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Magnetic Field-Flow Fractionation: Theoretical Basis

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Abstract

The application of high gradient magnetic fields to field-flow fractionation (FFF) is quite attractive based on the preliminary calculations presented here. The theoretical basis has defined the parameters of force on the individual particles and normal FFF parameters to evaluate the separation capability. The possible application of magnetic FFF to dynamic systems as well as a tool for separation of nominally paramagnetic and magnetically tagged molecules provides an impetus for further development.

INTRODUCTION

The need for separation methods for large molecules has led to the development of nonclassical separation techniques. The development of field-flow fractionation (FFF) has taken place over the last 15 years (*1–4*) and promises to be extremely useful in macromolecular separations. The theoretical and experimental descriptions of electric, thermal, gravitational, and chemical FFF demonstrate the generality of the technique.

As an extension of the scope of FFF techniques, the considerations for the application of a magnetic field are presented in this paper. Magnetic field-flow fractionation (MFFF) is compared to other FFF techniques. The concept of magnetic tagging of diamagnetic large molecules with paramagnetic ions for separation is presented and experimentally described for bovine serum albumin (BSA). This work presents a new facet of separation technology which promises to be extremely useful in not only separation but also characterization of metalloproteins.

THEORY

The concept of FFF has been presented elsewhere (*4*) and what is described here serves only as a basis of introduction to MFFF.

The FFF processes have been explained in terms of the field–solute interaction inducing a lateral drift velocity. This causes the solute molecules to accumulate near one wall of the laminar flow stream chamber. The accumulation essentially changes the flow streamline through which the solute moves. The streamlines near the wall are of lower velocity and, therefore, the stronger the interaction the more retardation is observed. This is shown diagramatically in Fig. 1.

PARTICLE-MAGNETIC FIELD INTERACTION

The interaction of a magnetic field gradient with a paramagnetic particle (or molecule) can be depicted as in Fig. 2. The assumption of a spherical geometry is only for mathematical convenience; different geometries would lead to more complex expressions than those below. Likewise, for simplicity the field gradient applied to a particle of magnetic susceptibility χ can be written in terms of a magnetic susceptibility experiment in a Guoy

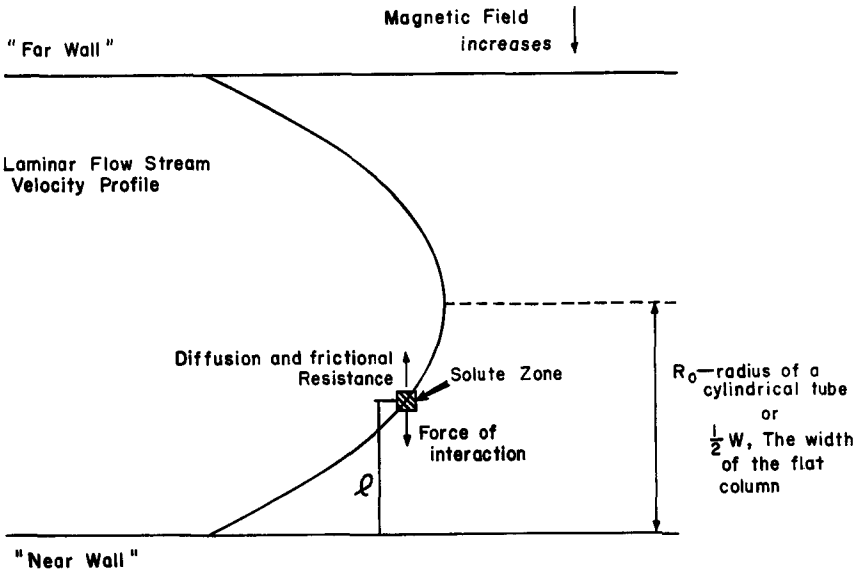

FIG. 1. A pictorial representation of the lateral shift in flow stream induced by the interaction of a magnetic field with a "plug" of paramagnetic solute.

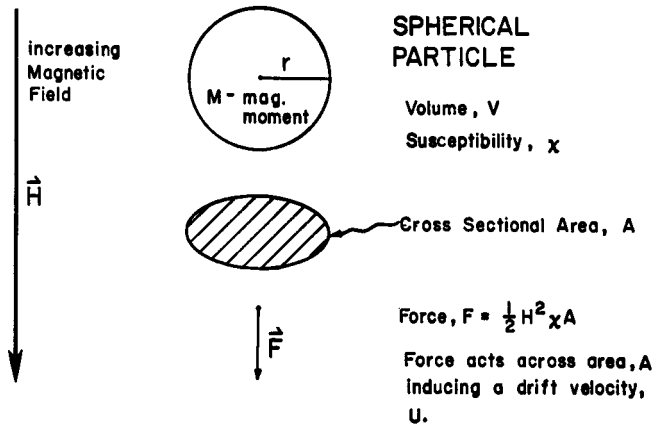

FIG. 2. Diagram of the driving force acting on a spherical particle with radius r, magnetic moment M, and magnetic susceptibility (per molecule) of χ through the cross-sectional area of the particle A. The force results in the drift velocity, U, in the direction of the "near" wall of the column.

balance (*5*, *6*). This description is in qualitative agreement with the preliminary experiments which follow.

The force per unit volume on the particle in the direction of increasing field is given by the product of the magnetization vector (I) times the magnetic field gradient ($\partial H/\partial x$):

$$F = I\,\partial H/\partial x = \chi H\,\partial H/\partial x \tag{1}$$

where I is in itself given by the product of the magnetic susceptibility times the magnetic field strength.

The force will then be given by

$$F\,dV = \chi H\,\partial H/\partial x\,dV \tag{2}$$

The work (w) done on the particle is the product of the force times the displacement:

$$w = dV \int F\,dx = dV \int \chi H\,\partial H/\partial x\,dx = dV\chi \int_{H_1}^{H_2} H\,dH$$

$$w = \frac{1}{2}\chi \Delta H^2\,dV \tag{3}$$

where $\Delta H^2 = H_2^2 - H_1^2$. Considering an element of volume dV being equal to the product of the area times the displacement, we have that

$$w = \frac{1}{2}\chi \Delta H^2 A \int dx = F \int dx \tag{4}$$

and the force is given by

$$F = \frac{1}{2}\chi \Delta H^2 A \tag{5}$$

However, the χ is susceptibility per unit volume on the molecular size. We can calculate χ from

$$\chi = \frac{\mu^2}{3kT}\Big/ V \tag{6}$$

when μ is in Bohr magnetons and V is the molecular volume in cubic centimeters. The molecular susceptibility for a spherical solute is (at 298°K)

$$\chi_{\text{molecular}} = 4.95 \times 10^{-4} \frac{\mu^2}{r^3} \tag{7}$$

where r is the molecular radius in Å.

The expression for the force on the particle by substitution is

$$F = \frac{1}{2}\frac{\mu^2 \Delta H^2 A}{3kTV} \tag{8}$$

$$= \frac{\mu^2 \Delta H^2}{6kT}\frac{A}{V} \tag{9}$$

$$= \frac{\mu^2 \Delta H^2}{8kTr}, \qquad \text{for spherical geometry} \tag{10}$$

For a spherical particle of radius 10 Å containing a Co^{2+} ion (3 spin system) and a 10,000 Gauss H drop, the force on the paramagnetic particle is

$$F = 4.46 \times 10^{-12} \text{ dyn} \tag{11}$$

Assumption of a diffusion coefficient (D) of 10^{-5} cm^2/sec yields the drift velocity (U) from the ratio of the force to the friction (f) of the solution (H_2O):

$$[U] = \frac{F}{f} = \frac{F \text{ dyn}}{kT/D} = 1.08 \times 10^{-3} \frac{\text{cm}}{\text{sec}} \tag{12}$$

If one assumes a column width (w) of 0.5 mm (as is typical), the following results are obtained for l, the distance from the near wall, and the ratio of l/w.

$$l = \frac{D}{[U]} = \frac{kT}{F} = \frac{6(kT)^2 V}{\mu^2 \Delta H^2 A} \tag{13}$$

or for a spherical particle

$$l = \frac{8r(kT)^2}{(\mu\Delta H)^2} = 0.09 \text{ mm for a } H \text{ drop of } 10{,}000 \text{ G}$$

$$l/w = 0.185$$

For the cylindrical or tubular column the retention parameter R may be calculated from (*1*)

$$R = \frac{8l}{w}\left[\coth\left(\frac{2l}{w}\right)^{-1} - 2l/w\right] \tag{14}$$

$$= 0.945 \text{ (for the example above)}$$

Resolution of different magnetic species can be written as

$$\text{Resolution } R_s = \frac{\Delta t_r}{1/2(W_1 + W_2)} \tag{15}$$

Since

$$R = t_0/t_r \tag{16}$$

$$1/R_1 = t_{r_1}/t_0$$

$$1/R_2 - 1/R_1 = \frac{(t_{r_2} - t_{r_1})}{t_0} \tag{17}$$

$$= \frac{\Delta t_r}{t_0} \tag{18}$$

$$\Delta t_r = \frac{t_0(R_1 - R_2)}{R_1R_2} \tag{19}$$

assuming a value of mt_0 = average peak width, then

$$R_s = \frac{\dfrac{t_0(R_1 - R_2)}{R_1R_2}}{mt_0} = \frac{(R_1 - R_2)}{mR_1R_2} = 1/m(1/R_2 - 1/R_1) \tag{20}$$

so the theoretical resolution of two species of different magnetic susceptibilities can be formulated for the cylindrical column (from 14 and 20). For an isothermal system, assuming the two molecules are of the same size, let

$$q = \frac{2kT}{wH^2A}; \qquad w = \frac{2kT}{qH^2A}$$

$$(l/w)_1 = q/\chi_1; \qquad (l/w)_2 = q/\chi_2 \tag{21}$$

In the limit $l/w \to 0$ and where the magnetic interaction is strong:

$$R_{\text{lim}} = 8\frac{l}{w} = 8\frac{q}{\chi} \tag{22}$$

$$\frac{1}{R_2} - \frac{1}{R_1} = \frac{1}{8q}(\chi_2 - \chi_1) \tag{23}$$

and

$$R_s = \frac{1}{m}(1/R_2 - 1/R_1) = \frac{1}{8mq}(\chi_2 - \chi_1) \tag{24}$$

This shows the separation can be performed based on the difference in magnetic susceptibility and/or diffusivity.

EXPERIMENTAL RESULTS

In order to test the proposed separation technique, a magnetic tagging experiment was performed. The apparatus is described in Fig. 3. The experiment involved the injection of a solution of bovine serum albumin, BSA, (9.9×10^{-5} M) and observation of the retention time in the presence and absence of the magnetic field. Nickel nitrate was added to the solution and the final concentrations were 5.0×10^{-5} M of both the BSA and the Ni^{2+}. The metal–BSA mixture was thus injected and eluted in the presence and absence of the field. The results are given in Table 1. Our l value for the Ni–BSA obtained from using Eq. (14) and a w value of 0.15 cm came out to be 0.027 cm. For a retention value of 1 we obtained an l value of 0.038 cm.

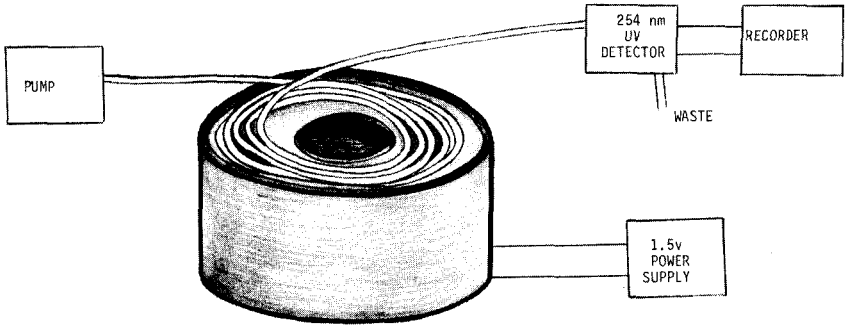

FIG. 3. Schematic diagram of MFFF apparatus, including Altex 110A pump, UV detector (254 nm excitation wavelength), Cemco magnet (400 Gauss, as measured by using a Bell Inc. Model 640 Gaussmeter), and the Teflon coiled column (5.6 cm diameter of the coil). Typical column parameters: flow rate, 0.1 mL/min; length, 304 cm; volume, 4.6 mL; i.d., 0.15 cm.

TABLE 1

Magnetic Tagging of Bovine Serum Albumin by Nickel Ions and Magnetic FFF Analysis[a]

Sample	$[Ni^{2+}](M)$	Magnetic field (G)	Retention time (min)
BSA	0	0	24.0
BSA	0	400	24.0
Ni + BSA	5.0×10^{-5}	0	24.0
Ni + BSA	5.0×10^{-5}	400	25.5

[a]Column: Teflon coiled column (5.6 cm diameter of the coil); length, 304 cm; volume, 4.6 mL; inner diameter, 0.15 cm. Mobile phase: Degassed deionized water; flow rate 0.2 cc/min. Magnetic field: 400 G, measured by using a Bell Inc. Model 640 Gaussmeter. Detector: Standard HPLC ultraviolet detector (254 nm excitation wavelength) with 8 μL cell volume and 20 μL injection port volume.

DISCUSSION

The high gradient magnetic field holds promise for MFFF. This is not surprising in light of the high magnetic field gradient "filtering" system which has been applied to removal of paramagnetic particulate from stack emissions and to removal of red blood cells from whole blood (*7*, *8*). The efficiency of both examples is quite remarkable. Examples of experimental MFFF systems should take advantage of the available high gradient high magnetic field technology.

Aside from the obvious purification and separation of paramagnetic metal-containing species, the possible utility of this technique as a characterization tool should be mentioned. There are many biochemical problems which involve the observation of a paramagnetic species during the course of an enzymatic reaction while the nonfunctioning enzyme is diamagnetic. The application of MFFF would complement ESR and magnetic susceptibility measurements because of the dynamic nature of the process. The fraction containing paramagnetic centers is removed from the nonreacting (and therefore diamagnetic) counterpart.

Another possible application is in the "tagging" of diamagnetic large molecules with a paramagnetic center (formation of a metal complex, for example) which can be removed after separation. This type of process is not found in other FFF techniques, and serves to make MFFF unique.

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