

THE POTENTIAL OF QUADRUPOLE MAGNETIC FIELD-FLOW FRACTIONATION FOR DETERMINING PARTICLE MAGNETIZATION DISTRIBUTIONS

[P. Stephen Williams](#),¹ [Lee R. Moore](#),¹ [Jeffrey J. Chalmers](#),² & [Maciej Zborowski](#)¹

¹ Dept. of Biomedical Engineering, The Cleveland Clinic Foundation, Cleveland, OH 44195, USA

² Dept. of Chemical Engineering, Ohio State University, Columbus, OH 43210, USA

INTRODUCTION: In recent years, Zborowski and co-workers [1,2] have developed the flow-through quadrupole magnetic separator (QMS) for isolating or enriching specific biological cell types for the purposes of medical diagnostics or therapy. In this system the quadrupole magnet produces a radially symmetric magnetic field. The flow channel has a thin annular geometry and is mounted concentrically in the field. Using an annular inlet flow splitter, a cell sample suspension is introduced close to the inner channel wall and pure suspending fluid introduced at the outer wall. Particular cell types, mobilized by labeling with immunospecific magnetic nanoparticles, are driven toward the outer channel wall as they are carried along the channel by the overall flow of suspending fluid. The mobilized cells are divided from the remaining cells by an annular splitter at the channel outlet. The theory for the annular channel operation [3] was based upon that previously developed for the parallel-plate systems invented by Giddings [4,5].

We have made use of the experience gained in developing this magnetic cell sorting system to develop quadrupole magnetic FFF. Magnetic FFF may be used to characterize particulate magnetic materials, such as the immunospecific particles used for labeling cells for magnetic separation. The principles of quadrupole magnetic FFF are explained below, and preliminary experimental results are presented.

THEORY: Field-flow fractionation (FFF) is a particle separation technique that takes place in a flow of suspending fluid in a thin channel. A field is applied across the channel thickness, perpendicular to the direction of flow. The particles to be separated interact with the field to a greater or lesser extent and are driven into the slower moving fluid close to one of the walls. Due to back diffusion from the resultant region of higher concentration next to the wall, a steady-state concentration profile is established for each (monodisperse) fraction of the sample. The diffusion rate of submicron-sized particles allows a fast exchange of particles within the thickness of the steady state distribution. Generally, the

stronger the interaction of the particles with the field, the thinner will be the steady-state distribution, and the more time that particles spend in the slowest moving streams next to the wall. Therefore, with the imposition of flow, an elution of the sample takes place with the particles that interact weakly with the field being carried to the channel outlet first, followed by particles that interact more and more strongly with the field. A consideration of the fluid flow velocity profile and the theory yielding steady-state particle distribution within this profile allows the prediction of elution time for a given strength of particle-field interaction. Conversely, an elution profile may be converted to a distribution in the sample property that interacts with the field. In the case of magnetic FFF, this yields a distribution in particle magnetic moment.

The velocity profile in an annulus is given by

$$v(\rho) = 2 \langle v \rangle (1 - \rho^2 - A_2 \ln \rho) / A_1 \quad (1)$$

in which $\langle v \rangle$ is the mean fluid velocity, ρ is the ratio of radial distance from the axis to the radius r_o of the outer wall, A_1 and A_2 are functions of ρ_i , the ratio of inner wall radius r_i to r_o . These are given by

$$A_1 = 1 - \rho_i^2 + A_2 \quad (2)$$

$$A_2 = (1 - \rho_i^2) / \ln \rho_i \quad (3)$$

It may be shown that the steady-state concentration profile for magnetically saturated particles in the quadrupole field is given by

$$c(\rho) = c_o \exp\left(-\frac{1}{\lambda} \frac{(1-\rho)}{(1-\rho_i)}\right) \quad (4)$$

in which c_o is the concentration at the outer wall, and λ is the ratio of thermal energy kT to the work required to move a particle against the magnetic force from r_o to r_i . The value of λ is given by

$$\lambda = \frac{kT}{V_m M_s B_o} \frac{1}{(1-\rho_i)} \quad (5)$$

in which V_m is the volume of magnetic material in the particle, M_s is the saturation magnetization of the material, and B_o is the magnetic induction at the outer wall.

The fast exchange of particles by diffusion across the thickness of a steady-state distribution leads to equalized sampling of velocity streamlines, and the migration of a small sample plug of monodisperse particles as a coherent zone along the length of the channel. The ratio of the velocity of such a zone to the mean fluid velocity is known as the retention ratio. The zone velocity corresponds to a weighted average of the stream velocities over the annular cross section, with the weighting being proportional to local particle concentration. The retention ratio is therefore given by

$$R = \frac{v_p}{\langle v \rangle} = \frac{\langle cv \rangle}{\langle c \rangle \langle v \rangle} \quad (6)$$

where v_p is the zone velocity, and the angle-brackets defines a quantity averaged over the annular cross section:

$$\langle x \rangle = \frac{2\pi \int_{r_i}^{r_o} r x(r) dr}{2\pi \int_{r_i}^{r_o} r dr} = \frac{s \int_{\rho_i}^1 \rho x(\rho) d\rho}{(1 - \rho_i^2)} \quad (7)$$

Therefore,

$$R = \frac{\int_{\rho_i}^1 \rho c(\rho) v(\rho) d\rho}{\langle v \rangle \int_{\rho_i}^1 \rho c(\rho) d\rho} \quad (8)$$

The retention ratio is therefore a function of both λ and ρ_i . Fig. 1 shows the dependence of R on λ for ρ_i of 0.5 and 0.9.

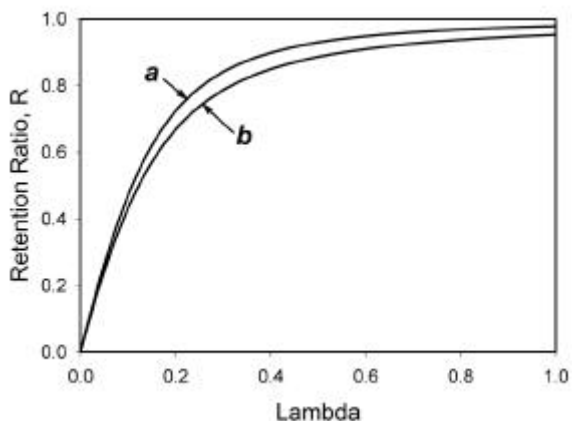


Fig. 1: Plots of Retention Ratio, R , versus λ for a) $\rho_i = 0.9$, and b) $\rho_i = 0.5$.

METHODS: A schematic of the quadrupole magnetic FFF channel and magnet is shown in Fig. 2. The sample and suspending fluid are delivered to the channel and collected at the channel outlet via a set of radial tubes at each end of the core rod. In this figure the channel thickness is exaggerated for clarity. It is, in fact, just 0.0508 cm in thickness, with a radius of 0.743 cm (so that $\rho_i = 0.932$), and length between inlet and outlet radial tubes of 14.6 cm.

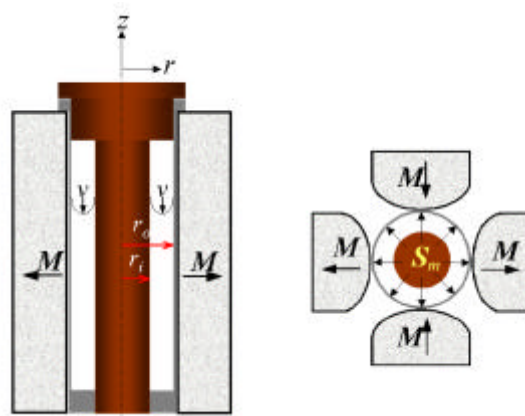


Fig. 2: Schematic diagram of the quadrupole magnetic FFF channel, shown in mid-axial section on the left and radial section on the right.

The quadrupole magnet was determined to exhibit a magnetic induction of 1.32 T at the inner wall of the channel cylinder.

A sample injection valve (Reodyne, Model 7725i) and syringe pump (Harvard Apparatus, Pump Model PhD2000) were used to introduce the sample and drive it through the channel. The pump was programmable, which allowed for the “stop-flow relaxation” of the sample once it was carried onto the channel. During this short interruption of flow, prior to the elution step, the sample particles attain their steady state distributions across the channel thickness. A HyperQuan VUV-10 HPLC detector (HyperQuan Inc., Colorado Springs, CO) was used to detect eluting particles.

RESULTS: A 33 μ L sample of unconjugated MACS beads (Miltenyi Biotec, Bergisch Gladbach, Germany) was introduced to the channel. Following a stop-flow time of 5 minutes, the sample was eluted at 1.0 mL/min. The sample was eluted in the presence of the magnetic field and with the channel removed from the magnet. The elution profiles (fractograms) are shown in

Fig. 3. The magnetic field is seen to cause the retardation of particle elution. This is consistent with the expectations for elution via the mechanism of magnetic FFF.

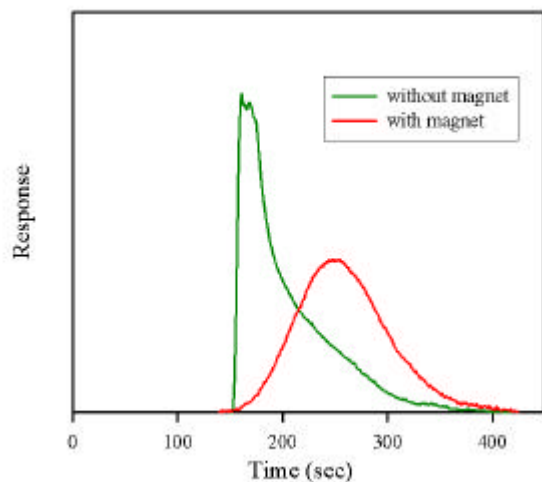


Fig. 3: Elution profiles (fractograms) for unconjugated MACS bead samples in the presence and absence of the quadrupole magnetic field.

DISCUSSION & CONCLUSIONS: MACS beads are expected to have a magnetic content approximately equivalent to a 10nm magnetite core (Miltenyi literature), although the polydispersity must be considered unknown. They are coated with dextran and have an overall diameter of about 50nm. (This overall size was confirmed in our laboratory by diffusion measurements.) If we assume a saturation magnetization of 480 kA/m for the magnetite, the expected mean λ would be approximately 0.18 for our system, corresponding to a retention ratio of about 0.6 (see Fig. 1). This is consistent with the moderate level of retention observed for the sample in the presence of the magnetic field. The moderate level of retention for this sample unfortunately does not allow us to extract any meaningful conclusions regarding their polydispersity of magnetic content.

The results, although preliminary, are an encouraging indication that quadrupole magnetic FFF will prove to be a powerful characterization tool for magnetic nanoparticles.

REFERENCES: ¹M. Zborowski, L.R. Moore, L. Sun, and J.J. Chalmers (1997) *Continuous-flow magnetic cell sorting using soluble immunomagnetic label* in *Scientific and Clinical Applications of Magnetic Carriers: An Overview* (eds. U. Häfeli, W. Schütt, J. Teller, M. Zborowski), Plenum Press, New York, pp. 247-260. ²M. Zborowski, P.S. Williams, L. Sun, L.R.

Moore, and J.J. Chalmers (1997) *Cylindrical SPLITT and quadrupole magnetic field in application to continuous-flow magnetic cell sorting*, *J. Liq. Chromatogr. & Rel Technol.* **20**:2887-2905. ³P.S. Williams, M. Zborowski, and J.J. Chalmers (1999) *Flow rate optimization for the quadrupole magnetic cell sorter*, *Anal. Chem.* **71**:3799-3807. ⁴J.C. Giddings (1992) *Optimization of transport-driven continuous SPLITT fractionation*, *Sep. Sci. Technol.* **27**:1489-1504. ⁵J.C. Giddings (1985) *A system based on split-flow lateral-transport thin (SPLITT) separation cells for rapid and continuous particle fractionation*, *Sep. Sci. Technol.* **20**:749-768.

ACKNOWLEDGEMENTS: This work was supported by NIH grant CA 62349 to M.Z. and by NSF Grant 0125657 to P.S.W.