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DEVELOPMENT OF H.G.M.S. TECHNIQUES FOR BIOLOGICAL CELL SEPARATION

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Abstract

High Gradient Magnetic Separation (HGMS) for biological specimens is reviewed. Selective tagging for the enhancement of cell capture is discussed. A detailed survey of major improvements in HGMS techniques -- filter matrix designs and magnet designs aimed specifically at bench-top experiments in bioseparation -- is also given.

INTRODUCTION

The earliest recorded work on magnetic separation of red blood

cells from whole blood (which had been carried out with limited success) involved the ingesting of iron granules by the cell[1-2]. The first successful direct capture of red blood cells from whole blood by high gradient magnetic separation (HGMS) was reported in 1975 by Melville et al[3]. The upshot of this work was a revival of interest in biological cell separation by magnetic techniques and to increase research on the technical details of the separation process.

In HGMS a suspension containing the specimen to be captured is passed through a filter (usually made of stainless steel wire) situated in a magnet -- see Figure 1. An applied magnetic field magnetizes the filter which then retains the desired specimen. The magnetic poles induced on the surface of the filter fibers, which are radially magnetized, form the basis of the magnetic dipolar traction force. The desired specimen is then washed off the filter when the applied magnetic field is removed.

For micron-size biological specimens, magnetic capture is based on the interaction between the magnetic force and the viscous drag force acting on the specimen:

Magnetic Force: $(\chi_s - \chi_o)VH(r)\nabla H(r)$

Viscous Drag Force: $6\pi\eta bv(r)$

where χ_s and χ_o are magnetic susceptibility per unit volume of the specimen and solution, respectively; V is the volume of the specimen of radius b; H(r) is the field strength at a location described by position vector $\underline{r}$ from the filter wire; η is the viscosity of the solution; and v(r) the velocity of the specimen.

Following a model developed by Watson[4], in which it is assumed that the wires in the filter are perpendicular to the applied field (those portions of filter wire parallel to the magnetic field create no field gradient), a magnetic velocity v_m is defined by equating the magnetic and viscous drag forces:

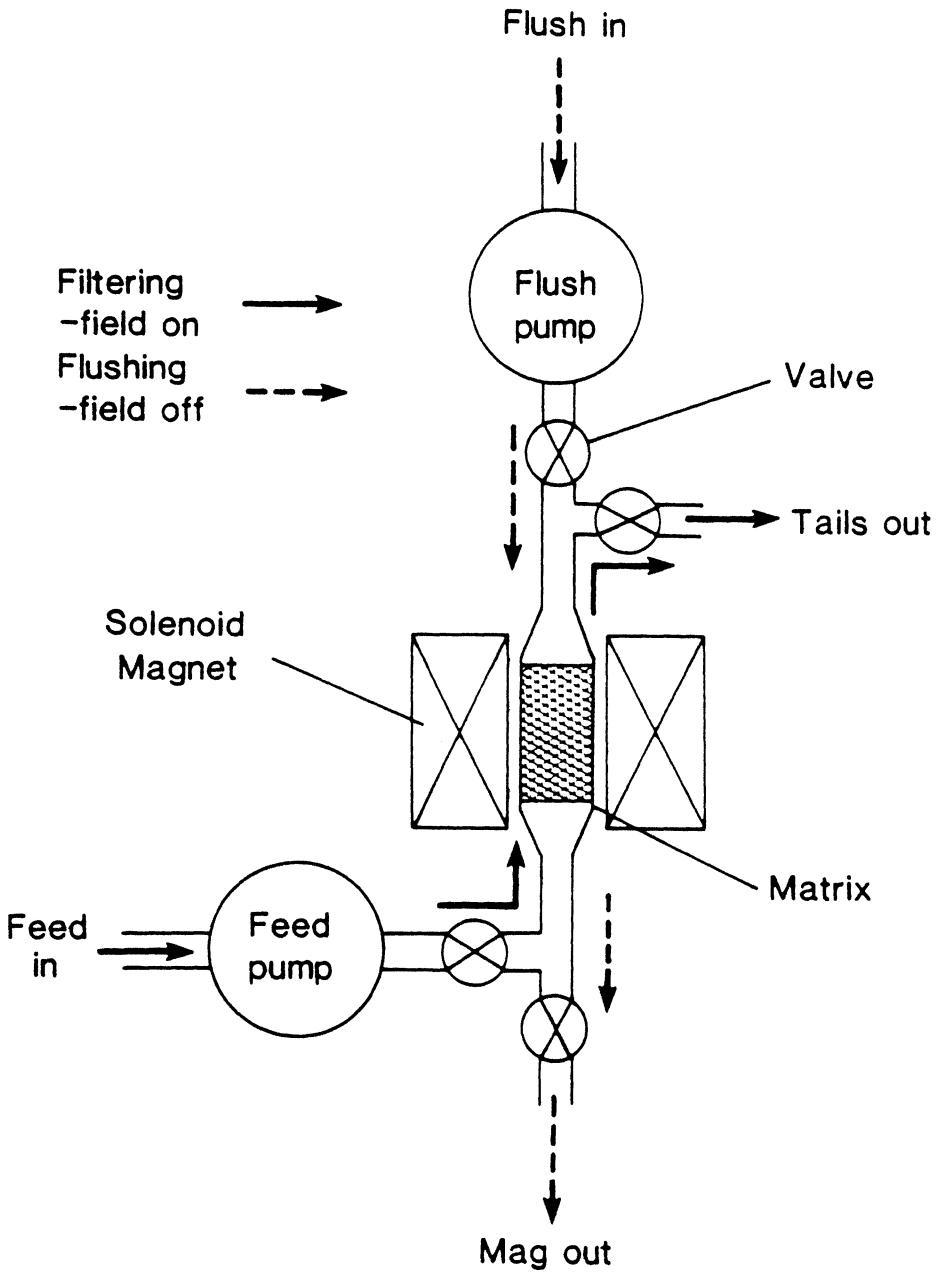


FIGURE 1 Schematic of equipment for HGMS.
(Courtesy of C.O. Too)

$$v_m = \frac{2\mu_o \chi M_s H_o b^2}{9\eta a}$$

where μ_o is the magnetic permeability of vacuum, M_s is the saturation magnetization (A/m) of the filter wire of radius a, and H_o the external magnetic field (A/m). As v_m is increased in relations to the fluid flow velocity v_o, magnetic capture of the specimen is enhanced. As can be seen from the above equation for v_m, capture efficiency depends on the magnetic properties and size of specimen, the size and arrangement of the filter, the magnetic field strength, flow rate, and flow direction relative to the direction of the applied magnetic field -- see section "Development of Filters for Magnetic Bioseparation" and equations (2) through (5) on the effects of filter arrangement and size, flow rate, and flow direction relative to field, on capture efficiency. (For larger specimens, diameter greater than (say) 30 microns, inertia and buoyancy forces have to be considered; for sub-micron size specimen, inter-particle forces must be considered.)

To enhance magnetic capture, Melville et al[3] used sodium dithionite to completely reduce red blood cells to the deoxygenated state, giving them a volume susceptibility of 3.88×10^{-6} (SI). This minute paramagnetism can be accounted for by a few iron atoms in hemoglobin being converted to the Fe^{3+} state, with a magnetic moment of 5.35 Bohr magnetons. The results indicated that the filter quickly saturates. Furthermore, the authors reported the significance of using "drawn" stainless steel wire instead of steel wool for the filter: Almost all of the red blood cells that were captured using steel wool were found to be ruptured when they were washed off the filter; with "drawn" wires, only a small portion of the cells showed slight indentations on their cell wall -- see also section on fliter development.

Another paramagnetic form of hemoglobin is methemoglobin,

which is obtained by oxidizing hemoglobin and has a magnetic moment of 5.80 and 4.47 Bohr magnetons for a pH less than 7 and a pH greater than 9.5, respectively[5]. HGMS for red blood cells from whole blood was attempted in the met-form by Owen[6] and Graham[7].

A significant development in experimental technique on magnetic separation during this period involved single wire HGMS study[8-9], later adapted to using a video camera to record the trajectories of magnetic particles[10], and then applied to red blood cells[11]. This provides an accurate and sensitive method to determine the magnetic properties of weakly paramagnetic biological specimens[11] and to perform sickle cell magnetic separation[12].

MAGNETIC LABELS

As mentioned above, efficiency of specimen capture depends on its magnetic properties. Therefore, HGMS can be extended to biological specimens which are non-magnetic by attaching them to a magnetic label. Owen et al[13-15] used the paramagnetism of methemoglobin and deoxyhemoglobin to label and separate leukocytes, white cells and other cellular classes. Paul et al[16] successfully isolated red blood cells infected with malarial parasites from oxygenated whole blood. This particular separation was achieved on account of the fact that, during its stay within the red blood cells, the malaria parasite (Plasmodium falciparum) digests hemoglobin and leaves behind a residue of oxidized haem product. These oxidized malarial cells are, in fact, slightly more paramagnetic (5.8 μ_B per cell) than deoxygenated red blood cells, and can be successfully separated from normal cells in oxygenated blood which are diamagnetic.

Magnetite (Fe_3O_4) has been successfully used to label and then removed bacteria [17] and viruses[18] in water pollution control, to enhance the capture of red blood cells[19-21], to remove planktons from Red Tide[22], and tumor cells[23] and B-lymphoma cells[24]

from human bone marrow. The biological specimen to be separated is usually attached to the Fe_3O_4 particles via a double layer of antibody -- see Figure 2.

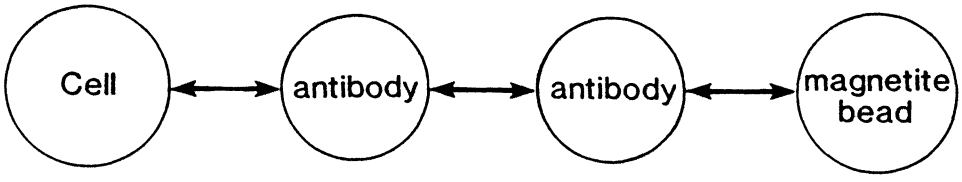


FIGURE 2 The biological specimen is attached to the magnetite bead via a double layer of antibody, Ref. 23 (or a cation, Ref. 18).

Erbium and dysprosium ions have magnetic moments nearly twice those of paramagnetic hemoglobin -- 9.5 and 10 μ_B for Er^{3+} and Dy^{3+}, respectively, compared with 5.8 and 5.35 μ_B for methemoglobin and deoxyhemoglobin. Also, these ions bind favorably to many biological specimens, sometimes simply by immersion in a solution of the chloride or nitrate. Furthermore, DNA has a high affinity for lanthanide ions, and living cells cannot normally be attached to these ions while dead cells readily do[25]. Enhancement of HGMS for cellulose[26] and red blood cells[27-29] by labeling with these lanthanide ions had been reported, as well as the separation of particles of bone and cartilage from human synovial fluid[30].

Ferritin, a iron-containing protein, was used by Owen to demonstrate HGMS of red blood cells at weaker magnetic fields of 0.3 to 0.45 Tesla[31]. Ferritin was also used to successfully separate Legionelle from other water bacteria[32].

Instead of using paramagnetic particles to attach to non-magnetic biological specimens in HGMS, microorganism Candida Utilis and Bacillus Subtilis have also been used successfully to

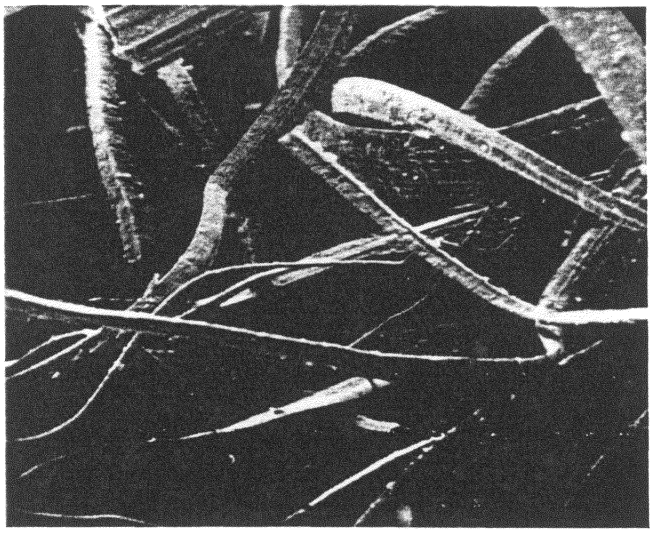
capture dissolved paramagnetic uranyl ions[33]. This process is practically useful in the removal of toxic metals from waste waters and radionuclides from solutions generated by the nuclear industry. Extremely low residual concentrations are obtainable.

To facilitate the application of magnetic separation for biological specimens, the magnetic properties of some biological specimens and the methods of determining these properties, and examples of magnetic labeling for HGMS are given in Tables 1 and 2, respectively, at the end of this paper.

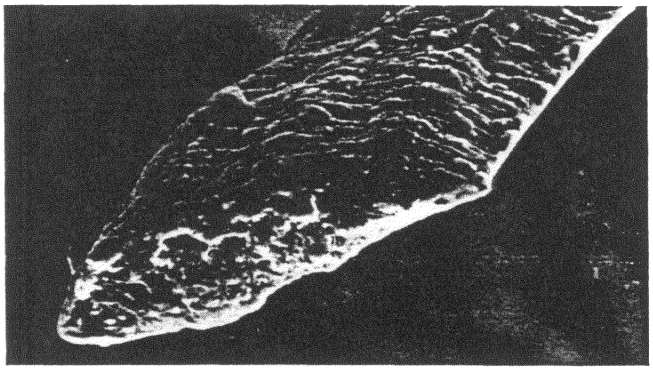
DEVELOPMENT OF FILTERS FOR MAGNETIC BIOSEPARATION

In its most primitive form, the filter matrix comprises a random assembly of filamentary stainless ferrite steel wool (Figure 3a) with lateral spatial fiber dimensions typically in the range of 50 to 100 microns. The disadvantages of this type of filter medium are obvious, with cell lysis being a highly probable occurence on the sharp serrated edges of the wool -- see Figure 3b. A distinct improvement on this is a matrix comprising a random assembly of lengths of continuous finely-drawn (cylindrical cross-section) ferrite wire -- see Figure 4. Fibrous material of this sort, with relatively weak ferromagnetic properties (largely due to precipitated martensite in the die-drawing process), is commercially available at fiber diameters as low as 8 microns. With fibers of this fineness there are two principle problems. The first of these is maintaining the physical integrity of the matrix because these fine fibers easily disintegrate. A second problem is the difficulty of washing entrapped cells from the matrix. Moreover, after washing, the matrix may not be reusable because of physical disintergration.

For a continuous fiber of a given size (with a minimum diameter of about 25 microns) significant enhancement of filter



(a)



(b)

FIGURE 3 Stainless steel wool for filter matrix. (a) Mag. 55x; (b) Mag. 600x. (Courtesy of Mr. G. Gaskin, Uni. of Salford, U.K.)

performance beyond that of the random assembly described above may be obtained by distributing the fibers as an ordered array. Ordered, here, means the fiber axis being orthogonal to (1) the external background field and (2) the fluid flow direction. The subsequent enhancement in performance is attributable to the fact that virtually all of the fibers of the ordered assembly are radially magnetized. These magnetic poles induced on the surface of the fiber magnetized radially form the basis of the magnetic dipolar traction force. In the random assembly, in rough terms, approximately only two-thirds of the total fiber length of the matrix is orthogonal to the external field. The performance is correspondingly reduced. (In fact, a detailed analysis shows magnetic depolarization effects from skew alignment of the fibers to cause even more serious limitation than anticipated by the simple reasoning given here[65].)

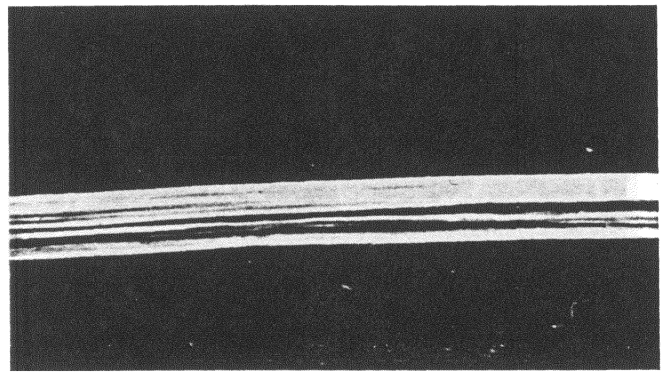


FIGURE 4 "Drawn" stainless steel fiber for matrix; mag. 1100x. (Courtesy of Mr. G. Gaskin, Uni. of Salford, U.K.)

The ordered-matrix filter is much easier to evaluate than its random fiber counterpart. In its simplest form it may comprise (say) N stacks of (say) woven or knitted (see Figure 5)

continuous cylindrically-shaped ferrite wire or even expanded metal screens. The latter are of less interest in bioseparation, once again because of potential cell wall damage on serrated edges. If these N layers contain a total wire length ℓ, and if the capture cross-sectional area per unit length of fiber is α, then for a filter of total cross-sectional area A, the capture efficiency, R_o, is[66]

$$R_o = 1 - [1 - 2\alpha\ell/A]^{N/2} \quad (1)$$

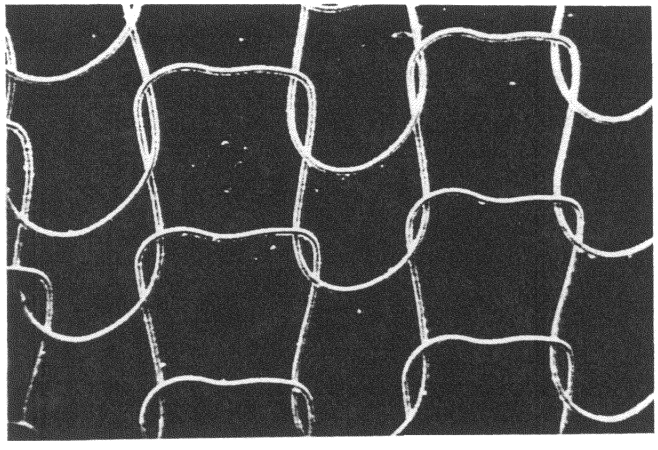


FIGURE 5 Knitted stainless steel ordered matrix; Mag. 20x. (Courtesy of Mr. G. Gaskin, Uni. of Salford, U.K.)

Empirical formulae have been devised by Wong[67] and Cowen et al[9] for α for both longitudinal (field parallel to flow) and transverse (field orthogonal to flow) configuration, respectively. These formulae are best expressed in terms of normalized capture radius, r_{ca}[68], with $\alpha = 2ar_{ca}$, where a is the fiber radius. They

are:

$$r_{ca} = |v_m/v_o|^{3/8} + \frac{K}{5}|v_m/v_o|^{2/5} \text{ (longitudinal)} \quad (2)$$

and $$r_{ca} = 1.2|v_m/v_o|^{3/8} - \frac{(1-K)}{5}|v_m/v_o|^{1/2} \text{(transverse)} \quad (3)$$

where v_o is the background fluid flow velocity, v_m is the magnetic velocity, and $K = M_s/2H_o$.

The above formulations are most appropriate where the separation process is designed with $v_m/v_o > 1$. For much smaller values of the ratio (say, <0.1), a much simpler formula

$$r_{ca} = 0.5(v_m/v_o) \quad (4)$$

can be derived analytically for the longitudinal case.

The disadvantages of woven or knitted ordered matrix filter are physical entrapment of the specimens during the wash-off phase at those locations where fiber overlap, and blind spots for magnetic capture created by fibers of an adjacent upstream layer.

The ultimate ordered matrix, an orthonomic matrix, is produced when the above-mentioned N layers are deployed coherently rather than randomly with respect to one another[69]. To express it another way, the N layers of the filter may be constructed as N/q groups of fiber sheet with the q layers of any of the groups arranged ideally with respect to the other layers of the group. A simple example of this principle is demonstrated in Figure 6 in which each layer is an array of regularly-spaced identical fibers each of radius a. In the case where q=2 (Figure 6a), the second layer is positioned geometrically to lie with its fibers parallel to those of the first layer but halfway between those of the first layer. In Figure 6b, a similar deployment is shown for q=3, but with, this time, the lateral stagger of the layers being one-

third of the inter-fiber spacing of the first layer.

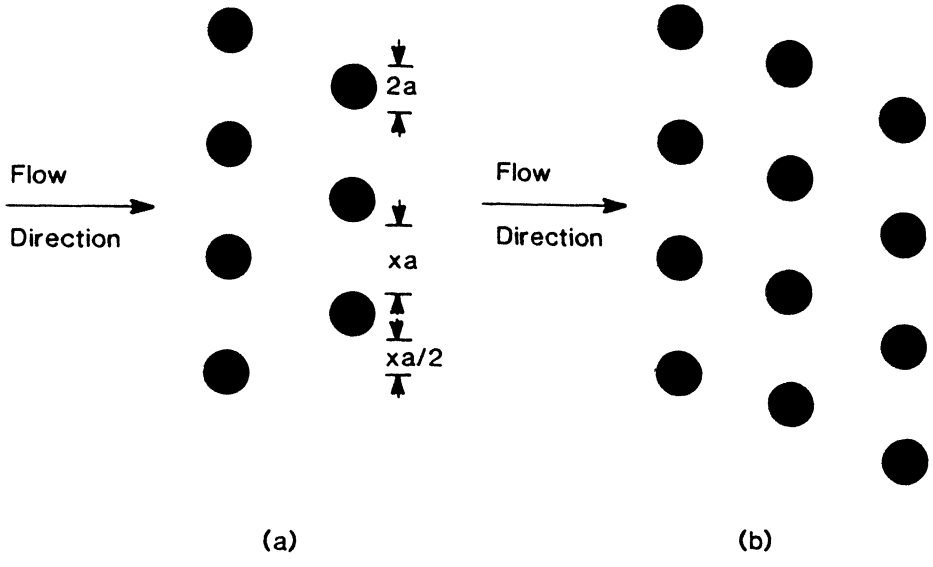


FIGURE 6 Orthonomic matrix with (a) q=2; (b) q=3.

In this type of filter arrangement, the filter capture efficiency is upgraded from the value given in equation (1) to

$$R_q = 1 - [1 - (q\alpha\ell/A)]^{N/q} \qquad (5)$$

Equation (5) can be re-expressed in terms of the inter-fiber spacing, xa, (see figure 6a) as

$$R_q = 1 - [1 - 2qr_{ca}/(x+2)]^{N/q} \qquad (6)$$

DESIGN OF AN ORTHONOMIC MATRIX FOR BIOSEPARATION

Any fundamental design exercise is, to some extent, arbitrary, and more than one solution is always possible. This notwithstanding we can proceed with an orthonomic matrix design for bioseparation along the following objective lines:

First we ask the question -- What is the mean radius, b, and magnetic volume susceptibility, χ, of the cell requiring magnetic entrapment? Next, we ask the question -- What capture efficiency (R_q) is required for the separation process? Then, for practical reasons we are forced to ask -- What is the length, L, of the magnetic filter? (The practical answer here is that the filter should be marginally smaller than the extent of the external magnetic field, see Figure 7.) The next design question is how thick should the fiber be? (The answer here, ideally, is a fiber radius 2.33 times the particle radius (i.e., 2.33b) for cells of positive susceptibility[68].) If this is of an impractical size, i.e., leading to a design value of fiber diameter less than 20 microns, then a choice in the range 5b to 10b can be made with little sacrifice in performance.

Next, we should ask the question -- How far apart ought the fibers to be? (In other words, what is x in equation (6)?) There are two answers to this question. First, if $|v_m/v_o| < 0.1$, then using equations (4) and (6), we get

$$x \simeq \frac{q(v_m/v_o)}{\{1-[1-R_q]^{q/N}\}} - 2 \qquad (7)$$

where we have already determined everything in equation (7), except q(our one arbitrary decision). Alternatively, if $v_m/v_o > 1$, we can use equations (2) and (6) to arrive at a very simple expression for x

$$x \simeq 2[q(v_m/v_o)^{3/8} - 1] \quad (8)$$

MAGNET SYSTEM FOR MAGNETIC BIOSEPARATION

These come in many shapes and sizes, but, for magnetic biofiltration, can be reduced to three main classes. By far the most common is the free-standing or bench-top electromagnet with the matrix canister wedged between the jaws of the pole pieces (see Figure 7). The disadvantage of using electromagnets for magnetic separation is the remanence field which exists when the power supply for the electromagnet is turned off, thereby inhibiting specimens wash-off.

A second group is the superconducting solenoid in which bench-top versions can be obtained commercially with axial fields ranging up to around 8 Tesla. These may be either cooled by liquid helium reservoirs, or, if user convenience is a prime concern, by refrigeration system. Superconducting magnets for bioseparation require an exceedingly long time period in the start-up and wash-off phases, and there is a high risk of quenching the magnet when power for the refrigeration system fails.

A third popular group of devices are permanent magnets, the commonest form of which is the C-magnet -- see, for example, Figure 1 of Ref. 16. Permanent magnets have the enormous advantage of being relatively low-cost (no power supply capital costs), robust and simple to use. Against that, the matrix cannot be rinsed in zero field without physical removal from the working field volume. This maneuver is not always a straightforward one.

Recent developments in high-tech permanent magnet materials have led to the development of various novel permanent magnet designs which deviate markedly from the conventional C-shaped

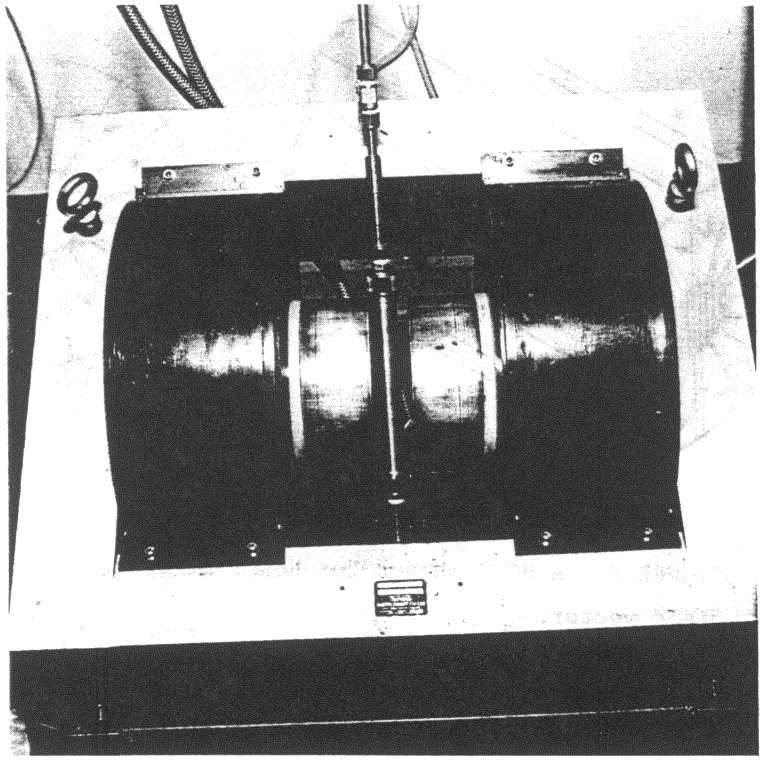


FIGURE 7 Electromagnet system for HGMS. Length L of filter matrix is slightly less than the dimension of the pole pieces. (Courtesy of Dr. T.J. Sheerer.)

princple. Among these is the hollow cylindrical flux source (HCFS) magnet recently considered by one of the authors[70] for bench-top high gradient magnetic filtration. Here, (see Figure 8) we have a multi-sectored solenoid geometry, the consequence of which is to produce, in the cylindrical working field volume, a uniform field whose field lines are orthogonal to the axis of the

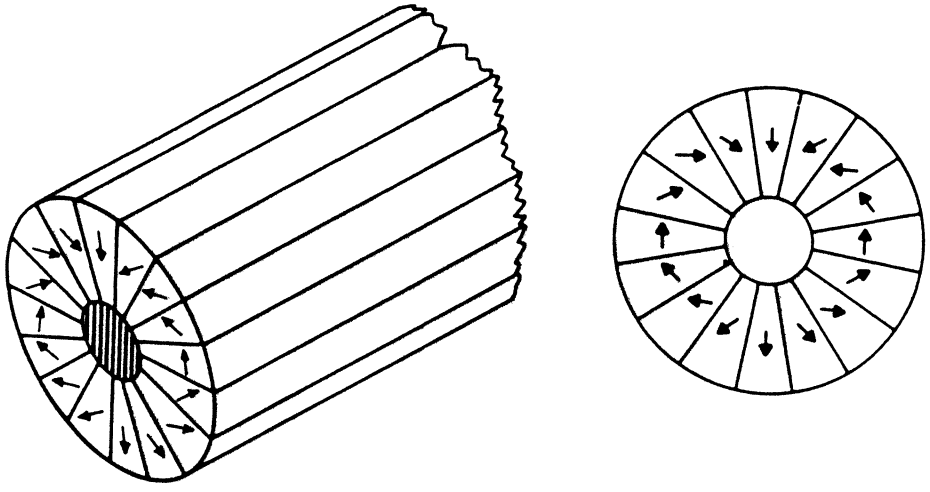


FIGURE 8 A HCFS magnet for HGMS. Arrow represents magnetic field vector.

system. As such, an orthonomic matrix of the type shown schematically in Figure 6 may be placed in the field volume with its fiber axis orthogonal to the field line. This arrangement will now capture particles in the transverse mode. If we wish to wash entrapped cells from the matrix, we simply rotate the matrix, coherently, through an angle of $\pm \pi/2$. The matrix fibers are now aligned with the field lines and, thereby, lose their dipolar attraction for the previously entrapped cells. The cells can then be removed from the system by backwashing. The filter can then be primed for a further sequence of operation simply by a further $\pi/2$ rotation of the matrix.

Table 1. Magnetic Properties of some Biological Specimens

Specimen		Magnetic Moment-Magnetization	Susceptibility	Ref.
Hemoglobin:				
Ferrohemoglobin			$\chi_V=1.08\times10^{-5}$ (SI)	34
			$=0.84\times10^{-5}$ (SI)	35
Deoxyhemoglobin		5.35	$\chi_M=0.15\times10^{-6}$ (SI)	34
		5.45	$\chi_M=0.16\times10^{-6}$ (SI) (a)	36
			$\chi_V=0.46\times10^{-6}$ (SI) (b)	37
Methemoglobin	pH<7	5.58	$\chi_M=0.18\times10^{-6}$ (SI)	5
	pH>9.5	4.47	0.10×10^{-6} (SI)	
			$\chi_V=0.54\times10^{-6}$ (SI) (b)	37
Methemoglobin Hydroxide		4.66		38
Oxyhemoglobin			$\chi_V=-0.75\times10^{-6}$(SI) (b)	37
Carbonmonoxyhemoglobin			$\chi_V=-0.58\times10^{-6}$(SI)	39
Cyanomethemoglobin			$\chi_V=0.31\times10^{-6}$ (SI) (c)	40
Red Blood Cell (size: r = 7.5 microns)				
Deoxygenated RBC			$\chi_V=3.00\times10^{-6}$ (SI) (b)	11
			3.90×10^{-6} (SI)	3
Plasma			$\chi_V=-7.7\times10^{-6}$ (SI) (b)	11
RBC in a reducing agent (isotonic sodium dithionite)			$\chi_V=3.50\times10^{-6}$ (SI) (b)	11
White Cell			$\chi_V=3.45\times10^{-6}$ (SI) (b)	11
Magnetotactic Bacteria				
Spirillum (0.25 micron wide, 1.75 microns long)		$M=1.3\times10^{-12}$(emu) (d)		41
		$M=0.9$ G-cm^3/gm (e)		42
		$M=3.6\times10^{-13}$(emu) (f)		43
Magnetosome, extracted from spirillum		M=13 G-cm^3/gm (e)		42

Table 1. (cont.)

Specimen	Magnetic Moment-	Susceptibility	Ref.
Magnetotactic Algae Anisonemer genus	$M=7.0x10^{-10}$ (emu) (per cell)		44
S. maxima, (20 microns long, 12 microns wide, and 4 microns thick) oxidized ferredoxin		$\chi_M=12x10^{-4}$ (cgs) (a)	45
reduced ferredoxin		$=36x10^{-4}$ (cgs) (a)	45
Escherichia Coli (1.5 microns long, 0.6 micron wide)		$\chi_V=1.0x10^{-6}$ (SI) (a)	46
Protein Metmyoglobin	5.95	$\chi_M=0.19x10^{-6}$ (SI) (a)	47
Carbonic Anhydrase, with substituted cobalt	4.40 (g)		48
Ferrimyoglobin Hydrate *	5.89	$\chi_M=15x10^{-3}$ (cgs) (h)	49
Ferrimyoglobin Cyanate *	4.17	$\chi_M= 2x10^{-3}$ (cgs) (h)	49
Ferrimyoglobin Azide (crystal) *	2.20	$\chi_M=5.5x10^{-3}$ (cgs) (h)	49
(solution) *	2.17	$\chi_M=4.1x10^{-3}$ (cgs) (h)	49
Ferrimyoglobin Imidazole *	2.31	$\chi_M=4.2x10^{-3}$ (cgs) (h)	49
Ferritin		$\chi_V=40x10^{-6}$ (SI) (i)	50
Laequer Tree Laccase, Rhusvernicifera		$\chi_M=2.6x10^{-3}$ (cgs) (a)	51
Fugal Laccase, Polyporus Versicolor		$\chi_M=2.8x10^{-3}$ (cgs) (a)	51
Human Ceruloplasmin		$\chi_M=7.38x10^{-3}$ (cgs) (a)	51

a) Faraday balance technqiue.

b) calculated from motion of cell in an inhomogeneous magnetic field around a single wire situated in an external magnet.

c) calculated from probability of capture with wire in HGMS matrix.

Table 1. (cont.)

d) Mossbauer technique.

e) Bulk magnetization measurement.

f) Light scattering technique.

g) magnetometer measurement.

h) magnetic torsion balance technique.

i) magnetic ferrography [52].

* Values at -10C.

Table 2. Magnetic Labels for some Biological Specimens

Labeling Agent	Specimen Labelled	Ref.
Fe_3O_4-containing polystyrene microspheres	Escherichia Coli, Bacteriophage T_6	18
	tumor cell from bone marrow	23
	B-lymphoma from bone marrow	24
	Caliform bacteria	17
	enzymes	53
	Red Tide, Chattonella type	22
	Cells based on Ganglisosides	54
	Leukocytes and other cellular	20,55 56,57
	Human RBC and lymphoid cells	21
Ferromagnetic Iron-dextran	Cells	20
	antigens in RBC and thymocytes antibody	19
Europium Chelates	Blood	26
	Bacteria Klebsiella pneumoniae	58
Europium and Dysprosium Chelates	Blood	27,28 29

Table 2. (cont.)

Labeling Agent	Specimen Labelled	Ref.
	biological cells, and bones and cartilage	59
Manganese Chloride	Blood	60,61
Synthetic Ferritin Particles	Legionelle from other water bacteria	32
Ferritin	Blood	62,63
Rosette Methemoglobin	Leukocytes and other cellular classes	14
Hemoglobin	Leukocytes, white cells	13,14 15
	Malaria parasite	16
Carbonyl Iron	Phagocytic Cells	64
Candida Utilis & Bacillus Subtilis	Uranyl ions in solution	33

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