size-based separation.

For cases in which electrophoretic displacements are selective, continuous separation can be realized by using (non-selective) flow rather than FFF for the second direction. Continuous flow or deflection electrophoresis is based on this combination[16,17]. However, when carried out in the space between parallel plates, the solute bands are smeared out (forming "crescent zones") because of the nonuniform parabolic flow[18]. If FFF is used in place of non-selective flow, it appears that this resolution loss can be largely avoided and that both discrete and continuous separation could be achieved[19].

The variability of the FFF × field displacement category is also large, reflecting the possible combination of the many FFF methods (along with extensions and variations) spelled out in Table II with a number of field-induced displacements, including sedimentation, electrophoresis, possibly isotachophoresis and dielectrophoresis, thermal diffusion, and so on.

Category 4: FFF × bulk flow displacement

There is a possibility of combining FFF and non-selective bulk or flow displacement to achieve separation. Thus an annular channel subject to slow rotation (bulk displacement) could yield continuous separation by the same general mechanism proposed for annular chromatography[20,21]. Separation on a rotating disc, with different components deflected along different trajectories to their unlike FFF velocities, could also be used.

In order to realize selective deflection using nonselective flow at right angles to FFF displacement, the flow profiles in the two directions would need to be different (*e.g.*, electroosmotic flow along the flow axis).

DEFLECTION AND SELECTIVITY

Some general aspects of 2D separation theory were discussed in the earlier 2D paper[5] and in some of the references noted therein[6,22]. Here we will focus on the deflection of components in 2D-FFF systems and on the resulting selectivity of deflection with respect to component properties.

In many cases[5] the coordinates of the component zones in a 2D separation system are most conveniently described in terms of radial distance R' and deflection angle θ (see Fig. 2). For resolving large numbers of components in the discrete (analytical) mode, separation must occur along both of these coordinates, although any two species can be separated by differences in only one coordinate[1,2]. However, for continuous separation, only differences in θ are required. Because a θ-based

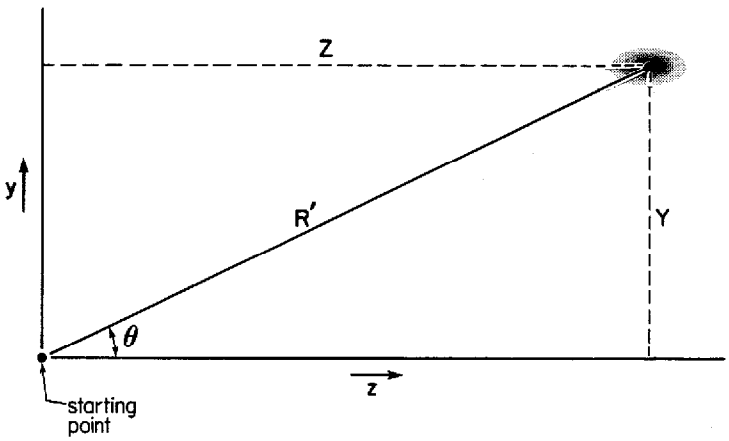

Fig. 2. Specification of component zone location by the polar coordinates R' and θ.

separation is central in both cases, we will describe some aspects of selectivity along this coordinate. (We note that an R'-based separation, governed by the increment $\delta R' = [(\delta X)^2 + (\delta Y)^2]^{1/2}$, depends directly on the individual increments δX and δY responsible for separation in the two contributing displacement steps.)

For theoretical simplicity, deflection will be referenced to $\tan\theta$ rather than θ. We have

$$v = \tan\theta = \frac{Y}{Z} \tag{1}$$

where Y and Z are the respective displacements along the y and z axes. When displacements occur uniformly along these axes for times t_y and t_z at the respective velocities v_y and v_z, we have

$$v = \frac{v_y t_y}{v_z t_z} \tag{2}$$

For simultaneous operation, for which $t_y = t_z$, v reduces to

$$v = \frac{v_y}{v_z} \tag{3}$$

The magnitude of deflection may depend upon several different component properties or upon a single property, depending on the nature of the sample and of the 2D separation system. The deflection selctivity with respect to some single property p (where p may be molecular weight M, particle diameter d, density ρ, etc.) is defined analogously to other selectivity expressions, namely[23]

$$S_p = \frac{\mathrm{d}\ln v}{\mathrm{d}\ln p} \tag{4}$$

For category 1 (FFF × FFF) operation, generally feasible only in the sequential mode, component velocities are expressed in terms of the respective retention ratios R_y and R_z in much the same way as in the one-dimensional case

$$Y = v_y t_y = R_y \langle v \rangle_y t_y \tag{5}$$

$$Z = v_z t_z = R_z \langle v \rangle_z t_z \tag{6}$$

where $\langle v \rangle$ is the cross-sectional average velocity. For the normal mode of FFF operation, each R is expressed in terms of its respective retention parameter λ, often according to the limiting equation[24]

$$R \approx 6\lambda \tag{7}$$

The parameter λ, in turn, is formulated in terms of property p $\lambda(p)$. Thus v can be expressed as

$$v = \frac{R_y\langle\upsilon\rangle_y t_y}{R_z\langle\upsilon\rangle_z t_z} \approx \frac{\lambda_y\langle\upsilon\rangle_y t_y}{\lambda_z\langle\upsilon\rangle_z t_z} \tag{8}$$

Retention parameter λ can be written in the general form[24]

$$\lambda = \frac{kT}{F_x w} \tag{9}$$

where kT is thermal energy, w is the channel thickness, and F_x is the x component of force exerted on the particle by the field. If we write $F_x = fU_x = fm_xS_x$, we get

$$\lambda = \frac{kT}{f}\frac{1}{wm_xS_x} = \frac{D}{wm_xS_x} \tag{10}$$

where U_x is the velocity of the particle along axis x due to the FFF field of strength S_x, m_x is the generalized mobility of the particle, f is the friction coefficient, and D the diffusion coefficient. When this λ is substituted back into eqn. 8, we get

$$v = \frac{(m_x)_z(S_x)_z\,\langle\upsilon\rangle_y t_y\,D_y}{(m_x)_y(S_x)_y\,\langle\upsilon\rangle_z t_z\,D_z} \tag{11}$$

where $(m_x)_z$ and $(m_x)_y$ are the mobilities and $(S_x)_z$ and $(S_x)_y$ the x-directed field strengths applicable during displacement along axes z and y, respectively, and D_z and D_y are the respective diffusion coefficients in effect during these two displacements. While D_z and D_y may be equal, they can differ substantially because the carrier liquid, pH, and temperature may not be the same in the two displacement steps.

In the case of category 2 (FFF × chromatography), generally involving sequential operation, v is obtained from eqn. 2 with the corresponding migration velocities (υ) inserted. Since the υ for chromatography is usually a complex function of most continuous properties p, no simple expressions for υ emerge. However, for FFF × size exclusion chromatography, υ can be readily calculated.

For category 3, involving FFF × direct field operation, we have for the field displacement

$$Y = U_y t_y = m_y S_y t_y \tag{12}$$

where U_y is the velocity induced along coordinate y by the field whose strength is S_y. The mobility of the particle due to the y-directed field is m_y.

To get the FFF displacement (operating in the normal mode) along z in comparable terms, we follow eqns. 6 and 7 to get

$$Z = R_z\langle\upsilon\rangle_z t_z \approx 6\lambda\langle\upsilon\rangle_z t_z \tag{13}$$

The substitution of λ from eqn. 10 into eqn. 13 gives

$$Z = \frac{6D\langle v\rangle_z t_z}{wm_x S_x} \tag{14}$$

This expression along with eqn. 12 yields

$$v = \frac{Y}{Z} = \frac{wm_x m_y S_x S_y\ t_y}{6D\langle v\rangle_z\ t_z} \tag{15}$$

Normally, operation would be simultaneous with $t_y = t_z$, giving

$$v = \frac{wm_x m_y S_x S_y}{6D\langle v\rangle_z} \tag{16}$$

When the same kind of field (*e.g.*, electrical or sedimentation) is used in both x and y directions, we have $m_x = m_y = m$ and this equation becomes

$$v = \frac{wm^2 S_x S_y}{6D\langle v\rangle_z} \tag{17}$$

which shows that deflection is very sensitive to mobility m, offering the possibility of high resolution, particularly in continuous operation. The mobility-based selectivity of such operation is found from eqn. 4 to be $S_m = 2$, an extraordinarily high value. This equation shows that charged species (*e.g.*, proteins) could be continuously separated in an ElFFF × electrophoresis system with a selectivity of two in electrophoretic mobility ($m = \mu$). A similar high promise applies to sedimentation, as will be shown in the next section.

If FFF is carried out in the steric rather than the normal mode, we can replace the R in eqn. 10 by

$$R = 6\gamma d/w \tag{18}$$

where γ is the steric correction factor and d is particle diameter. Eqns. 13 and 12 then lead to

$$v = \frac{wm_y S_y}{6\gamma d\langle v\rangle_z} \tag{19}$$

Finally, for FFF × bulk flow displacement (generally occurring simultaneously), the flow velocity v_y is constant and v becomes

$$v = \frac{v_y}{R_z\langle v\rangle_z} \tag{20}$$

All selectivity in this case originates from changes in R_z.

EXAMPLES OF POTENTIAL 2D SYSTEMS

It is interesting to apply these equations to several potential forms of 2D operation. For this purpose we assume that the sample consists of spherical particles of diameter d and density ρ_s. For such particles the sedimentation force is

$$F(\text{sed}) = \frac{\pi}{6} d^3 \; |\Delta\rho| \; G \tag{21}$$

where $\Delta\rho = \rho_s - \rho$, ρ is the carrier density, and G is the field strength expressed as acceleration. The mobility relative to G (the sedimentation coefficient) is

$$m(\text{sed}) = \frac{d^2 \; |\Delta\rho|}{18\eta} \tag{22}$$

For crossflow the force is

$$F(\text{flow}) = 3\pi\eta d U = 3\pi\eta d \dot{V}_c / Lb \tag{23}$$

where U is the velocity of crossflow, $\dot{V}_c$ is the volumetric crossflow rate, η is the carrier velocity, and L and b are the channel length and breadth, respectively. The mobility referenced to $\dot{V}_c$ becomes

$$m(\text{flow}) = \frac{1}{Lb} \tag{24}$$

The deflection for the 2D combination, sedimentation FFF × flow FFF, can be obtained by substituting eqns. 22 and 24 into eqn. 11

$$v(\text{SdNlFFF} \times \text{FlNlFFF}) = \frac{18\eta \dot{V}_c}{Lbd^2 \; |\Delta\rho| \; G} \frac{\langle v \rangle_y t_y}{\langle v \rangle_z t_z} \tag{25}$$

where η is now the viscosity of the flow FFF carrier liquid (instead of the carrier for sedimentation FFF, if different) by virtue of the D_y/D_z ratio in eqn. 11. The selectivity S_d of v with respect to d is two.

If sedimentation FFF at one carrier density is combined with that at another carrier density (thus falling in the $F_jFF(1) \times F_jFF(2)$ category of Table I), a similar analysis shows that the diameter-based selectivity vanishes and is replaced by a particle density selectivity.

When FFF is combined with direct field displacement, a variety of results are obtained. For the case of SdStFFF × gravitational sedimentation mentioned earlier, the substitution of eqn. 22 into eqn. 19 with S_y replaced by g (the acceleration of gravity) × cos θ(θ is the angle between the sedimentation path and a vertical axis) yields

$$v = \frac{wd \; |\Delta\rho| \; g \cos\theta}{108\gamma\eta\langle v \rangle_z} \tag{26}$$

The apparent diameter selectivity of this deflection is unity but the actual selectivity is somewhat higher because γ decreases somewhat with d.

If we now use the pair SdNlFFF × centrifugal sedimentation, v is obtained from eqn. 17 as

$$v = \frac{w \mid \Delta\rho \mid^2 d^4 G_x G_y}{1944\eta^2 D\langle v\rangle_z} \tag{27}$$

Since $D = kT/3\pi\eta d$, we have

$$v = \frac{\pi}{648}\frac{w \mid \Delta\rho \mid^2 d^5 G_x G_y}{kT\eta\langle v\rangle_z} \tag{28}$$

Eqn. 28 shows a remarkably high selectivity both with respect to diameter ($S_d = 5$) and density difference $\Delta\rho(S_{\Delta\rho} = 2)$. This high selectivity can, in theory, be realized using a sedimentation FFF channel "tilted" with respect to the rotating axis. The angle of tilt will specify the relationship between G_x and G_y. Unfortunately, preliminary efforts to utilize such a channel constructed in this laboratory have not proven successful, perhaps because of secondary flow.

The above cases, of course, represent only a few examples from the rich repertoire of potential 2D-FFF techniques. In most cases the calculation of deflection, selctivity and other separation properties is straightforward and can be carried out in much the same manner as illustrated above.

ACKNOWLEDGEMENT

This work was supported by Grant GM10851-32 from the National Institutes of Health.

REFERENCES

1 J. C. Giddings, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 10 (1987) 319.
2 J. C. Giddings, in H. J. Cortes (Editor), *Multidimensional Chromatography: Techniques and Applications*, Marcel Dekker, New York, 1990, Ch. 1.
3 H. J. Cortes, C. D. Pfeiffer and B. E. Richter, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 469.
4 J. A. Apffel and H. McNair, *J. Chromatogr.*, 279 (1983) 139.
5 J. C. Giddings, *Anal. Chem.*, 56 (1984) 1258A.
6 M. Zakaria, M-F. Gonnord and G. Guiochon, *J. Chromatogr.*, 271 (1983) 127.
7 B. R. Bochner and B. N. Ames, *J. Biol. Chem.*, 257 (1982) 9759.
8 N. L. Anderson and N. G. Anderson, *Anal. Biochem.*, 85 (1978) 341.
9 H. K. Jones and J. C. Giddings, *Anal. Chem.*, 61 (1989) 741.
10 J. C. Giddings, in I. M. Kolthoff and P. J. Elving (Editors), *Treatise on Analytical Chemistry*, Part I, Vol. 5, Wiley, New York, 1981, Ch. 3.
11 J. C. Giddings, *J. Chromatogr.*, 395 (1987) 19.
12 M. N. Myers and J. C. Giddings, *Powder Technol.*, 23 (1979) 15.
13 J. C. Giddings, M. Martin and M. N. Myers, *Sep. Sci. Technol.*, 14 (1979) 611.
14 J. C. Giddings and S. L. Brantley, *Sep. Sci. Technol.*, 19 (1984) 631.
15 J. C. Giddings, J. P. Chang, M. N. Myers, J. M. Davis and K. D. Caldwell, *J. Chromatogr.*, 225 (1983) 359.

16 C. J. O. R. Morris and R. Morris, *Separation Methods in Biochemistry*, Pitman, London, 2nd ed., 1976.
17 A. Strickler, *Sep. Sci.*, 2 (1967) 335.
18 A. Kolin, in Z. Deyl (Editor), *Electrophoresis, A Survey of Techniques and Applications. Part A: Techniques*, Elsevier, New York, 1979, Ch. 12.
19 J. C. Giddings, in J. D. Navratil and C. J. King (Editors), *Chemical Separations*, Vol. 1, Litarvan, Denver, CO, 1986, pp. 3–20.
20 J. C. Giddings, *Anal. Chem.*, 34 (1962) 37.
21 J. M. Begovich, C. H. Byers and W. G. Sisson, *Sep. Sci. Technol.*, 18 (1983) 1167.
22 P. C. Wankat, *AIChE J.*, 23 (1977) 859.
23 J. C. Giddings, *Pure Appl. Chem.*, 51 (1979) 1459.
24 J. C. Giddings, *Sep. Sci. Technol.*, 19 (1984) 831.