Quadrupole magnetic field-flow fractionation for the analysis of magnetic nanoparticles

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Abstract. Field-flow fractionation (FFF) is an analytical scale separation and characterization technique for macromolecules and particles. A quadrupole magnetic FFF device has been constructed for analyzing magnetic nanoparticles. It is shown to give reproducible results and be capable of distinguishing between different lots of a commercial magnetic nanoparticle material.

1. Introduction
The field-flow fractionation (FFF) techniques are analytical scale separation and characterization methods for macromolecules and particles (ranging from ~0.01 to 100 µm in diameter). The separation mechanism was conceived by Giddings in 1966 [1]. They are elution techniques similar to chromatography in which different components of a small sample elute from a separation channel at different times. Whereas chromatography exploits differences in partition between the mobile and stationary phases to separate sample components as they are carried along a column, FFF separation is achieved within the mobile phase alone. The separation device takes the form of a thin, parallel-walled channel, across the thickness of which is applied a field of some type. Due to viscous drag, the mobile phase velocity profile across the channel thickness is parabolic, or near-parabolic, with highest fluid velocity near the channel center and zero velocity at the walls. The field acts to drive susceptible sample components toward one of the walls, and therefore into relatively slowly moving fluid. For particles smaller than about a micron in diameter, a steady state exponential concentration profile results from the opposing influence of the field-driven and diffusive transport mechanisms. Particles that interact strongly with the field form thin zones adjacent to the wall, and are confined to the very slow moving fluid close to the wall. Particles that interact less strongly with the field form more diffuse zones, and they sample faster fluid streamlines in addition to those close to the wall. The net result is that particles elute from the channel in the order of increasing strength of interaction with the field. Furthermore, the quantitative theoretical foundation of FFF allows the determination of the strength of interaction of particles with the field as a function of their elution time.
FFF has been developed into a versatile family of techniques that exploits a range of different field types to characterize different particle properties [2,3]. The nature of the applied field determines the sample property that is characterized. For example, a gravitational or centrifugal field retains particles in the channel according to their buoyant mass, \( V_p \Delta \rho \), where \( V_p \) is the particle volume and \( \Delta \rho \) is the density difference between the particles and the fluid. An electrical field separates particles according to their electrical charge. There have been previous attempts to implement magnetic FFF, with varying degrees of success [4-20]. These have been reviewed in a recent publication [21]. The work presented here uses a quadrupole magnetic FFF instrument developed in our laboratory.

2. Particle Retention in FFF

In the case of classical FFF where the field imparts a constant cross-channel force on the particles, it may be shown that a population of identical particles in the channel approaches a steady state in which the particle concentration decays exponentially from one of the channel walls:

\[
c(x) = c_0 \exp\left(-\frac{xd}{D}\right) = c_0 \exp\left(-\frac{x}{w \lambda}\right)
\]

where \( x \) is the distance from the wall, \( c_0 \) is the concentration at the wall corresponding to \( x = 0 \), \( D \) is the particle diffusion coefficient, \( |u| \) is the field-induced velocity, \( w \) is the channel thickness, and dimensionless \( \lambda \) is the so-called retention parameter given by

\[
\lambda = \frac{D}{|u| w} = \frac{kT}{F_m w}
\]

in which \( k \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( F_m \) is the force on a single particle. When this steady-state zone is carried along the length of the channel by a flow of fluid, the elution time \( t_r \) is given by

\[
t_r = \frac{t^0}{R} = \frac{t^0}{6\lambda \left( \coth(1/2\lambda) - 2\lambda \right)} \approx \frac{t^0}{6\lambda (1 - 2\lambda)}
\]

where \( t^0 \) is the elution time for a non retained material and \( R \) is the retention ratio. For the elution of polydisperse samples, it is beneficial to gradually reduce the field strength during the analysis to hasten the elution of the more strongly retained fractions. Under such programmed field decay conditions, elution time may be obtained by solution of the equation

\[
t^0 = \int_0^{t_r} R \, dt
\]

Because the elution time of retained species may be predicted precisely from first principles, under conditions of constant or programmed field decay, it is possible to extract quantitative information concerning the strength of the interaction of the particles with the applied field.
3. Quadrupole Magnetic FFF
The quadrupole magnetic FFF device differs from all previous implementations of magnetic FFF in that it utilizes a radially symmetric magnetic field with the channel occupying a thin annular space within the field.

3.1. The quadrupole field.
The quadrupole magnetic field is radially symmetric, as shown in figure 1. The magnetic induction $B$ increases linearly with radial distance $r$ from the axis according to the equation

$$B = B_0 r / r_o$$  \hspace{1cm} (5)

where $B_o$ is the value of $B$ at the radial distance of the pole tips, $r_o$.

![Figure 1. The quadrupole magnetic field. The helical channel occupies a thin annular space between inner and outer radii, $r_i$ and $r_o$, respectively. The thickness of the annulus is exaggerated in the figure for clarity.](image)

It follows that within the aperture between the pole tips, the gradient in $B$ is constant:

$$|\nabla B| = B_0 / r_o$$  \hspace{1cm} (6)

The magnetic field must be as uniform as possible along the channel length. This is achieved by confining the channel within the quadrupole assembly such that the fringing effects close to the ends of the pole tips are avoided. The channel occupies an annular space between radial distance $r_i$ and $r_o$, as shown in figure 1, thereby avoiding the axial region where field strength is small.

3.2 Magnetic force on particles
The magnetic force on a particle in a magnetic field is a function of its magnetization and the gradient in the field strength:

$$F_m = (1/2) \mu_0 V_m |\nabla (M \cdot H)|$$  \hspace{1cm} (7)

where $\mu_0$ is the magnetic permeability of free space equal to $4\pi \times 10^{-7}$ H/m, $V_m$ is the volume of the particle, $M$ is the particle magnetization, and $H$ is the field strength. For paramagnetic particles, magnetization is proportional to the local field strength. However, at high field strengths particles may
approach magnetic saturation, and at saturation magnetization is constant and independent of field strength.

3.2.1. Paramagnetic particles. For paramagnetic particles, the magnetization $M$ is a linear function of field strength $H$, given by $M = \chi H$, where the constant $\chi$ is the magnetic susceptibility. The force on a particle differing in susceptibility from the suspending medium by $\Delta \chi$ is given by

$$F_m = V_m \Delta \chi \left(\frac{\nabla B^2}{2\mu_0}\right) = (V_m \Delta \chi) \frac{B_0^2}{\mu_0 r_o^2} r$$

where the final expression in equation (8) corresponds to the special case of a quadrupole magnetic field. The radial force therefore increases linearly with $r$.

3.2.2. Magnetically saturated particles. At high field strengths, magnetization approaches the saturation magnetization $M_s$. The radial force on a particle is then given by

$$F_m = (1/2)V_m M_s \left|\nabla B\right| = (1/2)V_m M_s B_o / r_o$$

and in a quadrupole field, radial force $F_m$ is therefore constant, as shown in the final expression in equation (10).

4. Experimental

A quadrupole electromagnet was constructed for the experiments, as shown in figure 2. This allows for setting field strengths to suit each sample, and for programming a field decay during analysis of polydisperse samples.

Figure 2. The quadrupole electromagnet assembly.

A channel was constructed by machining, to a depth of 500 µm, a spiral path into the surface of a Delrin cylinder which fitted tightly into a polished stainless steel tube. The advantages of such a helical channel have been described previously [21]. The rest of the system is similar to a typical liquid chromatography setup. An HPLC pump, injection valve (with 20 µL loop), and UV-detector were used.
The power supply for the electromagnet assembly was controlled by computer, which was also used to collect data from the UV-detector via an analog to digital converter. The carrier solution was phosphate buffered saline at pH 7.4. (A detailed description may be found elsewhere [21].)

5. Results and Discussion
Several elution curves (or fractograms) for various lots of Skold Technology nanoparticles are shown in figure 3. These have a mean diameter of approximately 230 nm, and are composed of dextran coated magnetite. All fractograms were obtained under identical conditions of carrier flow rate of 1.0 mL/min, initial field strength of 51 mT at the outer channel wall (monitored with a Hall effect probe), held constant for 2 minutes and then decaying to 2.7 mT over a period of 30 minutes. A stop flow time of 30 minutes was used before elution to allow the sample to approach its steady state distribution across the channel thickness.

![Figure 3](image_url)

**Figure 3.** Elution curves for various lots of Skold Technology magnetic nanoparticles obtained under identical conditions. a) two elution curves for lot 80, b) elution curves for lots 80 and 78, c) elution curves for lots 80 and 50-56, d) elution curves for lots 80 and 83.

The elution curves in figure 3a, obtained more than two weeks apart, demonstrate the reproducibility of the analysis. The narrow pulse toward the end of each fractogram indicates the time when the channel...
was removed from the magnetic field. The material eluting after this point may have aggregated and/or reversibly adsorbed to the channel wall. The elution curves shown in figure 3b indicate that lots 78 and 80 have very similar magnetic properties. The curves shown in figure 3c suggest that lot 50-56 might have a slightly higher magnetite content than lot 80, although the differences could be explained by slight differences in the applied magnetic field. The difference in the curves shown in figure 3d give a stronger indication that lot 83 has a higher magnetite content than lot 80. It is apparent that lots 50-56, 78, 80, and 83 are relatively consistent. A previous study has shown, however, that lot 40-43 has a far higher magnetite content than those shown here.

6. Conclusions
With the capability for programmed field decay during sample elution and the introduction of the helical channel design, the quadrupole magnetic FFF system is beginning to show its potential as a powerful separation and characterization tool for magnetic particles. Theory and software for data reduction are to be developed in the near future.

7. References
[1] Giddings J C 1966 A new separation concept based on a coupling of concentration and flow nonuniformities Sep. Sci. 1 123-5
[2] Giddings J C 1993 Field-flow fractionation: Separation and characterization of macromolecular-colloidal-particulate materials Science 260 1456-65
[3] Giddings J C 2000 The field-flow fractionation family: underlying principles Field-Flow Fractionation Handbook eds M E Schimpf, K D Caldwell and J C Giddings (New York, Wiley-Interscience) chapter 1 pp 3-30
[4] Vickrey T M and Garcia-Ramirez J A 1980 Magnetic field-flow fractionation: theoretical basis Sep. Sci. Technol. 15 1297-304
[5] Schunk T C, Gorse J and Burke M F 1984 Parameters affecting magnetic field-flow fractionation of metal oxide particles Sep. Sci. Technol. 19 653-66
[6] Gorse J, Schunk T C and Burke M F 1984-1985 The study of liquid suspensions of iron oxide particles with a magnetic field-flow fractionation device Sep. Sci. Technol. 19, 1073-85
[7] Semenov S N and Kuznetsov A A 1986 Flow fractionation in a transverse high-gradient magnetic field Russ. J. Phys. Chem. 60 247-50 (Trans. from 1986 Zh. Fiz. Khim. 60 424-28)
[8] Semenov S N 1986 Flow fractionation in a strong transverse magnetic field Russ. J. Phys. Chem. 60 729-31 (Trans. from 1986 Zh. Fiz. Khim. 60 1231-3)
[9] Mori S 1986 Magnetic field-flow fractionation using capillary tubing Chromatographia 21 642-4
[10] Ohara T, Mori S, Oda Y, Yamamoto K, Wada Y and Tsukamoto O 1994 FFF using high gradient and high intensity magnetic field: process analysis Presented at the Fourth International Symposium on Field-Flow Fractionation (FFF94), Sweden
[11] Tsukamoto O, Ohizumi T, Ohara T, Mori S and Wada Y 1995 Feasibility study on separation of several tens nanometer scale particles by magnetic field-flow-fractionation technique using superconducting magnet IEEE Trans. Appl. Superconductivity 5 311-4
[12] Ohara T, Mori S, Oda Y, Wada Y and Tsukamoto O 1995 Feasibility of using magnetic chromatography for ultra-fine particle separation Proc. IEE Japan-Power & Energy’95 161-6
[13] Ohara T, Mori S, Oda Y, Wada Y and Tsukamoto O 1996 Feasibility of magnetic chromatography for ultra-fine particle separation Trans. IEE Japan 116-B 979-86
[14] Wang X, Ohara T, Whitby E R, Karki K C and Winstead C H 1997 Computer simulation of magnetic chromatography system for ultra-fine particle separation Trans. IEE Japan 117-B 1466-74
[15] Ohara T 1997 Feasibility of using magnetic chromatography for ultra-fine particle separation *High Magnetic Fields: Applications, Generations, Materials*, ed H J Schneider-Muntau (New Jersey, World Scientific) pp. 43-55

[16] Ohara T, Wang X, Wada H and Whitby E R 2000 Magnetic chromatography: numerical analysis in the case of particle size distribution *Trans. IEE Japan* **120-A** 62-7

[17] Karki K C, Whitby E R, Patankar S V, Winstead C, Ohara T and Wang X 2001 A numerical model for magnetic chromatography *Appl. Math. Modelling* **25** 355-73

[18] Mitsuhashi K, Yoshizaki R, Ohara T, Matsumoto F, Nagai H and Wada H 2002 Retention of ions in a magnetic chromatograph using high-intensity and high-gradient magnetic fields *Sep. Sci. Technol.* **37** 3635-45

[19] Nomizu T, Nakashima H, Sato M, Tanaka T and Kawaguchi H 1996 Magnetic chromatography of magnetic fine particles suspended in a liquid with a steel-bead column under a periodically intermittent magnetic field *Anal. Sci.* **12** 829-34

[20] Nomizu T, Yamamoto K and Watanabe M 2001 Magnetic chromatography for magnetic fine particles using a periodically intermittent magnetic field *Anal. Sci.* **17** i177-80

[21] Carpino F, Moore L R, Zborowski M, Chalmers J J and Williams P S Analysis of magnetic nanoparticles using quadrupole magnetic field-flow fractionation *J. Magn. Magn. Mater.* accepted

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