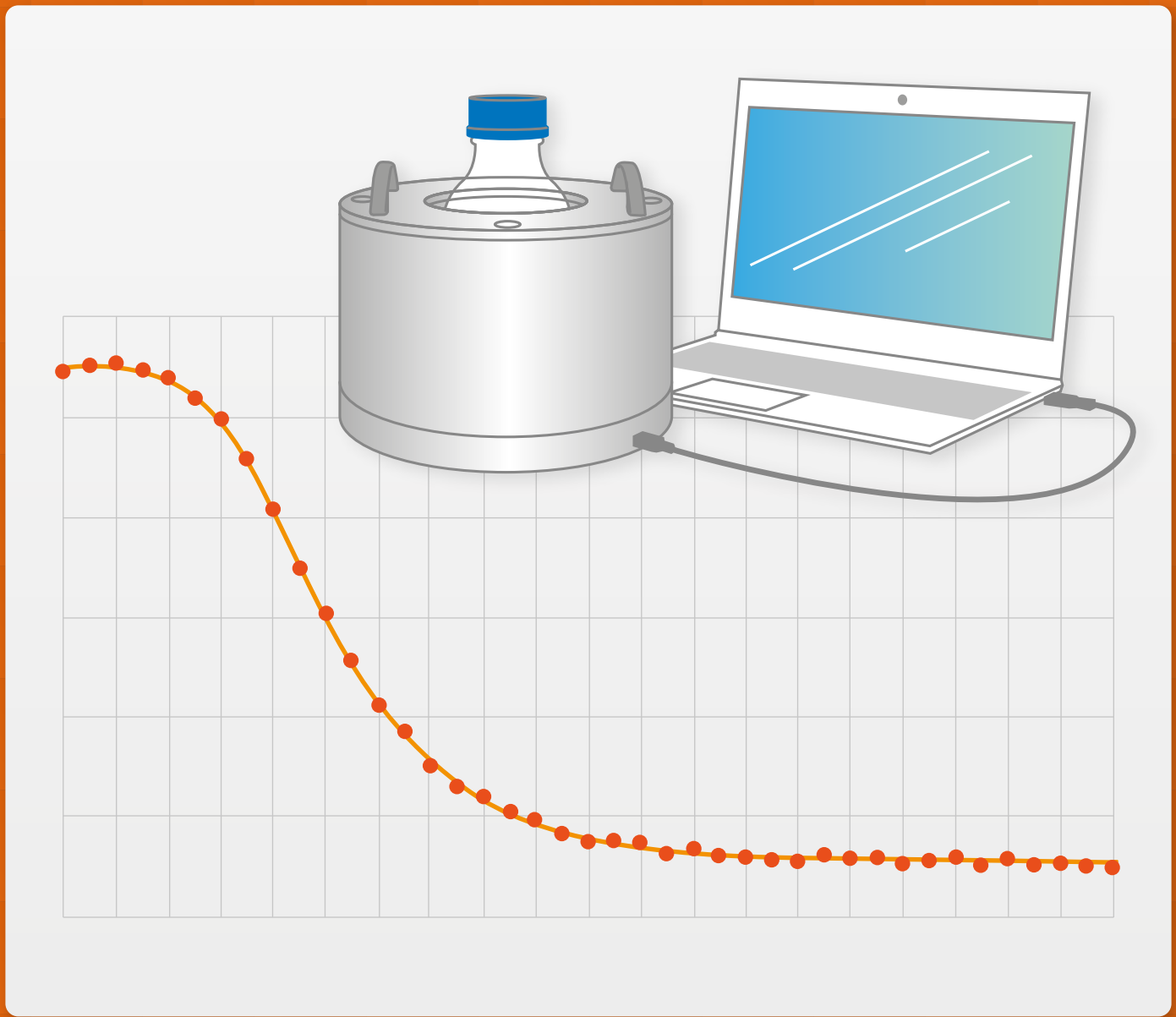
The Basic Guide for Monitoring Biomagnetic Separation Processes

Discover the importance of monitoring biomagnetic separation processes and the best techniques to do it



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SUMARY

Chapter 1
Biomagnetic Separation processes: how to detect inconsistencies?

It is hardly breaking news that separation technology is one of the most complex and important areas of biotechnology. Finding cost-effective separation techniques is a crucial factor for the growth of industrial biotechnology. Not only is it necessary at the application point (diagnostics, protein purification, cell sorting) but also to facilitate large-scale production.

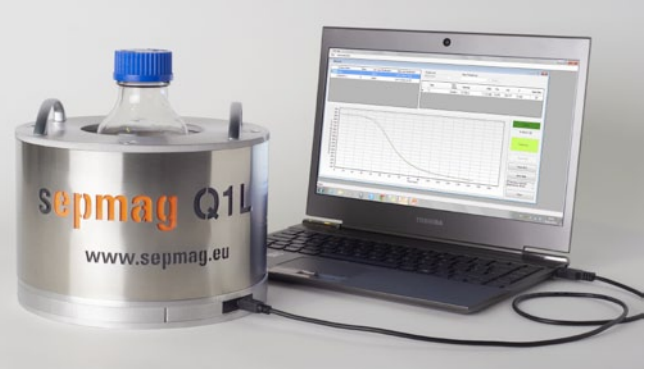
Biomagnetic Separation techniques are becoming increasingly important with a wide range of possible applications in the biosciences. Magnetic micro- or nanospheres can be separated easily and quickly by magnetic forces and can be used in conjunction with bioaffinity ligands, e.g. antibodies or proteins with a high affinity to the target.

Although scientists typically use these beads in relatively small quantities, once the kit hits the market and has proven itself useful, the production volume for the kit must necessarily increase to meet demand. There will be a need to supply kits to many hospitals and labs globally. A cost effective method of producing magnetic beads is therefore required.
As we will discuss in this eBook, the main problem with Biomagnetic Separation is how to characterize and monitor the process. The principle behind the process is simple: the effect of a magnetic force on particles in a solution. However, although it does not look like a complex process, many users mistakenly think it is no more than a mere block magnet exerting some degree of force over a nearby test tube.

The problem with this over-simplistic approach is the undefined process conditions: a block magnet does not establish the value of 'magnetic force'. As consequence, it is impossible to determine the key parameters of the Biomagnetic Separation process, how to validate them or what will happen when we transfer the process to a different volume or setup. In most cases, the 'END' of the process is determined simply by the technician visual observation, and sometimes 'standardized' by the observed time separation. Even when carefully executed, this approach only gives information about the situation after the process is finished, without monitoring what is happening during it.

The lack of validation/control tools causes serious problems when the separated product shows non-compliances or when experiment results are inconsistent. No objective data can be discussed and, because the exact separation conditions are not well defined, checking the potential problem causes is difficult, if not impossible.

This eBook explains how to approach the problem of monitoring Biomagnetic Separation processes. Firstly, homogeneous Biomagnetic Separation conditions must be established in order to optically monitor changes in the suspension. This methodology can be applied to both R&D, where technicians usually need to compare different samples in small volumes, and production, where the purpose is to ensure that successive batches are identical.



Chapter 2
Two simple concepts for a better understanding of your Biomagnetic Separation process.

The apparently simple nature of the Biomagnetic Separation process is the reason for its great popularity in Life Science, but it is also one the causes behind problems with its proper application. All users are aware of the working principle: the magnetic field applied generates a force over the magnetic beads or particles. It is very simple to perform a quick feasibility test: just take a small quantity of magnetic carriers in suspension, approach a magnet and – Voilà! – the beads/particles will speed towards the magnet.

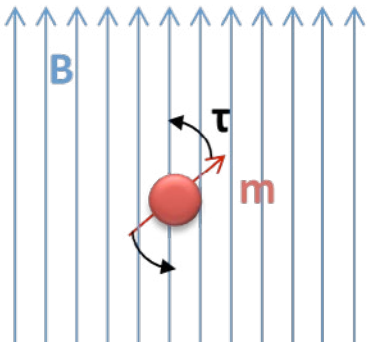
Success with initial experiments often leads the users to overlook the Biomagnetic Separation process itself. The need to focus on the biological and chemical aspects of the project (selecting the right bead, ligand and surface binding) delays deeper discussions about key factors governing the Biomagnetic Separation process. It is not unusual for users to be unaware of the physics governing the interaction between the magnetic beads and the applied magnetic field. This lack of understanding of the critical parameters of the process usually leads to inconsistent results as the project advances.

Although the details of the Biomagnetic Separation process involve a lot of Physics, for most users, two simple concepts are enough to give a clear picture and avoid problems.

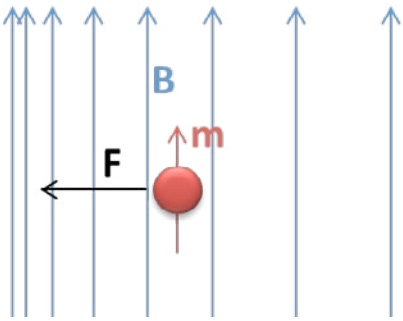
Magnetic force is generated by non-uniform magnetic fields

A constant magnetic field DOES NOT generate a magnetic force over a magnetic field, but only a torque. To generate a magnetic force we need a magnetic field changing the magnitude and/or direction in the space. A magnet block does not generate a magnetic force because it generates a high magnetic field, but because it creates a strong magnetic field gradient.

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

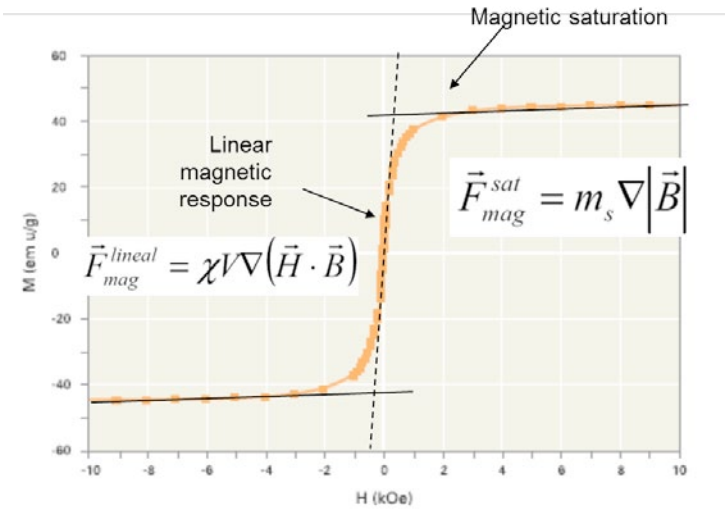


$$\mathbf{F} = \nabla (\mathbf{m} \cdot \mathbf{B})$$



The magnetic force depends on how changes in the magnetic moment of the bead/particle

Magnetic beads and particles are highly susceptible. That means that the magnetic moment changes significantly when a small magnetic field is applied, the magnetic response being linear. However, when the field increases beyond a certain value, the magnetic bead becomes saturated and the increase in magnetic moment is almost zero for additional increases in the magnetic field. The expression for the force is different in both cases.



These two concepts illustrate why classical separators do not define a single magnetic force value. As both the spatial magnetic field variation and the field itself change with the distance, the force is different at every point of the space. Far from the retention area, the beads are in the linear magnetic response region (low field) and the force is proportional to the gradient of B^2. If the magnet is strong enough, the beads are saturated in the retention area. Depending on the geometry, the gradient can be very high.

The Advanced Biomagnetic Separation systems manufactured by SEPMAG generate a magnetic field pattern with both a high magnetic field and gradient. This means that all the beads experience the same magnetic force and the separation process is performed under precisely defined and homogenous conditions. Working under these conditions means that the force is greater farther away from the retention area (the homogenous force is much higher than in classical separators), which reduces both separation time and losses. At the same time, the force in the retention area can be gentler that in non-homogeneous devices (the homogenous force is usually calculated to retain the beads when the supernatant is removed, but not higher), reducing the risk of irreversible aggregation and, in cell sorting, increasing the viability of the captured cells.

Under homogenous Biomagnetic Separation conditions, monitoring the process makes it possible to compare different conditions. The effects of magnetic beads characteristics, concentration or buffer viscosity are clearly identifiable. Given that the magnetic force is constant throughout the sample volume, changes in separation dynamics can be directly related to changes in the suspension.

Chapter 3
Why monitoring Biomagnetic Separation processes?

Biomagnetic Separation has numerous applications in Life Science. From cell sorting to molecular diagnostics, this technology can be used with volumes ranging from a few nanoliters (lab-on-chip) to tens of liters (production of IVD-reagents).

To control the process, technicians rely on the fact that the magnetic bead suspension is usually dark. During the separation process, the solid phase moves to the retention area and the buffer becomes transparent. This makes it clear when the process has finished either by sight or, when more precision is required, using a spectrometer.

The main limitation of these approaches is that they only allow analysis of the final result, telling us whether the separation is complete after a given time. A visual inspection provides no information about what happened during the process and it is not possible to detect a faster-than-expected process if the separation time is fixed.

Why monitor Biomagnetic Separation processes in R&D?

For technicians using traditional magnetic separators, real time-monitoring was not an issue, given that this kind of information would be very difficult to interpret. Because the magnetic field and the gradient change depending of the position of the magnetic bead it is difficult to analyze changes in the process dynamics. Variations in magnetic behavior due to changes in the suspension/beads are different at different points in the working volume, making the data difficult to interpret.

However, when a Biomagnetic Separation system with constant magnetic force is used, the information provided by real-time monitoring is far easier to analyze. Because the contribution of the applied field pattern is the same throughout the working volume, magnetic bead dynamics depend directly on magnetic moment and diameter (the latter affects the drag force). Changes in the viscosity of the buffer caused by variations in temperature or composition are clearly visible because the separation speed changes. Continuous monitoring also makes it possible to optimize protocols with curves that can be extrapolated and the results can be extrapolated to different concentrations and the interaction between the beads can be studied.

Why monitor Biomagnetic Separation production processes?

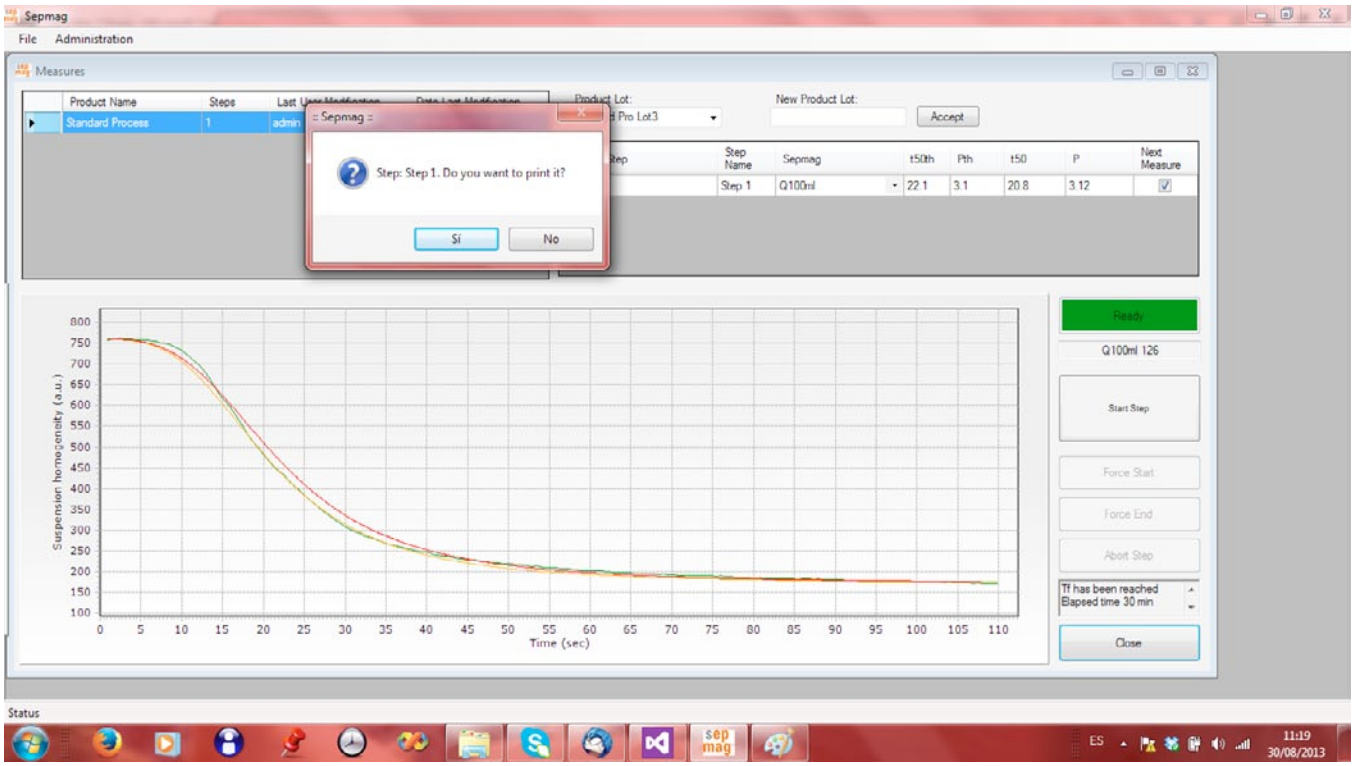
When the Biomagnetic Separation process is transferred from the R&D laboratory to the production facilities, Quality Control becomes a major concern. Validation of the process conditions is the first issue, but when a homogenous magnetic force is used this is not a problem. Once the value of the force has been tested in R&D or during the validation stage, the production system is given similar values to guarantee that there are no losses or irreversible aggregation problems.

With constant force systems, the separation time can be adapted to the new vessel diameter. Given that the speed is proportional to the magnetic force, the time needed for the farthest beads to reach the retention area is t=distance/speed.

However, in production processes, use of the separation time alone involves a risk of approving faster batches than those validated, because the suspension transparency will be correct at the separation time. For example, if magnetic beads are aggregated before starting the separation, the batch might appear to be correct, but the resulting product would cause quality issues in subsequent steps of the manufacturing process.

When the separation process is monitored, the situation is different. Among the quality issues detectable through real time monitoring are bead aggregation, incorrect concentration, variations in bead diameter and magnetic charge, buffer viscosity changes (caused by incorrect composition or by differing temperatures).

To conclude, even if Biomagnetic Separation users have not traditionally monitored the process, they are important reasons for doing so at both R&D and production level. In the past, the main constraint on monitoring was the lack of controlled conditions for the process. Using advanced Biomagnetic Separation systems with a constant force throughout the working volume overcomes this limitation and makes it possible to benefit from the information provided by real-time monitoring.



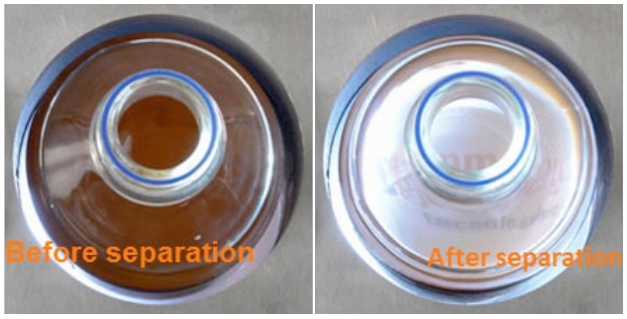
Chapter 4
Monitoring Biomagnetic Separation processes: options and choices.

The standard method for developing and validating Biomagnetic Separation processes is sampling the supernatant at different times. This sample is usually measured using a spectrophotometer. Bead concentration is determined by selecting the right wavelength to avoid interferences with the biomolecules presents in the buffer and comparing it with a calibration curve. The separation time is selected when the number of beads approaches zero. Magnetic susceptibility using the fundamental frequency or some of its harmonics has recently been proposed as alternative.

One of the problems with these approaches involves the sampling method, because the concentration differs at different points of the working volume. How and from where the sample should be taken is a controversial question.

Obtaining real-time information about the entire working volume (ml at the laboratory, liters at production) is a suitable option for checking the evolution of the process. To achieve this goal without interfering with the Biomagnetic Separation process, optical measurements seem to be a better candidate than magnetic techniques.

The starting suspension, in which the magnetic beads are homogenously distributed, is opaque (unless concentration is very low), however when the solid phase is separated, the buffer becomes transparent. Measuring the light transmitted through the vessel gives a real-time measurement of the evolution of the Biomagnetic Separation process. When constant magnetic force is used, the beads move radially from the center, so that measuring transmitted light gives a good picture of the process evolution.

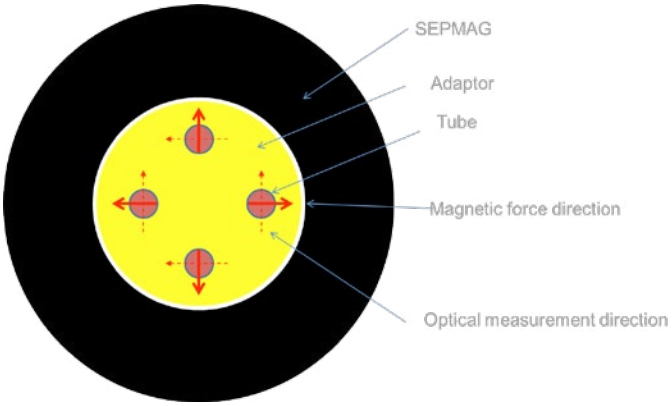


This method can be used with different wave lengths, using spectrophotometry if necessary. It is likely to improve accuracy, however, the system needs to be tailored to the bead/particle and suspension. The correct wavelength for a 50 nm magnetic particle is not the same as that for a 1 micron magnetic bead. A simpler approach, measuring white light transmittance, has proven effective for a wide range of diameters and buffers.

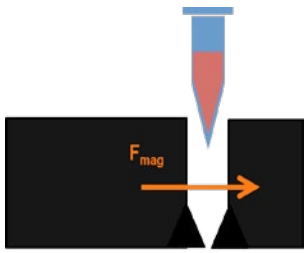
Using the constant magnetic force of Advanced Biomagnetic Systems makes it simple to optically monitor the evolution of the process. Also, as you will see in later chapters, these systems make it easy to interpret the information obtained and use it to obtain information about the process and/or control its performance.

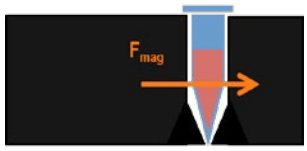
Chapter 5
Monitoring Biomagnetic Separation processes in small tubes

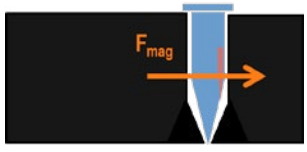
One of the problems of working with small tubes and classical magnetic separators (or simple magnets) is the lack of definition of the magnetic force. As the magnetic field and its gradient changes with distance, the force on the magnetic beads is not constant and variations in the behavior of the suspension are difficult to interpret.



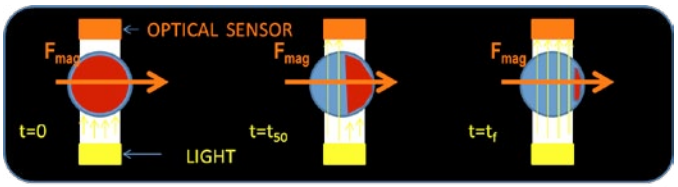
This problem is avoided when advanced Biomagnetic Separation systems are used. In these devices the magnetic force has constant magnitude and radial direction. Working conditions are fixed, therefore changes in the separation processes can be directly related to the characteristics of the magnetic beads/particles and/or the buffer. This is why academic researchers using SEPMAG systems call them 'precision magnetophoresis devices'.

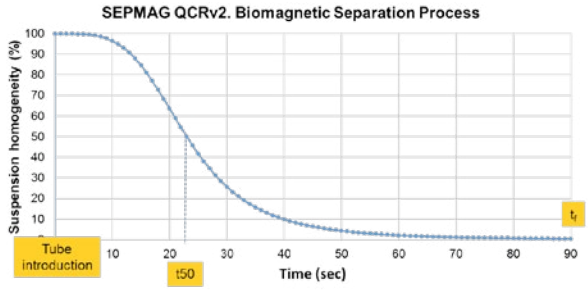






When using small tubes (we will discuss large volume in next chapter) the sample is inserted in





the Biomagnetic Separation systems using an adaptor. The typical geometry is revolver-like, and the tube holder is positioned in an area where the beads are magnetically saturated. The constant magnetic field gradient means that the force is constant.

As shown in the figure, the constant magnetic force causes all the beads to move at the same speed. The beads at the front move at a constant speed, generating a growing area that looks transparent and which contains buffer, not beads. Aligning the light emission and the sensor perpendiculars to the magnetic force direction, gives a response that varies from 100% opaque (in the beginning) to 0% (maximum transparency).

The resultant curve describes the Biomagnetic Separation process with an unparalleled level of detail. Researchers can ascertain the behavior of different parameters. For example, increasing the concentration means a shorter separation time, but also a steeper curve. These measurements have given us an understanding of the cooperative mechanisms governing the magnetic bead separation [JS Andreu et al, Physical Review E 84 (2), 021402, 2011]. Other researchers have used these measurements for

analyzing the different behavior of magnetic beads in different steps of the synthesis route [Benelmekki et al, Soft Matter, 2012, 8, 6039-6047].

Real-time monitoring of the Biomagnetic Separation process opens up a wide range of possibilities: from basic research questions to optimizing protocols, from checking the properties of new magnetic particles to testing whether a new coating protocol induces aggregation. Because the curve is continuous, it can be parameterized, parameters can be correlated with changes in the suspension, theoretical models can be built and the experimental hypothesis checked.

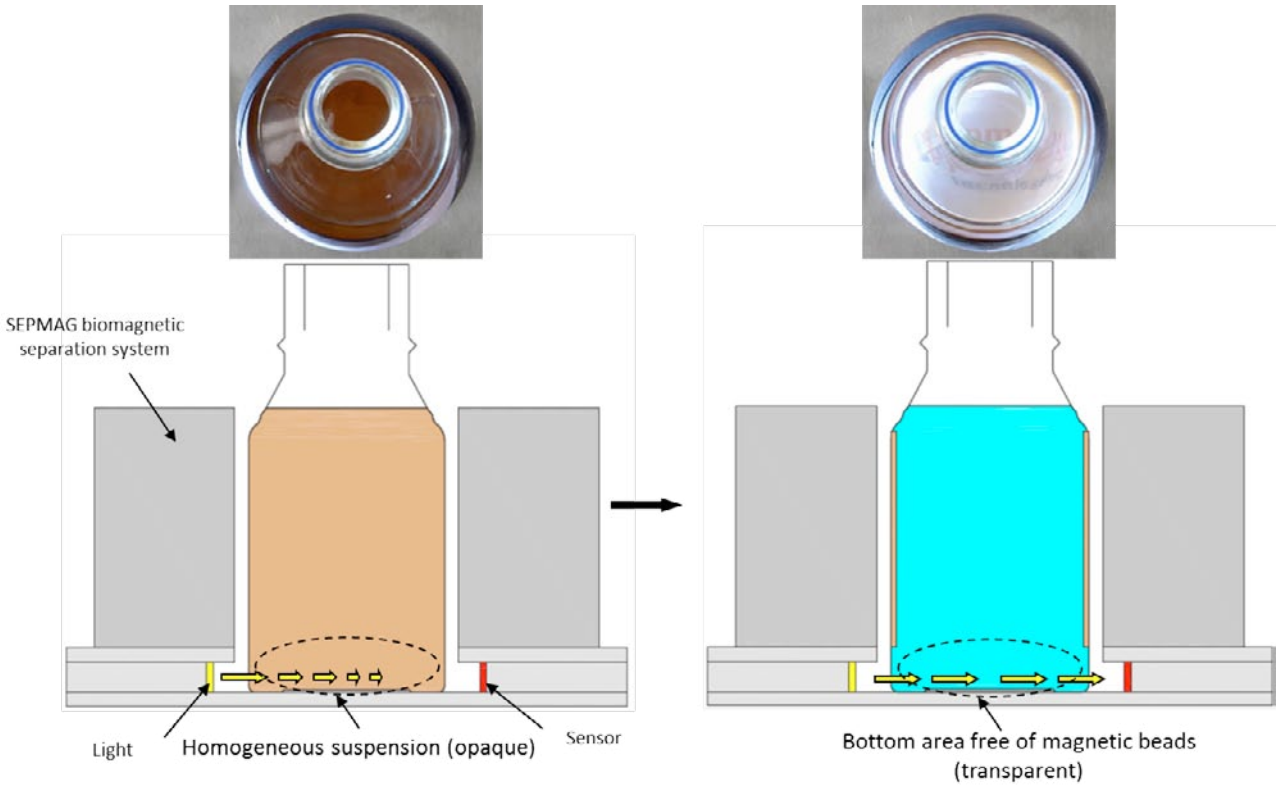
There are still many unanswered questions regarding the details of Biomagnetic Separation processes. This monitoring methodology gives researchers a new tool for answering those questions.

Chapter 6
Monitoring Biomagnetic Separation production processes

The traditional way to check whether a Biomagnetic Separation Production process is complete is by sight. The technician/researcher looks at the suspension. At the beginning of the process the suspension is homogenous and opaque. When the separation process is complete, the magnetic beads are left on the walls of the vessel and the supernatant is transparent. When the suspension is 'transparent', the technician stops the process by extracting the supernatant, leaving the magnetic beads in the bottle. After repeating the same process several times, a separation time can be defined and used as a benchmark. With the traditional method, the only quality control record is the OK/no OK signed by the person handling the vessel with no supporting data. In case of a quality issue with the product, this may not be detected until a later stage, and there is no data to show whether the problem occurred before, during or after the Biomagnetic Separation.

In contrast, by continuously monitoring the opacity of the suspension while inside the Biomagnetic Separation system, technicians have a record of its evolution during the process. Changes in opacity inside the biomagnetic separator should be the same if the suspension is the same. Changes to the properties of the magnetic beads (diameter, magnetic charge), concentration and/or viscosity of the buffer lead to different opacity, so any deviations from the expected pattern serve as an early warning system.

If the process is documented with printed reports for every stage, acceptance parameters can be defined objectively, without subjective operator judgement. Moreover, cumulated experimental data allows teams (technicians, researchers and managers) to audit, analyze, revise and improve the Biomagnetic Separation steps of their manufacturing processes.



Chapter 7
Parameterizing Biomagnetic Separation curves

The purpose of monitoring a Biomagnetic Separation is to obtain a record of different processes to be able to compare the results. Using homogenous magnetic force, changes can easily be related to any modifications made to the suspension. Changes to the magnetic bead specifications (diameter, magnetic moment), concentration, and variations in buffer viscosity suggest different separation process dynamics.

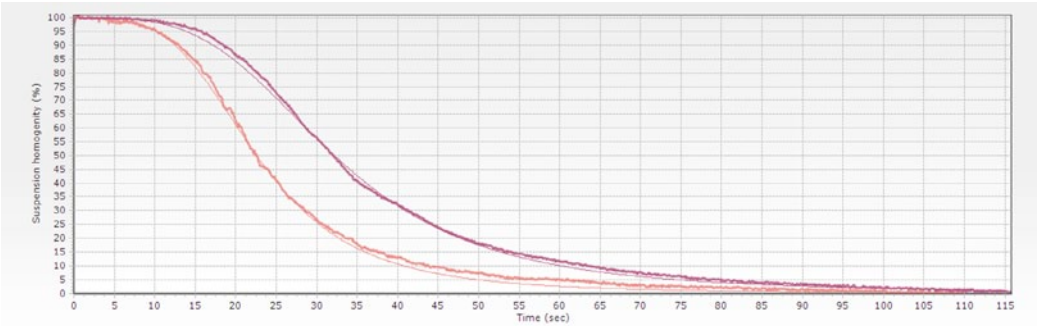
As discussed in previous chapters, the traditional method for monitoring the Biomagnetic Separation process is to measure the suspension opacity. However, in order to have a systematic, objective comparison method, it is desirable to parameterize the resultant curve.

When SEPMAG started monitoring Biomagnetic Separation processes a decade ago, we assumed that the behavior would be an exponential decay. But our early experiments revealed a poor fit between the experimental data with this expression. This discrepancy forced us to do more research into the mechanisms underlying the process. Based on the premise of the constant magnetic force, researchers of UAB and ICMAB-CSIC helped us to understand the cooperative nature of the Biomagnetic Separation process and why the separation does not decay exponentially [Andreu et al, Journal of Nanomaterials Article ID: 678581 (2012)].

Further experimental work demonstrated that the typical Biomagnetic Separation curve can be successfully fitted by a sigmoidal function. The behavior can then be described by just four parameters: opacity at the starting time (when t=0), +final opacity (at t=infinite), the slope (defined by the dimensionless exponent ‘p’) and the half-separation time (t50, expressed in seconds).

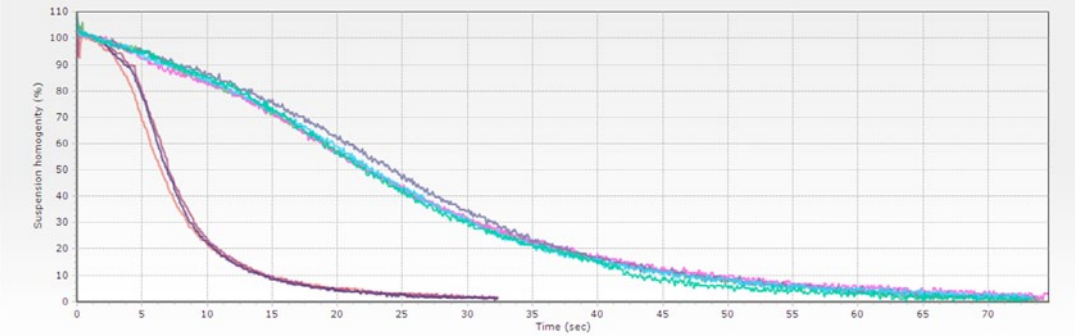
A typical example is to determine whether the magnetic beads are aggregated –something that may happen if they have not been stocked in the right conditions. If the transparency is only checked at the specified separation time, good and bad batches will seem both OK, as the final state is exactly the same. However, the monitoring curves tell a different story. The separation of the second batch (lighter line) is faster than the original one. We can quantify the difference by taking the t50 value of the sigmoidal fitting: for beads acting as expected, the t50 is 32 seconds, but for the batch with aggregates the t50=22 s, which is 30% faster! Notice that irreversible aggregates act like larger diameter magnetic beads, meaning quicker separation. The differences between both batches after 100 s are very difficult to detect, because they are within the experimental error, but parameterizing the curve, t50 gives us a simple way to compare both processes and identify irreversible bead aggregation in the faster separation.

Sigmoidal fitting also allows us normalize the measurements, making it easy to compare different magnetic beads concentrations. Using the experimental measurements, the starting opacity would be very different. Lower concentration means lower opacity. Using the opacity at t=infinite as zero and the opacity



at t=0 as 100%, the comparison is straightforward. When the Biomagnetic Separation process is due to cooperative behavior (each bead interacts magnetically with its neighbors), lower concentration means weaker interactions and a slower process. In the example we can see how when the same magnetic beads are diluted from 1% to 0.1%, t50 increases from 7 s to 24 s. The slope of the sigmoidal also changes, becoming smoother when diluted. It changes from 2.7 to 2.0 (the graph shows different assays for each concentration).

We can conclude that the curves obtained by optical monitoring of the biomagnetic+ separation at a constant magnetic force are a powerful tool for identifying changes in the process. But if the curves are fitted to a model, we can quantify the variations and use this information to correlate them with the changes in the suspension (beads or buffer).



Chapter 8
Using monitoring data for managing Biomagnetic Separation processes.

When Biomagnetic Separation is used in production processes, quality control becomes a priority. The first step is to define and validate the process, but then the key point is checking the repeatability of every single batch.

To define and validate the process it is important to know the working conditions. To exert a constant force over the entire batch volume, the magnetic field pattern needs to fulfill two conditions:

- All magnetic beads must be in the same state (saturated or constant susceptibility)
- The spatial magnetic field variation should be a constant gradient (if beads are saturated) or a constant gradient of the square of the field (if beads have constant susceptibility).

Having a constant force over the entire batch volume ensures in-batch consistency. If generated by permanent magnets, the magnetic field pattern is the same for every batch.

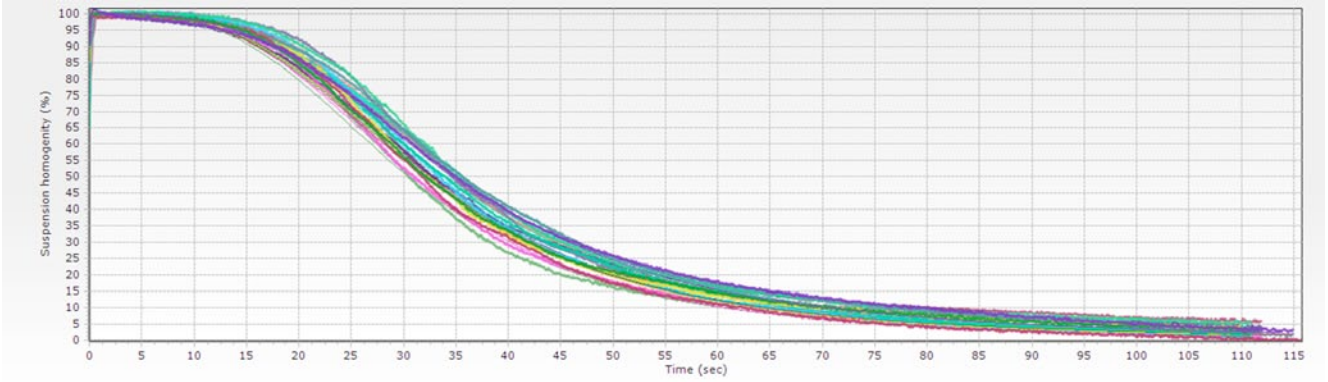
In such conditions, quality problems only appear in the magnetic bead suspension. As the characteristics of the beads and/or the buffer may change from batch to batch, the purpose of monitoring the process is to quickly detect any variations in the suspension through changes in the Biomagnetic Separation behavior. To do this, we need to define a 'standard' curve to serve as reference.

One way to define the standard curve for a given process is to collect the data from the first batches and use this data to define the process. The example shown in the figure plots 15 validated batches. The resultant parameters for the separation curve are t50=33.5 (σ=1.7) and p=3.25 (σ=0.08).

The acceptable tolerance for the parameters is still controversial. Some users consider that the obtained separation curves are more sensitive to variations than the final application of the beads (usually CLIA). This means that over-tightening the acceptance criteria may lead to batches being discarded that may be within the tolerances of the diagnostic test.

The issue is still open, but most industrial users of the monitoring technology prefer not to disclose details of this subject, considering the results are sensitive, proprietary information.

In any case, the parameterization of the curves makes it possible to build standards that enable production and quality assurance teams to discuss the acceptance criteria with quantitative data. A far less controversial method than the OK/ NO OK alternative used when Biomagnetic Separation depended on suspension transparency evaluated visually at the fixed separation time.



Chapter 9
Conclusions

This eBook looks at how to detect inconsistencies in Biomagnetic Separation process. We have reviewed the basic concepts, focusing on how to achieve homogenous separation conditions, in other words, how to exert a constant magnetic force over the entire working volume.

After defining Biomagnetic Separation conditions, we have discussed the reasons why the process should be monitored. We have seen that R&D needs to make an objective comparison of the behavior of different suspensions, and production facilities working with magnetic beads (as the CLIA-IVD kits manufacturers) to check that every batch is equal and does not deviate from the established quality acceptance criteria.

To fulfill the need to monitor Biomagnetic Separation, we have discussed the different options and concluded that optical methods seems to be the best option, as they do not interfere with the process. Opacity measurement seems simpler (and cheaper) than spectrophotometry, as well as being accurate enough for monitoring most of the magnetic beads and particles.

The proposed solution has been described not only for monitoring Biomagnetic Separation processes in small tubes (lab scale) but also for the large volumes used in production processes. These different applications require different approaches and setups that have been outlined.

Finally, we have discussed in some detail how to parameterize the Biomagnetic Separation curves obtained with the monitoring technology. We have concluded that if the curves are fitted to a model, we can quantify the variations and use this information to correlate them with the changes in the suspension (due to the beads and/or buffer). Parameterization also makes it possible to build standards that help production and quality assurance teams to discuss the acceptance criteria with quantitative data.

Summarizing and monitoring the Biomagnetic Separation are new tools for investigating, understanding and controlling the process, enabling researchers and engineers to approach the subject in an objective, quantitative manner.

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