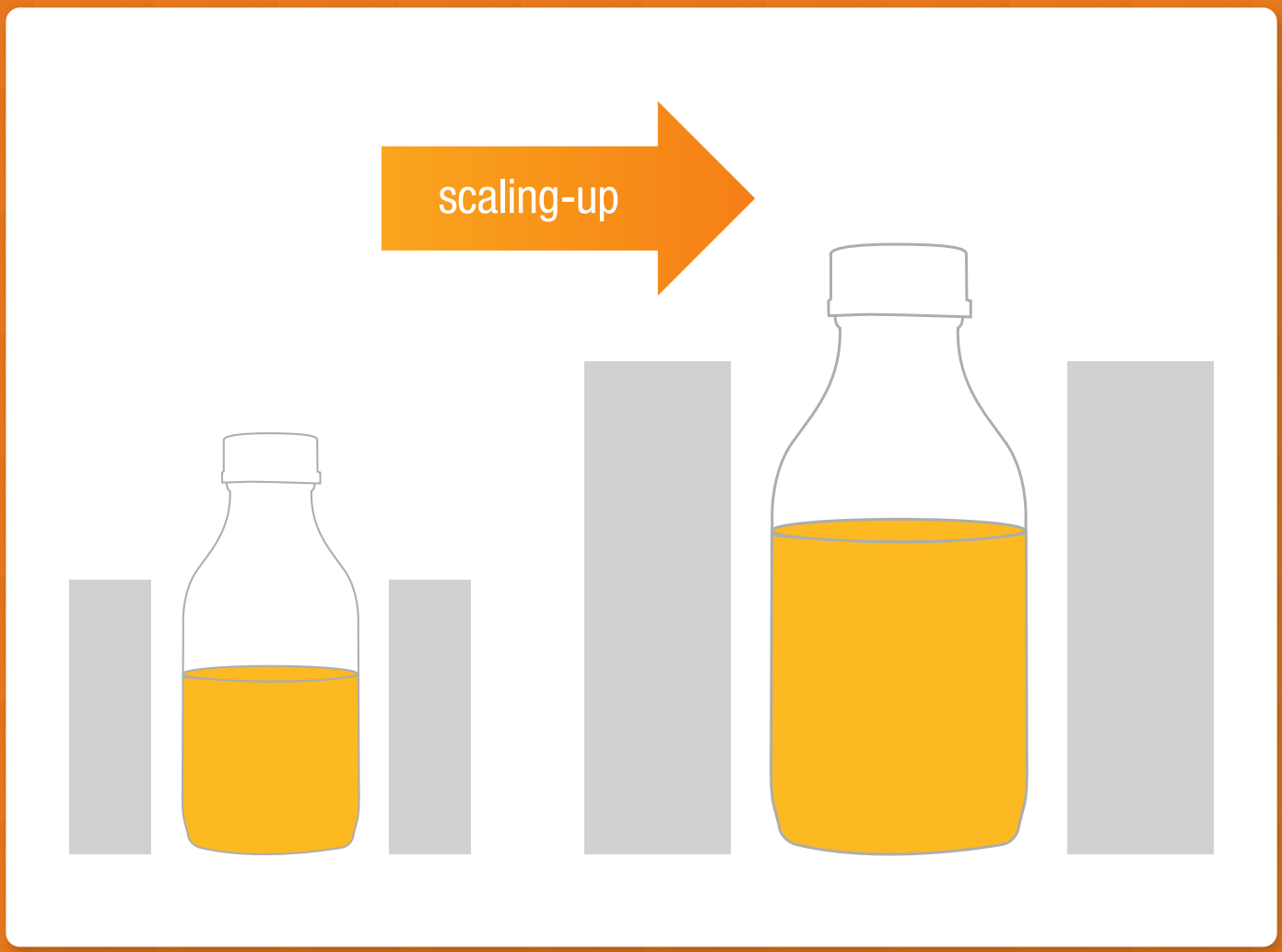
The Basic Guide to Scale-Up Biomagnetic Separation Processes



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SUMARY

1. Why do IVD Companies Scale Up Their Biomagnetic Separation Processes

When biomagnetic particle kits are initially developed, R&D companies work with small volumes in order to test and optimize a number of variables. When the kit is deemed successful, the company obviously wants to take the kit to market and consequently ramp up production. With just a few hundred milliliters of material, a company can generate a number of kits. However, as demand increases, the IVD company needs to be able to continually grow to cope with that demand. If the process cannot be scaled up, the biomagnetic separation process will become the bottleneck for the company.

At the point of slow market growth, the company can consider merely duplicating the number of lots. But having separate small lots can be problematic because the company must make sure the lots are consistent. They must quality control each lot and characterize each lot independently. If this type of production is pursued as the demand grows, resources such as those needed for the coating steps, resuspension steps and quality control steps will be prohibitively expensive for the company.

Increasing the lot volume once the demand increases is highly advantageous because it allows companies to:

- Minimize the number of quality control checks necessary – one would only have to QC check the large volume lot before it is aliquoted into kits.
- Decrease labor costs since the time necessary to produce a large volume of material is only slightly longer than or the same as the time necessary to produce a small volume of material.
- Easily trace kit problems to a particular lot since many kits come from the same batch.
- Enjoy all of the economies of scale.

A word of caution: All of the benefits of scaling up production of biomagnetic separation processes vanish if the conditions of the process are not well-defined.

Therefore, it is advantageous in many ways for a company to ramp up production as soon as they feel the market demands more supply of the product. This scale-up process must be done carefully so that large lots are not only internally consistent but as consistent as possible with each other.

2. Why Do Different Batch Volumes Require Different Magnetic Fields?

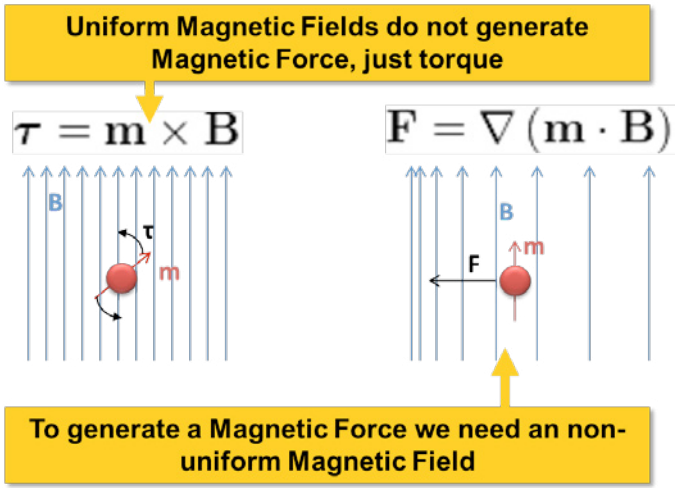
One of the most common mistakes people make when attempting to scale up non-homogeneous biomagnetic separation processes is that they tend to use the exact same magnetic field with different volumes. Simply put, this will not work.

Sometimes people relate the force generated by a powerful magnet directly with the high magnetic field that magnet produces. However, the intensity of the magnetic field is not the relevant parameter for determining the magnetic force. For example, if you have a homogeneous magnetic field that has high intensity such as the field generated by an MRI magnet, the assumption is that the intensity of this magnetic field determines the amount of magnetic force. If the field is homogenous in biomagnetic separation, no matter how high the field is, the force is equal to zero and particles will not move but will instead align with the field.

A magnet has a force because it generates a field that changes with distance. It is this change that produces the magnetic force. Depending on the magnetic characteristics of the beads the magnetic

force is related to either the gradient of the magnetic field or (if the field is very low) with the gradient of the magnetic field squared. Under any circumstance, however, the force will vary depending on the characteristics of the beads and the spatial variation of the magnetic field.

When a company needs to scale up a biomagnetic separation process, it is imperative that they do not pay attention to the magnetic field per se, but instead pay attention to how the magnetic field varies. For homogeneous biomagnetic separation systems, the force is constant and well-defined so scaling up is typically straightforward. For non-homogeneous systems, you need to take a close look at the exact conditions your system has (e.g. variation of the magnetic field, characteristics of the beads and characteristics of the magnetic field) before scaling up your process. When you do decide to scale up your process, you must scale up the magnetic force and not the magnetic field.



3. What are the Two Basic Questions You Must Answer Before Scaling Up a Biomagnetic Separation Process?

Scaling up a biomagnetic separation process without understanding the parameters of the process will lead to problems that cause the company to lose money and increase the product's time to market. Before scaling up any type of production process, it is important to know what parameters are relevant and important to the process itself so that you know which parameters you need to change or scale up.

For biomagnetic separation processes, the beads move because of the magnetic force which opposes the viscosity (i.e. drag force) of the buffer. Since the viscosity is well defined by the buffer, the key parameter is the magnetic force. So instead of trying to reproduce the same magnetic field for a scaling up process, you need to reproduce the same magnetic force.

The magnetic force depends on the change of the magnetic field over a distance (gradient of the magnetic field) and on the magnetization of the magnetic beads (magnetic charge). Therefore the two basic questions that one needs to answer before scaling up the process are as follows:

1. **What is the state of the beads?** Are the beads magnetically saturated with a fixed magnetic moment or do they have a constant susceptibility?
2. **What is the variation of the magnetic field on the working volume?**

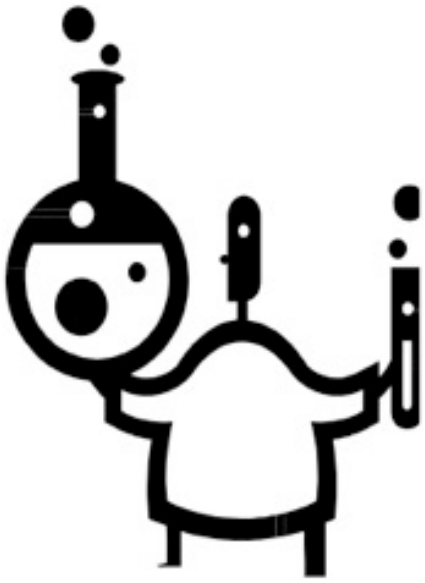
Unfortunately, if you are using a non-homogeneous biomagnetic separation system, it is very difficult to obtain a clear answer for these two questions. If you cannot clearly define the above parameters of the process, the scale-up is more likely to fail. A homogeneous system with homogeneous magnetic force is straightforward to scale up because the magnetic force is already well-defined.

4. Problems When Configuring a Large Volume Processing System?

Normally, when companies want to scale up a process, they try to simply use bigger systems. If they do not try to analyze and define the process well, there are four typical problems that they will encounter when attempting to scale up a non-homogeneous biomagnetic separation process.

1. Larger batches will not have the same characteristics as smaller lots. Often larger batches will have different bead surface areas activated, more beads that are clumped, and the density of beads in the final volume is different.
2. When you increase the volume without carefully defining the scale up process, you will have a greater loss of beads and biomolecules. Often you will find a 15-20% loss of material.
3. In larger volumes irreversible aggregation can be a serious issue, even if it was not an issue in smaller volumes.
4. Aliquots from the same batch for the individual IVD kits have characteristics that can vary much more than aliquots from smaller batches.

Therefore, it is important to understand the conditions of your process in order to avoid the above problems during scale-up. It is important to note that using a homogeneous biomagnetic separation system will help you avoid the above problems since the parameters are well-defined.



5. Why Can't You Simply Use a Bigger Magnet to Scale-Up Biomagnetic Separation Processes?

While it might be thought that 'bigger is better' during a scale-up process, merely using a larger magnet for larger volumes generates very different biomagnetic separation conditions which then leads to inconsistencies and other problems with the final product.

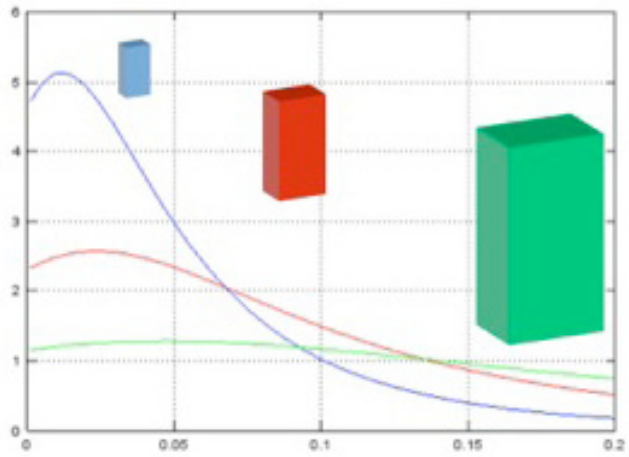
In small working volumes, acceptable conditions are worked out, but when scaling up, those conditions do not seem to work well. Specifically, when one looks at the gradient of the magnetic field, the magnitude of the gradient is related to the magnetic force when the beads are saturated. This gradient will vary greatly with the size of the magnet and the volume of the sample. For example, if the beads are in a 5 cm bottle (diameter), and a small magnet (with dimensions = 2 x 1 x 0.5 cm) is used for separation, the magnetic force would be between 3 and 5 T/m.

But if a larger volume is used such as a bottle with a diameter of 10 cm (~four times the volume of the above example), there is a temptation to use a larger magnet in order to counter the larger volume. If the magnet has dimension of, for example, 4 x 2 x 1 cm, the magnetic force experienced by the farthest beads from the magnet would be slightly more than 1.5 T/m and would never exceed 2.5 T/m. This

is always below the minimum value of the smaller volume example. Even if you increase the magnet size even further (e.g. 8 x 4 x 2 cm), this will not help because the force will be even weaker.

Therefore, just increasing the size of the magnet will not help separate beads in larger volumes. This is a problem that must not be solved by increasing the magnetic field, but must be solved by increasing the gradient of the magnetic field.

Because of this problem, scaling up of non-homogeneous biomagnetic separation systems is never straightforward. Thankfully, modern homogeneous biomagnetic separation systems solve this problem because the magnetic force is well-defined.

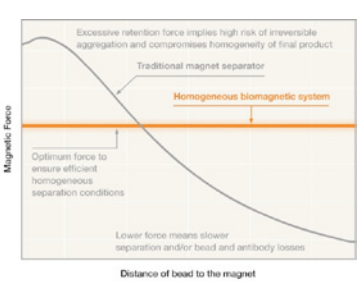


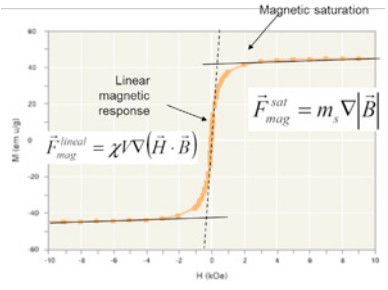
6. Why Do Larger Batches Not Have the Same Characteristics as Smaller Batches?

When a non-homogeneous magnetic separation device (a classical system) is used for scaling up a process, the conditions for a larger batch will completely change from the smaller batch. In these classical non-homogeneous separators, both the magnetic field gradient and the magnetic state of the beads (either linear or saturated) will vary depending on their position and distance relative to the magnet. Beads in a smaller field will have linear characteristics and beads in a higher field will be saturated. The magnetic field gradient also varies, often varying maximally at a particular distance and then becoming less variable again at a greater distance.

Even if the force distribution is good enough to generate optimal characteristics of the product (i.e. small losses with no irreversible aggregation), the use of a different device, either larger or smaller, all the parameters previously determined or observed will completely change for the new device. The force experienced, for example, by a particular percentage of beads will be different from one device to the other and will depend on their relative position to the magnets. This happens because the magnetic state of the beads will change due to the differences in the magnetic field intensity. It will be very difficult to reproduce the parameters over the entire new volume with a non-homogenous system. Because of this, the characteristics of the lot will be completely different from the initial lot. There will be greater aggregation and coating problems among other difficulties. And while it is possible that the final product's functionality will not be changed in any appreciable way, more often than not, variations of the magnetic force due to a different device has a huge impact on the performance of the final product.

In homogeneous biomagnetic separation systems, you have more control over the conditions since the parameters are well defined and easy to reproduce at various scales of production. Therefore the problems normally seen in scaling up with non-homogeneous systems do not manifest in homogeneous systems.



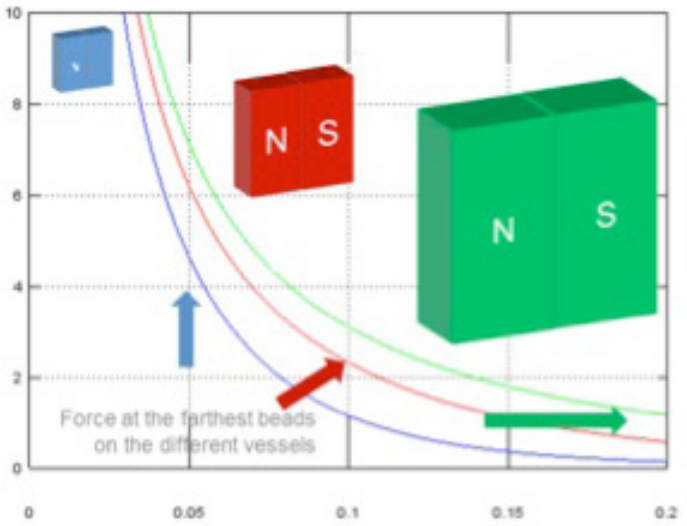


7. Why do Larger Non-Homogeneous Magnetic Separators Have Higher Losses of Magnetic Beads and Biomolecules?

During non-homogeneous separation, in order to generate a magnetic force, you need to use magnet arrangements to create a variation in the magnetic field. In this situation, the magnetic force will always vary with distance from the magnets.

For example, if you use a small system with a bottle diameter of 5 cm and small magnets, the farthest beads from the magnets experience forces of more than 4 T/m. If you increase the bottle diameter, the farthest beads will experience a smaller force. So in this situation you have the same magnetic field profile, but a longer distance which results in a smaller force at the outer boundaries of where the beads lie. If you then decide to increase magnet size, the force typically does not increase as quickly as volume size. Therefore, you will have less magnetic force, a slower speed of bead movement over a larger distance (i.e. larger volumes = longer distance the beads must travel to the retention zone). This situation requires either much longer separation times in order to get comparable losses to smaller production sizes or accepting larger losses at the same separation times.

On the other hand, homogeneous separation systems use a constant force throughout the entire volume of the vessel. Because of this it is much easier to determine how to scale up the process when one wants to use a larger vessel. In these systems, the separation time is then dependent only on the size of the bottle (i.e. the distance the beads must travel).



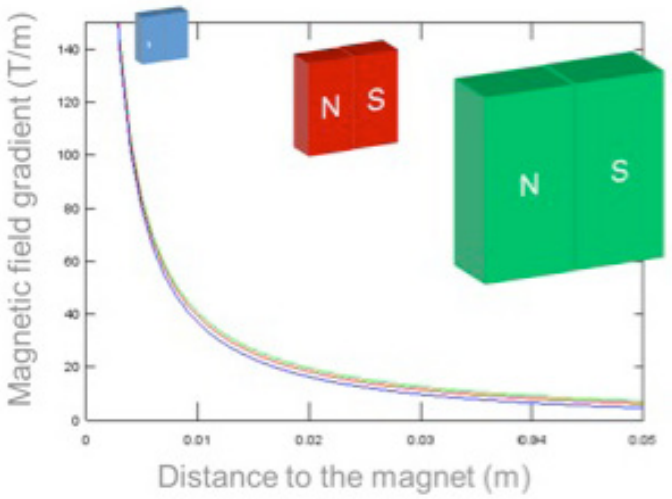
8. Why are there More Magnetic Bead Irreversible Aggregation Problems in Non-Homogeneous Magnetic Separators?

When companies desire to increase the volume and scale up their production using non-homogeneous systems, they use higher magnetic forces in order to separate the biomagnetic beads. As a result, the forces experienced by the beads nearest the magnet are extremely high. In addition, the time of separation also needs to be increased substantially when the volume is increased in order to collect an acceptable percentage of beads in a non-homogeneous system. In fact, the larger the magnet that is used in the system, the longer the separation time required to achieve the same or similar bead yield as in smaller production sizes.

Longer separation times combined with exposure to very high magnetic forces during that time results

in an increase in the formation of irreversibly aggregated bead clumps. This problem decreases yield and forces the use of extraordinary resuspension methods such as sonication. Sonication in order to resuspend these clumps adds steps to the separation process which results in a longer time to produce the lot, larger investment of time and money into the process, a longer time to develop the process and more resources necessary in order to validate the process.

You can try to avoid these problems by using a force that is low enough such that you will not be exposing beads to such a high magnetic force over time, but this cannot be done with non-homogeneous separation systems without greatly increasing magnetic bead losses or without using exponentially longer separation times. Using homogeneous separation systems, however, you can both decrease the force and decrease the separation time. The separation time in these systems will be proportional to the diameter of the vessel. Due to the mild force and short separation times irreversible aggregation problems are avoided when using homogenous biomagnetic separation systems.



9. When Scaling Up Biomagnetic Separation, Should You Use Electromagnets or Permanent Magnets?

At small volumes it is easy to create and use a quadripol electromagnet to generate high forces during biomagnetic separation processes, even to the point of making the process very close to homogeneous. The main advantage to using electromagnets for this type of process is the ability to easily modify the current passing through the magnetic coils, thus modifying the value of the magnetic field and force during the setup of your process.

At small volumes the number of ampere-turns (called the 'magnetomotive force') necessary to generate the force is quite small. The dimensions of the system coils are also small, so the resistance is small and the heat generated is negligible and can be easily dissipated (heat = (resistance of the coil) * (electrical current)^2.

As you increase volumes, the dimensions of the iron pole parts and the yoke increase proportionally to the working volume. In addition, the magnetomotive force (the number of ampere-turns) needs to increase in order to maintain a constant magnetic field gradient. This means that you need to increase the variation of the field proportionally with the radius of the vessel. It also means that you need to increase either the resistance or significantly increase the weight of the copper coil. Therefore the resulting electromagnet will be extremely heavy, would require a large amount of electrical power and the great amount of electrical power will generate a great deal of resistive heat that will need to be removed or dissipated. In order to remove the heat from a scaled up electromagnetic device, a cooling system will be necessary in order to control the temperature. Cooling systems require water flow, costing the lab more in development and maintenance costs.

In contrast, devices based on permanent magnets with modern materials are much lighter for the same working volume and magnetic force. No electrical power or cooling systems are needed with perma-

nent magnet systems, no maintenance, no power supply that can fail, and none of the other technical issues that are related to electromagnets (e.g. electromagnetic compatibility, interference, etc.) are seen with permanent magnets. The only reason one would want to use an electromagnet is if they do not know the conditions and will have to vary the conditions of their process on a small scale.

If you know the conditions, such as in a fixed homogeneous biomagnetic separation system and want to permanently scale up your process, however, permanent magnets are the most reasonable option.

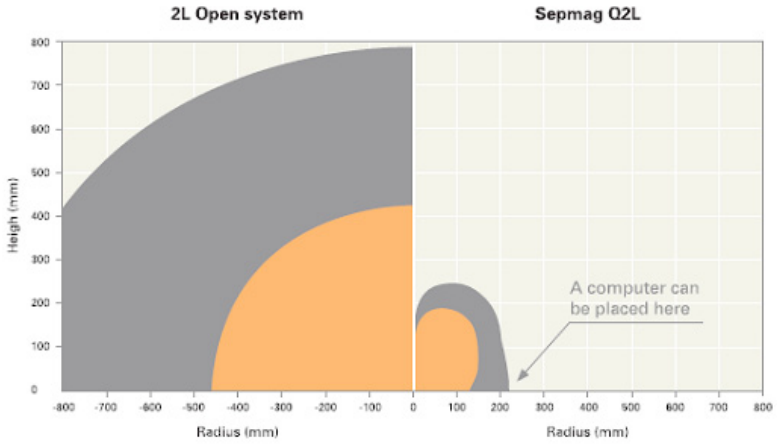
10. What are the Safety Issues with Large Magnetic Separation Systems?

When you have systems that have large standard open magnets that can generate stray fields, such as non-homogeneous biomagnetic separators, the devices can be very dangerous. The magnetic fields generated all around the device and are known to have caused laboratory accidents in the past. Scaling up a process means that the stray fields will increase quickly with the size of the device.

When you have a large standard non-homogeneous open magnet separator, you will need to have large 'Caution' areas around your device where certain people and items cannot go. People who have pacemakers, magnetic recording devices and devices with magnetic memory such as computers must be separated from this system.

In addition the 'Danger' area is also large and increases as the system increases. In this area, you will not be able to keep steel or magnetic materials in this zone (e.g. tools, scissors) because they can be attracted to the magnet and essentially be catapulted through the air, potentially harming individuals nearby.

By contrast, in a closed system such as in a homogenous biomagnetic system, where all of the magnetic field is confined in the working volume, there are only minimal stray fields no matter how large the system is. These closed systems take up much less counter space, do not require that you clear valuable lab space for large 'Danger' and 'Caution' zones and direct all of the magnetic energy toward separating beads instead of leaking large quantities of energy into the surrounding area. Therefore, a homogeneous biomagnetic system is safer and cheaper to house than a non-homogeneous magnetic system, especially when scaling up to much larger processing systems.



11. What would be my separation time at a larger volume?

Stablishing a separation time is the most usual way to specify a biomagnetic separation process. The problem is that this magnitude is not only related with the magnetic beads suspension, but also with the specific system used to perform the separation.

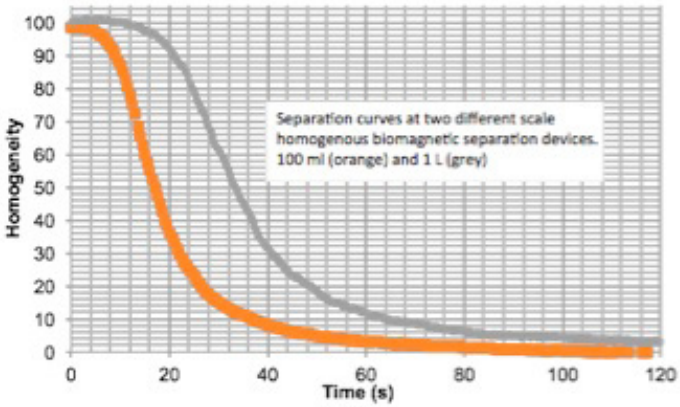
Notice that the separation time would depend on the distance travelled by the beads and its speed. Assuming a constant speed, the bigger the vessel, the larger the separation time would be. Then to keep separation time constant, the force should be higher, something not easy to achieve at a reasonable cost. Even if it would make economic sense to build the system with higher force for larger volume, using a higher magnetic force would imply a higher risk of irreversible aggregation and the separation conditions would be very different that the verified ones at small volumes. As consequence, is important to define the separation time for a given process, but trying to keep it constant at every scale is misleading. Separation time is a consequence of the applied magnetic force, that should be the parameter defining the behavior of the beads and avoid losses and/or irreversible aggregation problems.

The speed of the magnetic beads depends on the competition between the applied magnetic force and the drag force. The first would depend on the interaction between the magnetic moment of the beads and the profile of the applied magnetic field (remember you need an inhomogeneous magnetic field to have a force). The second depends on the viscosity of the buffer and the beads diameter.

For a given suspension (specific magnetic beads, at a given concentration in a defined buffer), we will need a constant magnetic force to have constant speed. Using classical magnetic separators, the force decrease with the distance, the beads closest to the magnet would move faster (higher force) than the beads farthest (lower force). Then, the separation time would be defined by the farthest beads. The bigger the vessel, thus the distance to the farthest beads. Still worst, as force decrease fast with the distance, the initial speed (force) would be lower as bigger is the vessel. For this reason, the separation time scales exponentially when the scale up is done using classical separators.

If we can generate a constant magnetic force, as advanced biomagnetic separation systems do, the separation time would scale proportionally to the distance travelled by the beads. That means it would be proportional to the diameter of the bottle is a cylindrical flask is used. For example, using the same magnetic force, the separation time using a 1 L borosilicate bottle (ø101 mm) would be 2.8 times the separation time using a 100 ml bottles (ø36 mm), even if the volume would be 10 times. Scaling from 100 ml to 10 L (ø227 mm) would imply to increase production volume by a factor 100 with a separation time 6.3 larger.

As the biomagnetic separation time is not the longer step on the process (for large volumes, pumping out the supernatant, adding the clean buffer and resuspending the beads are processes taking much longer than the few minutes of the separation), the magnetic force can be lower at larger volume systems. That helps to keep weight (and costs) of advanced biomagnetic separation systems. For this reason, when the plan is scaling up the process it is advisable not to use the maximal magnetic force available at small scale, but to replicate the conditions of the force available (at a reasonable cost) at very large volume.



12. How can I check if my scaling up has been successful?

The scaling up of a biomagnetic separation process is not an easy task if we use classical magnetic separators. In these devices, the magnetic force over the beads changes with the distance, thus the magnetic force profile is very different at different volumes. However, using advanced biomagnetic separation systems, the magnetic force becomes constant at all the working volume. Then the process scaling up is straightforward: you just need to use systems with the same magnetic force, and you would have the same separation conditions regardless the scale.

Even if physics give as a clear path for the scaling up, the requirements of the life-science industry requires experimental validation and, when possible, the monitoring of every single batch for guarantee the batch-to-batch consistency. Advanced biomagnetic separation systems incorporate electronic hardware and software to optically monitoring every single process. As the suspension is dark due the presence of the magnetic beads, when the separation is complete, the optical properties are the buffer's ones. Taking advantage of this change of transparency, the built-in sub-system allows checking if the separation is complete at the expected time (i.e. if the dark suspension has become transparent).

As discussed in previous chapters, for a given magnetic force and suspension, the separation time would depend on the vessel diameter. Monitoring the transparency changes of the suspension during biomagnetic separation process allow to determine if the separation is competed at the expected separation time. For a homogenous magnetic force system, the time-dependence of the optical changes has sigmoidal-like shape. Thus, the process can be parameterized and the expected behavior at larger volume can be checked.

With these tools (homogenous magnetic force and optical monitoring), the separation time of the larger volume can be determined by objective criteria -not depending on the subjectivity of a researcher/ process engineer- and the success/failure of the scaling-up can easily be validated.

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