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Analysis of magnetic nanoparticles using quadrupole magnetic field-flow fractionation

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Abstract

The new technique of quadrupole magnetic field-flow fractionation is described. It is a separation and characterization technique for particulate magnetic materials. Components of a sample are eluted from the separation channel at times dependent on the strength of their interaction with the magnetic field. A quadrupole electromagnet allows a programmed reduction of field strength during analysis of polydisperse samples.

Keywords: Field-flow fractionation; FFF; Magnetic nanoparticles; Magnetic separation; Electromagnet; Quadrupole; Characterization technique; Magnetophoresis

Field-flow fractionation (FFF) is a separation and characterization technique conceived by Giddings in 1966 [1]. It is an elution technique similar to chromatography in which different components of a sample mixture elute from the system at different times. As in liquid chromatography, a flow of carrier fluid is provided by a pump (commonly, a high performance liquid chromatography (HPLC) pump), the sample is introduced to this stream using an HPLC sample valve, and a detector (such as a UV-visible HPLC spectrophotometer) downstream of the separation device is used to detect the elution of sample components. Whereas chromatography exploits differences in partition between the mobile phase and a stationary phase to separate sample components as they are carried along a column, FFF separation is achieved within the mobile phase alone. In fact, there is no stationary phase present. Rather than the packed or open tubular column of HPLC, the separation device takes the form of a thin, ribbon-like channel, across the thickness of which a uniform, or close to uniform, field is applied. Due to viscous drag, the fluid velocity profile

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across the channel thickness is parabolic, or near-parabolic, with the highest fluid velocity near the channel center and zero velocity at the walls. For a channel cross section of high aspect ratio, and parallel channel walls, the fluid velocity profile may be assumed constant across the channel breadth with small perturbations at the edges due to the drag of the side-walls. The field acts to drive susceptible sample components toward one of the walls (the so-called accumulation wall), and therefore into relatively slowly moving fluid. For particles smaller than about a micron in diameter, the resulting increase of concentration close to the wall is opposed by diffusion. The result is a steady state where the concentration of each sample component decays exponentially away from the wall. 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 concentration profiles, and they may sample faster fluid streamlines in addition to those close to the wall. The outcome is that particles that interact less strongly with the field are carried to the channel outlet more quickly than those that interact more strongly. Particles therefore elute in the order of increasing strength of interaction with the field. Furthermore, the time of elution may yield quantitative information on the strength of interaction of the particles with the field.

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 carrier fluid. An electrical field separates particles according to their electrical charge. Field-flow fractionation has developed into a versatile family of techniques that exploits a variety of field types to characterize different particle properties [2,3]. There have been previous attempts to implement magnetic FFF, with varying degrees of success. These will be discussed briefly below. The advantages of the quadrupole magnetic FFF system that is under development in our laboratory will then be described.

In 1980, Vickrey and Garcia-Ramirez [4] attempted to demonstrate magnetic FFF by coiling a 304 cm Teflon tube of 0.15 cm internal diameter against the windings of a 400 Gauss (0.04 T) electromagnet. The coiled tube lay close to the plane of one end of the iron core. No retention of the Ni-BSA complex sample was observed, which is not surprising given the far from ideal tubular channel geometry [3], the low field strength, and the channel position in a region of low field gradient.

Schunk, Gorse, and Burke [5,6] reported in 1984 the use of a parallel plate channel with a magnetic field provided by an electromagnet. The channel was made of 0.5 in (1.27 cm) and 0.125 in (0.32 cm) thick glass plates separated by a 250 μm spacer. The thinner wall was placed in contact with one end of the iron core of the magnet to maximize field strength and field gradient in the channel. A maximum field strength of about 275 Gauss (0.0275 T) and field gradient of about 21 Gauss/mm (2.1 T/m) were obtainable, with less than 1% variation in field strength along the length of the channel. They were able to separate singlet from doublet 0.8 μm rod shaped iron oxide particles used in the recording industry (i.e., fairly large, high susceptibility particles).

Semenov and Kuznetzov [7], in 1986, proposed the use of a ferromagnetic wire at the axis of a tubular channel placed perpendicular to a magnetic field that magnetized both the wire and the particles to be separated. They presented calculations that suggested retention of both paramagnetic and diamagnetic particles was possible in such a system. The coaxial channel geometry is also far from ideal. The force on retained particles would rapidly increase as the wire is approached, which would tend to induce particle capture. Also, the small surface of the wire would have to serve as the accumulation wall, which would make the system highly susceptible to overloading. In the same year, Semenov [8] proposed a parallel plate channel in which the field would be provided by the induced magnetization of parallel ferromagnetic wires arrayed uniformly in the surface of one of the walls. The wires were to lie in the direction of flow and the field was to be applied across the channel breadth, perpendicular to the wires and

the flow direction. He presented a theoretical study of the proposed system and concluded that it would be more efficient than the coaxial system. Such an arrangement would help to alleviate overloading, although the strong spatial variation in force on the particles would remain.

Also in 1986, Mori [9] reported an attempt to demonstrate magnetic FFF using a system very similar to that of Vickrey and Garcia-Ramirez [4]. He used a Teflon tube of 0.5 mm internal diameter and length 270 cm, 210 cm of which was coiled against a pole of a 9000 Gauss (0.9 T) electromagnet. Very slight retardation of Ni^{2+} complexes with BSA, egg albumin, and EDTA was observed. Although Mori used a stronger field strength than in the earlier system [4], and the tube was placed against the pole piece rather than the electrical coil, the field gradient would not have been very high and the tubular channel geometry is not suited to FFF.

From 1994, Ohara and co-workers [10–18] have pursued an approach to magnetic FFF similar in concept to that proposed by Semenov [8]. The majority [10–17] of their publications describe theoretical studies and simulations. Tsukamoto et al. [11] considered a set of stacked channels with ferromagnetic wires embedded in the dividing walls. The stacking of channels was to maximize throughput of the system. The wires were assumed to be parallel to one another and in the direction of fluid flow. An external magnetic field was to be applied across the channel thicknesses, perpendicular to the wires and to the fluid flow. It was assumed that wires of 10 μm diameter would be embedded in 10 μm thick walls with 10 μm thick channels between them, and that a 10 T magnetic field would be applied. The feasibility of separating 20 nm particles of uranium from plutonium was examined. It is apparent from this, and from following publications, that the system would suffer from the very localized distribution in concentration close to the wires due to the rapidly increasing field gradient as the wires are approached. Subsequent papers by Ohara and coworkers [12–18] consider single channel systems, and refer to the technique as magnetic chromatography, even though no partition between phases is involved. Regularly spaced wires were assumed to be embedded within the two walls of the channel, each wire being matched by another directly across the channel thickness. In 2002, Mitsuhashi et al. [18] reported an experimental implementation. The channel was 45 cm long, 2.7 cm wide, and 190 μm thick. Wires were embedded in both channel walls and lay in the direction of fluid flow. They were of rectangular cross section, being 150 μm broad and 30 μm thick. They were embedded into the walls to a depth of 30 μm, so that they were flush with the wall surfaces, and spaced 150 μm apart. Rather than the wires of one wall being arranged exactly opposite the wires of the other, they were offset by 150 μm so that wires in each wall lay opposite spaces between wires in the other. An external field of 3 T was applied. They were able to show slight retention of some transition metal salts, although band spreading was extremely high.

In 1996, Nomizu et al. [19] introduced a technique they also referred to as magnetic chromatography that used a column packed with 0.7 μm carbon–steel beads and a periodically intermittent magnetic field. This technique has more in common with a form of FFF known as cyclical FFF [20,21] than chromatography. In cyclical FFF, a major part of the migration along the channel occurs when particles are driven away from the wall by a periodic reversal of the field direction. The particles therefore spend some time entrained in faster streamlines further from the flow boundaries. The distance migrated during a field reversal is proportional to the particle mobility, provided the opposite channel wall is not encountered. Higher mobility particles are therefore predicted to migrate into faster fluid regions and elute before those of lower mobility. (Giddings [20] showed that this is strictly true when the particle excursion is limited to 0.75 of the channel thickness.) Nomizu et al. [19] did not propose a mechanism for their technique, but we can provide the following explanation. When the field is applied, the rate of migration of particles to the packing surface would be proportional to their mobility. Those of higher mobility would be quickly captured and immobilized, while those of lower mobility would be carried further down the column before being captured. With interruption

of the field, the migration of particles from the packing surface would be due to the drag of the carrier fluid (aided by a continuous vibration applied to the column), rather than by the influence of the reversed field used in cyclical FFF. The migration away from the capture surface would be independent of mobility provided remanent magnetization of the packing can be ignored. According to this mechanism, the lower mobility particles would elute before those of higher mobility. Nomizu et al. [22] later presented an open tubular design where the separation tube was placed along the circular edge of an electromagnet pole piece. The mechanism of operation would correspond to that of the packed column system, except that the transport of particles away from the wall on removal of the field would depend on diffusion and hydrodynamic effects. They were able to baseline separate 0.7 μm hematite particles from 0.6 μm magnetite particles, with hematite eluting before magnetite as expected. An improved result was obtained by gradually increasing the zero field periods during elution.

The quadrupole magnetic FFF system being developed in our laboratory will now be described. In the region between the poles of a quadrupole magnet, the field strength $\boldsymbol{B}$ increases in magnitude linearly from the axis. The field gradient is therefore constant throughout this region. It follows that the radial force on a paramagnetic particle increases linearly with distance from the axis and is ideally independent of angle [23]. This is not a strong spatial variation of force. In asymmetrical flow FFF [24], the cross channel drag force increases from zero at the depletion wall to a maximum at the accumulation wall. Such a variation does not significantly affect the separation capability of asymmetrical flow FFF compared to the conventional, symmetrical variant. The relatively small variation of force across the thickness of a channel occupying a thin annular space, coaxial with the magnet assembly, can therefore be expected to be of little importance. Furthermore, particles that are magnetically saturated in the annular region occupied by the channel would experience a constant outward radial force, which is consistent with the ideal FFF model.

An early prototype channel was designed to occupy the full annulus between a polymer cylinder and an outer stainless steel tube. The channel ends were sealed and the tube held concentric to the cylinder with rubber o-rings. The carrier fluid and sample were introduced via several inlets around the circumference, and collected via several outlets feeding a single tube to the detector. Such a design has several drawbacks. Uniform carrier fluid flow demands a high degree of uniformity in annular thickness around the circumference and along the length. Any variation in annular thickness around the circumference would offer varying resistance to flow, and hence varying mean flow velocities along the length of the channel. Variation in annular thickness along channel length, due to outer tube or inner rod distortion or to non-uniform o-rings, could lead to carrier fluid velocity components around the circumference as fluid finds the path of least resistance. The multiple inlets and outlets would also be liable to partial or even complete blockage by bubbles or sample materials. All of these factors would lead to broadening of the range of elution time for each sample component, and hence a loss of separation efficiency. Most of these drawbacks could be avoided by restricting the channel to a fraction of the annulus. Multiple (say, up to four) channels could even be arranged in parallel, each with a single inlet and outlet. Since multiple channels would be unlikely to be used simultaneously, a better solution was conceived in which a single channel takes a helical path from a single inlet to a single outlet. This could be achieved by cutting the spiral channel into the surface of a polymer cylinder that fits tightly into a stainless steel tube. The tight fit eliminates the need for rubber o-rings and the problem of deviation from concentricity. The uniformity in channel thickness across its breadth and length is also ensured by the tight fit of the uncut surface of the cylinder within the tube. There are additional advantages to the helical channel design. Small non-uniformities in the magnitude of the magnetic field strength around the circumference are sampled equally by all sample particles as they migrate along the channel. In the case of an annular channel, these non-

uniformities would contribute to loss of separation efficiency. The helical channel may also be considerably longer than the quadrupole magnet, and hence any annular channel, and for fixed field strength and mean fluid velocity, separation efficiency is directly proportional to channel length.

The quadrupole electromagnet was constructed of 6.00 in (15.24 cm) wide, 1.00 in (2.54 cm) and 0.75 inch (1.91 cm) thick, 1018 low-carbon, cold rolled steel plate (Cumberland, Steel Division, Cleveland, OH), cut and machined at the Cleveland Clinic Foundation Prototype Laboratory. The cores for the electromagnet coils were 1.00 in (2.54 cm) thick, 6.00 in (15.24 cm) deep, and 6.76 in (17.16 cm) long, tapering inward at 15° on each side toward the pole tips for approximately 1 in. The pole tips were machined to the required hyperbolic function for the 1.60 cm diameter aperture. The square yoke, which provided the return path for magnetic flux, was made of the 0.75 in thick steel plate. The magnet cores were bolted to the yoke plates, which were in turn bolted to 1.00 in square steel supports at the corners. The coils of #18 AWG coated copper were wound by CWS Coil Winding Specialist (Santa Ana, CA). A cross sectional schematic of the electromagnet is shown in Fig. 1. The coils were found to have room temperature resistances of between 35.6 Ω and 35.8 Ω. A simulation of the magnetic field using Magneto 5.1 (Integrated Engineering Software, Winnipeg, Canada) predicted a maximum field strength at the pole tips of 0.818 T at a current of 1.25 A through each coil. A maximum field strength of 0.71 T was measured at 1.25 A using a Model 6010 Gauss/Tesla Meter (SYPRIS, Test & Measurement, F.W. Bell, Orlando, FL). Field strength was carefully measured during the elution of all samples at a position adjacent to the channel. A correction was applied to obtain the field strength inside the channel.

The coils were wired in parallel and the current provided by a Xantrex HPD60-5 Series Programmable Power Supply (Xantrex Technology Inc., Burnaby, British Columbia, Canada). This power supply was capable of supplying up to 5 A at up to 60 V, and was controlled by a computer using a GPIB interface (also from Xantrex Technology Inc.), and this allowed for the programmed decay of current (and hence field strength) during sample elution.

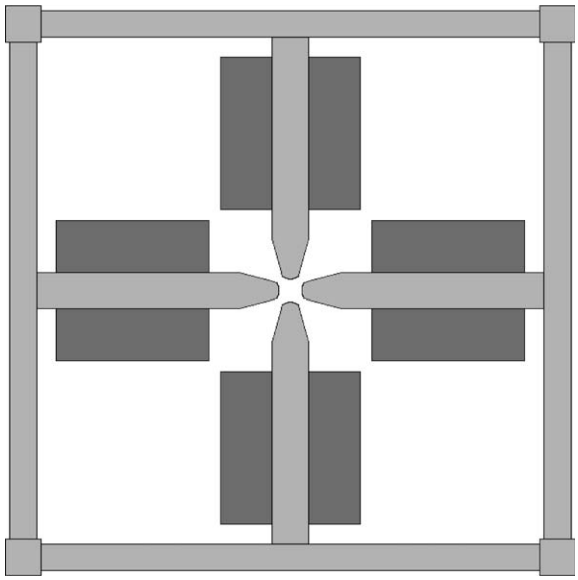

Fig. 1. Schematic of the quadrupole electromagnet cross section. The low-carbon steel pole pieces and yoke are shown in pale gray, and the copper coils in darker gray. The stainless steel tube that encloses the helical channel fits snugly between the four pole tips at the center of the system.

The channel was machined by the Cleveland Clinic Foundation Prototype Laboratory to a depth of 500 μm into the surface of a precision-turned Delrin™ (du Pont) cylinder of 0.586 in (1.488 cm) diameter and 6.20 in (15.75 cm) length. An internally polished, 6.10 in (15.49 cm) long, 304 stainless steel tube served as the accumulation wall. This had an outer diameter of 0.625 in (1.588 cm), and wall thickness of 0.020 in (0.051 cm). The channel was assembled by exploiting the difference in coefficient of thermal expansion of the two components with immersion in liquid nitrogen. An excellent channel seal was obtained on return to room temperature. The channel geometry is shown in Fig. 2. The channel made six complete spirals around the cylinder, had an overall length of 5.50 in (13.97 cm), with 5.00 in (12.70 cm) between inlet and outlet ports. The cutting tool had a diameter of 0.500 in (1.27 cm), resulting in a depletion wall breadth of 0.455 in (1.156 cm) perpendicular to the direction of flow.

Fig. 2. The helical channel geometry machined into the surface of the polymer cylinder. The original surface of the cylinder is shown in gray with the machined area white in order to highlight the spiral path of the channel. The inlet and outlet ports are shown as small circles at the ends of the channel.

The channel had an effective length of 31.4 cm, and a nominal volume of 1.90 ml.

The carrier fluid was driven by a Dionex GP40 Gradient Pump (Dionex Corporation, Sunnyvale, CA). The sample was introduced to the channel using a 7725i Rheodyne injection valve (Rheodyne, Cotati, CA) with a 20 μl injection loop, and a Model VUV-12 UV-detector (HyperQuan Inc., Colorado Springs, CO) was used for detecting sample elution. The detector output was fed to a PC using a 12-bit analog-to-digital converter (DI-154RS, Dataq Instruments, Akron, OH), and was displayed and saved to the PC using Windaq Lite software (Dataq Instruments).

Two samples of magnetic nanoparticles were kindly supplied by BD Biosciences Pharmingen. They had been procured from Skold Technology (lots 40–43 and 80, respectively) for preparation of their commercial line of IMag products. The particles are composed of dextran coated magnetite and have a nominal size range of $230\,\text{nm} \pm 150\,\text{nm}$. They were supplied in suspension in 10 mM MES (2-(N-morpholino)ethanesulphonic acid) buffer at pH 6.1, and were not conjugated with any antibodies or any other surface functional groups. They were supplied at nominal concentrations of 4.49 mg/ml (lot 40–43) and 4.72 mg/ml (lot 80). Phosphate buffered saline (PBS) at pH of 7.4 was used as the carrier fluid.

The samples were diluted with PBS carrier to $\frac{1}{7}$ of the supplied concentration. Sample volumes of 20 μl were introduced to the FFF channel at a sample loading flow rate of 0.10 ml/min for 2 min with an applied magnetic field of 56.5 mT at the accumulation wall, obtained at a total current of 0.30 A. (Magnetic field was measured at a point adjacent to the channel and a correction applied to obtain the field strength inside the channel, as explained earlier.) Stop flow relaxation was allowed to take place for 30 min. (This was to allow the particles to approach their steady state distributions close to the accumulation wall prior to elution.) The samples were eluted at a flow rate of 2.0 ml/min with the field strength held constant at 56.5 mT for 2.0 min, then decaying to 3.3 mT over a period of 7.0 min, after which it was held constant at 3.3 mT. The elution curves for the two samples are shown in Fig. 3. The field decay program used for the elutions is also included in the figure. This is shown by the dashed curve, which relates to the right hand scale. The two lots of nanoparticles are seen to be quite different. The elution times of the samples are expected to be functions of the magnetic content of the particles and to be independent of the quantity of dextran. It appears that since lot 80 elutes well before lot 40–43, it must contain significantly less magnetite than lot 40–43. The spike on the elution curve for lot 80, just beyond 20 min, indicates the time at which the channel was removed from the quadrupole magnet. The elution of the small amount of material following this shows that a small fraction of the sample does not elute at 3.3 mT, and this could indicate the presence of a small number of particles containing much more magnetite than

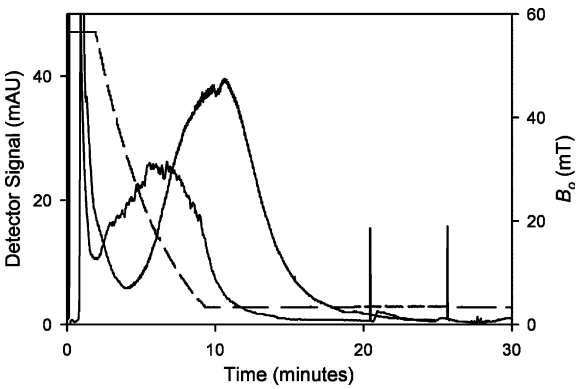

Fig. 3. Superimposed fractograms (detector response as a function of elution time) obtained for lots 40–43 and 80 of Skold magnetic nanoparticles. Lot 80 is less retained than lot 40–43 and the particles therefore contain less magnetite. The dashed curve shows the programmed field decay at the channel accumulation wall (right hand axis). The field strength is held constant at 56.5 mT for 2 min, and decays to 3.3 mT over a period of 7 min. Fluid flow rate was 2.0 ml/min for both samples.

others or some particle aggregates. The spike on the elution curve for lot 40–43 just beyond 25 min also indicates the point of removal of the channel from the magnet. In this case, very little material is seen to elute after this.

In conclusion, we have successfully demonstrated a novel approach to magnetic FFF that differs radically from earlier approaches. We have described the advantages that our approach has over earlier attempts. In short, our system possesses all of the qualities required for successful operation: (1) the channel is thin and of uniform thickness across its breadth; (2) the field strength and field gradient are relatively constant throughout the channel; (3) the field is sufficiently strong to retain many of the magnetic nanoparticles of interest; (4) the field strength may be adjusted to suit the magnetic properties of the sample, and may be programmed for the elution of broadly dispersed samples. The work described here is preliminary. There was no effort to optimize the flow rate and field decay program. This will be carried out in the future where there will also be the objective of extracting quantitative information regarding the magnetic properties of the particles.

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