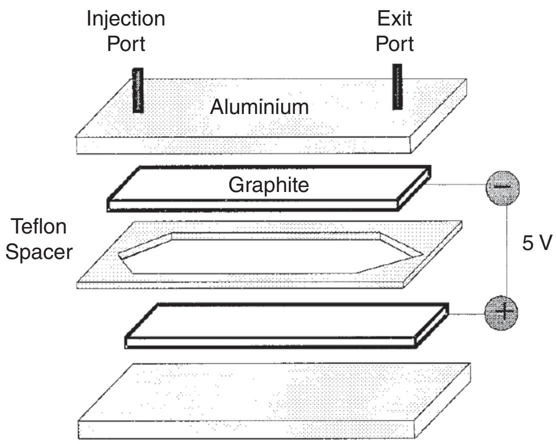

Figure 1.9 Exploded view of the main components of an ElFFF channel. *Source*: Tri et al. (2000) / American Chemical Society.

The retention parameter in ElFFF is given by

$$\lambda = \frac{D}{\mu_e E_{\text{eff}} w} = \frac{kT}{3\pi\eta d_h \mu_e E_{\text{eff}} w} \tag{1.36}$$

where μ_e is the particle electrophoretic mobility and E_{eff} is the effective electric field (potential gradient), and it was noted that the particle hydrodynamic diameter includes the double layer thickness which is significant at low ionic strength.

Palkar and Schure (1997a) studied the time dependence of the electrode polarization effect as well as the influence of flow rate. They also studied the influence of sample size on retention and the effect of sample conductivity (Palkar and Schure 1997b). It is clear that the precise prediction of retention times in ElFFF is not a simple matter.

Micro-machined ElFFF channels were introduced in 1998 by Gale et al. (1998). The channels were 4–6 cm long, just 20–30 μm thick, and 0.4–8 mm broad. Titanium followed by gold was sputtered onto the silicon wafer and glass plate walls to serve as electrodes (titanium was used for its good adhesion to silicon). Sample volumes were typically as small as 0.1 μL, injected through a septum close to the channel inlet. The advantages of miniaturization of ElFFF were later examined in theory and practice (Gale et al. 2001, 2002). The advantages lie in improved efficiency, faster sample relaxation, reduced steric inversion diameter, and reduced system time constant (the time for the system to stabilize on applying the potential gradient). The reduced time constant opened up the possibility of using alternating electric fields for cyclical operation. The electrode polarization problem could then be circumvented using a square-wave, cyclical electric field with optimized frequency (Lao et al. 2002; Gale and Srinivas 2005). In cyclical operation, species are separated according to differences in their electrophoretic mobilities μ_e, rather than in the ratios of D/μ_e. It is the mobility and the frequency of the cycling field that determines the fraction of time spent in the faster-flowing regions as compared to the regions close to the walls (Giddings 1986; Lee et al. 1988; Stevens 1990). Cyclical ElFFF has been shown to be an effective separation technique for submicron-charged particles. Improvements to modeling (Chen and Chauhan 2007; Kantak et al. 2006), experimental optimization (Gigault et al. 2011; Srinivas et al. 2010), and the advantage of operation with biased fields (Ornthai et al. 2015; Tasci et al. 2013) have yielded significant improvements.

1.8 Magnetic Field-Flow Fractionation

Different approaches to implementing magnetic FFF (MgFFF) have been considered through the years. Many of the early efforts were reviewed by Carpino et al. (2005b). Studies involving simple capillary tubes with transverse applied magnetic field gradients were reported by Mori (1986), Latham et al. (2005), and Vickrey and Garcia-Ramirez (1980). Tubular channel geometry with a transverse applied field is not well suited to the implementation of FFF (Giddings 2000), and the field gradients were relatively small. Mori (1986) demonstrated only slight retention of Ni^{2+} protein complexes, and Latham et al. (2005) obtained separation of 13-nm $CoFe_2O_4$ particles in hexane from 6-nm Fe_2O_3 particles that eluted with the void peak. Nomizu et al. (2001) used an intermittent transverse magnetic gradient, provided by an electromagnet, applied to a capillary tube to show separation between retained 0.6-μm magnetite particles from effectively non-retained 0.7-μm hematite particles in an aqueous carrier with 0.1% sodium oleate as a dispersive agent. It is not possible to determine whether retention of magnetite particles was in accord with magnetophoretic mobility as would be expected for the cyclical FFF mechanism, or whether it was simply a function of the time captured during periods of applied transverse field gradient.

Fukui, Ohara, and coworkers (Fukui et al. 2008, 2009; Takahashi et al. 2006) proposed the use of high-temperature superconducting magnets to obtain higher field gradients of 200 T/m or more. They carried out theoretical modeling of separation in capillaries subjected to such field gradients. They did not allow for relaxation to steady-state distributions before elution, however, and their simulations reflected separation due to differences in relaxation across the capillary cross-section. Therefore, the mechanism considered was not strictly that of FFF.

In 1984, Schunk, Gorse, and Burke (Gorse et al. 1984; Schunk et al. 1984) reported the use of a parallel-plate channel with a transverse field gradient generated by an electromagnet. They were able to separate singlet 0.8-μm-rod-shaped iron oxide particles used in the recording industry from doublets. Again, the field gradient was rather small. As a means of creating high field gradients in a channel, Semenov and Kuznetsov (1986) proposed mounting a ferromagnetic wire at the axis of a tubular channel and magnetizing the wire with an external magnetic field. The concept is taken from high-gradient magnetic separation (HGMS) technology (Oberteuffer 1973) widely used nowadays for immunomagnetic cell separation. The small surface of the wire would serve as the accumulation wall, which would make the system susceptible to overloading. The field gradient would also tend to increase rapidly with an approach to the wire which would tend to capture species which is the objective in HGMS. Semenov (1986) solved these problems by proposing that a uniform array of wires be embedded in one of the walls of a parallel-plate channel. The regular spacing of the wires was predicted to generate a fairly uniform field gradient in the channel. There have since been several modeling efforts and simulations of particle separations for such a design (Karki et al. 2001; Ohara et al. 1996; Ohara 1997; Ohara et al. 2000; Tsukamoto et al. 1995; Wang et al. 1997) and just one experimental implementation where there was shown to be slight retention of some transition metal salts (Mitsuhashi et al. 2002).

The most successful approach to MgFFF to date uses a quadrupole electromagnet and helical channel (Carpino et al. 2005a, 2005b, 2007; Williams 2012; Williams et al., 2009b, 2010c). A relatively small aperture (1–2 cm diameter) quadrupole electromagnet can generate uniform field gradients comparable to those found in the much bigger (10 cm diameter) superconducting quadrupoles (Takahashi et al. 2006), and these can be efficiently exploited using a helical channel mounted axisymmetrically to the field, close to the pole pieces. The helical channel has the advantage over a simple annular channel in that it is far simpler to maintain uniform thickness. It is also much easier to introduce fluid uniformly to the helical channel than to a full annular channel, and this is also true for the withdrawal of the fluid at the channel outlet. Also, the helical flow path carries all sample components through any small variations in field gradient around the annular space that would contribute to bandspreading in an annular channel. The use of an electromagnet also allows for very easy implementation of programmed decay of magnetic field gradient during sample analysis (Williams et al. 2010a). The quadrupole electromagnet and spiral channel are shown schematically in Figure 1.10 a and b, respectively, and a photograph of the system in Figure 1.10 c.

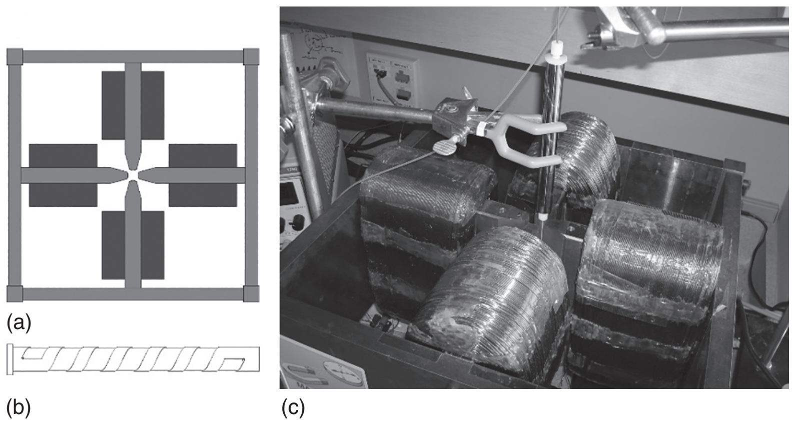

Figure 1.10 (a) Schematic of the cross-section of the soft iron pole pieces and yoke (pale gray) and electrical coils (dark gray); (b) Schematic of spiral channel machined into Delrin^{TM} (DuPont) rod that fits tightly into a stainless steel cylinder; *Source*: Carpino et al. (2005b) / with permission of ELSEVIER. (c) Photograph of the MgFFF system with the assembled spiral channel ready for an introduction to the quadrupole aperture.

The small deviations from parabolic of longitudinal and azimuthal velocity profiles in annular flow have been studied, as well as their influence on the retention ratio in a helical channel (Williams et al. 2009a, 2010b). However, if the channel thickness is relatively small compared to its radius of curvature, then the retention ratio and nonequilibrium bandspreading parameter are well approximated by the classical model equations (see Eqs. (1.2–1.6)).

The force F_m experienced by a magnetic particle in suspension placed in a magnetic field gradient is given by

$$F_m = V_m \Delta\chi \frac{B}{\mu_0} \nabla B = V_m M \nabla B \tag{1.37}$$

where V_m is the volume of magnetizable material contained in the particle, $\Delta\chi$ is the difference in magnetic susceptibility between the magnetizable material and the other materials present (the fluid and the other particle components, all assumed to have small susceptibility), μ_0 is the magnetic permeability of free space, M is the magnetization of the magnetizable material in the particles at the applied magnetic field B (other materials assumed to have negligible magnetization), and ∇B is the gradient in the magnitude of the magnetic field across the channel thickness. In an ideal quadrupole, the magnitude of the magnetic field B increases linearly with distance from the axis

$$B = \frac{r}{r_o} B_o \tag{1.38}$$

where r is the distance from the axis, r_o is the radius of the channel outer wall, and B_o is the magnitude of the field at r_o. The field gradient ∇B across the channel thickness is therefore constant and equal to B_o/r_o. Replacing F_G in Eq. (1.24) by F_m, the retention parameter λ is given by

$$\lambda = \frac{kT}{F_m w} = \frac{kT\, r_o}{V_m M B_o w} \tag{1.39}$$

The magnetization is a function of local field B as may be seen in Eq. (1.37), where $M = \chi B/\mu_0$, and for paramagnetic and diamagnetic materials, χ is constant. However, particles that are of particular interest for magnetic characterization are the superparamagnetic nanoparticles used for immunomagnetic labeling of specific biological cell types or used as drug carriers for magnetically targeted cancer treatment. These are composite particles where the iron oxide nanoparticles are coated with a biocompatible material such as dextran and which also may carry a chemotherapeutic drug and/or antibodies to a specific cell type. The magnetization M of the superparamagnetic component does not increase indefinitely with B. It approaches a saturation magnetization M_s at relatively low fields of 0.1–0.2 T. In this case, the concentration profile approaches an exponential, with λ given by Eq. (1.39) with $M = M_s$. Even at low field strength, where the magnetic material is approximately paramagnetic, the concentration profile does not deviate significantly from an exponential in the thin channels used.

For the characterization of superparamagnetic nanoparticles, it is not necessary for them to remain magnetically saturated during elution. If the full magnetization curve (M as a function of B from zero to saturation) is known for the magnetic material included in a sample of magnetic nanoparticles, then it is possible to predict retention times as a function of V_m, or the equivalent spherical diameter d_m, for any applied field or programmed field decay. There is one caveat. Although only the magnetic component responds to the field gradient, the steric exclusion correction, if it is considered, is dependent on the overall particle size. They do tend to be rather small (less than about 200 nm), however, and the steric correction will be correspondingly small. Using the general integral approach developed for data reduction (Williams et al. 2001), it is also possible to transform an elution profile into a distribution in V_m or d_m. Regarding the prediction of fractionating power, nonequilibrium bandspreading is a function of D and therefore of overall particle size (see Eq. (1.4) for the contribution to plate height). It would be necessary to make some assumptions concerning particle size. For example, particles may be assumed to have similar sizes (determined by light scattering or AsFlFFF, perhaps) but contain differing amounts of magnetic components.

It should be pointed out that MgFFF applies to the fractionation of superparamagnetic nanoparticles only if they have a nonmagnetic coating. Without the coating, the magnetic interaction between the magnetized particles causes aggregation, and fractionation is not possible (Williams et al. 2010c).

1.9 Novel Techniques

1.9.1 Combined Fields

The combination of more than a single transverse field can sometimes be advantageous. Various combinations have been explored. Chen et al. (1988) showed that a steric (or hyperlayer) separation of supramicron polystyrene particle standards by symmetrical FlFFF could be improved if gravity was imposed in the same direction as the cross-flow. They called the technique gravity-augmented FlFFF.

Liu and Giddings (1991) reported the use of thermal-electrical FFF for the separation of submicron polystyrene particle standards in acetonitrile. The retention of the particles due to the thermal gradient could be enhanced or reduced by the application of a voltage gradient across the channel.

Ultrasound-gravitational FFF has been described (Yin et al., 2013) in which the force due to a resonant acoustic field on microparticles toward the node at the channel midpoint is opposed by gravity. No experimental results were presented, but particles of different compositions were expected to be driven to different equilibrium positions across the channel thickness and be carried to the outlet at different times. The technique would not have the capability to separate particles by size alone because forces due to both the standing acoustic wave and gravity depend on particle volume.

Johann et al. (2015) constructed an AsFlFFF instrument in which an electrical field can be applied across the channel thickness. They were able to determine electrophoretic mobilities of nanoparticles and proteins by measuring elution times using cross-flow alone and then measuring the increase in elution times with the application of a voltage gradient. They were also able to show enhanced separation with the application of the electrical field. Further studies have been reported recently following the commercializing of this electrical AsFlFFF system (also referred to as EAF4) (Choi et al. 2020; Metzger et al. 2021; Kohl et al. 2021).

The technique of dielectrophoretic FFF (DEP-FFF) requires the opposition of dielectrical force and gravity (Huang et al. 1997; Markx et al. 1997; Wang et al. 1998, 2000; Yang et al. 2000). A negative dielectrophoretic force on microparticles drives them away from an array of microelectrodes in the lower channel wall, and this is opposed by gravity if they are denser than the fluid. The dielectrophoretic force varies strongly with distance from the electrodes, while gravity exerts a constant force. Both forces vary with the volume of the particles. The equilibrium position and elution time depend on both the density and the polarizability of the particle. The technique has been found to separate various types of biological cells based on differences in their polarizability.

An interesting technique proposed by Janča and Audebert combines an electrical field with gravity to implement a type of hyperlayer FFF based on isopycnic focusing (Janča and Audebert 1993, 1994). A colloidal density modifier, such as Percoll, is added to the carrier fluid. This is driven by the electrical field to form a density gradient across the channel thickness, and the microparticles to be separated find their isopycnic equilibrium positions within this gradient under the influence of gravity. They were able to show the influence of voltage gradient on the retention ratios of different particles.

1.9.2 Two-Dimensional, Continuous Fractionation

There has been some development of two-dimensional FFF instruments for continuous fractionations. Giddings discussed the theoretical aspects of continuous FFF separations (Giddings 1984, 1990b). It was explained that a selective FFF separation in one direction can be combined with a field-induced migration at right angles resulting in different trajectories to different collection points for different species. The field-induced migration may or may not be selective, but if it is selective, its selectivity must differ from that of the FFF separation. The migration at right angles to the FFF separation may also be provided by a non-selective flow or bulk displacement.

A continuous steric FFF device was developed that used a planar channel whose breadth was set at an angle to the horizontal so that particles sedimented across the channel breadth as they migrated in steric mode along the channel length in the horizontal direction (Myers and Giddings 1979; Schure et al. 1985). Particles of different sizes could be collected at different outlets along the lower edge of the channel. Ivory et al. (1995) constructed a continuous SdFFF instrument that used a centrifuge rotor housing a channel with a conic cross-section. The channel was therefore at an angle to the radius. It was intended that the migration in the direction of flow and rotation was to be *via* the mechanism of normal or steric mode SdFFF and the migration across the channel breadth by sedimentation. Unfortunately, separation appeared to be disrupted by flow instabilities in the rotating channel.

Pearlstein and Shiue (1995) presented a concept for continuous FFF separation in the annular space between concentric cylinders, one of which rotates while the other is held stationary. The carrier fluid flows axially within the annulus and the sample is introduced at a fixed point on the circumference at the channel inlet. Sample species were predicted to follow different spiral paths along the annular channel to be collected at different points around the circumference of the outlet. They presented only a mathematical model of the expected separation for such a system.

Vastamäki and coworkers (Vastamäki et al. 2014, 2001, 2003, 2005) have developed a continuous two-dimensional ThFFF instrument. It makes use of radial carrier fluid flow between two circular plates, the upper of which is stationary and heated, while the lower is slowly rotated and is cooled. The carrier fluid is introduced at the center of the upper heated disk, and the sample is continuously fed into a second inlet that is a small distance from the axis of the upper disk. Sample species relax to the lower rotating disk and migrate radially outward by the mechanism of ThFFF. At the same time, they are angularly displaced by the rotation of the lower disk. Different species follow different curved paths to the circumference where they are collected at several collection ports around the edges of the disks.

There are numerous potentially useful combinations of fields, flows, as well as field and flow directions that may be exploited for separations of different species of differing size or composition. There are likely to be many interesting developments in the future.

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