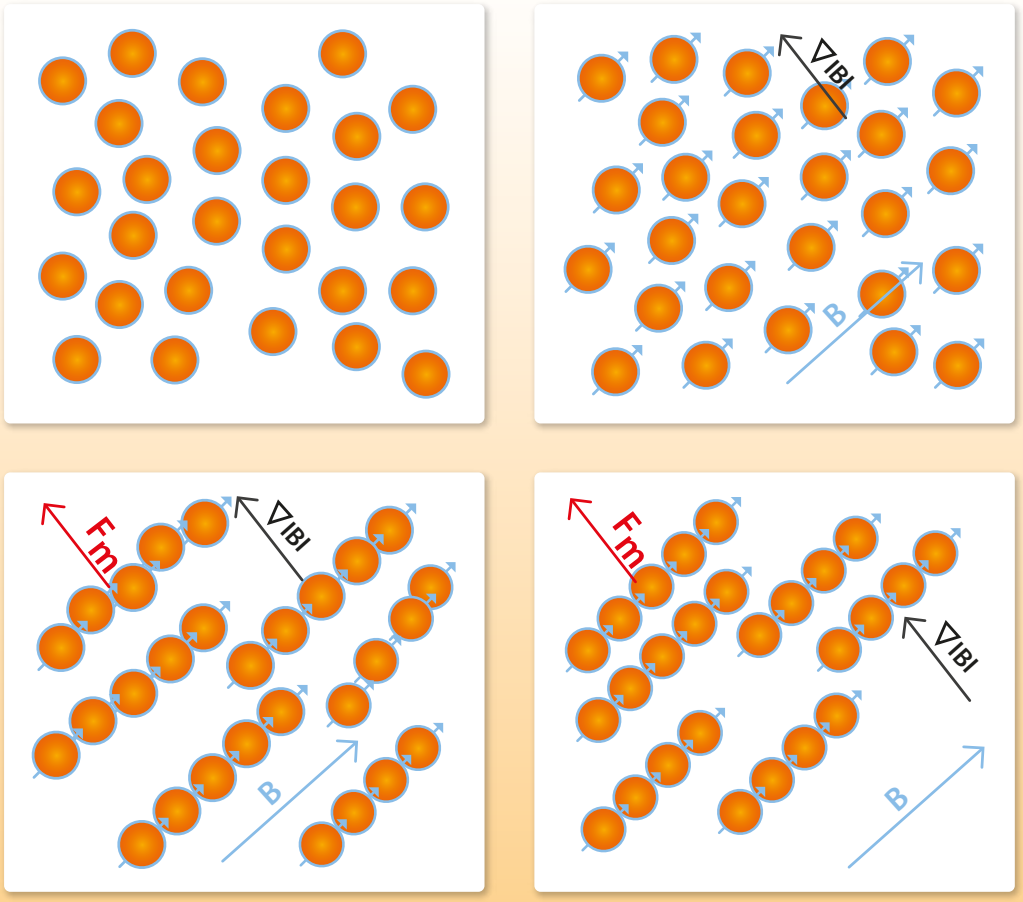
The Advanced Guide for the Validation of Biomagnetic Separation Processes



Sepmag®

SUMARY

1. Avoiding Reproducibility Issues in IVD Production

Magnetic beads are used by many biotech companies, typically for in vitro diagnostic applications. Because these beads are so widely used, it is very important that every aliquot of every batch has exactly the same properties.

One way to avoid variability is to make sure that the properties of the raw materials themselves are controlled from batch to batch. Scientists take great pains to make sure that the properties, for example, of the magnetic beads, the antibodies and the buffers are standardized and well defined.

The magnetic separation process itself should be controlled such that all of the beads behave exactly the same when the magnetic force is applied. If the beads do not behave in a uniform manner, the biomagnetic separation step can lead to a non-homogeneous final product. This non-homogeneity can lead to either an irreversible aggregation of material and/or costly loss of material. If the beads are too close to the magnets, the beads are subjected to high levels of magnetic force over a long period of time causing aggregated beads and the inability to resuspend the material. If the beads are farther from the magnets, they experience very low levels of magnetic force making it difficult to pull the beads into the desired force regime. In the former case, the batch suffers mainly from quality of product issues and in the latter case, the batch suffers mainly from loss of material. Both cases cause a loss of time and energy as scientists attempt to recover material

What is the solution?

For optimal reproducibility, biomagnetic separation conditions should be homogenous over the entire batch volume. A homogenous system such as Sepmag generates a magnetic field profile that provides homogenous magnetic force across the entire batch volume. Beads no longer experience variable magnetic fields depending on their distance from the magnet. This type of a system creates well defined bead behavior that then leads to optimal yield and reliable reproducibility.

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2. Homogeneity: The Key Factor in Biomagnetic Separation

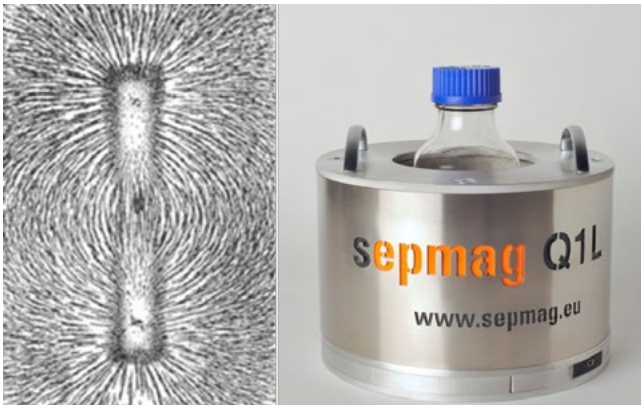
When a biotech company or other related industry decides to use biomagnetic separation technology, a great deal of time, energy and resources are typically spent by R&D and manufacturing departments to determine the optimal bead to use for their specific applications. Often, however, they overlook one of the major determinants of separation: homogeneity of separation conditions.

Traditional magnetic separators

When traditional magnetic separators are used (both classical open magnet and rod-like systems), the beads experience non-homogeneous magnetic forces and fields depending on the distance the beads are from the magnet. Some beads will be far from the magnets and some will be close, therefore the separation conditions and microenvironment that each bead 'sees' are different. Beads close to the magnet will experience magnetic saturation while beads farther from the magnet experience varying magnetic fields as they travel toward the magnets. In addition, beads farther from the magnet will be more difficult to attract due to the low magnetic forces. Beads close to the magnet experience excessive forces for longer times causing an increase in bead aggregation, loss of bead activity and problems with resuspension. Laborious sonication is necessary to recover aggregated beads. One can easily see that beads existing in the extreme magnetic force regimes (near or far) are generally difficult to recover completely.

Sepmag systems

In contrast to traditional systems, Sepmag systems are designed so that every bead experiences the same magnetic force, regardless of its distance from the magnetic ring. Since beads 'see' the same magnetic force, the conditions are well defined and do not suffer from the inability to parameterize the process as in the previous case. Results are reproducible because the magnetic forces are homogeneous, controlled and the details of the magnetic forces are understood. In addition, processes can be scaled up without disrupting the homogeneous conditions. Variability in the end product is ultimately eliminated, no matter the size of your batch.



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3. The Basic Principles of Generating Magnetic Forces for Life Sciences Applications

Often in life science research and clinical applications, magnetic carriers are utilized to separate or isolate biomolecules from suspension. A biomolecule is coated onto a magnetic bead or is 'captured' by a bead and then pulled to a given position in the solution via magnetic forces. In order to establish a robust and reproducible standard operating procedure (SOP), one needs to understand how the magnetic forces are generated and what key parameters need to be controlled.

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Magnetic Profiles and Forces

The magnetic force depends on the magnetic field profile provided by the instrument. A homogeneous magnetic field does not generate force on magnetic objects, but does generate torque. This means if you have a perfectly homogeneous magnetic field, your bead only rotate, but will not move in a given direction. In order to generate a magnetic force, one needs to first generate a non-homogeneous magnetic field. If you have a non-homogeneous magnetic field, any variations in the magnetic moment and/or field will give you a force.

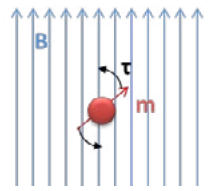
Therefore, the magnetic force is dependent on BOTH the variation of the magnetic field and the variation of the moment of the bead. Superparamagnetic particles have two very different behaviors at low magnetic and high magnetic field. At low magnetic field, the magnetic moment changes linearly with the field. At high magnetic field (i.e. saturation), the magnetic moment will be nearly constant.

Generating a Magnetic Force

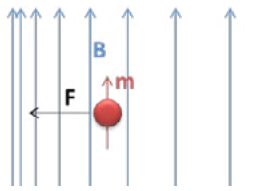
A single permanent magnet can be used to generate a magnetic force because the magnetic field decreases with distance. However, most of the variability of the field occurs out of the working volume. Most of the energy is lost to these stray fields.

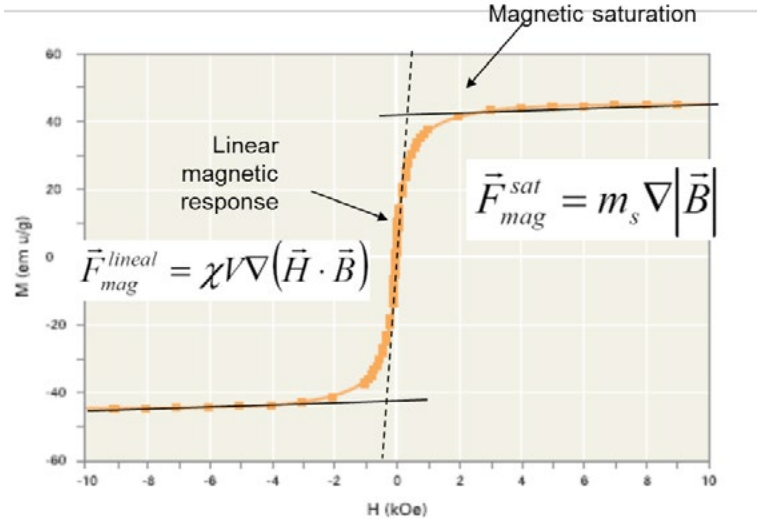
More modern biomagnetic separation devices such as Sepmag generate variable fields in a well defined working volume. This minimizes stray magnetic fields and focuses all of the energy of the permanent magnet on the generation of magnetic force over the beads. This is a much more efficient use of biomagnetic separation and results in a reproducible product.

$$\boldsymbol{\tau} = \mathbf{m} \times \mathbf{B}$$



$$\mathbf{F} = \nabla (\mathbf{m} \cdot \mathbf{B})$$





4. Setting Homogenous Biomagnetic Separation Conditions

Reproducibility is very important when considering production of in vitro diagnostic kits. As such, there should be good quality control in place that strictly defines the parameters of the assay's raw materials. For example, magnetic beads, antibodies and buffers should not vary from batch to batch. In addition, these raw materials should all act the same during biomagnetic separation.

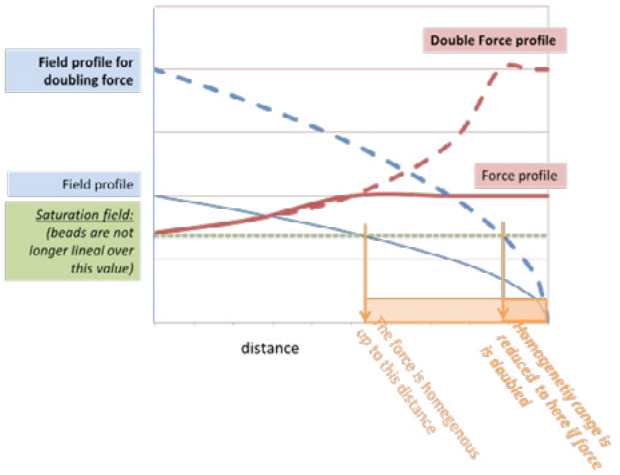
So, what is the problem?

There are several problems to be considered:

1. For desired homogenous conditions, one must ensure constant magnetic force across the entire batch volume.
2. In order to generate magnetic force, one must have a non-homogeneous magnetic field.
3. Some beads may experience a low magnetic field, where the magnetic moment changes linearly with the field.
4. Some beads experience a high enough magnetic field to saturate their magnetic moments, rendering it essentially constant.
5. Although it is difficult, the magnetic field pattern needs to be chosen carefully such that it has a constant magnetic force across all beads.

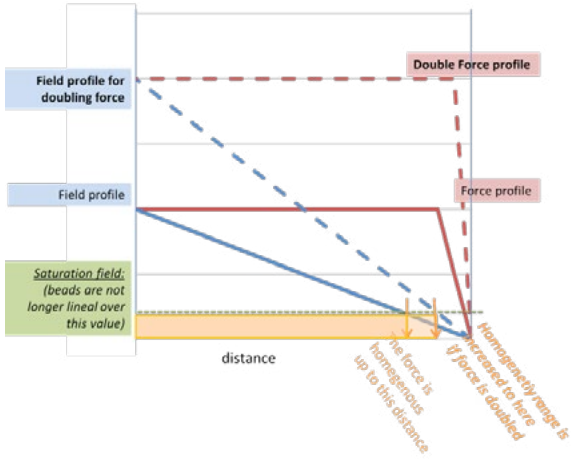
Solution at Low Magnetic Field

A constant magnetic force at low magnetic field is achieved by generating a field that is always below saturation. If one increases the force, this will reduce the region where the force is homogenous. If the field reaches magnetic moment saturation, the force will decrease. Large volumes complicate this problem by exacerbating the effect of the field profile on distance from the magnet. Therefore, it is not always easy to accomplish the desired goal.



Solution at High Magnetic Field

If one already has a high field, generating constant magnetic force, one needs only to develop a constant gradient to achieve a magnetic force. Cylindrical geometry of the vessel permits the generation of a quadripolar field. In this situation, only 1% of the sample volume experiences non-homogeneous forces. Radial gradients are high enough in cylinders to allow almost the entire volume of beads to experience saturation. Even if 10% of the central region in the cylinder is below the saturation field, only 1% of the entire batch volume will be affected. Since conditions in these cylinders are homogenous and consistent, the same conditions can be reproduced at higher batch volumes. Reproducibility is independent of scale.



5. Avoiding resuspension problems using biomagnetic separation technology

One of the major problems of traditional biomagnetic separation technology is aggregation of the magnetic beads. Aggregation decreases the yield and also contributes to variability between batches.

What causes aggregation?

Traditional biomagnetic separation creates a magnetic force that is dependent on the distance from the magnet. In other words, beads that are far from the magnet are difficult to attract because of the low magnetic force. Often, this results in a great deal of material loss and an increase in separation time. A common way to rectify these problems is to increase the magnetic force by adding permanent magnets to the separator in order to pull more beads toward the magnets in a shorter amount of time. Doing this, however, only increases the aggregation problem.

Beads that are close to the magnets experience excessive magnetic forces for a longer amount of time. Beads exposed to high magnetic force over time tend to aggregate, causing a loss of bead activity. Sometimes the loss of bead activity is irreversible. In addition, resuspension of the aggregated beads is very difficult, requiring laborious sonication to first break up the aggregates. Increasing the magnetic force to pull beads in from farther away would clearly exacerbate this problem.

How can one avoid aggregation?

A new technology used by Sepmag biomagnetic separation devices allows scientists and engineers to apply the optimum magnetic force to all the beads equally, ensuring homogeneous separation conditions. These conditions are gentle on the beads and allow for efficient separation without the aggregation and resuspension problems of traditional methods. The Sepmag separator also alleviates the problems of beads that are far from the magnet, since even these beads experience the same magnetic forces as the beads closer to the magnetic ring. No longer will scientists need to increase the force or the separation time to attempt to increase yield. No longer will scientists need to use sonication techniques to break up aggregated beads. Sepmag technology increases yield and decreases batch to batch variability without extra work.

6. Determining the Optimal Biomagnetic Separation Time

Those who use Life Sciences products rightly demand that these products show consistency from batch to batch. In other words, when comparing batches, one should find very little, if any, variability. In biomagnetic separation devices that use permanent magnets, the working conditions are generally very stable. The main parameter that needs to be determined in any given assay situation is the separation time, i.e. the time that the beads are exposed to defined biomagnetic separation conditions.

The problem

When one uses standard biomagnetic separation devices, the magnetic conditions under which the beads are separated are non-homogenous. In other words, beads that are far from the magnets experience very weak forces and magnetic fields. In order to prevent or minimize loss of material, typically the separation time needs to be extended for these beads.

Near the final retention position of the beads, the magnetic forces and fields are high. If the beads are exposed to high forces for a long period of time, there is a great risk of bead aggregation and inability to resuspend the beads.

These are two opposing situations that exist in the same vessel. If one decides to separate the beads over a longer period of time, the risk of irreversible aggregation increases. If one shortens the separation time, beads farther from the magnet will be lost.

How can this be resolved?

Newer Sepmag's biomagnetic separation technology uses homogenous conditions where the magnetic force is the same, regardless of the distance from the magnets. Since the force does not decrease with distance as in traditional devices, the gentle retention forces will hold the beads during buffer extraction. Since the forces on the beads at a distance are the same as the forces on the beads closer to the magnets, separation time will be much shorter than in non-homogenous systems. All of the beads move at the same speed, so separation times can easily be determined using optical methods. These optical techniques can also be utilized to make sure the separation is complete. Since the beads will be exposed to the magnetic force for a much shorter time overall, the risk of irreversible aggregation is greatly reduced.

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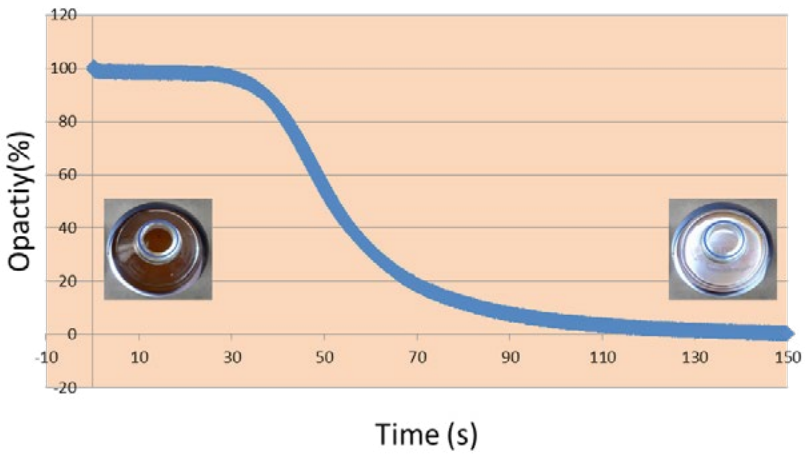

7. Biomagnetic Separation: Ensuring Complete Consistency From Lot to Lot

Consistent lot to lot results is achieved with biomagnetic technology only when separation is performed in defined and homogeneous conditions. When homogeneity is realized, separation is reproducible and scalable.

Under traditional biomagnetic separator conditions beads do not experience homogenous conditions. Beads close to the magnets experience strong magnetic forces for a long period of time leading to aggregation of the beads. This aggregation is sometimes irreversible, causing a loss of bead activity and an inability to resuspend the material. All of these problems cause problematic inconsistencies from lot to lot.

Beads that are far from the magnets experience weak or variable magnetic forces. Weak forces do not allow separation of the beads over typical separation times, causing a loss of material. The beads move toward the magnets slowly, under the auspices of the variable magnetic forces, causing the properties of the magnetic beads to be affected. The normal desire is to increase the separation time in order to pull the distant beads into the retention zone. However, increasing the time would also expose the beads near the magnets for a longer time. This ultimately increases the aggregation of the beads. There does not seem to be a good solution for a high yield using traditional separation techniques.

The latest breakthrough developments in biomagnetic separation technology consist of separators that create a homogeneous and well-defined magnetic force independent of distance from the magnets. With the Sepmag systems, every bead receives the same magnetic force. This results in highly reproducible product from lot to lot, no matter what volume is used.

Magnetophoretical conditions are reproduced faithfully for all of the Sepmag systems. Therefore, one can work with several different volumes and scale up at any time without the necessity of revalidating the process. Since permanent magnets retain their magnetic properties for hundreds of years, the Sepmag systems are timeless devices that will ensure complete consistency between lots for a very long time.

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8. Guaranteeing Time Stable Biomagnetic Separation Conditions

Of course, as in most industries, product consistency is key to the success of the Life Sciences industry. With biomagnetic separation, not only should working conditions be constant over time, but conditions should also be consistent from lot to lot, regardless of the time between production runs. One thing that should always be considered is the quality of the magnet used in the biomagnetic separation devices.

Modern separation devices use Rare Earth permanent magnets as the magnetic field sources. The normal assumption is that 'permanent' magnet properties are 'permanent' and constant over time, however these magnets can be demagnetized under certain conditions that are achievable in a lab situation. It is vitally important that production managers understand and avoid these adverse lab conditions in order to properly maintain their magnetic sources.

Permanent magnets (i.e. Rare Earth) can be demagnetized if they are exposed to magnetic fields of more than 2 T. A field of 2T is nearly five orders of magnitude greater than the Earth's magnetic field. Superconducting coils found in devices such as an MRI or in an Intense Pulsed Magnetic Field machine can achieve these values. Therefore, the biomagnetic separator should not be put in the working volume of this devices, when magnetic field can be high enough to demagnetize it.

Permanent Rare Earth magnets can also be demagnetized when exposed to temperatures greater than 80ºC. Under normal temperature fluctuations, magnetization can also vary, but only slightly and the effect is reversible. Between 20 and 40ºC, for example, the magnetic forces can decrease from 2-3%. If the lab temperature is not controlled, magnetic forces will reversibly drop approximately 0.1% per each oC increase in temperature. When temperatures are greater than 80ºC, however, the changes in magnetization can be irreversible.

Avoiding these two conditions in the lab will guarantee a long life for your biomagnetic separation device.

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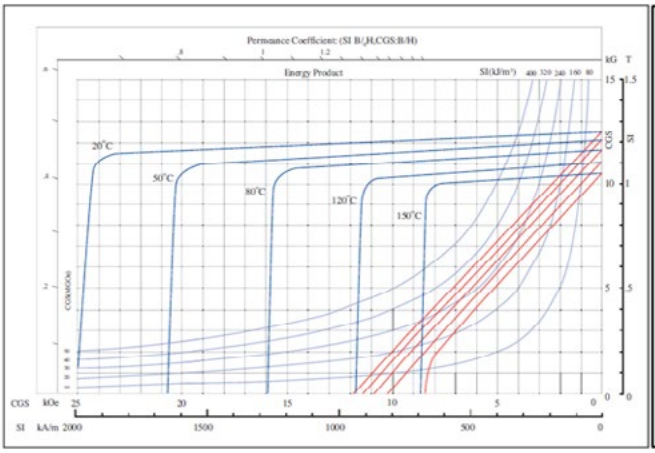

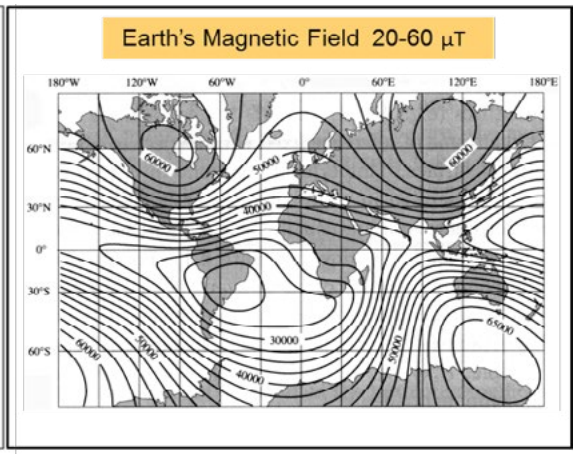

9. Fully Controlling Your Biomagnetic Separation Process

Because biomaterial is expensive, fragile, complex and sometimes rare, biotech companies spend a great deal of time and resources to develop and refine biomaterial production processes. Quality control and standard operating procedure demand that production managers make sure that all technicians and operators know and follow the exact procedures from batch to batch. Typically, the parameters of a process are painstakingly determined before production can proceed in a scaled-up manner. The exception to this seems to be in the case of magnetic separation processes.

When companies use traditional magnetic separators without fully understanding the critical aspects of separation, they are unable to understand the end product results and are unable to control the consistency of the process. Too often magnetic separation is not consistent or reproducible.

It is important for production managers to understand that in traditional magnetic separation, not all beads 'see' the same conditions. The beads close to the magnet experience very strong magnetic forces for a long period of time. These beads will likely have issues with aggregation, loss of activity and resuspension difficulties. The beads far away from the magnet experience variable magnetic forces or weak magnetic forces. Because of the variation in force as the beads travel closer to the magnets, the properties of the beads will be affected.
Sepmag biomagnetic separators are advanced systems that create homogenous and well-defined magnetic forces independent of the distance from the magnets. All beads 'see' the same magnetic force in these systems. Because of this, the process is highly reproducible and is very scalable.

Included with the Sepmag separators is a Quality Control Recording device that monitors the process in real time, allowing the production manager to keep track of performance for regulatory and quality control purposes. The recording device is an optical recorder that detects the turbidity of the liquid and comes with software for full analysis of the data in real time. The software supplied will run on any PC.

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10. Accelerating the Movement of Magnetic Beads

Because biomagnetic separation techniques are relatively simple, life science laboratories and industries are quite enamored with them. Indeed, using only magnetic beads and magnetic fields, biomolecules can be captured and extracted from complex media. However, if this application is to be considered practical, it should also be faster than other separation technologies such as chromatography, electrophoresis or centrifugation.

When there are small volumes (on the order of milliliters) and low viscosity suspensions, the separation is quite fast regardless of the biomagnetic separation conditions. When the volumes are large or the viscosity increases however, the time of separation can increase exponentially if inappropriate conditions are used. Furthermore, when non-homogenous biomagnetic separation devices are used, it is difficult if not impossible to replicate the optimal conditions when the volume is changed.

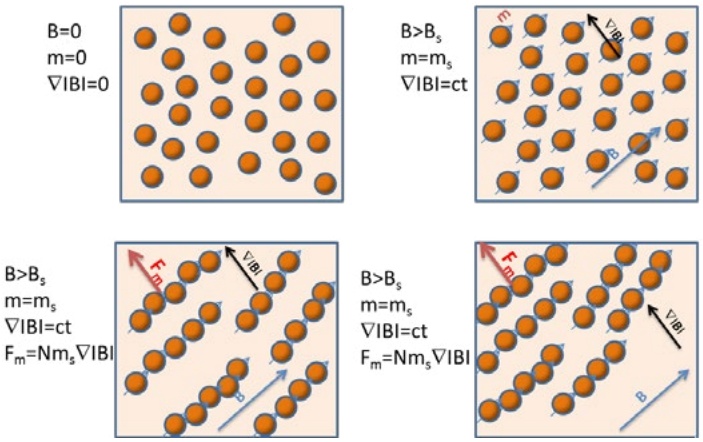
So how do we make this process faster without compromising the material?

Superparamagnetic beads have no magnetic moment when there is no magnetic field present. If one applies a magnetic field, it must be a field such that beads are saturated in that field and have a constant, fixed magnetic moment. In this case, the beads will act like small dipolar magnets and will interact with each other, forming chains. The higher magnetic moment of the chained cluster will cause the chains to move quickly in the direction of the magnetic gradient.

To decrease the separation time, the magnetic field profile should have:

1. A field high enough to saturate the beads causing the above mentioned dipolar interaction and chain formation and,
2. A steep magnetic field gradient.

If both of the above conditions are fulfilled, the chains that form, which have a higher magnetic moment than single beads and proportionally less drag force, will move faster than individual beads in the exact same field. The trick is to optimize the field such that chains form, but aggregation is not a serious problem.



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11. Monitoring Homogenous Biomagnetic Separation Processes

Biomagnetic separation used to take place in academic labs, but recently it has become a very industrial application. But as processes are scaled up and volumes increase, the investment required for each batch is larger; the expected economic return is also larger. When production mistakes happen, investments returns and the viability of the product on the market are both endangered. In order to minimize production mistakes, industries must establish Standard Operating Procedures (SOP), Quality Control Protocols (QCP) and Validation Audits (VA).

When a company uses biomagnetic separation, they usually only require that a technician signs off on the conformity of the process. No other records are typically produced for that process, so if something is wrong with the product, the production manager cannot backtrack using the production records and deduce the problem with the magnetic separation. This is not the optimal way to ensure consistency between lots.

When using homogenous biomagnetic separation technology, as Sepmag uses, all of the beads move at the same speed and so one can make optical measurements of each single batch over time and compare results between lots of the same product. Changes in the characteristics of the suspension will change the data gathered from the optical sensor (i.e. will detect changes in the behavior of the bead movement during production).

In the beginning, a standard curve can be validated and compared against the behavior of each lot. Objective parameters can be measured (such as the t50). If anything causes a deviation from the standard curve, it can be an early warning of quality problems in the batch. Some causes of deviation from the standard curve include:

- Magnetic bead property variations (i.e. size and magnetic content) which can change the speed of separation.
- Magnetic bead concentration variations which also can change the speed of separation.
- Suspension viscosity variations which will alter magnetic force.

Optical recorders, such as Sepmag's QCR system, will allow companies to not only be warned that there is a problem sooner, but will also be able to identify and rectify the specific problem with production.

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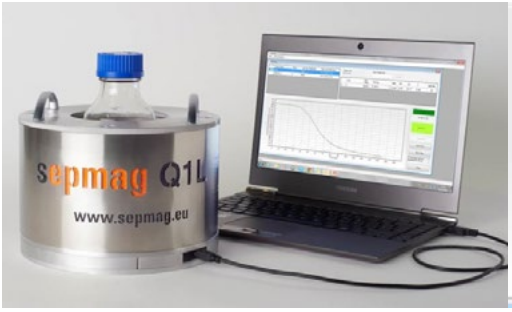
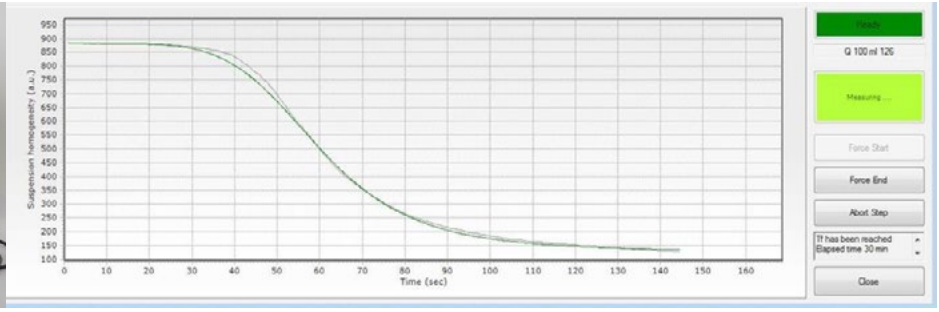




12. Settling on the Correct Biomagnetic Separation Conditions

When using biomagnetic separation, in order to ensure the consistency of the resulting product and the process itself, there must be some sort of validation procedure. Validation should be consistent within a given lot, from lot to lot and also when the process is scaled up. The validation procedure should optimally be related to the conditions of magnetic separation and not be dependent on any specific device that generates the magnetic field.

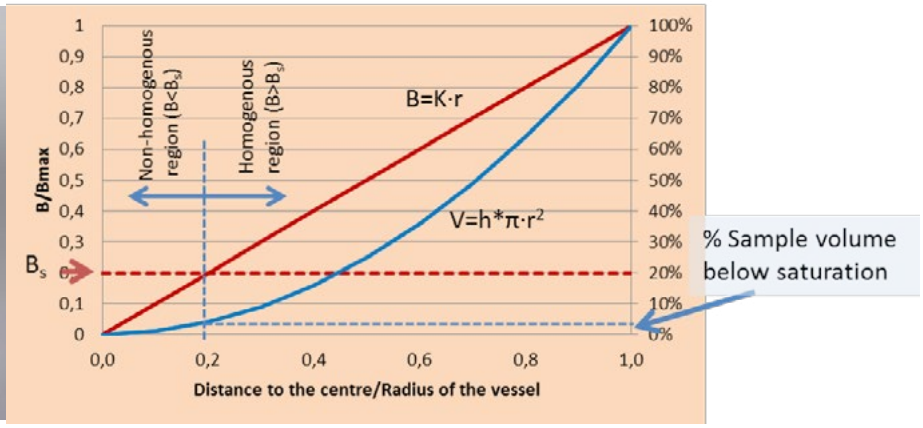
Why should validation be independent of the device?

If the process is validated in a specific magnetic separation device that generates an unvalidated, unknown magnetic field pattern, it will be virtually impossible to scale up the process without revalidating every single aspect of the production. Furthermore, if the magnetic field profile is known, but the biomagnetic separation conditions are not homogeneous, it will be very difficult to guarantee consistency in the lot in the case where batch size is changed. Within the lot itself, beads will experience very different conditions depending on their distance from the magnets. Beads close to the magnet will experience high forces over a long time and beads farther from the magnet experience variable forces. This leads to variability in the final product and an inability to translate the production data from one device to another.

Is there a way to make the validation process more straightforward?

Indeed, using a homogeneous separation system such as Sepmag, the parameters of the process are easily measured and monitored, allowing one to create standard curves and optically watch the process over a period of time in real time. Several parameters should be considered:

- The magnetic field should be high enough such that the beads are near their magnetic saturation point. Standard magnetite beads, for example, are saturated at B>0.1 T. Magnetic fields over 0.1 T are enough to magnetize the beads near ms, but realize that other types of paramagnetic material may require different field values.
- The magnetic field gradient should be steep enough to retain the beads when the liquid is removed, but not steep enough to induce irreversible aggregation.
- Finally, cylindrical geometries will allow regions with non-perfect conditions to be very small compared with the full volume. Any small force or agitation will easily push the beads that exist in this small non-perfect zone into the homogenous region.



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