

Geometric Scaling Effects in Electrical Field Flow Fractionation. 1. Theoretical Analysis

Bruce K. Gale,* Karin D. Caldwell,[†] and A. Bruno Frazier[‡]

Institute for Micromanufacturing, Louisiana Tech University, P.O. Box 10137, 911 Hergot Street, Ruston, Louisiana 71272

This work outlines the fundamental scaling laws associated with electrical field flow fractionation channels. Although general FFF theory indicates few advantages from miniaturization, EFFF theory indicates clear advantages to miniaturization of the EFFF channel. Retention, plate heights, resolution, equilibration times, and time constants are examined. The outlined theory predicts scaling advantages in each of these areas after miniaturization. Potential applications, such as the use of these systems for sample preparation in microscale total analysis systems, and improvements associated with these theoretical predictions are also discussed.

Over the past several years, a great interest in the development of microscale total analysis systems (μ -TAS) has emerged.¹ These μ -TAS integrate sample handling, analysis, detection, and signal processing as well as any other pertinent processes.² Significant progress has been made in miniaturizing some of the components of a total analysis system, and some progress has been made in integrating these components, though major work remains to be accomplished before a true μ -TAS is completed.³ The main component of many μ -TAS is a chromatography system with which the analysis is performed. One branch of chromatography of significant interest in this regard is field flow fractionation (FFF).

Early papers on the subject of microscale FFF concluded that there is no inherent advantage to miniaturizing FFF systems,⁴ but a closer look at the theory for FFF reveals that although most FFF systems do not benefit significantly from miniaturization, a few FFF subtypes could indeed benefit. These subtypes included electrical, thermal, and magnetic FFF, as well as other FFF subtypes that depend on a force gradient as the separation mechanism. In fact, miniaturized electrical,^{5–7} thermal,⁸ and

dielectrophoretic⁹ FFF systems have already been reported. This paper will focus on the scaling effects associated with electrical field flow fractionation (EFFF) systems.

Most chromatography systems naturally gain advantages from miniaturization. Some of these advantages include the following: reduced plate heights or increased resolution, reduced analysis times, increased parallelism of analysis, reduced sample size, reduced system size, and reduced power consumption. Additionally, when microfabrication technologies are used to miniaturize the chromatography systems, additional advantages are accrued such as the following: the potential for batch fabrication and the associated reduction in production costs, the possibility for integrated electronics, signal processing, and particle detection, and improved manufacturing precision. Most of the performance gains associated with miniaturized chromatography systems are connected to two phenomena. First, the field strength for a given system increases as the system is miniaturized. Thus, a higher field is available to drive the separation over a shorter distance which reduces analysis times and time-dependent plate height contributions. Second, the smaller channel dimensions reduce spatially related contributions to plate height.

A miniaturized EFFF system, the focus of this work, would potentially gain all of the advantages associated with miniaturization of general chromatography systems as well as a few unique advantages such as reduced time constants, reduced solvent consumption, reduced steric transition point, and reduced relaxation and equilibration times. This miniaturized EFFF system would be available to provide analysis in a number of different fields and for a range of applications. The main application of EFFF systems to date has been for the characterization of polymers,¹⁰ colloids,^{11,12} sugars¹³ and clays.¹⁴ Other applications for the system parallel applications for other FFF systems and include such diverse biological materials as cells,^{15,16} bacteria,¹⁷ viruses,¹⁸

* Corresponding author: (phone) (318) 255–5118; (fax) (318) 255-5104; (e-mail) bkgale@coes.latech.edu.

[†] Current address: Center for Surface Biotechnology, Department of Chemistry, Uppsala University, Sweden.

[‡] Current address: School of Electrical and Computer Engineering, Georgia Institute of Technology, Atlanta, GA 30332.

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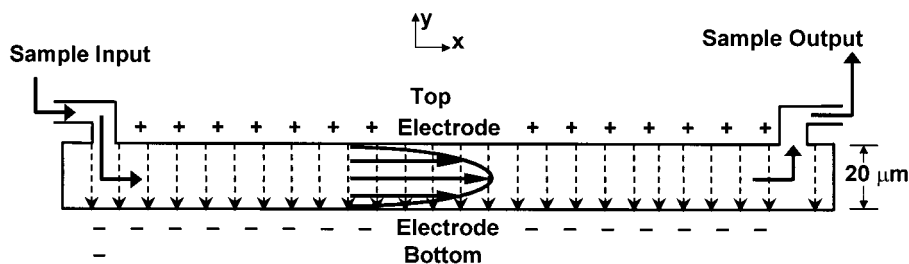


Figure 1. Diagram of an electrical field flow fractionation system showing the general configuration of the system, the application of the electric fields, and the laminar flow profile that performs the separation function.

proteins,¹⁹ DNA,²⁰ starches,²¹ lipid emulsions,²² liposomes,²³ micelles,²⁴ and vesicles.²⁵ Yet other biological and medical applications include studies of DNA and protein adsorption on surfaces, analysis of drug delivery vehicles such as vesicles, micelles, emulsions, and liposomes, organelle separation and characterization, and diagnostic tests (separation of viruses and bacteria). EFFF systems are not limited to biological applications, but are indeed available for particle analysis in a growing list of fields. A relatively well-developed field is environmental water monitoring where EFFF has been applied with some success,¹⁴ as well as other FFF systems.²⁶

Although the applications for EFFF are significant and increasing, the performance of current EFFF systems has been limited by factors such as fabrication technology. In order for EFFF to reach its full potential and prove successful commercially, more powerful EFFF systems will need to be developed. Miniaturization of the EFFF system appears to be one route for development of an improved EFFF system. To fully understand whether miniaturization would improve the system, a close look at EFFF theory is required along with the scaling effects in these systems.

GENERAL FFF THEORY

The general theory for retention and zone broadening in FFF systems is well developed.^{27,28} The EFFF channel, as shown in Figure 1, is a thin channel of rectangular cross section with an aspect ratio (the ratio of width to height) exceeding 80, as needed to closely approximate two infinite, parallel plates.^{29,30} Most FFF channels are of a similar design except that the applied field varies

between FFF subtypes. Flow between parallel plates separated by small distances is laminar for all flow velocities of interest. Laminar flow implies a parabolic velocity distribution such that the fluid velocity at the surface of the plates is zero, while it is at a maximum in the center of the channel. Thus, if a particle or group of particles were to maintain an average distance y from the wall, and this distance were different from another group of particles, their velocities through the channel would be different and they would exit the channel at different times. A variety of parameters affect the operation of any FFF system. Each of these parameters will be explored here in a systematic fashion.

Retention. Retention theory involves an understanding of what causes disparate particles to be retained to different degrees, i.e., move through the channel at different rates. The governing retention equation for all FFF systems is given by²⁷

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

where R is the retention ratio or the ratio between void time (volume) and elution time (volume). λ is a nondimensional parameter related to height, w , of the channel and the physical properties of the particles being retained and is given by

$$\lambda = D/Uw \quad (2)$$

where D is the diffusion coefficient of the particles and U , the drift velocity, is dependent on the applied field strength as shown by

$$U = S\phi/f \quad (3)$$

S is the applied field strength, ϕ is the field susceptibility of the particles and f is the sample friction coefficient. Therefore, the average thickness of the particle cloud will be determined by a balance between dispersive and concentrating forces.

The diffusion coefficient, D , can be calculated using the modified Einstein equation

$$D = kT/3\pi\eta d \quad (4)$$

where k is the Boltzmann constant, T is the absolute temperature, η is the viscosity, and d is the particle diameter.

At high retention levels (as λ and R approach zero), eq 1 is typically approximated by the simpler expression

$$R = 6\lambda \quad (5)$$

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Since most FFF systems are operated under high retention ($R < 0.2$), eq 5 is generally a reasonable approximation and is much more convenient for a performance analysis of the FFF systems.

The value of miniaturization on retention in FFF systems is highly dependent on the system subtype. As can be seen from eq 2, retention is an inverse function of channel height, w . Thus, as the channel height is reduced, the value of the retention ratio increases, indicating a loss of retention. Generally, the goal of most FFF instruments is to increase retention, as reducing retention can easily be accomplished by reducing the field strength. Thus, for general systems, miniaturization will actually prove a disadvantage. The only possible offset to this disadvantage occurs when the field strength in the system is also a function of channel height, as is the case with EFFF systems.

In EFFF, an electric field, E , is applied across the channel and particles are subjected to the applied field according to their electrophoretic mobility, μ . There is a difficulty, though, in estimating the effective field experienced by the particles in EFFF systems. The naïve approach would be to simply divide the applied voltage by the channel height to arrive at the electric field strength, as is appropriate for many nonaqueous applications. This approach, however, does not work for EFFF, since most of the applied voltage is dropped across the interface and polarization layer between the electrode and the bulk carrier solution. Thus, the actual effective field for retention in the channel is much less, typically around 1% of the applied field.¹⁰ This loss of effective voltage is caused by the effects of the ionic double layer present at the electrode–solution interface and is a function of many variables including the electrode material, the chemical composition of the carrier solution, the concentration of the carrier, and the temperature in the interface region. Another difficulty associated with EFFF concerns the aqueous carrier solutions used in most EFFF systems. Aqueous carrier solutions, which are especially of interest for biological separations, break down into hydrogen and oxygen at relatively low applied voltages (1.7 V and above). This electrolysis can eliminate retention in the system due to disruption of the flow characteristics by bubble formation. Thus, since it is not the field but the voltage that is the limiting factor, it is reasonable to discuss scaling in EFFF systems in terms of voltage rather than field strength. Conveniently, the product of E and w appears quite regularly in the equations characterizing EFFF, so replacing E and w with V often simplifies the equations. As with the electric field strength, the effective voltage, V_{eff} , in the channel is much less than the applied voltage and will be defined as

$$V_{\text{eff}} \equiv E_{\text{eff}}w \quad (6)$$

For a given applied voltage, V_{app} , E_{eff} scales inversely with w so that the effective field across the bulk of the carrier fluid increases with decreasing channel width. The V_{eff} is not affected by scaling. This condition is assumed to hold for channel widths greater than approximately twice the double-layer thickness and would likely only be challenged in the very smallest channels since double layers are typically measured in nanometers for the conditions found in EFFF channels. Thus, for EFFF

$$U = \mu E_{\text{eff}} = \mu V_{\text{eff}}/w \quad (7)$$

Most of the general FFF equations can be applied directly to EFFF by replacing U in the equation with the result of eq 7. For example, λ , the nondimensional parameter relating column and sample characteristics to R , is represented by

$$\lambda = D/\mu V_{\text{eff}} \quad (8)$$

Thus, when EFFF systems are operated at high retention, the level of retention becomes entirely independent of the channel dimensions, assuming all other parameters remain constant. The only scaling effect that can be deduced from this analysis is that if the length of the channel is reduced by a scaling factor, s , the elution time also drops by the same factor, s . This time reduction is potentially advantageous, but there are no special techniques or manufacturing processes required to obtain this advantage.

Of critical importance to all of these analyses is the assumption that all parameters involved in retention in EFFF systems are constant and do not vary with channel dimensions. For example, this analysis assumes that the voltage dropped across the bulk of the channel remains constant even while the distance is being reduced. In other words, the field in the bulk of the channel is increasing as the channel size is reduced. If for some reason, this assumption were not true (i.e., the double layer fills the channel eliminating the “bulk” of the channel), then the analysis will fail and the conclusions derived here will be invalid. Considering the typical dimensions of double layers and the fact that even FFF channels measured in micrometers are orders of magnitude larger than the atomic-scale effects that might disrupt the analysis, we are confident that this analysis will hold up under experimental analysis.

Another assumption with possible bearing on the scaling analysis presented here revolves around the assumption that the particle distribution in an EFFF system remains exponential. Since the voltage drop across the EFFF channel is clearly not constant, especially near the channel walls, there is some likelihood that the assumption of an exponentially distributed field is not entirely correct. The potential effect of this nonuniformity in the distribution may limit the proportionality between R and λ given in eq 5, but this effect is not well understood and will be examined in future communications based on experimental work.

Plate Heights and Resolution. Particle clouds in FFF systems are not generally limited to the volume into which they were initially injected, but tend to become dispersed across volume elements by mixing, diffusion, and other forces. As these particles spread in the volume and time dimensions, they can begin to overlap and cause a loss of separation efficiency. Thus, this spreading and loss of information must be limited to produce the best separations possible. The level of separation efficiency generated by a particular instrument can be quantified using the plate theory of chromatography. In plate theory, the length, L , of a separation column can be broken down into N theoretical plates of height H

$$H = L/N \quad (9)$$

The plate height, H , is a measure of variance (σ^2) that has been created by the separation system while the band of particles being separated moves through the channel. N therefore becomes

a measure of the separation efficiency of the system and indicates the number of times a certain separation level is accomplished in a channel. H can be closely approximated by³¹

$$H = \sigma^2 / L \quad (10)$$

The total plate height can be thought of as the sum of several contributing factors. One group of factors, known as instrumental factors, H_i , can be minimized by good instrument design and operation procedures. These factors include the structure of the instrument (wall roughness, section connections, etc), sample plug length, and factors related to extracolumn devices such as detectors and tubing. Once the instrumental factors have been minimized, the largest contributor to band broadening will be the nonequilibrium effects, H_n . These nonequilibrium effects are caused by the inherent distribution of the sample over a number of volume elements and the slow movement of particles between volume elements. A third group of factors, H_p , is related to the polydispersity, or variation, in the sample being tested or separated. The fourth factor, H_D , is caused by the diffusion of particles in the system. The terms H_p and H_D are not related to system parameters or are small enough to be neglected and can be ignored for optimization of FFF systems. Thus, for the purposes of a scaling argument, the relevant plate height terms are the nonequilibrium effects, H_n , and the instrumental plate height, H_i .

In FFF, the nonequilibrium component of plate height, H_n , is heavily dependent on the channel dimensions and the flow rate²⁷ as shown by

$$H_n = \chi(\lambda) w^2 \langle v \rangle / D \quad (11)$$

where function $\chi(\lambda)$ is generally represented by

$$\chi(\lambda) = 24\lambda^3(1 - 8\lambda + 12\lambda^2) \quad (12)$$

As retention increases, R and λ decrease, and H_n becomes progressively smaller due to the highly compact bands. In this case, the function $\chi(\lambda)$ closely approaches $24\lambda^3$ ³¹ and H_n can be reduced to

$$H_n = \frac{24\lambda^3 w^2 \langle v \rangle}{D} = \frac{24D^2 \langle v \rangle}{U^3 w} \quad (13)$$

using eq 2. This analysis assumes an exponential particle distribution, though, which may not be strictly correct as mentioned previously, but should be sufficient for the analysis presented here.

An examination of eq 13 indicates that reducing w will actually increase plate heights for general FFF systems. This result is true in FFF subtypes for which U is not a function of w . For example, in sedimentation FFF, there is no relationship between field strength and channel thickness and therefore no apparent rationale exists for miniaturizing the system. A similar result is obtained for flow FFF. For electrical and thermal FFF, though,

the applied field strength is dependent upon the channel thickness, assuming a constant or limited applied voltage or temperature difference.

The plate height equations for EFFF are the same as for general FFF systems, but the effect of miniaturization on the systems is somewhat different. The only contributor to plate height that varies with FFF subtype is the nonequilibrium plate height, H_n . In the case of EFFF systems, this contribution can be derived by substituting eq 7 into eq 13

$$H_n = \frac{24D^2 \langle v \rangle}{\mu^3 E^3 w} = \frac{24D^2 \langle v \rangle w^2}{\mu^3 V_{\text{eff}}^3} \quad (14)$$

Of immediate note is the fact that H_n is no longer inversely related to channel height, w , but is now directly related to the channel height to the second power. Thus, reductions in channel height decrease plate heights in EFFF systems in a quadratic manner.

The resolution of a chromatography system, R_s , is a measure of the relative separation efficiency of the system and is directly related to plate heights. Mathematically, a resolution equal to 1 is defined as two peaks separated by 4σ . The equation for resolution in FFF systems is easily derived and can be written as³¹

$$R_s = \left(\frac{\sqrt{L}}{4} \right) \frac{\Delta d \left(\frac{S_d}{\sqrt{H}} \right)}{d} \quad (15)$$

Replacing H with eq 13, we arrive at

$$R_s = \frac{(\Delta d / d)}{8D} \sqrt{\frac{LwU^3}{6\langle v \rangle}} S_d \quad (16)$$

In this equation, S_d is the size selectivity index for the system, d is the average diameter of the particles being compared, and Δd is the difference in particle diameters. In some FFF systems, S_d can be a very important parameter, while in others, S_d is close to 1 and of little importance to estimates of resolution or retention.

Examination of this equation reveals that, for any FFF system, the resolution of the system is proportional to the square root of channel height. Thus, a reduction in channel height would in general reduce resolution. Additionally, if the entire system is miniaturized, the length would also be reduced and would further reduce the resolution in the system. Thus, miniaturization of general FFF systems does not appear practical or reasonable from a resolution standpoint, as several researchers have pointed out.^{4,32}

For FFF systems in which U is dependent on the channel height (thermal and electrical), miniaturization may however result in resolution gain. The resolution for an EFFF system can be found by substituting eqs 6 and 7 into eq 16

$$R_s = \frac{(\Delta d / d) S_d}{8Dw} \sqrt{\frac{L\mu^3 V_{\text{eff}}^3}{6\langle v \rangle}} \quad (17)$$

In most cases for EFFF, S_d is assumed to be equal to 1 since the drift velocity, U , is independent of particle diameter and the sample selectivity is only due to the differences in diffusion coefficient,

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D .¹⁰ A size selectivity of unity has indeed been reported.¹² However, S_d has been experimentally measured in some EFFF systems where the size selectivity was found to be somewhat lower, so S_d will be left in eq 17 in case it is needed for future reference.

Note in eq 17 how the resolution now improves with channel height reductions while for general FFF systems that is not the case. The resolution varies inversely with channel height, indicating that miniaturization of EFFF systems is potentially beneficial. A closer look indicates that reducing channel heights is nearly the only way to improve the resolution in EFFF systems. D , Δd , d , and μ are all properties of the particles being separated and cannot be influenced by instrument design. Increasing L or decreasing $\langle v \rangle$ increases the time required for the separation faster than the resolution improves, and these changes can become impractical at certain levels. V_{eff} , while not limited in general, is limited in the predominately aqueous carrier fluids used in EFFF due to electrolysis at relatively low voltages. Thus, the only remaining option for increasing resolution in EFFF systems is to reduce w , the channel plate separation distance. Thus, while retention is not affected by channel height reductions in EFFF systems, both plate height and resolution are improved.

Other FFF Considerations. Steric Transition Point. An important effect encountered in FFF systems is the “steric transition” that occurs when particle elution times begin to reverse once the radius of the particles being separated exceeds the average particle distance from the channel wall. In normal FFF modes, smaller particles will elute ahead of larger particles. However, beyond the steric transition point, this elution order is reversed and larger particles elute ahead of smaller particles. The steric transition point is a measure of the amount of particle zone compaction and the effective field that operates in the channel. A knowledge of the location of the steric transition point can be extremely helpful, not only because it is necessary to avoid confusion when interpreting retention and separation data but also because steric separations can often be done at high flow rates and with high resolution.

The steric inversion diameter, d_i , can be found using

$$d_i = S_d \sqrt{2kT/3\pi\gamma\eta U} \quad (18)$$

where γ is a dimensionless number of order unity used for complications originating from wall repulsion and other effects.³³ Examination of eq 18, though, indicates that, for general FFF systems, there is no effect from channel height on the steric transition point. The only parameter that determines the steric inversion point is the sample drift velocity, which is a function of the applied field for most systems and is not sensitive to channel dimensions.

In EFFF, though, U is sensitive to the channel height and so there is potential for the steric inversion point to be changed by adjusting channel dimensions. Adjustment of the steric transition point using channel heights may be especially valuable for systems in which the magnitude of the applied field is limited. Therefore, using eq 7 in eq 18, the steric transition point in EFFF systems can be derived as

$$d_i = S_d \sqrt{2kTw/3\pi\gamma\eta\mu V_{\text{eff}}} \quad (19)$$

Examination of eq 19 shows that the steric transition point for EFFF systems is dependent upon channel height. Thus, by miniaturizing the system, it becomes possible to significantly reduce the steric transition point and make available the high-speed separations possible using the steric mode of separation. The separation mode cannot be changed to steric mode simply by increasing the applied voltage, as can be done in general systems, due again to the electrolysis of water at even moderate applied voltages. Thus, for EFFF systems, steric separations of smaller particles might be impossible unless channels with smaller dimensions are fabricated.

Stop Flow. After sample injection into an FFF system, time is required for particles to migrate toward the channel walls and establish an equilibrium position under the influence of the applied field. Equilibrium in the channel is not instantaneous and requires a relaxation time, τ_e , equal to the time required for a particle to migrate from one electrode to the other in the presence of the applied field. If the drift velocity, U , is constant, the maximum relaxation time will be found using

$$\tau_e = w/U \quad (20)$$

To permit sample equilibration in FFF systems, it is customary to allow for a period of stop flow in which the particles are allowed to migrate under the influence of the applied field to an equilibrium position. If this stop flow time is not practiced, high velocity flow lines are able to overly influence the migration of particles through the channel and cause peak fronting and losses in resolution, especially for small particles which tend to elute shortly after the void peak. For most FFF systems, stop flow times of at least 5 min are practiced and the stop flow time is often much longer. It is clear from eq 20 that reductions in channel height or miniaturization of FFF systems would have a positive effect on equilibration times and could significantly reduce or even eliminate the need for stop flow.

This reduction in equilibration time is even more significant for EFFF and TFFF since their fields are dependent on the plate separation distance. The equation for the time to equilibrate, τ_e , in EFFF systems is

$$\tau_e = w^2/\mu V_{\text{eff}} \quad (21)$$

and is found by applying eq 7 to eq 20. Note that in EFFF systems the equilibration time is an quadratic function of w .

UNIQUE EFFF EFFECTS

Field Time Constant. The time constant for variation of the applied electric field is important for two reasons. First, it determines the time required for the system to stabilize after the voltage is applied across the channel. Second, it determines the possibility of ever using alternating electrical fields in an EFFF system. Separations of this type would be valuable to the analytical community and would be based on transport rates in the fluid. A diagram of the interior of the EFFF channel is given in Figure 2a. Note that, in general, w is much greater than d ($w \gg x_{\text{DL}}$). The circuit in Figure 2b can be derived from Figure 2a, where

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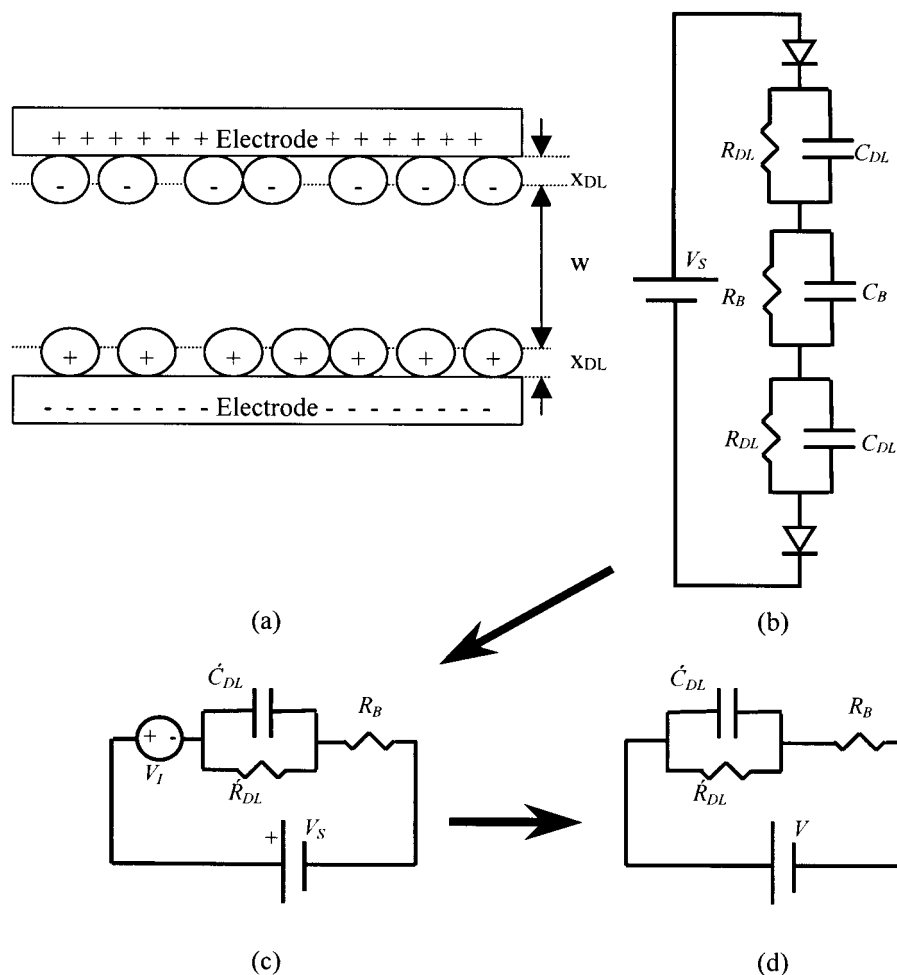


Figure 2. Electrical representation of the EIFFF channel. (a) Schematic of the double layer showing the double-layer capacitors along with the general capacitance of the channel. (b) A circuit derived from the double-layer model showing the capacitance of the double layer, C_{DL} , the resistance through the double layer, R_{DL} , the bulk channel resistance R_B , and the bulk capacitance C_B . The diodes represent the interfacial electrical properties of the system. (c) Simplified version of the circuit in (b). The double-layer capacitance and resistance can be lumped into \hat{C}_{DL} and \hat{R}_{DL} , and the bulk capacitance, which is much smaller than the double-layer capacitance, can be ignored. The diodes are replaced by a voltage source, V_i , which represents the circuit for positive applied voltages greater than the standard electrode potential. (d) The voltage source can be ignored for determination of the scaling of the electrical time constant.

the capacitance C_{DL} corresponds to the double-layer capacitance and R_{DL} corresponds to the interface resistance and the resistance across the electrode–solution interface. R_B represents the bulk solution resistance and C_B the bulk capacitance. The diodes represent the electrical properties of the electrode–solution interface that has characteristics similar to a diode. The model can be simplified as shown in Figure 2c. In this circuit, the double-layer capacitance and resistance at the two interfaces have been lumped together and are represented by \hat{C}_{DL} and \hat{R}_{DL} . Further simplification can be made by neglecting the relatively small bulk capacitance. The bulk capacitance can be modeled as an open circuit, especially for the low-frequency applied voltages (typically dc) used in EFFF systems. The magnitude of the bulk capacitance will increase as the channel height is reduced, and could eventually become significant, but at the level of miniaturization proposed in this work, it is still more than 3 orders of magnitude smaller than the double-layer capacitance. It is important to note also that the numerical values associated with the interfacial voltage source, V_i , and the double-layer resistance, R_{DL} , are strong functions of the materials used in the system and the geometry of the system. Since this analysis assumes that the materials are

not changing and that the geometry scales in all directions, R_{DL} will be considered to vary only as a function of area of the electrode (which is consistent with the assumption of a constant double layer thickness). While this does not include all effects, it will be sufficient for demonstrating the scaling effects expected in the μ -EFFF channel. The final simplified circuit is shown in Figure 2d. In this circuit, the interfacial voltage source is removed since it is irrelevant for estimates of the system time constant. The circuit in Figure 2d will therefore provide a basis for the electrical time constant analysis.

The double layer capacitance can be represented by

$$C_{DL} = \epsilon_{DL}(bL/x_{DL}) \propto s^2 \quad (22)$$

where ϵ_{DL} is the permittivity of the double layer, x_{DL} is the double-layer thickness (assumed to be constant), and b is the channel breadth. The bulk resistance is represented by

$$R_B = \rho_B(w/bL) \propto 1/s \quad (23)$$

where ρ_B is the resistivity of the bulk carrier solution. The double

Table 1. General FFF and EFFF Parameters Affected by Miniaturization

parameter	general FFF scale factor	advantage or disadvantage	EFFF scale factor	advantage or disadvantage
retention ratio (R)	1/s	disadvantage	1	potential advantage
analysis time	s^2	limited advantage	s	advantage
drift velocity (U)	1	neither	s	advantage
plate height (H)	1/s	disadvantage	s^2	advantage
resolution (R_s)	s	disadvantage	$1/\sqrt{s}$	advantage
steric transition (d)	1	neither	\sqrt{s}	potential advantage
equilibration time (τ_e)	s	advantage	s^2	advantage
field time constant (τ)	n/a	n/a	s	advantage
required sample size	s^3	advantage	s^3	advantage
solvent consumption	s^3	advantage	s^3	advantage
instrument size	s	advantage	s	advantage
separable particle size	s	relative	s	relative

layer or interface resistance can be represented by

$$R_{DL} = \rho_{DL}(x_{DL}/bL) \propto 1/s^2 \quad (24)$$

where ρ_{DL} is the resistivity through the double layer. The total impedance, Z_T of the circuit in Figure 2b can therefore be represented by

$$Z_T = \frac{R_{DL} + R_B + j\omega R_{DL}R_B C_{DL}}{1 + j\omega R_{DL}C_{DL}} \quad (25)$$

where ω is the frequency of the applied voltage.

Using standard dynamic system analysis, the electrical time constant, τ_{RC} , can be shown to be

$$\tau_{RC} = \frac{R_{DL}R_B C_{DL}}{R_{DL} + R_B} \quad (26)$$

Replacing each component in eq 26 with the scaling relationships presented in eqs 22–24 and simplifying, we arrive at a relationship for scaling of the electrical time constant in the EFFF channel

$$\tau_{RC} \propto \frac{\frac{1}{s^2} \frac{1}{s} s^2}{\frac{1}{s^2} + \frac{1}{s}} = \frac{s}{s+1} \approx s \quad (27)$$

On first glance, eq 27 does not appear to promise much of an improvement in scaling of the channel time constant. Closer inspection, though, indicates that since the system is being miniaturized, s will be much smaller than 1. Therefore, the dominant term in the denominator is the constant 1. The net effect is that the s in the denominator can be ignored indicating that the time constant is approximately proportional to s . In fact, the presence of the s in the denominator actually increases the effect since the denominator is composed of a sum. Thus, the total scaling of the electrical time constant will be slightly more than s alone. For example, a scale factor of ~ 0.1 produces a total scale factor of 0.09 for the electrical time constant. This means that the macroscale system time constant of ~ 40 s³² will be reduced to ~ 3.6 s. A reduction of this scale translates into a timesaving of

well over 3 mins or more in the actual operation of the system. This theoretical prediction will be verified in later communications.

SCALING EFFECTS

A number of other parameters are also important to consider when pondering miniaturization of an FFF device. Several of these factors have been discussed in other publications and will not be elaborated on here.⁴ Table 1 presents a summary of the scaling factors for both general FFF and EFFF systems for critical parameter equations that have been presented as well as for a few other parameters. In addition, Table 1 presents information regarding whether miniaturization would prove an advantage or disadvantage for that particular parameter.

Analysis Time. The total time required to perform a given analysis or separation is mainly a function of retention time for the most highly retained particles. The retention time, t_r , is directly linked to the retention ratio, R . The relationship between R and t_r at high retention is given by any of the following equivalent expressions

$$t_r = \frac{L}{R\langle v \rangle} = \frac{LUW}{6D\langle v \rangle} = \frac{L\mu V_{\text{eff}}}{6D\langle v \rangle} \quad (28)$$

For general FFF systems, the retention time appears to scale with s^2 , which may initially seem to be a significant advantage, but this advantage, as mentioned previously is made at the expense of retention and resolution. Additionally, previous work in thermal systems indicates that the theory with regard to time optimization does not always follow this scaling law.³⁴ Examination of eq 30 reveals that, in order to maintain resolution, the flow velocity would need to be reduced by s^2 , thus eliminating any retention or analysis time improvements. While there may be applications where time is more critical than resolution, there are easier methods for reducing retention time that have a less severe effect on resolution, most notably increasing the carrier flow velocity.

For EFFF systems, though, there is a gain in resolution with miniaturization. Thus, these reduced analysis times are an advantage that can be claimed by miniaturized EFFF systems without fear of concomitant disadvantages. Additional time is saved

(34) Giddings, J. C.; Martin, M.; Meyers, M. N. *J. Chromatogr.* **1978**, *158*, 419–435.

by miniaturization in the areas of stop flow and system time constants

CONCLUSION

While most chromatography systems benefit significantly from miniaturization, the reverse is often the case for more general FFF systems. Fortunately, EFFF systems are different enough in structure and operation from the general FFF systems that they benefit from miniaturization. It seems clear when examining the theory and a broad range of scaling effects in EFFF systems that miniaturization would be of critical importance, if not a necessity, in the design and development of improved EFFF systems. Using eq 17 as the model for the resolution of an EFFF system, several distinct advantages become apparent when micromachining technologies are used to miniaturize an EFFF system. For example, separating 127- and 252-nm polystyrene spheres in a 6-cm μ -EFFF system with channel height of 20 μ m and a flow rate of 0.25 cm/s would yield a theoretical resolution of 5.8 and a run time of \sim 5 min. To equal the same resolution using the current EFFF technology (with a channel thickness of 178 μ m, a channel width of 2 cm, and a length of 64 cm) would require a run time of 390 min or 6.5 h—nearly 2 orders of magnitude larger.

While predictions of this nature are compelling, there are some hurdles to overcome. Significantly, most of the advantages from miniaturization in EFFF systems hinge on the assumption that the field strength will improve with miniaturization. While this assumption appears valid for most cases, there is concern that in EFFF systems the double layer will limit the improvement in applied electrical field strengths and thus limit the value of miniaturization of these devices. Thus, the critical question to ask with regard to miniaturization is, will the field scale appropriately? This question of how the electric field scales, as well as how the experimental data from a miniaturized EFFF compares to the presented theory and scaling effects, will be answered in later communications.

ACKNOWLEDGMENT

This work was supported by a National Science Foundation (NSF) Graduate Research Fellowship, the Whitaker Foundation, and a University of Utah Technology Innovation Grant.

Received for review December 12, 2000. Accepted March 4, 2001.

AC001463Q