

The Attraction of Medical Magnets

Understanding Biomagnetic Separation

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Sophisticated medical technology is creating faster and more efficient processes to support today's health care needs. From nanotechnology developments to expanding knowledge of cellular biology, diagnostic practices are advancing the future of the medical field. Biomagnetic separation is a technique which, while dating back to the 1990's, continues to improve with advances in device design and component optimization. Utilizing magnets in bioseparation has proven to be reliable for such processes as DNA synthesis and laboratory testing. Grasping the science behind the magnets is vital in understanding the attraction to this technique.

Although the concept of magnetic attraction for biological purposes has been around for more than a decade, it is important to review and discuss the fundamentals. Figure 1 provides a basic concept of the process schematically.

The process steps are:

- 1) Fluid sample is placed in a vessel
- 2) Target-specific magnetic beads (shown in green) are added and bind with target entities (dark blue)
- 3) Vessel is brought to a magnetic separator
- 4) Beads and target entities (green and dark blue particles) are retained to the side of the vessel
- 5) Remaining fluid is removed
- 6) Rinse fluid is added to separate the beads and target entities and the magnet is removed
- 7) Magnet is reapplied to separate out the beads
- 8) Fluid containing target entities is removed for further processing.

The coated magnetic beads have an active surface chemistry specific to the target biological entities. Once added to the fluid sample, coated beads bind with the target entities. By placing the vessel into the magnetic separator, the targeted entities move to the side of the vessel because of the magnet field. The remaining fluid can then be removed and, once the vessel is removed from the separator device, a rinse fluid can be added to detach the beads from the target entities. When the vessel is placed back into the separator, the magnetic beads are retained and the remaining fluid containing the target entities can be removed for the next step in the diagnostic process.

To gain a better understanding of magnets in general, refer to the units below which are commonly used when describing magnetic quantities.

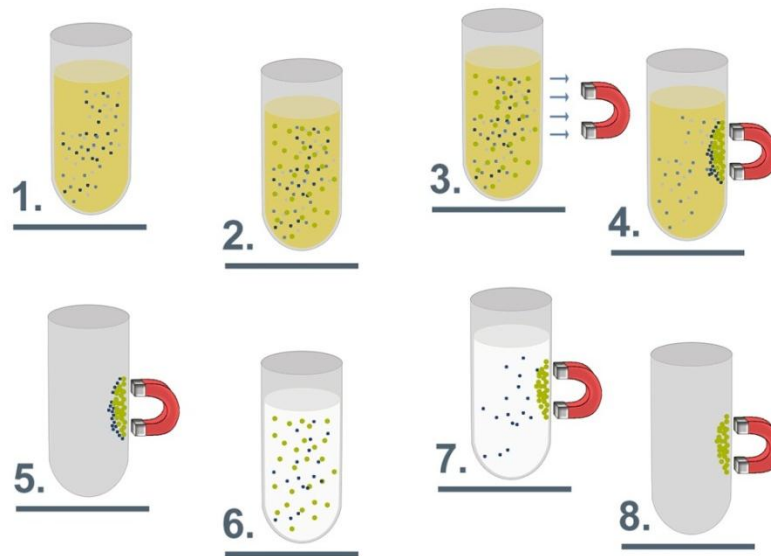


Figure 1: The biomagnetic separation process

Quantity	Symbol	Gaussian / c.g.s.	Conversion	Rationalized S.I.
Magnetic field strength	H	Oe	$10^3 / 4\pi$	$A \cdot m^{-1}$
Magnetic flux	Φ	Mx	10^{-8}	Wb
Magnetic flux density	B	G	10^{-4}	$Wb \cdot m^{-2}$
Magnetic induction	B	G	10^{-4}	T
Magnetomotive force	F	Gb	$10 / 4\pi$	A
Volume magnetization	M	$emu \cdot cm^{-3}$	10^3	$A \cdot m^{-1}$
Mass magnetization	M	$emu \cdot g^{-1}$	1	$A \cdot m^2 \cdot kg^{-1}$
Magnetic moment	m	emu	10^{-3}	$A \cdot m^2$
Volume susceptibility	X_v	dimensionless	4π	dimensionless
Mass susceptibility	X_G	$cm^3 \cdot g^{-1}$	$4\pi \times 10^{-3}$	$m^3 \cdot kg^{-1}$
Permeability	μ	dimensionless	$4\pi \times 10^{-7}$	$H \cdot m^{-1}$
Maximum energy product	BH_{max}	MG-Oe	$10^2 / 4\pi$	$kJ \cdot m^{-3}$

Table 1: Multiply the values of the quantity in Gaussian / c.g.s. units by the conversion factor to obtain the quantity in S.I. units

While it is not necessary for the users of biomagnetic separation systems to become experts in magnetism, it is useful to have an understanding of topical basics. Understanding how constituent magnetic fields affect the

final position of retained magnet beads, particularly in high throughput screening (HTS) applications, is important when developing protocols.

In practical terms, a magnetic field is a region of space in which an energy gradient can produce a force on a

magnetic dipole (such as a magnetic bead or a permanent magnet) or on a current-carrying conductor. Such fields are produced as a result of the movement of electrical charges, such as the flow of electric current in a conductor, or, in the case of permanent magnets, as a result of electron motion and spin within the material itself.

Magnetic fields are usually represented as H. The Biot-Savart law can be used to calculate H at a particular distance from a current-carrying conductor. The basic equation of the Biot-Savart law can be modified to enable calculation of H at the center of a coil and in this modified form can be represented as:

$$H = \sum \frac{1}{4\pi r^2} i \partial l \sin \Theta$$

Equation 1

Where H = magnetic field, r = radial distance, i = current flowing in a length ∂l of the conductor, and θ = angle between ∂l and the radial distance.

Since $\sum \partial l = 2\pi a$ and $r = a$, and since $\theta = 90^\circ$ (and hence $\sin \theta = 1$), the magnetic field strength will be

$$H = \frac{i}{2a}$$

Equation 2

From this equation, we can see that magnetic field strength is measured in amperes per meter, $A \cdot m^{-1}$, and that a single circular conductor carrying 1 A, with a diameter of 1m, produces a magnetic field strength of $1 A \cdot m^{-1}$, as shown in Figure 2.

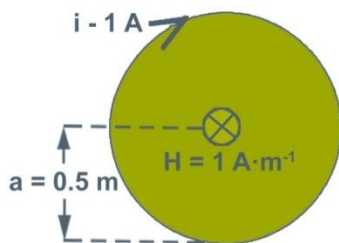


Figure 2: Value of a magnetic field, H, at the center of a circular conductor, with current i and radius a.

In the above scenario, there will be energy associated with the flow of current through the coil, which generates the magnetic field. This energy can be defined as:

$$E = \frac{1}{2} i \Phi$$

Equation 3

Where E = energy associated with the coil, i = coil current and Φ = magnetic flux generated by the coil. Φ can be seen as the total magnetic effect of the coil and is measured in webers (Wb). Since a change in Φ with time generates an electromotive force (e.m.f.) in a conductor, 1 Wb is defined as the amount of magnetic flux that generates an e.m.f. of 1 volt, in a conducting coil of one turn, when it is reduced uniformly to zero in 1 second.

More useful to the magnetic designer is the density of this flux (per unit cross-section), represented as B, because this represents the intensity of the generated flux. The magnetic flux density is therefore measured in $\text{Wb} \cdot \text{m}^{-2}$.

B represents the response of a medium to the presence of a magnetic field H. It is also known as the magnetic induction, whose definition is related to the force that it generates on an electric current or a moving electrical charge. Magnetic induction is measured in tesla, T, and thus $1 \text{ Wb} \cdot \text{m}^{-2} = 1 \text{ T}$.

In free space, the relationship between the applied magnetic field and the induction that it produces is defined thus:

$$\mu_0 = \frac{B}{H}$$

Equation 4

Where μ_0 is a constant and is known as the permeability of free space, with a value of $4\pi \times 10^{-7}$ henries per meter, $\text{H} \cdot \text{m}^{-1}$. However, in ferromagnetic and ferromagnetic systems (the kind of systems to be found in biomagnetic separators), B is no longer a linear function of H. Thus the permeability is no longer constant and may vary with H:

$$B = \mu H$$

Equation 5

The simple current loop described above is also known as a magnetic dipole since the magnetic field that is produced

by the current loop, at distances away from the center of the loop, generates a magnetic moment m . This moment is equivalent to that produced by two magnetic poles separated by a distance l , with pole strengths of p , such that

$$m=pl$$

Equation 6

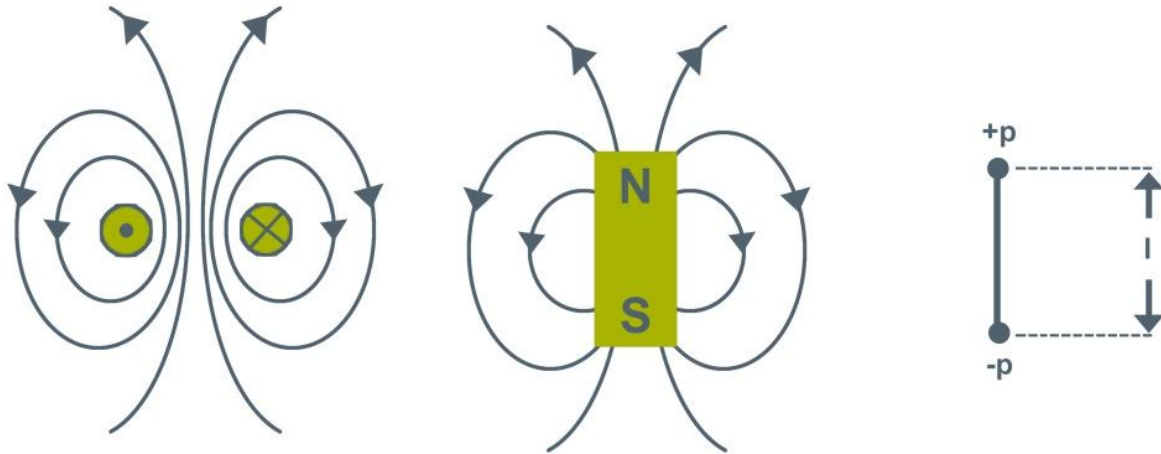


Figure 3: Comparison of the similar fields generated by a conducting coil (left) and a permanent magnet (center), with the equivalent magnetic dipole (right)

Because they are not already magnetized, dipoles of this kind are induced in magnetic beads during biomagnetic separation, because of the presence of permanent magnets in the system. Whether induced (as in the case of magnetic beads) or present as a result of residual magnetization (as in the case of permanent magnets), in the presence of a magnetic induction, a torque τ will act on the magnetic dipole such that

$$\tau = m \times B$$

Equation 7

And thus:

$$\tau = \mu_0 m \times H$$

Equation 8

In the presence of a magnetic field H (or magnetic induction B), a force acts on the magnetic dipole (such as that induced on a magnetic bead) to align the moment m , so that it is parallel to the applied field. Note that a larger volume of material (such as a magnetic bead) will lead to a larger magnetic moment, and thus the actual torque is also proportional to the volume of the bead.

There is another factor to consider here. All materials have an effect on the magnetic induction created by the application of a magnetic field, making it either larger or smaller. This effect is known as the magnetization of the material, represented by M .

For any system, the relationship between magnetic induction, applied magnetic field and magnetization is:

$$B = \mu_0 (H + M)$$

Equation 9

M is also measured in $A \cdot m^{-1}$. Since M is actually a material-specific response, it is defined as the overall magnetic moment per unit volume of material:

$$M = \frac{m}{V}$$

Equation 10

M arises as a response of electrons within the materials, in terms of spin and angular momentum, to the applied field. The relationship between magnetization M , in response to the applied field H , is known as the volume magnetic susceptibility of the material, and is defined thus:

$$X_V = \frac{M}{H}$$

Equation 11

In SI units, X_V is dimensionless. Similar to the permeability, μ , for many materials volume magnetic susceptibility X_V is not a constant. Sometimes it is also useful to specify the mass magnetic susceptibility X_G which is defined as:

$$X_G = \frac{X_V}{\rho}$$

Equation 12

where ρ = density. SI units for X_G are therefore $\text{m}^3 \cdot \text{kg}^{-1}$. In addition, it is sometimes useful to use relative permeability, μ_r , defined as:

$$\mu_r = \frac{\mu}{\mu_0}$$

Equation 13

It also follows that:

$$\mu_r = X_V + 1$$

Equation 14

Biomagnetic separation is a simple and effective tool because magnetic beads and the target biological entities are suspended in fluids which have low magnetic permeabilities and which therefore do not impede the applied magnetic field. In addition, the biological entities themselves have low susceptibilities and thus the intended actions of the magnetic beads are not hindered by any significant magnetic response from the biological entities themselves.

Bulk susceptibility is used to classify materials into various categories. Diamagnetic materials are those for which X_V is small and negative. Such materials respond weakly in such a way as to oppose the applied field. Paramagnetic and antiferromagnetic materials have X_V that is small and positive. The response is still weak but does slightly enhance the applied field. Ferromagnetic and ferrimagnetic materials have typically strong and positive values of X_V .

The susceptibilities of diamagnetic, paramagnetic and antiferromagnetic materials are linear over a relatively wide range of values of H . Ferromagnetic and ferrimagnetic materials do not exhibit this characteristic.

The reaction of these materials depends on the existence and behavior of magnetic dipole moments within the material. Diamagnetic materials do not have magnetic dipole moments. The other material types do. Figure 4 is a schematic representation of the ordering of magnetic dipoles within the various magnetic materials.

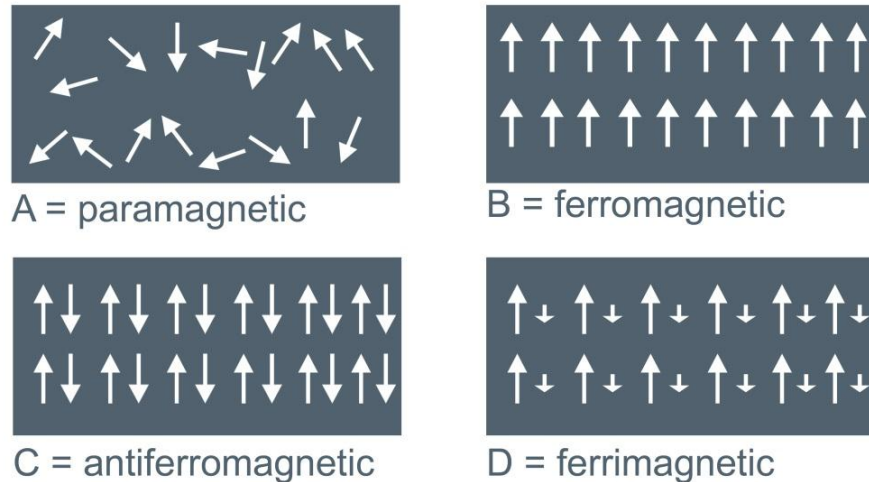


Figure 4: Schematic classification of materials as a function of magnetic dipole ordering: A = paramagnetic, B = ferromagnetic, C = antiferromagnetic and D = ferrimagnetic.

Having addressed the basic function of magnetic materials, we can delve deeper into the optimization of magnetic fields used for biomagnetic separation. As previously stated, when a magnetic bead encounters an applied magnetic field a magnetic dipole is induced in the bead which causes the particle to rotate so that the axis of this dipole is parallel to the applied field. However, in the absence of some other driving force, this will be the extent of the interaction of the bead with the magnetic field. This type of reaction is of little use during separation.

In order to cause the magnetic bead to move to a desired location within a container, it needs to experience a force that acts in the desired direction. This is accomplished by not only producing a significant magnetic field within the container, but also by designing the system such that there is a significant magnetic field gradient within it.

The applied force needs to overcome any friction or viscosity-related resistance caused by moving the bead within the fluid medium and it also needs to overcome any gravitational effects. While these additional effects (in particular the fluid viscosity) may not be insignificant, the force generated on the bead by the applied magnetic field is the dominant factor in such systems. When small

volumes are being separated, for example in high throughput screening (HTS) protocols, these additional effects can be neglected without negating the basic principles described herein.

Within a magnetic system, it has been shown that the force on a small particle such as a magnetic bead can be described as:

$$F_m = \frac{V \Delta_\chi}{2\mu_0} \nabla B^2$$

Equation 15

Where F_m = the magnetic force, V = the volume of the particle, Δ_χ = the difference in susceptibilities of the bead and the fluid medium, μ_0 = the permeability of free space and ∇B^2 = the magnetic energy density gradient. For magnetostatic fields, with no electric currents:

$$\nabla B^2 = 2 (\mathbf{B} \cdot \nabla) \mathbf{B}$$

Equation 16

Maximizing this product will maximize the force acting on the magnetic beads during separation. Rearranging the above terms, it can further be shown that:

$$F_m = V (M \cdot \nabla) B$$

Equation 17

Since:

$$(M \cdot \nabla) B = M_x \frac{\partial B}{\partial x} + M_y \frac{\partial B}{\partial y} + M_z \frac{\partial B}{\partial z}$$

Equation 18

the magnitude of the force acting on the magnetic beads, in the simplest one dimensional case where susceptibility is constant, can be defined as:

$$F_m = VM \frac{dB}{dx}$$

Equation 19

Thus, the magnetic force on the beads is proportional to the product of the magnetization of the beads induced by the magnets in the separator system and the gradient of the magnetic induction, with respect to distance, within the container used for separation. The magnetic force will always act in the direction of increasing B and parallel it.

Since B is a function of M as shown in $B = \mu_0 (H + M)$, the use of materials with higher intrinsic values of M (and therefore higher values of X_G) within the magnetic beads, will lead to an increased force acting upon them during separation. Commercially available magnetic beads are frequently specified by their mass magnetization value, which will be constant at or above the saturation level of the beads. The magnetic gradient is a commonly used figure of merit for comparing different biomagnetic separation systems, as is the value of B^2 .

It should be noted that the application of too much force on magnetic beads attached to biological entities can cause those entities to be damaged during the separation process and while the beads are retained at the container wall, possibly crushing or otherwise deforming delicate biological structures. Aside from causing the separation in the first place, there needs to be enough force acting on the beads once retained, so that the remaining fluid can be decanted effectively without losing any of the retained beads. A balance is required between these two factors and end users should investigate the effects of separator systems and the specific beads being used on the target entities to avoid this potential type of damage.

It can be seen that the behavior of magnetic beads within biomagnetic separation systems can be carefully controlled by matching the needs of the protocol to the right beads and the right separator devices. Understanding the basics of magnetic principles helps to define requirements in specifying biomagnetic separator devices. A wide variety of separator designs allows the user to select an appropriate means of separation, based on fluid sample volume, required speed of processing and other parameters.

The combination of a wide range of surface chemistries, bead morphologies and separator devices provides an extensive array of options for optimizing separation. As technology advances, so too does the possibility for achieving higher quality results with biomagnetic separation protocols.



Figure 5 : Dexter Magnetic Technologies' LifeSep® Series of Magnetic Separation Devices

Biomagnetic Separator Device Designs

Historically, Dexter Magnetic Technologies has been integral in the advancement of biomagnetic separator devices in the past decade. Engineers Dr. Hatch and Mr. Schilling created and patented a separator with multiple magnetic poles in 2003. The dimensions of the device were matched to particular integers of wells in a multi-well tray and with similar outer dimensions to the multi-well tray itself. The resulting design provided a consistent separation across the multi-well tray, with the magnetic beads being retained on the bottom of the wells. Later that year, Dr. Hatch and Mr Ras patented another Dexter separator design. This device was essentially based on a large, single magnet shaped in such a way as to produce a flat magnetic field onto which a multi-well tray could be placed. The field profile produced a highly uniform separation across the whole well tray, making the device particularly well suited to 1536-well trays. The current industry standard for separator devices is the Dexter LifeSep® series of off-the-shelf biomagnetic separator systems, available in both tube and tray designs.