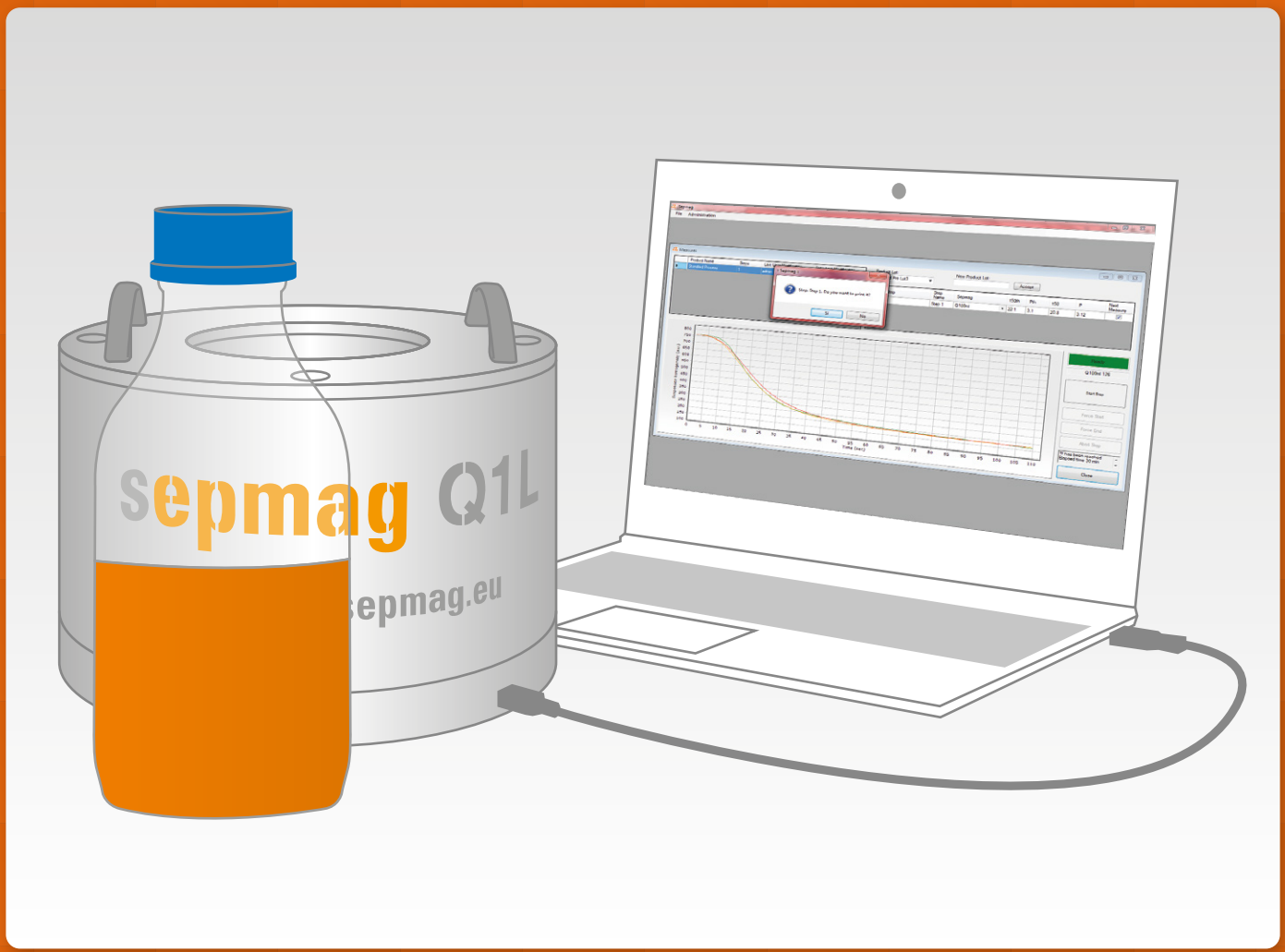
7 Keys to Successfully Scaling-up Biomagnetic Separation Processes



Sepmag®

SUMARY

Introduction

The last two decades have seen an explosive growth in the use of magnetic beads in Life Science, with sustained double figure sales increase all across the industry. The main driver of this success has been the use of magnetic beads as a solid phase on Chemiluminescence Immunoassays (CLIA) kits. Thanks to its easy automation, this technique has become the preferred choice for high throughput In Vitro Diagnostic.

The commercial success of CLIA analyzers has forced the IVD-kit manufacturers (and magnetic beads manufacturers) to ramp production for coping with the demand. The early strategy was to increase the number of lots by keeping them at the same volume and maintaining the same operational procedures. The problem with this approach, however, is that it needs a proportional increase of highly skilled workers. In addition to that, strict quality controls would be needed to ensure that each single lot falls within the acceptable range of functionality and yields. Producing 100 lots of 100 ml for getting a total of 10 L of magnetic bead suspension needs to prove that each single 100 ml lot is equivalent for guarantee that each single IVD-kit falls inside the accepted tolerances. The high costs of this approach force manufacturers to explore simpler and cost-effective ways to cope with the increasing demand.

The alternative approach is to keep the number of batches almost constant but increase the volume per batch. Increasing the lot volume allows companies to maintain labor and QC costs. If correctly implemented, the repercussion of labor and QC on the individual kits will decrease proportionally to the batch volume. However, in order to succeed, you will need in-lot consistency that makes every single aliquot (usually below 1 ml) compliant with the required tolerances. Scaling-up Biomagnetic Separation processes is not just simply using a bigger magnet. It is far more complicated.

The good news is that, at SEPMAG, we have helped many companies scale-up processes up to tens of liters. Since these companies keep on buying more of our systems to keep increasing the volume, we can assume that our work has proved to be successfully helpful and cost-effective.

In this ebook we try to summarize more than 10 years of experience in scaling up Biomagnetic Separation processes. Most of our customers are leading IVD-companies, and magnetic bead manufacturers. Nonetheless we also aid innovative start-ups and academic institutions. We hope the 7 points listed below can help you to develop new processes and break the incorrect paradigm that Biomagnetic Separation is efficient only at small volumes.

1. HAVE A RIGHT START

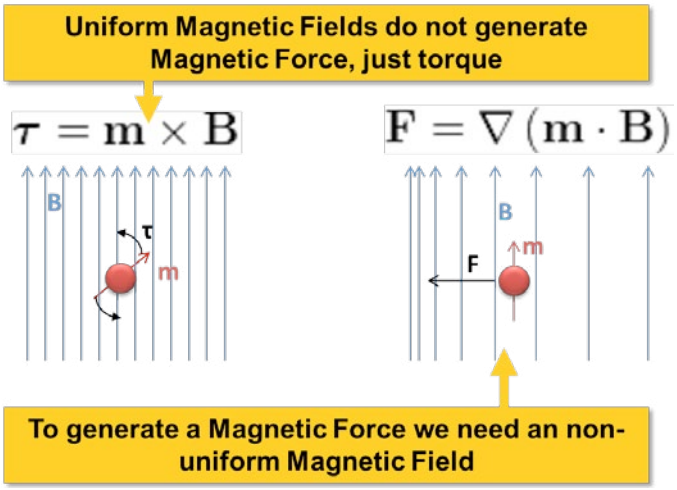
Developing magnetic beads based products is a long process. For a diagnostic kit, a protein purification product or a new application, it's necessary to select the right biomarker, the coating surface and the magnetic bead. These three aspects are usually checked with great detail while looking for the right characteristics and protocols. Unfortunately, many times, little attention is paid to the biomagnetic separation process itself. In most cases, all the development is performed using the available small volume magnetic separator or, sometimes, just a small magnet.

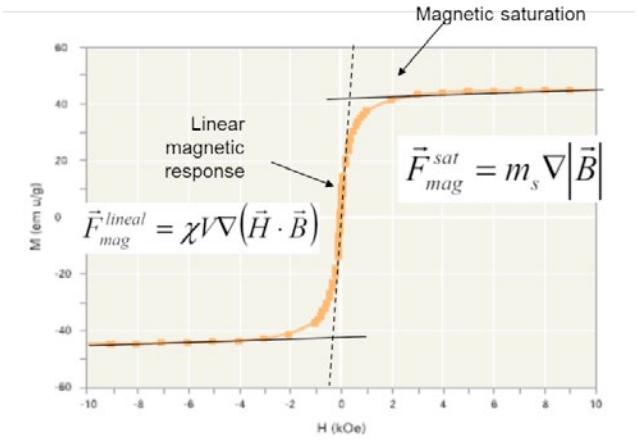
If the development is smooth, the magnetic separators seem to be an unimportant issue. The problems usually appear when the volume of the experiments needs to be scaled up from the usual few milliliters to the tenths of liters. The results seem to be inconsistent when the scale changes, losses increase, attempts of using 'bigger magnets' lead to irreversible aggregation problems, and resuspension becomes a nightmare. These problems are also present at the initial scale, but they tend to be attributed to the lack of performance of the biomarker or the magnetic bead.

The truth is that, in many cases, the problem is on the magnetic separator itself. Developers have paid great attention to specify the characteristics of the antibody/protein, bead size and magnetic content, surface properties, temperature and composition of the incubation buffer. But not to the biomagnetic separation process itself. In most of the cases, the only specified parameter for the process is the separation time for the specific device used.

The right start is to understand the biomagnetic separation process itself (as we did with all the other materials and methods used during the development), then fix the parameters governing it. This way, we will be able to specify the separation conditions, evaluate different values of the magnetic force and objectively validate biomagnetic separation process.

2. UNDERSTAND MAGNETIC FORCE





To successfully scale up a biomagnetic separation process is necessary to understand the key parameter governing it. To move a magnetic bead we need to apply a magnetic force over it. This force would make the bead move in a direction and be in equilibrium with the drag force generated by the viscosity of the buffer. The result would be a constant velocity (if the magnetic force is constant).

The first usual misunderstanding is the assumption that the force would be proportional to the magnetic field. Checking any physics handbook you will notice that a uniform magnetic force DOES NOT generate a magnetic force. A uniform magnetic field will only make the beads rotate, not move from their position. The confusion between magnetic force and magnetic field happens since generating a perfectly uniform magnetic is difficult. A permanent magnet generates a field varying on the space, and it's this variation the real cause of the magnetic force responsible of the magnetic beads displacement. However, as stronger magnets generate stronger gradients, many people interpret the observation as a direct correlation between force and field.

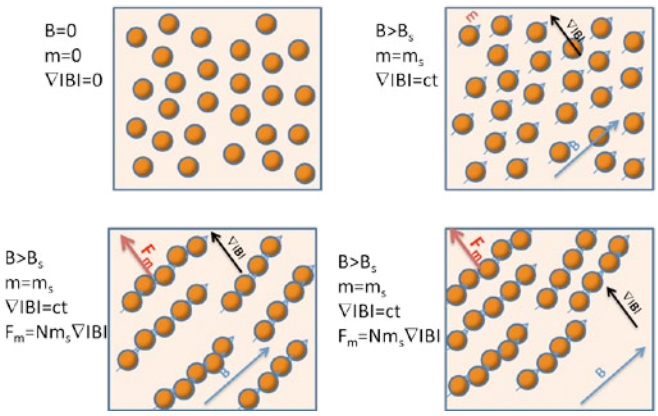
By taking a closer look to the expression of the magnetic force we realize that we need to take into account how it varies with the magnetic field and also consider the magnetic moment of the bead. The magnetic moment of magnetic beads or particles typically varies at low fields and is saturated at high fields. Thus, the expression of the force is different in both field regions. When the main characteristic is of constant susceptibility (the magnetic moment changes proportionally to the applied field), the magnetic force is proportional to the spatial variation of the square of the magnetic field. When the magnetic beads are saturated (i.e. the magnetic moment is constant), the magnetic force is proportional to the gradient of the magnetic moment.

Knowing the factors controlling the magnetic force is key for understanding the biomagnetic separation process, defining the right procedures and correctly validating the operational procedures.

3. KNOW HOW YOUR PARTICLE MAGNETIC BEHAVES

To successfully scale-up a biomagnetic separation process it is necessary to understand how the magnetic beads behave. The separation speed depends on the balance between the magnetic force (generated by the field pattern and the moment of the beads) and the drag force (caused by the buffer viscosity). Thus, it is important to understand how this two forces act on a real magnetic bead suspension.

The magnetic beads behavior is usually described in textbooks as the competing effect of the two described forces over a **single bead**. The problem with this approach is that it does not describe the separation process as it really occurs. Using a constant magnetic field gradient it's easy to calculate the separation speed, what is usually in the range of hours by centimeter. However, real experiments show a faster behavior, completing the separation in few seconds.



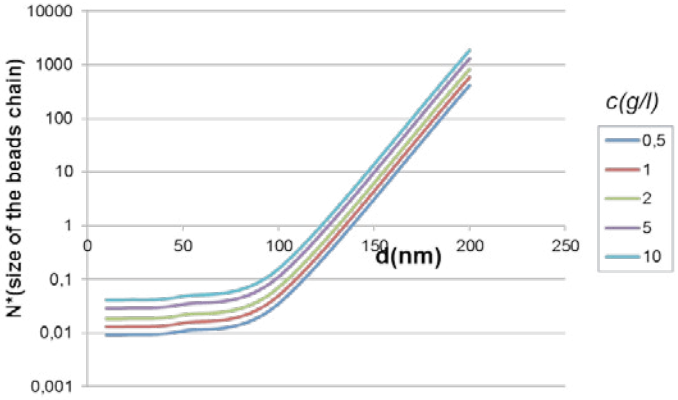
The flaw of the described interpretation resides in the assumption that magnetic beads move individually. This is only true for very small particles and/or beads with very low magnetic moment. However, most of the practical applications use beads with diameters over 500 nm and more than 30% magnetic pigment contents. With these characteristics, when a magnetic field is applied, the beads become magnetized. If the field is big enough (higher than the saturation field value, typically over 0.1 T for most magnetic iron oxides), the beads become fully magnetized and act as small permanent magnets. As a consequence, they tend to align north and south poles, forming chains.

The chains will move in the direction of the magnetic field gradient (that can be different of the magnetic field). As the chains have larger moment and **lower ratio drag/magnetic force** than single magnetic beads, the separation speed is faster, leading to the short separation times responsible for the popularity of magnetic separation in Life Science.

It should be noticed that the d ipolar magnetic interaction is competing against thermal agitation. When the magnetic moment is small, the thermal agitation would avoid the chains formation and the beads would move individually. That would happen when the magnetic moment of the individual beads is small. Having the same proportion of magnetic content over all the bead weight or volume, small beads will have smaller magnetic moment. A second reason for low magnetic moment can be if the applied field is below the saturation value. In this case, the magnetization of the beads changes with the field (the proportionality constant is the magnetic susceptibility) and would be less than saturation magnetic moment. If the value is small enough, the magnetic interaction energy would not be able to overcome the thermal agitation

The cooperative nature of the biomagnetic separation implies that the magnetic beads concentration matters. Higher concentrations mean closer neighbor beads, easier interaction and chain formation, thus faster separation. By contrast, very dilute samples difficult the dipolar magnetic interaction, slowing the separation.

Understanding the cooperative nature of magnetic bead movement is important when selecting the right separation conditions. The option of decreasing the beads' diameter to increase the specific area (and binding capacity) should be carefully weighed against its effect on the separation speed. The advantages of decreasing the magnetic pigment content to reduce density (and thus sedimentation) could compete against the reduction of the separation speed. By contrast, increasing the concentration is an easy way to accelerate separation without the need of changing the coating and/or magnetic beads. In any case, the deeper the knowledge about the magnetic beads' behavior is, the easier it will be to correctly define the separation process and establish the experimental protocols regardless of the scale.

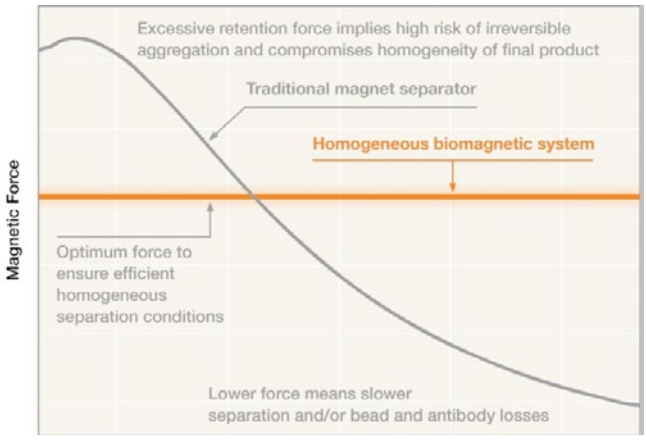


4. SELECT THE RIGHT BIOMAGNETIC SEPARATION CONDITIONS

To scale-up a Biomagnetic Separation process, selecting the correct working conditions is beyond paramount. Almost any set of conditions may appear to work well enough at very small volume. Classical magnetic separators generate inhomogeneous magnetic force, having some beads magnetically saturated on regions near the retention areas and non-saturated beads in the rest of the working volume. For tubes of one milliliter or less the separation may apparently seems working fine, as the irreversible aggregation problem would not be noticed, the separation time is short and the magnetic beads losses not appreciable.

However, when the volume scales-up to hundreds of milliliters or liters, the problems (high losses despite long separation times, irreversible aggregation of the beads) become evident. To avoid these unpleasant surprises it is very important to select the right biomagnetic separation conditions at the early steps of the project.

The best way to keep consistency at different volumes is to validate the process using homogenous magnetic force, i.e. having all the beads in the same biomagnetic separation. For this we need to fulfill two conditions: a constant magnetic field gradient and, simultaneously, a field big enough to saturate the magnetic beads. On this way, the process is defined by the field profile (the gradient value) and the conditions can easily be scaled-up.



Note that the exact force value would depend on the bead magnetic moment, thus the optimal value of the gradient would be different for small magnetic beads or for bigger one. The force should be big enough to retain the beads when the supernatant is extracted, but gentle enough to avoid irreversible aggregation.

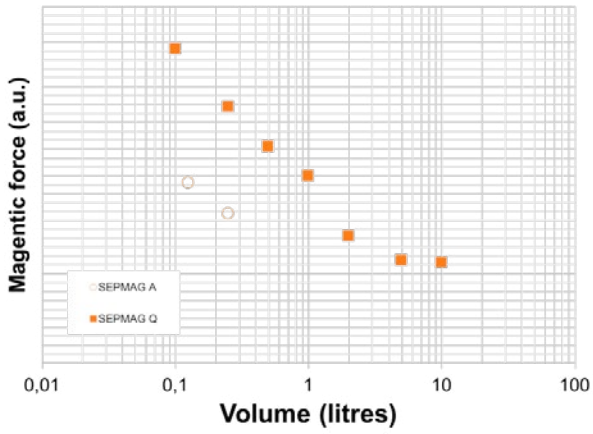
With this approach, all the magnetic beads in the working volume would experience the same force, regardless their initial position. Moreover, the separation time is shorter compared with classical separators, as the farthest beads experience a much higher force (remember: separation speed is proportional to the magnetic force)

If the force is kept constant when the process is scaled-up, the separation speed would be the same. Thus, the separation time will scale proportional to the distance travelled by the farthest magnetic bead. The only potential concern would be if increasing the time nearest beads would be retained can generate irreversible aggregation. As the retention force is gentler than in classical separators, the probability is almost zero. The effect of longer separation times can easily be experimentally assessed at small volume: extracting the supernatant can be performed not when the separation is complete, but delayed until the estimated separation time at large volume. As the magnetic beads experience the same force during the same time in both volumes, this simple experiment may reassure about the lack of irreversible aggregation problems before investing in the large volume equipment.

Selecting the biomagnetic conditions as described (homogenous magnetic force), the problem of scaling-up the process disappear. The behavior of the magnetic beads would be the same, regardless the working volume, as the conditions are well defined. In consequence, we can correctly validate the process at small scale and transfer to larger scale without more worries to reproduce the defined conditions.

5. SCALE UP THE CONDITIONS TO THE NEW VOLUME

Almost all life science magnetic beads projects start at small volume. The high cost of the biomolecules (antibodies, protein, nucleic acids….) and the uncertainties involved –what would be the right surface and protocol to coat the beads- make sound to work initially at scales of few milliliters.



As previously discussed, the problem is not working at small scale, but that we don't pay careful attention when defining the biomagnetic separation conditions. If we leave this task for later stages of the development, we may find important bottlenecks for the scaling-up and, many times, jeopardize the whole project, as the initial conditions may not be scalable at a reasonable cost.

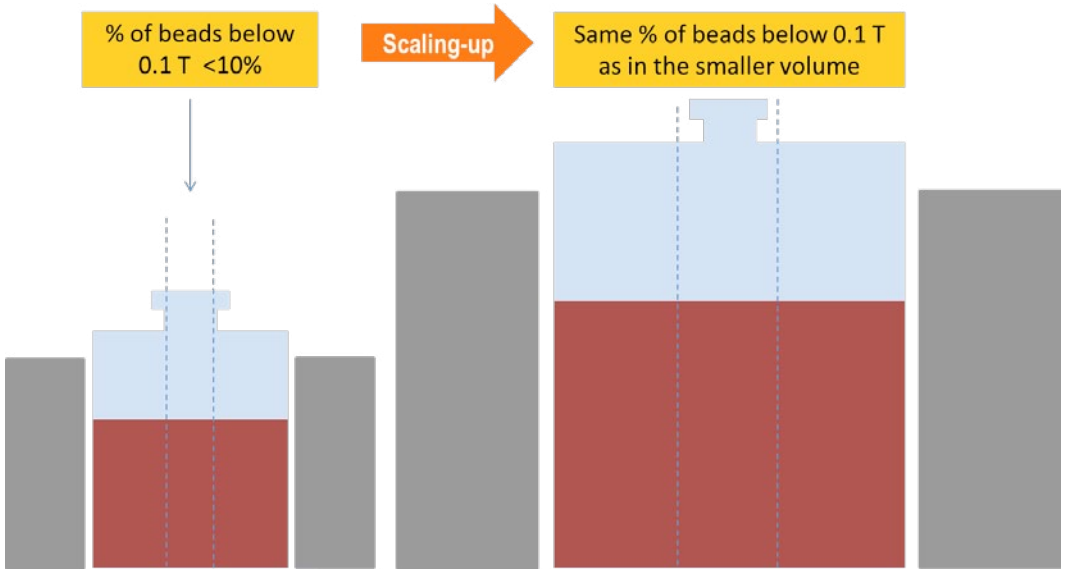
They exist two ways to scaling up the biomagnetic separation conditions from laboratory scale to production (or from millilitres to tens of liters). The first one is to use the same magnetic force at small volume than we will use at the final scale. Advanced biomagnetic systems are usually presented in different families. Is the case of SEPMAG® A and SEPMAG® Q. The A systems permits to work with volumes from ml to 125 or 250 ml (depending on the model) with the same magnetic force as 1 to 2 liters Q-series. Then pro-

cesses developed in SEPMAG® A systems in milliliter scale, can be tested at 125-250 ml using the same device and then directly transferred to volumes of 1-2 liters just using SEPMAG Q.

However, this strategy is not always possible. The weight of biomagnetic separation systems augments faster than the working volume, making sometimes not economically feasible keeping exactly the same magnetic forces at all the scales. This is a typical situation on R&D, where the need to make as many separation as possible often leads to choose fast separation speed as the main criteria for selecting the small volume biomagnetic separation conditions. When the R&D phase is completed and the focus moves to manufacturing, the productivity is mainly driven by the batch volume, not the separation time (the separation time is just a fraction of the process: inserting/extracting the bottle, pumping-out the supernatant, adding the clean buffer, perform Quality Control test… all this operations would consume much more time).

To keep the scaled-up process on a reasonable budget (and having a system with weight and dimensions fitting in the existing facilities) the key is to have the magnetic beads saturated. If the new force is enough to retain the beads while the supernatant is removed, the biomagnetic separation conditions would be the equivalent: the beads would be interacting with their neighbors in the same way as in the small volume, overcoming the thermal agitation. Having all the beads interacting as in the small volume guarantees that the chains would be formed –remember that fast separation is driven by this cooperative behavior- and the separation speed would be proportional to the gradient. Then separation time would be slower, but in the same range as the small volume. If we have a validated process with a separation time of 30 seconds, we can estimate the value when we scale-up to a 10 fold volume by using a vessel 2.5 higher and with double the diameter. Using a larger biomagnetic separation system, with half the magnetic field gradient (to keep weight and cost reasonable), the separation speed would be half the small scale. As the distance the beads need to travel is the double at large scale (the ration between vessel diameters), the new separation time would be 4 times the original one: 120 seconds (2 minutes). Note that the 90 seconds difference would be not relevant if you compare with 10-fold time you will need to pump-out the supernatant if you keep the extraction flow constant.

As discussed, using advanced biomagnetic separation systems, with constant force, makes the scaling up straight-forward, just by using the same force value at different volumes or, when not possible, keeping the same magnetic beads state. This allows avoid risks when change scale, and guarantees the process scalability from millilitres to tens of liters.

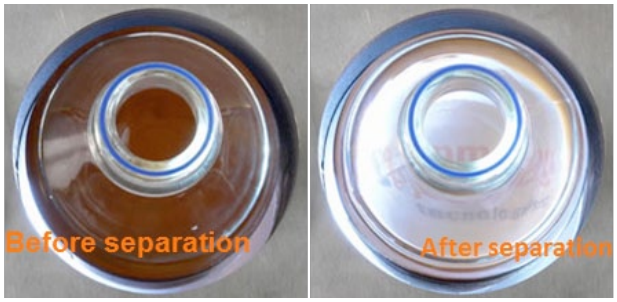


6. MONITOR THE PROCESS

Successfully scaling-up biomagnetic separation processes relies on determining the right working conditions. Having a constant magnetic force in the whole working volume guarantees the in-lot consistency, but manufacturing also needs to guarantee the lot-to-lot consistency.

The consistency of a lot is usually determined at the end of the manufacturing process, checking the functional properties of the product, as it can be the RLU in CLIA kits.

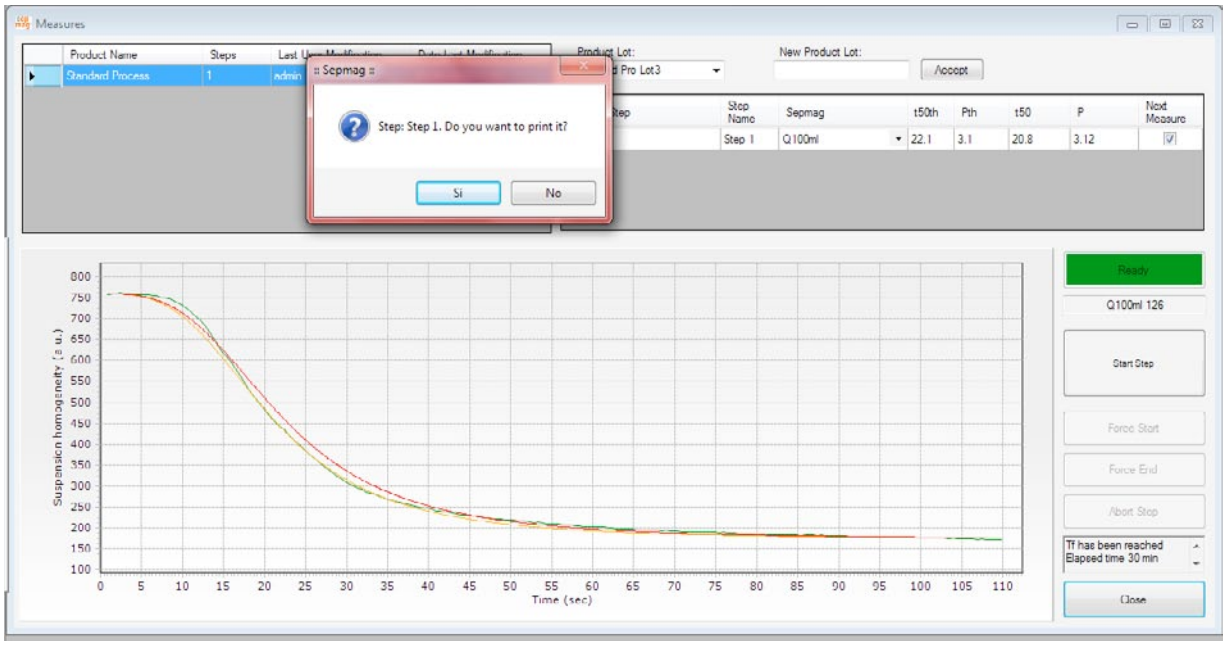
The control over the biomagnetic separation steps of the process is usually eyesight. The technician responsible of the process looks if the suspension has become transparent at the specified separation time. This approach has several flaws. First, it only gives information about the state of the suspension at separation time, but does not gather data about how the transparency evolves during the process. If the separation has been faster than it should (an indicator of wrong concentration, bead diameter or magnetic moment), the transparency at the separation time would

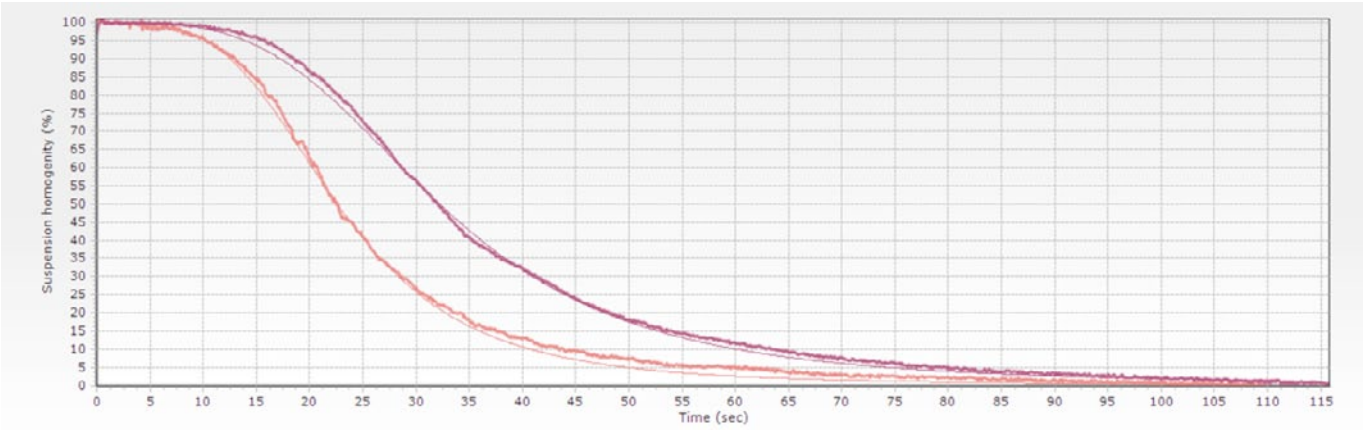


the same, making impossible to distinguish the wrong process from a correct one.

The second flaw is the subjectivity of the measurement. Two different persons may have different visual criteria for considering the 'transparency' achieved, leading to a higher variability for the acceptance of the batch, thus increasing the variability on the resultant product.

If it is a problem on the batch, it would probably not be detected until the latest steps of the process. But then, it would be difficult to determine at which step the problem occurred.





The ideal situation would be to monitor the whole biomagnetic separation process. Since the suspension is dark when homogeneous (before separation) but becomes transparent when the solid phase has been separated, we can record the optical changes during the process. The resulting curves can be used as an objective record of each single lot.

When the biomagnetic separation process is performed using advanced systems, the magnetic force is constant and the field pattern generated by permanent magnets. Using these conditions, changes in the behavior would be directly related to changes in the suspension, and the sigmoidal-like curves can be used as quality control. If the magnetic beads are irreversible aggregated in one separation step, in the next separation step would show faster separation than expected. In the example, the optical monitoring of two sequential washing steps reveals that the second one (lighter curve) shows a different behavior. A naked-eyed observation would probably give a 'pass' status, as both processes had achieved whole transparency at the separation time (115 seconds in the example). The sigmoidal curve, by contrast, quantifies the difference as the reduction of the $t_{50\%}$ (when 50% of the transparency is achieved) from 37 to 22 seconds. This big difference should rising an early quality alert, but also gives us some clues about the problem cause: magnetic beads move faster when the diameter is larger (concentration also can lead to quicker movement, but would also change the shape). As in the example the beads are the same –no new beads have been added between the washing steps-, the 'apparent' diameter increase is probably caused by irreversible aggregates acting 'bigger' beads.

For each process step, standard curves can be generated (defined by the expected sigmoidal shape). The transparency curve of every single biomagnetic separation step can then be compared with its standard. Thus, technicians have a powerful tool to detect if something is wrong during the separation process: wrong magnetic beads diameter/magnetic charge, incorrect beads concentration, problems with buffer viscosity.…).

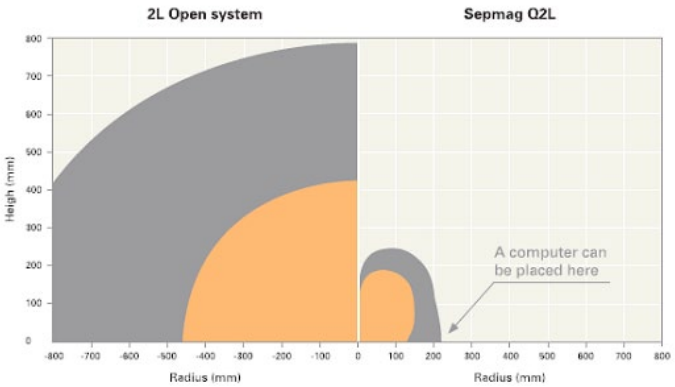
The transparency curves also provide the production team and the QC-manager with an audit tool to objectively discuss and review the issue on the process, saving time, money and discussions. The alerts generated by deviation from the biomagnetic separation standard curves permits earlier corrective actions or, if necessary, discard the batch. For large volume production that means big savings on the unnecessary wasted resources (antibodies, buffer, magnetic beads…) dedicated until final QC detects the problem, but also using the time saved to complete the production on time or with minimal delays. Moreover, all this benefits can be obtained at no cost, as monitoring option is included in all the advanced biomagnetic separation systems.

7. LOOK AT THE OPERATIONAL SAFETY

Scaling up biomagnetic separation process is not just about quantity and quality of the production. One of the main concerns is the operational safety of using 'big magnets'. As most of the classical magnetic separators are assemblies of permanent magnets in an open configuration, the use of large versions of these devices raises legitimate concerns about the risk for the operators and other laboratory/production equipment. As we will discussed later, the problem does not longer exist using advanced biomagnetic separation systems, but understanding the risks is also key for successfully implement in production environment.

Let us just review the definition and level of risk due to the presence of strong magnetic fields. The first level is related with the risk of affecting watches, instruments and magnetic recording materials (include here the hard disk of computers!), and it includes pacemakers and other electronic medical implants. For avoiding this risk, we should define a CAUTION AREA where people fitted with medical devices should never enter, and where you should not place the instruments or materials susceptible to be affected by the magnetic field (note that it includes credit cards and similar). The CAUTION AREA is defined as the region where the magnetic field exceeds 0.5 mT (5 Gauss), roughly 10 times the Earth magnetic field.

However, the main risk for most people is the potential injuries caused by the mechanical movement of magnetic or magnetizable objects due the interaction with separation device. Carrying around steel tools like scissors, screw-drivers, hammers… put a person in risk. The magnetizable object can be strongly attracted by the stray magnetic fields of the separators and harm the person if parts of the body are in the trajectory. As the force increases very quickly when approaching to the field source, when the carriers feels the force is usually too late to react. The magnetic field level to define the DANGER AREA is settled in 3 mT (30 Gauss).



For classical magnetic separators (and still worst for big magnet blocks) the DANGER and CAUTION areas are quite large. Note that you need to consider the CAUTION AREA as the effective footprint of your system, as you can't place any computer or instruments inside it, becoming the main limitation for using the space of your clean room or laboratory.

In the example, the magnetic footprint of the classical magnetic separator (left map) requires more than 3 m^2. By contrast, advanced biomagnetic separators show very little stray fields (right field map). For the same working volume (2 L vessel), the last has a magnetic footprint of just 0.15 m^2, occupying jus 1/20 of the space of the clean room or laboratory than the classical device. The reason of this different performance in safety is that advanced systems focus almost all the magnetic energy of the permanent magnets on the working volume, minimizing the magnetic field outside the device. This intrinsic safe design has allowed SEPMAG®'s customers to easily integrate biomagnetic separation systems up to tens of liters in their existing facilities without need of remodeling or special measures. This short danger distance also permits place different biomagnetic separators in the same lab, increasing production capability (duplicating the volume capacity) and/or flexibility (when different systems process use different volumes). Moreover, the ability to place instruments near each other has also allowed the development and integration of the optical monitoring system. Such option would have been more difficult to implement, especially for very large volume systems, if computers couldn't be placed near the permanent magnet assembly.

Conclusion

In this eBook we have compiled SEPMAG®'s experience in scaling up biomagnetic separation processes. Information has been accumulated since 2004, when the company began working on the first large volume system. SEPMAG® has summarized it's basic know-how in 7 key concepts. First and foremost, realizing that the importance of tackling biomagnetic separation issues at the very beginning of the development project, avoiding the common mistake of delaying the handling of the situation issues until further steps of the process. The awareness of the problems that can appear when scaling up would help to take the right decision at early steps and save troubles, time and money in later stages.

To make the right decisions, it is absolutely necessary to understand the magnetic force clearly and acknowledge that it's related with the spatial variation of the magnetic field and the magnetic moment of the beads, not just proportional to the magnetic field intensity. That leads to important considerations about the right magnetic field profile for having a constant magnetic force. It is also very important to fully understand that effective biomagnetic separation is a cooperative phenomenon: when the magnetic beads or particles move individually the movement is very slow (should be measured in hours). It is just when the dipolar magnetic interaction makes them move collectively that separation times are fast enough for practical applications (separated in seconds).

With this basic knowledge tools, we can select the right biomagnetic conditions for having a good in-lot consistency. Choosing the right magnetic field profile, the beads can be magnetically saturated and the force being constant over all the working volume. The absolute value of the force would depend on the specificities of the beads and its concentration. The separation speed would also depend on the buffer viscosity: separation on whole blood would be 3-4 times slower than water suspensions.

If the right conditions are applied at different scales, the magnetic beads will behave consistently (no losses, no irreversible aggregation) regardless of the working volume. The separation time only will need to be adjusted according the distance travelled by the farthest beads (i.e. vessel dimensions) and, for very large systems, corrected taking into account the different magnetic field gradient values.

Two more aspects are covered in this eBook as essential for successfully scaling up the biomagnetic separation processes. One is the monitoring of the process, as a key tool to objectivize the process validation and the lot acceptance. Measuring how the suspension transparency changes with time, we have the record of every single process, but also the possibility of generating reference curves. The deviations from the standard can be used as early Quality Control alert and the database of recorded curves for process auditory.

Lastly, the subject covered of safety during operation. The use of large amounts of permanent magnets rises legitimate concerns about the danger for the operators and the effect over equipment placed near the separator. By contrast with classical magnetic separators (or worst, big magnet blocks), advanced biomagnetic separators show very little stray fields, focusing almost all the magnetic energy of the permanent magnets on the working volume. That leads to very short danger area (where accidents can occur if people manipulates other magnetizable objects) and also small caution area (where magnetic record systems or pacemaker can be affected). This intrinsic safe design has allowed SEPMAG®'s customers to easily integrate biomagnetic separation systems up to tens of liters in their existing facilities.

SEPMAG® hopes this review of the basic issues regarding the scalability of the biomagnetic separation will help you to smoothly transition your process from the R&D to production. Our ultimate goal is that you may profitably cope with the current and future demand of your products, maintaining the highest quality standards.

About the author

Lluís M. Martínez - Chief Scientific Officer at SEPMAG®

Founder of SEPMAG®, Lluís holds a PhD in Magnetic Materials by the UAB. He has conducted research at German and Spanish academic institutions. Having worked in companies in Ireland, USA and Spain, he has more than 20 years of experience applying magnetic materials and sensors to industrial products and processes. He has filled several international patents on the field and co-authored more than 20 scientific papers, most of them on the subject of magnetic particle movement.

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