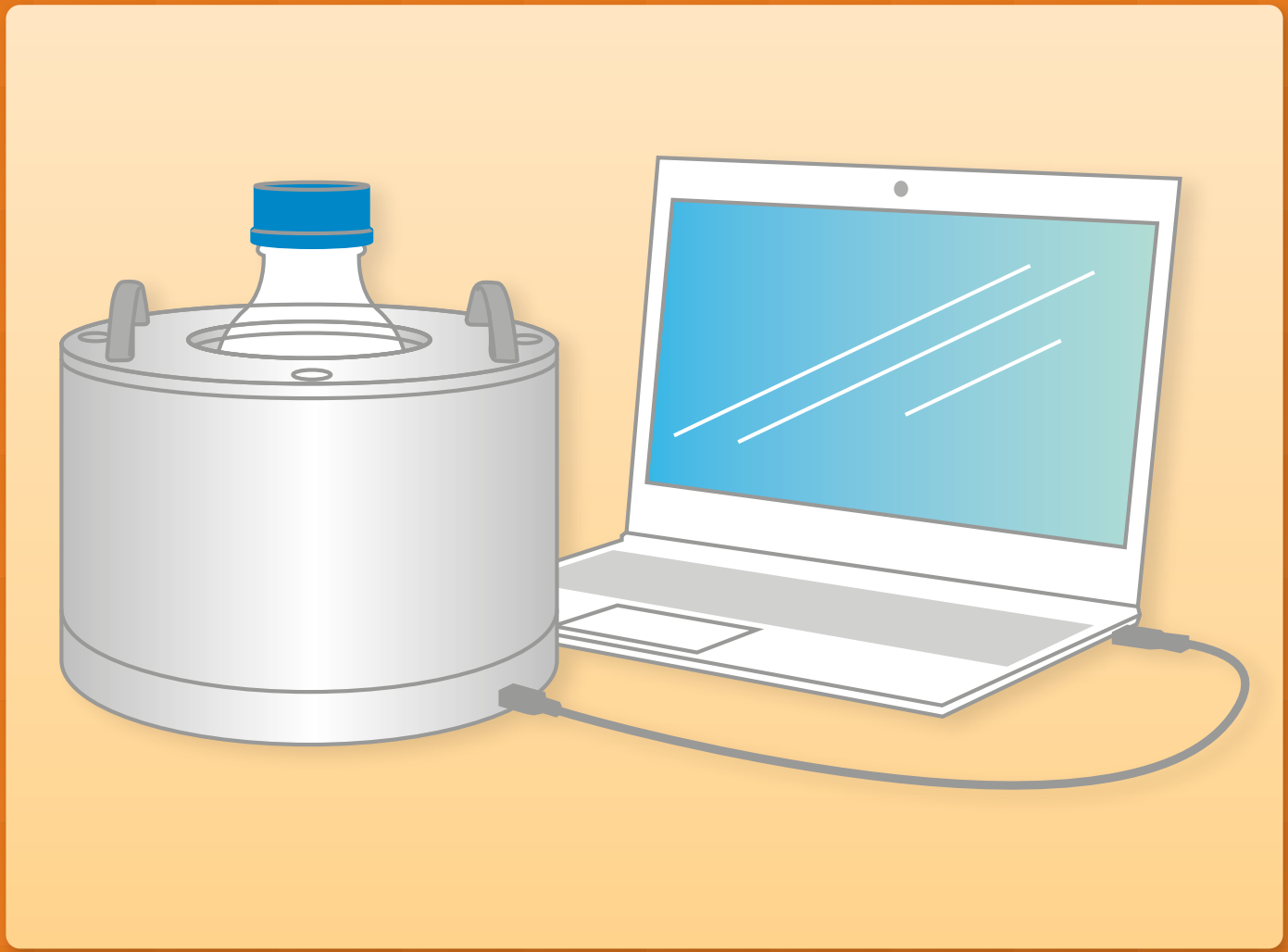
Quality Control: Real-Time Monitoring of Biomagnetic Separation Processes



Sepmag®

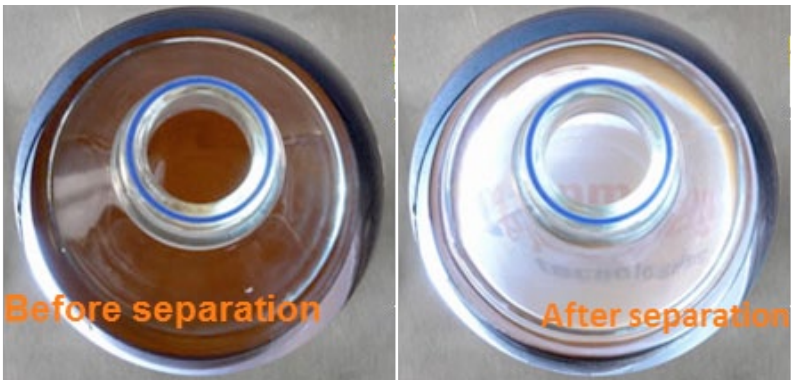
Quality Control: Real-Time Monitoring of Biomagnetic Separation Processes

The validation of each single production batch is a mandatory requirement at IVD-kits manufacturing. The lot-to-lot consistency is critical to assure consistent results when the reagents are used in the analyzers.

Since many steps are involved in the production process, the placement of **Quality Control points** is one of the more critical decisions, with large repercussion in the kits manufacturing costs. A single control point at the end of the process may seem cheap, but it implies that a 'no-pass' result would force the whole batch to discard, wasting all the time and resources invested on it. The other solution, having many QC along the different process steps would greatly increase the costs, as tests usually involve intensive labor and/or expensive analysis techniques. The key to have an efficient and cost effective QC protocol would be having the right number of test points, and whatever possible, use existing techniques not requiring intensive use of manpower.

As usually there are many separation steps (several washing before and after each conjugation), having them monitored provide inexpensive QC points along the whole process. To do it, however, we should update the way that Biomagnetic separation steps are usually validated. In many cases, these processes are validated solely by separation time. The technician checks by eye-sight the transparency of the suspension after the defined separation times, and if he/she found it OK, signs the form. No information about how the process has run is recorded. This single end-of time point control limits the possibility of audits if problems are detected in later steps.

For CLIA IVD-kits manufacturing (or any products involving magnetic beads), we can take advantage of the biomagnetic separation process itself to check the magnetic beads behavior

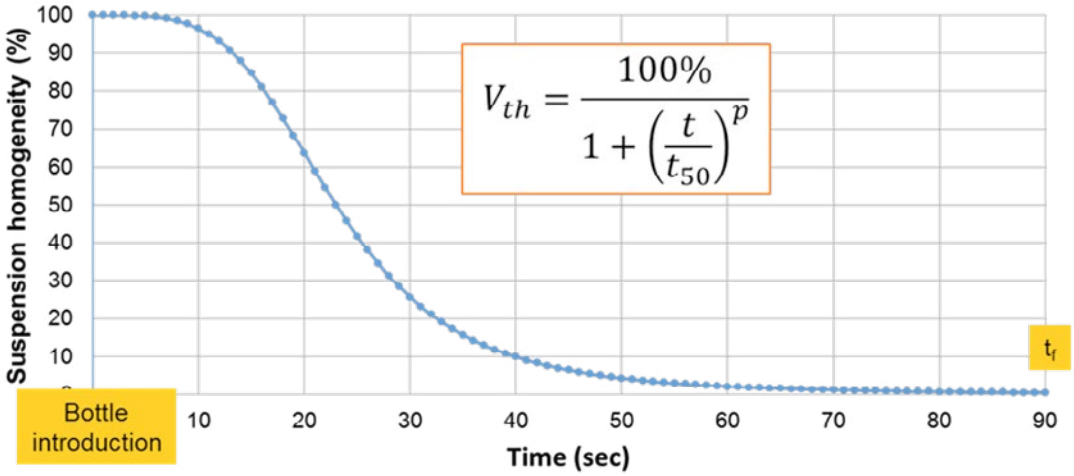


To address this issue, **Advanced Biomagnetic Separation systems** monitor transparency changes during the whole separation process. The resultant curves give a quantitative value of the transparency at the end of the process, and also show how it changes since the vessel is introduced in the magnetic separation rack. Properly recorded, these graphs would allow comparing the successive batches, and generating reference curves.

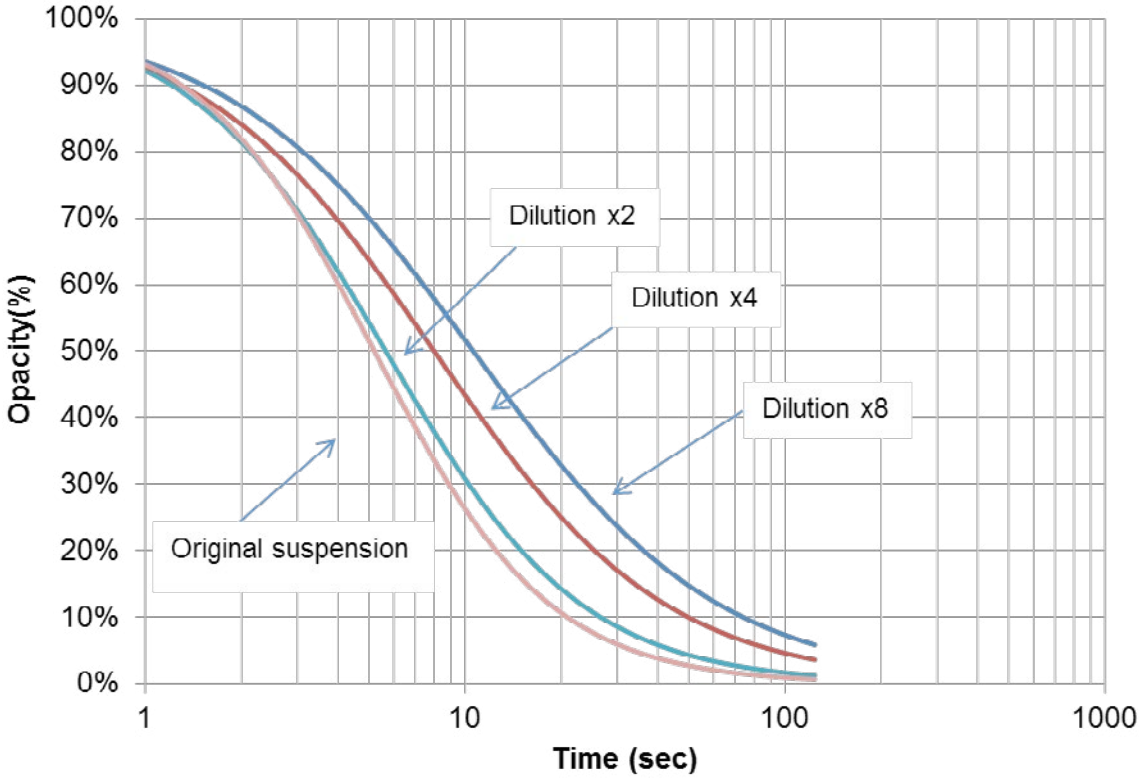
Having the Biomagnetic Separation System equipped with optical monitoring, implies having an additional QC point for each single washing step, without the need of external equipment or additional labor costs. Any change of the properties of the magnetic beads (diameter, magnetic charge), the buffer (viscosity, iconicity, beads concentration) would affect the separation behavior. Any deviation from the expected curve can be used as an alarm. It would allow stopping the batch before incurring in additional costs and/or make the corrective actions when possible.

When using a constant magnetic force in all the working volumes, the opacity versus time curve typically has a sigmoidal shape. The curve can be parameterized just by the two values that define this curve: the exponent *p* and the time t_{50}.The first reflects the *'steepness'* of the curve and the second reflects the time it takes to reach 50% of the difference between the maximum and minimal opacity. These two parameters change on a different way depending on what different magnetic bead (diameter, % magnetic content, magnetic material) or the suspension (buffer viscosity, beads concentration, ionicity) characteristics vary.

If the Biomagnetic Separation System has well-defined conditions (i.e. homogenous force), the recorded curves will not only indicate that something is wrong, but also help identify the specific problem.



On a typical example, the changes in the concentration would affect the curves modifying both the exponent *p* and the t_{50}. The biomagnetic separation is a cooperative behavior where the beads interact between them through magnetic dipolar interaction (overcoming the thermal agitation). The higher the concentration the nearer the magnetic beads would be to the nearest neighbor. As a consequence, diluting the sample slows down the separation. This behavior is reflected in the transparency changes by a lower is *p* (the curve is less 'stepper') and a higher t_{50} (slower separation) for diluted samples when compared with the original suspension.



How to check if magnetic beads clumps are formed during Biomagnetic separation processes?

When manufacturing CLIA IVD-kits, one of the main problems during the successive steps (coating, washing...) is the formation of irreversible aggregates, usually due to the excessive magnetic retention force during the separation process.

If the magnetic beads are not well re-suspended, clumps are formed and not all the surface is exposed. This leads to inhomogeneity in the coating if the problem appears in earlier manufacturing steps or in larger variability on the reagent reactivity if it happens in the latest. Clumps are also a big problem in magnetic beads analytical uses, as protein purification for screening, or any other application where the final product need to be aliquot in small volumes.

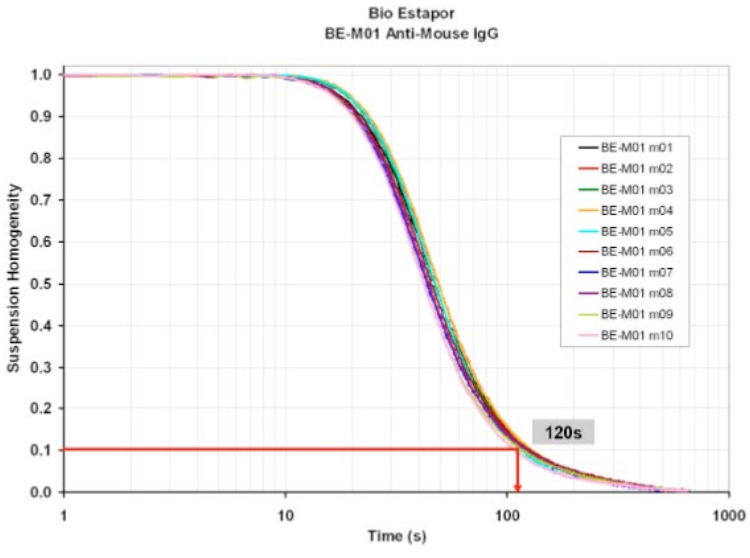
As discussed in previous eBooks, the first action should always be minimizing the risk of irreversible aggregation by using the right magnetic retention force. To avoid trade-offs between losses (or long separation time) and clumps formation, homogenous biomagnetic separation conditions is the best options, as it will increase the force far from the retention area –thus accelerating separation- without need of excessively high values at it.

However, even if the problem is theoretically eliminated by using advanced Biomagnetic separation systems, the existence or not of 'clumps' should be experimentally verified. The classical way is to check the RLU (**Relative Luminescence Units**) variability of the test after finishing all the manufacturing steps.

The already discussed alternative (or complementary) way is to monitor each biomagnetic separation step by itself.

The biomagnetic separation monitoring tools can been used to validate re-suspension protocols. One example is the experiment we did to show that small diameter Anti-mouse IgG magnetic beads can be used with **SEPMAG® Biomagnetic Separation Systems** without the need of any sonication step. Avoiding the use of ultrasound is key to simplify the scaling up of the process beyond the milliliters volume.

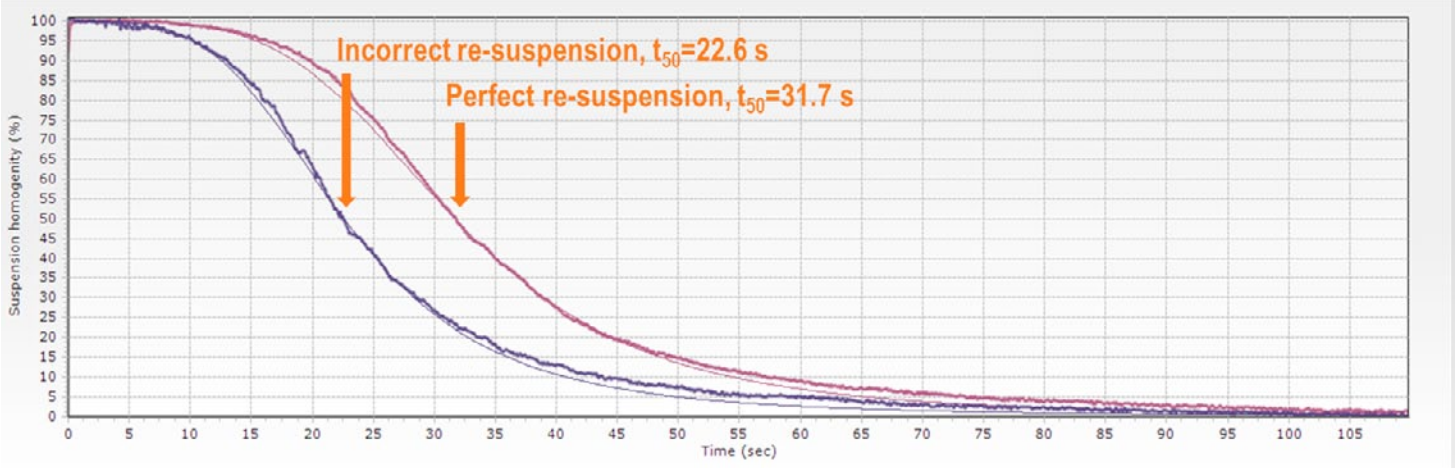
To check if the protocol generates clumps, the same suspension was separated, then re-suspended just by agitation, and then separated again, up to a total of 10 separation/suspension cycles. The SEPMAG® had a carefully chosen homogenous magnetic force which makes the separation fast, and simultaneously claims that the value is gentle enough to avoid clump formation. The recorded optical curve does not show changes. The monitoring process demonstrated the feasibility of the re-suspension protocol without sonication for this magnetic beads and magnetic separation rack.



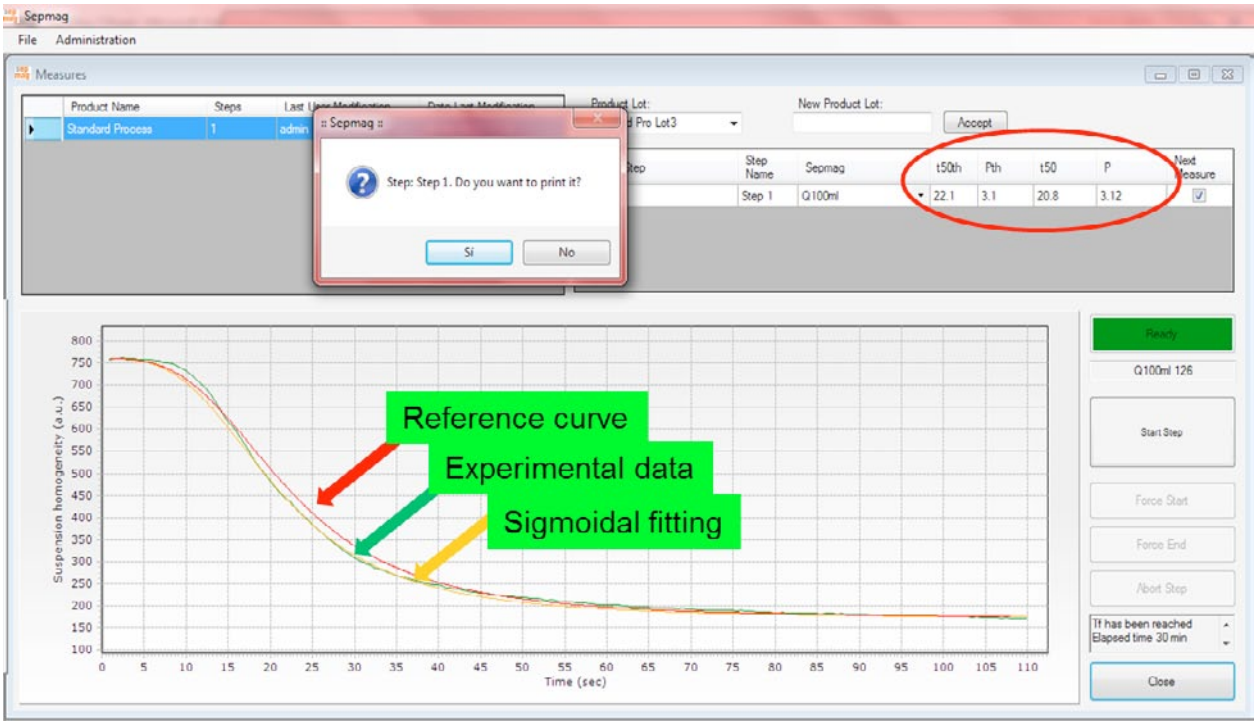
This experiment was important because the re-suspension problem is one of the biggest problems when manufacturing needs large batch volumes. When the vessels have more than a few milliliters, the use of sonication is complex as it is necessary to use probes to transmit the ultrasound energy to the suspension. Besides the risk of contamination, the repeatability of the process becomes complicated, making it necessary to double check if the clumps appear.

Basically, if you have clumps, the magnetic separation is equivalent to having beads of larger diameter and the process is faster. Unfortunately, the usual SOP (**Standard Operation Procedures**) only checks visually if the separation is complete at the defined separation time. Even if the clumps accelerate the separation time, it would be impossible to detect by eyesight at the final specified time, as both suspensions would be crystal clear.

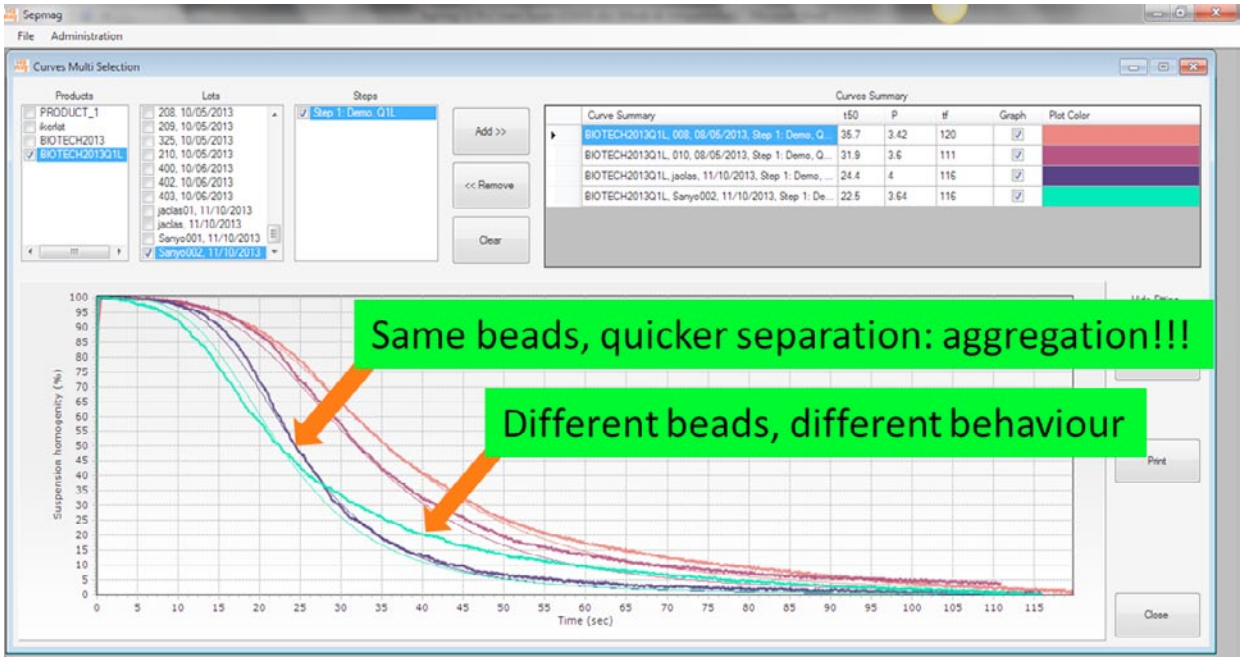
The dynamics of the magnetic beads are very different when the beads are well re-suspended and when clumps are formed.



By recording the transmitted light across the vessel, we can monitor the transparency changes during the separation process, showing the describer sigmoidal-like behavior. As shown in the figure (real case), using the same suspension in the same **SEPMAG® Q1L**, the time is reduced by almost a 30% when beads have become aggregated due to a bad storage protocol.



We can take advantage of this behavior to stablish quality control protocols that can indicate re-suspension problems during the biomagnetic separation steps. A reference curve can be generated for each step and the experimental curve obtained during the process. Any significant deviation from it would be an early indicator of quality problem. That may allow stopping the batch and/or take corrective actions before going ahead with the following steps, avoiding the cost and delays to detect the problem and later on the whole manufacturing process.



The optical monitoring of the biomagnetic separation process gives us much more information than just the separation time. Besides giving us a methodology to objectively determine this parameter –i.e., not dependent on subjective eye-sight-, the measured curve would be an early alert for many quality control problems. Its shape would change not only as a result of the clumps formation, but also viscosity or concentration, or if the magnetic beads have different diameter or content of magnetic content.

When the entire process is monitored rather than separation time alone, Quality Problems can be identified more quickly.

Deviations from the reference curves may reveal numerous production problems (aggregation, incorrect bead characteristics and incorrect concentration) that can be detected during the Biomagnetic Separation step. This means corrective actions can be taken sooner, thus reducing costs.

In future documents we will discuss with more detail how different production problems affect the optical curve, so that the reader can have a basic guide to detect the root cause of the non-conformity of the batch.

ABOUT THE AUTHOR

Lluís M. Martínez, Chief Scientific Officer at SEPMAG®

Founder of SEPMAG®, Lluis holds a PhD in Magnetic Materials by the UAB. He has conducted research in German and Spanish academic institutions. Having worked in companies in Ireland, USA and Spain, he cumulates more than 20 years of experience, applying magnetic materials and sensors to industrial products and processes. He has filed several international patents on the field and co-authored more than 20 scientific papers, most of them related with the movement of magnetic particles.
martinez@sepmag.eu

www.sepmag.eu

sepmag®

Follow us on:

SEPMAG SYSTEMS
contact@sepmag.eu
+34 935 820 161

Parc Tecnològic del Vallès
E-08290 Cerdanyola del Valles. Barcelona